# STATISTICAL ANALYSIS PROTOCOLS

for

# ASSESSMENT OF IMPACTS OF BRINE DISCHARGE FOR THE STRATEGIC PETROLEUM RESERVE PROGRAM

## FINAL REPORT

Submitted to:

**U. S. DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
**Environmental Data and Information Service**
**Center for Environmental Assessment Service**
**Brine Disposal Analysis Program Office**

Submitted by:

Ecology Simulations Inc.
Box 4200 Campus Station
Athens, GA 30602

and

Science Applications Inc.
Jackson Plaza Tower
Suite 1000
Oak Ridge, TN 37830

August 5, 1981

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# I. INTRODUCTION

The analysis of brine disposal impacts might be addressed by focusing one or both of the following questions:

(1) Are there any differences between or among measurements taken at a station before and after brine discharge?

(2) Are there any differences between or among measurements taken inside and outside of the region of brine discharge?

Addressing the first question involves the use of data taken at the same station (and in the same manner) at two different periods of time. These could be periods just prior to and after discharge, or the pre-disposal period could be the same time of the year as the discharge period, but during the previous year. Because brine discharge at Bryan Mound has begun slowly, the option of comparing stations just prior to and after discharge is not really feasible. That is, there was no clear cut and rapid transition from pre- to post-discharge. The other option for answering the first question (same month, different years) is also not feasible. Based on the analyses presented in Task I and II of this project, and the results presented in Appendix A, it appears that the great temporal variability in the composition of the biota of the nearshore gulf disallows any close correspondence of monthly data from year to year even though overall, seasonal trends are definitely distinguishable and generally recur from year to year. Prior analyses have shown that the temporal variability in the nekton and benthos communities at the offshore disposal site were far greater than the spatial differences (Comiskey et at. 1980). Even diurnal trends explain a considerable amount of the variance for some species in the nekton community.

The final report for the Bryan Mound predisposal studies (Hann and Randall 1980) indicated that there were some problems in coordination of diffuser and station locations and a number of sampling arrays (see benthos and phytoplankton methodologies discussed in Section III.) had to be relocated. Therefore, for at least these groups, no stations which are being monitored during the discharge phase of the program were sampled during the previous year.

In summary, the first question can be statistically addressed, but any significant pre- and post-discharge differences which are found cannot be conclusively attributed to brine discharge because, in all cases, the predischarge samples will include temporal variability that cannot be isolated from the effects of brine discharge.

The utility of the second question in addressing brine discharge impacts rests on there being an appropriate "control" station (or stratum) outside the area of brine impact. The offshore site at Bryan Mound does not show as much spatial (hydrographic and substrate) variability as does the nearshore site. For the benthos and nekton, the results of previous analyses (Comiskey et al. 1980) indicate that the offshore site is relatively homogeneous with respect to community distribution. Therefore it seems feasible to utilize sampling stations within and outside of the area of increased salinities to assess brine discharge impacts either for a specific cruise or over a number of cruises during the period of brine discharge. One must keep in mind, however, that the brine plume follows the ambient current regime, and, as such, could conceivably impact a larger area than that covered by the plume at any one time. For the plankton there would be no cumulative impact since these organisms are not permanent residents of the diffuser site and are only "passing through".

This dismissal of predischarge data for impact assessment should not be regarded as a lack of support for predischarge (baseline) monitoring. Baseline monitoring is regarded as an important component of the overall impact assessment scheme, supplying information upon which a sound impact assessment sampling design can be based. The analysis plan presented in Section IV represents a structured approach toward uniting baseline analysis and the more formal hypothesis testing activities associated with quantitative impact assessment.

## II. RELATIONSHIP OF THE ANALYSIS PROTOCOLS TO THE GOALS AND OBJECTIVES OF THE PROGRAM

As stated in the Task Descriptions, the objective of Task IV is:

> "The development of a set of statistical tests which, utilizing the framework of the Bryan Mound and regional models, will test the ecological significance of any outlying, post-brine discharge data observations..."

The Task Descriptions provide further guidance to the type of analytic tests appropriate to Task IV.

> "...Such tests might include: a) inference for difference of means and proportions of pre- and post-discharge population value estimates, b) multinomial tests for the significance of pre- and post-discharge changes in relative abundance within trophic levels of various trophic components where, for example, the trophic level would be defined as the "population" with each component species comprising a given proportion (or ratio) of the total, and c) analysis of variance techniques to test the significance of population changes over three or more years, intersite population similarity testing, etc... Significance levels, including Type II errors, should be chosen based on the ecological logic of the conceptual model and data quality and quantity..."

Several references are made in the Task Descriptions to the Bryan Mound and regional models as a framework for hypothesis testing. Unfortunately, no static or dynamic modeling outputs were available for guidance during the preparation of this document. However, SAI has performed a variety of (multivariate) analyses in the Strategic Petroleum Reserve (SPR) program that have as one of their major purposes, the guidance toward hypothesis testing. These are the types of analyses which should be conducted during any monitoring program where the purpose is to provide a pre-discharge baseline for impact assessment. These analyses have been conducted in the context of an overall analytic system which is responsive to the needs of baseline and impact assessment programs. Therefore, rather than rely on modeling results which are not available, we will present an overall analysis scheme which SAI has developed over the last four years in the SPR and other programs. This scheme is a hierarchical process where each level of analysis contributes information, data sets, and data products to direct subsequent analyses, with the end product being the testing of hypotheses concerning environmental impacts.

To facilitate the understanding of how the various types of analyses fit into the overall analysis scheme, we have attempted to carry several data sets collectively through the entire analytic procedure, and the results are presented in Appendix A. Some of these analyses were conducted especially for this study, while others were conducted by SAI during other SPR studies. Ideally, one data set should be carried through the whole scheme, but no Bryan Mound studies included the variables required to show examples of all aspects of the analysis scheme. For example, for the nekton, no ancillary environmental variables were available and for the benthos, no sediment parameters were available until very late in the program.

Although the analysis scheme includes five different levels or categories of analysis, only those dealing with basic descriptive statistics and hypothesis testing are directly germane to the objectives of Task IV. Therefore, in addition to presenting the overall analysis scheme with representative data products from the SPR program, we will outline the applications of basic descriptive statistics and hypothesis testing to the Bryan Mound biological data and will provide to NOAA a delineation of the software needed to conduct these analyses. This will be accomplished within the context of a statistical critique of the Bryan Mound monitoring efforts (Section V).

The parametric statistical techniques implicity called for in the Task Descriptions are as follows:

a) t-tests
b) discriminant function analysis
c) analysis of variance

Programs to conduct these analyses are included in STAT-PACK as discussed in Section VI. In our presentation of the SPR analysis protocols (Section IV), we have attempted to place these specific tests into the framework of an overall analysis scheme. The Basic Descriptive Statistics and Hypothesis Testing components of the analysis scheme include a number of techniques which, while not essential to fulfilling

the requirements of Task IV, are useful in defining the basic statistical character of the data and in providing guidance to hypothesis testing. We have delivered to the National Oceanic and Atmospheric Administration (NOAA) the software to conduct these analyses. We have provided these programs (as discussed in Section VI) in standard FORTRAN IV with complete documentation, detailing how the programs can be used and integrated into the NOAA system. Each program is delineated in detail in Appendix B. In summary, the software delineated by SAI consists of those applicable software already on NOAA's computing system, supplemented by software written by SAI.

There are many instances where meeting the goals of the analysis program requires the ability to subset the data temporally, spatially, and taxonomically (e.g., trophic groups as "populations"). This ability is, of course, realized in the data management system which we have also supplied to NOAA under Task II of this contract.

## III. THE DATA BASE

Four sets of biological data are currently being collected at the Bryan Mound brine disposal site. In this section these four data sets are discussed in greater detail with regard to those features of experimental design, collection methodology and sample processing that are pertinent to statistical characterization. Most of the discussions are taken directly from Texas A&M University (TAMU) reports for the Bryan Mound site (Hann and Randall 1980, 1981).

### III.1 Benthos

The station array at the offshore diffuser site is shown in Figures 1 and 2, and the coordinates for the 19 stations are given in Table 1. The original sampling design involved fifteen stations (1-15), with four stations added in October 1979. According to the TAMU final predisposal report (Harper and McKinney 1980), the rationale for choosing this pattern was to align the major transect (A) northeast and southwest, in accordance with the predominately longshore current flow. Stations 1-7 comprising Transect A were established to reflect the expected longshore salinity gradient or duration of exposure (greater effort to the west) once brine discharge begins. Station 5 is located at the discharge point. Stations 4, 3, 2, and 1 are 500, 1000, 2000, and 4000 meters downcurrent from Station 5. Stations 6 and 7 are 1000 and 2000 meters upcurrent from Station 5.

The lesser transects, D (Stations 8-10) and E (Stations 11-13), are oriented parallel with Transect A, while Stations 5, 9, and 12 along with the peripheral Stations 14 and 15 provide a WNW trending transect, which is approximately onshore-offshore. Figure 2 also illustrates that the stations are arranged roughly in two rings of 1000 m and 2000 m radius, respectively, from the center of the discharge area.

The offshore site was first sampled in December 1977, and was sampled monthly without interruption through November 1978, yielding a full year's data. No sampling was conducted during December of 1978 but sampling was resumed in January of 1979 and continues to the present.

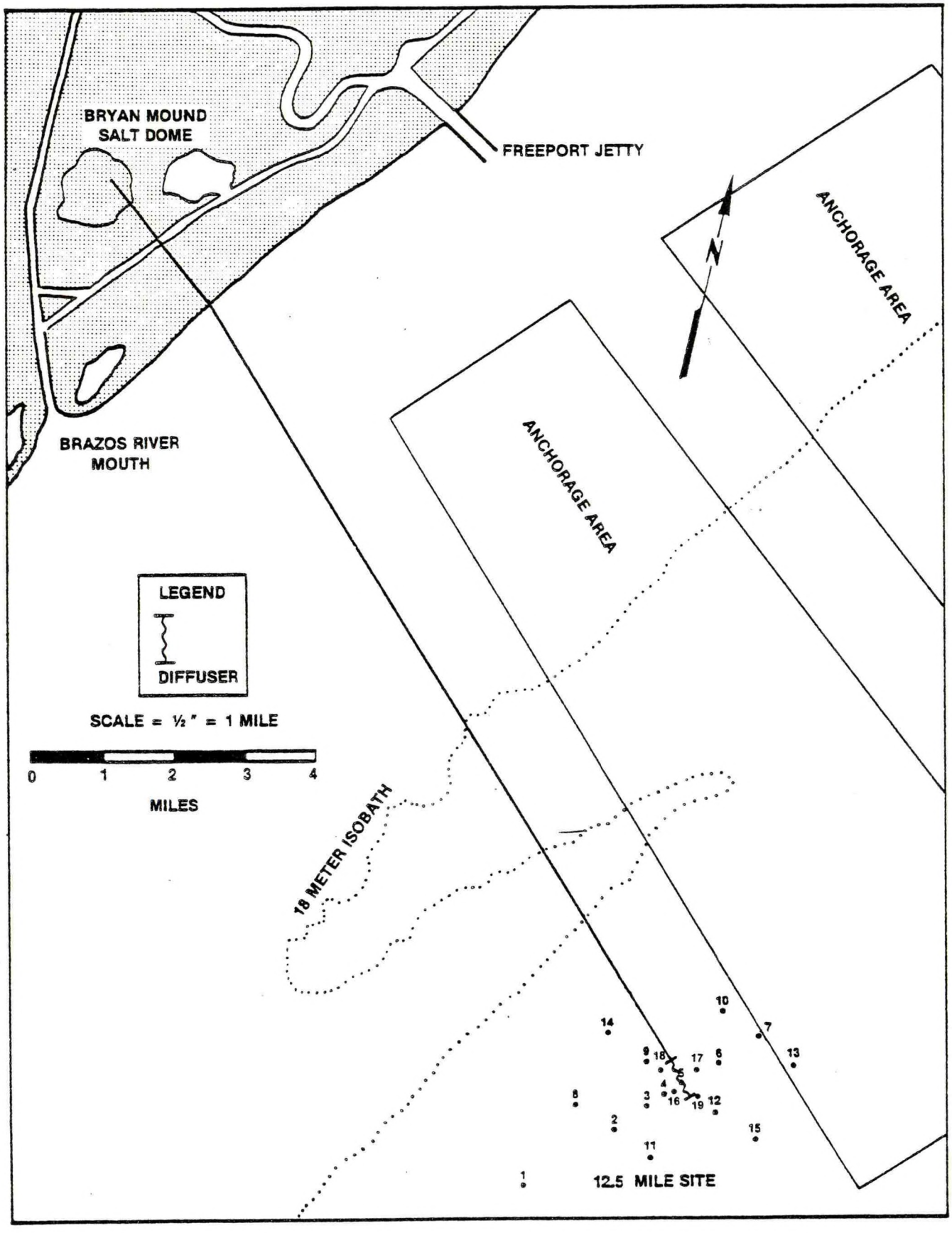


**Figure 1.** Map of the area offshore from Freeport, Texas showing the location of the offshore benthic study area.

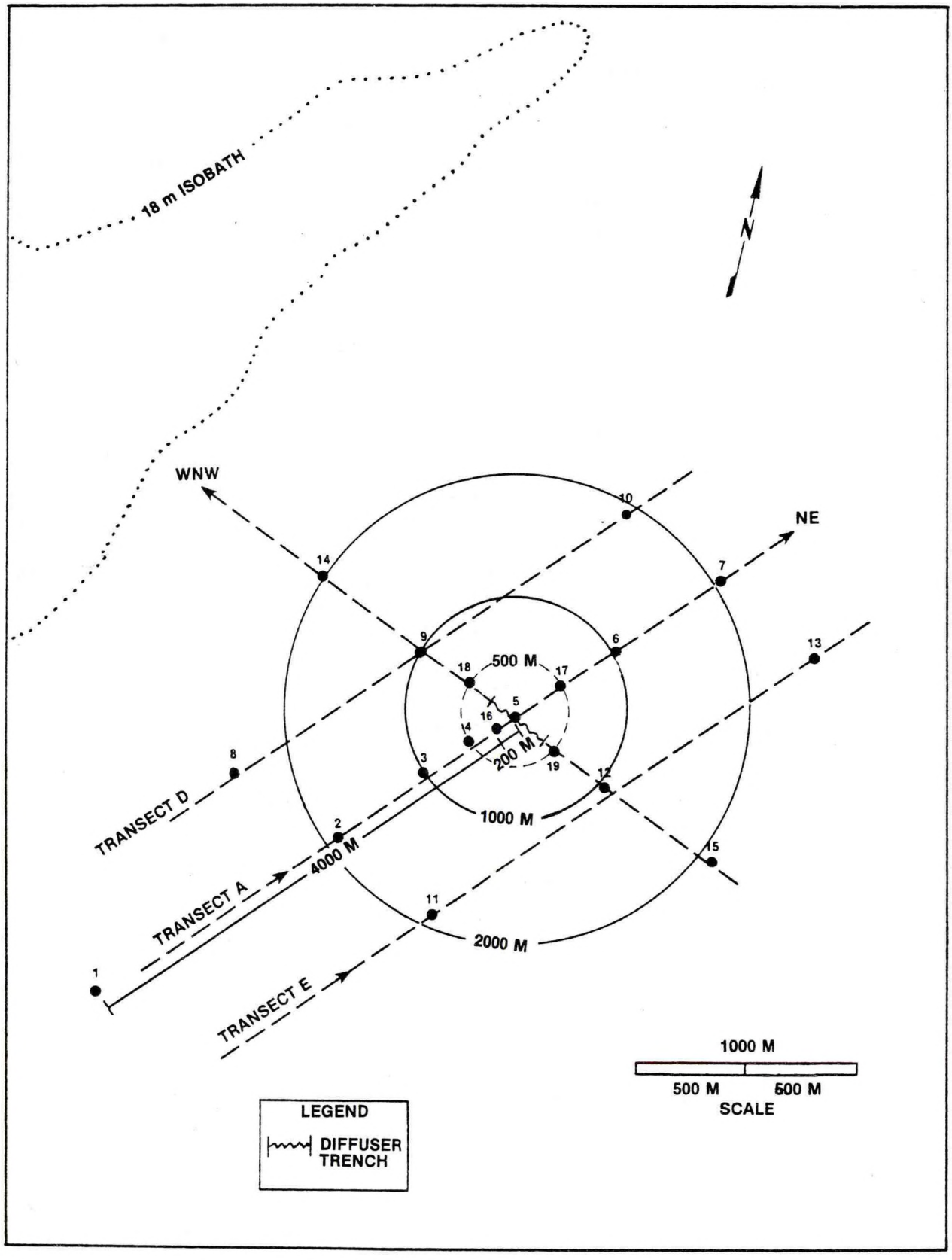


**Figure 2. Spatial display of benthic stations at the 12.5 mile diffuser site for Bryan Mound, indicating major transects.**

**Table 1. Summary of benthic station locations at the 12.5 mile diffuser site for Bryan Mound.**

| | Pre-October 1979 | | Post-October 1979 | |
|---|---|---|---|---|
| Station Number | Latitude (N) | Longitude (W) | Latitude (N) | Longitude (W) |
| 1 | 28°42'32" | 95°16'29" | 28°42'42" | 95°16'38" |
| 2 | 28°43'12" | 95°15'12" | 28°43'27" | 95°15'32" |
| 3 | 28°43'30" | 95°14'49" | 28°43'46" | 95°15'03" |
| 4 | 28°43'38" | 95°14'30" | 28°43'58" | 95°14'48" |
| 5 | 28°43'54" | 95°14'15" | 28°44'08" | 95°14'28" |
| 6 | 28°44'10" | 95°13'51" | 28°44'28" | 95°14'04" |
| 7 | 28°44'26" | 95°13'09" | 28°44'48" | 95°13'35" |
| 8 | 28°43'24" | 95°15'30" | 28°44'03" | 95°15'47" |
| 9 | 28°43'51" | 95°14'32" | 28°44'33" | 95°14'57" |
| 10 | 28°44'32" | 95°13'27" | 28°45'06" | 95°14'01" |
| 11 | 28°43'02" | 95°14'42" | 28°43'09" | 95°15'05" |
| 12 | 28°43'37" | 95°13'57" | 28°43'41" | 95°14'10" |
| 13 | 28°44'09" | 95°13'57" | 28°44'12" | 95°13'18" |
| 14 | 28°44"32" | 95°15'40" | 28°44'59" | 95°15'19" |
| 15 | 28°43'06" | 95°13'00" | 28°43'17" | 95°13'47" |
| 16 | | | 28°44'05" | 95°14'37" |
| 17 | | | 28°44'18" | 95°14'18" |
| 18 | | | 28°44'20" | 95°14'45" |
| 19 | | | 28°43'53" | 95°14'23" |

From Hann et al. (1980)

Harper and McKinney (1980) report that from December 1977 through November 1978, the relatively low resolution (±400 m) LORAN A navigation system was used to locate stations. Since December 1978 a LORAN C navigation system has been used. Between December 1978 and September 1979 the LORAN C coordinates were based on LORAN A coordinates.

When the diffuser was installed, it was determined that Station 5 (center station) was about 600 m seaward of its intended location (the center of the diffuser) and the entire sampling array was shifted inshore. These "new" stations have been sampled since October 1979. Concurrent with the station relocation, four additional stations were added to the 15 existing offshore stations. Stations 18, 17, and 19 were located 500 m inshore, northeast (upcurrent) and offshore of Station 5 (at the diffuser), respectively. Station 16 was located 200 m southwest (downcurrent) of Station 5 (Figure 2). The reason for the addition of these stations was to enhance the ability to detect nearfield biological stress.

Sampling utilized three Ekman grabs (232 $cm^2$ each) attached to a line having an anchor at one end and a buoy at the other. When the vessel came on station, the line with the attached grabs was dropped overboard while the vessel was in reverse. This caused the anchor to dig into the bottom and the grabs to spread apart. The vessel was brought near the buoy and the divers entered the water and descended the line to the bottom. The grabs each representing 0.023 $m^2$ or 1/43 $m^2$ were pushed into the bottom by hand, triggered, and the vent flaps secured with a strong, elastic band. While on the bottom, the divers collected a water sample (Harper and McKinney 1980).

The temperature and salinity of the bottom water samples were recorded as soon as the divers returned to the vessel. The temperature and salinity of a surface water sample were also recorded. Since October 1978 dissolved oxygen content of bottom water was measured at every station.

When the grabs were brought aboard the vessel, the contents were placed in plastic tubs and the sediment temperature was recorded. The samples were then washed on a 0.5 mm mesh sieve to remove sediments, and the material remaining on the sieve was preserved in 5 percent seawater formalin.

Sediment texture was only qualitatively assessed during the 1977 and 1978 collections. Since January 1979, sediment samples have been collected at stations along transect A monthly, and at all stations quarterly using a fourth Ekman grab (collected in a manner identical to that for the biological samples).

In the laboratory, the benthic samples were washed with fresh water to remove the formalin and any remaining sediment. Most samples contained very little shell hash, and the entire sample was preserved in rose Bengal-stained 70 percent ethanol. The material was subsequently examined microscopically and all stained organisms were removed, identified to lowest possible taxon, classified as adult or young, and counted. Because several persons made initial identifications, all samples were examined by the principal investigator to standardize the nomenclature and size classifications.

The sediment samples were refrigerated at 4°C until analysis, if not air dried immediately. Each sample was air dried and then analyzed for grain size using the methods of Folk (1974), including sieve analysis for sand fractions and pipette analysis for silts and clays. Organic and carbonate carbon content were also determined. A dried, weighed sediment sample was subjected to 10% hydrogen peroxide digestion. When effervescence ceased the sample was rinsed, dried and reweighed to determine the amount of organic carbon. The sample was then treated with concentrated hydrochloric acid to determine the carbonate carbon content.

### III.2 Demersal Nekton

Collections of nekton were made aboard chartered shrimp trawlers off Freeport, Texas, beginning in October 1977, but sampling at the offshore

diffuser site did not begin until July 1978. In July 1978 replicate trawl sampling was initiated at four offshore diffuser site stations (trawls 20-27) while trawls 28-43 were added in October 1978. Sampling has occurred approximately monthly since October 1978 at all stations, with day and night sampling occurring during some months.

Because of the addition of trawls during the study, the trawl numbers do not consistently increase offshore. The trawls furthest offshore were coded 16-18 in the initial design (numbers increasing offshore) and only those stations at the nearshore diffuser site were initially replicated. On the later cruises, 53 trawls were collected, most of the new ones being located inshore of trawls 16-18. In the final design there were two trawls at each station. Coordinates for the trawls are given in Table 2. The following is a breakdown of the trawl depths:

| | |
|---|---|
| 2 fm | 46 and 47 |
| 3.5 fm | 1 and 48 |
| 5 fm | 2 and 49 |
| 8 fm | 3-14 (nearshore diffuser) |
| 10 fm | 15, 19 and 50 |
| 12 fm | 20-43 (offshore diffuser) |
| 13.5 fm | 44 and 45 |
| 15 fm | 16 and 51 |
| 20 fm | 17 and 52 |
| 25 fm | 18 and 53 |

These trawl locations are shown in Figure 3 in reference to the nearshore and offshore diffuser sites, and the sampling design in the vicinity of the offshore diffuser site is shown in Figure 4. The twelve stations in the vicinity of the offshore diffuser site (Figure 4), each of which was sampled with duplicate trawls, form the basis for the statistical analyses presented by Chittenden et al. 1981.

Collections were made using two 34-foot Hollis-Special commercial trawls equipped with tickler chains and 1 3/4 inch stretch-mesh netting in the cod-end. Tows were made at a speed of about 2.75 knots for ten minutes bottom time duration, so that about 0.5 nautical miles of bottom were swept. Tows were made in straightline fashion except during October and December 1977 when circular tows were made at each station (Hann et al. 1979).

**Table 2. Summary of station locations for trawl collections at the Bryan Mound offshore study area.**

| Trawl Code | Depth(fm) | Latitude | Longitude |
|---|---|---|---|
| 46 | 2 | 28 53.61N | 95 20.91W |
| 47 | 2 | 28 53.61N | 95 20.91W |
| 1 | 3.5 | 28 52.57N | 95 19.56W |
| 48 | 3.5 | 28 52.57N | 95 19.56W |
| 2 | 5 | 28 50.60N | 95 18.82W |
| 49 | 5 | 28 50.60N | 95 18.82W |
| 3 | 8 | 28 48.46N | 95 19.59W |
| 4 | 8 | 28 48.46N | 95 19.59W |
| 5 | 8 | 28 49.72N | 95 18.39W |
| 6 | 8 | 28 49.72N | 95 18.39W |
| 7 | 8 | 28 49.44N | 95 18.10W |
| 8 | 8 | 28 49.44N | 95 18.10W |
| 9 | 8 | 28 49.55N | 95 17.88W |
| 10 | 8 | 28 49.55N | 95 17.88W |
| 11 | 8 | 28 49.71N | 95 17.61W |
| 12 | 8 | 28 49.71N | 95 17.61W |
| 13 | 8 | 28 50.43N | 95 16.43W |
| 14 | 8 | 28 50.43N | 95 16.43W |
| 15 | 10 | 28 54.78N | 95 15.93W |
| 50 | 10 | 28 54.78N | 95 15.93W |
| 16 | 15 | 28 36.09N | 95 12.30W |
| 51 | 15 | 28 36.09N | 95 12.30W |
| 17 | 20 | 28 20.00N | 95 08.70W |
| 52 | 20 | 28 20.00N | 95 08.70W |
| 18 | 25 | 28 14.80N | 95 07.80W |
| 53 | 25 | 28 14.80N | 95 07.80W |
| 19 | 10 | 28 46.58N | 95 15.94W |
| 20 | 12 | 28 43.20N | 95 16.12W |
| 21 | 12 | 28 43.20N | 95 16.12W |
| 22 | 12 | 28 44.08N | 95 14.59W |
| 23 | 12 | 28 44.08N | 95 14.59W |
| 24 | 12 | 28 44.19N | 95 14.41W |
| 25 | 12 | 28 44.19N | 95 14.41W |
| 26 | 12 | 28 45.04N | 95 12.89W |
| 27 | 12 | 28 45.04N | 95 12.89W |
| 28 | 12 | 28 44.02N | 95 15.17W |
| 29 | 12 | 28 44.02N | 95 15.17W |
| 30 | 12 | 28 44.59N | 95 14.21W |
| 31 | 12 | 28 44.59N | 95 14.21W |
| 32 | 12 | 28 44.08N | 95 15.68W |
| 33 | 12 | 28 44.08N | 95 15.68W |
| 34 | 12 | 28 44.55N | 95 14.85W |
| 35 | 12 | 28 44.55N | 95 14.85W |
| 36 | 12 | 28 44.99N | 95 14.05W |
| 37 | 12 | 28 44.99N | 94 14.05W |
| 38 | 12 | 28 43.23N | 95 15.01W |
| 39 | 12 | 28 43.23N | 95 15.01W |
| 40 | 12 | 28 43.78N | 95 14.22W |
| 41 | 12 | 28 43.78N | 95 14.22W |
| 42 | 12 | 28 44.14N | 95 13.40W |
| 43 | 12 | 28 44.14N | 95 13.40W |
| 44 | 13.5 | 28 41.11N | 95 13.42W |
| 45 | 13.5 | 28 41.11N | 95 13.42W |

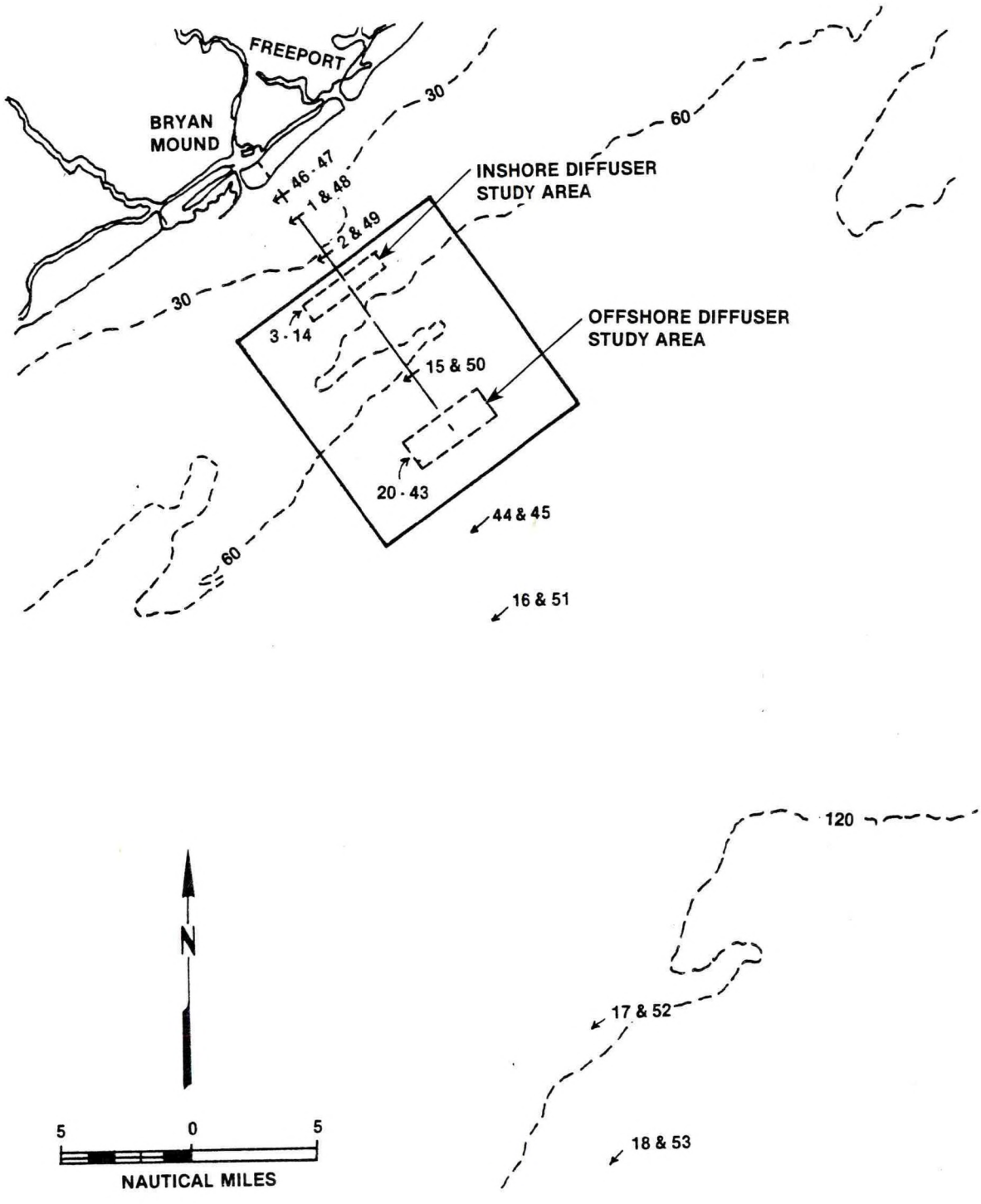


**Figure 3.** **Location of nekton trawls for the Bryan Mound offshore study area.**

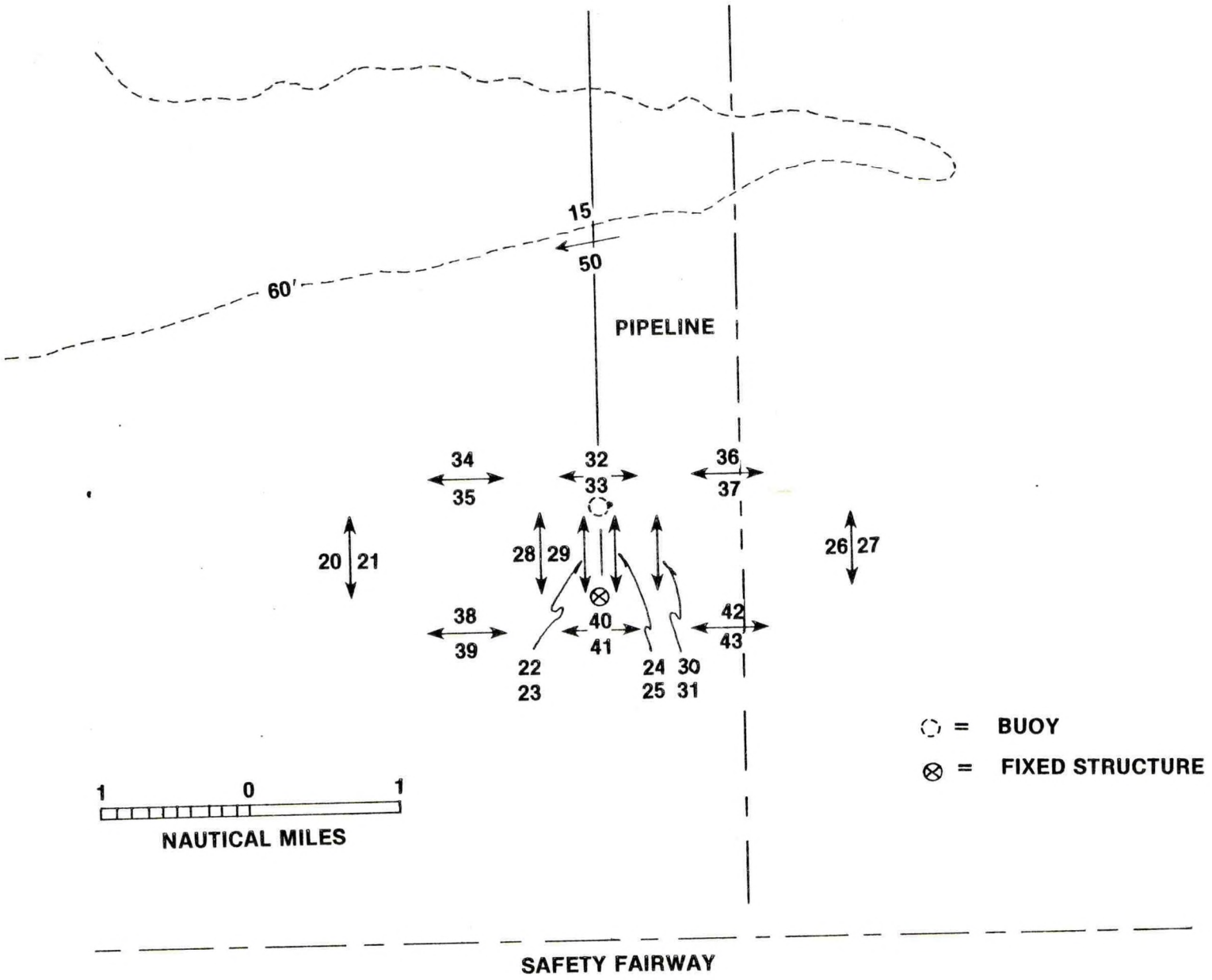


Figure 4. Detail of nekton trawl locations in the region of Bryan Mound diffuser.

Catches of nekton were processed in the field and/or in the laboratory depending upon weather conditions, size of the catch, and available manpower. Nekton processed in the laboratory had been preserved in 10% formalin solution in the field. The nekton species were identified, enumerated, and measured for the first trawl of each station. Only identification and enumeration were performed on the second trawl at stations where duplicate collections were made.

### III.3 Phytoplankton

Thirteen phytoplankton stations were initially established in the study area. The nine stations located approximately 150 m apart in the vicinity of the diffuser (Figure 5) are collectively referred to as the experimental stations, and the four stations each approximately 7.5 km from the diffuser area (Figure 6) are referred to as control stations in anticipation of future discharge of brine. Stations MM and D2 through D9 were sampled from June 1979 through January 1980. These stations were southwest of the diffuser area, and the grid was shifted in February 1980 to center the grid along the diffuser line. The new stations were designated D10 through D18. The change involved a shift of 450 m (center to center). Table 3 gives the coordinates of the sampling stations utilized from June 1979 to September 1980. Beginning with cruise 17 (15 September 1980) this array was abandoned, with only the center station (D14) and the nearshore and offshore control stations (15 and 36) being retained (Figures 5 and 6). Three other stations were added at this time. Based on the methodologies presented by Loeblich and Hall (1980), these final stations (DN1, DN2, UP) are apparently not fixed in space but are oriented with reference to the direction of the prevailing bottom current at the time of sampling. The three new stations (Figure 6) are located 500 m and 1000 m downcurrent and 500 m upcurrent of Station D14.

Samples were collected monthly between 0830 and 1800 hours. Until September 1980, a water sample was collected from one meter below the surface and one meter above the bottom at each station using van Dorn water bottles. Beginning in September 1980, single van Dorn samples were also collected from the 10 and 18 meter depths at each station.

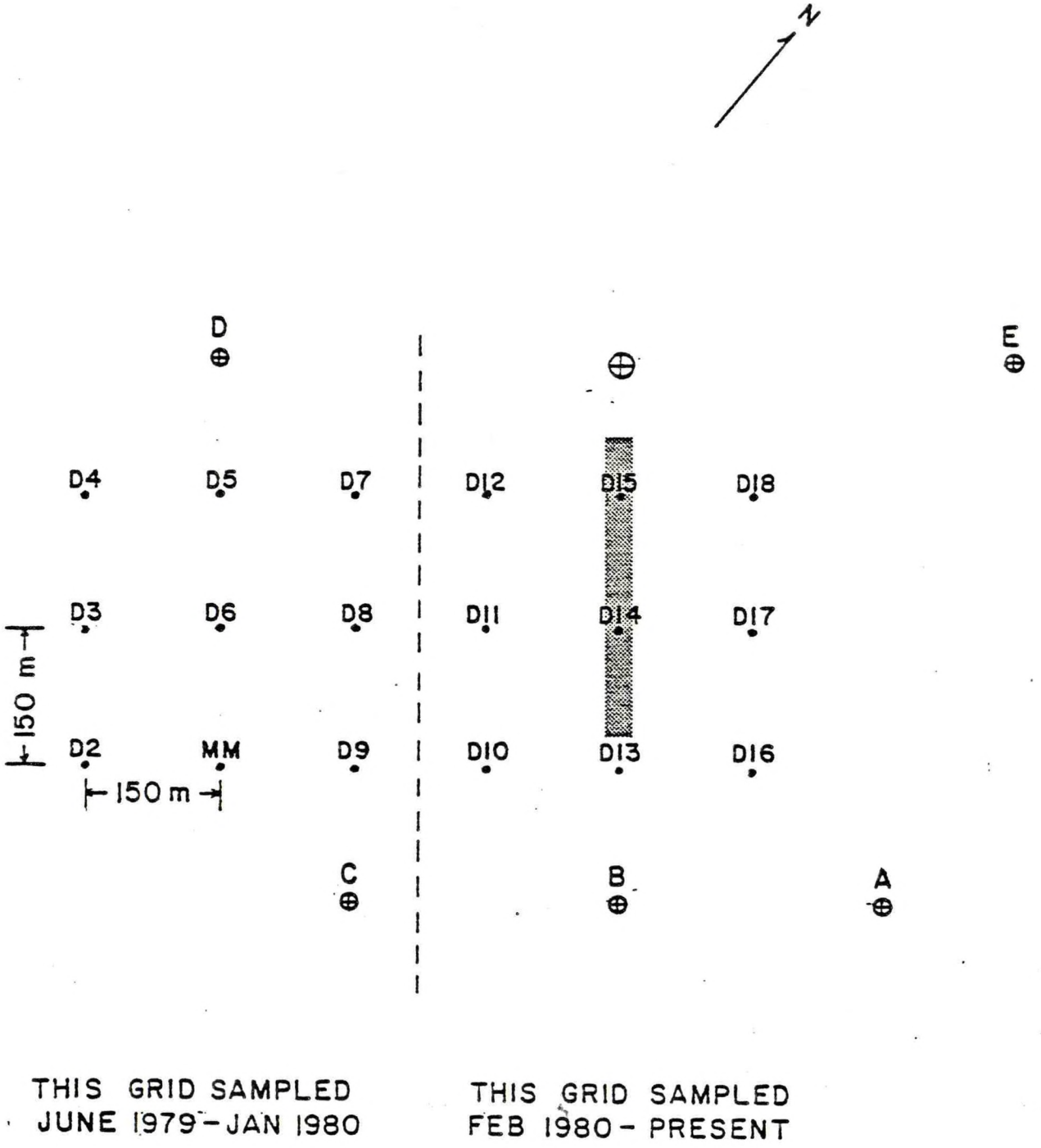


Figure 5. Location of sample stations with regard to the diffuser in the experimental stations. = NORDA buoy, located at shoreward end of diffuser trench. Buoy B is located at other end of diffuser trench. Buoys A, C, D, and E mark the outside corners of the study area. Diffuser shown in stippled region. All stations 150 m apart (from Hann et al. 1980).

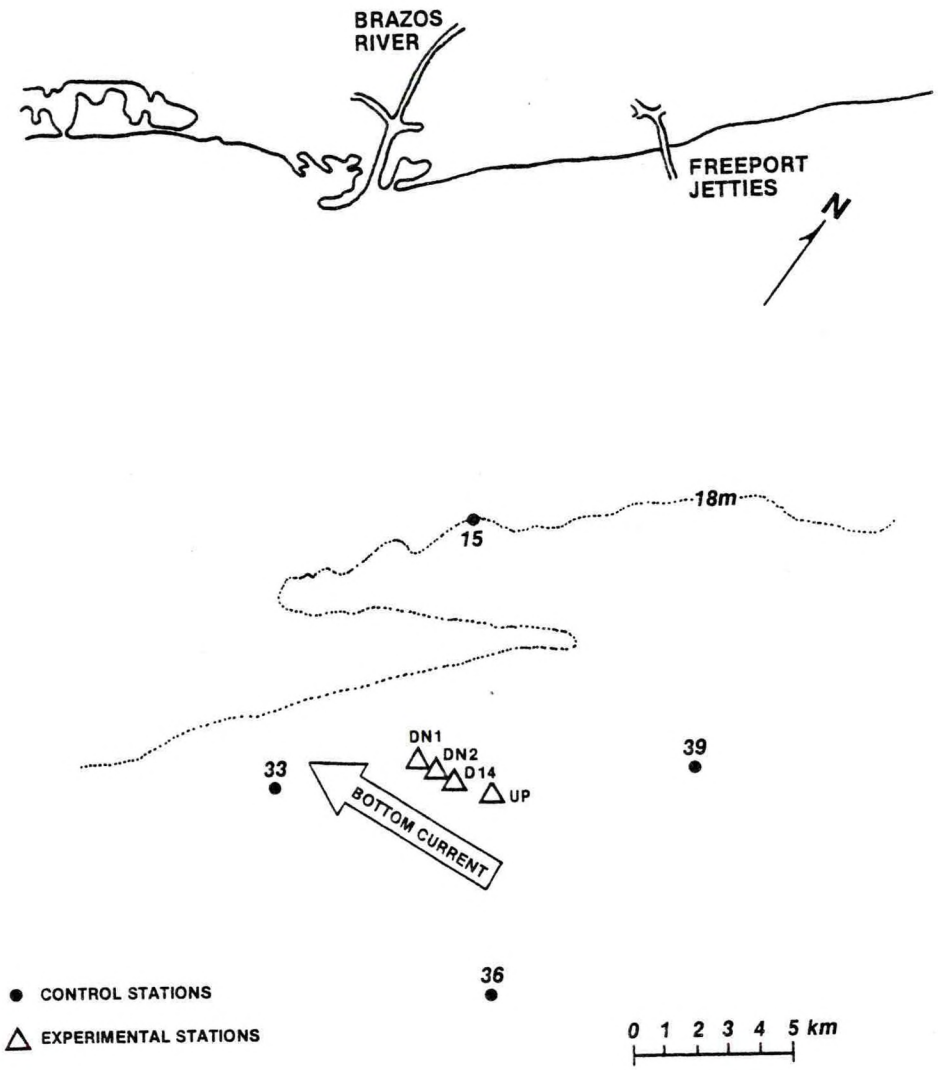


**Figure 6.** **Location of the phytoplankton experimental and control stations around the diffuser. Station D14 is centered over the diffuser site. Orientation of the other experimental stations is dependent on the direction of the bottom current (adapted from Hann and Randall 1981).**

**Table 3. Coordinates for the phytoplankton sampling stations in the Bryan Mound brine discharge study area off Freeport, Texas (from Hann et al. 1980).**

| Station Number | Latitude | Longitude |
|---|---|---|
| Experimental-June 1979 to Jan. 1980 | | |
| MM | 28°44.50' | 95°14.67' |
| D2 | 28°48.51' | 95°14.61' |
| D3 | 28°48.45' | 95°14.54' |
| D4 | 28°48.39' | 95°14.46' |
| D5 | 28°48.33' | 95°14.53' |
| D6 | 28°48.39' | 95°14.60' |
| D7 | 28°48.27' | 95°14.59' |
| D8 | 28°48.32' | 95°14.66' |
| D9 | 28°48.38' | 95°14.73' |
| Experimental - February 1980 | | |
| D10 | 28°44.06' | 95°14.56' |
| D11 | 28°44.11' | 95°14.61' |
| D12 | 28°44.18' | 95°14.67' |
| D13 | 28°44.08' | 95°14.49' |
| D14 | 28°44.17' | 95°14.55' |
| D15 | 28°44.22' | 95°14.59' |
| D16 | 28°44.13' | 95°14.41' |
| D17 | 28°44.20' | 95°14.46' |
| D18 | 28°44.27' | 95°14.51' |
| Control | | |
| 15 | 28°48.44' | 95°17.11' |
| 33 | 28°42.30' | 95°17.70' |
| 36 | 28°41.40' | 95°11.45' |
| 39 | 28°47.00' | 95°10.40' |

From Hann et al. (1980)

Temperature was measured using a glass-mercury immersion thermometer immediately upon bringing the samples onboard. A 1-liter cubitainer was filled with water from each van Dorn bottle and stored in an insulated cooler to insure minimum temperature change before analyses could be made in the laboratory.

Upon return to the laboratory, salinity of each sample was measured using a direct-reading American Optical refractometer. The remainder of each sample was stored in a constant temperature room overnight. Within 24 hours of collection, live cell counts and identification of the phytoplankton in each sample were made. Triplicate subsamples from the cubitainer were analyzed. An analysis (one of the triplicates) consisted of mixing the sample, withdrawing roughly 1 ml and filling a Palmer-Maloney counting chamber (0.1 ml). All phytoplankton in an area crossing the central portion of the chamber (0.014 ml) were identified to genus or to the lowest taxon possible.

From June 1979 through November 1980 _in vivo_ chlorophyll _a_ fluorescence was measured on triplicate aliquots of each water sample using a Turner Model 110 fluorometer following a procedure similar to that of Lorenzen (1966). Beginning in May 1980, a 200 ml subsample from each cubitainer was filtered and extracted with acetone for fluorometric analysis of chlorophyll _a_ and phaeophytin, using the method of Strictland and Parsons (1968).

### III.4 Zooplankton

The locations of the three zooplankton stations currently being sampled near the brine diffuser are shown in Figure 7. Station B (28°44.0' N, 95°14.4'W) was located near the end of the diffuser and Station A (28°42.8' N, 95°16.2'W) and station C (28°45.3' N, 95°12.5' W) were located two nautical miles from Station B, one upcoast and the other downcoast. Twenty one series of monthly zooplankton collections were made from an 80 ft shrimping vessel between June 1979 and February 1981.

On each cruise, three replicate oblique tows were taken from near the surface to near the bottom at each station. An additional oblique tow

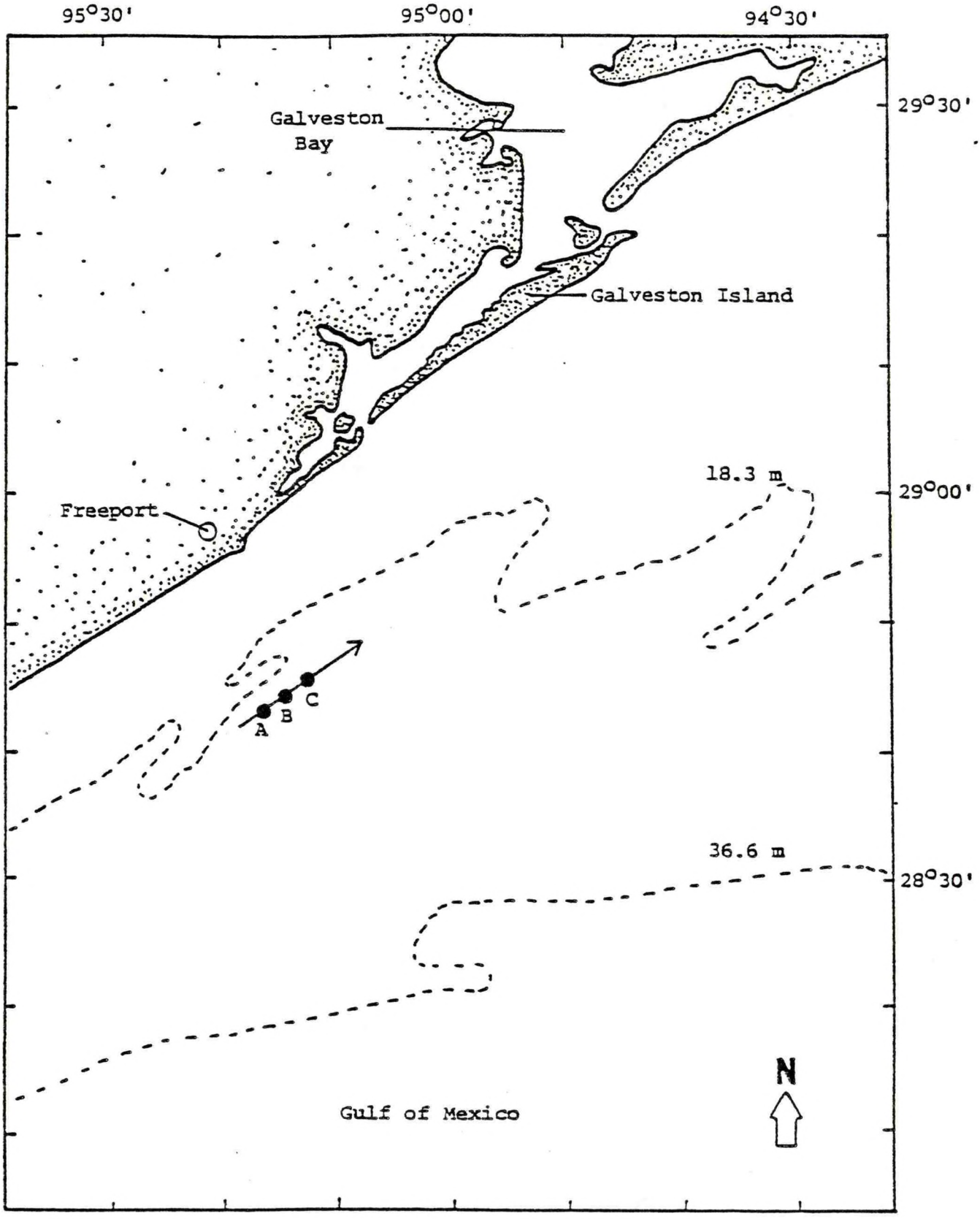


**Figure 7.** **Location of zooplankton sampling stations off Freeport, Texas. Brine diffuser is located near Station B (from Hann et al. 1980).**

(from the surface to the 1/2 depth level) was also taken at each station in order to estimate the vertical distribution of the zooplankton. The sampling gear consisted of a one meter mouth diameter Nitex net (length = 3.7 m) with a mesh size of 240 μm. A digital flowmeter positioned in the center of the net mouth was used to estimate the amount of water filtered during each tow. The amount of wire out was measured with a wire meter and this value along with the wire angle was used to determine the tow depth. All sampling was generally completed between 1100 and 1400 hours. This was done in an attempt to minimize variability resulting from the vertical migrations of animals. Tow durations averaged around two minutes and the amount of water filtered in each tow generally ranged between 50 and 150 $m^3$, with an average of 86 $m^3$. Samples were preserved on board in a solution of 5-10% formalin. On some of the cruises, temperature and salinity profiles were made at each station using a Hydrolab Model TC-2 Conductivity Meter.

Twelve samples were obtained on each cruise. Subsampling was necessary in the laboratory due to the large number of organisms collected in the tows. Subsamples were taken with a Hensen-Stempel pipette. One subsample from each sample was used to determine biomass. Biomass was measured as displacement volume using the mercury immersion method of Yentsch and Hebard (1957). Three smaller subsamples of equal size were also taken from each sample zooplankton counts. The subsample size was adjusted so that each contained approximately 500 organisms. Aliquot size ranged from 1/200 to 1/6000 of a sample. Major zooplankton groups were identified and counted. Since the copepods generally dominated the zooplankton, adult female copepods were identified to the species level.

Results of the eight predisposal collections (June 1979 to February 1980) were reported by Park and Minello (1980), while the results of collections made during thirteen cruises in the disposal period (March 1980 to February 1981) are discussed by Park and Minello (1981).

# IV. Analysis Plan

## IV.1 Overview of the Analysis Plan

Figure 8 shows the overall structure of the data analysis system, indicating five main "levels" of analysis. These are:

- Exploratory Analysis
- Basic Descriptive Statistics
- Bivariate Measures of Association
- Classification and Pattern Analysis
- Hypothesis Testing

The five levels are, of course, somewhat arbitrary and there is certainly some overlap among them, but they do represent an attempt to structure the analytic approach so that activities result in a progression of evaluations with a minimum of redundant effort. The plan incorporates most aspects of biological and geochemical data analysis and thus should provide an adequate context for impact assessment.

Exploratory analysis has, as its main goal, the characterization of the overall community composition at the various stations, and, from this initial characterization, a determination of what parameters (particularly species, higher taxonomic groups, etc.) will be utilized in subsequent analyses. It is most useful in initial (baseline) stages of an impact assessment program where large suites of variables (taxa) are involved. Any and all analyses requiring the total species assemblage at each station (or replicate) such as diversity, rarefraction curves, or total number of species and individuals are calculated during this stage, and descriptive statistics for these derived parameters (mean, variance, n) are calculated and stored. Through exploratory analysis, the investigator can define the criteria for subsetting of the original data set.

In the second stage of analysis, for those chosen data sets and parameters, basic descriptive statistics are calculated. The basic descriptive statistics help define the statistical nature of the data (especially the variation), define the need for data transformation (to satisy the criteria of additivity, normality and equality of variance),

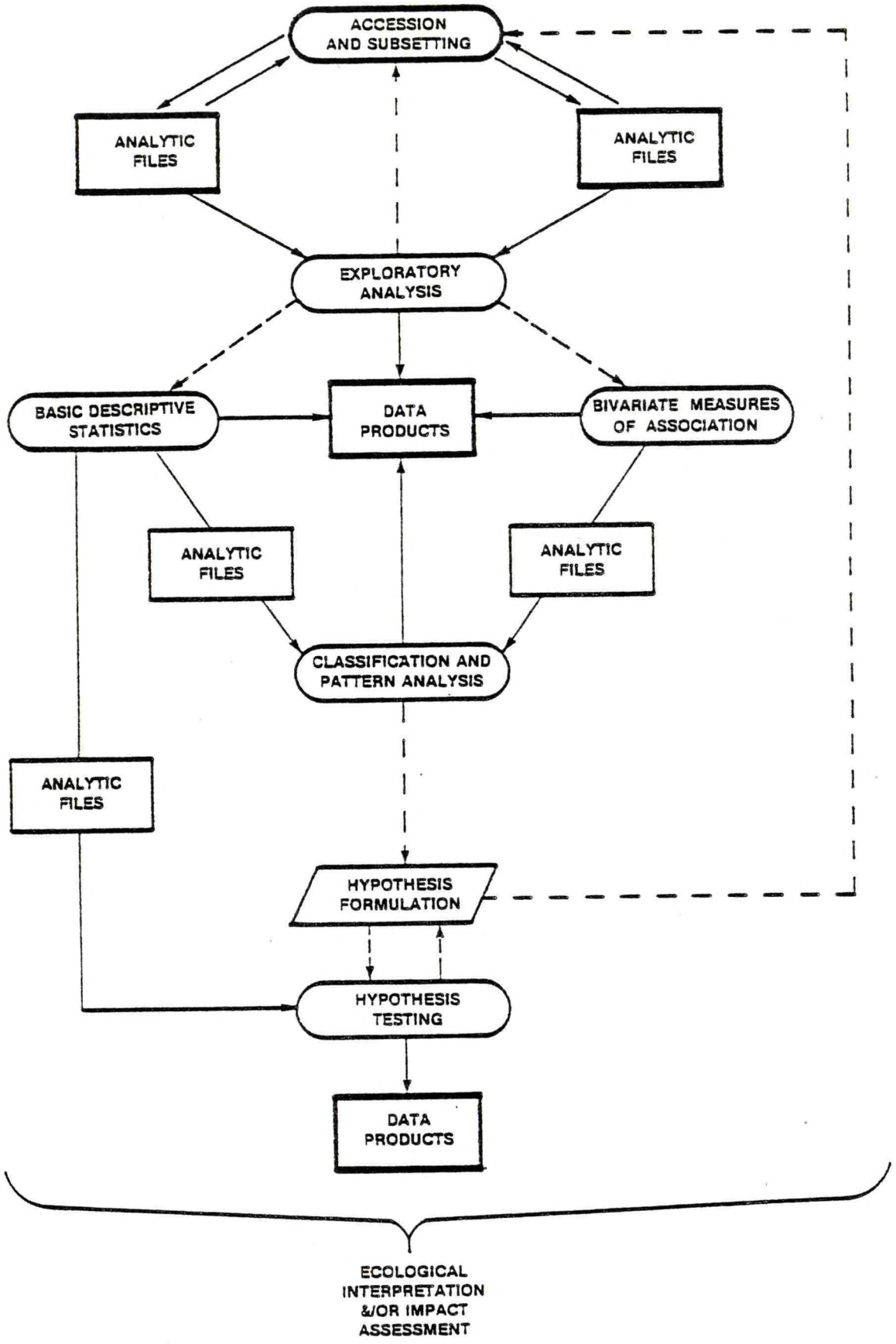


**Figure 8. Flow chart of the SAI data analysis system.**

and serve as inputs to hypothesis testing (concerning natural variation and/or impacts). Bivariate measures of association are calculated in the third phase of the analysis scheme, yielding spatial and temporal groupings of samples and species. These measures suggest community associations and serve as input data (matrices) to classification and pattern analyses.

In the fourth stage of the analysis system, higher order (community and habitat level) analyses begin. Because ecological data are often voluminous, the results of many large scale baseline monitoring programs are often little more than compilations of data. This obviates the fundamental objectives of baseline studies and renders the assessment of impacts more difficult. Classification and pattern analysis techniques are statistical tools for reducing these complex data to a set of dominant trends which should be further studied using hypothesis testing techniques. Classification and pattern analysis, therefore, are used to find the major trends in the data and also aid in the formulation of hypothesis to be tested. They are also very useful in identifying outlier samples which should then be examined more closely. Without the "Occham's Razor" of classification and pattern analysis, the most important trends in the data are often ignored, leading to erroneous hypotheses and conclusions.

In the final stage of the analysis scheme, all the information from previous stages is utilized for formulation of hypotheses which can then be tested by a number of techniques (ANOVA, ANCOVA, regression) which represent applications of the general linear model. Actually, by virtue of the experimental design of the monitoring program, some initial and general hypotheses have already been formulated. These initial hypotheses are refined, expanded, or truncated, depending on the results from previous application of the data analysis scheme. Many of the parameters required for hypothesis testing will have been calculated earlier (in basic descriptive statistics) and stored. Analysis of variance and t-tests can be used to test differences for class variables while analysis of covariance can be used to remove extraneous variance from the main effects. Hypothesis testing has, as additional features,

the capability for calculating the number of samples required for significance testing and also the optimization of the sampling design based on analyses of variances. A very important component is its predictive capability, expressed through multiple regression analysis and discriminant function analysis. In this way samples can be classified or dependent variables of interest can be predicted from sets of independent variables.

Below, each of these major areas is discussed in greater detail. It is important to stress that each stage of the data analysis system provides information to the next stage and is also a decision point regarding the direction of the next level of analysis. This scheme has been applied to a number of benthic characterizations in the Gulf of Mexico, Puget Sound and also in Chesapeake Bay studies, and has provided a very timely flow of information for program plan development.

## IV.2. Components of the Analysis Plan

### IV.2.1 Exploratory Analysis

The potontial size of a data set resulting from a large scale monitoring program is truly mind-boggling. Hundreds of samples, with (cumulatively) 400 to 500 species will yield hundreds of thousands of data points per cruise. These data must not only be adequately summarized, but they must be reduced to a more workable size so that detailed analyses can be efficiently performed. Therefore, the exploratory analysis should have as its objectives those given in Figure 9.

Components or data products resulting from the Exploratory Analysis are given in Figure 10. The taxonomically-based tabular display (Table 4) and the relative importance table (Table 5) complement each other well in providing a comprehensive summarization of the data by sample or station. The taxonomically-based display provides a vehicle for the user to quickly identify a particular species of interest (via the taxonomic hierarchy) and its importance at each of the replicates at a station.

## EXPLORATORY ANALYSIS

## OBJECTIVES

- USED TO SUMMARIZE RAW FIELD DATA BY SAMPLE AND STATION
- GAIN AN OVERALL DESCRIPTION OF TAXONOMIC COMPOSITION AND THE RELATIONSHIPS OF NUMBERS OF SPECIES TO NUMBER OF INDIVIDUALS
- CALCULATE THOSE "COMMUNITY" OR "ASSEMBLAGE" PARAMETERS FOR WHICH THE ENTIRE TAXONOMIC SUITE ARE REQUIRED
- PROVIDE GUIDANCE REGARDING DATA SUBSETTING FOR SUBSEQUENT ANALYSES (e.g., DEFINITION OF "MOST IMPORTANT" SPECIES)



Figure 9. Objectives of exploratory analysis.

# EXPLORATORY ANALYSIS

## COMPONENTS

- TAXONOMICALLY-BASED TABULAR DISPLAY OF NUMERIC DATA FOR REPLICATES AT A STATION
- RELATIVE IMPORTANCE TABLES INCLUDING
  - PERCENT AND CUMULATIVE PERCENT OF COMPOSITION
  - FREQUENCY OF OCCURRENCE
  - CUMULATIVE NUMBER OF INDIVIDUALS
  - DIVERSITY, RICHNESS, EVENNESS AND NUMBER OF SPECIES (MEAN AND VARIANCE)
  - POPULATION MEANS AND INDICES OF DISPERSION
- SPECIES - AREA CURVES
- RAREFACTION AND DOMINANCE—DIVERSITY CURVES
- NON-HIERARCHICAL CLUSTERING



Figure 10. Components of exploratory analysis.

**Table 4. Taxonomically-based tabular display of macrobenthic data collected at the Weeks Island brine disposal site during the June 1978 NMFS cruises.**

SAMPLE DATE: 6/28/78 SITE-STATION: 10- 401 ASSEMBLAGE 4 DEPTH 8.0 METERS

| TAXON | REP1 | REP2 | REP3 | TAXON TOT. | % OF TOT. |
|---|---|---|---|---|---|
| Cnidaria | | | | | |
| Zoantharia Actiniaria Nynantheae Athenaria | | | | | |
| ZOANTHARIA ACTINIARIA NYNANTHEAE ATHENARIA | 1 | 2 | 2 | 5 | 1.5 |
| Rhynchocoela | | | | | |
| Heteronemertea | | | | | |
| Lineidae | | | | | |
| CEREBRATULUS LACTEUS | 0 | 0 | 2 | 2 | 0.6 |
| Nematoda | | | | | |
| NEMATODA | 1 | 0 | 0 | 1 | 0.3 |
| Annelida | | | | | |
| Polychaeta | | | | | |
| Polynoidae | | | | | |
| POLYNOIDAE | 2 | 0 | 0 | 2 | 0.6 |
| Sigalionidae | | | | | |
| PHOLOE MINUTA | 1 | 0 | 0 | 1 | 0.3 |
| Phyllodocidae | | | | | |
| PHYLLODOCE ARENAE | 3 | 0 | 0 | 3 | 0.9 |
| Pilargidae | | | | | |
| SIGAMBRA SP | 3 | 0 | 1 | 4 | 1.2 |
| Syllidae | | | | | |
| EUSYLLIS SP | 1 | 0 | 0 | 1 | 0.3 |
| Nephtyidae | | | | | |
| AGLAOPHAMUS VERRILLI | 1 | 5 | 1 | 7 | 2.2 |
| Glyceridae | | | | | |
| GLYCERA AMERICANA | 1 | 0 | 0 | 1 | 0.3 |
| Orbiniidae | | | | | |
| HAPLOSCOLOPLOS FRAGILIS | 0 | 1 | 0 | 1 | 0.3 |
| Spionidae | | | | | |
| PARAPRIONOSPIO PINNATA | 7 | 6 | 19 | 32 | 9.9 |
| Magelonidae | | | | | |
| MAGELONA SP | 3 | 2 | 17 | 22 | 6.8 |
| Cirratulidae | | | | | |
| CIRRIFORMIA SP | 3 | 0 | 2 | 5 | 1.5 |
| Capitellidae | | | | | |
| MEDIOMASTUS CALIFORNIENSIS | 100 | 18 | 11 | 129 | 39.9 |
| Oweniidae | | | | | |
| OWENIA FUSIFORMIS | 0 | 1 | 2 | 3 | 0.9 |
| Terebellidae | | | | | |
| PISTA PALMATA | 0 | 1 | 0 | 1 | 0.3 |
| Bivalvia | | | | | |
| Veneroida | | | | | |
| Cardiidae | | | | | |
| TRACHYCARDIUM MURICATUM | 0 | 0 | 1 | 1 | 0.3 |
| Mactridae | | | | | |
| MULINIA LATERALIS | 19 | 6 | 41 | 66 | 20.4 |
| Tellinidae | | | | | |
| TELLINA VERSICOLOR | 1 | 1 | 1 | 3 | 0.9 |
| Pholadomyoida | | | | | |
| Pandoridae | | | | | |
| PANDORA TRILINEATA | 1 | 0 | 0 | 1 | 0.3 |
| Arthropoda Mandibulata Crustacea | | | | | |
| Peracardia Mysidacea Mysida | | | | | |
| Mysidae | | | | | |
| MYSIDOPSIS SP | 1 | 0 | 0 | 1 | 0.3 |
| Peracarida Cumacea | | | | | |
| Diastylidae | | | | | |
| DIASTYLIS SP | 1 | 2 | 1 | 4 | 1.2 |
| Peracardia Isopoda valvifera | | | | | |
| Idoteidae | | | | | |
| IDOTEA SP | 1 | 0 | 0 | 1 | 0.3 |
| Peracardia Amphipoda Gammaridea | | | | | |
| AMPELISCA SP | 0 | 0 | 1 | 1 | 0.3 |
| Dodicerotidae | | | | | |
| MONOCULODES SP | 4 | 7 | 0 | 11 | 3.4 |
| Eucardia Decapoda Pleocyemata Caridea | | | | | |
| Ogyrididae | | | | | |
| OGYRIDES LIMICOLA | 1 | 7 | 1 | 9 | 2.8 |
| Echinodermata | | | | | |
| Ophiuroidea Ophiurida Gnathophiurina | | | | | |
| Amphiuridae | | | | | |
| MICROPHOLIS ATRA | 0 | 3 | 1 | 4 | 1.2 |
| Chaetognata | | | | | |
| SAGITTA SP | 1 | 0 | 0 | 1 | 0.3 |
| NUMBER OF INDIV. PER REPLICATE: | 157 | 62 | 104 | | |
| NUMBER OF TAXA PER REPLICATE: | 22 | 14 | 16 | | |
| CUMMULATIVE NUMBER OF SPECIES ACROSS REP: | 22 | 26 | 29 | | |

TOTAL NUMBER OF TAXA FOR STATION: 29
TOTAL NUMBER OF INDIVIDUALS FOR STATION: 323

SPECIES DIVERSITY (H) = 2.1012 SPECIES EVENNESS (E) = 0.6240

## Table 5. Relative composition table for the macrobenthos collected at Station 22 in Mississippi Sound during the fall, 1980.

MEAN ABUNDANCE TABLE FOR STATION 220157, FALL CRUISE, 1980

| TAXON NAME | MEAN PERCENT COMPOSITION | CUMULATIVE PERCENT COMPOSITION | POOLED PERCENT COMPOSITION | FREQ. OF OCCURRENCE | CUMULATIVE ABUNDANCE | MEAN ABUNDANCE | INDEX OF DISPERSION |
|---|---|---|---|---|---|---|---|
| Magelona cf. phyllisae | 12.18 | 12.18 | 10.74 | 1.00 | 159 | 19.9 | 2.57 |
| Diopatra cuprea | 12.07 | 24.25 | 8.92 | 1.00 | 291 | 16.5 | 5.23 |
| Myriochele oculata | 10.10 | 34.35 | 9.80 | 1.00 | 436 | 18.1 | 8.01 |
| Copepoda | 6.35 | 40.69 | 6.96 | 0.88 | 539 | 12.9 | 19.87 |
| Mediomastus | 6.25 | 46.94 | 6.28 | 1.00 | 632 | 11.6 | 5.33 |
| Paraprionospio pinnata | 5.64 | 52.58 | 5.47 | 1.00 | 713 | 10.1 | 5.35 |
| Rhynchocoela | 5.57 | 58.15 | 11.89 | 0.25 | 889 | 22.0 | 173.73 |
| Rhynchocoela sp. D | 4.17 | 62.32 | 4.39 | 1.00 | 954 | 8.1 | 11.20 |
| Hemipholis elongata | 3.23 | 65.55 | 2.91 | 1.00 | 997 | 5.4 | 2.23 |
| Paleanotus heteroseta | 2.78 | 68.33 | 2.97 | 0.75 | 1041 | 5.5 | 6.34 |
| Pinnixa | 2.32 | 70.65 | 3.18 | 0.63 | 1088 | 5.9 | 11.26 |
| Pinnixa pearsei | 2.23 | 72.88 | 1.22 | 0.13 | 1106 | 2.3 | 18.00 |
| Pinnixa chaetopterana | 2.04 | 74.92 | 1.28 | 0.25 | 1125 | 2.4 | 13.23 |
| Cossura soyeri | 1.64 | 76.55 | 1.69 | 1.00 | 1150 | 3.1 | 1.78 |
| Ophiuroidea | 1.62 | 78.17 | 1.42 | 0.88 | 1171 | 2.6 | 1.30 |
| Micropholis atra | 1.57 | 79.74 | 1.42 | 0.88 | 1192 | 2.6 | 1.52 |
| Lumbrineris | 1.55 | 81.29 | 1.28 | 0.75 | 1211 | 2.4 | 1.32 |
| Ampelisca sp. A | 1.36 | 82.65 | 1.76 | 0.75 | 1237 | 3.3 | 6.40 |
| Harmothoe sp. E | 1.30 | 83.95 | 1.22 | 0.88 | 1255 | 2.3 | 1.49 |
| Lepidasthenia cf. varia | 1.22 | 85.17 | 0.81 | 0.75 | 1267 | 1.5 | 1.33 |
| Notomastus lobatus | 1.16 | 86.33 | 0.74 | 0.75 | 1278 | 1.4 | 1.03 |
| Rhynchocoela sp. A | 1.03 | 87.36 | 0.88 | 0.75 | 1291 | 1.6 | 1.22 |
| Sigambra tentaculata | 0.97 | 88.33 | 0.68 | 0.75 | 1301 | 1.3 | 1.31 |
| Gastropoda | 0.89 | 89.22 | 1.15 | 0.25 | 1318 | 2.1 | 8.93 |
| Pelecypoda | 0.72 | 89.94 | 0.81 | 0.63 | 1330 | 1.5 | 2.67 |
| Ancistrosyllis jonesi | 0.61 | 90.56 | 0.68 | 0.63 | 1340 | 1.3 | 1.09 |
| Polynoidae A | 0.40 | 90.95 | 0.34 | 0.38 | 1345 | 0.6 | 1.34 |
| Mysidopsis bigelowi | 0.36 | 91.31 | 0.34 | 0.38 | 1350 | 0.6 | 1.34 |
| Armandia maculata | 0.35 | 91.67 | 0.27 | 0.50 | 1354 | 0.5 | 0.57 |
| Oxyurostylis smithi | 0.34 | 92.01 | 0.47 | 0.50 | 1361 | 0.9 | 1.45 |
| Nassarius acutus | 0.34 | 92.35 | 0.27 | 0.25 | 1365 | 0.5 | 2.29 |
| Sthenelais limicola | 0.33 | 92.68 | 0.27 | 0.50 | 1369 | 0.5 | 0.57 |
| Amphiuridae | 0.30 | 92.98 | 0.20 | 0.25 | 1372 | 0.4 | 1.48 |
| Gyptis vittata | 0.27 | 93.25 | 0.27 | 0.38 | 1376 | 0.5 | 1.14 |
| Rhynchocoela sp. I | 0.27 | 93.52 | 0.34 | 0.50 | 1381 | 0.6 | 0.89 |
| Cyclostremiscus pentagonas | 0.26 | 93.77 | 0.41 | 0.13 | 1387 | 0.8 | 6.00 |
| Saturnia corpulenta | 0.25 | 94.03 | 0.20 | 0.25 | 1390 | 0.4 | 1.48 |
| Ninoe nigripes | 0.23 | 94.26 | 0.20 | 0.38 | 1393 | 0.4 | 0.71 |
| Gyptis brevipalpa | 0.23 | 94.49 | 0.27 | 0.38 | 1397 | 0.5 | 1.14 |
| Phascolion strombi | 0.21 | 94.70 | 0.14 | 0.25 | 1399 | 0.3 | 0.86 |
| Podarke obscura | 0.21 | 94.91 | 0.14 | 0.25 | 1401 | 0.3 | 0.86 |
| Nuculana acuta | 0.21 | 95.12 | 0.41 | 0.25 | 1407 | 0.8 | 2.95 |
| Nereis micromma | 0.21 | 95.33 | 0.20 | 0.38 | 1410 | 0.4 | 0.71 |
| Cerebratulus lacteus | 0.20 | 95.53 | 0.14 | 0.25 | 1412 | 0.3 | 0.86 |
| Owenia fusiformis | 0.19 | 95.72 | 0.14 | 0.25 | 1414 | 0.3 | 0.86 |
| Nematoda | 0.19 | 95.92 | 0.14 | 0.25 | 1416 | 0.3 | 0.86 |
| Mitrella lunata | 0.19 | 96.11 | 0.20 | 0.25 | 1419 | 0.4 | 1.48 |
| Automate evermanni | 0.19 | 96.30 | 0.20 | 0.38 | 1422 | 0.4 | 0.71 |
| Phoronis sp. A | 0.19 | 96.49 | 0.14 | 0.25 | 1424 | 0.3 | 0.86 |
| Mulinia lateralis | 0.18 | 96.67 | 0.20 | 0.25 | 1427 | 0.4 | 1.48 |
| Aglaophamus verrilli | 0.18 | 96.85 | 0.20 | 0.25 | 1430 | 0.4 | 1.48 |
| Oligochaeta | 0.16 | 97.01 | 0.14 | 0.25 | 1432 | 0.3 | 0.86 |
| Xanthidae | 0.16 | 97.18 | 0.14 | 0.25 | 1434 | 0.3 | 0.86 |
| Maldanidae | 0.16 | 97.33 | 0.14 | 0.25 | 1436 | 0.3 | 0.86 |
| Hesionidae | 0.16 | 97.49 | 0.14 | 0.25 | 1438 | 0.3 | 0.86 |
| Alpheus | 0.12 | 97.61 | 0.07 | 0.13 | 1439 | 0.1 | 1.00 |
| Ampelisca cf. verrilli | 0.12 | 97.73 | 0.07 | 0.13 | 1440 | 0.1 | 1.00 |
| Chaetozone gayheadia | 0.12 | 97.85 | 0.07 | 0.13 | 1441 | 0.1 | 1.00 |
| Nephtys | 0.12 | 97.97 | 0.07 | 0.13 | 1442 | 0.1 | 1.00 |
| Ancistrosyllis papillosa | 0.12 | 98.09 | 0.14 | 0.25 | 1444 | 0.3 | 0.86 |
| Actiniaria sp. A | 0.11 | 98.20 | 0.07 | 0.13 | 1445 | 0.1 | 1.00 |
| Chaetognatha | 0.11 | 98.31 | 0.14 | 0.25 | 1447 | 0.3 | 0.86 |
| Ogyrides limicola | 0.11 | 98.42 | 0.14 | 0.25 | 1449 | 0.3 | 0.86 |
| Glycinde solitaria | 0.11 | 98.52 | 0.14 | 0.25 | 1451 | 0.3 | 0.86 |
| Pagurus | 0.09 | 98.61 | 0.07 | 0.13 | 1452 | 0.1 | 1.00 |
| Nephtys sp. D | 0.09 | 98.70 | 0.07 | 0.13 | 1453 | 0.1 | 1.00 |
| Eulima cf. bilineatus | 0.09 | 98.79 | 0.07 | 0.13 | 1454 | 0.1 | 1.00 |
| Hexapanopeus angustifrons | 0.07 | 98.86 | 0.07 | 0.13 | 1455 | 0.1 | 1.00 |
| Phascolion | 0.07 | 98.94 | 0.07 | 0.13 | 1456 | 0.1 | 1.00 |
| Glycera americana | 0.07 | 99.01 | 0.07 | 0.13 | 1457 | 0.1 | 1.00 |
| Arcidae | 0.07 | 99.09 | 0.07 | 0.13 | 1458 | 0.1 | 1.00 |
| Nereis lamellosa | 0.07 | 99.16 | 0.07 | 0.13 | 1459 | 0.1 | 1.00 |
| Loimia viridis | 0.07 | 99.24 | 0.07 | 0.13 | 1460 | 0.1 | 1.00 |
| Mysidacea | 0.07 | 99.31 | 0.07 | 0.13 | 1461 | 0.1 | 1.00 |
| Prionospio | 0.06 | 99.37 | 0.14 | 0.13 | 1463 | 0.3 | 2.00 |
| Sigalionidae | 0.06 | 99.44 | 0.14 | 0.13 | 1465 | 0.3 | 2.00 |
| Abra aequalis | 0.04 | 99.48 | 0.07 | 0.13 | 1466 | 0.1 | 1.00 |
| Polynoidae | 0.04 | 99.52 | 0.07 | 0.13 | 1467 | 0.1 | 1.00 |
| Cabira incerta | 0.04 | 99.56 | 0.07 | 0.13 | 1468 | 0.1 | 1.00 |
| Magelona cf. cincta | 0.04 | 99.61 | 0.07 | 0.13 | 1469 | 0.1 | 1.00 |
| Onuphis | 0.04 | 99.65 | 0.07 | 0.13 | 1470 | 0.1 | 1.00 |
| Leucosilidae | 0.04 | 99.69 | 0.07 | 0.13 | 1471 | 0.1 | 1.00 |
| Flabelligeridae | 0.04 | 99.74 | 0.07 | 0.13 | 1472 | 0.1 | 1.00 |
| Carazziella hobsonae | 0.04 | 99.78 | 0.07 | 0.13 | 1473 | 0.1 | 1.00 |
| Callianassa latispina | 0.03 | 99.81 | 0.07 | 0.13 | 1474 | 0.1 | 1.00 |
| Cirratulidae | 0.03 | 99.84 | 0.07 | 0.13 | 1475 | 0.1 | 1.00 |
| Nucula cf. proxima | 0.03 | 99.87 | 0.07 | 0.13 | 1476 | 0.1 | 1.00 |
| Rhynchocoela sp. G | 0.03 | 99.91 | 0.07 | 0.13 | 1477 | 0.1 | 1.00 |
| Ampharetidae | 0.03 | 99.94 | 0.07 | 0.13 | 1478 | 0.1 | 1.00 |
| Macoma | 0.03 | 99.97 | 0.07 | 0.13 | 1479 | 0.1 | 1.00 |
| Gastropoda sp. A | 0.03 | 100.00 | 0.07 | 0.13 | 1480 | 0.1 | 1.00 |

| SAMPLE SUMMARY: 8 CASES | TAXA | INDIVIDUALS | DIVERSITY | EVENNESS |
|---|---|---|---|---|
| MEAN | 34. | 185. | 2.748 | 0.791 |
| VARIANCE | 73. | 11146. | 0.068 | 0.005 |

The relative importance tables (Table 5) contain a vast amount of summary information. Species are arranged in decreasing order of importance, with percent and cumulative percent composition, number and cumulative number of individuals, percent frequency, and number of occurrences. In addition, summary parameters, requiring the entire data set, are produced at this stage. These include diversity, richness, and evenness as well as the number of species (and total number of individuals). Means and standard errors of the means for these parameters as well as for number of individuals are given in summary tables for any aggregation above the sample (replicate) level. Also an index of dispersion ($\frac{S^2}{\bar{X}}$) is routinely calculated for each taxonomic group to be used along with other summary statistics to define the distribution of the populations.

These summary tables serve several purposes. They allow delineation of numerically "important species" for each station or data block (several stations). Typical criteria would include a certain threshold percent composition or number of occurrences. By identifying important species, the investigator can "whittle" the number of taxonomic variables down to a manageable level. Also, the data contained in the relative importance tables (especially cumulative percent composition and number of individuals) can be used directly as input to generate various descriptive plots (e.g., cumulative species-area curves, cumulative mean vs number of replicates, and cumulative percent composition vs number of species). Species-area curves are useful in determining the number of replicates required for an adequate qualitative characterization of the community (Figure 11).

Because of the very large number of samples involved, it is important to have a method which allows early classification of samples and species. However, most hierarchical clustering programs cannot handle the number of samples and/or species required for exploratory analysis. The cost of manipulating large data matrices quickly becomes prohibitive for hierarchial clustering programs. COMPCLUS, a computer program for rapid initial clustering of large data sets (Gauch 1979) can solve this dilemma. COMPCLUS derives clusters in a non-hierarchical manner and the

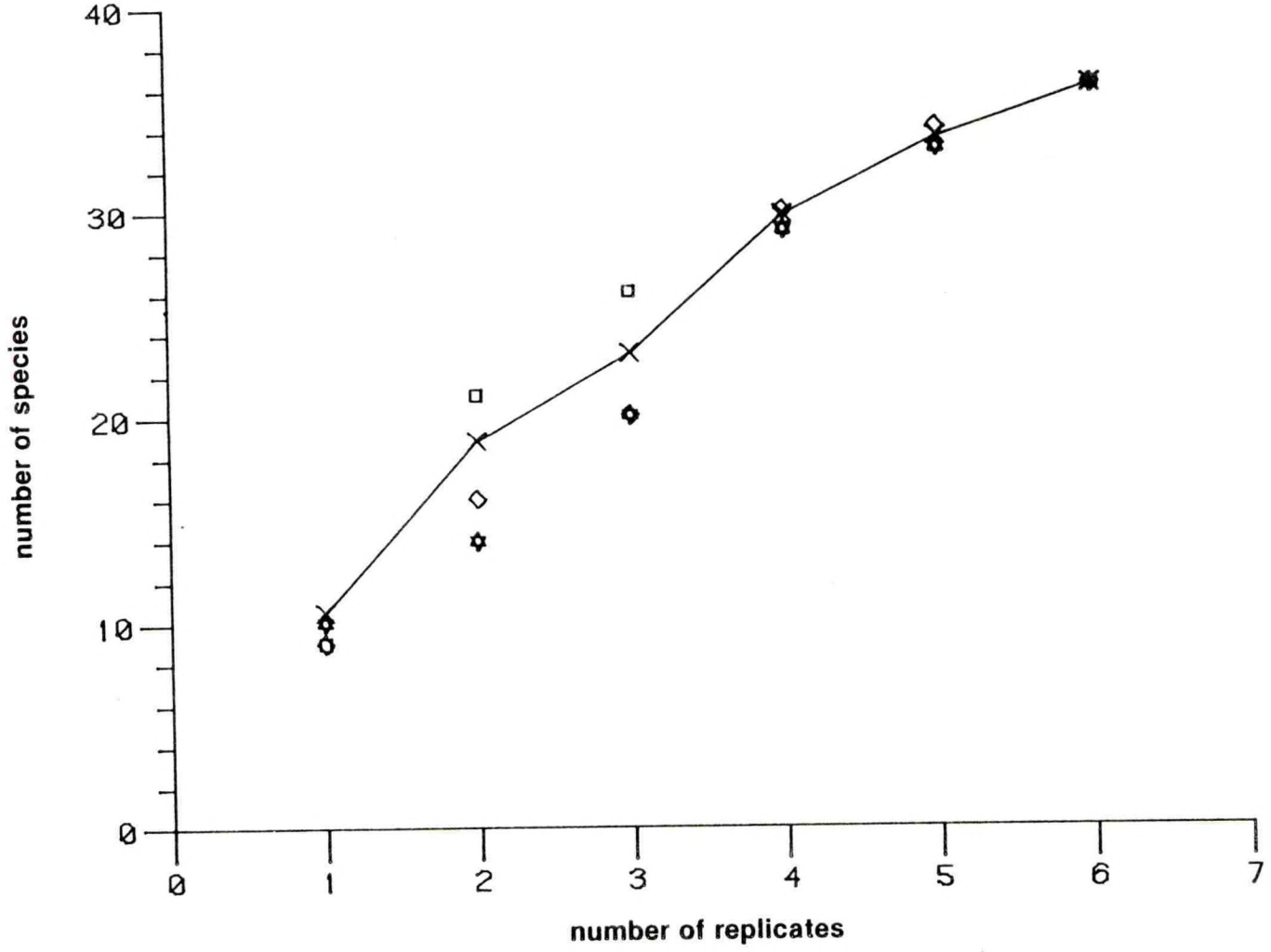


**Figure 11. Species area curve for six replicate samples from STOCS fall 1977 macrobenthos collections for station 2, transect 3.**

user can specify the number of clusters and the level of within - group similarity. In COMPCLUS, samples are first picked randomly to serve as cluster centers, with all samples within a given distance (in species space) of each center sample included in the particular cluster. Samples from small clusters are then reassigned into the nearest large cluster (within a user specified radius). The resulting composite samples are much fewer in number, and can be used as input for more detailed cluster and/or ordination analyses. Each cluster of samples is characterized by a composite species composition. Gauch (1979) points out that this sequence of (COMPCLUS) clustering followed by more detailed analyses of a defined number of between group or among group differences (using the samples within clusters as replicates) is a very efficient approach to analysis.

Sander's (1968) rarefaction method allows direct diversity comparisons between analogous faunal groups from similar habitats regardless of sample size or geographic location. This technique assumes that the populations are randomly or evenly distributed. Essentially, this method "rarifies" the sample, giving the reduced number of species to be expected in progressively smaller samples, while still preserving the same apportioning of numbers of individuals to the species. The resulting plot is a curvilinear interpolation of the number of species expected given the number of individuals in the sample. Comparisons using this technique require that the sampling techniques be similar. Figure 12 shows rarefraction curves for data from four monthly cruises (February, May, August, and November, 1978) for the Bryan Mound offshore site, data summed over all replicates and stations. A recent paper discussing the use of rarefaction in impact assessment is that by Simberloff (1977).

Dominance diversity is the numerical percentage composition of the various species present in the sample. The more the constituent species are represented by equal numbers of individuals, the more diverse the fauna. Dominance diversity values may be determined by plotting the percentage composition of the species along the ordinate and ranking the species by abundance along the absissa. The resultant frequency curve

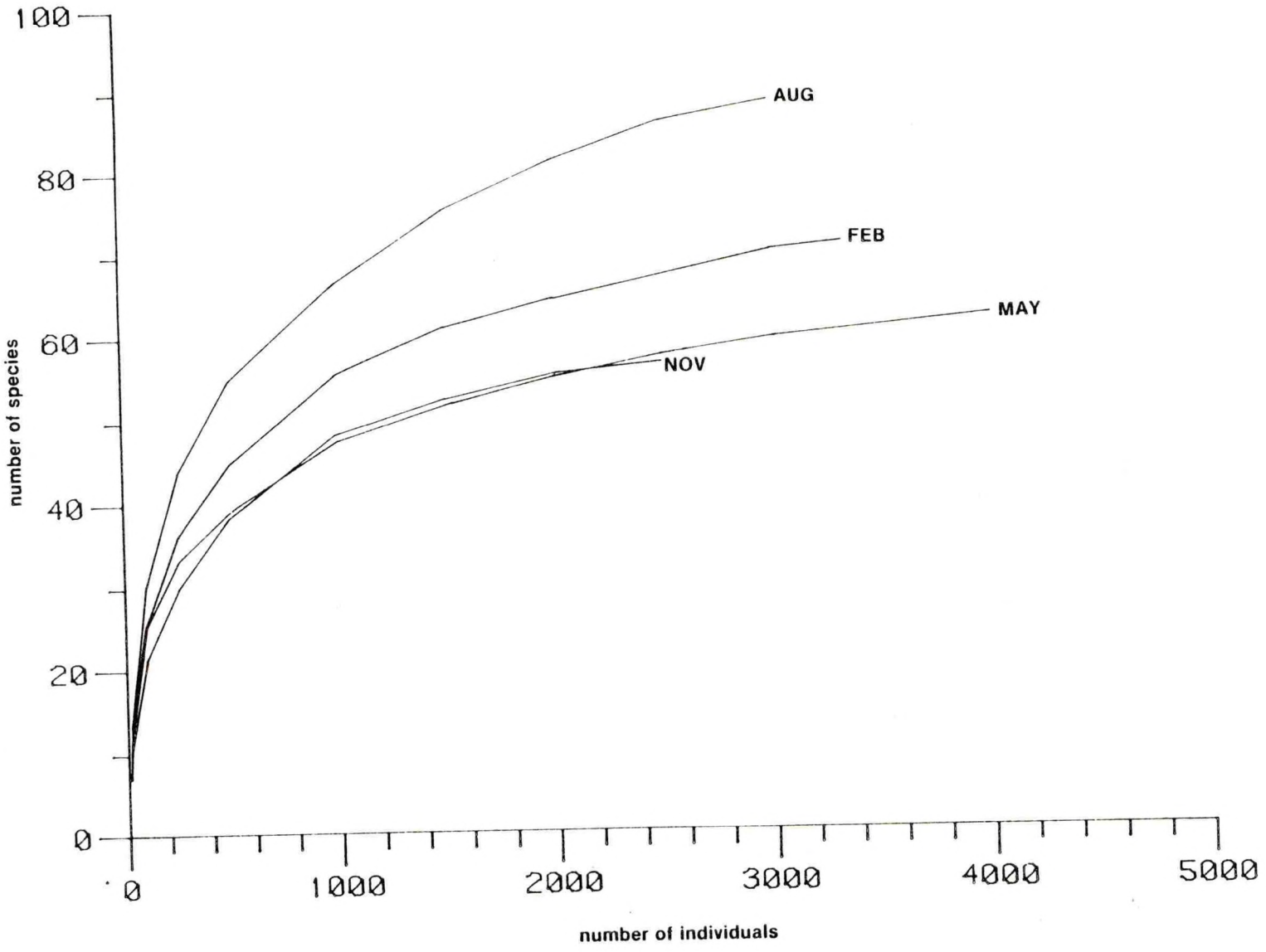


**Figure 12.** **Rarefaction curves for data from four monthly cruises at the offshore brine disposal site at Bryan Mound.**

is used as a measure of dominance diversity. Maximum diversification occurs when all the species in a sample are represented by exactly the same number of individuals, and the cumulative frequency curve is a straight diagonal line that represents a baseline. The larger the degree of departure from the baseline, the greater the dominance and, therefore, the smaller the diversity. These curves are useful in detecting stressed communities, where diversity is usually low.

### IV.2.2 Basic Descriptive Statistics

The objectives of basic descriptive statistics are shown in Figure 13. No tests of hypotheses should be conducted until the basic descriptive statistics for individual variables are calculated, displayed, and examined. It is this basic analytic stage that is often treated lightly in biological characterizations. This is unfortunate because these basic statistical procedures not only allow for testing the assumptions of the analyses and determining the need for data transformations, but they also allow initial estimation of pattern in the spatial distribution of biological or geochemical populations.

#### IV.2.2.1 Distribution of Data and Populations

The components of basic descriptive statistics are shown in Figure 14. The simple univariate statistics are the building blocks of the analysis. For each variable, the mean, variance (or standard deviation), n, range (maximum-minimum), skewness, kurtosis, coefficient of variation, and standard error of the mean are calculated, and outputted to a data file for use in subsequent procedures.

A basic characteristic of a distribution is skewness, a measure of the extent to which a distribution is symmetric about its mean. Skewness is calculated according to the following expression:

$$\text{Skewness} = \frac{\sum_{i=1}^{N} (X_i - \bar{X})^3}{N(\sigma)^3}$$

## BASIC DESCRIPTIVE STATISTICS

## OBJECTIVES

- STATISTICALLY CHARACTERIZE INDIVIDUAL VARIABLES (e.g., SPECIES, HIGHER TAXONOMIC GROUPS, ENVIRONMENTAL VARIABLES)
- TEST ASSUMPTIONS OF ANALYSES
- TRANSFORM DATA IF NECESSARY
- DETERMINE FIRST-ORDER PATTERN IN THE SPATIAL DISTRIBUTION OF BENTHIC ORGANISMS



**Figure 13. Objectives of basic descriptive statistics.**

# BASIC DESCRIPTIVE STATISTICS

## COMPONENTS

- SIMPLE UNIVARIATE STATISTICS
  - MEAN
  - VARIANCE
  - NUMBER OF REPLICATES
  - RANGE
  - MAXIMUM
  - MINIMUM
  - SKEWNESS
  - KURTOSIS
  - COEFFICIENT OF VARIATION
  - STANDARD ERROR OF THE MEAN
- PLOT OF CUMULATIVE MEAN VS NUMBER OF REPLICATES
- SPATIAL DISTRIBUTION OF BENTHOS
  - COEFFICIENT OF DISPERSION
  - MORISITA'S INDEX
- TESTS OF ASSUMPTIONS OF ANALYSES
  - HOMOGENEITY OF VARIANCE
    - BARTLETT'S TEST
    - F-max TEST
    - COCHRAN'S C
    - BURR FOSTER Q-TEST OF HOMOGENEITY
  - NON-NORMALITY
    - SHAPIRO-WILKS W TEST
    - KOLMOGOROV-SMIRNOV
    - $\chi^2$ TESTS
  - ADDITIVITY
    - TUKEY'S ONE DEGREE OF FREEDOM TEST
- DATA TRANSFORMATIONS

**Figure 14.** Components of basic descriptive statistics.

A skewness of 0, indicates the distribution is symmetric as is the normal distribution. If the value is positive, then the tail to the right of the mean is drawn out relative to the tail to the left. The opposite is true for negative skewness values. Therefore, an important use of a measure of skewness is to determine if a distribution is normal in shape. A normally distributed population will have a skewness value equal to 0. Table 6 (from Snedecor and Cochran 1980) can be used to test whether an obtained skewness value is significantly different from 0. Skewness is significantly different from 0 if the absolute value of a calculated skewness exceeds the table value for the particular sample size and desired significance level.

Another characteristic of a distribution is kurtosis. Kurtosis is a measure of the relative peakedness or flatness of a distribution, and is calculated as:

$$\text{Kurtosis} = \frac{\sum_{i=1}^{N} (X_i - \bar{X})^4}{N(\sigma)^4} - 3$$

As with skewness a normal distribution will have a kurtosis of 0. If the kurtosis is positive then the distribution is more peaked (narrow) than would be true for a normal distribution, while a negative value means that it is flatter. To test kurtosis, the appropriate sample size is entered in Table 7 (from Snedecor and Cochran 1980) for the desired level of significance. A negative kurtosis value is significantly different from 0 if it is less than the tabled negative kurtosis value. A positive kurtosis is significantly different from 0 if it exceeds the positive kurtosis value in the table.

Without a pilot study, there will be questions concerning the adequacy of the number of replicates collected at each station during the first sampling. One way to address this, at a preliminary level, is to plot the cumulative mean for the particular parameter vs the number of replicates (Figure 15). Very often the curve reaches an asymptote, indicating that the estimate of the mean is stabilizing, and an adequate number of replicates are being collected. In the example from the STOCS

**Table 6. Percentage points for testing skewness of data (from Snedecor and Cochran 1967).**

(one-tailed percentage points of the distribution of $\sqrt{b_1} = g_1 = m_3/m_2^{3/2}$)

| Size of Sample $n$ | Percentage Points 5% | Percentage Points 1% | Standard Deviation | Size of Sample $n$ | Percentage Points 5% | Percentage Points 1% | Standard Deviation |
|---|---|---|---|---|---|---|---|
| 25 | 0.711 | 1.061 | 0.4354 | 100 | 0.389 | 0.567 | 0.2377 |
| 30 | .661 | 0.982 | .4052 | 125 | .350 | .508 | .2139 |
| 35 | .621 | 0.921 | .3804 | 150 | .321 | .464 | .1961 |
| 40 | .587 | 0.869 | .3596 | 175 | .298 | .430 | .1820 |
| 45 | .558 | 0.825 | .3418 | 200 | .280 | .403 | .1706 |
| 50 | .533 | 0.787 | .3264 | 250 | .251 | .360 | .1531 |
| 60 | .492 | 0.723 | .3009 | 300 | .230 | .329 | .1400 |
| 70 | .459 | 0.673 | .2806 | 350 | .213 | .305 | .1298 |
| 80 | .432 | 0.631 | .2638 | 400 | .200 | .285 | .1216 |
| 90 | .409 | 0.596 | .2498 | 450 | .188 | .269 | .1147 |
| 100 | 0.389 | 0.567 | 0.2377 | 500 | 0.179 | 0.255 | 0.1089 |

Since the distribution of $\sqrt{b_1}$ is symmetrical about zero, the percentage points represent 10% and 2% two-tailed values. Reproduced from Table 34 B of *Tables for Statisticians and Biometricians*, vol. 1, by permission of Dr. E. S. Pearson and the *Biometrika* trustees.

**Table 7. Percentage points for testing kurtosis of data (from Snedecor and Cochran 1967).**

(percentage points of the distribution of $b_2 = m_4/m_2^2$)

| Size of Sample $n$ | Percentage Points | | | | Size of Sample $n$ | Percentage Points | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Upper | | Lower | | | Upper | | Lower | |
| | 1% | 5% | 5% | 1% | | 1% | 5% | 5% | 1% |
| 50 | 4.88 | 3.99 | 2.15 | 1.95 | 600 | 3.54 | 3.34 | 2.70 | 2.60 |
| 75 | 4.59 | 3.87 | 2.27 | 2.08 | 650 | 3.52 | 3.33 | 2.71 | 2.61 |
| 100 | 4.39 | 3.77 | 2.35 | 2.18 | 700 | 3.50 | 3.31 | 2.72 | 2.62 |
| 125 | 4.24 | 3.71 | 2.40 | 2.24 | 750 | 3.48 | 3.30 | 2.73 | 2.64 |
| 150 | 4.13 | 3.65 | 2.45 | 2.29 | 800 | 3.46 | 3.29 | 2.74 | 2.65 |
| | | | | | 850 | 3.45 | 3.28 | 2.74 | 2.66 |
| 200 | 3.98 | 3.57 | 2.51 | 2.37 | 900 | 3.43 | 3.28 | 2.75 | 2.66 |
| 250 | 3.87 | 3.52 | 2.55 | 2.42 | 950 | 3.42 | 3.27 | 2.76 | 2.67 |
| 300 | 3.79 | 3.47 | 2.59 | 2.46 | 1000 | 3.41 | 3.26 | 2.76 | 2.68 |
| 350 | 3.72 | 3.44 | 2.62 | 2.50 | | | | | |
| 400 | 3.67 | 3.41 | 2.64 | 2.52 | 1200 | 3.37 | 3.24 | 2.78 | 2.71 |
| 450 | 3.63 | 3.39 | 2.66 | 2.55 | 1400 | 3.34 | 3.22 | 2.80 | 2.72 |
| 500 | 3.60 | 3.37 | 2.67 | 2.57 | 1600 | 3.32 | 3.21 | 2.81 | 2.74 |
| 550 | 3.57 | 3.35 | 2.69 | 2.58 | 1800 | 3.30 | 3.20 | 2.82 | 2.76 |
| 600 | 3.54 | 3.34 | 2.70 | 2.60 | 2000 | 3.28 | 3.18 | 2.83 | 2.77 |

Reproduced from Table 34 C of *Tables for Statisticians and Biometricians*, by permission of Dr. E. S. Pearson and the *Biometrika* trustees.

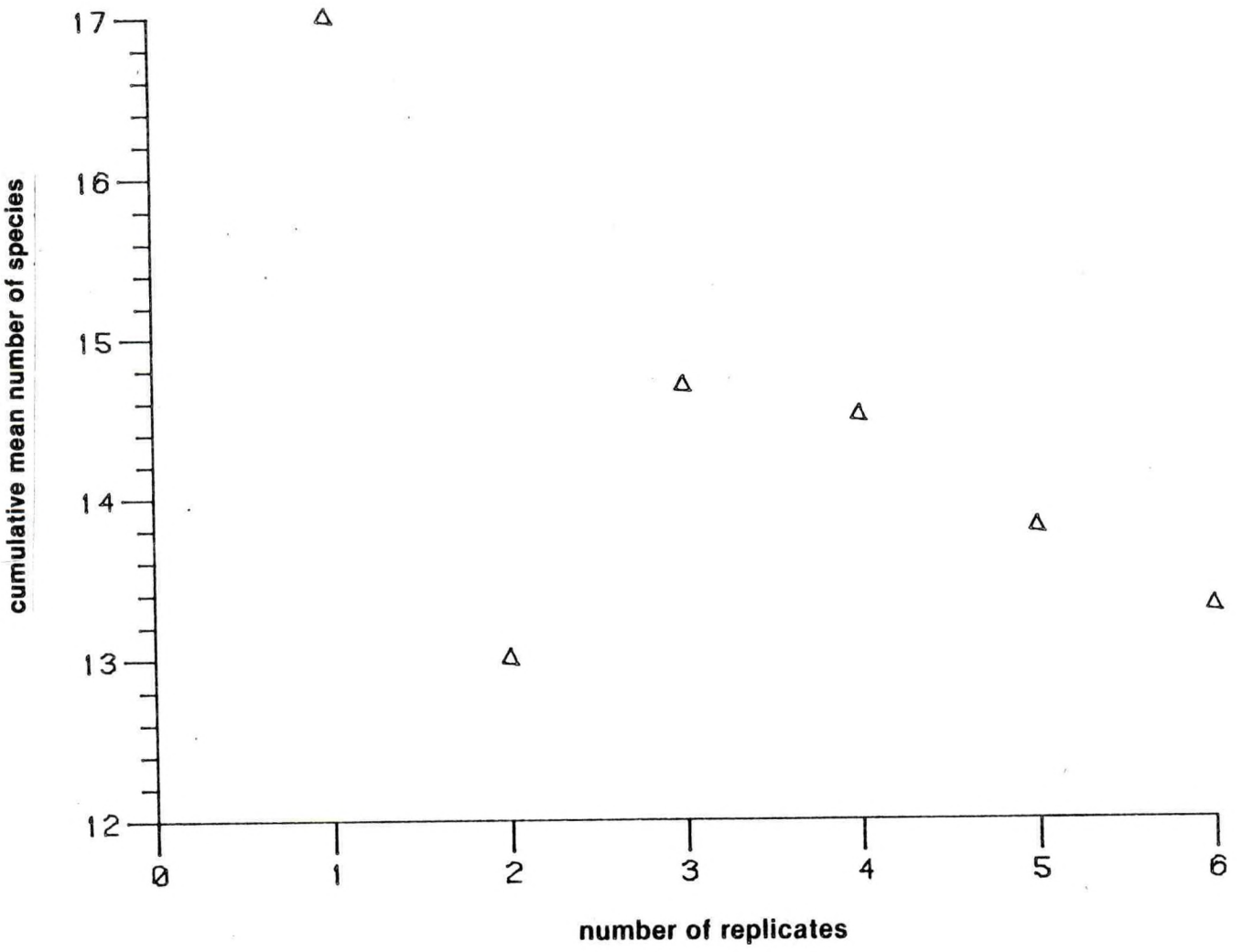


**Figure 15.** Plot of cumulative mean number of species of macrobenthos vs. number of replicates for samples collected at station 2, transect 3 in the STOCS fall 1977 collections.

study (Figure 15) it appears that several more replicates may be required for adequate stabilization of the mean. More refined tests to answer this question about the adequacy of replicates are given under Hypothesis Testing (see Section IV.2.5).

One of the most important aspects of basic descriptive statistics is the determination of the frequency distribution of the data. If the frequency distribution of counts from a sample fits one of several well-known mathematical frequency distributions, a number of important questions can be addressed (Elliot 1971).

1. Spatial dispersion of the population can be understood.
2. Errors of population parameters can be estimated.
3. Temporal and spatial changes in density can be compared.
4. The effects of environmental factors can be assessed.

The individuals of a population can follow three basic types of spatial dispersion, each of which influence the relationship between the variance ($\sigma^2$) and the arithmetic mean ($\mu$):

1. Random distribution $\sigma^2 = \mu$
2. Regular distribution $\sigma^2 < \mu$
3. Contagious distribution $\sigma^2 > \mu$

There are, in turn, suitable models (mathematical distributions) for these three relationships. The Poisson distribution is suitable for randomly distributed data, the positive binomial is an approximation for a regular distribution and the negative binomial ($\sigma^2 > \mu$) is often used for contagious distributions, although other models may be more appropriate. Since few bottom samples have a variance significantly less than the mean, the positive binomial is seldom an appropriate model.

The Poisson series is associated with events which occur randomly in a continuum of time or space (e.g., distribution of counts of benthic invertebrates per sampling unit in a bottom sample). A critical condition for applicability of the Poisson distribution is that there is equal chance of an individual occupying any point in the sampling area and this condition is fulfilled only if the individuals are distributed

completely at random on the bottom. If this condition is not met, the variance of the population is usually greater than the arithmetic mean ($\sigma^2 > \mu$) and the population is clumped or aggregated. The negative binomial distribution is often a suitable model in these cases.

The probability series for the negative binomial is given by $(q - p)^{-K}$ where $p = \mu/k$ and $q = 1 + p$, with $\mu$ and k the parameters of the distribution. The variance of the population $\sigma^2 = \mu + \mu^2/k$. The reciprocal of k (i.e., 1/k) is a measure of the excess variance or clumping of the individuals of the population. As 1/k approaches o and k approaches $\infty$, the distribution converges to the Poisson, while, with increased clumping, 1/k approaches $\infty$ (i.e. $k \to 0$) and the distribution converges to a logarithmic series. Agreement with the Poisson series is usually used as a criteria for assuming a random distribution, but this will not be true in all cases.

A very common test for the Poisson distribution is the ratio of the sample variance to the sample mean, this ratio being known under several names, including coefficient of dispersion or index of dispersion,

$$I = \frac{S^2}{\bar{x}} = \frac{\Sigma(x - \bar{x})^2}{\bar{x}\,(n - 1)}$$

which should approach unity for a random distribution. The significance of departures of this ratio from unity are assessed using $\chi^2$ (chi-squared) tables; where $I(n - 1)$ approximates $\chi^2$ with $n - 1$ degrees of freedom, and

$$\chi^2 = \frac{\Sigma(x - \bar{x})^2}{\bar{x}}$$

Agreement with the Poisson series is accepted at the 95% probability level ($P > .05$) if $\chi^2$ value lies between the appropriate 5% significant levels for $n - 1$ degrees of freedom. If $\chi^2$ is less than expected, the distribution is probably regular ($S^2 < \bar{X}$), while $\chi^2$ greater than expected points to a contagious distribution ($S^2 > \bar{X}$).

Another technique is to plot the log $s^2$ vs. log $\bar{x}$, and calculate regression models using $s^2$ and $\bar{x}$ for the important species in the data set (as determined under exploratory analysis) as the dependent and independent variables, respectively. Regression models are calculated for stations representative of the various habitats in the study area (as derived from cluster groups). The slope of the least squares regression of the log/log plot should be one for a Poisson distribution and greater than one for the negative binomial. The slopes for assemblages in different "habitat types" can be tested for significant differences to determine the influence of environmental parameters on spatial dispersion of the benthic populations. See Gage and Geekie (1973) for examples of the application of these techniques.

For large samples the results of this test should be checked by the $\chi^2$ test of goodness of fit. This test require enough samples to enable construction of a frequency distribution. The observed frequency distribution of the data for a parameter is then compared to the expected frequency distribution.

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

Frequencies are usually combined so no expected values are less than 5. Degrees of freedom are given by V = # frequency classes - number of estimated parameters - 1. Agreement with the Poisson series is accepted at the 95% probability level ($P > 0.05$) if the calculated $\chi^2$ is less than the 5 percent point for $\chi^2$ with V degrees of freedom in the appropriate tables.

In most cases, the spatial dispersion of populations of benthic invertebrates is contagious, with $\sigma^2 > \mu$. There are a whole series of "contagious" distributions, depending on the size of the aggregates (clumps), the distribution of the individuals of the population within the clumps, the spatial pattern of the clumps, and the distance between clumps. Sampling gear can influence the distribution of the data. Although a number of mathematical models have been used for description

of various contagious distributions, the negative binomial is most frequently applied.

For large samples, the $\chi^2$ goodness of fit test can be applied to determine agreement with the negative binomial distribution. For small samples, $\chi^2$ tests can be used if a frequency distribution can be arranged from the data. Other tests are used as a check on the goodness of fit test and also as independent tests when goodness of fit tests are not appropriate.

One common test is based on a comparison of observed and expected moments. First U is calculated, where U is the difference between the sample estimate of variance ($s^2$) and the expected variance in a negative binomial.

$$U = s^2 - \left(\bar{x} + \frac{\bar{x}^2}{\bar{k}}\right)$$

Another statistic, T, is then calculated. T is the difference between the sample estimate and expected values for the third moment (skewness).

$$T = \frac{\Sigma x^3 - 3\bar{x}\,\Sigma x^2 + 2\,\bar{x}^2\,\Sigma x}{n} - \frac{s^2(2\,s^2 - 1)}{\bar{x}}$$

If there is perfect agreement between U & T, then the expected values of U & T are 0. Agreement is accepted if the values of U or T differ from zero by less than the standard error. A large positive value of U or T indicates greater skewness than that described by the negative binomial, implying that the discrete log-normal distribution is more suitable. The discrete log-normal distribution is more skewed than the negative binomial. When logs of count data follow a normal frequency distribution, the original counts followed a discrete log-normal distribution. Large negative values indicate less skewness, pointing toward the Neyman distribution as being more suitable.

Indices of dispersion are parameters which are also used to assess the spatial dispersion of the data. Elliot (1971) lists four characteristic of the ideal index of dispersion: (1) it should provide real and continuous values over the range from maximum regularity through randomness to maximum contagion; (2) it should not be influenced by size of sample, number of replicates, sample mean or total number of counts in the sample; (3) it should provide ease in calculation; and (4) it should allow significant testing from difference between samples. Elliot points out that no ideal index of dispersion exists. He states that while indices based on variance to mean ratio can serve as tests for agreement with a Poisson series, the fact that they can be used as comparative indices only when $\Sigma x$, $\bar{x}$ and n are the same in each sample disallows their use as measures of the degree of clumping in natural populations.

Two related indices, the Charlier coefficient

$$= 100 \sqrt{\frac{s^2 = \bar{x}}{\bar{x}}}$$

and Green's coefficient

$$= \frac{(s^2/\bar{x}) \ -1}{\Sigma x \ -1}$$

overcome some of these problems, with only Green's coefficient independent of n, $\bar{x}$ and also $\Sigma x$. It ranges from 0 (random dispersion) to 1 (maximum contagion), however, it appears that a large n is necessary for this test.

Other indices are available. For the negative binomial, k [from the probability series of the negative binomial $(q - p)^{-k}$ ] is an index of dispersion. This index is not independent of n and the values of k are often influenced by sample size. Since, in the present program, these factors are held constant for a station, this index can be utilized.

The parameter b of Taylor's power law and Morisita's index of dispersion are other important indices. The parameter b is a measure of degree of clumping that is apparently independent of n, $\bar{x}$ and $\Sigma x$. Since calculation of b requires several estimates of $s^2$ and $\bar{x}$, it cannot be calculated for one station.

Morisita's index of dispersion

$$I_\delta = n \frac{\Sigma[x(x-1)]}{\Sigma x(\Sigma x-1)} = n \frac{\Sigma(x^2)-\Sigma x}{(\Sigma x)^2-\Sigma x}$$

is independent of $\bar{x}$ and $\Sigma x$ but is a strong function of n. It is a good comparative index of dispersion when each station contains the same level of replication. When the distribution is random, $I_\delta = 1$. For contagion, $I_\delta > 1$ and for a regular distribution $I_\delta < 1$. Significance tests for departures from randomness are made at $\alpha = 0.05$. When $I_\delta (\Sigma x-1) + n-\Sigma x$ lies outside the 5% significance levels of $\chi^2$ for n-1 degrees of freedom there is a significant departure from the random distribution. We suggest Morisita's index of dispersion be used in the SPR study.

IV.2.2.2 Tests for Assumptions of Parametric Statistical Analysis

The normal distribution is usually not appropriate for count data, being more useful for continuous variables. However, a large number of statistical methodologies have the following requirements:

(1) The data must follow the normal distribution (normality).

(2) The variance and mean should be independent (homoscedasticity).

(3) The components of variance should be additive.

Several procedures are available for testing for normality, but we recommend the W test developed by Shapiro and Wilks. While chi-squared tests, G-tests and the Kolmogorov-Smirnov test are also available, the W test does not require that the mean and variance be included in the hypothesis. Coefficients necessary to compute W are given in Table 8

**Table 8. Coefficients to calculate W in the Shapiro-Wilk test for normality (from Anderson and McLean 1974).**

| i \ n | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.7071 | 0.7071 | 0.6872 | 0.6646 | 0.6431 | 0.6233 | 0.6052 | 0.5888 | 0.5739 |
| 2 | | .0000 | .1677 | .2413 | .2806 | .3031 | .3164 | .3244 | .3291 |
| 3 | | | | .0000 | .0875 | .1401 | .1743 | .1976 | .2141 |
| 4 | | | | | | .0000 | .0561 | .0947 | .1224 |
| 5 | | | | | | | | .0000 | .0399 |

| i \ n | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.5601 | 0.5475 | 0.5359 | 0.5251 | 0.5150 | 0.5056 | 0.4968 | 0.4886 | 0.4808 | 0.4734 |
| 2 | .3315 | .3325 | .3325 | .3318 | .3306 | .3290 | .3273 | .3253 | .3232 | .3211 |
| 3 | .2260 | .2347 | .2412 | .2460 | .2495 | .2521 | .2540 | .2553 | .2561 | .2565 |
| 4 | .1429 | .1586 | .1707 | .1802 | .1878 | .1939 | .1988 | .2027 | .2059 | .2085 |
| 5 | .0695 | .0922 | .1099 | .1240 | .1353 | .1447 | .1524 | .1587 | .1641 | .1686 |
| 6 | 0.0000 | 0.0303 | 0.0539 | 0.0727 | 0.0880 | 0.1005 | 0.1109 | 0.1197 | 0.1271 | 0.1334 |
| 7 | | | .0000 | .0240 | .0433 | .0593 | .0725 | .0837 | .0932 | .1013 |
| 8 | | | | | .0000 | .0196 | .0359 | .0496 | .0612 | .0711 |
| 9 | | | | | | | .0000 | .0163 | .0303 | .0422 |
| 10 | | | | | | | | | .0000 | .0140 |

| i \ n | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.4643 | 0.4590 | 0.4542 | 0.4493 | 0.4450 | 0.4407 | 0.4366 | 0.4328 | 0.4291 | 0.4254 |
| 2 | .3185 | .3156 | .3126 | .3098 | .3069 | .3043 | .3018 | .2992 | .2968 | .2944 |
| 3 | .2578 | .2571 | .2563 | .2554 | .2543 | .2533 | .2522 | .2510 | .2499 | .2487 |
| 4 | .2119 | .2131 | .2139 | .2145 | .2148 | .2151 | .2152 | .2151 | .2150 | .2148 |
| 5 | .1736 | .1764 | .1787 | .1807 | .1822 | .1836 | .1848 | .1857 | .1864 | .1870 |
| 6 | 0.1399 | 0.1443 | 0.1480 | 0.1512 | 0.1539 | 0.1563 | 0.1584 | 0.1601 | 0.1616 | 0.1630 |
| 7 | .1092 | .1150 | .1201 | .1245 | .1283 | .1316 | .1346 | .1372 | .1395 | .1415 |
| 8 | .0804 | .0878 | .0941 | .0997 | .1046 | .1089 | .1128 | .1162 | .1192 | .1219 |
| 9 | .0530 | .0618 | .0696 | .0764 | .0823 | .0876 | .0923 | .0965 | .1002 | .1036 |
| 10 | .0263 | .0368 | .0459 | .0539 | .0610 | .0672 | .0728 | .0778 | .0822 | .0862 |
| 11 | 0.0000 | 0.0122 | 0.0228 | 0.0321 | 0.0403 | 0.0476 | 0.0540 | 0.0598 | 0.0650 | 0.0697 |
| 12 | | | .0000 | .0107 | .0200 | .0284 | .0358 | .0424 | .0483 | .0537 |
| 13 | | | | | .0000 | .0094 | .0178 | .0253 | .0320 | .0381 |
| 14 | | | | | | | .0000 | .0084 | .0159 | .0227 |
| 15 | | | | | | | | | .0000 | .0076 |

(from Anderson and McLean 1974). The percentiles of W are given in Table 9 (also from Anderson and McLean). Shapiro, Wilk and Chen (1968) demonstrated, through comparisons of several tests, that the W test was generally superior in detecting nonnormality over a range of sample sizes from 10 to 50. To perform this test the following procedure is employed:

(1) Order the n observations in the sample $Y_1 \leq Y_2 \leq Y_3 \leq Y_4$

(2) Compute $\Sigma(Yi - \bar{Y})^2$

(3) If n is even, n = 2k, compute

$$b = \sum_{i=1}^{k} A_{n-i+1} (Y_{n-i+1} - Y_i)$$

where the values of $A_{n-1+i}$ are taken from Table 8

(4) With n odd, n=2k+1, and one omits the sample median, $Y_{k+1}$, and calculates

(5) $b = \sum_{i=1}^{k} A_{n-i+1} (Y_{n-i+1} - Yi)$

(6) Compute $W=b^2/\Sigma(Yi-\bar{Y})^2$

(7) Compare W to the percentage points given in Table 9. W is generally tested at the $\alpha$=.05 level.

For this test, small values of W indicate departures from normality. This test should be applied to each level of classification separately. If several of the hypotheses are rejected, a transformation should be made. If a relative few are rejected use the data in the untransformed state.

Anderson and McLean (1974) set up some rules to follow for making transformations to stabilize variances.

1. If the test for homogeneity is accepted at $\alpha$ = 0.01 level, do not transform.

**Table 9. Percentiles of W for Shapiro-Wilk test for normality (from Anderson and McLean 1974).**

| | Level | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| n | 0.01 | 0.02 | 0.05 | 0.10 | 0.50 | 0.90 | 0.95 | 0.98 | 0.99 |
| 3 | 0.753 | 0.756 | 0.767 | 0.789 | 0.959 | 0.998 | 0.999 | 1.000 | 1.000 |
| 4 | .687 | .707 | .748 | .792 | .935 | .987 | .992 | .996 | .997 |
| 5 | .686 | .715 | .762 | .806 | .927 | .979 | .986 | .991 | .993 |
| 6 | 0.713 | 0.743 | 0.788 | 0.826 | 0.927 | 0.974 | 0.981 | 0.986 | 0.989 |
| 7 | .730 | .760 | .803 | .838 | .928 | .972 | .979 | .985 | .988 |
| 8 | .749 | .778 | .818 | .851 | .932 | .972 | .978 | .984 | .987 |
| 9 | .764 | .791 | .829 | .859 | .935 | .972 | .978 | .984 | .986 |
| 10 | .781 | .806 | .842 | .869 | .938 | .972 | .978 | .983 | .986 |
| 11 | 0.792 | 0.817 | 0.850 | 0.876 | 0.940 | 0.973 | 0.979 | 0.984 | 0.986 |
| 12 | .805 | .828 | .859 | .883 | .943 | .973 | .979 | .984 | .986 |
| 13 | .814 | .837 | .866 | .889 | .945 | .974 | .979 | .984 | .986 |
| 14 | .825 | .846 | .874 | .895 | .947 | .975 | .980 | .984 | .986 |
| 15 | .835 | .855 | .881 | .901 | .950 | .975 | .980 | .984 | .987 |
| 16 | 0.844 | 0.863 | 0.887 | 0.906 | 0.952 | 0.976 | 0.981 | 0.985 | 0.987 |
| 17 | .851 | .869 | .892 | .910 | .954 | .977 | .981 | .985 | .987 |
| 18 | .858 | .874 | .897 | .914 | .956 | .978 | .982 | .986 | .988 |
| 19 | .863 | .879 | .901 | .917 | .957 | .978 | .982 | .986 | .988 |
| 20 | .868 | .884 | .905 | .920 | .959 | .979 | .983 | .986 | .988 |
| 21 | 0.873 | 0.888 | 0.908 | 0.923 | 0.960 | 0.980 | 0.983 | 0.987 | 0.989 |
| 22 | .878 | .892 | .911 | .926 | .961 | .980 | .984 | .987 | .989 |
| 23 | .881 | .895 | .914 | .928 | .962 | .981 | .984 | .987 | .989 |
| 24 | .884 | .898 | .916 | .930 | .963 | .981 | .984 | .987 | .989 |
| 25 | .888 | .901 | .918 | .931 | .964 | .981 | .985 | .988 | .989 |
| 26 | 0.891 | 0.904 | 0.920 | 0.933 | 0.965 | 0.982 | 0.985 | 0.988 | 0.989 |
| 27 | .894 | .906 | .923 | .935 | .965 | .982 | .985 | .988 | .990 |
| 28 | .896 | .908 | .924 | .936 | .966 | .982 | .985 | .988 | .990 |
| 29 | .898 | .910 | .926 | .937 | .966 | .982 | .985 | .988 | .990 |
| 30 | .900 | .912 | .927 | .939 | .967 | .983 | .985 | .988 | .900 |
| 31 | 0.902 | 0.914 | 0.929 | 0.940 | 0.967 | 0.983 | 0.986 | 0.988 | 0.990 |
| 32 | .904 | .915 | .930 | .941 | .968 | .983 | .986 | .988 | .990 |
| 33 | .906 | .917 | .931 | .942 | .968 | .983 | .986 | .989 | .990 |
| 34 | .908 | .919 | .933 | .943 | .969 | .983 | .986 | .989 | .990 |
| 35 | .910 | .920 | .934 | .944 | .969 | .984 | .986 | .989 | .990 |
| 36 | 0.912 | 0.922 | 0.935 | 0.945 | 0.970 | 0.984 | 0.986 | 0.989 | 0.990 |
| 37 | .914 | .924 | .936 | .946 | .970 | .984 | .987 | .989 | .990 |
| 38 | .916 | .925 | .938 | .947 | .971 | .984 | .987 | .989 | .990 |
| 39 | .917 | .927 | .939 | .948 | .971 | .984 | .987 | .989 | .991 |
| 40 | .919 | .928 | .940 | .949 | .972 | .985 | .987 | .989 | .991 |
| 41 | 0.920 | 0.929 | 0.941 | 0.950 | 0.972 | 0.985 | 0.987 | 0.989 | 0.991 |
| 42 | .922 | .930 | .942 | .951 | .972 | .985 | .987 | .989 | .991 |
| 43 | .923 | .932 | .943 | .951 | .973 | .985 | .987 | .990 | .991 |
| 44 | .924 | .933 | .944 | .952 | .973 | .985 | .987 | .990 | .991 |
| 45 | .926 | .934 | .945 | .953 | .973 | .985 | .988 | .990 | .991 |
| 46 | 0.927 | 0.935 | 0.945 | 0.953 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |
| 47 | .928 | .936 | .946 | .954 | .974 | .985 | .988 | .990 | .991 |
| 48 | .929 | .937 | .947 | .954 | .974 | .985 | .988 | .990 | .991 |
| 49 | .929 | .937 | .947 | .955 | .974 | .985 | .988 | .990 | .991 |
| 50 | .930 | .938 | .947 | .955 | .974 | .985 | .988 | .990 | .991 |

2. If the test is rejected at $\alpha = 0.001$, transform.

3. If the result lies between $\alpha = 0.01$, and $\alpha = 0.001$, try to find some practical reason to transform, otherwise do not.

There are a number of relatively simple tests available to test homogeneity of variances:

$H_0$: $\sigma_1^2=\sigma_2^2=\sigma_3^2=\sigma_\varepsilon^2$

$H_1$: not all $\sigma_i^2$ are equal.

1. Bartlett's test (Barlett 1937) is given by

$$B = \frac{2.30259}{C} \left[ V \log_{10} MS_{error} - \sum_{j=1}^{k} (V_j \log_{10} \hat{\sigma}_j^2) \right]$$

where $$C = 1 + \frac{\sum_{j=1}^{k} \frac{1}{V_j} - \frac{1}{V}}{3(k-1)}$$

$V_j$ = degrees of freedom for $\hat{\sigma}_j^2$

$v$ = degrees of freedom for $MS_{error} = \sum_{j=1}^{k} V_j$,

and $\hat{\sigma}_j^2$ = unbiased estimate of population variance for the jth population, given by,

$$\hat{\sigma}_j^2 = \left[ \sum_{i=1}^{n} X^2 = \frac{(\sum_{i=1}^{n} X)^2}{n} /(n-1) \right]$$

$MS_{error} = \sum_{j=1}^{k} \hat{\sigma}_j^2/V$, and k = number of variances.

For values of $V_j \geq 5$, $\chi^2$ tables are used with k-1 degrees of freedom.

2. Cochran (1941) suggested a simple test of homogeneity of variance given by

$$C = \frac{\hat{\sigma}_{j\ \text{largest}}^2}{\sum_{j=1}^{k} \hat{\sigma}_j^2}$$

where $\hat{\sigma}_j^2$ largest is the largest of the k treatment variances and $\sum_{j=1}^{k} \hat{\sigma}_j^2$ is the sum of all the variances. Degrees of freedom are k and n-1 (as in $F_{max}$ test), and the sampling distribution of C is given in Table 10.

3. Hartley's $F_{max}$ test (Hartley 1940, 1950) is a relatively simple statistic and is given by

$$F_{max} = \hat{\sigma}^2_{\text{largest}} / \hat{\sigma}^2_{\text{smallest}}$$

with k and n-1 degrees of freedom, where k is the number of variances and n is the number of observations within each treatment level. The distributions of $F_{max}$ are available in tables and the hypotheses of homogeneity of variances is rejected if $F_{max}$ is greater than the tabled value for $F_{max(\alpha)}$.

SPSS, in the ONEWAY procedure, provides these three tests for homogeneity of variance. We have run a sample problem for the STOCS benthic infaunal data, for four stations on Transect 3 during the 1977 fall cruise (Figure 16). These stations were chosen to give as wide an array of habitat (sediment, depth) and biological communities as possible, as revealed by classification analysis. Six replicates were collected at each station on the cruise. For number of species and number of individuals (Tables 11 and 12, respectively) the results clearly show heteroscedsticity, requiring some sort of data transformation before hypothesis testing.

4. Burr-Foster Q-test. Box and Anderson (1955) have shown that all three tests described above are sensitive to departures from normality as well as heterogeneity of variances. The Burr-Foster Q-test of homogeneity is based on a statistic that is a monotone function of the coefficient of variation of the sample variances, and is robust to nonnormality. If this is a problem, this test should be run in lieu of those provided in SPSS. For equal sample sizes (n) for each of p parent populations, let $S_i^2$ = the ith sample variance (i=1,...,P). Then

## Table 10. Upper percentage points of Cochran's test for homogeneity of variance (from Kirk 1968).

$$C = \frac{\text{largest } \hat{\sigma}_j^2}{\Sigma \hat{\sigma}_j^2}$$

| df for $\hat{\sigma}_j^2$ | $\alpha$ | $k$ = number of variances | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 15 | 20 |
| 1 | .05 | .9985 | .9669 | .9065 | .8412 | .7808 | .7271 | .6798 | .6385 | .6020 | .4709 | .3894 |
| | .01 | .9999 | .9933 | .9676 | .9279 | .8828 | .8376 | .7945 | .7544 | .7175 | .5747 | .4799 |
| 2 | .05 | .9750 | .8709 | .7679 | .6838 | .6161 | .5612 | .5157 | .4775 | .4450 | .3346 | .2705 |
| | .01 | .9950 | .9423 | .8643 | .7885 | .7218 | .6644 | .6152 | .5727 | .5358 | .4069 | .3297 |
| 3 | .05 | .9392 | .7977 | .6841 | .5981 | .5321 | .4800 | .4377 | .4027 | .3733 | .2758 | .2205 |
| | .01 | .9794 | .8831 | .7814 | .6957 | .6258 | .5685 | .5209 | .4810 | .4469 | .3317 | .2654 |
| 4 | .05 | .9057 | .7457 | .6287 | .5441 | .4803 | .4307 | .3910 | .3584 | .3311 | .2419 | .1921 |
| | .01 | .9586 | .8335 | .7212 | .6329 | .5635 | .5080 | .4627 | .4251 | .3934 | .2882 | .2288 |
| 5 | .05 | .8772 | .7071 | .5895 | .5065 | .4447 | .3974 | .3595 | .3286 | .3029 | .2195 | .1735 |
| | .01 | .9373 | .7933 | .6761 | .5875 | .5195 | .4659 | .4226 | .3870 | .3572 | .2593 | .2048 |
| 6 | .05 | .8534 | .6771 | .5598 | .4783 | .4184 | .3726 | .3362 | .3067 | .2823 | .2034 | .1602 |
| | .01 | .9172 | .7606 | .6410 | .5531 | .4866 | .4347 | .3932 | .3592 | .3308 | .2386 | .1877 |
| 7 | .05 | .8332 | .6530 | .5365 | .4564 | .3980 | .3535 | .3185 | .2901 | .2666 | .1911 | .1501 |
| | .01 | .8988 | .7335 | .6129 | .5259 | .4608 | .4105 | .3704 | .3378 | .3106 | .2228 | .1748 |
| 8 | .05 | .8159 | .6333 | .5175 | .4387 | .3817 | .3384 | .3043 | .2768 | .2541 | .1815 | .1422 |
| | .01 | .8823 | .7107 | .5897 | .5037 | .4401 | .3911 | .3522 | .3207 | .2945 | .2104 | .1646 |
| 9 | .05 | .8010 | .6167 | .5017 | .4241 | .3682 | .3259 | .2926 | .2659 | .2439 | .1736 | .1357 |
| | .01 | .8674 | .6912 | .5702 | .4854 | .4229 | .3751 | .3373 | .3067 | .2813 | .2002 | .1567 |
| 16 | .05 | .7341 | .5466 | .4366 | .3645 | .3135 | .2756 | .2462 | .2226 | .2032 | .1429 | .1108 |
| | .01 | .7949 | .6059 | .4884 | .4094 | .3529 | .3105 | .2779 | .2514 | .2297 | .1612 | .1248 |
| 36 | .05 | .6602 | .4748 | .3720 | .3066 | .2612 | .2278 | .2022 | .1820 | .1655 | .1144 | .0879 |
| | .01 | .7067 | .5153 | .4057 | .3351 | .2858 | .2494 | .2214 | .1992 | .1811 | .1251 | .0960 |
| 144 | .05 | .5813 | .4031 | .3093 | .2513 | .2119 | .1833 | .1616 | .1446 | .1308 | .0889 | .0675 |
| | .01 | .6062 | .4230 | .3251 | .2644 | .2229 | .1929 | .1700 | .1521 | .1376 | .0934 | .0709 |

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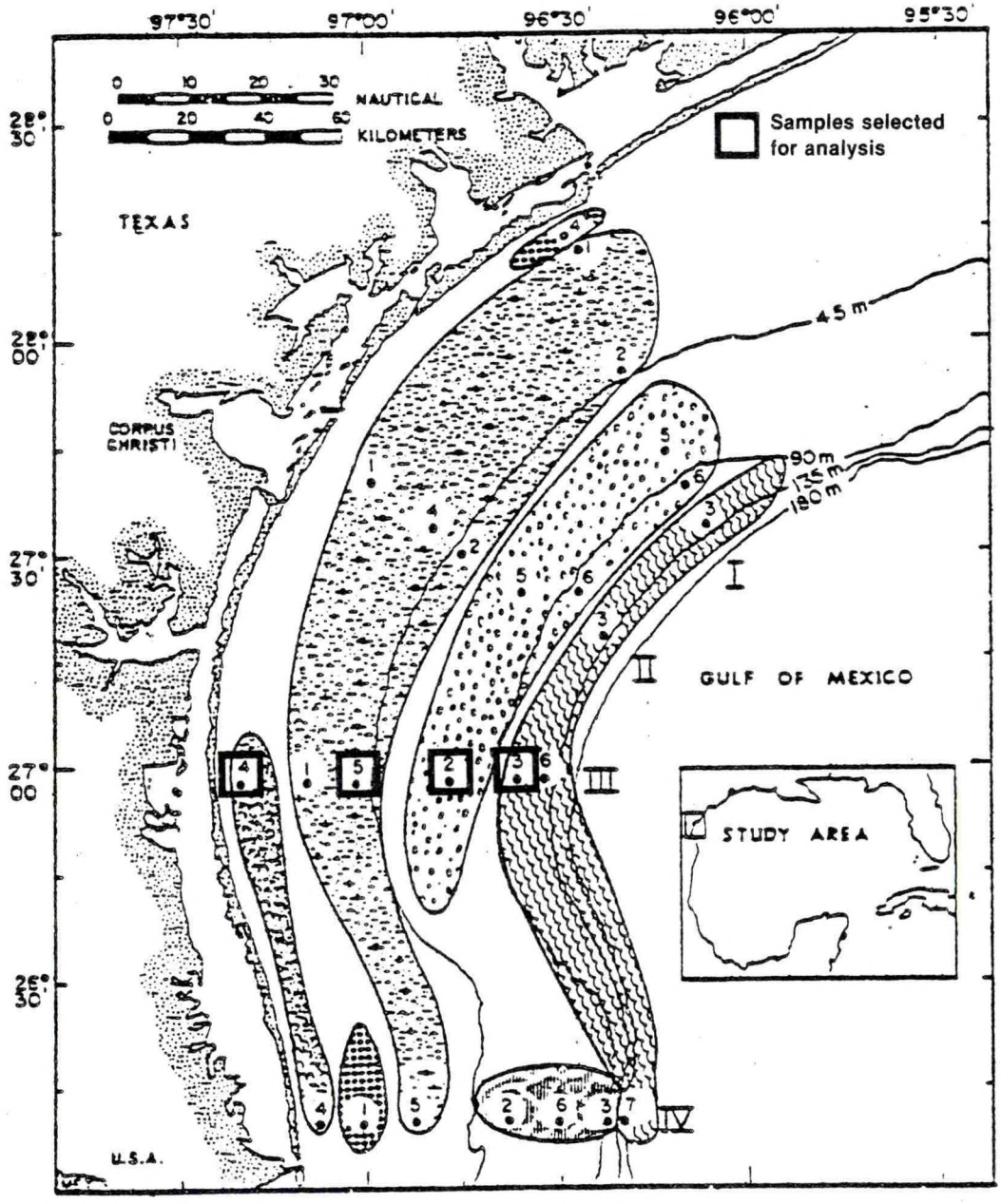



**Figure 16. STOCS macrobenthos sampling stations for fall 1977 including those used for testing homogeneity of variance.**

**Table 11. Results of homogeneity of variance tests for macrobenthos data (number of species) from four stations along transect 3 for the STOCS fall 1977 collections.**

ONEWAY

VARIABLE NSP NUMBER OF SPECIES

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 3 | 28386.0010 | 9462.0000 | 107.198 | 0.0000 |
| WITHIN GROUPS | 20 | 1765.3332 | 88.2667 | | |
| TOTAL | 23 | 30151.3340 | | | |

| GROUP | COUNT | MEAN | STANDARD DEVIATION | STANDARD ERROR | MINIMUM | MAXIMUM | 95 PCT CONF INT FOR MEAN | |
|---|---|---|---|---|---|---|---|---|
| GRP02 | 6 | 10.6667 | 2.2509 | 0.9189 | 9.0000 | 14.0000 | 8.3045 TO | 13.0288 |
| GRP03 | 6 | 14.6667 | 6.6533 | 2.7162 | 7.0000 | 25.0000 | 7.6845 TO | 21.6488 |
| GRP04 | 6 | 91.3333 | 17.1775 | 7.0127 | 69.0000 | 117.0000 | 73.3069 TO | 109.3597 |
| GRP05 | 6 | 10.6667 | 2.9439 | 1.2019 | 6.0000 | 14.0000 | 7.5773 TO | 13.7561 |
| TOTAL | 24 | 31.8333 | 36.2067 | 7.3907 | 6.0000 | 117.0000 | 16.5446 TO | 47.1221 |

TESTS FOR HOMOGENETIY OF VARIANCES
COCHRAN'S C = MAX. VARIANCE/SUM(VARIANCES) = 0.8357, p = 0.000 (approx.)
BARTLETT-BOX F = 7.363, p = 0.000
MAXIMUM VARIANCE / MINIMUM VARIANCE = 58.237

**Table 12. Results of homogeneity of variance tests for macrobenthos data (number of individuals) from four stations along transect 3 for the STOCS fall 1977 collections.**

ONEWAY

VARIABLE NIND NUMBER OF INDIVIDUALS

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 3 | 2365497.0340 | 788499.0000 | 37.302 | 0.0000 |
| WITHIN GROUPS | 20 | 422765.4791 | 21138.2734 | | |
| TOTAL | 23 | 2788262.5000 | | | |

| GROUP | COUNT | MEAN | STANDARD DEVIATION | STANDARD ERROR | MINIMUM | MAXIMUM | 95 PCT CONF INT FOR MEAN | |
|---|---|---|---|---|---|---|---|---|
| GRP02 | 6 | 13.3333 | 3.6148 | 1.4757 | 9.0000 | 18.0000 | 9.5399 TO | 17.1268 |
| GRP03 | 6 | 23.0000 | 14.8324 | 6.0553 | 9.0000 | 46.0000 | 7.4346 TO | 38.5654 |
| GRP04 | 6 | 745.8333 | 290.2588 | 118.4977 | 412.0000 | 1177.0000 | 441.2300 TO | 1050.4366 |
| GRP05 | 6 | 26.3333 | 8.3586 | 3.4124 | 20.0000 | 43.0000 | 17.5616 TO | 35.1050 |
| TOTAL | 24 | 202.1250 | 348.1793 | 71.0716 | 9.0000 | 1177.0000 | 55.1018 TO | 349.1482 |

TESTS FOR HOMOGENETIY OF VARIANCES
COCHRAN'S C = MAX. VARIANCE/SUM(VARIANCES) = 0.9964, p = 0.000 (approx.)
BARTLETT-BOX F = 27.848, p = 0.000
MAXIMUM VARIANCE / MINIMUM VARIANCE = 6447.717

$$Q = (S_1^4 + \ldots + S_p{}^4)/(S_1^2 + \ldots + S_p^2)^2.$$

Calculated value of Q are compared to tabular values (Table 13), with large values of Q lead to a rejection of the hypothesis of equal population variances.

In general, the F distribution is somewhat robust with respect to violations of the assumptions of homogeneity of variance provided that the number of observations in the samples are equal.

Although the problem of additivity is most important in analyses involving unreplicated data (where the interaction term must be used as the error mean square), it should be considered in all ANOVA's where interaction terms are present.

Tukey (1949) has devised a test (Tukey's one degree of freedom test for nonadditivity) to ascertain whether the interaction found in a given set of data could be explained in terms of multiplicative main effects (e.g. $Y_{ijk} = \alpha_i\beta_j\alpha\beta_{ij}e_{ijk}$). As an example, assume a two-way factorial ANOVA design with replication, where: $Y_{ijk} = u + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$ and the variance components are assumed additive. Tukey's test partitions the interaction sum of squares into one degree of freedom due to multiplicative effects (sum of squares non-additivity) and a residual sum of squares.

For each level i of factor A (where i=1 to a) compute

$$Q_i = \sum^{b} (\sum^{n} Yij)\ (n\bar{Y}_B - \bar{\bar{Y}})$$ where b = levels of factor B and j = 1 to b.

Now compute

$$Q = \sum^{a} Q_i\ (n\bar{Y}_A - \bar{\bar{Y}})$$

and

$$K = [\sum^{a} (n\bar{Y}_A - \bar{\bar{Y}})^2] \times [\sum^{b} (n\bar{Y}_B - \bar{\bar{Y}})^2].$$

Then SS nonadditivity = $Q^2/Kn$

And SS residual = SS interaction - SS nonadditivity

**Table 13. Percentile points for Q-test for equal degrees of freedom ($\nu$), and for samples (p) (from Anderson and McLean 1974).**

| | $\nu = 1$ | | $\nu = 2$ | | $\nu = 3$ | | $\nu = 4$ | |
|---|---|---|---|---|---|---|---|---|
| p | .99 | .999 | .99 | .999 | .99 | .999 | .99 | .999 |
| 3 | * | * | .863 | * | .757 | .919 | .684 | .828 |
| 4 | .920 | * | .720 | .898 | .605 | .754 | .549 | .675 |
| 5 | .828 | * | .608 | .773 | .512 | .644 | .443 | .552 |
| 6 | .744 | .949 | .539 | .690 | .430 | .546 | .369 | .461 |
| 7 | .671 | .865 | .469 | .606 | .372 | .471 | .318 | .394 |
| 8 | .609 | .793 | .412 | .537 | .325 | .411 | .276 | .342 |
| 9 | .576 | .750 | .371 | .481 | .287 | .363 | .244 | .300 |
| 10 | .528 | .694 | .333 | .433 | .257 | .324 | .218 | .267 |
| 12 | .448 | .598 | .276 | .358 | .211 | .265 | .179 | .217 |
| 14 | .391 | .522 | .234 | .303 | .178 | .222 | .151 | .181 |
| 15 | .365 | .490 | .217 | .280 | .165 | .205 | .140 | .167 |
| 16 | .343 | .460 | .202 | .261 | .154 | .190 | .130 | .155 |
| 18 | .304 | .409 | .178 | .228 | .135 | .165 | .114 | .135 |
| 20 | .273 | .367 | .158 | .202 | .120 | .146 | .101 | .119 |
| 22 | .246 | .332 | .142 | .180 | .108 | .130 | .090 | .106 |
| 24 | .224 | .302 | .129 | .162 | .098 | .117 | .082 | .096 |
| 26 | .206 | .276 | .118 | .148 | .090 | .107 | .075 | .087 |
| 28 | .190 | .254 | .108 | .135 | .082 | .098 | .069 | .080 |
| 30 | .176 | .234 | .100 | .124 | .075 | .090 | .064 | .074 |
| 32 | .163 | .218 | .093 | .115 | .070 | .083 | .060 | .068 |
| 36 | .143 | .189 | .082 | .100 | .062 | .072 | .052 | .060 |
| 40 | .127 | .167 | .072 | .088 | .055 | .064 | .047 | .053 |
| 45 | .111 | .145 | .063 | .076 | .048 | .055 | .041 | .046 |
| 50 | .098 | .127 | .056 | .067 | .043 | .049 | .037 | .041 |
| 60 | .080 | .102 | .045 | .053 | .035 | .039 | .030 | .033 |
| 64 | .074 | .094 | .042 | .049 | .033 | .037 | .028 | .031 |

*These entries exceeded 1 using the approximating distribution. Since $Q \geq 1$, they are omitted.

The F test is:

$$F_s = \text{MS non-additivity/MS residual},$$

with 1 and [(a - 1) · (b - 1)-1] degrees of freedom for the numerator and denominator respectively; a and b are the numbers of levels of the two main effects. The F value is then compared to the table F for the given $\alpha$ level (type I error), and the hypothesis of nonadditivity is rejected if $F_s > F_{tabular}$.

IV.2.2.3 Transformations

If the above tests lead one to reject the hypothesis of normality, equality of variances or additivity, then a transformation applied to the original data may make the hypotheses acceptable on the transformed data allowing parametric statistical tests (e.g., ANOVA) to be performed. Under certain conditions, the Poisson positive and negative binomial distributions will approach the normal distribution. However, in all cases, the variance and mean are not independent. If the variance is a function of the mean then a transformation can be made on the data to stabilize the variance, i.e., make it a constant regardless of the size of the mean. One transformation often corrects several conditions. Thus the same transformation can, at once, normalize the data, eliminate heteroscedasticity and restore additivity of the variance components. The following are our choices, should the data assume any of the following characteristics:

| Relationship of Mean to Variance | Transformation |
|---|---|
| Mean proportional to variance | Square root |
| Variance proportional to mean (1-mean) (proportional data, i.e., percentages) | Arcsine ($\sqrt{P}$) |
| Standard deviation proportional to $(\text{mean})^2$ | Reciprocal |
| Standard deviation proportional to mean | Log |
| Mean negatively correlated with variance | $\sqrt{B} - \sqrt{B-y}$ where $B = \max \{y_j\}$. |

For biological samples, where the variance is usually greater than the mean, the log (x + 1) transformation is usually prefered.

When transformed counts are used in statistical hypothesis testing, the arithmetic mean of the transformed counts are generally retransformed back to the original scale. These derived means are almost always smaller than the arithmetic means of the original counts (before transformation). An adjustment factor can be used to make the retransformed means comparable to the original means. For log transformation, 1.15 times the variance of the transformed counts can be added to the mean (transformed) before retransforming.

### IV.2.3 Bivariate Measures of Association

The objectives behind calculation of bivariate measures of association are given in Figure 17 and the components of these analyses are shown in Figure 18. This is essentially the stage where the first intervariable relationships are established, and these fall into four categories:

(1) Interspecies relationships

(2) Relationships between species and environmental variables

(3) Relationships between environmental variables

(4) Relationships between samples based on species composition or environmental characteristics.

In general, Pearson product moment correlation coefficients are used to show relationship in (1)-(3) above, while numerous other indices of association are used to establish relationships between samples. In correlation our concern is whether two variables/entities are inter-dependent (do they covary). It may well be that in some instances one variable is the cause of the variation in the other, but we neither know or assume this. Simple variable interrelationships may be examined (using scattergrams) by plotting one variable against another (Figure 19).

Not only are these simple bivariate correlations important in indicating variable groupings, but they are also used in the data matrix utilized

## BIVARIATE MEASURES OF ASSOCIATION

## OBJECTIVES

- ESTABLISH INITIAL BIVARIATE RELATIONSHIPS
  - BETWEEN SPECIES
  - BETWEEN SPECIES AND ENVIRONMENTAL PARAMETERS
  - BETWEEN ENVIRONMENTAL PARAMETERS
  - BETWEEN SAMPLES
- ESTABLISH SECOND ORDER COMMUNITY LEVEL GROUPINGS
- SERVE AS INPUT DATA TO HIGHER LEVEL ANALYSES



Figure 17. Objectives of bivariate measures of association.

## BIVARIATE MEASURES OF ASSOCIATION

## COMPONENTS

- CORRELATION COEFFICIENT
- INDICES OF ASSOCIATION
    - JACCARD
    - CZEKANOWSKI OR BRAY CURTIS
    - SORENSON'S INDEX
    - VARIOUS METRIC MEASURES (e.g. CANBERRA METRIC)
- $\chi^2$ TESTS OF ASSOCIATION



Figure 18. Components of bivariate measures of association.

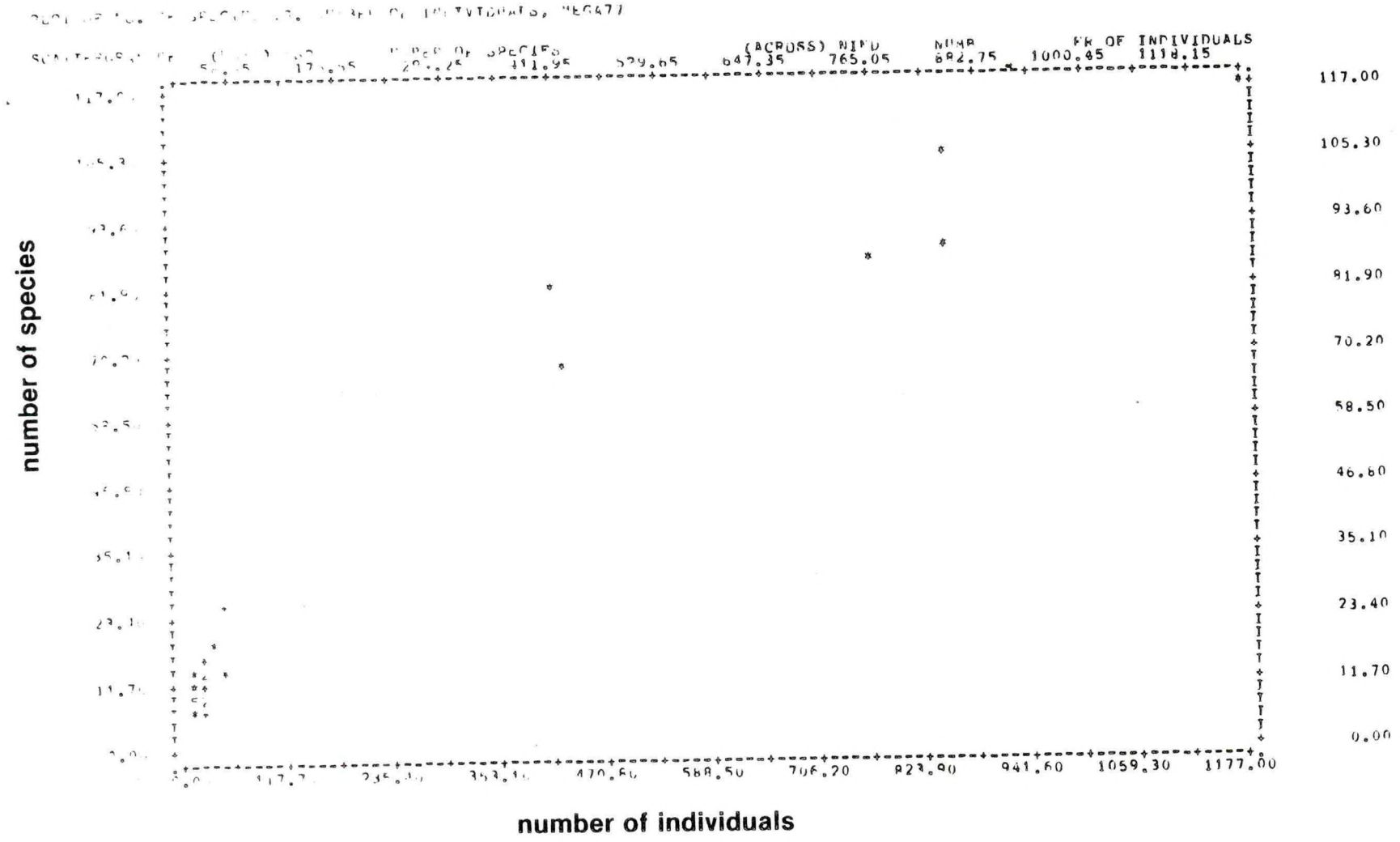



**Figure 19. Preliminary scattergram from SPSS showing the relation between the number of individuals and number of species for STOCS fall 1977 macrobenthos collections at four stations on transect 3.**

by higher level pattern analyses. While these correlations are instructive in identifying the strongest intervariable relationships, the myriad of relationships are difficult to comprehend when a large number of species are involved.

Another common technique used to elucidate species associations is the $\chi^2$ test of association. A 2x2 contingency table can be used to represent the frequencies of presence or absence of two taxa across a series of samples (Figure 20). The frequencies in the contingency table can be tested for positive, negative and random associations. The $\chi^2$ test of association may also be used to test the equality of a series associations with a 2 x N Table (Zar 1974).

A number of indices of association are available which establish the relationship between pairs of samples (Q-mode) and these have also been applied to pairs of species (over a number of samples) in the R-mode. Several of these are listed in Figure 18. The Bray Curtis measure, given by

$$\alpha_{c_{1,2}} = \frac{\sum_{i=1}^{N}(X_{1_j} - X_{2_j})}{\sum_{i=1}^{N}(X_{1_j} + X_{2_j})}$$

where

$X_{1_j}$ = the number of species j in sample 1,

$X_{2_j}$ = the number of species j in sample 2, and

N = number of different species in samples 1 and 2 combined.

is commonly employed and is typical of these measures. All double zero matches are excluded.

| | | Taxa A | | |
|---|---|---|---|---|
| | | Absent | Present | Row Totals |
| Taxa B | Absent | 100 | 30 | 130 |
| | Present | 25 | 75 | 100 |
| Column Totals | | 125 | 105 | 230 |

$\chi^2 = 61.41$

$\chi^2_{(d.f. = 1,\ \alpha = 0.05)} = 3.841$

reject hypothesis that taxa A and B are independently distributed

**Figure 20. Example of a 2 x 2 contingency table of frequencies used in $\chi^2$ test of association.**

The STOCS program used the Canberra-Metric measure (Lance and Williams 1967) to index dissimilarity between two samples (Q-mode) or two species (R-mode). Given two samples ($_1$ and $_2$), the Canberra Metric has the form:

$$CM_{12} = \frac{1}{N} \sum_{j=1}^{N} \frac{X_{1j} - X_{2k}}{X_{1j} + X_{2k}}$$

where $X_{1j}$, $X_{2j}$, and N are as defined above.

Zero entries cannot be used, but can be substituted for by very small numbers. These measures of association are extremely important because of their use in higher-level pattern analyses.

IV.2.4 Classification and Pattern Analysis Techniques

Five multivariate analysis tools which are used in environmental analyses have been applied to the SPR project (Comiskey et al. 1978, 1979, and 1980). These are cluster, factor, ordination, canonical correlation, and discriminant analysis. Because of their great utility in deriving major assemblage-level trends from large data sets, multivariate analyses are very useful in the baseline phase of an impact assessment monitoring program. Because each technique reveals unique information about the data set(s), they supplement each other, and we have found that it is very useful to apply at least several of these techniques to each environmental problem. The use of several of the analyses for particular data sets are demonstrated in Appendix A.

Due to the complexity of ecological communities and geochemical "assemblages," the patterns of variation are often much too intricate to be sorted out, at least initially, by univariate methods. The variables are too interrelated to directly apply "experimental techniques." That is, for any variable the number of covariates is so great that any univariate hypothesis testing procedure requires an unacceptable number of assumptions or support statistics (e.g., analysis of covariance). These problems with experimental design are particularly troublesome when a posteriori hypothesis testing is applied to what is essentially survey data. That is, survey data is by nature multivariate. Thus,

before any rigid hypothesis testing can be conducted, the basic pattern (s) in the data set(s) must be elucidated. Therefore multivariate techniques can be utilized to determine temporal and spatial trends in the data, and also serve as a guide to design of subsequent hypothesis testing activities.

The objectives of classification and pattern analysis are summarized in Figure 21, while Figure 22 outlines the data products expected from the application of these five types of multivariate analyses in the SPR program. Each is discussed in more detail below. One of the major purposes of the analyses of biotic assemblage data and ancillary environmental variables is to define species groupings (communities), station grouping (habitat) and relationship between species groupings and groups of environmental parameters. These results can be realized from the application of multivariate pattern analyses.

Classification and pattern analyses are actually two distinct ways of looking at biotic assemblage data. In classification analysis (e.g., cluster), the theoretical basis implicitly recognizes the occurrence of distinct communities of organisms or groupings of stations (habitats), while in pattern analysis, and especially in ordination analysis, the objectives are to display or order the species assemblage and stations over the ranges of important environmental trends (e.g., gradients). Communities as such are not defined, but the relationship of any one sample to any other is retained. This is often lost when species or samples are classified into discrete groupings. Distinct groupings of species in the ordination display would suggest more discrete communities. Factor analysis, canonical correlation analysis and discriminant function analysis all define major trends (groupings) in the data but allow for particular species or samples to be shared by several communities.

Multivariate classification or pattern analysis can assume either of two modes, "Q" or "R". Q-mode techniques analyze the pattern of samples (observations) in species (variable) space, while R-mode analysis searches for trends in the pattern of species in sample space (Kim

# CLASSIFICATION AND PATTERN ANALYSIS

## OBJECTIVES

- DEFINE MAJOR TRENDS WITHIN AND BETWEEN LARGE MULTIVARIATE DATA SETS
    - DEFINITION OF SPECIES GROUPINGS (COMMUNITIES)
    - DEFINITION OF STATION GROUPINGS (HABITAT)
    - DEFINITION OF THE RELATIONSHIP OF SPECIES GROUPINGS TO STATION GROUPINGS
- CLASSIFY SAMPLES INTO HOMOGENEOUS GROUPS
- DISPLAY SAMPLE / SPECIES ALONG GRADIENTS OF ENVIRONMENTAL FACTORS
- PROVIDE GUIDANCE TO HYPOTHESIS TESTING



**Figure 21.** Objectives of classification and pattern analysis.

# CLASSIFICATION AND PATTERN ANALYSIS COMPONENTS

## CLUSTER ANALYSIS

- PRODUCES DENDROGRAMS THAT ALLOW THE INVESTIGATOR TO DEFINE, BASED ON SPECIES COMPOSITION, SIMILAR GROUPINGS OF SPECIES (R-MODE) OR SAMPLES (Q-MODE) FOR A GIVEN LEVEL OF ASSOCIATION.

## RECIPROCAL AVERAGING ORDINATION ANALYSIS

- PRODUCES DIRECTLY COMPARABLE DISPLAYS OF SPECIES AND SAMPLES ALONG MAJOR AXES OF (ENVIRONMENTAL) VARIATION.

## FACTOR ANALYSIS

- YIELDS FACTORS (VECTORS OF VARIANCE) WITH EITHER SPECIES (R-MODE) OR SAMPLE (Q-MODE) LOADINGS. EACH VECTOR OR FACTOR REPRESENTS A MAJOR MULTIVARIATE TREND (e.g., SUMMER, HIGH SALINITY COMMUNITY), AND THE LOADINGS OF THE SPECIES OR SAMPLES REPRESENTS THEIR AFFINITY TO THE FACTOR.

- Q-MODE ANALYSIS RESULTS IN STATION (HABITAT TYPE) GROUPINGS
- R-MODE ANALYSIS RESULTS IN SPECIES (COMMUNITY) GROUPINGS

## DISCRIMINANT ANALYSIS

- DEFINES A VECTOR THAT MAXIMALLY DISCRIMINATES BETWEEN TWO OR MORE SETS OF DATA. THE PROCEDURE OF VARIABLE SELECTION FOR THE DISCRIMINANT FUNCTION IS STEP-WISE AND ALLOWS TESTING FOR SIGNIFICANT DIFFERENCES IN GROUP CENTROIDS. THE DISCRIMINANT FUNCTION ALLOWS ONE TO CLASSIFY SUBSEQUENT SAMPLES FOR GROUP MEMBERSHIP.

## CANONICAL CORRELATION ANALYSIS

- DEFINES PAIRS OF VECTORS (CANONICAL VARIABLES) FOR A PAIR OF DATA SETS THAT REPRESENT STRONG MULTIVARIATE TRENDS BETWEEN THE TWO DATA SETS.

- ANALYSIS OF A MACROBENTHIC SPECIES DATA SET WITH A DATA SET FOR ENVIRONMENTAL VARIABLES WILL YIELD GROUPS OF SPECIES (COMMUNITIES) WHICH ARE RELATED SIGNIFICANTLY TO GROUPS OF ENVIRONMENTAL VARIABLES (HABITAT).

**Figure 22. The components of classification and pattern analysis.**

1975). Obviously comparison of Q- and R-mode analyses of the same data set is very instructive. Q- and R-mode analyses are also called direct and inverse analysis in some environmental applications.

Figure 23 outlines the way that multivariate techniques have been used in the SPR program. Discriminant analysis has been routinely employed only for Q-mode analysis, while canonical correlation analysis has been used only for R-mode analysis. Cluster and factor analyses have been employed in our work for both Q- and R-mode analysis, but there is not necessarily a direct relationship between the sample and species groupings. This has been demonstrated by the failings of many ordered two-way tables (nodal analyses) to provide an interpretable arrangement of the data matrix. Ordination provides the link between the two modes by doing a simultaneous Q- and R-mode analysis, the graphical results of which are directly comparable.

A second way of looking at multivariate analyses is whether the analysis is conducted on one data set or between or among several data sets. If the analysis is conducted on one data set, ordination, cluster, and factor analyses can be used, while comparisons between two sets of data (e.g., species and environmental variables) are performed using discriminant and canonical correlation analysis.

It should again be stressed that these analyses should be performed in concert. Quite often, a cluster analysis will reveal a different attribute of the data set than will ordination, with both being needed for proper understanding and characterization of the natural system. For example, there are important analytic linkages between cluster analysis and discriminant analysis. Cluster analysis can be used to reveal "homogeneous" groupings of stations or variables (e.g. species). The stations within these "homogeneous" groupings can then be used as groups in discriminant analysis, and discriminant functions can be derived that best segregate the samples in these two groups. In the process, the species most important for group discrimination are revealed. These discriminating species could be "indicators" of one of the groups, and their absence in a sample may be used as an "indicator"

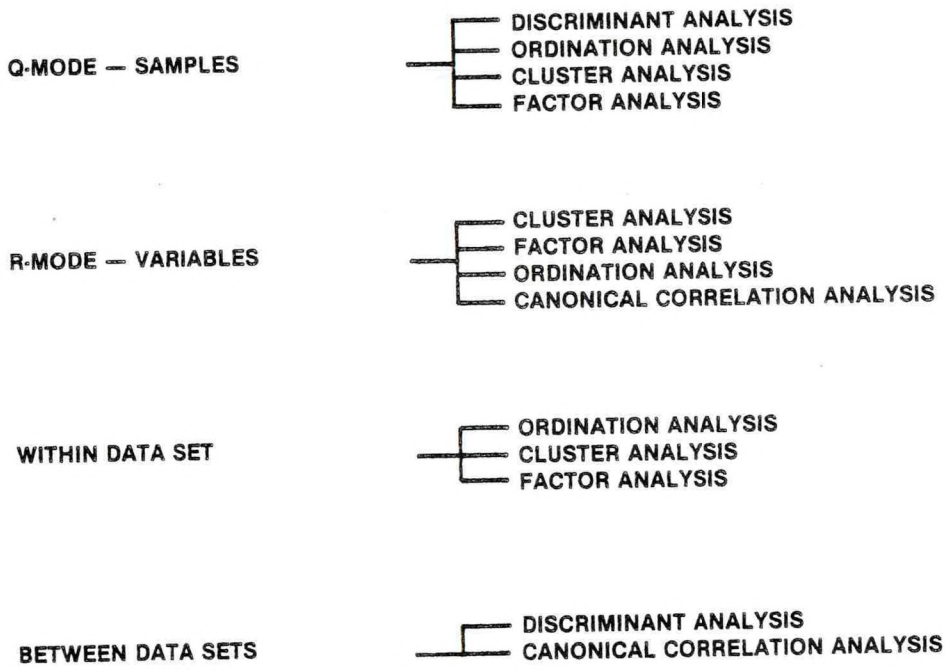



**Figure 23. Uses of multivariate analyses in previous marine studies from the NW Gulf of Mexico.**

of the other group. This is, therefore, another method whereby species groups can be related to station groups. Similarly, Q-mode cluster analysis, based on environmental variables, could be used to define station groups, and then the discriminant functions (based on species composition) derived for the cluster groups. In this way species groups (as indicated by the discriminant function scores) can be related more directly to environmental parameters (habitat).

A chronic problem involved in the a posteriori application of pattern and classification analyses is an inappropriate sample size. Table 14 gives some general guidelines for sample size based on several analytic parameters.

IV.2.4.1 Cluster Analysis

Cluster analysis can be used in two very important ways in the SPR program. They are as follows:

1. Because of the ability of clustering techniques to sort out community types, they have been applied in numerous pollution studies (e.g. Stephenson et al. 1974 and Roback et al. 1969). Due to pollution stresses, perturbed communities will be segregated from community types in healthy areas, thus facilitating detection of pollution stresses and assessment of ecological impacts. Walker (1974) gives a detailed discussion of the application of clustering techniques to benthic ecology.

2. Because cluster analysis is a powerful tool for identifying homogeneous groups of stations (based on the particular variables used), the technique provides valuable insight regarding the assumptions of statistical techniques and provides guidance in hypothesis testing. If distinct subgroups are not revealed by cluster analysis the data set can be taken to be essentially homogeneous. Since many of the classical methods of parametric statistical analysis are predicated on an assumed homogeneity within certain groups of the data set, cluster analysis provides an opportunity to investigate empirically the degree of success achieved in attempts to fulfill assumptions of homogeneity. The requirements of homogeneity are especially true for discriminant analysis and ANOVA. The clustering should be performed using potential covariates in order to assess whether or not the groupings of the data are homogeneous with respect to those variables, which are

**Table 14. Sample size needed to perform various multivariate statistical tests given by analytic method.**

| Analytical Method | Sample Size |
|---|---|
| Regression Analysis | $N \geq 2p+25$ |
| | $*N \geq 4p+25$ |
| Polynomial Regression | $N \geq 10q$ |
| Principle Components Analysis | $N \geq 5p+15$ |
| Factor Analysis | $N > 5p$ |
| Discriminant Analysis | $N > 3(p)-2$ |
| | $N \geq 2(p+k)15$ |
| Canonical Correlation | $N \geq 4(p+q)+15$ |
| Multivariate Analysis of Variance | $N \geq 2(p+\text{Number Cells})+15$ |

N = Number of samples.

P = Number of variables.

q = Number of independent variable.

k = Number of groups.

* = Multiples of 4 may be reduced to 2 if the normality assumption holds.

not included in the statistical design. The value of cluster analysis as an aid to factor and discriminant analysis is discussed further by Anderberg (1973).

There are a series of choices to make in performing cluster analysis. The several techniques available can give somewhat different results. Pielou (1977) has summarized the process into five basic decisions that must be reached in order to classify data. Clustering (classifications) strategies have also been discussed by Williams and Dale (1965), Williams (1971), and Sneath and Sokal (1973).

Clustering has been performed in the SPR program using an hierarchical, agglomerative, unweighted pair-group method (Lance and Williams 1967) with Jaccard's quantitative coefficient (Sepkoski 1974) as a distance measure. Other measures of association can be used including Czekanowski's coefficient and Otsuka's coefficient. Program options should include the ability to standardize values of each variable to a zero to one-hundred (0-100) scale. The scaling removes the effect of the relative differences in the size of the variables. Thus, the numerically dominant species are no more important than the less abundant species. Clustering programs utilizing flexible fusion strategies should also be considered for use in the program.

Due to cost (core usage) limitations associated with hierarchical clustering, these analyses should be performed on smaller (e.g., 120 samples) subsets of the data, both with regard to number of samples and number of variables (e.g. sediment variables or species counts). A non-hierarchical clustering program, designed specifically to handle very large data sets is discussed under Exploratory Analysis.

Some investigators utilize various types of ordered two-way (species by sample) tables to characterize the relationships resulting from Q- and R-mode cluster analysis. An example of this, from the STOCS study is presented in Figure 24. Because of the nature of cluster analysis discussed above, this two-way ordered display (nodal analysis) is often not very successful at defining Q- and R-mode relationships. There are

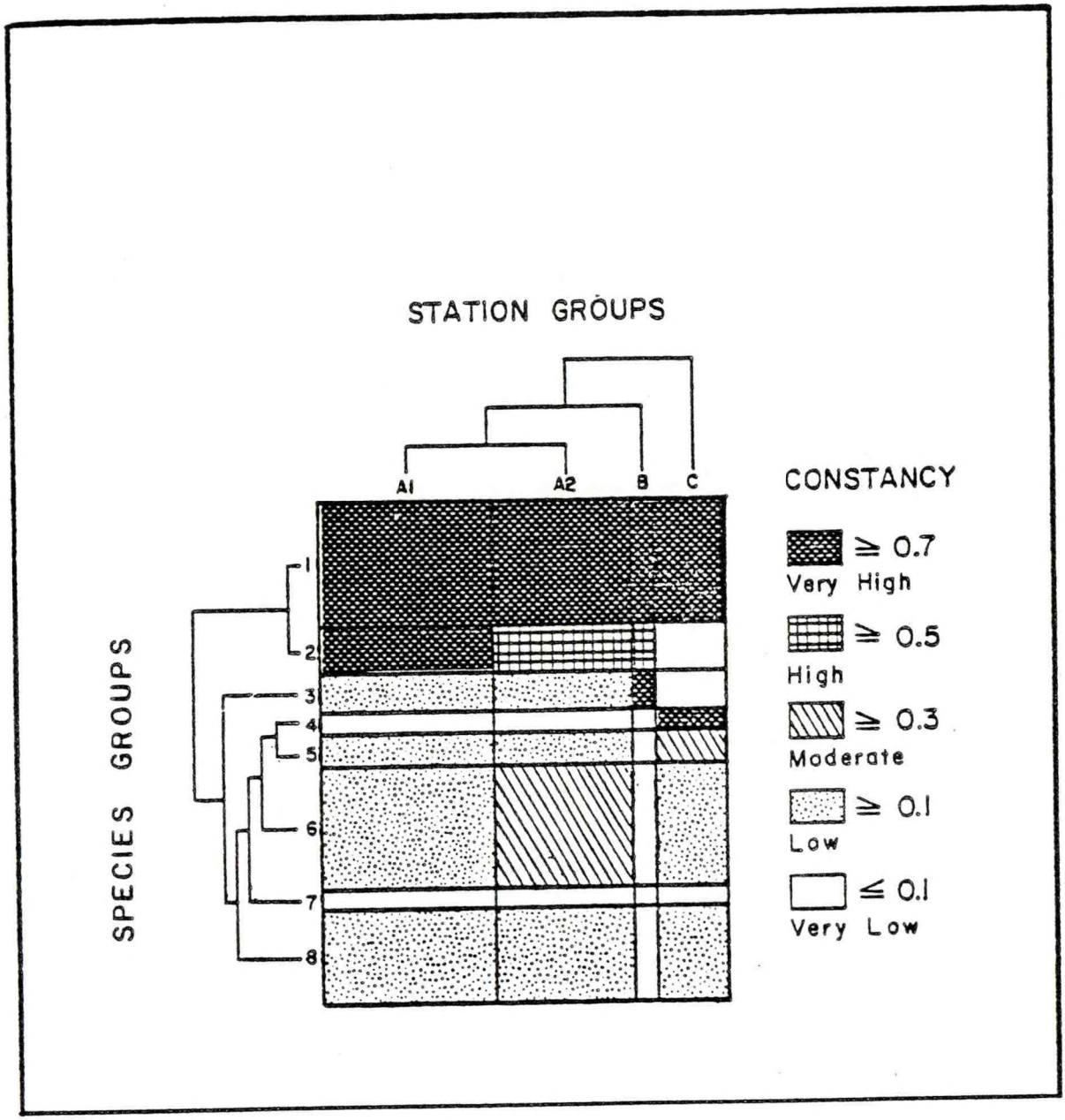



**Figure 24. Ordered two-way table resulting from Q- and R-mode cluster analysis (from Holland et al. 1977).**

other ways which are possibly superior to nodal analysis, as will be discussed below under ordination analysis.

IV.2.4.2 Ordination Analysis

Another technique that can be utilized to determine spatial and temporal patterns in community composition is ordination analysis. Results of ordination analysis are presented in a spatial display of species and/or sample relationships from a data matrix to geometric axes, thereby reducing complex multidimensionality of a many species by many sample data matrix to the dimensionality of two or three ordination axes which account for a majority of the variance in the data. This aids in defining major gradient trends in the data, which can then be related inductively to ecological gradients (Whittaker 1967; Whittaker and Gauch 1973).

The main purposes of using ordination techniques in ecological analyses are:

- To reduce a data matrix of multidimensionality to fewer dimensions revealing major trends.
- To perform an indirect gradient analysis by relating species and/or sample trends to environmental gradients such as sediment grain size or hydrographic gradients.

Ordination techniques include mathematically simple procedures such as weighted average (Whittaker 1948, Curtis and McIntosh 1951) and polar ordination (Bray and Curtis 1957). The former requires considerable knowledge of species characteristics in order to calculate "weights" to place points along an environmental gradient. The latter allows for the systematic (geometrical array) placement of samples between defined end points along an axis. The distance between pairs of samples may be calculated by percentage distance, Euclidean distance or some other commonly used distance measure (Beals 1960).

More mathematically sophisticated techniques are also utilized. These generally fall into the category of eigenanalysis problems. A commonly

used method is principal components analysis. The algorithm is found in statistics texts such as Tatsuoka (1975), Pielou (1977), and Morrison (1967). In essence, principal components is a mathematical method of projecting a multidimensional cloud of points into fewer dimensions. This usually involves a rigid rotation to derive a series of orthogonal axes which account for most of the common variance in the data set. Computations are performed on a secondary matrix such as a variance-covariance matrix to derive eigenvectors and eigenvalues. A good discussion of the technique is found in Gittins (1969).

A related technique receiving wide use is known as reciprocal averaging ordination analysis (Hill 1973). It is closely related to principal components analysis, being an eigenanalysis technique. Hill (1973) provides a comparison of reciprocal averaging to principal components with examples. Reciprocal averaging provides advantages over other ordination techniques in that no prior knowledge of the species is required to assign weights (scores are derived from the primary data matrix) and simultaneous species and sample ordinations are performed. This allows examination of species trends in sample space and sample trends in species space, thus providing an excellent set of graphics to be used in data trend or pattern analysis. In addition, species and/or sample relative locations on the ordination axes may be interpreted as "ecological distances" (Gauch 1977). The size of the eigenvalue for each axis (determining the amount of variance explained by each axis) will often determine the number of axes which should be examined.

Each ordination technique has its advantages and disadvantages. Gauch (1977) has reviewed several of the techniques and has developed a computer program package called ORDIFLEX which has been routinely employed in previous marine analyses by SAI staff to perform reciprocal averaging ordination. As suggested by Gauch (1977), an approach of successive refinement should be used. More than one type of analysis is usually performed so that the interpretations of patterns in the data are put into the framework of the computational characteristics of each procedure. The advantage of using a package such as ORDIFLEX is the efficiency by which more than one technique may be executed, saving

computer time and cost. We have recently implemented an improved version of reciprocal averaging ordination (Hill 1979a) called DECORANA, and will employ it in the present study.

In many cases, the data matrix resulting from Q and R mode cluster analysis ordering will not readily show clear two-way (species and sample) trends. This occurs because the two-way classification is not done in a coordinated manner, and the orderings of species or samples in the dendrograms usually do not represent a gradient of trends. One attempt to get around these problems is the TWINSPAN (two-way Indicator Species Analysis) program from Cornell (Hill 1979b). The program first dichotomously classifies samples, and then, classifies species within the context of the sample classification. This two-way classification is used to rearrange the derived data matrix so that the resulting ordered two-way table expresses the species' synecological relations as succinctly as possible. This table is then used to identify "differential species." A differential species is one with clear ecological preferences, so that its presence can be used to identify particular environmental conditions.

The dichotomous classifications in TWINSPAN are accomplished through a series of three ordination routines. The primary ordination is a reciprocal averaging technique similar to that used in ORDIFLEX, yielding both samples and species ordinations. This primary ordination is then dichotomized and, after identification of "differential species" (based on which side of the dichotomy they resided) a refined ordination is derived from the primary ordination, and it too is dichotomized. Finally an indicator ordination, based on a few of the most highly preferential species is constructed and compared to the refined ordination.

TWINSPAN is useful at a relatively early stage in the analysis since it can handle relatively large data sets. The output from TWINSPAN is an ordered two-way table expressing the relationship between species and station groupings.

### IV.2.4.3 Canonical Correlation Analysis

Canonical correlation analysis takes as its basic input two data sets, each of which represents multiple measurements (variables) from a particular domain of traits. Typically one group consists of predictor or independent variables and the other group contains criterion or dependent variables. An example might be species composition data as one data set and environmental variables as the other set. The basic strategy of canonical correlation analysis is to derive linear combinations from each set of variables in such a way that the correlation between the two linear combinations is maximized. In this manner the analysis technique accounts for the maximum linear relationship between the two sets of variables (Hotelling 1935, 1936). The linear combinations of the original variables formed in the process of obtaining the canonical correlations are termed canonical variates. Geometrically, the canonical correlation analysis method may be considered an exploration of the extent to which individuals occupy the same relative positions in one variable-set measurement space as in the other. After the first pair of canonical variates is extracted from the data sets, further linear combinations may be discovered that help define the relationship between the two variable sets. This further extraction of canonical correlations is subject to the constraints of orthogonality (i.e., independence) with all previous correlations extracted, and the combinations of the original variables must be linear.

The canonical correlation model reduces the dimensionality of the data to a few linear functions of the measures under study, by selecting those functions that have maximum covariances between domains. In other words, it tells how well the variation observed in one set of variables corresponds to the variation observed in the other set of variables. In the case of comparison of a species composition data set with a data set of important environmental variables (e.g., benthos vs. sediment parameters) canonical correlation analysis can be utilized to define the "habitat" of particular species groupings.

Of course, the univariate correlations between pairs of measures taken one from each domain are of interest, but there may be a great many of these. For example, if there are ten species and ten environmental factors there are one hundred bivariate correlations between pairs of species and environmental variables, in addition to the many species-species and environmental-environmental correlations. To try to think about all these correlations simultaneously is very difficult if one is attempting to generalize about the extent and nature of interrelationships of the domains.

IV.2.4.4 Factor Analysis

Factor analysis demonstrates relationships within or between sets of variables, and does not make the distinction between independent and dependent variables. Just as with canonical correlation analysis, factor analysis finds principal axes, each of which accounts for a smaller amount of variation in the data than the axes previously derived. The initial linear combinations of variables (factors) are independent and uncorrelated with one another. Also in both factor analyses and canonical correlation analysis, the loadings on the factors or canonical variates can be used to generate scores for each observation in the data set. The difference between the two techniques is that factors are usually rotated to simple structure, while rotation would not make sense in the context of canonical correlation analysis. Factor analysis has a record of successful application in marine community characterization (Lie and Kelley 1970; Angel and Fasham 1973; Comiskey et al. 1978, 1979, 1980).

The first step in factor analysis involves the calculation of appropriate measures of association for a set of relevant variables. Most factor analyses procedures require product-moment correlation coefficients, but in certain instances, variance-covariance matrices are more appropriate. Granted that some type of association matrix is used as the basic input to factor analysis, correlations between variables (or attributes) or "association" between individuals or objects may be calculated. If factor analysis is applied to a correlation matrix of units (objects, individuals, communities, or the like), it is called

Q-factor analysis, while the more common variety, based on correlations between variables, is known as R-mode factor analysis.

Factor analysis is actually a general term, which encompasses a wide variety of methods. Essentially, the particular factor analysis method employed depends on whether or not the original inter-variable association matrix (correlation matrix) is altered. If not, principal components analysis is performed. In principal components analysis, no assumptions are made regarding the underlying structure of the data. One simply extracts factors (linear combinations of variables), the first one extracted accounting for more variance than any subsequently derived axis. The next, orthogonal to the first, accounts for the next highest amount of variance, and so on until the factors are explaining an unacceptably small amount of the variance to be considered important.

Scree tests (eigenvalue plots) are used, along with the comparison of factors derived from the application of several techniques to the same data set to determine the number of factors to retain for rotation to simple structure in the final analysis. An example of a scree test is given in Figure 25. A point on the scree curve representing a distinct transition to less steep slope is used to determine the number of factors to retain.

The final factor pattern matrix will have "loadings" from each species on each factor. The absolute size of the loading on a factor is indicative of its relationship to the factor. The investigator selects a minimum loading, such that all loadings above this level are considered "salient" and worthy of further investigation. The species with salient loadings are considered indicative of that factor, and the factor is usually defined on the basis of the spatial/temporal characteristics of the species with salient loadings. Thus, a factor might indicate a spring community characteristic of clay bottoms. In this way, the species group is related directly to the sample group.

More quantitative assessment of factor associations comes from the use of factor scores, produced by the scoring coefficient matrix. These

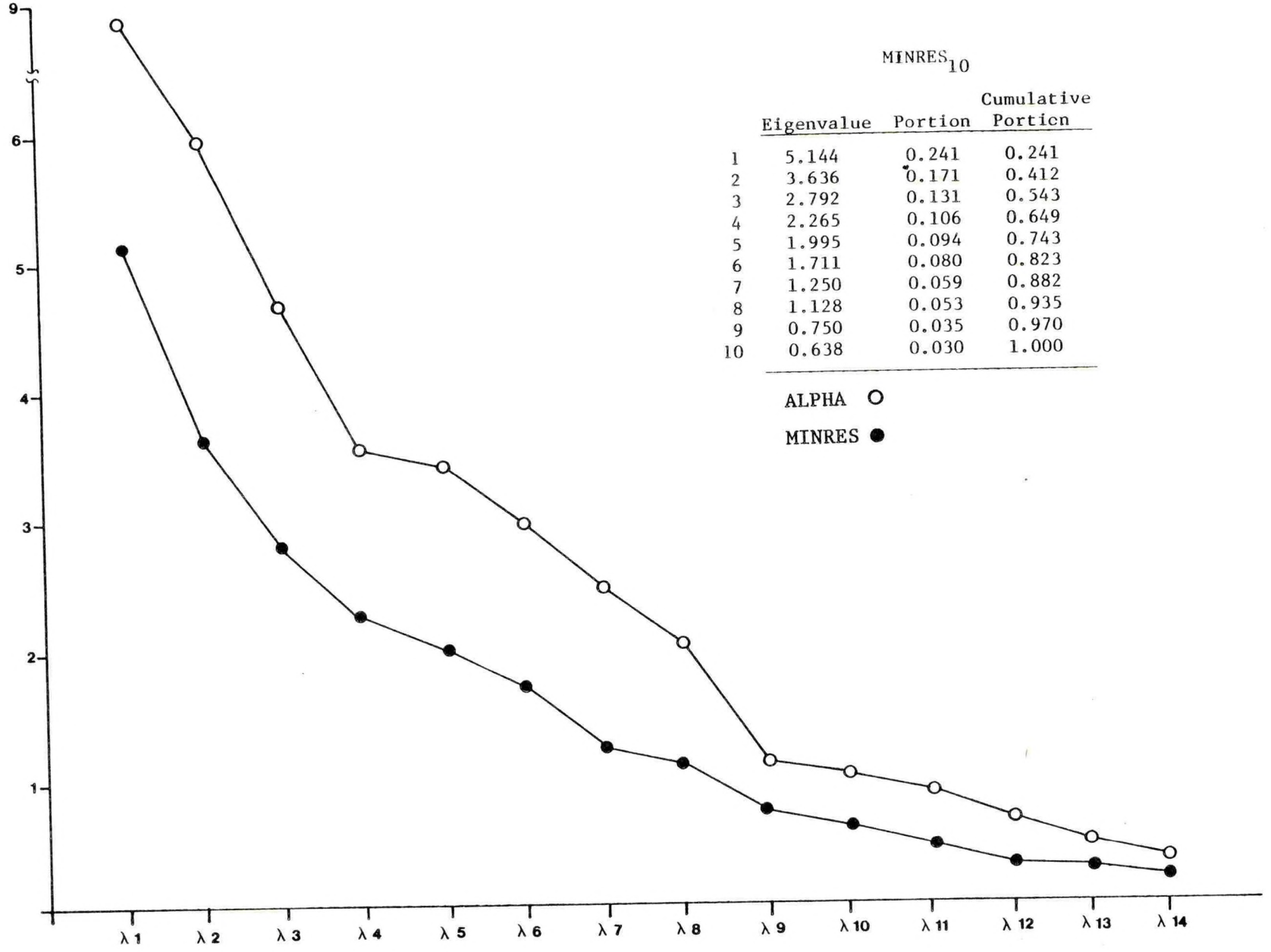



**Figure 25. An example of a scree test used to determine the number of factors to retain for rotation to simple structure for phytoplankton data collected at the Texoma brine discharge sites (October 1977-May 1978).**

factor scores can be used as variables to relate to important environmental variables through either bivariate measures of association or other classification or pattern analyses.

One of the cornerstones of factor analysis is inter-study factor comparisons. If the same or similar factors are extracted from several analyses (e.g., pre- and post-impact samples) the scientist can make more sweeping and general statements concerning trends in the ecosystem to which the techniques have been applied.

### IV.2.4.5 Discriminant Analysis

Discriminant analysis (sometimes called canonical variate analysis) has several characteristics that are useful in analysis of ecological data. It is one of several multivariate techniques described in such works as Cooley and Lohnes (1971), Tatsuoka (1971), Pielou (1977) and Morrison (1967).

The basic purpose of discriminant analysis is to allow the researcher to distinguish between two or more groups of observations. The groups are defined by the particular research situation and to distinguish between them, the researcher selects a set of discriminating variables on which the groups are expected to differ. For example, a marine ecologist might define the groups as the clusters of samples that define such categories as pre-impact (baseline) versus post-impact observations. Several types of discriminating variables may be used to distinguish between those groups, depending on the purpose of the analysis. Using species densities, one can test for differences in groups before and after impact. Using environmental parameters, he can test for differences in pre- and post-impact (e.g., brine disposal) conditions, ultimately testing the success of his measured environmental variables in discriminating between the defined groups.

The mathematical objective of discriminant analysis is to statistically define groups as discretely as possible by weighting and forming a linear combination of the variables used as discriminators. In theory, the variables are assumed to have a multivariate normal distribution and

equal variance-covariance matrices within each group. However, these assumptions need not be strictly adhered to in practice, as the method is robust (Nie et al. 1975). Discriminant functions are formed in such a way as to provide the maximum separation of the groups on that function.

Discriminant function analysis has not been used extensively in marine ecological analyses. Kercher and Goldstein (1977) have used discriminant analysis to distinguish between plant community types on the basis of environmental variables. Noy-Meir and Whittaker (1977) view the multivariate techniques such as discriminant (canonical variate) analysis as promising and relevant to ecological community description. The technique is certainly applicable to marine data (Comiskey et al. 1978; see examples in Appendix A).

Discriminant analysis has been used in the SPR Program to evaluate the discreteness of groups of samples using geochemical and environmental parameters as discriminant variables. The analysis provides three things that are useful for determining discreteness and displaying the relative degree of difference between groups:

1. It provides a plot of the centroid of the samples in a group on discriminant function axes (canonical variate axes) which gives a geometric representation of group relationships.

2. It provides an F-matrix for testing the difference between groups.

3. It provides a classification success table detailing the success of placing samples into the groups to which they are most related.

Possibly the greatest importance of discriminant analysis is its applicability to post-impact classification. Once a discriminant function is developed, new observations can easily be classified. If samples from a previously unimpacted area are now classified with a group from a degraded area, the researcher can then turn to the variable scores for the discriminant function to see which variables are most responsible for the aberrant classification.

IV.2.5 Hypothesis Testing

If an impact assessment program has been structured properly, the historical data for the study area and/or data from a pre-impact baseline sampling program will be utilized to optimize the design for the actual impact assessment phase, where hypotheses regarding the significance of changes attributable to the particular impact are tested.

Essentially, there are two goals of hypothesis testing. One is to detect true differences between treatment (pre vs. post or impact vs. control) means. The other is to estimate the true differences by stating the width of the confidence interval we desire for the true differences.

Figure 26 summarizes the objectives of hypothesis testing as related to impact assessment, while Figure 27 shows the components of these analyses. It should be noted that the means, variances and number of replicates will have already been calculated and stored (under Basic Descriptive Statistics) for use in hypothesis testing. Basic Descriptive Statistics will have also provided the necessary tests of assumptions of parametric hypothesis testing (normality, homoscedasticity and additivity), and the procedures for conducting any necessary transformations of the data.

Analysis of variance and t-tests can be used to test for station differences for data from individual cruises. When data for several cruises are analyzed together, two-way ANOVA's can be used to test for significant temporal and spatial differences, as well as the significance of the (station by cruise) interaction of these two class variables.

Multiple regression and discriminant function analysis are two predictive techniques that can be utilized in baseline studies. Multiple regression is used to relate, in a predictive manner, standing stock parameters to pertinent environmental variables (hydrographic parameters and sediment characteristics). Confidence intervals can be

# HYPOTHESIS TESTING

## OBJECTIVES

- TEST HYPOTHESES REGARDING THE STANDING STOCK (BIOMASS AND ABUNDANCE) FOR VARIOUS HABITAT TYPES AND COMMUNITIES
- STATISTICALLY CONTROL EXTRANEOUS ERROR VARIANCE AND INFLUENCE OF COVARIATES ON GROUP MEANS
- DEVELOP PREDICTIVE TOOLS TO ALLOW STATISTICAL CLASSIFICATION OF POST-IMPACT OBSERVATIONS
- DETERMINE NUMBER OF SAMPLES REQUIRED FOR GIVEN $\alpha$ ; $\beta$ LEVELS AND DIFFERENCES IN MEANS
- DRAW INFERENCES CONCERNING REAL-WORLD PATTERNS
- PROVIDE RECOMMENDATIONS FOR DESIGN CHANGES TO OPTIMIZE INFORMATION / COST RATIOS



Figure 26. Objectives of hypothesis testing.

# HYPOTHESIS TESTING

## COMPONENTS

- T-TEST AND ANALYSIS OF VARIANCE - TEST SIGNIFICANCE OF SPATIAL AND / OR TEMPORAL DIFFERENCES, FOLLOWED BY MULTIPLE MEANS TESTS TO IDENTIFY SPECIFIC DIFFERENCES IN MAIN EFFECT MEANS.
- SIMPLE AND MULTIPLE REGRESSION - RELATE, IN A PREDICTIVE MANNER, STANDING STOCKS TO IMPORTANT ENVIRONMENTAL PARAMETERS.
- ANALYSIS OF COVARIANCE - ADJUST, THROUGH "STATISTICAL CONTROL" EFFECT OF ANCILLARY SOURCES OF VARIATION (COVARIATES) SUCH AS DEPTH OF PENETRATION OF GRAB.
- DISCRIMINANT ANALYSIS - DEVELOP PREDICTIVE VECTORS TO MAXIMALLY DIFFERENTIATE GROUPS OF SAMPLES (e.g., SOFT VS. HARD BOTTOM) BASED ON TAXONOMIC VARIABLES (SPECIES COMPOSITION).
- CALCULATION OF THE REQUIRED NUMBER OF SAMPLES FOR GIVEN LEVELS OF $\alpha$ AND $\beta$ ERRORS AND PRECISION, BASED ON VARIANCE FROM HISTORICAL OR PROJECT GENERATED BASELINE DATA.
- OPTIMIZE SAMPLING DESIGN BASED ON VARIANCE RATIOS FROM BASELINE DATA.

Figure 27. Components of hypothesis testing.

calculated to allow classification of post-impact samples. Discriminant function analysis will allows for significance tests of station groupings and provides the basis by which post-impact samples can be classified to determine if substantive changes in community structure have occurred.

As in any field program there will be variables that cannot be controlled "experimentally" and must be controlled "statistically" through the use of analysis of covariance. Analysis of covariance can be used in the ANOVA mode (qualitative or class variables) or in the regression mode (quantitative variables) of the general linear model. Analysis of covariance allows extraneous variability resulting from such factors as different "depth of grab" to be removed from the effects that the investigator wishes to test (in this case stations differences) by lowering the error mean square, thereby increasing the power of the model. This often has the same effect as increasing the number of samples. Of course, the covariate must be quantitatively measured for each sample.

An important component of any statistical characterization is an assessment of the sampling plan (e.g., number of replicates per station). Often, the choice of number of replicates per station is made with little or no site-specific data to guide the decision-making process. After an initial sampling, baseline data are available for analysis. With these data or equivalent historical data, one can assess the adequacy of the level of replication using accepted techniques, including consideration of the power of the test (1-B). In this way both Type I and Type II errors can be considered.

The sampling design can also be assessed using the results of hypothesis testing and other calculations involving variance ratios. It is important to determine the ratio of between-station to within-station variance for stations at each site. This will shed light on the degree of homogeneity of each area. If an area is relatively homogeneous (ratio of between station/within station variance close to 1) then the whole area can be considered a stratum, and random sampling can be

conducted within these areas rather than within stations within each area. This will allow more efficiency and cost savings since many fewer samples will have to be collected. The process essentially involves the transition from randomly sampling within stations to random sampling within a much fewer number of strata (stratified random sampling).

The results from the hypothesis testing will allow inferences to be drawn concerning the real world system. When these inferences are considered in light of the life history work and other studies being conducted concurrently, the impact assessment program will hopefully provide the scientific basis for an environmentally sound use of the marine resource for at least one component of man's activities.

IV.2.5.1 Simple Hypothesis Testing

In the SPR study, the null hypothesis for station comparisons is that there is no significant difference in the means of the two populations.

$$H_0: \mu_1 = \mu_2$$

Depending on the alternative hypothesis, either a two-tailed or one-tailed analysis is performed. The two tailed test has, as its alternative hypothesis that the two means are significantly different.

$$H_1: \mu_1 \neq \mu_2$$

For ordered means, the investigator may only be interested in whether one of the means is significantly larger than the other. This results in a one-tailed test with the alternative hypothesis being

$$H_1: \mu_1 > \mu_2$$

The first step in simple hypothesis testing involves calculation of confidence limits for the mean. Assuming sample means are normally distributed (from the Central Limit Theorem) the 95 percent confidence limits are given by

$$([\bar{X} - t(s^2/n)^{\frac{1}{2}}] < X < [\bar{X} + t(s^2/n)^{\frac{1}{2}}])$$

where $(s^2/n)^{\frac{1}{2}}$ is the standard error of the mean as calculated earlier in Basic Descriptive Statistics and stored for later use. The "t" value comes from Student's t-distribution and is used to compare two means when a sample variance must be used in lieu of a population variance. The value of t is a decreasing function of the number of degrees of freedom.

Assuming that the data are normally distributed, the test statistic for the difference of two means is:

$$t_{(\alpha)df} = \frac{\bar{Y}_1 - \bar{Y}_2}{S_{(\bar{Y}_1 - \bar{Y}_2)}}$$

where $\bar{Y}_1$ and $\bar{Y}_2$ are the population means, $S_{(\bar{Y}1 = \bar{Y}2)}$ is the standard error of the difference of the two means and $t_{(\alpha)df}$ is the calculated t-value corresponding to $\alpha$ level of significance (type I error) and $n_1+n_2-2$ degrees of freedom, with $n_i$ = number of replicates per group.

The standard error of the difference of two means $S_{(\bar{Y}_1 - \bar{Y}_2)}$ is given as follows:

$$S_{(\bar{Y}_1 - \bar{Y}_2)} = \frac{\sum_{i=1}^{n_1}(Y_{1i} - \bar{Y}_1)^2 + \sum_{i=1}^{n_2}(Y_{2i} - \bar{Y}_2)^2}{n_1 + n_2 - 2} \left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{\frac{1}{2}}$$

and is derived from a pooled estimate (over both groups) of the variance:

$$S^2_{pooled} = \frac{\sum_{i=1}^{n}(Y_{1i} - \bar{Y}_1)^2 + \sum_{i=1}^{n}(Y_{2i} - \bar{Y}_2)^2}{n_1 + n_2 - 2} \left(\frac{1}{n_1} + \frac{1}{n_2}\right)$$

This calculation requires the assumption of homogeneity of variances. The value $t_{(\alpha)df}$ is then compared to the tabular t value for the same

degrees of freedom, and if the calculated t exceeds the tabular, there is a significant difference in means at the particular $\alpha$ level.

Rewriting the equation for the standard error of two means, we get

$$S_{(\bar{Y}_1 - \bar{Y}_2)} = S_{(pooled)} \cdot \left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{\frac{1}{2}}$$

where $S_{(pooled)}$ is called the standard error per unit variance.

Since, in the SPR program, there was generally equal replication at all stations ($n_1 = n_2 = \ldots = n_i$ where i varies from study to study and from assemblage to assemblage), then

$$S_{(\bar{Y}_1 - \bar{Y}_2)} = S_{(pooled)} \cdot \sqrt{\frac{2}{n}}$$

$$= \sqrt{S^2_{(pooled)}} \cdot \sqrt{\frac{2}{n}}$$

$$= \sqrt{\frac{2S^2_{(pooled)}}{n}} = \sqrt{\frac{2S^2_{(pooled)}}{n}}$$

Since $t_{(\alpha)df} = \bar{Y}_1 - \bar{Y}_2 / S_{(\bar{Y}_1 - \bar{Y}_2)}$

then $\bar{Y}_1 - \bar{Y}_2 = t_{(\alpha)df} \cdot S_{(\bar{Y}_1 - \bar{Y}_2)}$

$$= \sqrt{\frac{2}{n}} \cdot S_{(pooled)} \cdot t_{\alpha(df)} \cdot$$

For $\bar{Y}_1 - \bar{Y}_2$ to be significant,

$$\bar{Y}_1 - \bar{Y}_2 \geq \sqrt{\frac{2}{n}} \cdot S_{(pooled)} \cdot t_{(\alpha)df}$$

The trick now is to find the probability of this happening (the power of the test).

Letting $\delta$ = the true difference we wish to detect, then

$$t_{(\beta)df} = \frac{(\bar{Y}_1 - \bar{Y}_2) - \delta}{\sqrt{\frac{2}{n}} \cdot S_{(pooled)}}$$

It should be clear from this equation that the power of a t-test is:

(1) A positive function of the group mean differences $(\bar{Y}_1 - \bar{Y}_2)$,

(2) A negative function of measurement error (as expressed by $S_{pooled}$) and,

(3) A positive function of sample size.

When $t_{(\beta)df}$ is calculated, it is entered into a t table, to get the probability (P) that a value will be outside $\pm t_2$. The power of the analysis is then calculated from $1 - \frac{1}{2}(p)$. The power is the probability that a significant result will be seen if it exists.

The probability of obtaining a significant result depends on the true standard error per unit ($\sigma$), the number of replicates (n) and the number of degrees of freedom for estimating the error variance.

Since $$\frac{(\bar{Y}_1 - \bar{Y}_2) - \delta}{\sqrt{\frac{2}{n}} \cdot S_{(pooled)}} = t_{(\beta)df}$$

then $$\delta = (\bar{Y}_1 - \bar{Y}_2) - t_{(\beta)} \sqrt{\frac{2}{n}} \cdot S_{(pooled)}$$

but for $(\bar{Y}_1 - \bar{Y}_2)$ to be significant

$$(\bar{Y}_1 - \bar{Y}_2) \geq \sqrt{\frac{2}{n}} \cdot t_{(\alpha)df} \cdot S_{(pooled)}$$

Therefore, substituting, we get

$$\delta = \sqrt{\frac{2}{n}} \cdot S_{(pooled)} t_{\alpha(df)} - \sqrt{\frac{2}{n}} \cdot S_{(pooled)} \cdot t_{(\beta)df}$$

or

$$\delta = \sqrt{\frac{2}{n}} \cdot S_{(pooled)} \cdot (t_{(\alpha)df} + t_{(\beta)df})$$

The true difference which we desire to detect is equal to the standard error of the difference of the two means times the sum of the t values (corresponding to the Type I and Type II errors). Therefore, a basic

equation relating Type II error to Type I error, sample size (n), standard error of the mean and $\delta$ has been generated. This equation can be used to calculate $t_{(\beta)df}$ and from this the power of the analysis, P, can be calculated as

$$P = 1 - \tfrac{1}{2}p_2$$

where $p_2$ is the probability corresponding to $t_{(\beta)df}$ in the ordinary t table. The degrees of freedom for $t_{(\alpha)}$ and $t_{(\beta)}$ are those for the estimate of the error variance.

This equation is then solved for n, the number of replicates required to detect $\delta$ (true difference in means), given a sample estimate ($S_{pooled}$) of $\sigma$ (the true standard error per experimental unit), and significance levels $t_{(\alpha)df}$ and $t_{(\beta)df}$ where $t_{(\alpha)}$ is the significance value for t in tests of significance (usually 0.05) and $t_{(\beta)}$ is the value of t in the ordinary table corresponding to 2(1-p). The equation is,

$$n = 2(\frac{\sigma}{\delta})^2 \ (t_{(\alpha)df} + t_{(\beta)df})^2$$

This basic equation can be found in a number of statistics texts (e.g., Cochran and Cox 1957; Sokol and Rohlf 1969; and Anderson and McLean 1974). Many tabular variations based on this equation are available as aids in determining n, one of which is shown in Table 15 (from Cochran and Cox 1957.)

Historical or baseline data will allow an estimate of $\sigma$ [S(pooled)] for any pair of station means. In order to calculate n (required number of replicates) we must specify $\delta$, the true difference we wish to be able to detect (expressed as a percentage of the mean). To find a suitable n, one arbitrarily chooses a preliminary n (say 20 replicates), which determines the values of $t_\alpha$ and $t_\beta$ by controlling the degrees of freedom. The $\alpha$ and $\beta$ levels and the tabular values for $t_{(\alpha)df}$ and $t_{(\beta)df}$ are inputted into the equation by the user. A first order n is then calculated. This value is then rounded off (upwards) to the nearest whole number and the formula is then recalculated, substituting the new values for $t_{(\alpha)df}$ and $t_{(\beta)df}$ into the equation. These new values for $t_{(\alpha)df}$ and $t_{(\beta)df}$ result from different degrees of freedom

**Table 15.** **Table for finding number of replicates required for a given probability of obtaining a significant result for two-mean tests of significance (from Cochran and Cox 1957).**

Upper figure: Test of significance at 5% level, probability 80%
Middle figure: Test of significance at 5% level, probability 90%
Lower figure: Test of significance at 1% level, probability 95%

One-tailed tests

| $P_1$ = smaller % success | $\delta = P_2 - P_1$ = larger *minus* smaller percentage of success | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 |
| 5 | 330 | 105 | 55 | 35 | 25 | 20 | 16 | 13 | 11 | 9 | 8 | 7 | 6 | 6 |
| | 460 | 145 | 76 | 48 | 34 | 26 | 21 | 17 | 15 | 13 | 11 | 9 | 8 | 7 |
| | 850 | 270 | 140 | 89 | 63 | 47 | 37 | 30 | 25 | 21 | 19 | 17 | 14 | 13 |
| 10 | 540 | 155 | 76 | 47 | 32 | 23 | 19 | 15 | 13 | 11 | 9 | 8 | 7 | 6 |
| | 740 | 210 | 105 | 64 | 44 | 33 | 25 | 21 | 17 | 14 | 12 | 11 | 9 | 8 |
| | 1370 | 390 | 195 | 120 | 81 | 60 | 46 | 37 | 30 | 25 | 21 | 19 | 16 | 14 |
| 15 | 710 | 200 | 94 | 56 | 38 | 27 | 21 | 17 | 14 | 12 | 10 | 8 | 7 | 6 |
| | 990 | 270 | 130 | 77 | 52 | 38 | 29 | 22 | 19 | 16 | 13 | 10 | 10 | 8 |
| | 1820 | 500 | 240 | 145 | 96 | 69 | 52 | 41 | 33 | 27 | 22 | 20 | 17 | 14 |
| 20 | 860 | 230 | 110 | 63 | 42 | 30 | 22 | 18 | 15 | 12 | 10 | 8 | 7 | 6 |
| | 1190 | 320 | 150 | 88 | 58 | 41 | 31 | 24 | 20 | 16 | 14 | 11 | 10 | 8 |
| | 2190 | 590 | 280 | 160 | 105 | 76 | 57 | 44 | 35 | 28 | 23 | 20 | 17 | 14 |
| 25 | 980 | 260 | 120 | 69 | 45 | 32 | 24 | 19 | 15 | 12 | 10 | 8 | 7 | .. |
| | 1360 | 360 | 165 | 96 | 63 | 44 | 33 | 25 | 21 | 16 | 14 | 11 | 9 | .. |
| | 2510 | 660 | 300 | 175 | 115 | 81 | 60 | 46 | 36 | 29 | 23 | 20 | 16 | .. |
| 30 | 1080 | 280 | 130 | 73 | 47 | 33 | 24 | 19 | 15 | 12 | 10 | 8 | .. | .. |
| | 1500 | 390 | 175 | 100 | 65 | 46 | 33 | 25 | 21 | 16 | 13 | 11 | .. | .. |
| | 2760 | 720 | 330 | 185 | 120 | 84 | 61 | 47 | 36 | 28 | 22 | 19 | .. | .. |
| 35 | 1160 | 300 | 135 | 75 | 48 | 33 | 24 | 19 | 15 | 12 | 9 | .. | .. | .. |
| | 1600 | 410 | 185 | 105 | 67 | 46 | 33 | 25 | 20 | 16 | 12 | .. | .. | .. |
| | 2960 | 750 | 340 | 190 | 125 | 85 | 61 | 46 | 35 | 27 | 21 | .. | .. | .. |
| 40 | 1210 | 310 | 135 | 76 | 48 | 33 | 24 | 18 | 14 | 11 | .. | .. | .. | .. |
| | 1670 | 420 | 190 | 105 | 67 | 46 | 33 | 24 | 19 | 14 | .. | .. | .. | .. |
| | 3080 | 780 | 350 | 195 | 125 | 84 | 60 | 44 | 33 | 25 | .. | .. | .. | .. |
| 45 | 1230 | 310 | 135 | 75 | 47 | 32 | 22 | 17 | 13 | .. | .. | .. | .. | .. |
| | 1710 | 430 | 190 | 105 | 65 | 44 | 31 | 22 | 17 | .. | .. | .. | .. | .. |
| | 3140 | 790 | 350 | 190 | 120 | 81 | 57 | 41 | 30 | .. | .. | .. | .. | .. |
| 50 | 1230 | 310 | 135 | 73 | 45 | 30 | 21 | 15 | .. | .. | .. | .. | .. | .. |
| | 1710 | 420 | 185 | 100 | 63 | 41 | 29 | 21 | .. | .. | .. | .. | .. | .. |
| | 3140 | 780 | 340 | 185 | 115 | 76 | 52 | 37 | .. | .. | .. | .. | .. | .. |

based on the new calculated n. The values for $\sigma$ and $\delta$ do not change during this procedure. A second value of n is now calculated. If this new value of n is greater than the previous calculated value but between n and n+1, the value is rounded off to the nearest whole number and the analysis is complete. If the new value for n is less than the previous n but greater than n-1, then the n which was calculated in the previous step is used. Two iterations are usually sufficient for convergence.

In a similar manner, one can determine the number of replicates required for given limits of error in the difference between two means (that is, the number of replicates required to estimate the difference in the effects of two means for a given level of precision).

IV.2.5.2 Analysis of Variance

Analysis of variance (ANOVA) is used to determine the significant main effects (e.g., time and space) and interactions involving selected biotic and abiotic parameters. Once these significant trends have been identified through the use of analysis of variance, a clearer understanding of the importance of brine disposal to the nearshore NW gulf ecosystem can be developed. Several equivalent approaches are available to analyzing these data, including both multiple regression analysis and analysis of variance, which are both aspects of the general linear model. There are four important assumptions in the general linear model:

1. The error terms of the model are independently and normally distributed with a constant variance ($\delta^2$), a mean of zero and

$$\varepsilon_{ij} \sim IN(0,\sigma^2) \text{ for all } i \neq j.$$

2. The treatment effects, the overall mean, and the interaction are all additive.

3. The variances of the observations for each treatment or treatment combination are equal.

4. The observations are normally distributed.

The procedures for testing assumptions have been discussed under Basic Descriptive Statistics (see Section IV.2.2.2). Very often, one transformation will achieve compliance with a number of assumptions including normality, homogeneity of variances and additivity of treatment effects.

For individual cruises, one-way ANOVAs are appropriate, with fixed effect models of the form

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}$$

$$i = 1,2\ldots,B$$

$$j = 1,2\ldots,n$$

where $Y_{ij}$ = jth response for the ith treatment

$\mu$ = overall mean

$\beta_i$ = the effect of the ith treatment (station)

$\varepsilon_{ij}$ = the random error of the jth response to the ith treatment

The model is fixed effects because the stations are fixed and are the only ones of interest.

Obviously, it is not efficient to run a one-way ANOVA on data from all stations. These data will have to be reduced in size before ANOVA is performed. This is precisely the purpose of the analyses which preceded hypothesis testing in the overall analysis scheme. Certain subdivisions of the original data set that look promising as bioindices for impact assessment will be identified in the early stages of the impact assessment process (in baseline phase studies). We might want to test the null hypothesis that some stations are significantly different based on important bioindices.

$H_o$: $\mu_1 = \mu_2 = \ldots = \mu_r$

$H_1$: not all $\mu_i$ are equal.

where i = station, and i = 1,...,r, where r = number of stations (number of levels of whatever main effect is under study).

The ANOVA table for this design would be as follows:

| Source | df | SS | MS | F | EMS |
|---|---|---|---|---|---|
| Among Station | r-1 | A | A/n-1 | $\frac{A/n-1}{B/n-r}$ | $\sigma^2+n\phi(\beta)$ |
| Within Station | n-r | B | B/n-r | | $\sigma^2$ |
| Total | n-1 | A+B | | | |

and station effects would be tested using the F ratio of

$$F = \frac{\text{Among station mean square}}{\text{Within station mean square}}$$

and compared to table F for $\alpha$ level of significance ($\alpha = .05$) with (r-1) and (n-r) degrees of freedom for the numerator and denominator of the ratio, respectively.

Since one-way ANOVA's do not involve interaction terms, individual station differences can always be compared using Duncan's New Multiple Range Test or a similar a posteriori multiple means procedure. Examples of one-way ANOVAs and subsequent multiple means tests for nekton species are presented in the Appendix to this report. A posteriori mutliple means testing is often most applicable in the baseline phases of the monitoring program where relatively little is initially known about the study area. In the hypothesis testing stage of the analytic scheme, after historical and baseline data are thoroughly analyzed, the stage should be set for design of an optimized impact assessment sampling program utilizing a priori procedures.

The most obvious two-way design is one involving cruise and station as main effects. This model, in its simplest form, is also a fixed effects model with factorial design. In factorial designs the main effects are completely crossed (every possible combination of levels of the two main effects are included). It should be noted however, that a cruise

generally does not represent a fixed point in time but rather an interval of time. That is, due to practical considerations, only a certain number of samples can be collected on any one day. This problem could introduce bias into the station comparisons, with stations collected early and late possibly showing some temporal differences within a cruise. The importance of this involves the fact that tests for station differences may not show significance due, in part, to factors other than spatial ones. This extraneous variance which increases the size of the error mean square must be recognized in the one-way as well as the two-way designs, but is especially confounding when trends across both space and time are being investigated.

The obvious way to compensate for this temporal within-cruise effect is to use a randomized complete block design ANOVA, where the blocks are internally "homogeneous" units of time. The problem in applying this to the Bryan Mound study is that the sampling randimization required for a randomized complete block design (each block must contain at least one replicate for each treatment level (station) in the analysis and the order of collection of samples must be random), does not accommodate the practical problems associated with sampling the Gulf of Mexico.

For a two-way fixed effects and factorial design ANOVA, the model is

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{(ij)k}$$

where $\alpha_i$ = effect of ith level of treatment $\alpha$, i = 1,2,...,a

$\beta_j$ = effect of jth level of treatment $\beta$, j = 1,2,...,b

$\alpha\beta_{ij}$ = effect of interaction of $\alpha$ and $\beta$

$\varepsilon_{(ij)k}$ = error, k = 1,2,...,n

The ANOVA table for this analysis would look as follows:

| Source | df | Sum Squares | Mean Square | F | EMS |
|---|---|---|---|---|---|
| Station | a-1 | A | A/a-1 | $\frac{A/a-1}{E/ab(n-1)}$ | $\sigma^2+bn\phi(\alpha)$ |
| Cruise | b-1 | B | B/b-1 | $\frac{B/b-1}{E/ab(n-1)}$ | $\sigma^2+an\phi(\beta)$ |
| Station x Cruise | (a-1)(b-1) | C | $\frac{C}{(a-1)(b-1)}$ | $\frac{C(a-1)(b-1)}{E/ab(n-1)}$ | $\sigma^2+n\phi(\alpha\beta)$ |
| Error | ab(n-1) | E | E/ab(n-1) | | $\sigma^2$ |
| Total | abn-1 | A+B+C+E | | | |

All F tests are conducted by dividing the main effect or interaction of interest by the error mean square and comparing the calculated F to a tabular F for the $\alpha$ level of significance and $v_1$ and $v_2$ degrees of freedom, where $v_1$ = degrees of freedom of numerator and $v_2$ = EMS degrees of freedom.

Because most two-way designs include an interaction term, it is very important to test for significance of interaction before running a posteriori multiple means tests. If interaction is significant, the means for the dependent variable (e.g., total biomass of benthos) for the levels of one main effect (e.g., station comparisons) are not behaving similarly over the levels of the other main effect (e.g., cruise). In other words, if we were comparing biomass of total nekton at two stations for two cruises, significant interaction would mean that the trends for station means were not consistent over the two cruises (Station 1 might be higher on Cruise 1 and Station 2 higher on Cruise 2). Significant interaction can often hide significant main effects. If the interaction term in a two-way ANOVA is significant, no multiple mean testing should occur for either main effect. If the interaction term in the two-way ANOVA is not significant, then multiple means tests should be performed for all significant main effects.

In most commonly employed randomized design experiments, if the null hypothesis is true, $100(1-\alpha)$ percent of the time this decision will be reached. The probability of rejecting the null hypothesis when, in fact, it is false is determined by the power $(1-\beta)$ of the F in ANOVA.

For ANOVA, assuming a one-way completely random design, power and number of samples required to attain this power (given $\alpha$ and $\sigma^2$) can be calculated. The procedure for calculating power was developed by Tang (1938). Assumptions include normal distribution of observations and homoscedasticity of error variances. Given the null hypothesis and its alternate

$$\text{Ho: } \beta_j = 0 \text{ for all } j$$
$$\text{Hi: } \beta_j \neq 0 \text{ for all } j$$
$$\text{where } \beta_j = {}_j\text{th treatment effect,}$$

the appropriate variance ratio for testing for treatment effects is $F = MS_{among}/MS_{within}$, and the F distribution depends only on $v_1$ and $v_2$. If the null hypothesis is rejected, the distribution of the ratio $v_1$ and $v_2$, and also on a non-centrality parameter $\delta$, where

$$\delta = \sqrt{\sum_{i=1}^{k} n\beta_j{}^2/\sigma_\varepsilon{}^2}$$

The value of the noncentrality parameter increases as the treatment effects get larger. Therefore, in order to select the appropriate non-central F distribution required to calculate power, it is necessary to know $\delta$. Charts are available to simplify the calculation of power (Tang, 1938; Pearson and Hartley 1951) an example of which is shown in Table 16.

The parameter $\phi$, which is closely related to $\delta$, is calculated by

$$\phi = \frac{\sqrt{\sum_{i=1}^{k} \beta_j{}^2/k}}{\sigma_\varepsilon/\sqrt{n}}$$

and $\phi$ is entered in Table 16. The user must specify the following for this procedure:

$\alpha$ = Type 1 error (probability of rejecting the null hypothesis when it is true)

$\sum_{j=1}^{k} \beta_j^2$ = sum of squares due to treatment effects

k = number of treatment levels

n = size of jth sample

$\sigma_\varepsilon^2$ = error variance

$v_1$ & $v_2$ = degrees of freedom for treatment and error effects, respectively.

In many cases $\sigma_\varepsilon^2$ is not known exactly and must be estimated from the analysis. If a baseline assessment program has been properly structured, a very good estimates of $\sigma_\varepsilon^2$ can often be attained (the error can at least be bounded).

In cases where an estimate of the error variance is known and the investigator can assign minimum treatment effects he wishes to detect, a similar procedure can be employed to determine power and sample size. Let

$$\phi = \frac{\sqrt{\sum_{i=1}^{k} (U_j - U)^2/k}}{\sigma_\varepsilon/\sqrt{n}}$$

where $U_j - U$ is the minimum treatment effect an experimenter is interested in detecting

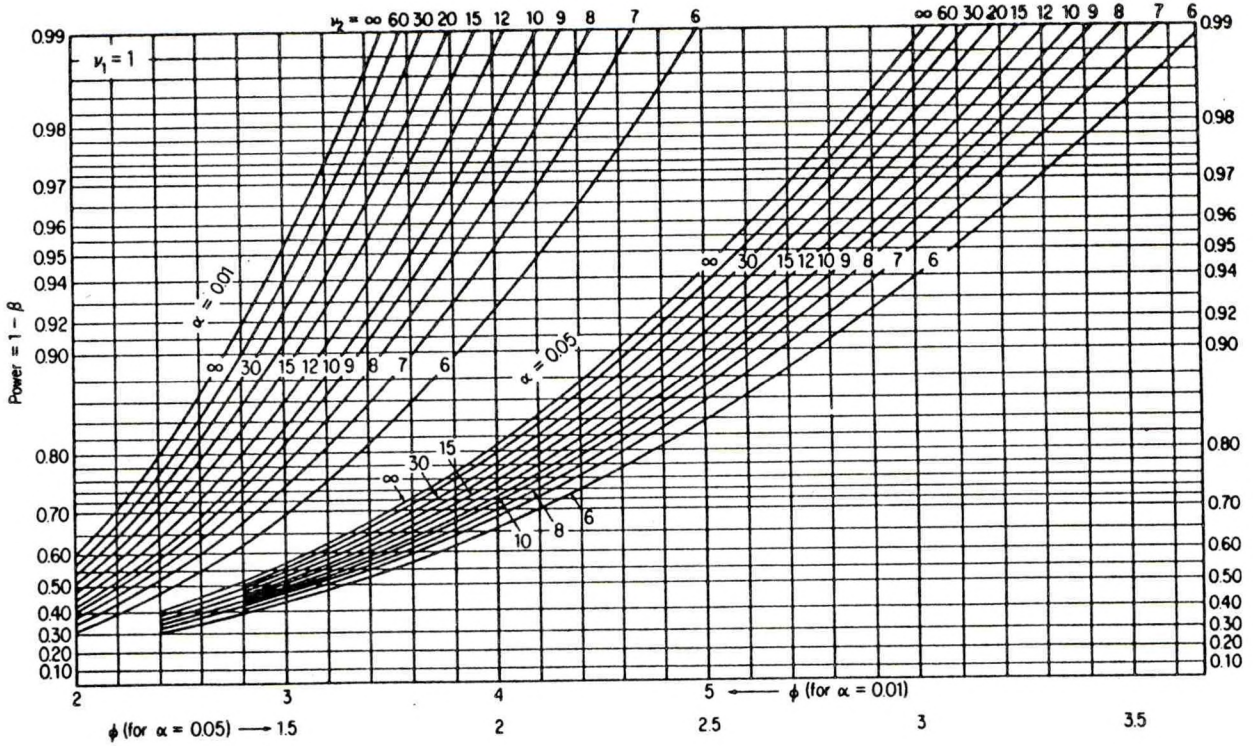
k = the number of treatment levels,

n = size of jth sample,

$\sigma_e$ = square root of population error variance,

N = total number of observations in the experiment.

## Table 16. Power function for analysis of variance.




Reproduced with permission from E. S. Pearson and H. O. Hartley, Charts of the power function for analysis of variance tests, derived from the non-central *F*-distribution, *Biometrika*, 1951, **38**, 112–130.

with k-1 and N-k degrees of freedom. $\phi$ is calculated and Table 16 is used to find the power. The sample size needed for a required power can be calculated by trial and error. The same basic approach can be applied to determine sample size for any ANOVA design.

To compare two ANOVA designs for optimum allocation of resources, the relative efficiency (RE) of one design with respect to another can be computed. RE is given by a ratio of variances of group means (treatment effects not included)

$$RE = \frac{S^2_{\bar{y}(1)}}{S^2_{\bar{y}(2)}} \times 100$$

where (1) and (2) represent the two designs. In the equation above, the relative efficiency of group 2 relative to group 1 is being assessed. The smaller the "without treatment" group mean variance, the easier it will be to determine significant treatment effects.

IV.2.5.3 Multiple Regression

A technique such as multiple regression analysis can be used to determine the predictability of selected dependent variables (i.e., total nekton density) using independent variables (i.e., environmental factors and possibly other species) as predictor variables. The multiple regression first order linear model has the general form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \cdots + \beta_k X_k + \varepsilon$$

where Y = dependent variable (e.g., total density of nekton)

$\beta_0$ = y intercept

$\beta_j$ = regression coefficients, j = 1 to k

$X_i$ = independent predictor variable (e.g., temperature or sediment texture), i = 1 to k.

$\varepsilon$ = error term

Higher order linear models might have as a typical form

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_1^3 + \beta_4 X_2 + \beta_5 X_3^2 + \varepsilon$$

where j = 1 to t, and t = =the number of $\beta_j X_i$ terms in the model

Expressing this equation in matrix notation, we have

$$\underline{Y} = \underline{X}\ \underline{B} + \underline{\varepsilon}$$

where $\underline{Y}$ is a (nx1) vector of observations (dependent variables)

$\underline{X}$ is a (nxp) matrix of known form (data matrix)

$\underline{B}$ is a (px1) vector of parameters

$\underline{\varepsilon}$ is a (nx1) vector of errors

And, it is assumed that $E(\underline{\varepsilon}) = 0$ and $V(\underline{\varepsilon}) = \underline{I}\sigma^2$, where $E_{(\varepsilon)}$ is the expected value of $\varepsilon$, and $V_{(\varepsilon)}$ is the variance of $\varepsilon$, and $\underline{I}$ is an identity matrix

therefore

$$\underline{\varepsilon} = \underline{Y} - \underline{XB}$$

and

$$\underline{\varepsilon}'\ \underline{\varepsilon} = (\underline{Y}-\underline{XB})'\ (\underline{Y}-\underline{XB})$$
$$= \underline{Y}'\underline{Y} - 2\underline{B}'\underline{X}'\underline{Y} + \underline{B}\underline{X}'\underline{X}\underline{B}$$

The least squares estimate of $\underline{B}$ is b. When b is substituted in the above equation, $\varepsilon'\varepsilon$ is minimized. By differentiating the equation with respect to $\underline{B}$ and setting the resulting equation equal to zero, we get the normal equation

$$(\underline{X}'\underline{X})\underline{b} = \underline{X}'\underline{Y}$$

and, solving for $\underline{b}$, we get

$$\underline{b} = (\underline{X}'\underline{X})^{-1}\underline{X}'\underline{Y}$$

As noted above, the model can include higher order (cubic and quadratic) terms in the independent variables (e.g., $X^2$) as well as linear components. This is not true for the exponents.

After obtaining this solution, a number of parameters can be calculated (Draper and Smith 1966).

- The fitted values ($\hat{\underline{Y}}$) are calculated from $\hat{\underline{Y}} = \underline{X}\underline{b}$
- Vector of residuals, $\underline{e}$ equals $\underline{Y} - \hat{\underline{Y}}$
- $V(\underline{b}) = (\underline{X}'\underline{X})^{-1}\sigma^2$ provides the variance-covariance matrix for the estimates
- $\hat{\underline{Y}}_0 = \underline{X}'_0\underline{b} = \underline{b}'\underline{X}_0$ is the fitted value at a specific point $X_0$
- $V(\hat{\underline{Y}}_0) = \underline{X}'_0 V(\underline{b})\underline{X}_0 = \underline{X}'(\underline{X}'\underline{X})^{-1}\underline{X}_0\sigma^2$ is the variance of the predicted value at $\underline{X}_0$.
- $1 - \alpha$ confidence limits for the mean value of Y at $X_0$ are obtained from $\hat{\underline{Y}} \pm t_{(v,1-\frac{1}{2}\alpha)} \cdot s \cdot \sqrt{\underline{X}'_0(\underline{X}'\underline{X})^{-1}\underline{X}_0}$
- $1 - \alpha$ confidence limits for an individual observation of Y at $X_0$ are obtained from $\hat{Y} \pm t_{(v,\ 1-\frac{1}{2}\alpha)} \cdot s \cdot \sqrt{1+\underline{X}'_0(\underline{X}'\underline{X})^{-1}\underline{X}_0}$

The basic ANOVA table has the following form.

| Source | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Regression | $\underline{b}'\underline{X}'\underline{Y}$ | p | MSR |
| Residual | $\underline{Y}'\underline{Y}-\underline{b}'\underline{X}'\underline{Y}$ | n-p | MSE |
| Total | $\underline{Y}'\underline{Y}$ | n | |

If a $B_0$ term is in the model, we can segregate the sum of squares due to regression and the sum of squares for the intercept (bo)

$$SS(bo) = n\bar{Y}^2$$

$$SS(\text{Regression/bo}) = SS(R/bo) = \underline{b}'\underline{X}'\underline{Y} - \frac{(\Sigma Y_i)^2}{n}$$

Further if repeated observations are available, the residual sum of squares can be decomposed into a sum of squares due to pure error (analagous to MS within) and a sum of squares due to lack of fit. In this way the model can be tested for lack of fit by

$$\frac{SS(\text{lack of Fit})/(n-p-n_e)}{SS(\text{pure error})/n_e}$$

where n=total number of observations
p=number of parameters (independent variables)
$n_e$=degrees of freedom for pure error

and compared to a table F value for $(n-p-n_e)$, and $n_e$ degrees of freedom.

An analysis of variance table could then be constructed as:

| Source | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| bo | $n\bar{Y}^2$ | 1 | |
| Regression/bo | $SS(R/bo)=\underline{b}'\underline{X}'\underline{Y}-n\bar{Y}^2$ | p - 1 | MS(R/bo) |
| Lack of fit | $SS(l.o.f.)=\underline{Y}'\underline{Y}-\underline{b}'\underline{X}'\underline{Y}-SS(p.e.)$ | n - p - ne | MS(l.o.f.) |
| Pure error | SS(p.e.) | ne | MS(p.e.) |
| Total | $\underline{Y}'\underline{Y}$ | n | |

Unfortunately to apply the test for lack of fit, the suite of values for the independent variables must be identical for all repeated values of the dependent variable. It would be fortuitous, in the SPR characterization, if the entire vector of "independent variables" (e.g., sediment and hydrographic parameters) were identical for several of the replicates at a station.

We can test the overall regression equation

$$H_o: \ B_1=B_2=\ldots.=B_{p-1} = 0$$
$$H_i: \ \text{not all } B_j=0$$

where p=number of parameters, by testing the ratio

$$\frac{[(SSR/bo)/(p-1)]}{s^2}$$

as an F variate with p-1 and v (equals n-p) degrees of freedom for the numerator and denominator, respectively.

Even though a significant F is found, this does not mean that the regression has predictive value. A general rule is that the observed F-ratio (regression mean square)/(residual mean square) should exceed the selected percentage point of the F distribution by four times (Draper and Smith 1966).

The coefficient of multiple determination ($R^2$) which is the square of the multiple correlation coefficient, can be calculated. It is a measure of the usefulness of the terms other than $b_0$ in the model, and is the sum of square due to regression divided by the total sum of squares, the latter corrected for the mean. It measures the proportion of the total variation about the mean explained by regression.

$$R^2 = \frac{SS(R/bo)}{\underline{Y}'\underline{Y}-SS(bo)}$$

The closer $R^2$ is to one, the better the fit of the model.

In performing regression analysis, the same assumptions are made about the errors (residuals) as are made for other applications of the general linear model:

(1) errors are independent
(2) mean zero
(3) constant variance ($\sigma^2$)
(4) are normally distributed

There are numerous ways in which residuals ($e_i = Y_i - \hat{Y}_i$, $i=1,2,\ldots n$, where $Y_i$ is an observation and $\hat{Y}_i$ is the fitted value from the regression) are examined. The principal ways of plotting include:

(1) overall (essentially a frequency distribution of residuals)
(2) in time sequence
(3) against the fitted $\hat{Y}_i$
(4) against the independent variables $X_{ji}$

A number of conditions can cause trends in these plots. These include:

(1) The variance is not constant but increases with time, implying that a weighted least squares analysis should be used or the data should be transformed.

(2) Either a linear or higher order effect in time is present for some independent variable, indicating that this term or terms should be entered into the regression model.

(3) A systematic error in the data collection has occurred.

A stepwise forward multiple regression procedure is suggested. This method is quite useful in that it allows the researcher to systematically enter the independent variables into the regression model based on certain statistical criteria (i.e., F ratios of variables to be included into the regression; Nie, et al. 1975). The independent variables are entered into the regression model in the order of their importance based on their contribution toward explaining residual variance. The stepwise regression procedure, at each step in the analysis, provides the necessary statistics to test the significance of the overall model, including all predictor variables, and to perform partial F tests to decompose each piece of the model ($B_1X_1$), while holding all other parts of the model constant.

The use of regression equations for impact assessment centers around developing confidence limits (usually 95 percent) for estimated values of the dependent variable, Y, for an individual post impact observation (see equation above for calculating confidence limits). If the observed value of Y falls outside the 95 percent confidence limits of $\hat{Y}$, it can be stated that the null hypothesis of no significant change has been rejected, and factors not in the model (impact) are causing changes in the response of the dependent variable.

### IV.2.5.4 Analysis of Covariance

Statistical control (e.g., covariance adjustment) and direct experimental control are alternative approaches to achieving the same goal of increasing the precision of the experiment. Indirect (statistical) control relates to the analysis of the experiment rather than to its design. Statistical control is achieved by quantitatively

measuring covariates that cannot be experimentally controlled along with the independent variables of interest. The portion of the variability in the dependent variable accounted for by the covariates can be partitioned out, thereby allowing the researcher to more accurately assess the effects of the independent variables of interest. Very often use of covariance can have the same effect as an increase in the number of replicates. In a covariance analysis each treatment mean ($\bar{Y}_i$) is adjusted so that it takes the value it would be expected to have if all the treatments had the same covariate (X) mean. In this way the technique removes the effect of variations of $\bar{X}_i$.

Analysis of covariance is usually considered appropriate when:

(1) There are one or more extraneous sources of variation believed to affect the dependent variable.

(2) Direct experimental control of extraneous sources of variation is either not possible or not feasible.

(3) The functional form of the relationship between the dependent variable and the extraneous variable is known.

(4) It is possible to obtain a measure of the extraneous variation that does not include effects of the treatment.

The gain in precision from the use of the covariance adjustment depends upon the degree of correlation between the covariate and the dependent variable. The higher the correlation, the larger will be the variation attributable to the covariate, and the more effective the analysis of covariance procedure will be in reducing the error variance.

Covariance analysis can be used for the following purposes:

(1) Test whether the slopes of several regression lines could have come from populations with the same slope (homogeneity of slope).

(2) Test if the means of dependent variables are significantly different among groups and whether this difference is due to differences in the independent variable(s) among groups.

(3) Fit a common regression line to group means of Y and test for significant heterogeneity among these means around the regression line.

(4) Compare this common regression line to a pooled regression of all Y on X. The means may not have the same regression slope as the items on which they are based.

In the SPR program, covariance analysis can be utilized to remove the effects of extraneous variables, such as the differential penetration of the box corer at stations with different sediment texture.

IV.2.6 Summary of Analysis Protocols

In summary, the analyses system, as discussed herein, would proceed through five distinct phases.

(1) Exploratory analysis
(2) Basic descriptive statistics
(3) Bivariate measures of association
(4) Classification and pattern analysis
(5) Hypothesis testing

IV.2.6.1 Exploratory Analysis

Exploratory analysis should be conducted on data from each cruise, and might consist of the following:

(1) For each sample (replicate) a relative composition table could be produced.

(2) For each station, a mean relative composition table could be produced.

(3) For each station, a taxonomically - based table could be produced, showing the number of individuals of each species in each replicate sample at the station.

(4) All measures which require the entire species assemblage at each replicate or station (e.g. total number of species, diversity, evenness, etc.) could be calculated for each sample at this stage, and summary statistics for these measures could be calculated for each station. These results could be included in the taxonomically - based tables.

(5) Large scale non-hierarchical clustering could be performed, expecially if the data set is large as a first attempt at grouping of stations and determining species assemblages associated with these station groupings.

(6) Graphical display of data resulting from exploratory analysis should be presented including:
- o rarefaction curves
- o dominance - diversity curves
- o species - area curves.

At the end of the exploratory analysis, the investigator should be in a position to determine:

(1) Appropriate "taxonomic" subsets of the data including "important" species and major trophic groupings.

(2) Appropriate spatial subsets of the data (e.g. station groups as identified in non-hierarchical clustering).

These taxonomic/spatial subsets then serve as input data for more detailed analyses to follow.

IV.2.6.2 Basic Descriptive Statistics

It is assumed that the (taxonomic) variables to be studied in detail have already been identified in exploratory analysis. Basic descriptive statistics should be calculated for:

(1) Taxonomic variables [e.g. species and higher (taxonomic or trophic) groups, and other parameters based on taxonomic variables such as diversity and eveness]

(2) Environmental variables

- o Sediment texture variables and organic carbon
- o Hydrographic variables

The following information should be calculated by station for these variables and stored for later use in statistical analyses and plotting:

- o mean
- o variance
- o number of replicates

- range
- maximum - minimum
- skewness
- kurtosis
- coefficient of variation
- standard error of the mean

In order to statistically characterize the data sets, the following should also be provided for selected data subsets, which represent the range of characteristics in the study area (e.g. stations from diverse "habitat" types):

- plots of cumulative mean vs number of replicates
- tests of assumptions of analysis
  - additivity
  - homogeneity of variance
  - normality
- determination of appropriate data transformations
- calculation of spatial distribution of biota as derived from:
  - coefficient of dispersion
  - Morisita's Index

IV.2.6.3 Bivariate Measures of Association

Again it is assumed that taxonomic and spatial subsetting of the data have already occurred. Correlation coefficients should be calculated for all important taxonomic and environmental variables to establish first-level relationships and to serve as input to higher level analyses.

In addition to the use of the correlation coefficient, various measures of association which are used to determine station (and species) groupings and which serve as imputs to higher lever pattern and classification analyses should be employed.

IV.2.6.4 Classification and Pattern Analysis

Five multivariate classification and pattern analysis techniques can be utilized to determine "community - level" and "habitat - level" relationships of the biological communities. These techniques are:

(1) Hierarchical clustering. This is used to identify station (Q - mode) and species (R - mode) groupings. Station groupings can be determined on the basis of either environmental data and species composition, and the results can be compared.

(2) Reciprocal averaging ordination. The ordination plots produced by this technique allow direct comparison of sample and species behavior, allowing identification of gradients in the environment and identification of the behavior of the "important species" along the gradient.

(3) Factor analysis. Factor analyses, run in both the Q- and R-mode, allow determination of station and species groupings based on taxonomic count data. Factor analysis can also be run on an environment data set in the Q-and R- modes. The importance of each grouping (factor) can be quantitatively determined (size of eigenvalue), and those species or environmental variables which share affinity with several groups can be readily identified.

(4) Canonical correlation analysis. This technique can be used to relate biotic data to environmental (sediment and/or hydrographic) data. Groups of species can be related to groups of environmental factors, thereby relating "communities" to "habitat".

(5) Discriminant function analysis. Discriminant function analysis can be utilized to develop criteria for segregating stations into groups, based on either taxonomic or environmental variables. Station groups will be determined from prior analyses (e.g. cluster analysis). Discriminant analysis will not only allow segregation of groups but will show those variables (attributes) that are most responsible for group segregation and which might be considered "indicators" of the habitat or station group.

In addition to these five techniques, Indicator Species Analysis can be utilized to provide two-way tables which show, in the clearest manner possible, the relationship between Q-mode (station) and R-mode (taxonomic) analysis results.

IV.2.6.5 Hypothesis testing.

The following are components of hypothesis testing.

(1) Analysis of variance and t-tests can be utilized to determine spatial and temporal effects for important variables (taxonomic and geochemical parameters). One and two-way fixed effects, factorial models can be utilized to assess temporal and spatial effects, while those analyses involving laboratory subsampling can use nested factorial models. For those cases where significant main effects are found with no significant interaction, multiple means testing can be conducted to determine which stations or cruises are responsible for the significant differences.

(2) Simple and multiple regression. These analyses can be utilized to develop predictive models relating standing stock of biota to environmental variables. Confidence intervals can be calculated for the estimates from these models, and based on these algorithms, the particular variable of interest can be predicted using newly collected (post discharge) data.

(3) Analysis of covariance. Covariance control of extraneous variability can be used in the SPR program if certain covariates (e.g. depth of grab) cannot be experimentally controlled but can be quantitatively measured.

(4) Discriminant function analysis. As discussed earlier, discriminant function analysis can be utilized to develop predictors, based on the taxonomic composition of samples or important geochemical variables, thereby allowing post-impact observations to be classified for group membership.

(5) Numbers of samples required for hypothesis testing. Based on the results of the initial sampling or analysis of an adequate historical data base, the number of samples needed for given levels of precision in tests of significance can be computed. The equation for determining number of samples can also be used to calculated the Type II error for any given number of samples.

(6) Sample design optimization. Variance ratios can be used to compare various sampling designs and give recommendations for improvement or optimization of the experimental design.

# V. EVALUATION OF THE BRYAN MOUND SAMPLING EFFORTS

## V.I Introduction

Based on the spatial, temporal and taxonomic attributes of the various biological data sets and ancillary environmental data sets resulting from the Bryan Mound studies, this section summarizes the utility of these data for testing hypotheses related to brine discharge impact assessment. Obvious advantages or disadvantages associated with the methodologies which were utilized in the Bryan Mound program are discussed. Of course, a complete assessment of the adequacy of the data can only be made through the use of basic descriptive statistics and hypothesis testing, and these analyses should be performed on each data set as a routine procedure in the Bryan Mound program. Basic descriptive statistics have generally not been utilized in the Bryan Mound program, even though they constitute an essential first step toward design optimzation for impact assessment monitoring.

In the present discussion the hypothesis testing portions of the analysis system are stressed. Based on the discussion in Chapter I, it appears that the only feasible way to approach impact assessment in the temporally variable nearshore Gulf of Mexico is to utilize control and impact stations collected at the same point in time. Therefore, our evaluation of the Bryan Mound methodologies stresses the use of the data in this context.

The hydrographic variables collected in the baseline phase of the Bryan Mound study in conjunction with the various biological collections have very little value for impact assessment because the hydrography does not differ enough across the extent of the offshore diffuser area to be important in determining the spatial distribution of biota. The importance of the hydrographic data which have been generated is to identify covariates contributing to temporal variability and to identify catastrophic events, such as an area-wide dissolved oxygen depletion, which will impact the benthos directly, and which could act synergistically with brine effects to impact the area as a whole.

Virtually all investigators involved in the Bryan Mound study utilized pre-discharge brine plume modeling scenarios (NOAA, 1977) or post discharge plume monitoring results to design or confirm the design of their impact assessment sampling scheme. One team generated very important pre- and post-discharge information for sediment salinity (Harper and McKinney 1980, 1981), and these data guided their grouping of stations into brine (influenced) and control strata.

In Chapter II, three types of analyses were identified as being germaine to the goals of this Task IV assessment. These are:

(1) t-tests
(2) analyses of variance
(3) discriminant analysis

Based on Table 14 (see Chapter IV), it is obvious that in no case is the level of replication at any station sufficient to allow utilization of discriminant analysis to distinguish impacted from unimpacted stations. The experimental design for these studies, where stations are located varying distances from the outfall, should yield a gradient of effects (greatest effects closest to the outfall, decreasing away from the outfall). Therefore, stations should not behave as groups and the data are not as amenable to group classification as they are to gradient analysis. In this situation, the only way that discriminant analysis could be used would be where there were enough replicates at each impacted or unimpacted station to satisfy the requirements of the analysis (see Table 14). One way around this problem of lack of replication is to treat the study area as being composed of several strata, as was done in the benthos study (Harper and McKinney 1981).

Therefore, the design which appears most applicable to the Bryan Mound data involves the testing for difference in station means between impacted and unimpacted stations (or a series of stations along a gradient of impact) using the (univariate) t-test and/or analysis of variance. Analysis of covariance could be used to remove the effects of uncontrollable environmental variation (e.g. that contributed by

sediment type), and regression could be used to establish relationships between biota, environmental variables and distance from the diffuser, assuming there are enough stations along the gradient for the analysis.

## V.2. Benthos

In general the nekton and benthos sampling designs were similiar except for the level of replication at each station (two for nekton vs three for the benthos). The entire benthos field sample (grab) was processed, so there were no subsampling considerations. It should, however, be emphasized that the three grab samples collected at each station were essentially contiguous, especially when viewed in the context of the expanse of the station array at the offshore diffuser site. Loran A navigation, which was used early in the program, allows relatively poor accuracy in station location and relocation. Therefore, it seems quite plausible that exactly the same area (i.e., station) was seldom sampled from cruise to cruise during the early phases of the program. In conditions where accuracy in station relocation is poor, and, hence, the actual "size" of the station is quite large, closely placed replicate samples are representative of only a small, isolated patch of the station. The samples collected on the next cruise would represent another isolated patch, and so on. This concern becomes especially critical when the sediment distribution in the study area is patchy, as it appears to be about the offshore diffuser site at Bryan Mound. This problem should be expressed by certain statistical characteristics of the data (e.g. small within-station variance, spuriously significant main effects and interactions with higher order trend characteristics), which contribute to a greater possibility for a Type I error. That is, in a one or two-way factorial design fixed effects ANOVA, the contiguous nature of the samples at each station will artificially decrease the denominator in all F tests (main effects and interactions), leading to a spurious rejection of the null hypothesis. Although the situation was obviously improved with the use of Loran C navigation beginning in the later stages of the predisposal period, the replicate grabs at each station were still essentially contiguous, and some real within-station variance was certainly masked.

Harper and McKinney (1980) utilized two-way factorial ANOVA's to determine the significance of spatial (station) and temporal (cruise) differences in diversity at the offshore diffuser site during the predisposal period. The analysis included 11 cruises (January 1979 to November 1979) and the original 15 stations (see Section III.1), with three replicates per station. While this is the correct ANOVA design for the data which were collected, the investigators have violated a cardinal rule in the application of a posteriori multiple means tests. Their results showed that the station x cruise interactions were significant. In cases like this multiple means tests should not be employed. The results of these ANOVA's must be considered in light of the contiguous nature of the replicates as described above.

Harper and McKinney (1981) report the results of the benthos study at the Bryan Mound offshore site through the first twelve months of the discharge period. They utilize two different ANOVA designs in their analyses. A fixed effects, factorial design two-way ANOVA, with cruise as one main effect and brine vs control area (strata) as the levels of the "treatment" main effect, was employed, utilizing the sum of the three replicates at each station as a replicate sample. This differs from the analyses presented above (Harper and McKinney 1980) where the integrity of the individual replicate samples was retained and station, not stratum differences were tested. In these post discharge analyses the investigators subdivided the array of stations in the study area (see Figure 2) into two groups, which generally formed concentric rings around the diffuser site. Those stations closest to the diffuser (Stations 4, 5, 12, 16, 17, 18, and 19 in Figure 2) were grouped as brine (influenced) stations, while those furthest away (Stations 1, 7, 10, 11, 13, 14, and 15 in Figure 2) were designated as control stations. Both the brine and control area had seven stations (equal replicates) in this design. The stratification of the design was guided by sediment salinity measurements and by the results of brine plume modeling and tracking studies.

The results of these ANOVA's generally showed very strong cruise effects and small treatment effect, the indication being that there were no

significant effects attributable to brine discharge. While there may, in fact, not be any impact on the benthos due to brine discharge, there are several problems with this ANOVA design which could render a conclusion of no significant impact invalid. The major problem involves the use of stations which may or may not all be impacted in an identical manner as replicates. The ANOVA design assumes that the differences in replicates within a treatment level (e.g., brine or control) are unrelated to the treatment itself. That is, when a design is modified from sampling at specific stations to sampling within larger strata (e.g., brine vs control strata) the implicit assumption is that all parts of each stratum are homogeneous with respect to both the degree of impact and influence of important covariates. In the benthos study, this was obviously not the situation. The "brine" group of stations (replicates) are most likely not all being impacted to a similar degree. Therefore, the treatment (brine exposure) is not being applied uniformly over the replicates (stations) in this level of the main effect. This leads to larger within-cell variability, reducing the power of the analysis and making it less likely that a significant effect will be seen if it does occur.

The other major problem associated with the design also involves this particular use of strata for impact assessment. While the basic concept of using strata as opposed to more numerous stations as part of the natural evolution of a well designed baseline sampling program is encouraged, sampling within a stratum should be random. That is, once control and brine strata are identified, the fixed stations within each stratum should be abandoned, and the locations of the stations should be selected randomly from the whole population of sample locations. Unless this is done, there will be a problem with correlation of errors and, more important, the inference space will be restricted to only those stations which are sampled. In the Bryan Mound benthos program, the fixed nature of the stations (replicates) within each stratum disallows the expansion of the inference space to the entire brine or control stratum.

In an attempt to utilize pre and post disposal information in the same design to assess brine impacts, Harper and McKinney (1981) employed a nested factorial design ANOVA, with treatment (brine vs control) and period (pre- and post-discharge) as crossed (factorial) effects, and cruise nested in period. This appears to be a typical case of trying to utilize data that have not been collected with any particular design in mind for formal hypothesis testing. There are a number of reasons why the model employed is not correct. The most obvious, in addition to the heterogeneity within the "brine" treatment group that was discussed above, is the lack of random selection of cruises within each period. A nested effect in an ANOVA model is never a fixed effect, as are cruises in the benthos sampling scheme. Not only are the cruises not randomly selected within each period, but they are not independent of each other. That is, the mean for any particular cruise is dependent to some degree on the mean for the previous cruise. This would reduce the mean square for cruise effects, increasing the probability of a Type I error for testing period effects (which are tested by MS period/MS cruise). Finally "period" and "cruise" effects are hopelessly confounded.

Adequate sediment characterization should be included as an integral aspect of the benthos impact assessment because sediment composition (especially texture) is a dominant environmental covariate in determining spatial trends in macrobenthos distribution. In order to utilize the benthos standing stock data for hypotheses testing (t-tests and one way ANOVA's) the impact of sediment composition must be assessed. Analysis of covariance should be applied to predisposal data to identify the importance of sediment parameters in determining station differences. Once the effect of sediment parameters are isolated, stations or strata which can be utilized for impact assessment can be identified. Unfortanately, only one sediment sample was collected at each station (none within station replication).

The use of divers to collect the benthic grab samples results in all the replicates grabs having the same depth of penetration and being otherwise similar, thereby removing a number of sources of extraneous variation in the study. This careful handling and collection of samples is a very positive aspect of this program.

Recommendations:

- Determine the effects of the contiguous distribution of replicates on station means and variability using predisposal data.
- Run a gear comparison test to determine if these small Ekman grab samples are the most efficient way to sample. Comparison of results using the Ekman grab with a larger ($0.05m^2$ or larger) grab should be made.
- Determine the number of replicates required to see desired differences in station or stratum means. Increase the replication at the stations or strata as determined.
- Determine the covariate effect of sediment composition on station means.
- If strata are to be utilized (i.e., if it can be shown that all "brine" stations are being exposed to similar levels of salinity overage) then samples should be taken at random within the strata and not at fixed stations.
- Type II error levels should be expressed for all analyses conducted.

## V.3 Nekton

As in the benthos study, the nekton study usually involved identifying and enumerating all organisms in the trawl sample, and thus subsampling problems (see the zooplankton and phytoplankton discussions) need not be considered. Because of the mobility of the nekton and the large scale spatial homogeneity of the sediments in the area of the offshore diffuser site, stations should be relatively similar, and it should be possible to select stations inside and outside the area of impact for comparison using t-tests or ANOVA's. Results presented in Appendix A of this report support the notion of the relative homogeneity of the nekton assemblages at any one time in the vicinity of the offshore diffuser site at Bryan Mound.

Chittenden et al. (1981) utilized a two-way ANOVA design for predischarge and discharge phase analyses. In both cases, two-way fixed effects and factorial design ANOVA's were employed, with cruise and station as main effects. These were followed by a priori single degree

of freedom means comparisons. Since station effects were not found to be quantitatively important during the brine discharge period, Chittenden et al. (1981) concluded that brine discharge was having no significant impact on nekton abundance.

Essentially, the same problem exists with the nekton analyses as was apparent for the benthos analyses. In a factorial, fixed effects design, is it implicitly assumed that each treatment level (i.e., degree of brine influence at each station) is constant over the entire period. This was certainly not the case in the Bryan Mound nekton study. Continuous brine discharge was initiated on March 13, 1980, but at relatively low levels, with a major increase in brine discharge occurring some months later. The point of this discussion is that the station effect was not operative at the same level during the entire period of discharge and this would introduce a large amount of within-station variation that would decrease the power of the ANOVA. This might help to explain why Chittenden et.al. (1981) found few significant station differences.

No information is given regarding the Type II error in these ANOVA's. While nekton sampling involved replication at all stations, it is suspected that this level of replication (two replicates/station) is not adequate to detect more subtle impacts to the nekton standing stock. This is due to well-known spatial variability in trawl catches. Therefore, parametric statistical methods which test for significant differences in station means might have little power (ability to see a true difference) and would be of less value to impact assessment. A decision regarding no significant impact could be meaningless if the power of the analysis is poor.

Twenty-four samples (trawls 20-43) were collected in the area of the offshore diffuser site for impact assessment (see Figures 3 and 4 in Section III.2). It is very likely that there is a more optimal distribution of stations and replicates than is being used in the present study (12 stations x 2 replicates/station). Perhaps strata should be chosen on the basis of a methodology similar to that employed

in the benthos study. It appears that the nekton study would have much to gain from a close examination of the results of the benthos study.

The nekton sampling program reflects an apparent preconceived notion that the initial design used in the predischarge phase was the optimal design for impact assessment. Nowhere is there any indication that the principal investigators ever questioned the adequacy or efficiency of the design. The predischarge monitoring information was not utilized to optimize the impact assessment sampling design.

Recommendations:

- Determine the number of replicates required for a given level of precision or to detect a given true difference in station means.
- Assess the feasibility of establishing strata for brine and control areas.
- Adjust the sampling design in the area of the diffuser (Trawls 20-43) by increasing the replication and either decreasing the number of stations or establishing spatial strata (assuming fixed costs).

## V.4 Phytoplankton

There is little question that the phytoplankton studies at the Bryan Mound offshore site are not adequate to assess brine impacts. The most obvious problem involves the lack of species-level identification of the organisms. Since several of the common genera in the study area are each represented by several species, this level of taxonomic identification is not acceptable.

From the methodologies presented in the most recent Bryan Mound monitoring report (Loeblich and Hall 1981) it appears that field sampling for phytoplankton counts and pigment concentrations did not involve replication. In the initial design, there was a dense array of stations in the area of the diffuser (see Figure 5), but no information on within-station variability is available because of the lack of replication. Therefore no basic descriptive statistics or hypothesis testing techniques requiring replicated data (t-test and one-way ANOVA)

can be utilized to determine station differences. Since there are no replicated data, little can be done with these baseline data regarding optimization of design for impact assessment. Numbers of replicates required to see certain percent differences in group means cannot be determined. The only thing that can be done statistically with these data is to consider all near bottom samples collected on one predischarge cruise (from the various stations) to be replicates. While this would introduce some spatial and temporal (short-term) variation into the error mean square, at least an upper limit could be set on the error term.

Since laboratory subsampling of each taxonomic sample was conducted in triplicate, the error introduced by laboratory subsampling could be assessed using a nested factorial ANOVA. This is not true for the pigment subsamples, which were not replicated. Therefore, the error terms in the denominator of the F ratios would be artifically inflated for pigments, thereby reducing the power of the ANOVA.

If brine discharge causes an impact to the phytoplankton that is exhibited as a gradient away from diffuser (down current) then it is conceivable that regression analysis could be used (with distance from the diffuser as the independent variable) with these unreplicated data to test for trends in standing stock or pigment concentrations. The change which was instituted in September 1980 in the design of the phytoplankton sampling program makes it more suited to this type of consideration. However, a number of other environmental variables should also be monitored along the gradient for use as predictor variables, and the number of stations along the gradient should be increased.

The sampling scheme included collection of discrete near bottom (and near surface) samples. Since brine impacts are expected to occur near the bottom (if at all), this discrete depth sampling is supported. Given the predischarge differences in the results for the near surface and near bottom samples, it is difficult to see how these near surface samples could be used to assess impacts. A similar question arises

regarding the utility of the 10 and 18 meter stations which were added in September 1980.

Loeblich and Hall (1981) indicate that cluster and discriminant analysis were conducted to aid in impact assessment, but no results are presented. The applicability of these techniques to the assessment of brine discharge related impacts is difficult to understand in light of the experimental design for the study.

Recommendations:

- The taxonomic efforts are not adequate. There must be a concerted effort made to identify the diatoms and dinoflagellates to species. Analyses and interpretations based on genus-level determinations may be ill-founded.

- Discontinue collection of nearsurface samples and also those at 10 meter depths. These samples cannot be used as controls because of known vertical differences in phytoplankton densities and community composition. While some water column sampling was justified in the baseline (pre-discharge) study, this information is of little value in assessment of brine impacts.

- Discontinue laboratory subsampling. By now the investigators should have a good handle on the within-sample (within-replicate) variability attributable to laboratory subsampling.

- The decrease in the number of stations from 13 to 5 was warranted and the attempt to align these stations according to the prevailing current conditions is commendable.

- Collect as many replicates at each station as reductions in other aspects of the program will allow (assuming fixed resources). After the first field collection involving replication, determine the number of replicates needed for the desired level of precision for the particular ANOVA design. Use this information to optimize the design for impact assessment sampling.

- Collect more ancillary parameters (e.g. nutrients) in order to explain spatial and temporal trends in the phytoplankton community.

## V.5 Zooplankton

There are a number of disturbing aspects to the zooplankton program at Bryan Mound. The major problem involves the fact that the tows are integrated over the upper 17 meters water column. Park and Minello (1981) report that, based on the results of brine plume tracking studies, the plume does not penetrate the water column vertically to the heights sampled by the zooplankton tows. If the objective of the sampling is to determine the significance of brine related impacts, this type of sampling should be immediately abandoned, and sampling should be restricted to the near bottom waters (as for the phytoplankton). Otherwise impacts near the bottom (if they do occur) will not be detected because of "dilution" of the effect of brine discharge by the contributions from the unimpacted portions of the water column.

The zooplankton efforts included replication in both field sampling and in laboratory processing for counts, but no replication accompanied laboratory subsampling for biomass. Therefore, for taxonomic counts, spatial variability in the field could be separated from the variability introduced by the need for subsampling (samples too large to count) using a nested factorial ANOVA design (with laboratory subsamples nested in each field replicate). The contribution of laboratory subsampling to the variance was apparently not assessed in the Bryan Mound study, since simple one-way ANOVA's (for each cruise, with station as the main effect) with 2 and 6 degrees of freedom for the numerator and denominator of the F ratio (mean square among stations/mean square within stations) respectively, were conducted. Apparently the three replicate subsamples were combined to give one value for each field replicate. This represents a loss of information which could otherwise have been used to increase the power of the analysis (i.e., used to reduce the (error) mean square among field replicates), making it easier to detect significant station differences if they did occur.

The use of a series of one way ANOVA's followed by a posteriori multiple means tests to test station differences for the individual months does not adequately reflect the field and laboratory experimental design criteria which are most applicable to the problem. A two-way nested

factorial ANOVA, with cruise and station as factorial effects and with laboratory subsamples nested within replicates would be the most applicable design. This design would be more powerful than that used by Park and Minello (1981) since the error mean square would be less. Since the field and laboratory procedures were consistent throughout the study, the analysis of the results of the zooplankton study could be treated as a priori hypothesis testing. In this case, single degree of freedom means comparisons could be made, again increasing the power of the analysis, and avoiding some of the problems with Type I error levels inherent in a posteriori multiple comparison tests (Kirk 1968).

Park and Minello (1981) indicated that the brine plume never reached Stations A and C (see Figure 7, Section III.4) and they considered these stations to adequately represent controls. While the extent of the brine plume is certainly one criteria for designation of control stations, the fact that planktonic organisms are essentially free floating would indicate that downcurrent stations might show some residual effect from the passage of the water mass through the brine field, especially if the effects on the zooplankton of the exposure to brine were delayed. The attempts of the principal investigators to limit the effects of vertical movement in the water column (by restricting sampling to the mid-day period) are applauded, with the additional note that there are statistical methods (e.g. blocking) which can be used to attain the same goals (removing the effect of time of day). The possibility of utilizing the approach which was taken in the phytoplankton sampling (orienting the station axis parallel to the near bottom current direction on a cruise by cruise basis) should be investigated.

In addition to the possible inclusion of several more stations along the longshore axis and the need for discrete near bottom samples, there is a suspected need for greater replication to see desired station differences. The replication problem is probably substantial, and it is suggested that data which were collected during the pre-disposal phase of the program be critically examined using basic descriptive statistics and hypothesis testing, so Type II errors can be assessed, and design

optimization can be achieved. Unless this is done, a finding of no significant difference in one-way (fixed effect) ANOVA's or t-tests will have little meaning (especially if the data will allow no differences smaller than 200% to be detected). The problem seems further complicated because results from pre-discharge studies indicate significant spatial differences in zooplankton abundance during certain cruises.

One of the most disturbing aspects of the zooplankton program was that predischarge data was not used to optimize the design for impact assessment sampling. Certainly, the fact that predischarge analyses showed significant station differences during some months (Park and Minello 1980) could be looked on as a signal that the design was in need of some refinement. It is unfortunate that the initial data were not utilized to optimize the design of the impact assessment sampling since the initial design accomodated most of the major sources of variation. Clearly, the zooplankton methodology utilized in the Bryan Mound study was not adequate to measure or detect post-discharge impacts.

Recommendations:

- Restrict sampling to the near bottom waters.
- Utilize the predischarge and postdischarge data in a nested factorial design ANOVA with a priori means testing to define components of variance and determine the adequacy of the present level of replication.
- Consider discontinuing or greatly restricting laboratory subsampling. The investigators should have enough data on hand to quantify this component of variance.
- Possibly increase the number of stations on the longshore axis, and adopt the procedure of orienting sampling stations with the ambient near bottom currents, as in the phytoplankton study.
- Discontinue analyses of data from individual cruises. The design of the analyses should take into account all main effects.

## V.6 SUMMARY

In general, the methodologies employed at the Bryan Mound site are either not particularly appropriate for impact assessment or do not represent an optimum allocation of resources. The major problem is that the predisposal data were not utilized statistically to determine even the most basic pieces of information (e.g., required degree of replication) disallowing their use in structuring an efficient discharge-phase sampling design. In the phytoplankton study there was a change in sampling design from predischarge to the discharge phase, but this apparently occurred without the aid of any statistical guidance. For the benthos, design modification did occur and, assuming that the overages of brine are similar over all parts of the "brine" stratum, the move was a positive step. However, there was little increase in efficiency since the same number of samples (stations) were collected before and after establishment of strata. The fact that the original designs were more or less retained throughout the Bryan Mound program assumes that the original design was optimal, which was, of course, not the case. In those cases where an ANOVA design was utilized, it appeared to be chosen to conform to the data already collected.

Any parametric statistical testing for differences in station means that does not indicate the Type II ($\beta$) error and therefore the power of the analysis ($1-\beta$) should be looked on with caution. A finding of no significant impact, which will probably result from the Bryan Mound studies, will be easily embraced by DOE. However, a finding of no significant impact in this case may be due to the fact that the poor power of the analysis (due to the small number of replicates or inefficient design) disallows anything but the most obvious and severe impacts to be detected.

The above discussion centers on an evaluation of the Bryan Mound sampling program and especially what was done with the data to develop an optimal impact assessment sampling design. While some fault was found in virtually all Bryan Mound biological studies, it is very easy for one to sit back and criticize. It should be emphasized that several of the studies (especially the nekton study) are generating a vast

amount of basic ecological information on the communities of the NW Gulf of Mexico. This type of baseline data is vital to a proper assessment of impacts, and the long pre-discharge sampling program generated an excellent baseline for the area.

While the scope and results of at least some of the individual studies are impressive, one becomes quickly aware that there was very little coordination among studies to integrate the field collection efforts. Obvious phytoplankton-zooplankton and benthos-nekton couplings were not formulated, with every investigator more or less working on his or her own. It does not appear that there was any attempt to utilize the same stations for the different studies, or to coordinate the temporal occurrence of the field sampling efforts for the different communities. Given the opportunity presented by the SPR program for a major synthesis of the regional marine ecology, the independent nature of each study was unfortunate.

While this evaluation was relatively negative, we agree with the overall conclusions that no significant short-term ecological effects from brine discharge are occurring. Given the variability of the nearshore Gulf ecosystem and the euryhaline nature of many of the species inhabiting the area, brine discharge appears to be just one more factor in a very dynamic and physically stressed ecosystem. Our criticisms were mainly aimed at the lack of efficiency in the experimental design and the resulting elevation of costs for the impact assessment program. While the SPR monitoring program at the Bryan Mound site was conducted in an extremely inefficient manner, it has generated a large volume of information. When this vast amount of data is evaluated in its entirety, indications are that no severe impacts are occurring.

# VI. COMPUTER PROGRAMS

## VI.1 Introduction

The large volume of ecological data coupled with the computational complexity of many of the analyses require computer programs to effectively implement the analysis plan outlined in Section IV. The NOAA UNIVAC computer system includes a software library called STAT-PACK (Sperry Rand Corporation 1973) which contains ninety-one basic statistical subprograms. An overview of the structure and use of the STAT-PACK library is provided in Section VI.2.1. Section VI.2.2 identifies the STAT-PACK subprograms which are useful in implementing those portions of the analysis plan which are germane to the objectives of Task IV.

Although the STAT-PACK library provides subprograms for most important statistical tests, there are several tools important in the statistical characterization of a data set which are not found in this software package. SAI has written several subprograms to supplement the STAT-PACK library in the area of basic descriptive statistics. Section VI.3 presents an overview of the SAI subprograms and how they are integrated into the STAT-PACK library. Appendix B provides a detailed description and code listing of each subprogram discussed in Section VI.3

## VI.2 STAT-PACK Statistical Library

### VI.2.1 Overview

STAT-PACK is a comprehensive library of statistical subprograms coded in UNIVAC FORTRAN V (Sperry Rand Corporation 1973). Each subprogram is a module of code which performs a well defined set of calculations. All of the subprograms, with the exception of the plotting routines, are completely independent of any specific input or output device. Tranfers of data into and results out of the subprograms are accomplished by arguments associated with each subprogram call. This modular design of STAT-PACK permits the user to link the appropriate subprograms together to perform a series of analyses.

The STAT-PACK user must write a driver program to input the data, set any option switches, call the appropriate subprograms, and output the desired results. In general, the driver program will not be complicated since STAT-PACK uses a limited number of data structures to transfer information to and from the subprograms. If several subprograms are linked together in the driver program, the user must ensure that the arguments for each subprogram are properly initialized before calling the subprogram. Figure 28 is an example of a driver program which inputs a data set of sample observations, calculates the mean, standard deviation, skewness and kurtosis, and outputs the results.

### VI.2.2 Subprograms Relevant to Task IV Objectives

The following STAT-PACK subprograms are discussed in this section:

| Name | Description |
|---|---|
| HIST | Histogram |
| AMEAN | Arithmetic mean |
| RANGE | Range of data |
| STDEV | Standard deviation |
| CVAR | Coefficient of variation |
| KURSK | Kurtosis and skewness |
| SIGPRP | Significance of proportions |
| SIGDMS | Significance of mean difference |
| SIGDVR | Significance of variance ratio |
| CFDMUV | Confidence limits of mean (unknown variance) |
| CFDMSU | Confidence limits of mean difference |
| ANOV1 | One-way analysis of variance |
| ANOV2 | Two-way analysis of variance |
| RESTEM | Stepwise multiple regression |
| DISCRA | Discriminant analysis |

The order of the discussion of these subprograms is the same as presented in the STAT-PACK manual.

Subroutine: HIST (X, N, STEP, NG, XMIN, XMAX, F, IND) Page 2-6

**Figure 28. Example of a STAT-PACK driver program.**

```
C
C       THIS IS AN EXAMPLE OF A STAT-PACK DRIVER PROGRAM.  THE PROGRAM IS
C       COMPOSED OF THREE SECTIONS; AN INPUT SECTION, A STAT-PACK SUBPROGRAM
C       CALLING SECTION, AND AN OUTPUT SECTION.
C
        DIMENSION X(100), G(4), IDSAMP(12)
C
C       INPUT SECTION: READ SAMPLE IDENTIFICATION (IDSAMP), THE NUMBER OF
C                      SAMPLE OBSERVATIONS (N), AND THE SET OF OBSERVATIONS
C                      (X).  THE MAXIMUM NUMBER OF SAMPLES IS 100.
C
        READ (5,5000,END=10) IDSAMP
5000    FORMAT (12A6)
        READ (5,5010) N
5010    FORMAT (I5)
        READ (5,5020) (X(J),J=1,N)
5020    FORMAT (8F10.0)
C
C       STAT-PACK SUBPROGRAM CALLS: AMEAN - ARITHMETIC MEAN
C                                   STDEV - STANDARD DEVIATION; UNBIASED
C                                           ESTIMATE OPTION
C                                   KURSK - SKEWNESS AND KURTOSIS; ESTIMATED
C                                           FROM MOMENTS OPTION
C
C       SEE THE STAT-PACK PROGRAMMER REFERENCE MANUAL FOR A DETAILED
C       DISCUSSION OF THESE SUBPROGRAMS.
C
        CALL AMEAN (X,N,XBAR)
        XND = -1.0
        CALL STDEV (X,N,XND,S)
        IND = 1
        CALL KURSL (X,N,IND,G,$10)
C
C       OUTPUT SECTION: WRITE THE SAMPLE ID (IDSAMP), THE SAMPLE MEAN (XBAR)
C                       THE STANDARD DEVIATION (S), THE SKEWNESS (G(1)), AND
C                       THE KURTOSIS (G(2)).
C
        WRITE (6,6000) IDSAMP
6000    FORMAT (35H DESCRIPTIVE STATISTICS FOR SAMPLE ,12A6)
        WRITE (6,6010) XBAR
6010    FORMAT (15X,7HMEAN = ,F15.4)
        WRITE (6,6020) S
6020    FORMAT (22H STANDARD DEVIATION = ,F15.4)
        WRITE (6,6030) G(1)
6030    FORMAT (11X,11HSKEWNESS = ,F15.4)
        WRITE (6,6040) G(2)
6040    FORMAT (11X,11HKURTOSIS = ,F15.4)
10      STOP
        END
```

Function:

This subroutine generates frequency values and a histogram plot of a variable with specified "size" classes or grouping values.

Application:

This procedure can be used to graphically portray the distribution of a data set. The histogram plot is helpful in determining if a data set meets the assumption of a normal distribution. (Section IV.2.2.1)

Requirements of the driver program:

The driver program must input the data into a real number array called X. The midpoint of the lowest and highest group must also be inputted into XMIN and XMAX respectively. N is the number of values in the X array and NG in the number of groups to be formed for the histogram. The size of the groups is inputted into the variable STEP. The frequencies are returned in the array F. The subroutine also permits the input frequencies of grouped data to be used. The use of the grouping option, IND, is explained in the STAT-PACK manual.

The test program in the manual is set up to read a single data set consisting of a title card and a control card which specifies the parameters (e.g. N) of the data. It should be noted that the test program is set up to do either the grouped or ungrouped option. Another interesting aspect of the test program is that there is no "WRITE" statement for the output of the subroutine. Apparently the subroutine contains its own output capabilities. The test program is also set up to handle more than one set of data.

Subroutine: AMEAN (X, N, XBAR) Page 3-1

Function:

This subroutine calculates the arithmetic mean of a variable.

Application:

The mean of a variable is one of the basic statistics that is used to summarize data. (Section IV.2.2.1)

Requirements of driver program:

The driver program for this subprogram is very simple. The data must be inputted into the real number array X and the number of observations into variable N. The mean is outputted as variable XBAR.

The test program is set up to handle several sets of data. It also reads and writes a title with the array SH. The use of the title is optional and may be disregarded by the user.

Subroutine: RANGE (X, N, R) Page 3-32

Function:

This subroutine identifies the range of a set of measurements.

Application:

The range is a descriptive statistic that is useful in providing a preliminary index of the variability of a variable. It can also be used to detect outlier values which may generate an abnormally large range. (Section IV.2.2.1)

Requirements of the driver program:

The driver program must input the data into the real number array X. The number of values in X must be inputted as N. The range is outputted as the variable R.

The test program uses a title card and a separate record to input N.

The following statements inserted after the WRITE statement in the test program could generate the minimum and maximum of this range. (Note: the AMAX1 function of the FORTRAN subprogram library is used.)

```
      XMAX = 0.0
      DO 100 K=1,N
      XMAX = AMAX1(XMAX,X(K))
  100 CONTINUE
      XMIN = XMAX - R
      WRITE(6,0000) XMIN,XMAX
 6000 FORMAT(8HMINIMUM=,F10.0,3X,8HMAXIMUM=,F10.0)
```

The maximum and minimum values can assist in the identification of erroneous values.

Subroutine: STDEV (X, N, XND, S) Page 3-39

Function:

This subroutine calculates the standard deviation of a variable.

Application:

The standard deviation is a summary statistic used to represent the variability of a variable (Section IV 2.2.1). It is also used in other statisical computations (e.g., standard error of the mean).

Requirements of the driver program:

The driver program must read the data into the real number array X and the number of observations into the variable N. The option of using N or N-1 in the computational formula must also be specified in the program. If N is used then XND = 1.0. If N-1 is used XND = -1.0. The standard deviation is returned to the driver program as S, and the arithmetic mean of the array X is returned as XND.

The test program uses title (array SH) and control cards to input the data attributes (N,XND). It is also set up to handle more than one set of data.

Subroutine: CVAR (X, N, V, XBAR) Page 3-43

Function:

This subroutine calculates the coefficient of variation of a variable.

Application:

The coefficient of variation is the ratio of the standard deviation to the mean of a variable. This statistic provides an index of the variability that is not dependent on the scale of measurements. (Section IV 2.2.1)

Requirements of the driver program:

The driver program must input the data into the X array and the number of values of X into the variable N. The coefficient of variation is outputted as variable V. The variable XBAR is used on input to control the calculations of the standard deviation. On output XBAR is the mean of X. The STAT-PACK manual explains these options.

As before the test program in the STAT-PACK manual is set up to use title cards and control cards. It is also set up to handle more than one set of data.

Subroutine: KURSK (X, N, IND, G, $k) Page 3-71

Function:

This subroutine calculates skewness and kurtosis of a variable.

Application:

The skewness indicates the degree of asymmetry in the distribution of replicated values for a variable. The kurtosis indicates whether or not the peak or central tendency of the data set is greater than or less than a normal distribution. Both of these descriptive statistics can be used to investigate the normality of the data. (Section IV.2.2.1)

Requirements of the driver program:

The data must be inputted into the real number array X and the number of observations in the array into the variable N. The driver program must also specify which options are to be used for the calculation. The options are controlled by the value of IND. The STAT-PACK manual explains the options of the subroutine. The last argument of the subroutine must be a statement number in the driver program that the subroutine will return to if it overflows during computations. The test program illustrates the use of this feature. The value of skewness and kurtosis are outputted in the array G.

The test program is set up to use title cards and control cards. The value of N for each group of values is on the first record of each set in the test program. Statement 99 is used for the overflow return of the subroutine.

Subroutine: SIGPRP (H, N, P, AL, BE, ETA, D, $k) Page 6-1

Function:

This subroutine performs a two-tailed significance test on the difference between the actual and hypothesized binomial proportion of a sample.

Application:

The test of proportions is useful if the expected and sample proportion of successes (e.g. values over a certain condition) are known. This test could also be applied to changes in the relative frequency of occurrence of a species or other taxa (Section IV.2.5.1)

Requirements of the driver program:

The driver program requires the input of the sample proportion as H, the number of observations as N, the test proportion as P and the significance level, ALPHA, as AL. Similar to the KURSK subroutine, an overflow return statement number must be provided. The outputs of the subroutine are D (the difference between proportions), ETA (the probability of the difference), and BE (the rejection level for AL).

The driver program must provide formats for all of the input and output. The test program given in the STAT-PACK manual is one example of how this may be handled.

Subroutine: SIGDMNS (X1, N1, X2, N2, XBAR, U1, S1, U2, S2, T, ETA, NDF) Page 6-5

Function:

This subroutine performs a two-tailed t-test on the difference of two sample means.

Application:

The t-test is a standard statistical procedure used to test the significances of the difference between two sample means. The means in the SPR impact assessment program could be from predischarge and discharge phase sampling. (Section IV.2.5.1)

Requirements of the driver program:

The driver program must input the data from the two sets of replicate samples into arrays X1 and X2. The numbers of replicates in the two arrays are variables N1 and N2. The test criterion for minimum difference required is input as XBAR. If XBAR = 0.0, then the test will determine the equality of the means. The output of the subroutines are the means (U1 and U2), the sample standard deviations (S1 and S2), the t statistic (T), the probability of t (ETA), and degrees of freedom (NDF).

The test program used title and control cards. It is also set up to handle more than one set of data. In the test program, the data values are first read in for X1 and then for X2. It is possible to format the data so that the value of X1 and X2 for each observation are read off the same record. Once again, the output must be formatted by the main program. The test program provides an example of this formatting.

Subroutine: SIGDVR (X1, N1, X2, N2, R, U1, S1, S2, F, ETA, NDF1, NDF2, L) Page 6-13

Function:

This subroutine perform the basic F-test of significance on the ratio of two variances.

Application:

The ratio of two variances may be of interest if an impact event is suspected to change the variability of a variable. Other sources or causes of variation must be accounted for in this analysis. A major use of this significance testing is discussed in Section IV.2.5.2. More complex patterns of variance can be analyzed with the analysis of variance procedure discussed later.

Requirements of the driver program:

The data arrays, X1 and X2, and the number of observations in X1 and X2 (N1 and N2), must be inputted by the driver program. The hypothesized ratio must be inputted as R. The outputs of the subroutine are the means of the two samples (U1 and U2), the sample variances (S1 and S2), the ratio of variances (F), the probability of F (ETA), and the degrees of freedom for the ratio (NDF1 and NDF2). A control variable, L, is also outputted to the driver program to indicate which variance is greater. The use of control variables is shown in the test program in the STAT-PACK manual. The data for the test program are setup in the same way as in the SIGDMN procedure.

Subroutine: CFDMUV (X, N, ALPHA, XBAR, S, UL, UU, $k) Page 7-5

Function:

This subroutine calculates the confidence limits for a sample mean when the true variance is unknown and the estimated sample variance must be used.

Application:

Confidence limits can be used to define the range within which the value of a variable is likely to occur with a specified probability. If confidence limits were determined for pre-impact observations, post impact data could be evaluated based on those limits (Section IV.2.5.1).

Requirements for driver program:

The driver program must read the data into array X, the number observations into N, and the Type I error level as ALPHA. The output is the mean (XBAR), standard deviation (S based on N), upper confidence limit (UL), and the lower confidence limit (UU). The subroutine also requires a return statement number in the driver program if an overflow condition is encountered.

The test program and test data for this procedure are similar to those previously discussed. Once again input and output formats must be specified by the main program.

Subroutine: CFDMSU (X1, N1, X2, N2, ALPHA, AB1, AB2, S1, S2, UL, UU, $k) Page 7-10

Function:

This subroutine calculates the confidence limits of the difference between two sample means.

Application:

This procedure can provide an estimate of the likely range of the differences between two sample means. If similar sets of variables were obtained before and after impact then the confidence limits of the difference of means could be used to evaluate possible changes. Note: this procedure requires the assumption of equal sample variances. This may not be true for environmental data. (Section IV.2.5.1)

Requirements of the driver program:

The driver program must read the data for the two samples into arrays X1 and X2 and the number of samples in each array into N1 and N2. The value of Type I error is inputted as ALPHA. The outputs of the program are the two sample means (XB1 and XB2), the standard deviations (S1 and S2), the lower confidence limit (UL), and the upper confidence limit (UU). An overflow return statement number must also be provided by the main program. Examples of input/output formats are given in the test program in the STAT-PACK manual.

Subroutines: ANOV1 (Y, N, J, I, R, S, SS, NDF, SM, F, PF, $k) Page 8-1

Function:

This subroutine performs a one-way analysis of variance.

Applications:

Analysis of variance (ANOVA) is used to test the equality of three or more means. Note: this procedure is limited to replicated data. (Section IV.2.5.2)

Requirements of driver program:

The data for all samples of all groups must be inputted into the Y array. The values from the first group are the first set of values in the Y array. The second group of values immediately follows the first and the pattern is repeated through all groups. The driver program must also input the total number of samples for all groups as N and the number of samples in each group as the array J. The number of groups is inputted as I. The output from the subroutine is R, the array of residuals for all N observations. The estimates for each group comprise the array S. The sum of squares, degrees of freedom, and mean squares are in the arrays SS, NDF and SM. The STAT-PACK manual explains the organization of these arrays. The F-statistic is outputted as F. The probability of F is returned as PF. This subroutine also requires an overflow return statement.

The test program uses several control cards including one for multiple data sets in one run. After the number of samples and replicates per group are entered, the data are entered sequentially. It should be noted that if the data were not formatted sequentially but were coded, they could be read into a two dimensional array and then transferred to the X array. An example of this is:

```
          DIMENSION      YT(10,20)
C 10 is the number of groups and 20 is the maximum value of the
          J array.
          ----- read other data attributes
          -----
10        READ(5,5000,END=20) L,M,YTT
5000      FORMAT(2I,F)
C     L=group number, M = replicate number in group L
          YT(L,M) = YTT
          GO TO 10
20        IA = 1
          DO 100 KA=1,I
          DO 100 KB=1,J(I)
                  Y(IA)=Y(KA,KB)
                  I=I+1
100       CONTINUE
-----remainder of test program
```

The test program also contains an example of the format for the output of the results.

Subroutine: ANOV2 (Y, N, NI, NJ, NK, NB, B, R, SS, NDF, SM, F, PF, $k) Page 8-7

Function:

This subroutine performs a 2-way crossed (factorial) analysis of variance.

Application:

Two-way analysis of variance is used to assess the effects of more than one factor assumed to have fixed effects. The ANOVA model specified here also assumes a complete design with all combinations of treatments present. The results provide information about the significance of the main effects and their interaction. Note: the subroutine is also

limited to performing analyses with an equal number of replicates in each treatment combination. (Section IV.2.5.2)

Requirements of the driver program:

The driver program must input the data into array X in a manner similar to the ANOV1 subroutine. All of the values for the first level of the first factor and the first level of the second factor are the first set of numbers in the Y array. The next set of numbers is for the first level of the first factor and the second level of the second factor. The pattern is continued until all levels of the second factor are completed. Then the array continues in that pattern with the second through N levels of the first factor.

The driver program must also input the total number of data points as N, the number of levels in the first factor as NI, the number of levels in the second factor are NJ, and the number of replicates in each treatment combination as NK. The outputs of the subroutine are the number of estimated effects (NB) and the means of the main effects levels, the interaction means and the overall mean (all in the array B). The residuals for all estimated effects are in the array R. The sum of squares, degrees of freedom, mean squares, F-statistics, and probability of F are in arrays SS, NDF, SM, F and PF respectively. The STAT-PACK manual explains the organization of all of these arrays. An overflow return statement number must also be included in the main program.

The test program also illustrates how the output format may be handled. Note: If the replication level is 1, then the ANOVA table is different because no true error term would exist, and the interaction term must be used to test for significant differences in main effect means.

Subroutines: RESTEM (X, N, NP1, MAXN, MAXNP1, W, IW, EFIN, EFOUT, XBAR, A, SIG, CONST, NVAR, FLEVEL, SY, NOIN, IVAR, B, SB, R, IND) Page 9-1

Function:

Performs stepwise multiple regression.

Application:

Stepwise multiple regression can be used to establish predictive relationships between a dependent variable (e.g., a biological attribute) and a set of independent variables (e.g. from the physical environment). The relative importance of the independent variables in explaining variation in the dependent variable is determined in stepwise multiple regression through the order of entry of variables into the model. Post-impact observations can be tested for significance of departure from predicted values. (Section IV 2.5.3)

Requirements of the driver program:

The driver program must provide two kinds of information to the RESTEM subroutine. It must input the data and their attributes and also the control specifications for the regression model. The data for multiple regression is inputted into a 2-dimensional X array. The row dimension is determined by the number of observations and the column dimension is equal to the number of independent variables plus the dependent variable. The number of observations is inputted as N and the number of variables (dependent and independent) is inputted as NP1. The maximum dimension of N and NP1 are inputted as MAXN and MAXNP1 respectively. An array of weighting factors for the observations maybe inputted in array W. IW controls the use of the weighting factors. The uses of W and IW are explained in the STAT-PACK manual. The regression model control variables include F-criteria for the entry and removal of each variable. These values are inputted as EFIN and EFOUT. The output of the RESTEM subroutine includes the array of weighted variable means (XBAR), the matrix of sums of squares, correlation coefficients and inverse matrix for all variables (A), and the array of standard deviations for the variables (SIG).

For each step in the stepwise multiple regression procedure, the following information may be obtained from the subroutine: (1) the

constant term of the regression model, CONST; (2) which variable was entered or removed in that step, NVAR; (3) the F-statistic for the model, FLEVEL; (4) the standard error of Y, SY; (5) the number of variables in the current regression model, NOIN; (6) an array of values showing the subscripts of variables in the regression, IVAR; (7) the regression coefficent, B; (8) the standard error of the regression coefficients, SP; (9) and residuals from the predicted values of Y, R.

The subroutine provides for control of the number of steps that are to be performed by way of the variable IND. The STAT-PACK manual provides an explanation of the use of IND. Since the amount of information available at each step is large, the user may not want to print out the complete results of every step. The test program in the STAT-PACK manual prints some of the descriptive statistics (e.g. correlation coefficient) in the first step, while for the remaining steps, the F-values, regression coefficients, variables included in the model, variable entered and removed in the particular step, and the test statistics are printed. After the final step, the residuals of the predicted results are printed. Control of the output of the regression subroutine can be obtained by adding or removing variables from the WRITE statements in the test program. The test program is also set up with control cards and the data is inputted as a two dimensional data set with the variable for each observation on one record or set of records.

Subroutine: DISCRA (X, NVAR, NPTS, NCLS, XTEST, NTEST, XBAR, S, DET, AZERO, ALIN, PROB, JC, MAXVAR, MAXPTS, MAXCLS, MAXTST, $k) Page 11-25

Function:

This subroutine performs a classification of ungrouped multivariate observations into defined groups using a discriminant analysis function to determine the classfication probabilities.

Application:

The DISCRA subroutine can be used to classify ungrouped observations. If several sets of pre-impact, multivariate observations (e.g. biological collections) were used to form defined groups (i.e. based on time or location) then the classification of post-impact observations can be attempted. The resulting pattern of misclassifications, if any, can be interpreted in the perspective of impact assessment. It should be noted that a complete set of measurements must be obtained for each multivariate observation if it is to be included in the analysis. (Section IV.2.4.5)

Requirements of the driver program:

Similar to the multiple regression procedure, the driver program must input the data and its attributes. The data is inputted as a three dimensional array called X. The first dimension of X is the number of variables, the second dimension of X is the number of observations per class (group) and the third dimension is the number of classes (or defined groups). While the number of variables for each class is constant, the number of observations per class may differ. The number of variables is inputted as NVAR and the number of classes (defined groups) is inputted as NCLS. The number of observations per class is inputted in the array NPTS. The ungrouped observations are inputted into the two dimensional array called XTEST. The row dimension of this array is the number of test observations (input as NTEST), and the numbers of variables determine the number of columns in the matrix. The maximum dimensions of the X array and XTEST array must be inputted as MAXVAR for the maximum number of variables, MAXPTS for the maximum number of observations per class, MAXCLS for the maximum number of classes and MAXTST for the maximum number of test observations. The main program must also have an error overflow return statement number.

The output of the DISCRA subroutine includes descriptive statistics for the variables and defined groups, the coefficients and constant terms of the classification equations, and the classfication probabilities of the ungrouped (test) points. The means by variable and class are outputted

in the array XBAR. The variance-covariance matrix by class resides in the three dimensional array S. The natural logarithm of determinants of these arrays are in the DET array. The constants and coefficients of the classification equations are in arrays AZERO and ALIN, respectively. Each class has its own constant and set of coefficients. The probability of classification of each test (ungrouped) observation is outputted as array PROB. The rows of PROB are the observations and the columns of PROB are the defined groups.

The output of all of these items is optional and should be selected by the user. The minimum information needed for interpretation are the classification probabilities for the ungrouped observations. A summary of the classification frequencies may be generated from the PROB array with the following statements.

```
C     DIMENSION IGP and PGP to NCLS Values
           DO 501 KA = 1,NTEST
           MAXP = 0.0
           DO 502 KB =1,NCLS
           MAXP = AMAXI(MAXP,PROB(KA,KB))
502        CONTINUE
           DO 503 KC = 1,NCLS
           IF(PROB(KA,KC).EQ.MAXP) IGP(KC) = IGP(KC)+1
           IF(PROB(KA,KC).EQ.MAXP) WRITE(6,1100) KA,PROB(KA,KC),KC
1100       FORMAT(5HOBS#=,I2,6HPROB.=,F5.3,6HGROUP=,I2)
503        CONTINUE
501        CONTINUE
           DO 504 KD=1,NCLS
           PGP(KD)=((100.0*IGP(KD))/NTEST
           WRITE(6,1200) KD,IGP(KD),PGP(KD)
1200       FORMAT(6HGROUP#,I2,3X,12HNO.IN GROUP,
      1    I4,3X,17H PERCENT OF OBS =, F6.2)
504        CONTINUE
           WRITE (6,1300)
1300       FORMAT(60HIF THE TOTAL OF PERCENTAGES IS
      2    GREATER THAN 100, ONE OR MORE/37H SAMPLES HAS
           EQUAL MAX.  PROBABILITIES.)
```

The test program in the STAT-PACK manual is set up to read control cards and a sequential data format. The sequential data assume that all variables from an observation are on one record or set of records. The observations are arranged by group with group 1 data being first in the sequence, followed by the group 2 data and so forth. The ungrouped observations follow the groups in a format similar to that for the

groups. If the data were not sorted, then array positions could be determined from code values on the records. Each observation would have to have its group number (including an ungrouped code) and observation number within groups.

It should be noted that the DISCRA subroutine uses the GJR subroutine from the MATH-PACK library on the UNIVAC Computer. This will not affect the driver program, but it may require changes in the job control commands during the execution of the program.

### VI.2.3 Other Subprograms Relevant to the Analysis Plan

Several other subroutines in STAT-PACK could be utilized to implement other parts of the data analysis plan discussed in Section IV.

Analysis of covariance (ANCOVA) is a useful hypothesis testing procedure (Section IV.2.5.4), and is available in STAT-PACK subroutine ANOCO. ANCOVA can be used to test the importance of uncontrollable sources of variation on group means. However, the subroutine ANOCO requires the entry of several transposed matrices and does not appear simple to use.

Bivariate measures of association (Section IV.2.3) such as correlation and Chi-square can be obtained from STAT-PACK subroutines CORAN, and CHI21S respectively. Factor analysis (Section IV.2.4.4) is available in the FACTAN subroutine. Different models of analysis of variance (Section IV.2.5.2) such as 3-way factorial, split plots and nested designs are discussed in subroutines in Section 8 of the STAT-PACK system. Time series techniques, which are useful in analyzing patterns in temporally sequential data, are discussed in Section 10 of the STAT-PACK manual.

## VI.3 Project Generated Software

Although the UNIVAC STAT-PACK is a fairly comprehensive software library, there are several statistical tools which are germane to Task IV and which have been utilized in the analysis plan that are not available in STAT-PACK. SAI has written several FORTRAN subroutines and

programs to increase the utility of STAT-PACK to perform the statistical tests set forth in the analysis plan. The nine subroutines written by SAI and discussed in detail in Appendix B are:

| Name | Description |
|---|---|
| STDERR | Standard error of the mean |
| CUMMN | Cummulative means vs. number of replicates |
| WTEST | Shapiro - Wilk test of normality |
| IDXDIS | Index of disperson |
| MORISIT | Morrsita's index of dispersion |
| TRANS | Data transformations |
| COCR | Cochran's test for homogeneity of variance |
| QTEST | Burr-Foster Q-test for homogeneity of variance |
| FMAX | Hartley's $F_{max}$ test for homogeneity of variance |

The subroutines follow the design and argument naming conventions used in STAT-PACK. The subroutines are completely independent of any specific input or output device since input and output variables required by a subroutine are passed as arguments. Variable dimensions are used in argument arrays to permit the subroutines to be called from any program and to conserve memory. By using the same argument naming conventions as are used in the STAT-PACK subroutines, the user of STAT-PACK can more easily incorporate the SAI subroutines into the analysis system.

One self-contained program is also discussed in Appendix B. This program, SMPNUM, calculates the number of samples required for given Type I and Type II error levels.

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# APPENDIX A

# EXEMPLARY ANALYSIS

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# APPENDIX A
# EXEMPLARY ANALYSIS

## A.1 INTRODUCTION

In this section, a structured exemplary analysis is presented to demonstrate the utility of the analysis protocols delineated in this report. Unfortunately, none of the data sets in the Bryan Mound data base are in themselves sufficient to display all aspects of the analysis system. The major limitations are as follows:

- Data for phytoplankton and zooplankton studies were unavailable at the time these analyses were performed.
- The integrity of the individual replicate samples of macrobenthos has not been maintained. As received from NODC, the data for the individual replicates (3) have been summed for each species at each cruise/station stratum. This limitation disallows assessment of within-station variability for these data and limits its utility for quantitative impact assessment.
- For a portion of the demersal nekton data set in the Project Data Base (July - December 1978) only fourteen species were enumerated, with the remaining taxa being only qualitatively assessed. However, individual trawls (replicates) are distinguishable, allowing these data to be utilized for calculation of basic descriptive statistics and hypothesis testing.
- Neither of the above studies involved the collection of sufficient ancillary environmental data (esp. sediment parameters) for analysis of covariance or multiple regression analysis. In fact, no ancillary hydrographic data were collected with the nekton samples.

Based on the above considerations, the following plan was developed for exemplary analysis:

(1) The demersal nekton data set for the period July 1978 - April 1979 would serve as the main data base.

(2) Where these data were insufficient for exemplary analysis, Texoma data (megabenthos and sediment chemistry) are utilized.

Locations of sampling stations for the Bryan Mound study site were presented earlier in this document (Figures 3 and 4). The Texoma study sites and stations are shown in Figure A1.

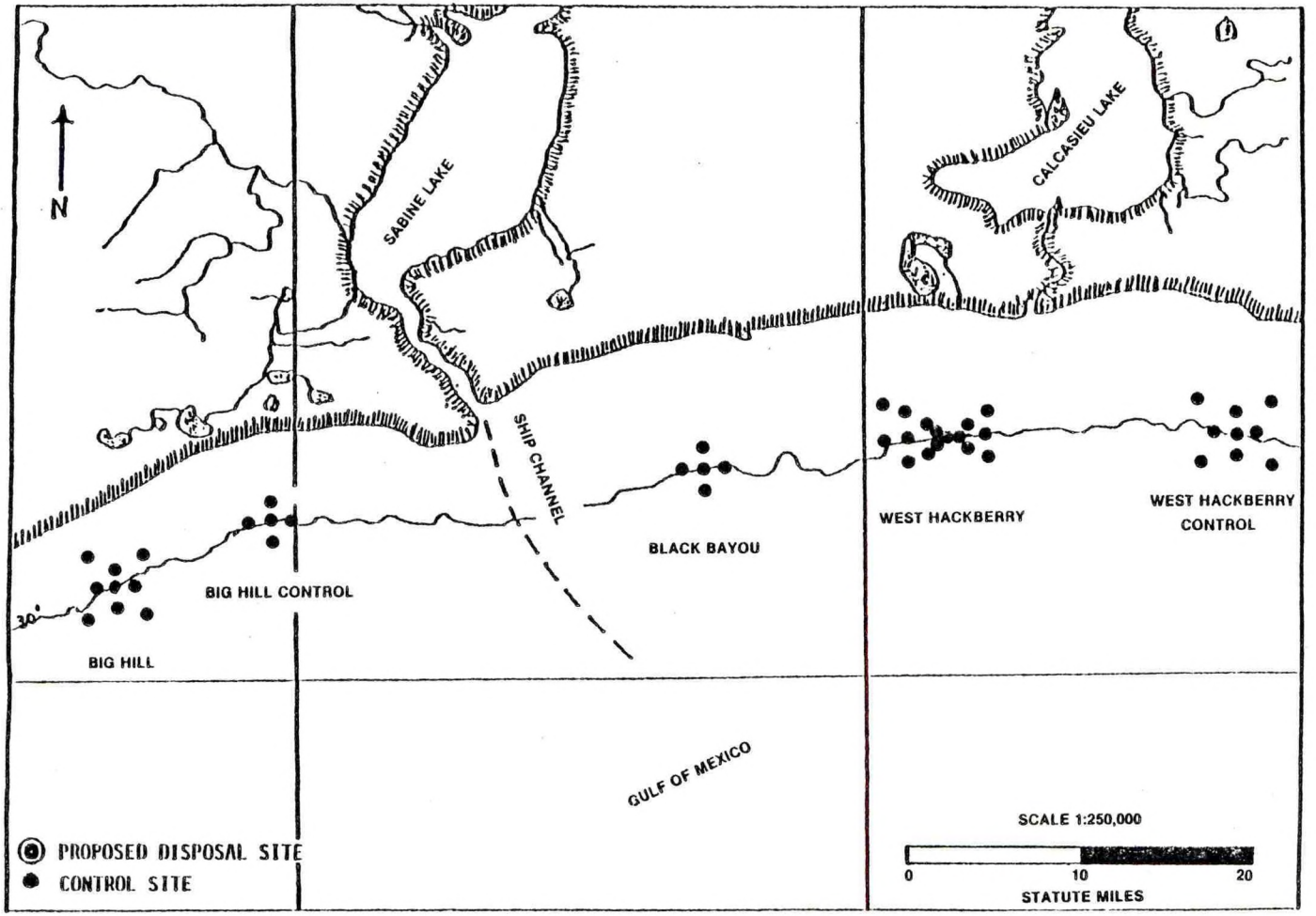


**Figure A1. Approximate location of Texoma benthic study sites and stations.**

For the Bryan Mound demersal nekton data base, two data sets were utilized in these exemplary analysis.

(1) For exploratory analysis, calculation of basic descriptive statistics and discriminant function analysis, the data set utilized included two sets of twenty-four trawl samples collected in April 1979. The first collection, occurring on April 5, 1979, was conducted at night, while the later collection, occurring on April 20, 1979, was conducted during daylight hours. It should, therefore, be remembered that any comparisons of data from these two cruises will involve consideration of diurnal as well as seasonal patterns. Because of the dynamic nature of the nearshore Gulf ecosystem, changes in the nekton community over a two week period (especially in the spring) can be significant.

(2) For calculation of bivariate measures of association, pattern and classification analysis, and hypothesis testing the daytime data set collected over five or six cruises from July 1978 through April 1979 was utilized. Separate analyses are presented for samples from the offshore diffuser area as well as for samples from all depths (3.5 to 25 fm).

It should also be remembered that the data utilized in this exemplary analysis are pre-discharge data and thus, no hypotheses testing concerning brine impacts can be conducted. What will be presented, therefore, is the path by which predisposal monitoring data can be utilized to:

- understand the basic processes inherent in the ecological system;
- define the major sources of variance in the system;
- determine the statistical nature of the data;
- determine predisposal temporal and spatial differences in biological parameters.

## A.2 EXPLORATORY ANALYSIS

In the exploratory phase of the analysis system we are attempting to summarize field data temporally, spatially and taxonomically and utilize this summary information as a guide to reducing the multidimensionality of the data set by reducing the number of variables (species) which will

be utilized in subsequent analyses. This summary information is used to describe the overall taxonomic composition of the samples and to determine the relationship of the number of species to the number of individuals. Since the data set will be taxonomically subsetted after exploratory analyses, it is in the exploratory phase that parameters such as diversity, evenness, total number of species, etc. are calculated and stored for use in descriptive statistics and hypotheses testing.

The most important data product in the exploratory analysis phase of the program is the relative composition table (Tables A1 to A3). Both mean percent composition and pooled percent composition are given in these tables. The differences in the results for the two types of percent composition depend on the evenness of the distribution of a particular taxa among samples, which may or may not be reflected by the frequency of occurrence. That is, two species may be present in all samples (frequency of occurrence equal to 1.00) but still show differences in mean and pooled percent composition due to different distributions among these samples. Species which are highly clumped in the samples will have higher pooled than mean percent compositions. The mean percent composition, by reducing each sample to a 0-100 (percent) scale, reduces the influence of outlier (in this case very high) values in one or several samples. Note that cumulative percent composition and cumulative abundance are also presented. These values are used to generate various descriptive curves discussed below.

Also presented on the percent composition tables is mean abundance for each taxa and some index of variation. In the case of Tables A1 to A3, the index of dispersion ($s^2/\bar{X}$) is used. By including these parameters, the transition from exploratory analysis to basic descriptive statistics has begun. Note that Tables A1 to A3 also include means and variances for number of taxa, number of individuals, diversity, and evenness. These parameters all require the entire species assemblage for calculation and this descriptive information is stored for later use.

**Table A1. Relative composition table for the demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site for April 5 1979 (night).**

| TAXON NAME | MEAN PERCENT COMPOSITION | CUMULATIVE PERCENT COMPOSITION | POOLED PERCENT COMPOSITION | FREQ. OF OCCURRENCE | CUMULATIVE ABUNDANCE | MEAN ABUNDANCE | INDEX OF DISPERSION |
|---|---|---|---|---|---|---|---|
| Penaeus aztecus | 22.34 | 22.34 | 14.42 | 0.96 | 194 | 8.1 | 4.07 |
| Cynoscion nothus | 13.67 | 36.01 | 31.15 | 0.67 | 613 | 17.5 | 95.40 |
| Syacium gunteri | 7.76 | 43.77 | 7.66 | 0.63 | 716 | 4.3 | 8.48 |
| Etropus crossotus | 6.84 | 50.61 | 7.29 | 0.71 | 814 | 4.1 | 6.34 |
| Penaeus duorarum | 6.64 | 57.25 | 5.28 | 0.88 | 885 | 3.0 | 7.57 |
| Cynoscion arenarius | 5.25 | 62.51 | 3.94 | 0.67 | 938 | 2.2 | 2.99 |
| Urophycis floridanus | 4.63 | 67.14 | 3.94 | 0.71 | 991 | 2.2 | 2.83 |
| Peprilus burti | 3.07 | 70.21 | 1.64 | 0.54 | 1013 | 0.9 | 1.32 |
| Anchoa hepsetus | 2.87 | 73.08 | 2.31 | 0.38 | 1044 | 1.3 | 4.81 |
| Urophycis cirratus | 2.87 | 75.95 | 2.23 | 0.42 | 1074 | 1.3 | 2.66 |
| Penaeus setiferus | 2.83 | 78.78 | 2.53 | 0.58 | 1108 | 1.4 | 1.65 |
| Trichiurus lepturus | 2.76 | 81.54 | 2.53 | 0.38 | 1142 | 1.4 | 5.21 |
| Halieutichthys caribbaeus | 2.58 | 84.13 | 1.86 | 0.50 | 1167 | 1.0 | 2.96 |
| Symphurus civitatus | 2.03 | 86.16 | 2.38 | 0.46 | 1199 | 1.3 | 4.41 |
| Centropristis philadelphicus | 1.89 | 88.05 | 1.86 | 0.54 | 1224 | 1.0 | 1.71 |
| Lepophidium graellsi | 1.44 | 89.49 | 1.19 | 0.46 | 1240 | 0.7 | 1.13 |
| Porichthys porosissimus | 1.31 | 90.80 | 0.89 | 0.38 | 1252 | 0.5 | 1.57 |
| Ogcocephalus sp | 1.13 | 91.93 | 0.37 | 0.21 | 1257 | 0.2 | 0.83 |
| Synodus foetens | 0.87 | 92.80 | 0.30 | 0.17 | 1261 | 0.2 | 0.87 |
| Bregmaceros atlanticus | 0.84 | 93.64 | 0.59 | 0.25 | 1269 | 0.3 | 1.22 |
| Brevoortia patronus | 0.84 | 94.48 | 0.22 | 0.08 | 1272 | 0.1 | 1.61 |
| Chloroscombrus chrysurus | 0.83 | 95.32 | 0.59 | 0.25 | 1280 | 0.3 | 1.48 |
| Archosargus probatocephalus | 0.83 | 96.15 | 0.67 | 0.17 | 1289 | 0.4 | 2.04 |
| Sphoeroides parvus | 0.80 | 96.95 | 0.67 | 0.21 | 1298 | 0.4 | 1.81 |
| Serraniculus pumilio | 0.71 | 97.66 | 0.89 | 0.29 | 1310 | 0.5 | 2.09 |
| Symphurus plagiusa | 0.26 | 97.92 | 0.37 | 0.08 | 1315 | 0.2 | 3.34 |
| Lutjanus campechanus | 0.25 | 98.16 | 0.07 | 0.04 | 1316 | 0.0 | 1.00 |
| Harengula pensacolae | 0.24 | 98.41 | 0.15 | 0.08 | 1318 | 0.1 | 0.96 |
| Orthopristis chrysoptera | 0.23 | 98.63 | 0.15 | 0.04 | 1320 | 0.1 | 2.01 |
| Monacanthus hispidus | 0.19 | 98.82 | 0.15 | 0.08 | 1322 | 0.1 | 0.96 |
| Anchoa mitchilli | 0.16 | 98.98 | 0.15 | 0.08 | 1324 | 0.1 | 0.96 |
| Ophidion welshi | 0.15 | 99.13 | 0.15 | 0.08 | 1326 | 0.1 | 0.96 |
| Ancylopsetta quadrocellata | 0.14 | 99.27 | 0.15 | 0.08 | 1328 | 0.1 | 0.96 |
| Hippocampus erectus | 0.13 | 99.40 | 0.07 | 0.04 | 1329 | 0.0 | 1.00 |
| Diplectrum bivittatum | 0.13 | 99.53 | 0.15 | 0.08 | 1331 | 0.1 | 0.96 |
| Prionotus salmonicolor | 0.11 | 99.64 | 0.07 | 0.04 | 1332 | 0.0 | 1.00 |
| Citharichthys spilopterus | 0.11 | 99.75 | 0.45 | 0.08 | 1338 | 0.3 | 3.22 |
| Prionotus tribulus | 0.10 | 99.85 | 0.22 | 0.13 | 1341 | 0.1 | 0.91 |
| Raja texana | 0.06 | 99.91 | 0.07 | 0.04 | 1342 | <0.1 | 1.00 |
| Paralichthys lethostigma | 0.05 | 99.97 | 0.07 | 0.04 | 1343 | <0.1 | 1.00 |
| Scorpaena calcarata | 0.02 | 99.98 | 0.07 | 0.04 | 1344 | <0.1 | 1.00 |
| Gymnothorax nigromarginatus | 0.02 | 100.00 | 0.07 | 0.04 | 1345 | <0.1 | 1.00 |

| SAMPLE SUMMARY: 24 REPLICATES | TAXA | INDIVIDUALS | DIVERSITY | EVENNESS |
|---|---|---|---|---|
| MEAN | 13. | 56. | 2.036 | 0.835 |
| VARIANCE | 20. | 3536. | 0.093 | 0.013 |

**Table A2. Relative composition table for the demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site on April 20 1979 (day) collection.**

| TAXON NAME | MEAN PERCENT COMPOSITION | CUMULATIVE PERCENT COMPOSITION | POOLED PERCENT COMPOSITION | FREQ. OF OCCURRENCE | CUMULATIVE ABUNDANCE | MEAN ABUNDANCE | INDEX OF DISPERSION |
|---|---|---|---|---|---|---|---|
| Chloroscombrus chrysurus | 40.36 | 40.36 | 43.44 | 1.00 | 4482 | 186.8 | 125.41 |
| Cynoscion nothus | 14.68 | 55.04 | 15.40 | 0.92 | 6071 | 66.2 | 69.83 |
| Anchoa hepsetus | 11.68 | 66.72 | 9.61 | 1.00 | 7062 | 41.3 | 9.37 |
| Peprilus burti | 11.42 | 78.14 | 11.40 | 1.00 | 8238 | 49.0 | 32.94 |
| Trichiurus lepturus | 9.86 | 88.00 | 9.37 | 1.00 | 9205 | 40.3 | 16.76 |
| Harengula pensacolae | 3.60 | 91.60 | 3.26 | 1.00 | 9541 | 14.0 | 6.21 |
| Diplectrum bivittatum | 2.43 | 94.03 | 2.04 | 0.96 | 9751 | 8.8 | 5.24 |
| Syacium gunteri | 1.63 | 95.66 | 1.58 | 0.88 | 9914 | 6.8 | 7.04 |
| Opisthonema oglinum | 1.10 | 96.76 | 0.93 | 0.67 | 10010 | 4.0 | 6.26 |
| Cynoscion arenarius | 0.99 | 97.75 | 0.93 | 0.79 | 10106 | 4.0 | 4.46 |
| Etropus crossotus | 0.36 | 98.11 | 0.26 | 0.54 | 10133 | 1.1 | 1.65 |
| Penaeus aztecus | 0.33 | 98.44 | 0.28 | 0.42 | 10162 | 1.2 | 3.74 |
| Centropristis philadelphicus | 0.24 | 98.68 | 0.21 | 0.54 | 10184 | 0.9 | 1.51 |
| Citharichthys spilopterus | 0.18 | 98.87 | 0.17 | 0.42 | 10202 | 0.8 | 1.65 |
| Penaeus setiferus | 0.18 | 99.05 | 0.20 | 0.46 | 10223 | 0.9 | 1.82 |
| Anchoa mitchilli | 0.17 | 99.21 | 0.14 | 0.33 | 10237 | 0.6 | 1.93 |
| Urophycis floridanus | 0.14 | 99.35 | 0.12 | 0.29 | 10249 | 0.5 | 1.57 |
| Vomer setapinnis | 0.12 | 99.47 | 0.15 | 0.42 | 10264 | 0.6 | 1.50 |
| Lelostomus xanthurus | 0.06 | 99.53 | 0.07 | 0.21 | 10271 | 0.3 | 1.33 |
| Sphyraena guachancho | 0.05 | 99.58 | 0.03 | 0.13 | 10274 | 0.1 | 0.91 |
| Halieutichthys caribbaeus | 0.05 | 99.63 | 0.05 | 0.17 | 10279 | 0.2 | 1.25 |
| Penaeus duorarum | 0.05 | 99.68 | 0.05 | 0.13 | 10284 | 0.2 | 1.66 |
| Synodus foetens | 0.04 | 99.72 | 0.04 | 0.17 | 10288 | 0.2 | 0.87 |
| Prionotus tribulus | 0.04 | 99.76 | 0.05 | 0.17 | 10293 | 0.2 | 1.25 |
| Prionotus rubio | 0.04 | 99.79 | 0.04 | 0.13 | 10297 | 0.2 | 1.39 |
| Echeneis naucrates | 0.03 | 99.83 | 0.02 | 0.08 | 10299 | 0.1 | 0.96 |
| Rhizoprionodon terraenovae | 0.02 | 99.85 | 0.02 | 0.08 | 10301 | 0.1 | 0.96 |
| Scomberomorus maculatus | 0.02 | 99.86 | 0.02 | 0.08 | 10303 | 0.1 | 0.96 |
| Porichthys porosissimus | 0.02 | 99.88 | 0.02 | 0.08 | 10305 | 0.1 | 0.96 |
| Raja texana | 0.02 | 99.90 | 0.02 | 0.08 | 10307 | 0.1 | 0.96 |
| Lagodon rhomboides | 0.02 | 99.92 | 0.02 | 0.08 | 10309 | 0.1 | 0.96 |
| Caranx hippos | 0.01 | 99.93 | 0.01 | 0.04 | 10310 | <0.1 | 1.00 |
| Ogcocephalus sp | 0.01 | 99.94 | 0.01 | 0.04 | 10311 | <0.1 | 1.00 |
| Chaetodipterus faber | 0.01 | 99.95 | 0.01 | 0.04 | 10312 | <0.1 | 1.00 |
| Peprilus paru | 0.01 | 99.96 | 0.01 | 0.04 | 10313 | <0.1 | 1.00 |
| Sardinella anchovia | 0.01 | 99.97 | 0.01 | 0.04 | 10314 | <0.1 | 1.00 |
| Gymnothorax nigromarginatus | 0.01 | 99.98 | 0.01 | 0.04 | 10315 | <0.1 | 1.00 |
| Urophycis cirratus | 0.01 | 99.99 | 0.01 | 0.04 | 10316 | <0.1 | 1.00 |
| Lagocephalus laevigatus | 0.01 | 100.00 | 0.01 | 0.04 | 10317 | <0.1 | 1.00 |

| SAMPLE SUMMARY: 24 REPLICATES | TAXA | INDIVIDUALS | DIVERSITY | EVENNESS |
|---|---|---|---|---|
| MEAN | 15. | 430. | 1.648 | 0.620 |
| VARIANCE | 14. | 31355. | 0.124 | 0.013 |

**Table A3. Relative composition table for the demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site on April 5 (night) and April 20 (day) 1979 collections.**

| TAXON NAME | MEAN PERCENT COMPOSITION | CUMULATIVE PERCENT COMPOSITION | POOLED PERCENT COMPOSITION | FREQ. OF OCCURRENCE | CUMULATIVE ABUNDANCE | MEAN ABUNDANCE | INDEX OF DISPERSION |
|---|---|---|---|---|---|---|---|
| Chloroscombrus chrysurus | 20.60 | 20.60 | 38.50 | 0.63 | 4490 | 93.5 | 217.38 |
| Cynoscion nothus | 14.18 | 34.77 | 17.22 | 0.79 | 6498 | 41.8 | 88.07 |
| Penaeus aztecus | 11.34 | 46.11 | 1.91 | 0.69 | 6721 | 4.6 | 6.53 |
| Anchoa hepsetus | 7.27 | 53.39 | 8.76 | 0.69 | 7743 | 21.3 | 28.22 |
| Peprilus burti | 7.24 | 60.63 | 10.27 | 0.77 | 8941 | 25.0 | 55.32 |
| Trichiurus lepturus | 6.31 | 66.94 | 8.58 | 0.69 | 9942 | 20.9 | 34.52 |
| Syacium gunteri | 4.69 | 71.64 | 2.28 | 0.75 | 10208 | 5.5 | 7.72 |
| Etropus crossotus | 3.60 | 75.24 | 1.07 | 0.63 | 10333 | 2.6 | 6.07 |
| Penaeus duorarum | 3.34 | 78.58 | 0.65 | 0.50 | 10409 | 1.6 | 8.25 |
| Cynoscion arenarius | 3.12 | 81.70 | 1.28 | 0.73 | 10558 | 3.1 | 4.12 |
| Urophycis floridanus | 2.38 | 84.09 | 0.56 | 0.50 | 10623 | 1.4 | 3.10 |
| Harengula pensacolae | 1.92 | 86.01 | 2.90 | 0.54 | 10961 | 7.0 | 13.06 |
| Penaeus setiferus | 1.51 | 87.52 | 0.47 | 0.52 | 11016 | 1.1 | 1.75 |
| Urophycis cirratus | 1.44 | 88.96 | 0.27 | 0.23 | 11047 | 0.6 | 3.13 |
| Halieutichthys caribbaeus | 1.32 | 90.27 | 0.26 | 0.33 | 11077 | 0.6 | 2.90 |
| Diplectrum bivittatum | 1.28 | 91.55 | 1.82 | 0.52 | 11289 | 4.4 | 9.43 |
| Centropristis philadelphicus | 1.06 | 92.62 | 0.40 | 0.54 | 11336 | 1.0 | 1.59 |
| Symphurus civitatus | 1.02 | 93.63 | 0.27 | 0.23 | 11368 | 0.7 | 5.00 |
| Lepophidium graellsi | 0.72 | 94.35 | 0.14 | 0.23 | 11384 | 0.3 | 1.45 |
| Porichthys porosissimus | 0.67 | 95.02 | 0.12 | 0.23 | 11398 | 0.3 | 1.60 |
| Ogcocephalus sp | 0.57 | 95.59 | 0.05 | 0.13 | 11404 | 0.1 | 0.90 |
| Opisthonema oglinum | 0.55 | 96.14 | 0.82 | 0.33 | 11500 | 2.0 | 8.17 |
| Synodus foetens | 0.45 | 96.59 | 0.07 | 0.17 | 11508 | 0.2 | 0.85 |
| Bregmaceros atlanticus | 0.42 | 97.01 | 0.07 | 0.13 | 11516 | 0.2 | 1.36 |
| Brevoortia patronus | 0.42 | 97.43 | 0.03 | 0.04 | 11519 | 0.1 | 1.62 |
| Archosargus probatocephalus | 0.42 | 97.85 | 0.08 | 0.08 | 11528 | 0.2 | 2.19 |
| Sphoeroides parvus | 0.40 | 98.25 | 0.08 | 0.10 | 11537 | 0.2 | 1.96 |
| Serraniculus pumilio | 0.35 | 98.61 | 0.10 | 0.15 | 11549 | 0.3 | 2.30 |
| Anchoa mitchilli | 0.16 | 98.77 | 0.14 | 0.21 | 11565 | 0.3 | 1.96 |
| Citharichthys spilopterus | 0.15 | 98.92 | 0.21 | 0.25 | 11589 | 0.5 | 2.13 |
| Symphurus plagiusa | 0.13 | 99.04 | 0.04 | 0.04 | 11594 | 0.1 | 3.38 |
| Lutjanus campechanus | 0.12 | 99.17 | 0.01 | 0.02 | 11595 | <0.1 | 1.00 |
| Orthopristis chrysoptera | 0.11 | 99.28 | 0.02 | 0.02 | 11597 | <0.1 | 1.98 |
| Monacanthus hispidus | 0.09 | 99.37 | 0.02 | 0.04 | 11599 | <0.1 | 0.98 |
| Ophidion welshi | 0.07 | 99.45 | 0.02 | 0.04 | 11601 | <0.1 | 0.98 |
| Ancylopsetta quadrocellata | 0.07 | 99.52 | 0.02 | 0.04 | 11603 | <0.1 | 0.98 |
| Prionotus tribulus | 0.07 | 99.59 | 0.07 | 0.15 | 11611 | 0.2 | 1.10 |
| Hippocampus erectus | 0.07 | 99.65 | 0.01 | 0.02 | 11612 | <0.1 | 1.00 |
| Vomer setapinnis | 0.06 | 99.71 | 0.13 | 0.21 | 11627 | 0.3 | 1.79 |
| Prionotus salmonicolor | 0.06 | 99.77 | 0.01 | 0.02 | 11628 | <0.1 | 1.00 |
| Raja texana | 0.04 | 99.81 | 0.03 | 0.06 | 11631 | 0.1 | 0.95 |
| Leiostomus xanthurus | 0.03 | 99.84 | 0.06 | 0.10 | 11638 | 0.1 | 1.45 |
| Paralichthys lethostigma | 0.03 | 99.87 | 0.01 | 0.02 | 11639 | <0.1 | 1.00 |
| Sphyraena guachancho | 0.03 | 99.89 | 0.03 | 0.06 | 11642 | 0.1 | 0.95 |
| Prionotus rubio | 0.02 | 99.91 | 0.03 | 0.06 | 11646 | 0.1 | 1.46 |
| Echeneis naucrates | 0.02 | 99.93 | 0.02 | 0.04 | 11648 | <0.1 | 0.98 |
| Gymnothorax nigromarginatus | 0.01 | 99.94 | 0.02 | 0.04 | 11650 | <0.1 | 0.98 |
| Rhizoprionodon terraenovae | 0.01 | 99.95 | 0.02 | 0.04 | 11652 | <0.1 | 0.98 |
| Scomberomorus maculatus | 0.01 | 99.96 | 0.02 | 0.04 | 11654 | <0.1 | 0.98 |
| Scorpaena calcarata | 0.01 | 99.97 | 0.01 | 0.02 | 11655 | <0.1 | 1.00 |
| Lagodon rhomboides | 0.01 | 99.97 | 0.02 | 0.04 | 11657 | <0.1 | 0.98 |
| Caranx hippos | 0.01 | 99.98 | 0.01 | 0.02 | 11658 | <0.1 | 1.00 |
| Peprilus paru | 0.00 | 99.99 | 0.01 | 0.02 | 11659 | <0.1 | 1.00 |
| Sardinella anchovia | 0.00 | 99.99 | 0.01 | 0.02 | 11660 | <0.1 | 1.00 |
| Chaetodipterus faber | 0.00 | 100.00 | 0.01 | 0.02 | 11661 | <0.1 | 1.00 |
| Lagocephalus laevigatus | 0.00 | 100.00 | 0.01 | 0.02 | 11662 | <0.1 | 1.00 |

| SAMPLE SUMMARY: 48 REPLICATES | | TAXA | INDIVIDUALS | DIVERSITY | EVENNESS |
|---|---|---|---|---|---|
| | MEAN | 14. | 243. | 1.842 | 0.727 |
| | VARIANCE | 18. | 52755. | 0.145 | 0.025 |

Taxonomic subsetting of the data set is a somewhat subjective process, and various criteria can be established to facilitate the subsetting. Examples of commonly employed criteria include:

- o all species greater than a certain percent composition;
- o all species occurring with a frequency greater than a certain level;
- o all species with a mean greater than a certain threshold value;
- o some combination of the above.

The data in Table A3 were utilized to determine the taxonomic subset for discriminant function analysis described below. In this case, the fifteen most abundant species were utilized. These fifteen species cumulatively accounted for 90% of the total number of individuals over both cruises and all stations.

Several other components of exploratory analysis are important in determining the relative composition of the samples across stations or cruises, and in determining the adequacy of the sampling design. Dominance diversity curves for the two April 1979 cruises are shown in Figure A2, where cumulative percent composition (based on pooled percent composition in Tables A1 and A2) is plotted vs. number of species. These two curves in Figure A2 are directly comparable since the number of samples was the same for both sets of collections. The results are interesting and show that the samples from the night collections in early April displayed more even abundance of a smaller number of taxa than the samples from the daytime cruise in late April. In fact, the late April samples showed that the first twenty-five percent of the species accounted for virtually all the individuals, with the last forty species being quite rare. The early April curve reaches an asymptote only after more than 50% of the species are accounted for. Information like this provides valuable insight into subsetting strategies.

Figure A3 includes plots for the cumulative number of species vs. the number of replicates for the April collections. In many monitoring

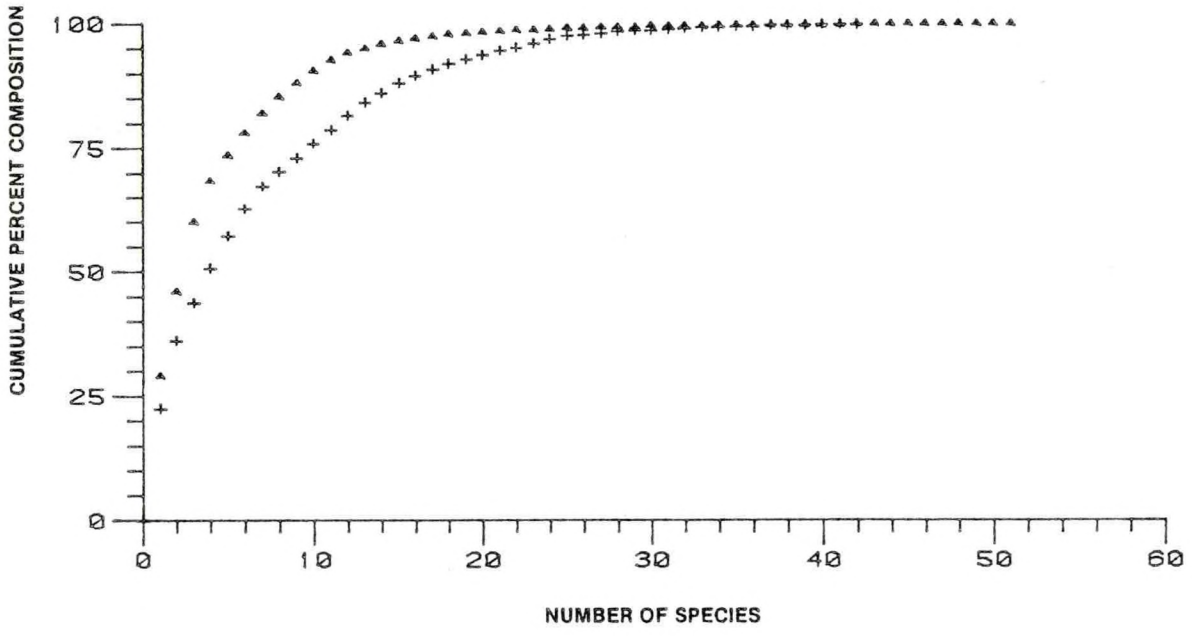


**Figure A2.** **Dominance diversity curves plotting cumulative percent composition vs. number of species for dominant demersal nekton species collected in trawl samples at the Bryan Mound offshore diffuser site on April 5th (plus) and 20th (triangle) 1979.**

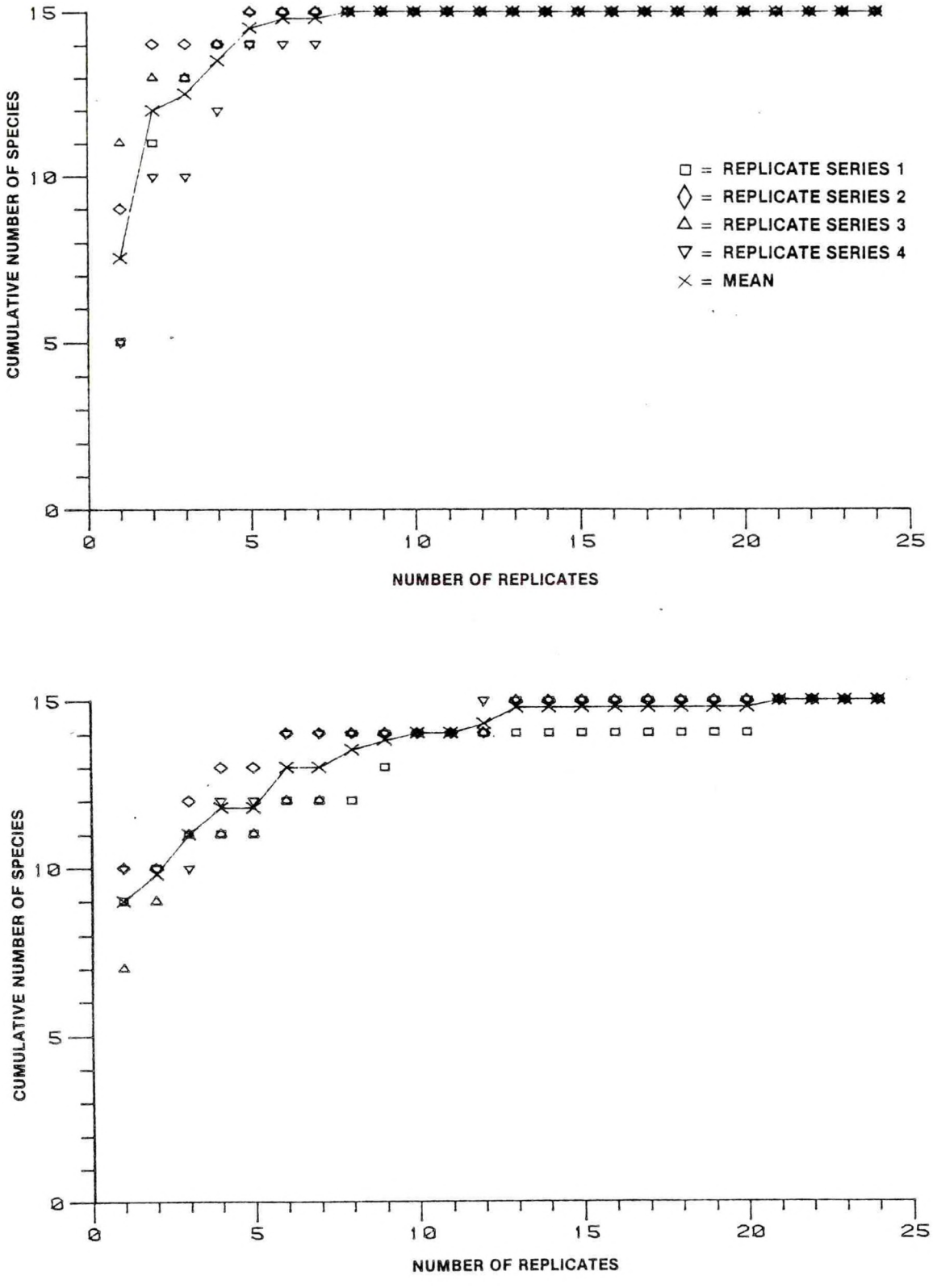


**Figure A3.** **Species area curves plotting cumulative number of species vs. number of replicates for demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site on April 5th (top) and April 20th (bottom) 1979.**

studies, these curves are utilized to determine the adequacy of intensity of sample replication. If the purpose of the study is to qualitatively describe the species assemblage, this technique is very valuable, but is of limited value in determining the adequacy of the data for statistical treatment of individual parameters (e.g. total number of individuals of the whole assemblage or of particular taxa). These problems are addressed below under basic descriptive statistics and hypothesis testing. Note in Figure A3 that for each level of replication (X axis) a series of values are plotted for cumulative number of species. The reason for this is that the shape of the curve will depend on the order that the replicates are entered. If, for example, the first sample plotted just happened to have a large number of species the curve would be very different than if another sample (with fewer species) was plotted first. The April 5, 1979 curve in Figure A3 shows a range of values for the first sample entered from five to eleven species. For this reason, four randomly selected sequences, and the mean for these four sequences are plotted, with the "average" curve (X's on Figure A3) used to determine the relationship of species to degree of replication. By plotting the random sequences as well as the mean, the change (i.e. decrease) in the range of the values of cumulative number of species with degree of replication can also be determined.

Finally, rarefaction curves (Figure A4) should also be routinely produced. These curves allow direct comparison of "diversity" among the same or analogous faunal groups collected in a similar manner from similar habitats, regardless of sample size. Figure A4 interpolates the number of species expected given the number of individuals in the sample. When compared to the results of the dominance diversity analysis (Figure A2) a very interesting trend is uncovered. While the number of species for the April 20 collection is less than that for the April 5 collection (Figure A2), for the same number of individuals collected (e.g. 1000) a greater number of species would be expected for the later collection (Figure A4). When compared to the calculated means for evenness (see Tables A1 and A2), where the April 5 collections had

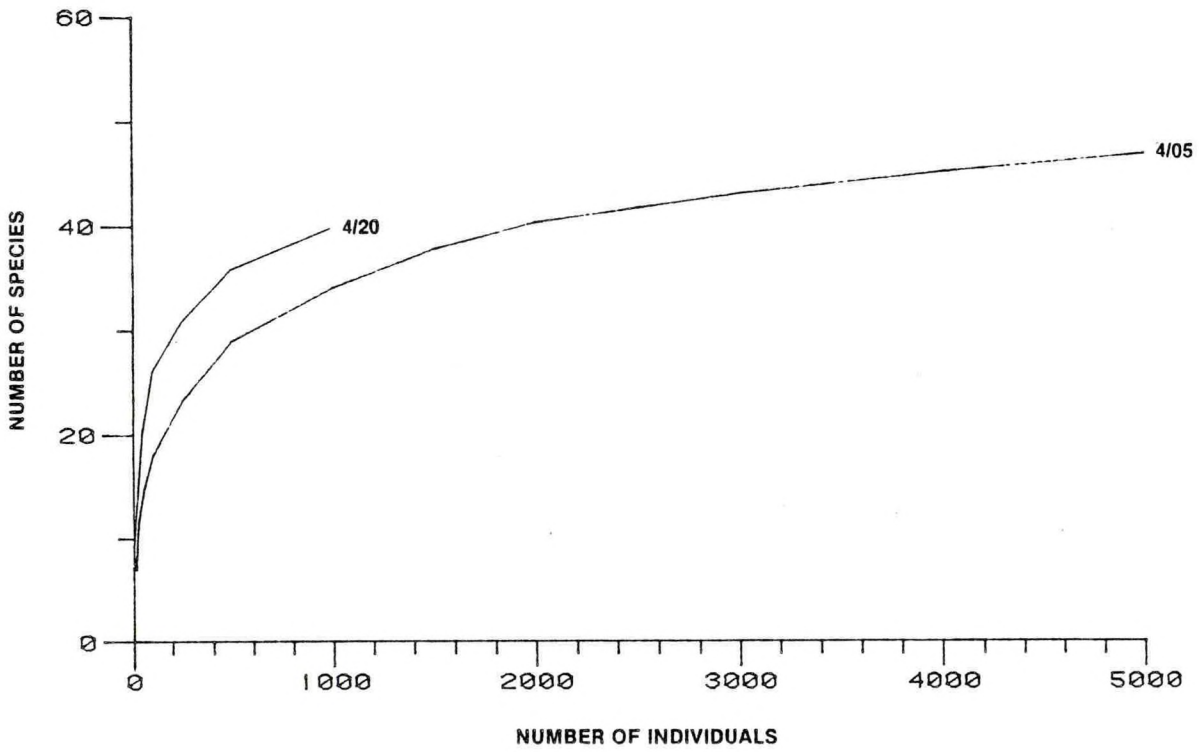


**Figure A4. Rarefaction curves for demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site on April 5th and April 20th 1979.**

the higher mean for both diversity and evenness, it becomes quite obvious that each type of exploratory analysis displays a different aspect of the data.

## A.3 BASIC DESCRIPTIVE STATISTICS

The emphasis of the exploratory analysis was on the community composition and its use in determining important variables for further analysis. In the basic descriptive statistics portion of the analysis scheme, the emphasis shifts from community composition to detailed characterization of specific variables (univariate emphasis) selected from the results of exploratory analysis. As described above, some of this work has already been accomplished (see Tables A1 to A3), with means and indices of variation calculated for numbers of individuals of each species, total number of individuals, total number of species, evenness, diversity, etc. That is, all parameters requiring the entire data set for calculation have been calculated before subsetting.

In the basic descriptive statistics component of the scheme, for those variables chosen for subsequent analysis, more detailed univariate statistics are calculated (Table A4), including range (maximum to minimum), skewness, kurtosis, and other parameters expressing variation of the particular variable at a station (or other set of replicates). This information helps identify outliers as well. Skewness and kurtosis values can be compared to tabular values to determine significant departures from the normal distribution.

Calculation of basic descriptive statistics is a very important prerequisite to hypothesis testing. It not only allows the assumptions of parametric hypothesis testing to be tested and transformations to be made if the data do not conform to the assumptions, but also gives initial direction regarding quantitative criteria for intensity of replication. Finally basic descriptive statistics can be utilized to determine univariate patterns in the spatial distribution of the organisms under consideration.

Table A4. **Basic descriptive statistics for selected variables (untransformed and log $_{10}$ transformed) for July 1978 nekton collections at the Bryan Mound offshore diffuser site.**

| VARIABLE | VALID N | SUM | MEAN | ST-DEV | MAXIMUM | MINIMUM | SKEWNESS | KURTOSIS |
|---|---|---|---|---|---|---|---|---|
| Number of individuals | 8 | 152.00 | 19.00 | 12.84 | 34.00 | 4.00 | 0.20 | -2.21 |
| Number of species | 8 | 23.00 | 2.88 | 0.64 | 41.00 | 2.00 | 0.07 | 0.74 |
| Number of *P. aztecus* | 8 | 66.00 | 8.25 | 3.69 | 14.00 | 3.00 | 0.21 | -0.85 |
| Number of *C. nothus* | 8 | 1.00 | 0.13 | 0.35 | 1.00 | 0.00 | 2.83 | 8.00 |
| $Log_{10}$ number of individuals | 8 | 9.63 | 1.20 | 0.33 | 1.54 | 0.70 | -0.29 | -1.58 |
| $Log_{10}$ number of species | 8 | 4.66 | 0.58 | 0.07 | 0.70 | 0.48 | -0.37 | 0.22 |
| $Log_{10}$ number of *P. aztecus* | 8 | 7.46 | 0.93 | 0.19 | 1.18 | 0.60 | -0.52 | -0.25 |
| $Log_{10}$ number of *C. nothus* | 8 | 0.30 | 0.04 | 0.11 | 0.30 | 0.00 | 2.83 | 8.00 |

Table A5. **Morisita's index and significance of departure from randomness for selected variables for demersal nekton collected at the Bryan Mound offshore diffuser site during two cruises in April 1979.**

| Sampling period | Variable | Morisita's index | Expected F | Tabular F |
|---|---|---|---|---|
| 4/05 | Number of individuals | 2.155 | 60.05 | 1.65 |
| 4/05 | Number of species | 0.974 | 0.74 | 1.65 |
| 4/05 | Number of *P. aztecus* | 1.365 | 4.02 | 1.65 |
| 4/05 | Number of *C. nothus* | 6.194 | 91.35 | 1.65 |
| 4/20 | Number of individuals | 1.174 | 72.29 | 1.65 |
| 4/20 | Number of species | 0.943 | 0.43 | 1.65 |
| 4/20 | Number of *P. aztecus* | 3.251 | 3.25 | 1.65 |
| 4/20 | Number of *C. nothus* | 1.997 | 68.68 | 1.65 |

One method of assessing the patchiness in the distribution of populations of organisms in a community (degree of nonrandomness) is to compare the variance and the mean. For a random distribution, the data should follow the Poisson distribution where the variance equals the mean. Tables A1 to A3 in the exploratory analysis section include a coefficient of dispersion, which is a variance to mean ratio. As can be seen most species have values greater than one indicating clumped distributions. A more concise summary of the spatial distribution of the "important" species in the Bryan Mound nekton community in April 1979 (as selected in the exploratory analysis phase) is the plot of log $s^2$ vs log $\bar{X}$ (Figure A5). Regression equations can be calculated based on these data. The coefficient of the independent variable can then be used to assess departures from randomness. In Figure A5, the coefficient of $\bar{X}$ is much higher than 1, again indicating departures from the random distribution for the community as a whole. If samples are collected at several stations in different sediment type/ depth strata (representing the range of conditions expected in the study area) differences in the degree of aggregation at these several strata can be assessed by statistically comparing the slopes of the log $s^2$/log $\bar{X}$ curves.

Plots of cumulative means vs. cumulative number of replicates are shown in Figure A6 for three commonly utilized biotic variables which might be expected to require considerably different numbers of replicates to attain the same precision of the estimate. These plots also demonstrate how erroneous the mean from just several samples can be. Again we are using the nekton data for April 5 and 20, 1979 at the Bryan Mound offshore diffuser site. Number of species, which might be expected to be the least variable of the three, is, in fact, quite consistent across all levels of replication, with a range of means of ~8.7 to 10.0. This indicates that relatively few samples would be needed for adequate quantitative characterization of number of species.

One might expect P. aztecus density to be more variable than those for total number of individuals of all species in the trawl samples, and, to

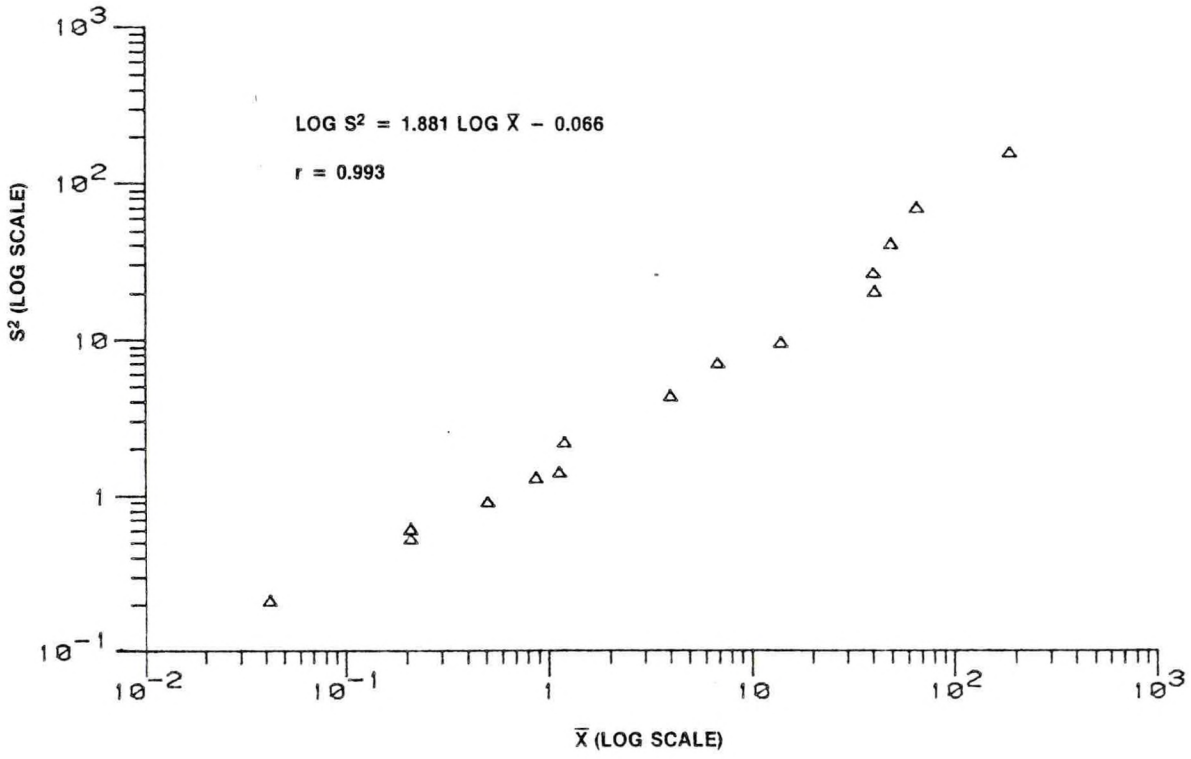


**Figure A5. Plot of log $s^2$ vs. log x for fifteen numerically dominant species of demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site on April 20 1979.**

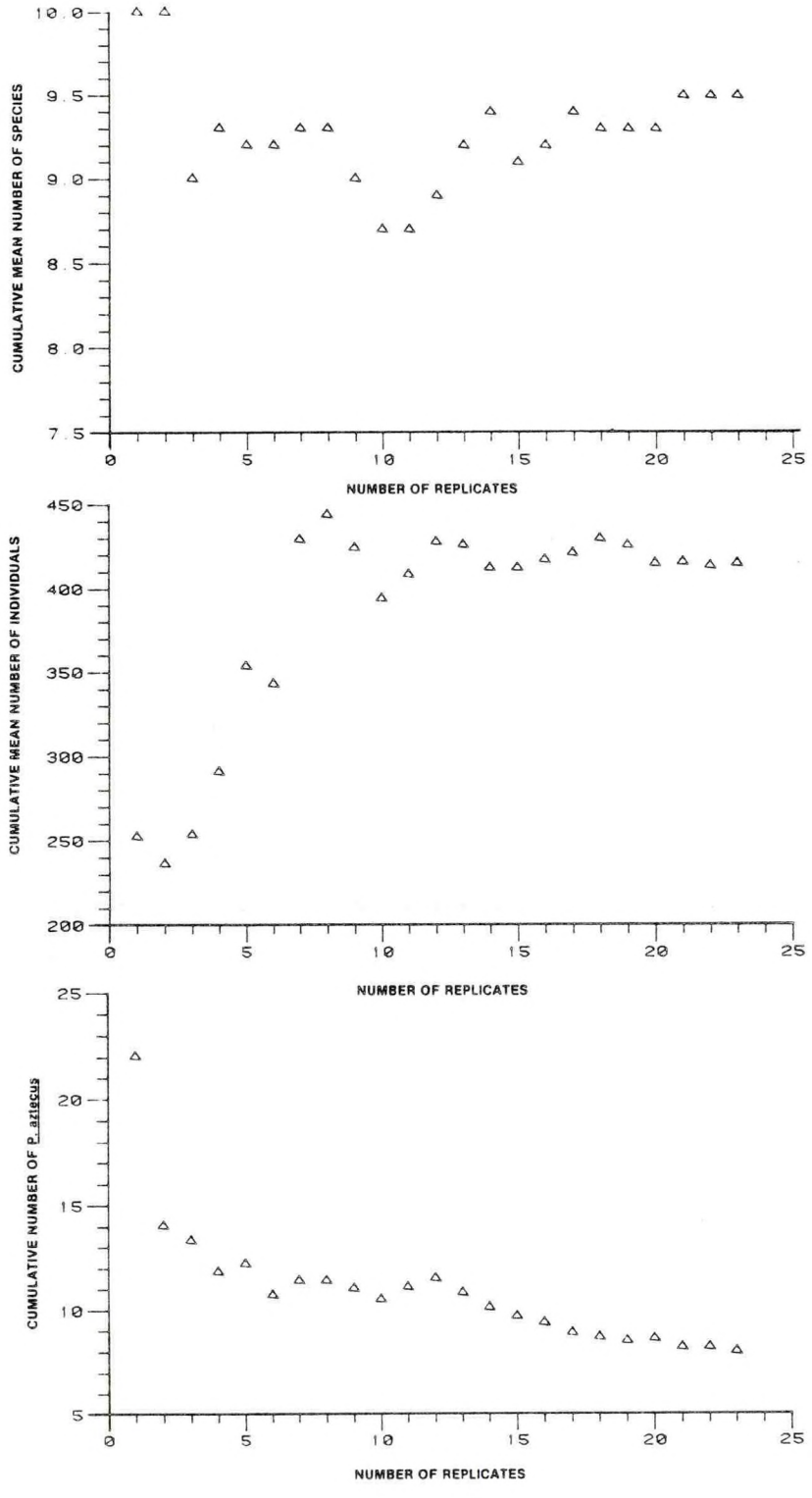


**Figure A6. Plots of cumulative means vs. number of replicates for demersal nekton collected in trawl samples at the Bryan Mound offshore diffuser site. Top - number of species, 4/20/79; middle - number of individuals, 4/20/79; bottom - number of Penaeus aztecus, 4/5/79.**

some degree, this is borne out by the plots (Figure A6). For total number of individuals, a plateau is reached on the seventh sample, with excellent repeatability after the twentieth replicate. For P. aztecus, the curve only begins to level off after the twentieth sample. The results, while they are not in themselves definitive, should be kept in mind when deciding on "how many replicates". The number of replicates desired will depend not only on the differences which we wish to be able to detect, but also on the variability of the particular parameter we are measuring. The topic is discussed further in the hypothesis testing portion of the analysis scheme (see Section 2.5.2).

Another important measure of non-randomness is Morista's Index (Morista 1959). This index, $I_\sigma$, is independent of the mean of the samples and will tend toward unity for random distribution and toward q for aggregated distributions (where q is equal to the number of samples). Values less than 1 indicate an even distribution. According to Morisita (1959) the departure from randomness can be tested by

$$F_o = \frac{I_\sigma (N - 1) + q - N}{q - 1}$$

$$\text{with } N = \sum_{i=1}^{q} n_i$$

$$F_o \text{ compared to } F_\infty^{q-1},$$

The results of the application of Morisita's Index to the Bryan Mound nekton data set is shown in Table A5. The results for the two sampling periods (April 5 and April 20) are quite similar. Numbers of individuals for the total community, and numbers of C. nothus and P. aztecus all exhibited significant aggregation. P. aztecus was much more evenly distributed than either of the other two biotic groupings. In contrast, the number of species showed a relatively even spatial distribution which, as can be seen from Table A5, was not significantly different from random.

Several of the assumptions of parametric hypothesis testing, including homogeneity of variance, normality, and additivity are tested under basic descriptive statistics. Table A6 shows the results of the application of three commonly employed tests of homogeneity of variance, the Bartlett-Box F, F-max test and Cochran's C, to untransformed and log transformed ($\log_{10}$ + 1) data. Two variables, total number of species/sample and total number of individuals/sample were used. For this analysis, data for eight replicate samples at the offshore diffuser site during five monthly cruises (July 1978 to April 1979) were utilized, with the homogeneity of variances computed across these seasonal groups of samples. The results (Table A6) clearly show that while these different tests sometimes give different results, the log transformations generally removed the heterogeneity of the variances. Therefore parameter hypothesis testing should be conducted on these transformed data ($\log_{10}$ + 1) rather than on the original untransformed data.

Results of the Shapiro-Wilk W test for non-normality are shown in Table A7 for selected variables for the same nekton data set, but in this case, all samples were pooled (over all cruises) to yield forty replicates. Results (Table A7) show that for these data normality is not a problem and that little difference is seen for untransformed and log transformed data.

Results of Tukey's test for nonadditivity are shown in Table A8. Because additivity involves the interaction of several class variables (e.g. cruise and station), the data set consisted of two replicate samples collected at four stations at the offshore diffuser site for five monthly cruises from July 1978 to April 1979. The results (Table A8) clearly show that for total number of individuals there is evidence for nonadditivity in the untransformed data, (at $\alpha = .05$, $F_{(1,11)} = 6.72$ compared to calculated F of 23.94) In fact, this calculated F exceeds the tabular $F_{(1,\ 11)}$ for $\alpha = .001$ (23.7). However, when the data are log transformed ($\log_{10}$+1), the nonadditivity disappears (F = 0.13).

Table A6. **Homogeneity of variance tests for total number of species and total number of individuals for demersal nekton data collected in trawl samples at the Bryan Mound offshore diffuser site during five daytime cruises from July 1978 to May 1979.**

TOTAL NUMBER OF INDIVIDUALS

COCHRANS C = MAX. VARIANCE/SUM(VARIANCES) = 0.5569, P = 0.004 (APPROX.)
BARTLETT-BOX F = 10.147, P = 0.000
MAXIMUM VARIANCE / MINIMUM VARIANCE = 154.128

$LOG_{10}$ TOTAL NUMBER OF INDIVIDUALS

COCHRANS C = MAX. VARIANCE/SUM(VARIANCES) = 0.3551, P = 0.324 (APPROX.)
BARTLETT-BOX F = 1.626, P = 0.165
MAXIMUM VARIANCE / MINIMUM VARIANCE = 7.735

TOTAL NUMBER OF SPECIES

COCHRANS C = MAX. VARIANCE/SUM(VARIANCES) = 0.7059, P = 0.000 (APPROX.)
BARTLETT-BOX F = 4.950, P = 0.001
MAXIMUM VARIANCE / MINIMUM VARIANCE = 15.130

$LOG_{10}$ TOTAL NUMBER OF SPECIES

COCHRANS C = MAX. VARIANCE/SUM(VARIANCES) = 0.4314, P = 0.083 (APPROX.)
BARTLETT-BOX F = 1.378, P = 0.239
MAXIMUM VARIANCE / MINIMUM VARIANCE = 4.950

Table A7. **Results of the Shapiro-Wilk's W test for non-normality for selected nekton variables collected in trawl samples at the Bryan Mound offshore diffuser site during five daytime cruises from July 1978 to May 1979.**

| Variable | Shapiro Wilks' W* |
|---|---|
| Number of individuals | 0.8247 |
| Number of species | 0.8211 |
| Number of *P. aztecus* | 0.7361 |
| Number of *C. nothus* | 0.5984 |
| $Log_{10}$ number of individuals | 0.9705 |
| $Log_{10}$ number of species | 0.9154 |
| $Log_{10}$ number of *P. aztecus* | 0.8262 |
| $Log_{10}$ number of *C. nothus* | 0.9066 |

* Values greater than 0.940 indicate nonnormality at alpha=0.05, n=40 samples.

**Table A8. Tukey's one degree of freedom test of non-additivity for total number of individuals (untransformed and $\log_{10}$ transformed data) collected at four stations at the Bryan Mound offshore diffuser site during five cruises from July 1978 to April 1979.**

$$Q = \sum^{a} Q_i \, (n\bar{Y}_A - \bar{\bar{Y}})$$

$$K = [\sum^{a} (n\bar{Y}_A - \bar{\bar{Y}})^2] \times [\sum^{b} (n\bar{Y}_B - \bar{\bar{Y}})^2]$$

SS nonadditivity = $Q^2/kn$

SS residual = SS interaction - SS nonadditivity

$F_s = M_s$ nonadditivity/$M_s$ residual

(see Section IV.2.1 for additional explanation)

Number of individuals

Q = 2043448.9
K = 147394290
SS nonadd. = 19010

| Source | df | SS | MS |
|---|---|---|---|
| (from SPSS) A x B | 12 | 38256 | |
| nonadditivity | 1 | 19010 | 19010 |
| residual | 11 | 19246 | 1749.6 |

$F_s = 10.86$, $F_{(1,11),\ \alpha = 0.05} = 6.72$

nonadditivity is significant.

$\log_{10}$ number of individuals

Q = 0.07928

K = 0.19655

SS nonadd. = 0.01598

| Source | df | SS | MS |
|---|---|---|---|
| (from SPSS) A x B | 12 | 1.110 | |
| nonadditivity | 1 | 0.01598 | 0.01598 |
| residual | 11 | 1.0940 | 0.09945 |

$F_s = 0.16076$, $F_{(1,11),\ \alpha = 0.05} = 6.72$

nonadditivity is not significant.

In comparing these results for nonadditivity with those obtained earlier for homogeneity of variance, a major and well-recognized trend emerges. Very often, a transformation that will solve one problem (e.g., heterogeneity of variance) will solve other problems (e.g., nonadditivity) as well.

## A.4 BIVARIATE MEASURES OF ASSOCIATION

Because very little ancillary environmental data were collected in the Bryan Mound nekton studies, exemplary bivariate measures of association are restricted to species - species correlations (R-mode). An example of a common data product associated with bivariate analyses is the correlation matrix (Table A9). In this case, the correlation matrix consists of all the simple bivariate correlations between eleven species of demersal nekton at the offshore diffuser site. This six cruise (July 1978 to April 1979 ) data set is essentially the same data set used in earlier analyses, with the addition of one more cruise (October). October was excluded from some other analyses because it was missing one sample and this would have introduced an unnecessary level of complexity to certain aspects of this exemplary exercise. The correlation matrix in Table A9 is relatively small compared to most resulting from community analyses. More species would have been included, but for much of the time period covered, there were only fourteen species being enumerated in the Bryan Mound study. Of these, two species (Stellifer lanceolatus and Menticirrhus littoralis) were not collected during the daytime cruises at the offshore site and P. duorarum occurred in low numbers.

Of special note in Table A9 are several correlations greater than 0.70. These include P. burti x Trichiurus lepturus (0.71), and those between all possible combinations of Arius felis, Polydactylus octonemus and Micropogon undulatus. Other species highly correlated with this trio include P. aztecus and M. americanus (all correlations for both species with members of the trio are greater than 0.45). However, the correlation between P. aztecus and M. americanus was not significant ($\alpha = 0.05$).

**Table A9. Correlation matrix for important demersal nekton species collected in trawl samples at the Bryan Mound offshore diffuser site during six daytime cruises from July 1978 to April 1979.**

| | *Peprilus burti* | *Trichiurus lepturus* | *Stenotomus caprinus* | *Penaeus setiferus* | *Cynoscion arenarius* | *Cynoscion nothus* | *Penaeus aztecus* | *Menticirrhus americanus* | *Polydactylus octonemus* | *Arius felis* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Micropogon undulatus* | 0.0240 | -0.0587 | -0.0312 | -0.1836 | 0.0581 | 0.1922 | 0.5255 | 0.4612 | 0.7354 | 0.7585 |
| *Arius felis* | -0.0681 | -0.0227 | 0.0200 | -0.0582 | 0.1926 | 0.2645 | 0.4681 | 0.5136 | 0.7322 | |
| *Polydactylus octonemus* | -0.1030 | -0.0226 | -0.0187 | 0.0214 | 0.2644 | 0.2655 | 0.4569 | 0.5883 | | |
| *Menticirrhus americanus* | -0.0420 | -0.0479 | -0.0315 | -0.0450 | 0.0416 | 0.1867 | 0.1960 | | | |
| *Penaeus aztecus* | -0.3701* | -0.2960 | 0.2775 | 0.1005 | 0.2197 | 0.1496 | | | | |
| *Cynoscion nothus* | -0.0943 | 0.1941 | -0.5305 | 0.5316 | 0.6931 | | | | | |
| *Cynoscion arenarius* | -0.0279 | 0.3658 | -0.2675 | 0.4780 | | | | | | |
| *Penaeus setiferus* | -0.2773 | -0.2811 | -0.3505 | | | | | | | |
| *Stenotomus caprinus* | 0.0510 | -0.1866 | | | | | | | | |
| *Trichiurus lepturus* | 0.7078 | | | | | | | | | |

* Those correlations underlined are significant at $\alpha = 0.05$

Another identifiable group included P. setiferus, C. arenarius and C. nothus, with all correlations between these taxa being 0.48 or greater, and the correlation between the two species of sea trout being especially high (0.69). The only negative correlation greater than 0.40 was between S. caprinus and C. nothus. P. aztecus and P. setiferus, the dominant penaied shrimp in the Gulf fishery, were not significantly correlated.

Some major relationships can be drawn from this table, but even with these few variables, the overall "community level" structure of the information is not completely comprehendable to most people. Therefore, this R-mode bivariate measure of association, like the Q-mode measures (Bray - Curtis, Mahabanoblis' D, Jaccard coefficent), serve a primary role as inputs to multivariate analyses, where higher level order is imposed on these simple bivariate measures.

As mentioned previously, the nekton data and for that matter the macrobenthos data from Bryan Mound were not accompanied by appropriate charaterization of environmental parameters. In the Texoma study, ancillary environmental data were collected with the biological samples. For phytoplankton for example, the environmental suite consisted of water column parameters, including hydrographic variables (salinity, temperature and dissolved oxygen) and nutrients (phosphate, nitrate, silicate, sulfate). For macrobenthos (Table A10) these would include near bottom hydrographic parameters as well as sediment parameters (grain size and derived statistics, organic carbon, carbonate carbon). Again, inspection of Table A10 will yield insight into the major bivariate relationships between individual species and particular environmental parameters but will generate little understanding of the effects of these environmental variables on the community as a whole. These latter insights are gained in the pattern analysis phase of the program by canonical correlation analysis (see below). Since it is not common practice to stress a discussion of single Q-mode relationships, but instead, use them as inputs to cluster analysis and certain types of discriminant analysis, they are discussed in Section A5.

**Table A10. Results of correlation analyses[a] between numerically dominant benthic megafauna and environmental variables for the Texoma sites during November 1977 to May 1978.**

| Taxonomic Group | Salinity | Dissolved Oxygen | Temperature | % Organic Carbon | % Carbonate Carbon | % Fine | % Clay | Mϕ | $M_D$ | Skewness | Kurtosis | σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nuculana concentrica | NS[b] | NS | NS | NS | -0.19 | NS | NS | 0.13 | NS | NS | NS | NS |
| Callianassa latispina | NS | NS | -0.10 | NS | -0.20 | -0.17 | -0.17 | NS | -0.16 | NS | NS | NS |
| Ancistrosyllis papillosa | -0.10 | NS | -0.27 | NS | NS | -0.11 | NS | -0.17 | NS | NS | -0.15 | NS |
| Nematoda | -0.29 | 0.22 | -0.29 | -0.19 | NS | -0.14 | NS | -0.15 | NS | NS | NS | NS |
| Owenia fusiformis | NS | 0.12 | -0.11 | NS | NS | -0.38 | -0.31 | -0.27 | -0.38 | 0.26 | NS | NS |
| Acetes americanus | NS | NS | NS | NS | NS | -0.14 | -0.19 | NS | -0.17 | 0.14 | NS | NS |
| Drilonereis longa | NS | 0.10 | 0.15 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Magelona sp. | 0.17 | 0.17 | -0.38 | -0.18 | NS | -0.12 | NS | NS | NS | 0.20 | -0.18 | 0.15 |
| Neanthes succinea | 0.15 | 0.15 | -0.26 | -0.14 | NS | -0.47 | -0.41 | -0.21 | -0.46 | 0.39 | NS | 0.17 |
| Lumbrineris tenuis | 0.14 | NS | -0.22 | -0.21 | NS | -0.39 | -0.26 | -0.18 | -0.33 | 0.26 | -0.19 | 0.14 |
| Clymenella torquata | NS | NS | -0.17 | -0.23 | -0.17 | -0.51 | -0.42 | -0.33 | -0.51 | 0.37 | -0.13 | 0.12 |
| Cossura delta | NS | 0.12 | -0.36 | -0.13 | 0.32 | NS | NS | -0.13 | NS | -0.14 | -0.19 | -0.17 |
| Total Megafauna | NS | NS | -0.22 | -0.20 | 0.24 | NS | -0.13 | -0.12 | NS | NS | -0.20 | NS |
| Micropholis atra | NS | NS | NS | -0.14 | NS | -0.26 | -0.23 | NS | -0.24 | 0.21 | NS | 0.19 |
| Paraprionospio pinnata | NS | NS | -0.16 | NS | 0.32 | -0.26 | -0.18 | NS | 0.25 | -0.30 | NS | -0.17 |
| Sabellides oculata | -0.23 | NS | NS | NS | NS | NS | NS | NS | NS | -0.15 | NS | NS |
| Paranthus rapiformis | -0.27 | -0.21 | 0.12 | NS | NS | NS | NS | NS | NS | -0.11 | NS | NS |
| Cirratulis sp. | NS | -0.35 | 0.30 | NS | NS | 0.28 | 0.19 | 0.30 | 0.30 | NS | NS | 0.14 |
| Sigambra tentaculata | NS | -0.30 | 0.20 | NS | 0.15 | 0.34 | 0.27 | 0.21 | 0.33 | -0.19 | NS | NS |
| Cerebratulus lacteus | NS | -0.34 | 0.23 | -0.13 | 0.17 | 0.14 | NS | NS | NS | NS | NS | NS |
| Glycera dibranchiata | NS | -0.23 | 0.19 | NS | 0.19 | NS | NS | NS | NS | NS | NS | NS |
| Mulinia lateralis | NS | -0.21 | 0.27 | -0.13 | NS | NS | NS | NS | NS | NS | NS | NS |
| Venerid larvae | NS | -0.24 | 0.12 | NS | NS | NS | NS | NS | NS | NS | 0.20 | NS |
| Nassarius acutus | NS | NS | 0.18 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Diopatra cuprea | NS | NS | NS | NS | -0.27 | -0.26 | -0.19 | NS | -0.25 | 0.31 | NS | 0.23 |
| Aglaophamus verrilli | NS | NS | NS | NS | NS | -0.20 | -0.18 | -0.17 | -0.19 | NS | NS | NS |
| Ampelisca sp. | NS | NS | NS | -0.18 | NS | -0.34 | -0.29 | -0.29 | -0.34 | 0.20 | -0.18 | NS |
| Carinoma tremaphoros | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a] Significant at the 0.05 level

[b] NS - Not Significant

## A.5 CLASSIFICATION AND PATTERN ANALYSIS

Five classification and pattern analysis techniques are generally employed in ecological assessment and these are discussed in Section IV.2.4 of this report. One technique, canonical correlation analysis is utilized to identify relationships between two dissimilar but related groups of variables [e.g., between a group of species (densities) and a group of environmental variables (sediment and hydrographic variables)].

Presented in this section are results of analyses utilizing the techniques of cluster analysis, reciprocal averaging ordination, factor analysis and discriminant function analysis for the demersal nekton data at Bryan Mound. These first three techniques were utilized for analyses of two data sets. These are:

(1) Five cruise data set over all depths

(2) Six cruise data set at the offshore diffuser site.

Discriminant function analysis was utilized to differentiate between the April 5 (night) and April 20 (daytime) collections at the offshore diffuser site based on taxonomic composition of the samples.

Neither the nekton or macrobenthos studies at the Bryan Mound site included the collection of important environmental variables. Therefore we cannot utilize the nekton data to show the application of canonical correlation analysis. We can, however, show the use of this technique through analysis of the megabenthos data from the Texoma study (Comiskey et al. 1979). Finally, the combined use of cluster analysis, canonical correlation analysis and discriminant function analysis is shown for the sediment geochemical data set collected at the Texoma sites (Comiskey et al. 1979).

### A5.1 Basic Daytime Data Set Over 3.5 to 25 Fathom Depths Off Freeport Texas

The multivariate analyses for the Bryan Mound nekton data set collected over all depths were expected, a priori, to yield certain significant

spatial trends not exhibited in the analysis of the data set for the offshore diffuser site itself. That is, they were expected to add "regional" persective to the analyses conducted using only the data from the area of the offshore diffuser site. Sixteen stations spread over depths of 3.5 fathoms to 25 fathoms were included in the five cruise (July, September and December 1978 and February and April 1979) data set. P. duorarum was dropped after the initial factor analysis, due to low communality. It has, however, been retained in other analyses (cluster and ordination).

A.5.1.1 Factor Analysis

Results of the factor analysis are shown in Tables A11 and A12, which are the correlation and (Promax-rotated) factor pattern matrices, respectively. Table A11 indicates that there were a number of correlations whose absolute values were greater than 0.50. Included among these were the correlations of Menticirrhus americanus with both Cynoscion arenarius (0.50) and Stellifer lanceolatus (0.65). C. arenarius also showed high correlations with Penaeus setiferus and Cynoscion nothus (0.53 and 0.55, respectively). P. setiferus and C. nothus were also highly correlated (0.63), as were P. setiferus and S. lanceolatus (0.59). P. burti and T. lepturus formed a group (correlation of 0.58) that was positively associated with the P. setiferus-Cynoscion group. Micropogon undulatus formed the center of a group which included Arius felis ($r = 0.65$) and Polydactylus octonemus ($r = 0.71$). These correlations were the highest in the data set. Polydactylus octonemus and Arius felis were not as strongly correlated ($r = 0.33$). This group was also positively related to the Penaeus setiferus-Cynoscion group.

As in other analyses for this data set, the only significant negative correlations involved S. caprinus. These included negative relationships with all species except P. aztecus and P. burti. The largest negative correlations were with members of the P. setiferus-Cynoscion group (especially P. setiferus and C. nothus, with the correlations being -0.55 and -0.41, respectively).

**Table A11. Correlation matrix for important demersal nekton species collected in trawl samples over all depths in the Bryan Mound study area for five daytime cruises from July 1978 to April 1979.**

| | Stenotomus caprinus | Cynoscion arenarius | Penaeus setiferus | Cynoscion nothus | Peprilus burti | Trichiurus lepturus | Penaeus aztecus | Arius felis | Polydactylus octonemus | Micropogon undulatus | Menticirrhus americanus |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stellifer lanceolatus | -0.20695 | 0.36335 | 0.58852 | 0.35414 | 0.07273 | 0.31947 | -0.02842 | 0.43995 | 0.02313 | 0.33433 | 0.64862 |
| Menticirrhus americanus | -0.23074* | 0.50397 | 0.45968 | 0.30694 | -0.11759 | 0.13381 | -0.16671 | 0.16724 | -0.07469 | 0.18106 | |
| Micropogon undulatus | -0.24004 | 0.33019 | 0.28902 | 0.42856 | 0.21091 | 0.19607 | 0.23207 | 0.65185 | 0.70849 | | |
| Polydactylus octonemus | -0.16895 | 0.29614 | 0.11921 | 0.36125 | 0.08128 | 0.04980 | 0.31392 | 0.33441 | | | |
| Arius felis | -0.20273 | 0.12236 | 0.32730 | 0.34080 | 0.15703 | 0.33760 | 0.14893 | | | | |
| Penaeus aztecus | 0.21175 | 0.15243 | -0.04309 | 0.12492 | 0.01767 | -0.16303 | | | | | |
| Trichiurus lepturus | -0.26050 | 0.20219 | 0.04586 | 0.38610 | 0.57681 | | | | | | |
| Peprilus burti | 0.05438 | 0.10636 | -0.16954 | 0.07488 | | | | | | | |
| Cynoscion nothus | -0.55048 | 0.53270 | 0.62525 | | | | | | | | |
| Penaeus setiferus | -0.41096 | 0.54950 | | | | | | | | | |
| Cynoscion arenarius | -0.20915 | | | | | | | | | | |

* Those correlations underlined are significant at $\alpha = 0.05$

The rotated factor pattern matrix (obtained from the iterated principal axis procedure in SAS, with Promax rotation), shown in Table A12, revealed that four important factors account for virtually all the common variance in the data set. Three species, Stellifer lanceolatus, Menticirrhus americanus, and Penaeus setiferus had salient loadings on Factor 1 (0.93, 0.72, and 0.46, respectively). However, P. setiferus had a somewhat higher loading on Factor 4 (0.56) indicating a tread for more ubiquitous distribution for this species. Arius felis also showed an affinity to this group (loading of 0.31). Although all three species with loadings greater than 0.45 were generally present in high numbers during December through April, the only real agreement is the high mean in December. S. lanceolatus was at peak density in April, while P. setiferus and M. americanus were present only in relatively low numbers. M. americanus abundance peaked in February when the other two species were found in relatively low numbers. Since the highest loading on this factor was for Stellifer lanceolatus, and since this species showed the most constricted onshore-offshore range of any species in the data set, it is concluded that Factor 1 is predominantly a spatial factor defining the most inshore community. It should be remembered however that overall temporal similarity was seen in the occurrance for these species, with lowest populations in July and October. It should also be remembered that P. setiferus had a higher loading on Factor 4 than on Factor 1. As will be seen (below) the major difference in trends represented by these two factors (1 and 4) is spatial.

Factor 2, with salient loadings from M. undulatus (0.86), P. octonemus (0.78), Arius felis (0.47) and P. aztecus (0.46), appears to be mainly a temporal factor. The two species with the heaviest loadings occurred predominantly in September, as did Arius felis. P. aztecus, did not show a clear cut temporal similarity to the other species except for the fact that October was among the months with highest means. There did not seem to be any overall spatial similarity of these species, with two (Arius felis and P. octonemus) being found no further offshore than the offshore and diffuser site and two species (P. aztecus and M. undulatus) found at all depths. The conclusion therefore is that Factor 2 is a temporal factor with those species with heaviest loadings having peak populations in July and September and low numbers in February.

**Table A12. Promax rotated factor pattern matrix for important demersal nekton species collected in trawl samples over all depths in the Bryan Mound study for five daytime cruises from July 1978 to April 1979.**

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| Stellifer lanceolatus | 0.92651 | 0.01593 | -0.09869 | -0.08940 |
| Menticirrhus americanus | 0.72029 | -0.17520 | 0.09321 | 0.13782 |
| Micropogon undulatus | 0.18396 | 0.85620 | -0.09119 | -0.00402 |
| Polydactylus octonemus | -0.22260 | 0.77615 | 0.04764 | 0.18736 |
| Arius felis | 0.31005 | 0.46561 | -0.20582 | -0.05287 |
| Penaeus aztecus | -0.09667 | 0.46182 | 0.13852 | -0.09296 |
| Trichiurus lepturus | 0.02667 | -0.18062 | -0.97791 | 0.19167 |
| Peprilus burti | -0.07641 | 0.10275 | -0.63245 | -0.13734 |
| Cynoscion nothus | -0.04643 | 0.11138 | -0.08666 | 0.87548 |
| Penaeus setiferus | 0.45990 | -0.01752 | 0.24870 | 0.55549 |
| Cynoscion arenarius | 0.26483 | 0.11854 | 0.03979 | 0.40147 |
| Stenotomus caprinus | 0.01842 | 0.08846 | 0.06376 | -0.61671 |

Factor 3 has only two species, Trichiurus lepturus and Peprilus burti with salient loadings (-0.98 and -0.63, respectively). An examination of the cruise and station means for these two species indicated that both had peak numbers in the Bryan Mound study area in April, although present during the entire study. Station means were somewhat similar but not strikingly so. There were obvious differences (e.g., Stations 26 and 27) in the spatial pattern of the two species. It appears then that Factor 3 defines a temporal trend in the data, with both species present year round in the study area, but with peak numbers occurring in April.

Factor 4, shows three salient positive loadings (C. nothus (0.88), P. setiferus (0.56), and C. arenarius (0.40)), and one salient negative loading (Stenotomus caprinus (-0.62)). This factor appears to represent both temporal and spatial variability. Comparing S. caprinus pattern to that for the other species, it can be seen that S. caprinus was unique in both space and time, with maximum populations in July at the furthest offshore stations (15-25 fathoms). The other three species were characterized by peak numbers in December, but C. nothus, which had the highest loading for this factor, also had high numbers in April. Both P. setiferus and C. nothus had low populations in July, the month when S. caprinus had the highest mean. None of the three species with positive loadings had any representation at the furthest offshore stations, at which S. caprinus had peak numbers. The station means for P. setiferus and C. arenarius indicate quite clearly that peak populations occurred at the nearshore stations. The data for C. nothus do not show this as well. However, the high values for Stations 20-27 (offshore diffuser site) were greatly influenced by the large catches in one month (December). It is concluded therefore that Factor 4 defines a group of species with generally similar spatial and temporal distribution, that is different from that for S. caprinus which is also defined by the factor. The three species with heaviest positive loadings on this factor were distributed over the entire study area out to Station 16 (15 fathoms), but with generally higher means inshore. They occurred in lowest numbers when S. caprinus (which was only found at the deepest stations) was at peak abundance (July).

This factor analysis, therefore identified five major trends in the data set over all depths for the period July 1978 to April 1979. These are:

(1) S. caprinus behaved uniquely with respect to both space and time, with peak populations in July and at Stations 16-18 (15-25 fathoms).

(2) An inshore group, generally restricted to the less than 10 fathoms stations, consisted of Stellifer lenceolatus and Menticirrhus americanus. P. setiferus was closely related to this group temporally and spatially except that it occurred somewhat further offshore.

(3) A group closely related to the inshore group, including P. setiferus C. nothus and C. arenarius, had a seasonal distribution with peak numbers in December and lowest numbers in July. Species belonging to this group generally had higher numbers at the nearshore stations and no representation at Stations 17-18 (20 and 25 fathoms). Thus, the greatest difference in this group and the one with S. lanceolatus and M. americanus (defined by Factor 2) appeared to be the onshore-offshore constriction of the latter group. This group was defined by the same factor as was S. caprinus (bipolar factor) and these species showed distributions in space and time that were negatively correlated with those of S. caprinus.

(4) The Micropogon-Polydactylus-Penaeus aztecus group was spread widely over the study area, occurring mainly in July and October, with low population in February.

(5) T. lepturus and P. burti formed a distinct group characterized by peak numbers in April, and, with the exception of the absence of T. lepturus at the 20 and 25 fathoms depth stations, both species were distributed over the entire study area. This group defines a factor in virtually all our analyses where March-April data are used.

A.5.1.2 Cluster Analysis

The dendrogram showing results of the cluster analysis of the basic daytime data set (July, September, December, February, and April) at stations from 3.5 to 25 fathoms depth (Stations 1-4, 13, 20-27, 16-18) is shown in Figure A7. In this analysis Jaccard's quantitative coefficient (Sepkoski 1974) was used as the bivariate measure of Q-mode association.

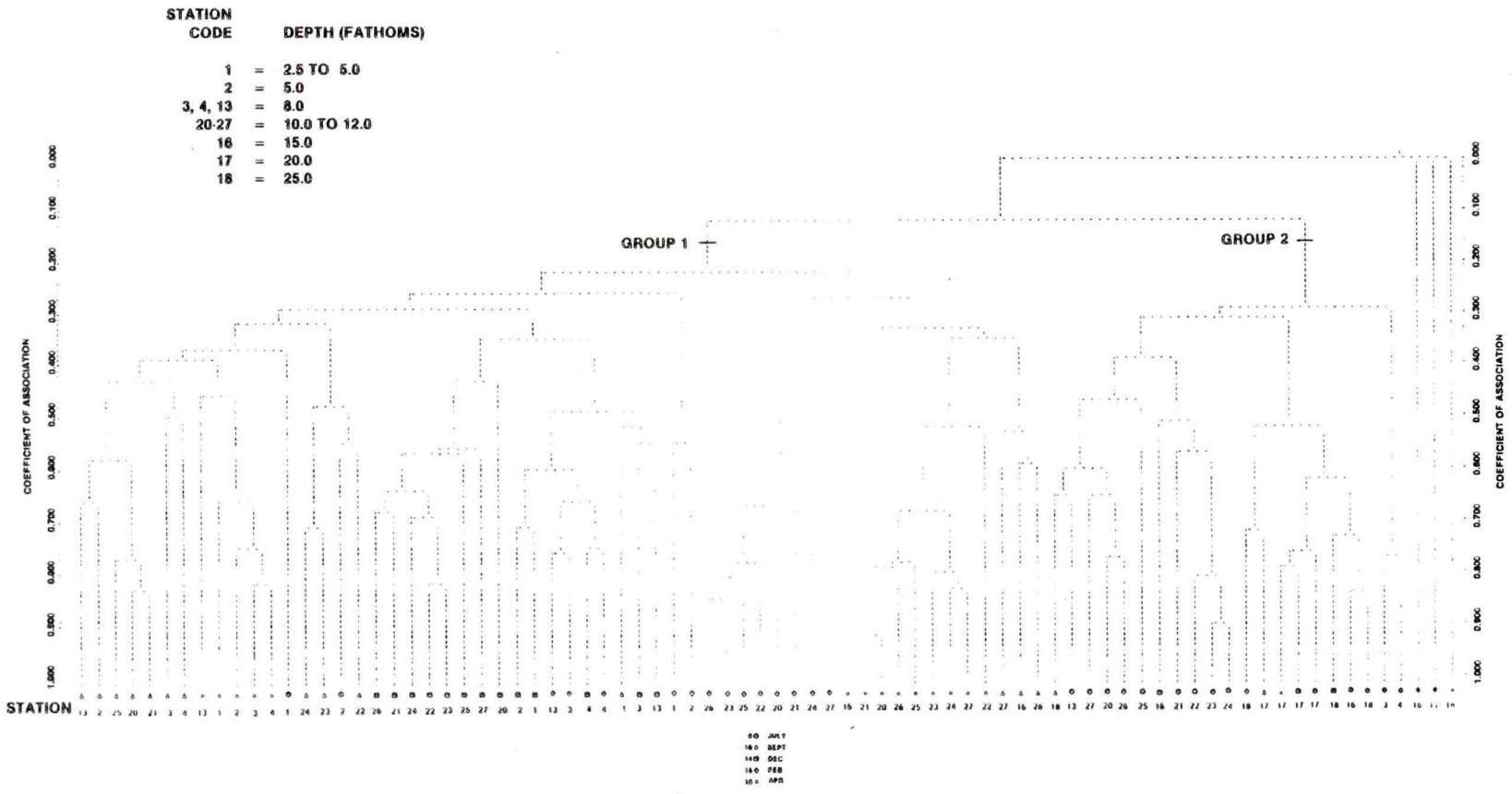


Figure A7. Dendrogram of cluster analysis of nekton samples (selected inshore to offshore stations) from July 1978 to April 1979 in the vicinity of the Bryan Mound offshore diffuser site.

Besides three outlier samples from the furthest offshore stations (15-25 fathoms) in February and April (each containing only one species), two major groups of samples are defined. One (far right) is very revealing. It contains two major subunits, samples from July at all but the shallowest depths (Stations 1 and 2), and samples from the deepest stations (16-18) from all months. This indicates that inshore movement of offshore populations occurs in summer, probably in response to changing salinity and temperature gradients.

The other major cluster of stations in the dendrogram shows mainly a seasonal pattern with secondary spatial trends also evident. Of special interest is the close association of September and April samples in two portions of the dendrogram. To the far left, the inshore stations of April and the inshore to offshore diffuser stations for September are closely related, while on the other side of the sample grouping, samples from the the offshore diffuser site for April are similar to a small group of offshore stations in September. This mixture of temporal and spatial patterns is extremely interesting. Related to the September-April group (far left) are samples from July at the shallowest stations (Stations 1 and 2), further indicating the inshore movement of the offshore populations in July. February samples collected at the offshore stations were more closely related to the April and September offshore group than to the shallow stations in February. There is a clear distinction in February between samples from the nearshore and offshore diffuser sites. The December samples at the offshore diffuser site also compose a very distinct group and are more closely related to the samples from the inshore stations for December and February than to the February offshore diffuser site stations. This might indicate that the community characterzistic of the inshore area during most of the year was migrating offshore in December, perhaps in response to dropping temperature.

### A.5.1.3 Reciprocal Averaging Ordination Analysis

The sample plots (Figures A8 and A9) and species plot (Figure A10) showing the results of the ordination analysis for this same daytime data set over all depths indicate both temporal and spatial features.

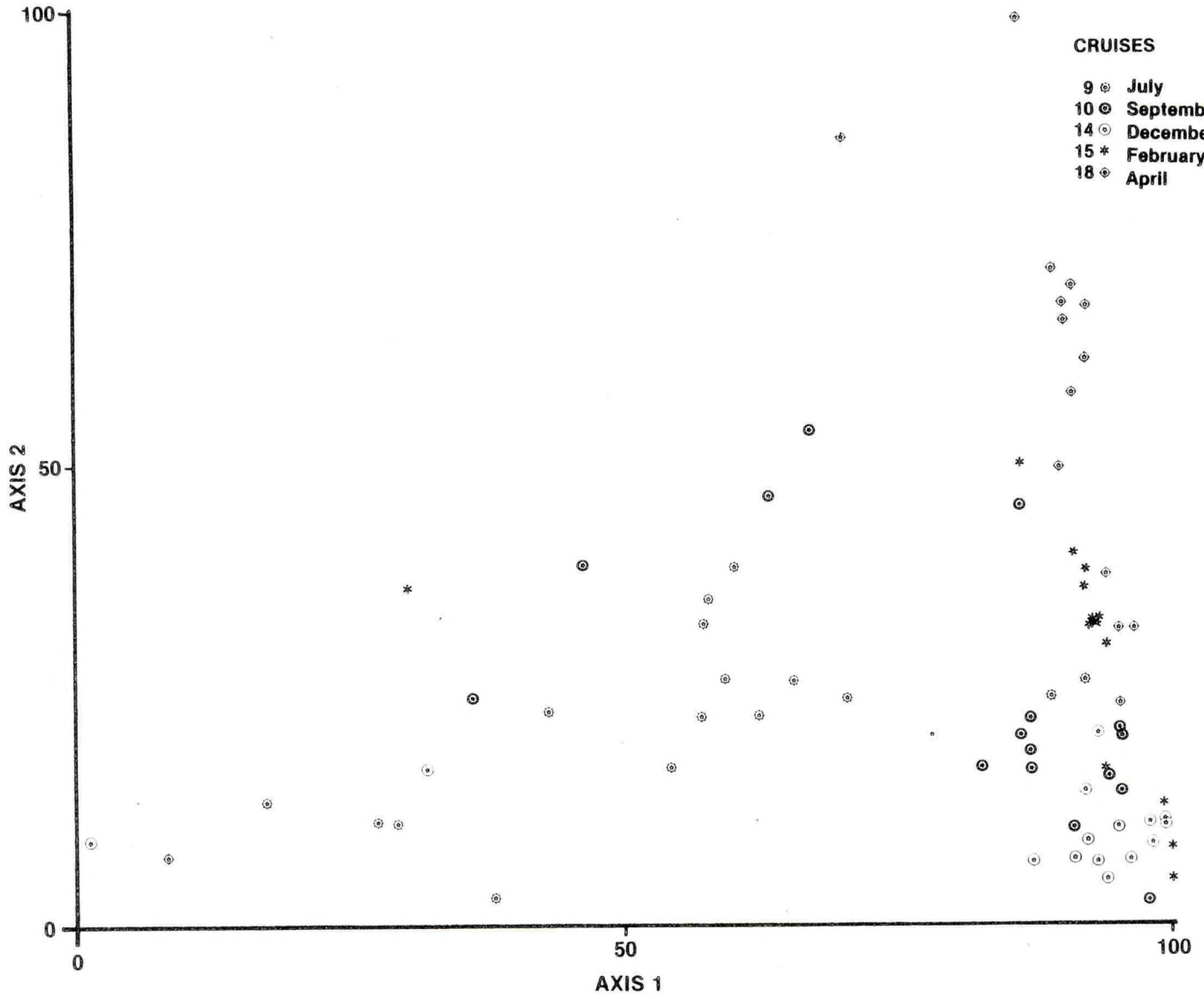


**Figure A8.** Reciprocal averaging ordination of nekton samples labeled by cruise collected over all depths (selected inshore to offshore stations) from July 1978 to April 1979 in the vicinity of the Bryan Mound offshore diffuser site.

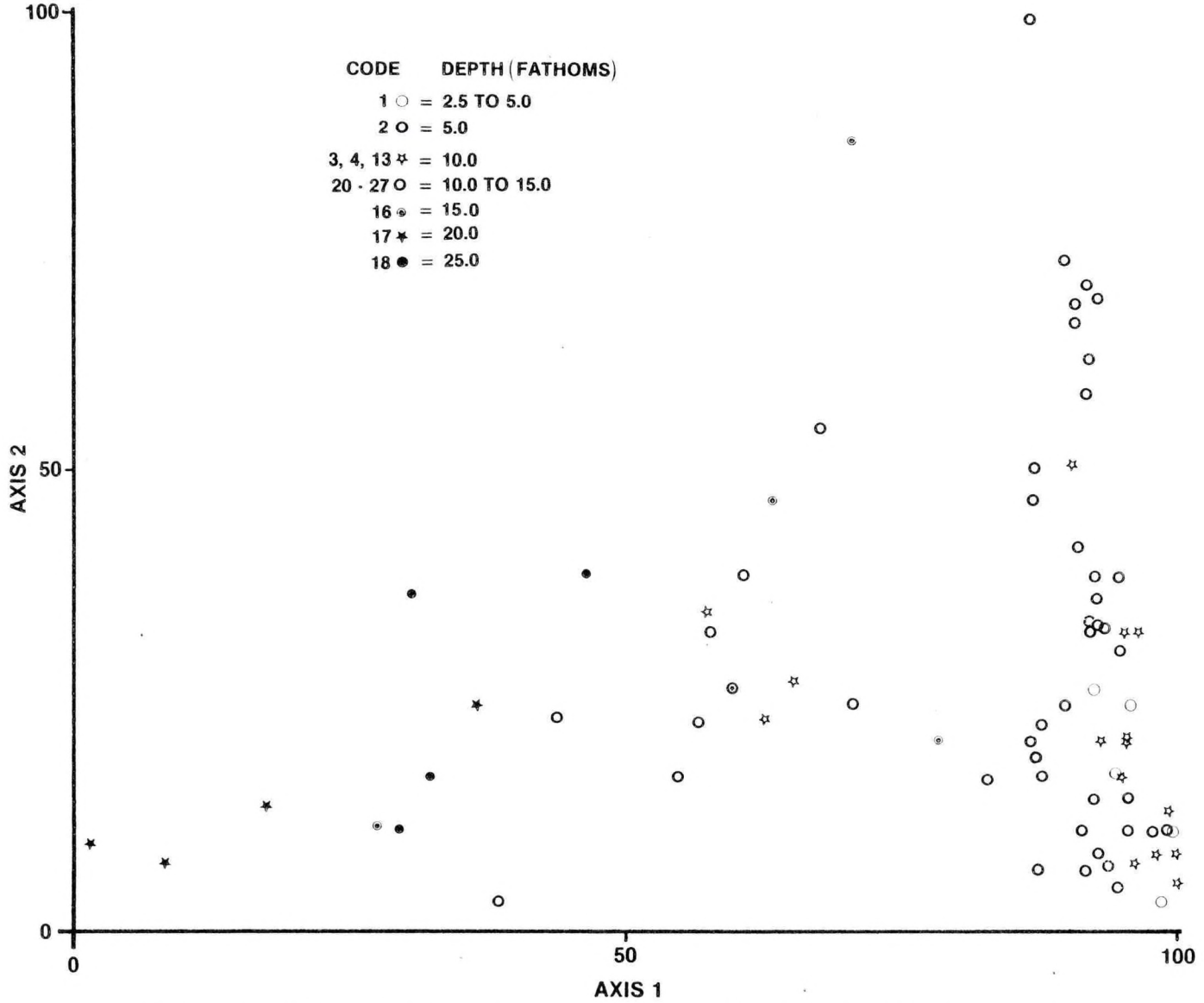


**Figure A9.** **Reciprocal averaging ordination of nekton samples labeled by depth collected over all depths (selected inshore to offshore stations) from July 1978 to April 1979 in the vicinity of the Bryan Mound offshore diffuser site.**

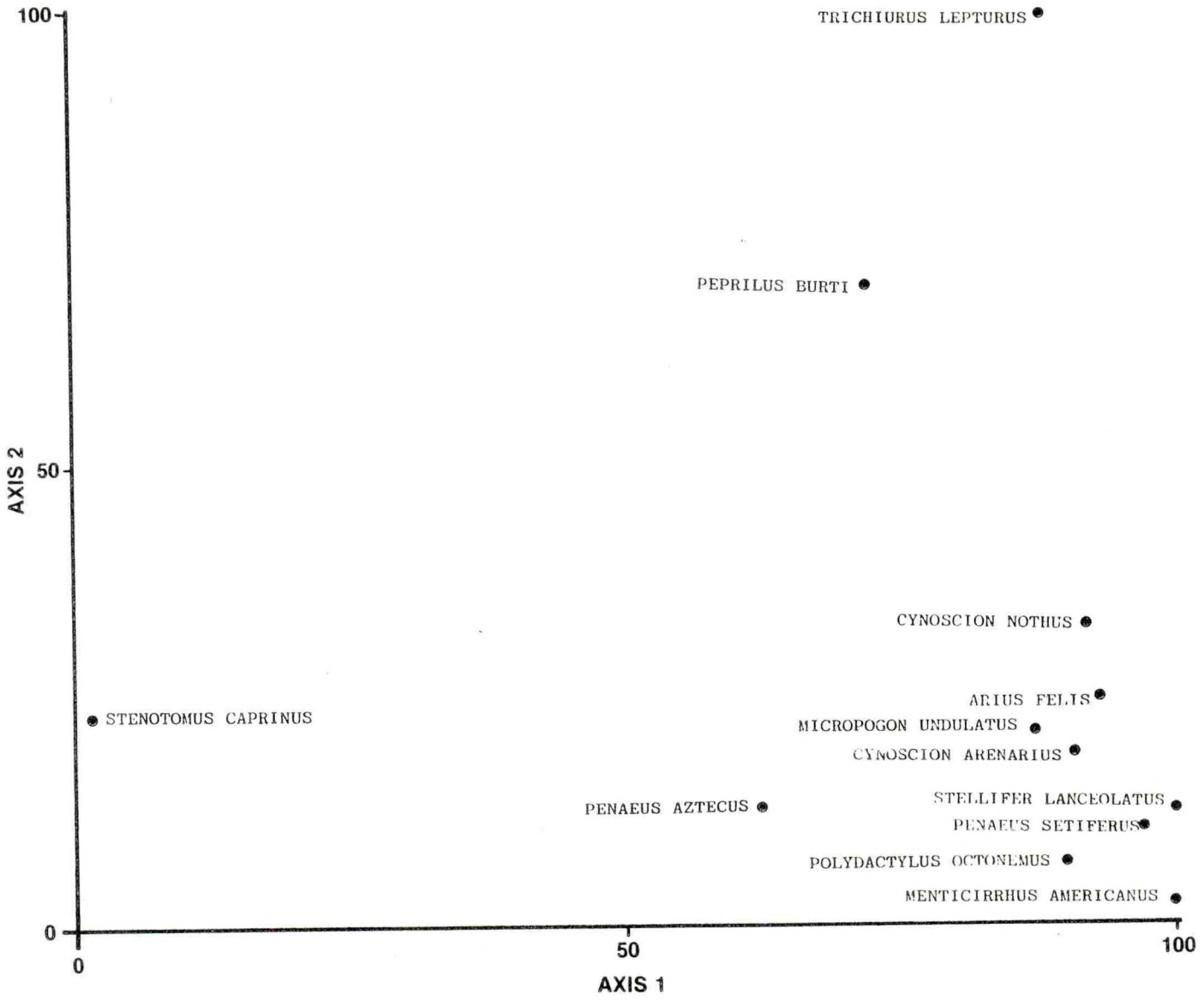


**Figure A10. Reciprocal averaging ordination of nekton samples labeled by species collected over all depths (selected inshore to offshore stations) from July 1978 to April 1979 in the vicinity of the Bryan Mound offshore diffuser site.**

July samples span the length of the Axis 1, the axis of maximum variance. Axis 1 appears to represent primarily a depth gradient. Note samples from all cruises (Figure A8) located in the lower left corner. Comparison with Figure A9 shows that these samples are predominately from the stations further offshore. A line perpendicular to Axis 1 at about the 80 mark denotes a clear line of demarcation. All samples to the left of the line are either (1) from the stations furthest offshore (16-18) or (2) from July. July samples from most of the inshore stations, from the offshore diffuser site, and from deeper stations are located in this area. No samples from Stations 1 or 2 are present to the left of this line of demarcation. Comparing this with results shown for the species in sample space (Figure A10), one can see that these samples are dominated by P. aztecus and S. caprinus. Figure A10 gives conclusive evidence that the offshore brown shrimp community makes its maximum nearshore penetration during mid-summer. The conclusion, then, is that Axis 1, the axis explaining the maximum variance in the data, represents primarily a spatial factor related to inshore-offshore variation in the demersal community. It is this ability to link Q- and R-mode analyses that makes reciprocal averaging ordination a very useful tool.

Axis 2 appears to represent an intricate combination of temporal and spatial factors. Note the position of T. lepturus and P. burti in the species display (Figure A10). They characterize the April samples, with those samples from the offshore diffuser site located in the upper portion of the cluster. It is interesting to note that the most nearshore samples from April are grouped with the large February cluster of mainly offshore diffuser site samples, indicating that the populations at the offshore diffuser site in February may migrate shoreward in April. This central group of February samples is most clearly characterized by C. nothus.

As was the situation in the lower left of the sample display, the lower right corner is a mixture of samples from the different months, and generally exhibits a second major trend in the data set. It can be quite clearly seen that this corner of the display contains the majority

of the samples collected at the inshore stations. Most December samples are mainly clustered in this area, indicating that there is strong offshore penetration of the nearshore community occurring during December. Many September samples are clustered here also, indicating that a number of species had peaks occurring in both months. The locations of the samples collected in September at the offshore diffuser site are certainly due to the presence of M. undulatus (Figure A10). As can be seen from Figure A12, the species characterizing this inshore group are those same species which showed the most restricted onshore-offshore distributions in the factor analysis, and include S. lanceolatus, P. setiferus, P. octonemus, and M. americanus. These species make their maximum offshore excursions in the September to February period, possibly in response to decreasing and low temperatures.

### A.5.2 Daytime Data Set for the Offshore Diffuser Site at Bryan Mound

Since there is very little spatial separation of the nekton sampling stations at the offshore diffuser site (in the context of the Gulf of Mexico continental shelf) it was expected a priori that mainly seasonal patterns would emerge from the pattern analyses of the data set for the offshore diffuser site. Trawls 20-27, collected during July, September, October, and December 1978 and February and April, 1979, were included in the analyses. The locations of these trawls (collected at four stations) are shown in Figure 4 of this report.

#### A.5.2.1 Factor Analysis

Results of the factor analysis for the daytime sampling at the offshore diffuser site are shown in the obliquely rotated factor pattern matrix presented in Table A13. The correlation matrix, upon which the factor analysis is based (see Table A9), was described above. The three factors with eigenvalues greater than one were retained and were rotated to this simple structure using the iterated principal axis solution. Taken together, these three factors adequately describe the main trends for seasonal dynamics at the offshore diffuser site. Of the total variance in the system, 71.4% is accounted for by the three factors.

**Table A13. Promax rotated factor pattern matrix for important demersal nekton species collected in trawl samples over all depths at the Bryan Mound offshore diffuser site for six daytime cruises from July 1978 to April 1979.**

| | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| Micropogon undulatus | 0.88731 | -0.10082 | 0.03620 |
| Arius felis | 0.86203 | 0.01546 | 0.00645 |
| Polydactylus octonemus | 0.85609 | 0.08039 | -0.01760 |
| Menticirrhus americanus | 0.56880 | -0.00300 | 0.00548 |
| Penaeus aztecus | 0.53798 | 0.01106 | -0.34301 |
| Cynoscion nothus | 0.15212 | 0.88755 | 0.02660 |
| Cynoscion arenarius | 0.10472 | 0.72952 | 0.11472 |
| Penaeus setiferus | -0.19602 | 0.70066 | -0.39096 |
| Stenotomus caprinus | 0.10730 | -0.52330 | -0.08524 |
| Trichiurus lepturus | -0.03801 | 0.20792 | 0.98121 |
| Peprilus burti | -0.04460 | -0.12306 | 0.73580 |

The five species which showed high bivariate correlations (see Table A9) (Micropogon undulatus, Arius felis, Polydactylus octonemus, Menticirrhus americanus and Penaeus aztecus) had salient loadings on Factor 1. An examination of the cruise and station means for these species shows that Factor 1 is characterized by species occurring in largest numbers in September and also December. The more a species deviates from this temporal trend, the lower its loading on this factor.

Factor 2 was characterized predominantly by the two sea trout (C. nothus and C. arenarius) and also Penaeus setiferus. Upon examining the cruise and station means for these species, it becomes apparent that Factor 2 defines a group with generally higher means sometime during the December through April period, and also with an indication of higher means in September compared to July or October. All three species had highest means in December. P. setiferus means were also relatively high in March, while both C. nothus and C. arenarius showed a minor peak in April. This winter and early spring dimension to Factor 2 is confirmed by the salient negative loading for S. caprinus. Examination of the spatial and temporal pattern of the means for S. caprinus shows that the peak population occurred during July with lower numbers in September and October. No specimens of S. caprinus were taken from December through April. These trends are very similar to the temporal (and spatial) trends revealed in the analysis over all depths (see Section A.5.1.1).

The third factor was characterized by Trichiurus lepturus (0.98) and P. burti (0.74). Both species had by far their highest populations in April at the offshore diffuser site. A very minor peak in September is also indicated. These two species also defined a factor in the analyses over all depths.

In summary, results of the factor analysis for the daytime collections at the offshore diffuser site can be summarized as follows:

(1) The two sea trout species (C. nothus and C. arenarius) and the white shrimp (Penaeus setiferus) characterize the group with high numbers during the December through April period, and a minor peak in September. Peak populations

occurred in December. It was the pattern of this group that best approximated that of the total nekton (fourteen selected species). S. caprinus was negatively related to this group, having peak populations in July at the offshore diffuser site.

(2) The major group (greatest number of species), consisting of M. undulatus, A. felis, P. octonemus, M. americanus, and P. aztecus, was characterized by species with peak abundance in September, and minor peaks in abundance in December.

(3) Two species (P. burti and T. lepturus) were characterized by maximum numbers at the offshore diffuser site in April.

A.5.2.2 Cluster Analysis

Results of the cluster analysis for the six daytime cruises at the offshore diffuser site (Stations 20-27) are shown in Figure A11. As expected, temporal patterns prevailed. At the level indicated in Figure A11, seven groups of samples are apparent. Each month forms a relatively well-defined cluster of samples. There is a very distinct July group, containing one sample from October. The unique nature of the July samples is undoubtedly due to the dominance of the brown shrimp community (and especially S. caprinus) at the offshore diffuser in July. The other groups are more closely related to each other than any is to the July group, providing further indication of the unique character of the July samples. The October cluster shows one other station (27) as being very different, but still more like the other October samples than like any other month. The February samples, which form a complete group, are more similar to the April samples than to any others. The April cluster is also well defined, except that it contains two samples from September. Otherwise, the September and December samples are more similar to each other than to those from any other months. The grouping of the September and December samples is similar to the trends implied in the factor analysis.

The overall conclusion from this analysis is that temporal trends were strong, with good within-month community similarity in the area of the offshore diffuser site. Essentially, there appears to be two major communities, one in July and the other present during the rest of the

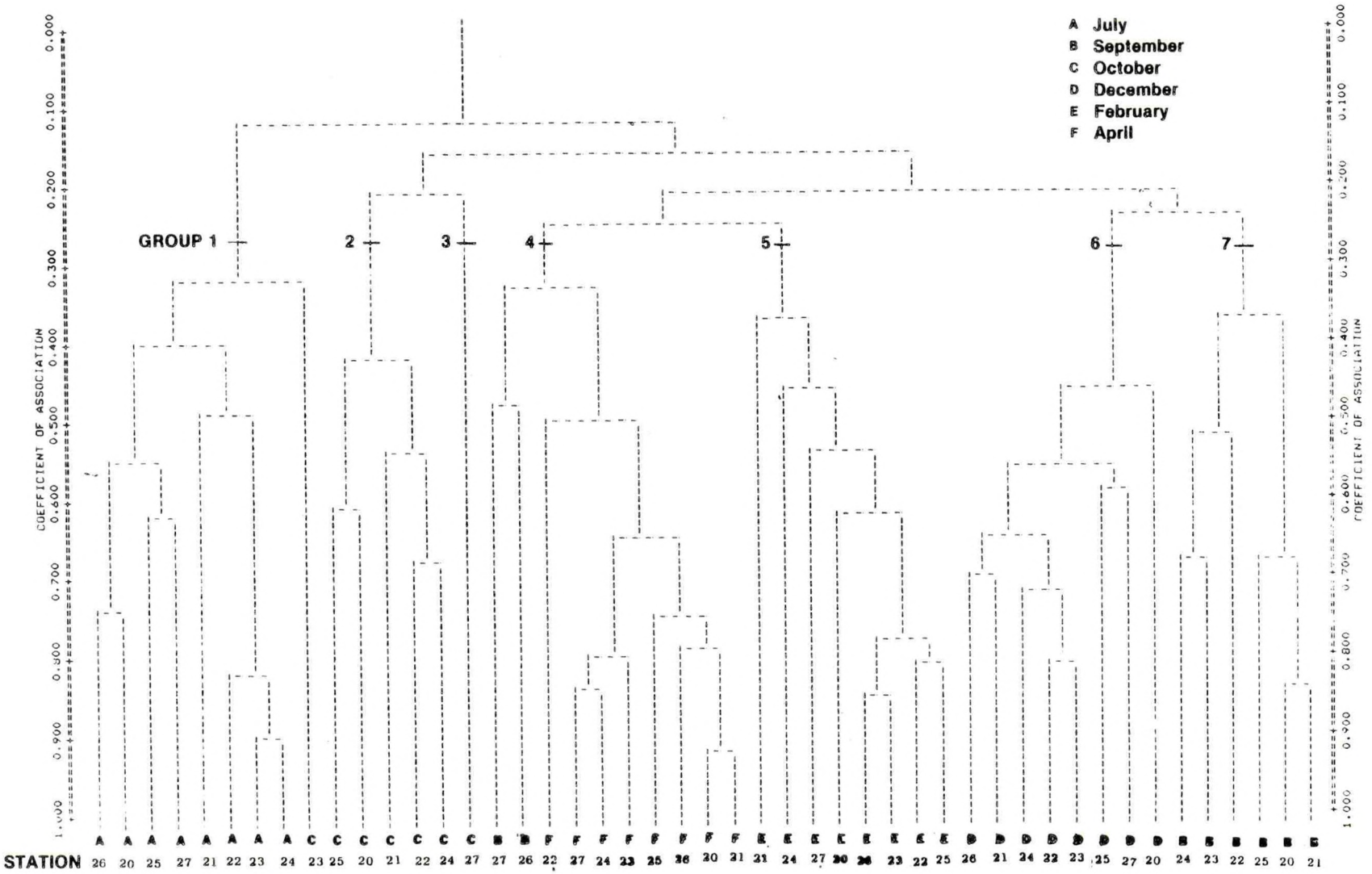


**Figure A11. Dendrogram of the cluster analysis of nekton samples labeled by cruise and station collected at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

year. However, distinct temporal trends were apparent for the species comprising the main group, and several major components of the "white shrimp" community can be identified.

A.5.2.3 Reciprocal Averaging Ordination Analysis

Results of the ordination anslysis for the daytime data set (six cruises) at the offshore diffuser stations (20-27) are presented in Figures A12 and A13, which are the plots of samples and species, respectively. Only the sample display labelled by cruise is presented, since the previous analysis showed little spatial pattern.

The analysis shows that the July samples are the most unique, occupying a large area of the ordination display. There is a lot of spread in the July data due to the low diversity of the "community" (fourteen selected species) at this time. These July samples are dominated by the members of the more offshore brown shrimp community (especially S. caprinus), which make their maximum shoreward intrusion in July. This can be seen by comparing Figures A12 and A13.

Relationships among the fall samples were previously shown by the results of the factor analysis. Factor 1 was characterized by a group of species which had largest numbers in September and December. These species were M. undulatus, A. felis, P. octonemus, M. americanus, and P. aztecus. This was the dominant trend in the data set according to the factor analysis, accounting for the largest portion of the common variance. It is clearly the reason why the ordination plot of samples in species space (Figure A12) shows the September samples to be more closely related to the December samples than to those collected in October. It should be recalled that October was a period of both low numbers of nekton and low diversity (only five of the fourteen selected species were present). The wide spread in the October data is due to low diversity and equitability, with C. nothus accounting for eighty of ninety-one total individuals collected, and being absent from only one station. The divergent October sample at bottom center of the display is the one with C. nothus missing. This is a good example where ordination analysis can help explain the grouping of samples in the

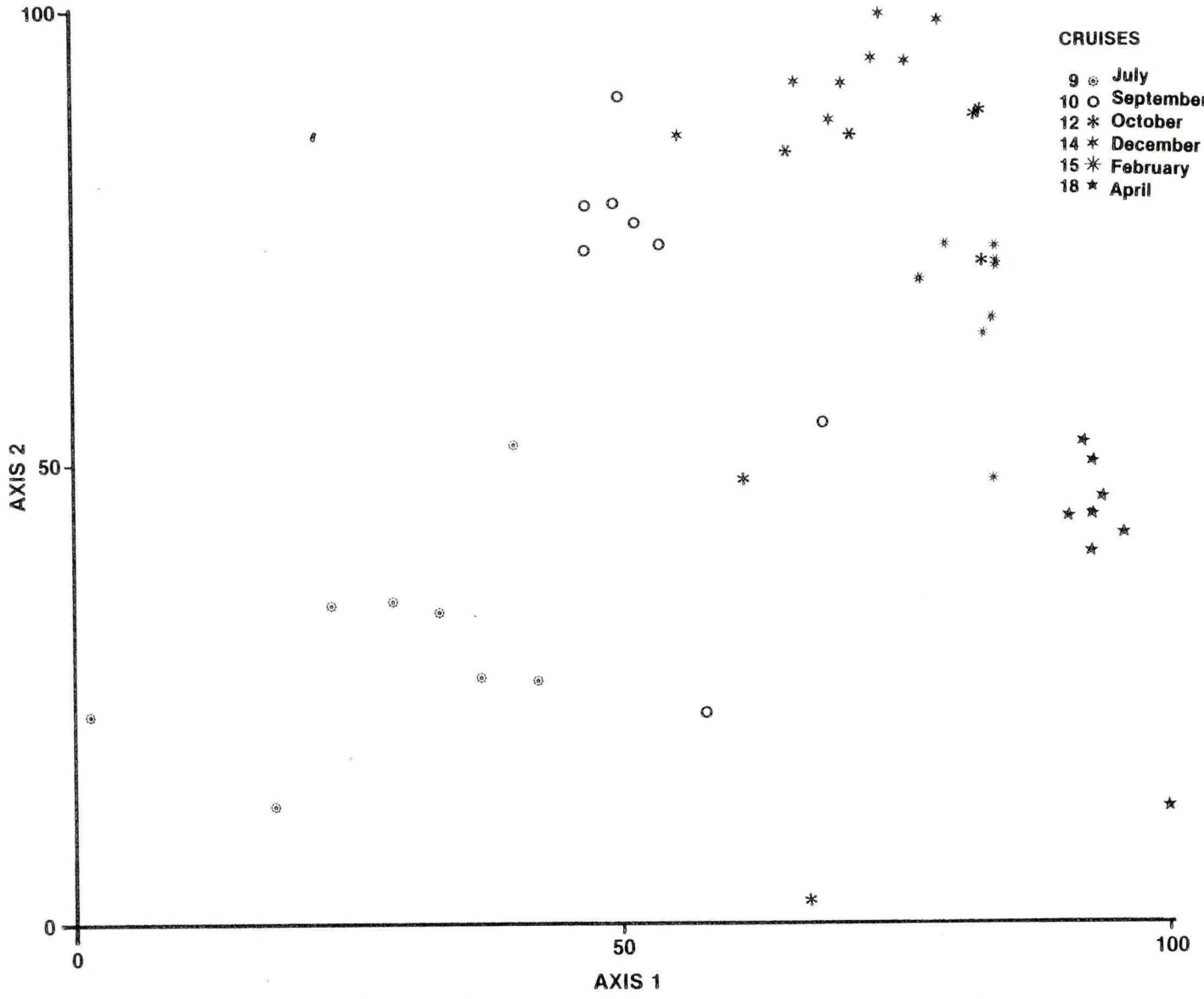


**Figure A12. Reciprocal averaging ordination of nekton samples labeled by cruise collected at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

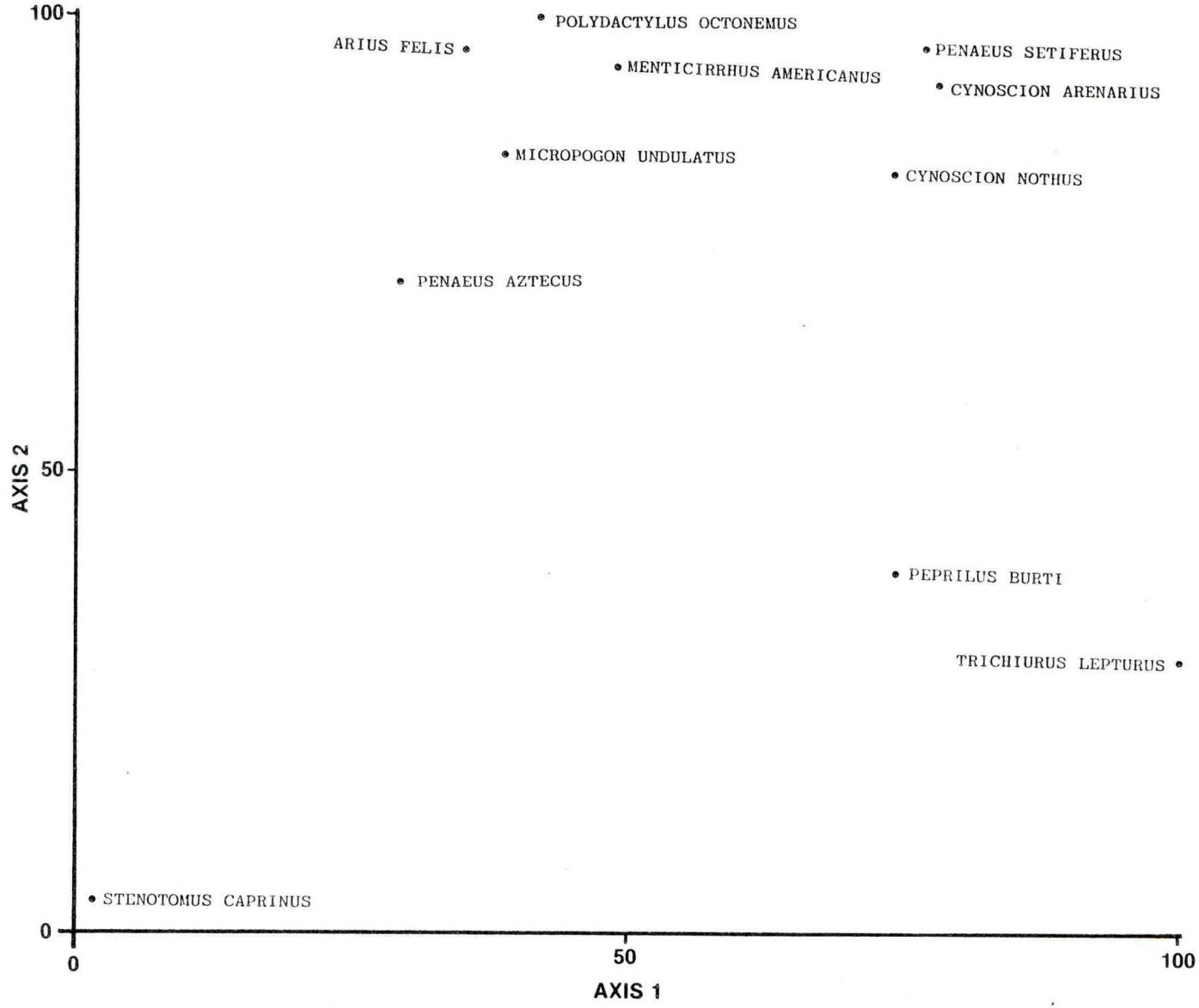


**Figure A13. Reciprocal averaging ordination of nekton samples labeled by species collected at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

cluster analysis. The transition from the July community to the September community is fairly distinct, with the appearance of M. undulatus and the increased importance of C. nothus. It is the dominance of C. nothus in October that pulls these samples off to one side of the display. December showed a return to a more equitable distribution of individuals among species in the samples at this time.

Results of the factor analysis showed P. setiferus and the two sea trout, C. nothus and C. arenarius, with highest loadings on Factor 2. It was concluded that Factor 2 was a temporal factor, representing species with generally higher means sometime during the December to April period, but mainly in December. Note also that the factor analysis showed that S. caprinus, which had virtually the opposite seasonal distribution (peak abundance in July and none collected in December and April), had a negative loading on this factor. The fact that the members of the Penaeus setiferus-Cynoscion spp. group are located in the upper right corner of the ordination display (Figure A13) and S. caprinus is in the lower left indicates that the dominant trend shown by the ordination of data at the offshore diffuser site is temporal. The spread of February samples to the lower right is probably due to the greater importance of P. burti in February as compared to December.

Factor 3 in the factor analysis of data collected at the offshore diffuser site was characterized by P. burti and T. lepturus. Both had by far highest populations at the offshore diffuser site in April. The position of the outlier samples from September (Figure A12) is probably due to these species also, as they showed minor peaks during that month. These results again help to explain the results of the cluster analysis, where two September samples were clustered with the April group (see Figure A11).

### A.5.3 Discriminant Function Analysis of the April 1979 Day/Night Nekton Data Set at the Bryan Mound Offshore Diffuser Site

The results of the application of discriminant function analysis to the Bryan Mound nekton data are now discussed. In this example, the fifteen

most abundant species (based on percent composition over all 48 samples for April 5 and April 20 collections at the offshore diffuser site), were chosen for inclusion in the analysis (see Table A3).

The design of the analysis involved defining two groups of twenty-four samples each, with each group consisting of the samples collected on on of the two cruises. Therefore the discriminant analysis will test whether or not there are significant differences for this fifteen species assemblage between the two cruises (April 5 night vs April 20 day). If significant differences are found, they would be attributable to both diurnal and temporal changes in the composition of the assemblage.

In discriminant function analysis, the procedure is to first assign samples to groups and then develop functions that best disciminate between or among these groups. Based on these discriminant functions, samples are classified into groups and the results compared to the initial classification to ascertain the integrity of the initial groupings. Most discriminant function analysis programs include hypothesis testing capabilities, where there is a parametric test for determining whether any pair of group centroids (grand multivariate means for the groups) differ significantly on the particular discriminant function.

Because there are only two groups, only one discriminant function is calculated in this exemplary analysis. As can be seen in Table A14, the discriminant function developed in this example was highly significant, indicating that there was a significant multivariate difference in the centroids for the two groups of samples.

The criteria chosen for determining the entry of variables into the discriminant function is the variable that minimizes the residual variance among the data sets. Univariate F test results are shown in Table A15. In this case it is clear that Chloroscombrus chrysurus, with a univariate F value of 691.7 will be the first variable entered. Other criteria (Wilk's lambda, Rao's V, Mahalonobis' distance) could have been utilized in lieu of the minimum residual variance criterion.

Table A14. **Summary table of results of discriminant function analysis of important nekton species ($log_{10}$ transformed) collected in forty-eight trawls on April 5 (night) and April 20 (day) 1979 at the Bryan Mound offshore diffuser site.**

| FUNCTION | EIGENVALUE | CANONICAL CORRELATION | AFTER FUNCTION | WILKS' LAMBDA | CHI-SQUARED | D.F. | SIGNIFICANCE |
|---|---|---|---|---|---|---|---|
| | | | 0 | 0.0343604 | 144.95 | 6 | 0.0000 |
| 1* | 28.10323 | 0.9826696 | | | | | |

* MARKS THE 1 CANONICAL DISCRIMINANT FUNCTION(S) TO BE USED IN THE REMAINING ANALYSIS.

Table A15. **Wilk's lambda (U-statistic) and univariate F-ratios (1 and 46 degrees of freedom) for fifteen numerically dominant nekton species collected in trawl samples at the Bryan Mound offshore diffuser site in April 1979.**

| VARIBLE | WILKS' LAMBDA | F | SIGNIFICANCE |
|---|---|---|---|
| Penaeus aztecus | 0.48086 | 49.66 | 0.0000 |
| Penaeus duorarum | 0.54222 | 38.84 | 0.0000 |
| Penaeus setiferus | 0.96527 | 1.655 | 0.2047 |
| Harengula pensacolae | 0.11976 | 338.1 | 0.0000 |
| Anchoa hepsetus | 0.15299 | 254.7 | 0.0000 |
| Halieutichthys caribbaeus | 0.86745 | 7.029 | 0.0110 |
| Urophycis cirratus | 0.79007 | 12.22 | 0.0011 |
| Urophycis floridanus | 0.79378 | 11.95 | 0.0012 |
| Chloroscombrus chrysurus | 0.06235 | 691.7 | 0.0000 |
| Cynoscion nothus | 0.68374 | 21.28 | 0.0000 |
| Cynoscion arenarius | 0.95091 | 2.375 | 0.1302 |
| Trichiurus lepturus | 0.19363 | 191.6 | 0.0000 |
| Peprilus burti | 0.19082 | 195.1 | 0.0000 |
| Etropus crossotus | 0.88015 | 6.264 | 0.0159 |
| Syacium gunteri | 0.92798 | 3.570 | 0.0652 |

The standardized and unstandardized discriminant functions are shown in Table A16. The unstandardized function is used to calculate the disciminant scores using the raw data set, while the standardized function, because it is standardized to a unit variance, expresses the contribution of each variable in differentiating the two groups. These numerical entries are not simple bivariate relationships between the variables and the function, but are more analagous to multiple regression coefficients or partial correlation coefficients, in that they represent conditional relationships. That is, as each variable enters the function, it contributes to explaining residual variance.

Based on the standardized discriminant functions (Table A16), all species except P. setiferus had positive coefficients. When the scores for each sample are plotted on a histogram (Figure A14) there is a clear separation of the two groups, with those from the April 20 daytime cruise with negative discriminant scores and those from April 5 nighttime cruise with positive scores. Those variables with negative coefficients in Table A16 are generally those with higher means in group 2, leading to the high negative scores for group 2 on the discriminant function.

The classification table (Table A17) indicates that of 48 original samples included in 2 groups, all are classified successfully by the discriminant function. This can be seen also in the histogram (Figure A14) where no overlap in observations in discriminant space is evident.

One of the main values of discriminant analysis is in providing a mechanism by which impacted stations can be differentiated from control stations. For example, if the stations in the area of the brine plume (24 trawls) can be looked on as representing a homogenous group in pre-discharge analyses (as was seen in the cluster analyses), when discharge occurs, they would still be expected to classify as a group if no impact is occurring in any part of the region. Stations which deviate from this might be expected to show impact.

**Table A16. Standardized and unstandardized discriminant function coefficients for $log_{10}$ densities of fifteen numerically dominant nekton species collected in trawl samples at the Bryan Mound offshore diffuser site in April 1979.**

| | DISCRIMINANT FUNCTION 1 | |
|---|---|---|
| VARIABLE | STANDARDIZED COEFFICIENT | UNSTANDARDIZED COEFFICIENT |
| Penaeus setiferus | 0.32354 | 0.5300729 |
| Harengula pensacolae | -0.46637 | -0.9994866 |
| Anchoa hepsetus | -0.41281 | -0.6127792 |
| Chloroscombrus chrysurus | -0.64194 | -1.026365 |
| Trichiurus lepturus | -0.20506 | -0.2742222 |
| Syacium gunteri | -0.34596 | -0.3396931 |
| (CONSTANT) | | 5.915137 |

**Table A17. Classification success matrix from discriminant function analysis of nekton species ($log_{10}$ densities) collected in trawl samples at the Bryan Mound offshore diffuser site during two cruises in April 1979.**

| ACTUAL GROUP | NO. OF CASES | PREDICTED GROUP MEMBERSHIP 1 | 2 |
|---|---|---|---|
| GROUP 1: 4/05/79 CRUISE | 24 | 24<br>100.0% | 0<br>0.0% |
| GROUP 2: 4/20/79 CRUISE | 24 | 0<br>0.0% | 24<br>100.0% |

PERCENT OF PREDICTED CASES CORRECTLY CLASSIFIED: 100.00%

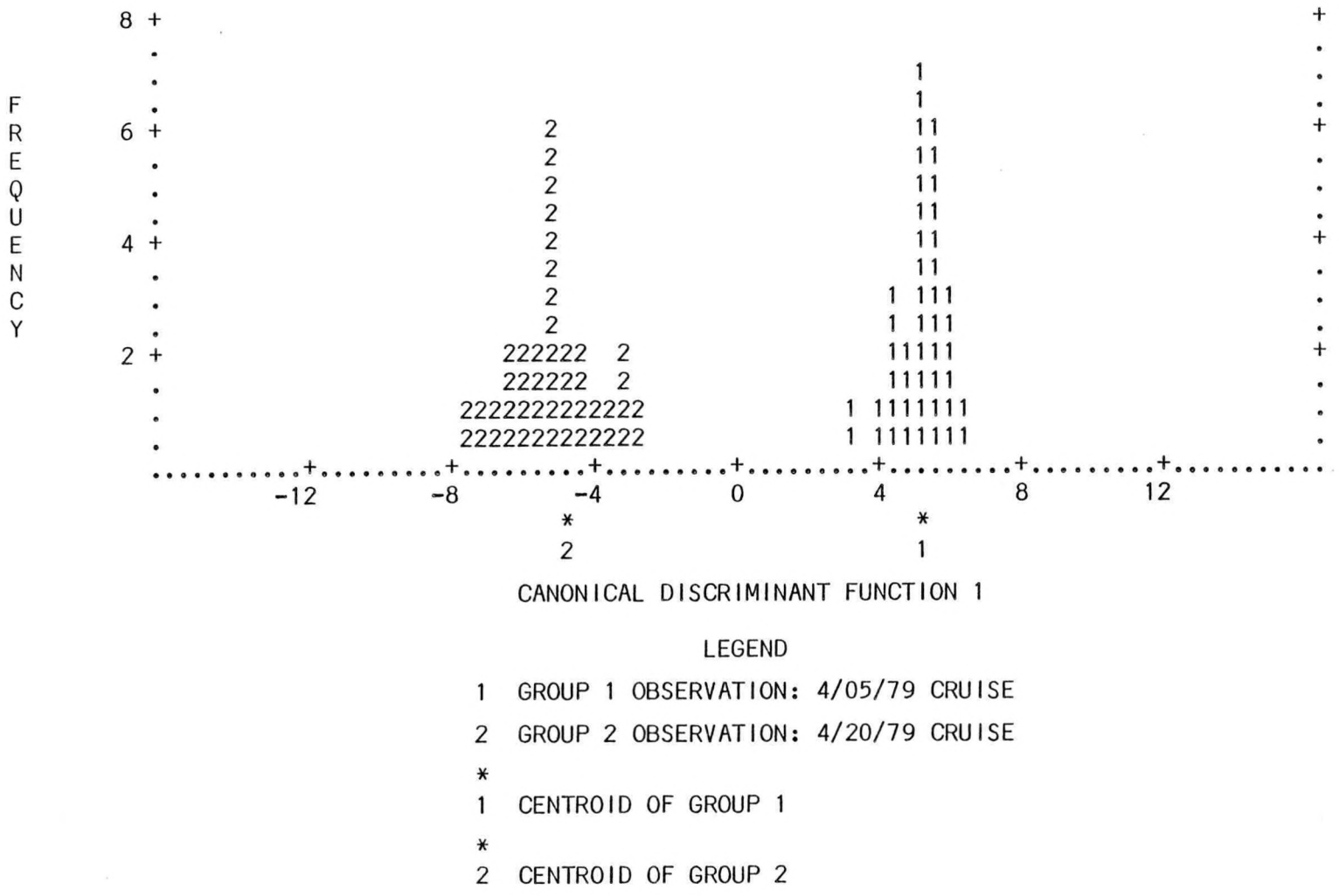


**Figure A14. Histogram plot of scores of forty eight nekton samples on discriminant function 1 for fifteen numerically important species collected on two cruises in April 1979.**

The results of this analysis point out a major problem in quantitative impact assessment in the Gulf of Mexico. Although only fifteen days separated these collections (recognizing that one set was night and one set day), all the samples for each group were classified correctly and were distinct from those of the other group. This shows the great temporal variability in the nearshore Gulf ecosystem and points toward the fact that pre- and post discharge sampling for impact assessment is risky at best, unless the time period between the two collections is minimal. There is virtually no hope of using monthly data from a previous year to assess impacts for the same month the following year.

### A.5.4 Canonical Correlation Analysis of the Texoma Megabenthos Environmental Data Sets

Megabenthos and ancillary environmental data from the Texoma study area were employed to exemplify the use of canonical correlation analysis. The collection sites and stations are shown in Figure A1. The $\log_{10}$ transformed densities of dominant benthic megafaunal species and selected physical and chemical data (hydrographic data such as temperature, salinity and dissolved oxygen, and sediment data including organic carbon, carbonate carbon, silts, clays, fines, $M_D$, $M_\phi$, skewness, kurtosis, and sigma) were submitted to canonical analysis to determine the relationship between species groups and habitat factors for the period November 1977-May 1978 over all sites and at the West Hackberry group of sites, and for the period November 1977 to October 1978 at the Big Hill site.

The first three canonical variates derived in this analysis are highly significant based on the probability of being greater than Chi-square (Table A18). Based on the eigenvalues, the set of environmental variables explains a large portion of the variation in the sets of species defined in each of first three canonical variates with the first two comprising most of this variance. For example, the set of environmental variables which are important on the first canonical variate explains over 71 percent of the variance in the species abundance.

**Table A18. Summary table of canonical correlation analysis using benthic megafaunal species densities (Group 1) and selected CTD/GEOSED variables (Group 2). Samples collected from five Texoma study sites for the period of November 1977 to May 1978.**

| Canonical Variable | Mean of Group 1 Canonical Variable | Mean of Group 2 Canonical Variable | Canonical Correlation | Eigenvalue | Chi-Square | DF | Prob<Chi-Sq |
|---|---|---|---|---|---|---|---|
| 1 | -0.01951070 | -0.17026635 | 0.84368796 | 0.71180937 | 783.57666 | 338 | 0.0001 |
| 2 | 0.03343443 | -0.43504804 | 0.74345844 | 0.55273045 | 558.38857 | 300 | 0.0001 |
| 3 | 0.08240495 | -0.85279762 | 0.66692635 | 0.44479075 | 412.75708 | 264 | 0.0001 |
| 4 | 0.01755805 | -0.30742929 | 0.58965975 | 0.34769862 | 306.25484 | 230 | 0.0006 |
| 5 | 0.03054392 | 0.76882362 | 0.52658213 | 0.27728873 | 228.92284 | 198 | 0.0652 |
| 6 | -0.03289136 | -0.95536665 | 0.50048179 | ------ | 170.14391 | 168 | 0.4393 |
| 7 | 0.06188500 | -0.31436387 | 0.41516946 | ------ | 117.95709 | 140 | 0.9120 |
| 8 | 0.06305997 | 0.12138631 | 0.38214988 | ------ | 83.71481 | 114 | 0.9850 |
| 9 | 0.03173906 | 0.67245461 | 0.31052630 | ------ | 55.14048 | 90 | 0.9986 |
| 10 | -0.03189181 | -0.61918527 | 0.28565814 | ------ | 36.78746 | 68 | 0.9993 |
| 11 | -0.03645653 | -0.03983660 | 0.24849773 | ------ | 21.38022 | 48 | 0.9997 |
| 12 | 0.04801954 | -0.48817734 | 0.17977381 | ------ | 9.84327 | 30 | 0.9998 |
| 13 | -0.03965976 | 0.52859348 | 0.14594587 | ------ | 3.89699 | 14 | 0.9957 |

From Comiskey et al. (1979)

The relative size of the correlation coefficients between each canonical variate of a group and the variables of that group gives an indication of those variables that are most important in explaining patterns in the data. Species correlations on the first three canonical variates are shown in Table A19 and the environmental variable correlations are shown in Table A20.

Hydrographic variables are most highly correlated with canonical variable 1 (Table A20), and the correlations indicate that temperature is related to this canonical variable in the opposite way than salinity and dissolved oxygen. The seasonal implication is clearly for a late spring period, when temperatures are rising rapidly and salinity and dissolved oxygen are relatively low. Sediment textural parameters have intermediate and positive loadings on canonical variable 1, while skewness and sigma have negative correlations. Note that for these sediment parameters, the relatively higher loadings on canonical variable 2 are of the opposite sign as those on canonical variable 1. Clearly, sediment texture and sorting variables are important in defining canonical variable 1 as well as canonical variable 2. Temperature and dissolved oxygen are also important for canonical variable 2, with the same relationship they had with canonical variable 1. This also indicates a spring occurrence for those species correlated with canonical variable 2, but not with strongest trends during the months of high regional discharge.

The correlation coefficients for species and canonical variables 1 and 2 (Table A19) essentially characterize the two communities defined by Factors 1 and 2 in a factor analysis of these same data (Comiskey et al. 1979). Both assemblages have maximum abundance in the spring, but show different sediment preferences. Sigambra tentaculata, Cirratulis sp., Sabellides oculata, Paraprionospio pinnata, Carinoma tremaphoros, Paranthus rapiformis, and Cerebratulus lacteus are characterized by positive correlations with canonical variable 1 and low correlations with canonical variable 2. Another group, including Lumbrineris tenuis,

**Table A19. Correlation coefficients between each canonical variable of Group 1 (benthic megafaunal densities) and the variables of Group 1 for samples collected from five Texoma study sites for the period November 1977 to May 1978.**

| Taxonomic Group | Can Var 1 | Can Var 2 | Can Var 3 |
|---|---|---|---|
| Paranthus rapiformis | 0.550 | 0.188 | 0.002 |
| Cerebratulus lacteus | 0.295 | 0.115 | 0.156 |
| Carinoma tremaphoros | 0.335 | 0.077 | -0.195 |
| Diopatra cuprea | -0.331 | 0.298 | -0.149 |
| Neanthes succinea | -0.397 | -0.437 | 0.262 |
| Owenia fusiformis | -0.272 | 0.341 | 0.330 |
| Glycera dibranchiata | 0.115 | 0.143 | 0.304 |
| Clymenella torquata | -0.253 | 0.669 | -0.123 |
| Lumbrineris tenuis | -0.420 | 0.290 | 0.244 |
| Nassarius acutus | 0.033 | -0.016 | -0.060 |
| Mulinia lateralis | 0.169 | 0.354 | -0.060 |
| Nuculana concentrica | -0.015 | -0.233 | -0.103 |
| Callianassa latispina | -0.181 | 0.102 | -0.074 |
| Acetes americanus | -0.137 | 0.185 | -0.004 |
| Nematoda | 0.082 | 0.119 | 0.716 |
| Aglaophamus verrilli | -0.065 | 0.179 | -0.050 |
| Ancistrosyllis papillosa | 0.044 | 0.174 | 0.261 |
| Cossura delta | 0.020 | -0.076 | 0.556 |
| Magelona sp. | -0.457 | -0.058 | 0.286 |
| Paraprionospio pinnata | 0.422 | -0.082 | 0.212 |
| Sigambra tentaculata | 0.385 | -0.031 | 0.214 |
| Ampelisca sp. | 0.086 | 0.563 | 0.053 |
| Micropholis sp. | -0.294 | 0.374 | 0.108 |
| Sabellides oculata | 0.580 | 0.266 | 0.051 |
| Cirratulis sp. | 0.607 | 0.202 | 0.187 |
| Venerid larvae | -0.012 | -0.105 | 0.132 |

From Comiskey et al. (1979)

**Table A20. Correlation coefficients between each canonical variable of Group 2 (CTD/GEOSED data) and the variables of Group 2 for samples collected from five Texoma study sites for the period November 1977 to May 1978.**

| Variable | Can Var 1 | Can Var 2 | Can Var 3 |
|---|---|---|---|
| Temperature | 0.658 | 0.531 | -0.438 |
| Salinity | -0.562 | -0.082 | -0.421 |
| Dissolved Oxygen | -0.527 | -0.579 | 0.339 |
| Organic Carbon | 0.051 | -0.358 | -0.179 |
| Carbonate Carbon | 0.072 | -0.179 | 0.522 |
| Silts | 0.435 | -0.334 | -0.036 |
| Clays | 0.305 | -0.780 | -0.006 |
| Fines | 0.497 | -0.812 | -0.066 |
| $M_D$ | 0.516 | -0.811 | 0.038 |
| $M_\phi$ | 0.118 | -0.548 | -0.105 |
| Skewness | -0.422 | 0.662 | 0.039 |
| Kurtosis | 0.065 | -0.105 | -0.211 |
| Sigma | -0.387 | 0.312 | -0.072 |

From Comiskey et al. (1979)

Clymenella torquata, Nereis succinea, Diopatra cuprea, Owenia fusiformis, Magelona sp., and Micropholis sp., are generally characterized by negative correlations with canonical variable 1 and positive loadings with canonical variable 2. Species of the first group, therefore, show some preference for the finer textured stations, while the other group shows a distinct aversion to the finest textured sediments. Because no species is strongly and negatively correlated with canonical variable 2, it is concluded that those species which are positively correlated with the first canonical variable and show a positive relationship to sediment fines do not show a strong preference for the stations with the finest textured sediments.

The third pair of canonical variables (Tables A19 and A20) essentially defines winter conditions, with low temperature and salinity, high dissolved oxygen in the water column, and high carbonate carbon in the sediments. No texture variable is significantly correlated with the canonical variable 3, indicating the lack of spatial dimension to the third pair of cannonical variables. The taxa with highest correlations with the variable included Nematoda, Cossura delta, Magelona sp., L. tenuis, Glycera dibranchiata and O. fusiformis.

### A.5.5 Multivariate Analyses of the Texoma Sediment Chemistry Data Set

The final example of the use of multivarate analyses in combination to reveal major trends in the data involves sediment geochemistry data collected in the Texoma study. This set of analyses involve the use of cluster, canonical correlation and discriminant analysis.

Sediments are good integrators of environmental effects because, unlike the water column which is spatially transient, the substrate remains in place, at least over certain periods. The sediments are in constant contact with the overlying water column, and chemicals are either taken up (adsorbed) by the sediments or released by the sediments to the water column across the water sediment interface. The direction of exchange will be determined by many factors including the redox states of the water and sediments and the degree of saturation of the sediment exchange complex.

The water that will be used to leach or dissolve the caverns to store the SPR oil will be taken from the Intracoastal Waterway which is contaminated with (among other pollutants) various trace metals. Thus, when the brine solution resulting from cavitation is disposed into the Gulf, it may bear a load of trace metal contaminants. Because many organisms living in the sediments feed on the sediments, the transfer of trace metals from water column to sediments and ultimately to higher trophic levels (shrimp and man) is a concern. Therefore, one of the tasks in the Texoma baseline study was to determine the existing burden of trace metals in the sediments before discharge began and to relate those burdens to sediment properties. This information could then be compared to trace metal loads during periods after brine discharge was initiated to see if any buildup in trace metals was occurring. The analyses persented below were conducted to accomppish these tasks.

The two groups of variables which were utilized in the analyses are as follows:

| Trace Metal Suite | Sediment Texture and Major Metal Suite |
|---|---|
| Copper (Cu) | Iron (Fe) |
| Chromium (Cr) | Aluminum (Al) |
| Lead (Pb) | Percent fines (silt and clay) |
| Manganese (Mn) | Total Organic Carbon (TOC) |
| Nickle (Ni) | |
| Zinc (Zn) | |
| Cadmium (Cd) | |

Data were collected on four cruises (September 1977, February 1978, June 1978, and August 1978) at the Big Hill site, but all three Texoma sites (West Hackberry and West Hackberry Control sites as well as the Big Hill site) were sampled only in September 1977 and February 1978. All analyses involving all three sites were, therefore, restricted to data from these two cruises.

Cluster analysis utilized both trace metal and sediment texture variables for samples collected at these three sites during the cruises in Fall 1977 and Winter 1978. The dendrogram (Figure A15) demonstrates both temporal and spatial patterns in the data. The group of samples at

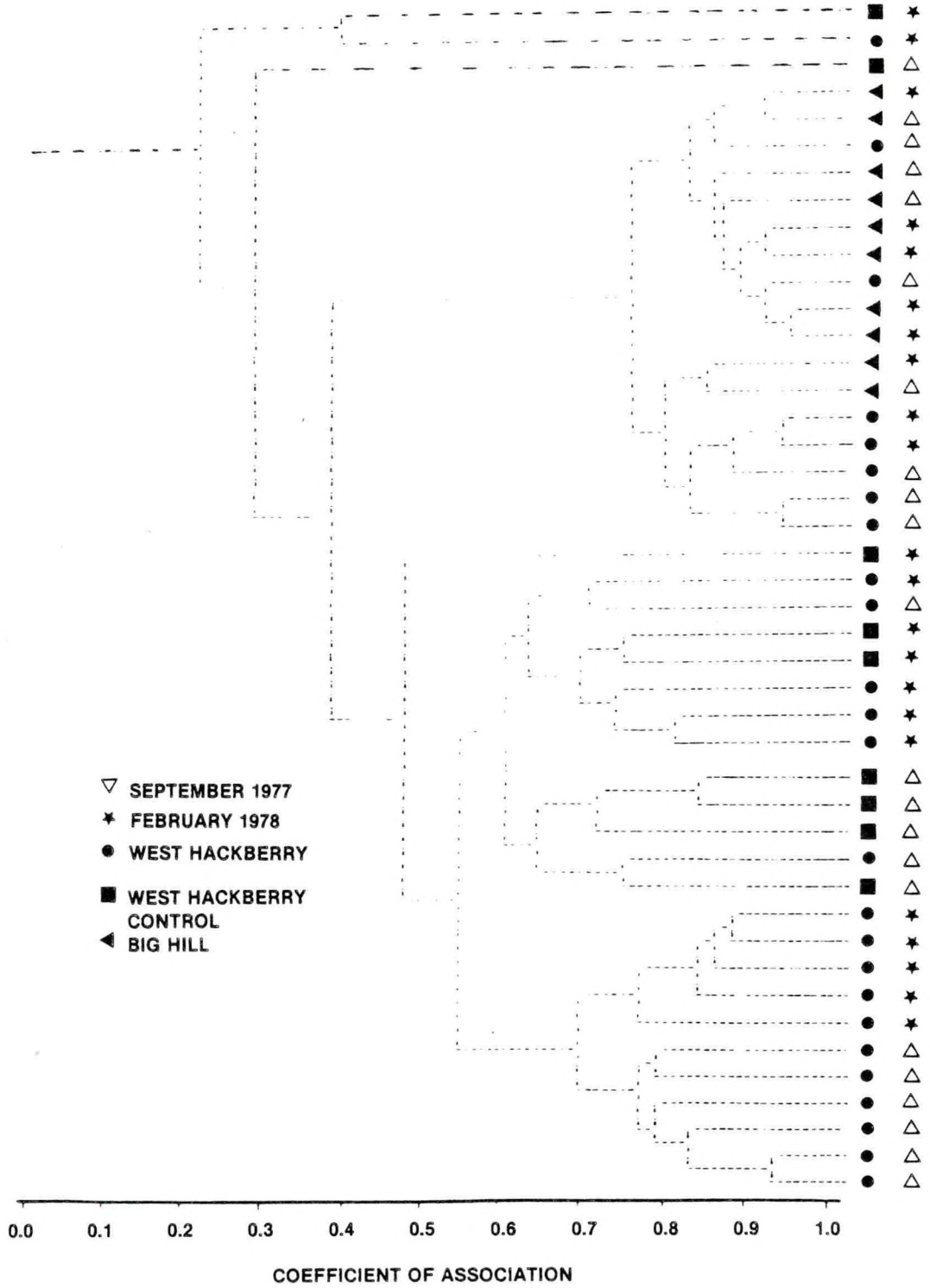


**Figure A15. Cluster dendrogram of trace metal/texture variables for sediment samples collected during September 1977 and and February 1978 in the Texoma study area.**

From Comiskey et al. (1979)

the bottom of the dendrogram is composed entirely of West Hackberry samples, with those from September 1977 (cruise 1) distinct (at the next level) from those of February 1978. All along the dendrogram there is a mixture of seasonal and spatial pattern. The West Hackberry samples are spread over the length of the dendrogram demonstrating the heterogeneity of the sediment regime at this site.

The canonical correlation analysis between the two sets of variables (textural and geochemical) for this same sediment-geochemical data set demonstrates the textural factors responsible for the trends in geochemical composition (Tables A21 to A23). The close relationship between increasing fineness of texture and increasing geochemical burden is clearly demonstrated. That is, comparing canonical variable 1 for the two data sets, we see that all trace metal variables are positively related to percent fines, TOC, iron, and aluminum. Chromium, lead, manganese and zinc are most closely related to these textural and major metal variables.

The use of discriminant analysis and the close relationship between discriminant analysis and canonical correlation analysis can be seen by comparing the results of canonical correlation analysis and discriminant analysis for the same sediment/geochemical data set. By determining discriminant functions and classification functions for the predisposal period a sample taken after initiation of disposal can be classified to see if its group classification changes. The three sites chosen for this analysis were selected because they exhibited a fairly wide range of sediment texture and organic matter properties. Since these factors influence the trace metal burden of sediments (that is the ability of sediments to take up trace metals), these three sites, two of which were potential disposal sites, showed fairly substantial differences in trace metal composition.

It would have been ideal if enough samples were collected on one cruise for multivariate site comparisons. Because the goal was to determine seasonal patterns as well as site patterns, and because each trace metal analysis is quite expensive, it was deemed most desirable to sample

**Table A21. Summary table of canonical correlation analysis of trace metal geochemistry (Group 1) and percent fines, total organic carbon, iron and aluminum (Group 2), for samples collected from the West Hackberry, West Hackberry Control and Big Hill site during September 1977 and February 1978.**

| Canonical Variable | Mean of Group 1 Canonical Variable | Mean of Group 2 Canonical Variable | Canonical Correlation | Chi-Square | DF | Prob < Chi-Sq |
|---|---|---|---|---|---|---|
| 1 | 0.502 | 0.511 | 0.984 | 287.11 | 28 | 0.0001 |
| 2 | 0.427 | 0.247 | 0.793 | 106.20 | 18 | 0.0001 |

**Table A22. Correlation coefficients between each canonical variable of Group 1 (trace metals) and variables Group 1 for samples collected from West Hackberry, West Hackberry Control, and Big Hill sites during September 1977 and February 1978.**

| Canonical Variable | Cu | Cr | Pb | Mn | Ni | Zn | Cd |
|---|---|---|---|---|---|---|---|
| 1 | 0.503 | 0.950 | 0.981 | 0.931 | 0.760 | 0.930 | 0.276 |
| 2 | 0.646 | 0.050 | 0.094 | -0.048 | 0.569 | -0.032 | 0.393 |

**Table A23. Correlation coefficients between canonical variable of Group 2 (texture variables of geochemical samples) and the variables of Group 2 for samples collected from the West Hackberry, West Hackberry Control and Big Hill sites during September 1977 and February 1978.**

| Canonical Variables | Fines | Total Organic Carbon | Iron | Aluminum |
|---|---|---|---|---|
| 1 | 0.936 | 0.951 | 0.978 | 0.743 |
| 2 | 0.016 | -0.274 | 0.047 | -0.568 |

several times over the year and sample less intensively during any one cruise. Therefore, for the analyses over three sites, September and February data for each site were combined, the seasonal factor being disregarded. Thus the data set for each spatial group (k = 3) includes both September and February samples. With this in mind the results of the analysis should give some insight into the relative effect of seasonal vs. site factors.

The number of samples (replicates) for each group are as follows:

| Site | September Cruise | February Cruise | Total for Site Group |
|---|---|---|---|
| West Hackberry | 13 | 12 | 25 |
| West Hackberry Control | 5 | 4 | 9 |
| Big Hill | 5 | 5 | 10 |

For all analyses equal prior probabilities of group occurrence were assumed since an individual case or sample has equal probability of falling into any one of the groups.

In addition to running a discriminant analysis on trace metal data for the three sites, a second discriminant analysis was performed on the sediment texture parameters for these same sites. A third analysis was subsequently performed using Big Hill data for four seasonal cruises to further demonstrate the discriminating power of the technique.

Both sets of variables (trace metals and texture/major metals) proved quite effective in discriminating the three sites for the combined Fall 1977 and Winter 1978 data set, with the trace metals being slightly better discriminators than the texture parameters. The results of these analyses are presented in Tables A24 to A29 and in Figures A16 to A17.

For the analyses for trace metals, the overall F test shows that there is a rejection of the null hypothesis of equal group centroids,

Table A24. **Relative importance of discriminant functions based on relative percent of eigenvalue and canonical correlation using trace metal variables collected from three Texoma study sites during September 1977 and February 1978.**

| Discriminant Function | Eigenvalue | Relative Percentage | Canonical Correlation |
|---|---|---|---|
| 1 | 5.355 | 72.7 | 0.917 |
| 2 | 2.006 | 27.3 | 0.816 |

Table A25. **Relative importance of discriminant functions based on relative percent of eigenvalue and canonical correlation using texture variables for samples collected from three Texoma study sites during September 1977 and February 1978.**

| Discriminant Function | Eigenvalue | Relative Percentage | Canonical Correlation |
|---|---|---|---|
| 1 | 2.905 | 85.9 | 0.862 |
| 2 | 0.474 | 14.1 | 0.567 |

**Table A26. Standardized discriminant function coefficients for copper, chromium, lead, manganese, nickel, zinc, and cadmium during September 1977 and February 1978 at three Texoma study sites.**

| | Standarized Discriminant Function Coefficients | |
|---|---|---|
| | Function 1 | Function 2 |
| Copper | -0.439 | 0.351 |
| Chromium | 0.283 | 2.340 |
| Lead | 2.042 | -0.814 |
| Manganese | 0.475 | -0.336 |
| Nickel | -0.678 | 0.844 |
| Zinc | -1.419 | -2.274 |
| Cadmium | 0.0001 | 0.322 |

**Table A27. Classification success matrix of chemical geosediment samples (trace metals) for samples collected during September 1977 and February 1978 at three Texoma study sites.**

| | | Predicted Group Membership | | |
|---|---|---|---|---|
| Actual Group | No. of Cases | West Hackberry | West Hackberry Control | Big Hill |
| Group 1-WH | 25 | 23<br>92% | 0<br>0% | 0<br>0% |
| Group 2-WHC | 9 | 2<br>8% | 9<br>100% | 0<br>0% |
| Group 3-BH | 10 | 0<br>0% | 0<br>0% | 10<br>100% |

Percent of "Grouped" Cases Correctly Classified: 95%

Table A28. **Standardized discriminant function coefficients of iron, aluminum, percent fine and total organic carbon for chemical geosediment samples collected during September 1977 and February 1978 at three Texoma study sites.**

| | Standarized Discriminant Function Coefficients | |
|---|---|---|
| | Function 1 | Function 2 |
| Iron | 2.347 | -0.600 |
| Aluminum | 0.734 | 0.668 |
| % Fines | 0.610 | 1.337 |
| Total Organic Carbon | 1.066 | -1.842 |

Table A29. **Classification success matrix of chemical geosediment samples (texture variables) for samples collected during September 1977 and February 1978 at three Texoma study sites.**

| | | Predicted Group Membership | | |
|---|---|---|---|---|
| Actual Group | No. of Cases | West Hackberry | West Hackberry Control | Big Hill |
| Group 1-WH | 25 | 20<br>80% | 0<br>0% | 0<br>0% |
| Group 2-WHC | 9 | 3<br>12% | 9<br>100% | 0<br>0% |
| Group 3-BH | 10 | 2<br>8% | 0<br>0% | 10<br>100% |

Percent of "Grouped" Cases Correctly Classified: 88%

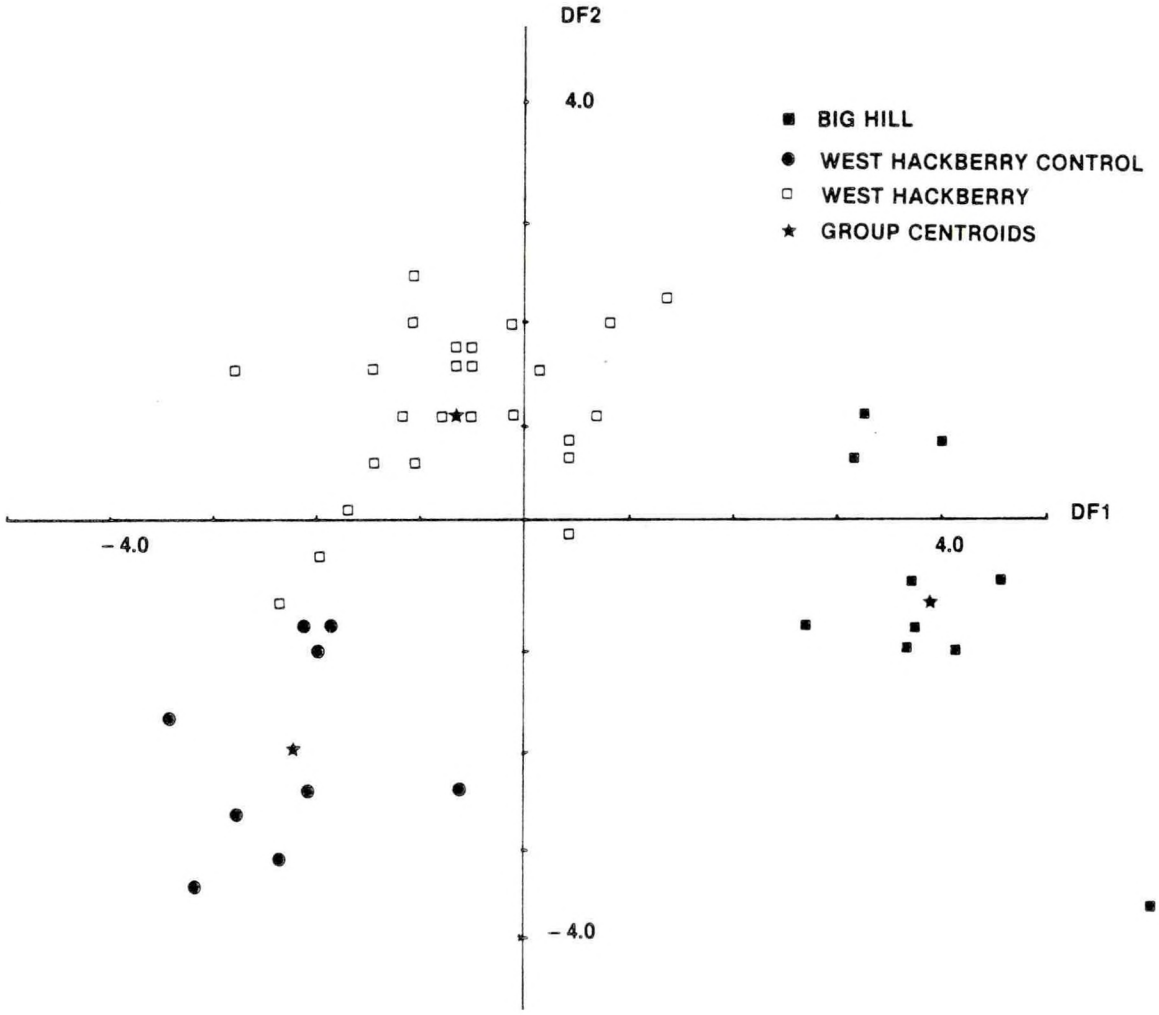


**Figure A16.** **Plot of chemical geosediment samples on discriminant functions 1 and 2 using trace metal variables (copper, chromium, lead, manganese, nickel, zinc, and cadmium) for samples collected at the West Hackberry, West Hackberry Control and Big Hill sites during September 1977 and February 1978.**

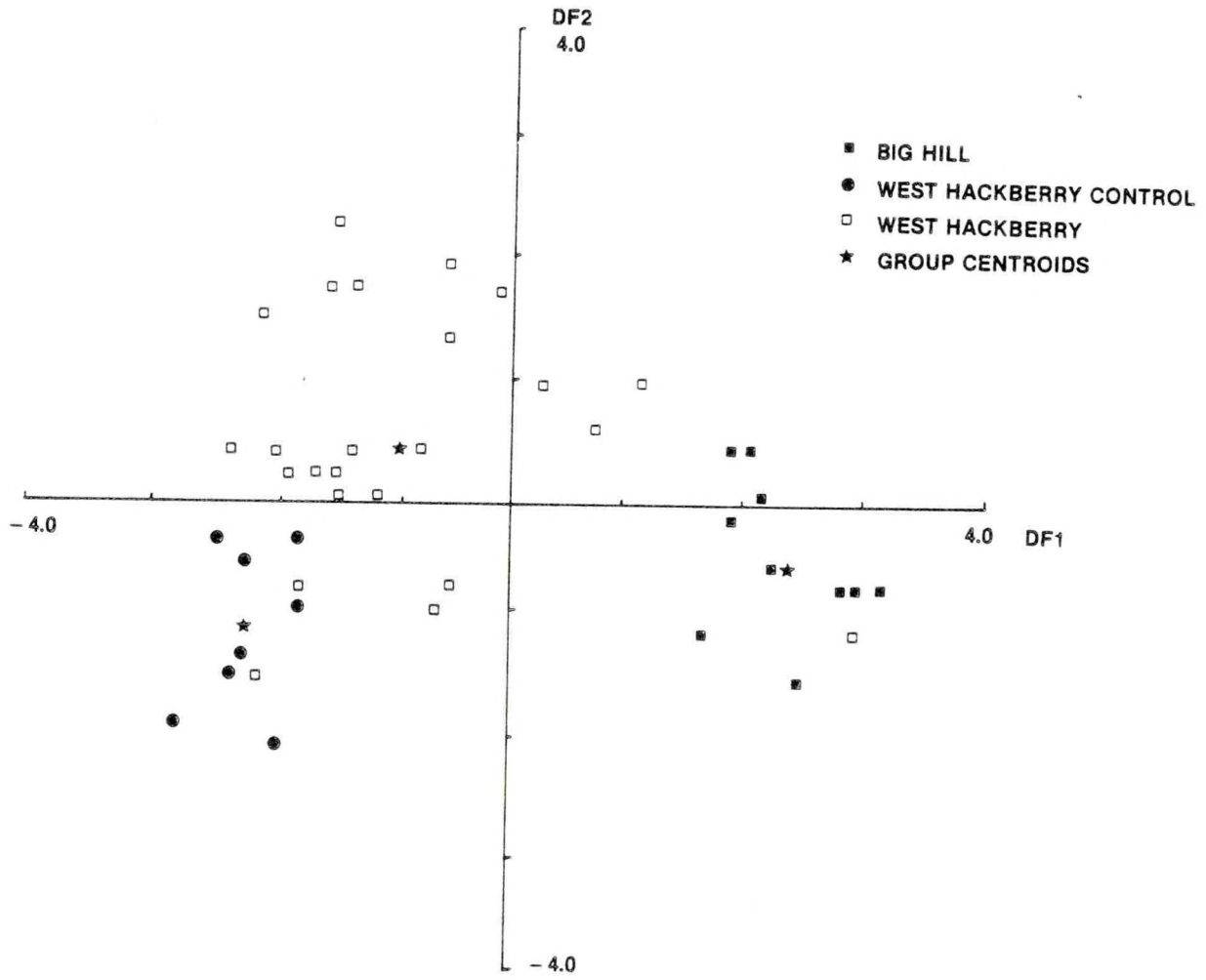


**Figure A17.** **Plot of chemical geosediment samples on discriminant functions 1 and 2 using texture variables (iron, aluminum, %fine, %TOC) for samples collected at the West Hackberry, West Hackberry control and Big Hill sites during September 1977 and February 1978.**

$$H_o: \quad \mu_1 = \mu_2 = \mu_3,$$

where $\mu_i$ equals the centroid of group i (i = 1, 2, 3) and $F_{.001}$ (14,70) = 3.31 compared to calculated F of 16.885. Thus, the group centroids of the three sites are significantly different. The position of the group centroids along the first discriminant function (axis DF1 in Figure A16) suggests that the West Hackberry and West Hackberry Control samples are more similar to each other than either is to the Big Hill samples. Typically, the West Hackberry samples are distinguished from those at Big Hill by lead and zinc concentrations (Table 26).

The classification success matrix (Table A27) shows that the chemical variables are very successful in discriminating the three sites with 95% correct classification. All West Hackberry Control and Big Hill samples were properly classified. For West Hackberry, two samples were misclassified as belonging to the West Hackberry Control group. This fits the geographical layout of the sites quite well. Big Hill samples are clearly distinct from the other sites while West Hackberry samples grade into West Hackberry Control samples. It appears that the first two discriminant functions account for virtually all the variance between groups.

Very similar results are seen for the sediment texture analysis (Tables A25, A28 and A29). Here again, all the Big Hill and West Hackberry Control samples were classified correctly (Table A29). However, of the 25 West Hackberry samples, only 20 (80%) were classified in the original group. Three were classified in the West Hackberry Control group and two in the Big Hill group, and these misclassifed samples can be identified in Figure A17. The variable which was the best discriminator of groups was total organic carbon. Again only the first two canonical correlations (between the sediment variables and the dummy criterion of group variables) are of interest, with little variance explained by subsequent discriminant functions.

Note that of the two discriminant functions which were identified as being statistically significant in these analyses (Tables A24 and A25), the first (based on eigenvalues) explained an overwhelming amount of variance, making it an extremely effective discriminator of geochemical groupings. Also note the very good correspondence between site groupings (Figures A16 and A17) for the "textural" and trace metal analyses. That is, of course, due to the close functional correspondence between the two suites of parameters as seen in the canonical correlation results (Tables A22 and A23).

As a last example, the power of discriminant analysis is extended by determining temporal differences at the Big Hill site. For this analysis, the data from June 1978 and August 1978 collections are included with those for September 1977 and February 1978, yielding four (temporal) groups. The results (Tables A30 to A32, and Figure A18 show that the four seasonal collections at the Big Hill site could be discriminated even though there was clearly greater between site variability than temporal variability in the data (as seen by the fact that the previous analyses over three sites showed the Big Hill data from September 1977 and February 1978 to be in the same group). The overall F test leads to a rejection of the null hypothesis that there are no significant differences in group centroids

$$H_0: \quad \mu_1 = \mu_2 = \mu_3 = \mu_4$$

with $F_{.001}\ (8,76) = 3.87$, compared to calculated F of 13.30.

The greatest strength of the discriminant function, and its most useful application to impact assessment is its predictive capability. Once a discriminant function is established, it can be used to classify new obeservations. That is, if we develop a discriminant function that successfully delineates the pre-discharge baseline, we could then apply it to postdischarge samples to assess change. In the process the variables which were most important for predictive purposes would be defined.

Table A30. **Relative importance of discriminant functions based on relative percent of eigenvalue and canonical correlation using trace metal and textural data for samples collected from the Big Hill study site during four cruises in 1977 and 1978.**

| Discriminant Function | Eigenvalue | Relative Percentage | Canonical Correlation |
|---|---|---|---|
| 1 | 97.44 | 93.65 | 0.995 |
| 2 | 5.86 | 5.63 | 0.924 |

Table A31. **Standardized discriminant functions coefficients of copper (Cu), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni), cadmium (Cd), percent fines, and calcium carbonate ($CaCo_3$) for samples collected from the Big Hill study site during four cruises in 1977 and 1978.**

| | Standardized Discriminant Function Coefficients | |
|---|---|---|
| | Function 1 | Function 2 |
| Cu | 0.6287 | -0.0958 |
| Cr | 0.2684 | -0.7458 |
| Pb | -0.2780 | -0.2826 |
| Mn | 0.1366 | -0.1670 |
| Ni | -0.1706 | 1.4491 |
| Cd | 0.1102 | 0.0743 |
| Fines | 0.1168 | 0.3601 |
| $CaCO_3$ | 0.5173 | -0.1571 |

**Table A32. Classification success matrix of samples collected during four cruises at the Big Hill site during 1977 and 1978 based on trace metal and textural variables.**

| Actual Group | No. of Cases | Predicted Group Membership | | | |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Group 1-Sept 1977 | 5 | 5<br>100.0% | 0<br>0.0% | 0<br>0.0% | 0<br>0.0% |
| Group 2-Feb 1978 | 5 | 0<br>0.0% | 5<br>100.0% | 0<br>0.0% | 0<br>0.0% |
| Group 3-June 1978 | 7 | 0<br>0.0% | 0<br>0.0% | 7<br>100.0% | 0<br>0.0% |
| Group 4-Aug 1978 | 7 | 0<br>0.0% | 0<br>0.0% | 1<br>14.3% | 6<br>85.7% |

Percent of "Grouped" Cases Correctly Classified: 95.8%.

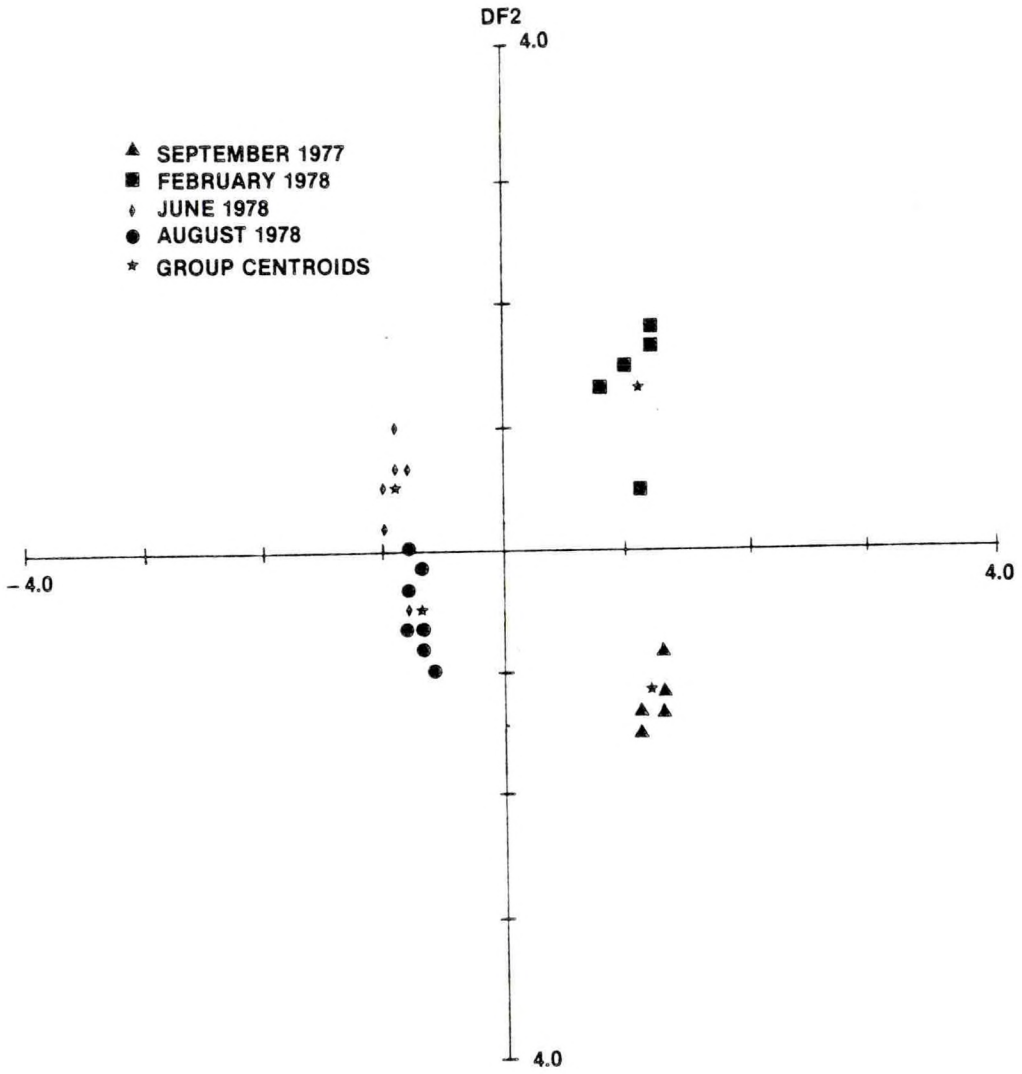


**Figure A18.** **Plot of chemical geosediment samples on discriminant functions 1 and 2 using texture and trace metal variables for samples collected at the Big Hill site during September 1977, and February, June, and August 1978.**

From Comiskey et al. (1979)

Since the discriminant analysis procedure which we employ is a step-wise procedure, the last variable entered into the analysis (least significant) could be eliminated from future analyses, with little loss in discriminating power. This could save many dollars in a sampling program by eliminating sampling and analyses not important or necessary to achieve the goals of the program. Thus, only those variables most responsible for group segregation would be monitored, with variables which are of little value eliminated from the program.

In summary, the three Texoma sites used in the discriminant analysis are shown to be very discrete based on either the suite of leachable trace metals in the sediments or the sediment properties themselves. The within site similarity far exceeds that among sites, even over several cruises, indicating that spatial patterns are stronger than temporal patterns in the sediment system. However, when the spatial factor is restricted, as in the analyses involving only Big Hill samples from four cruises, the analysis was able to discriminate the seasonal groupings. In fact, 100% success in seasonal discrimination was achieved on the basis of trace metal composition of the sediments.

## A.6 HYPOTHESIS TESTING

The process by which hypothesis testing can be utilized to determine the significance of differences in main effects for the Bryan Mound nekton data is now discussed. The design of the hypothesis testing procedure is obviously restricted by the limitations of the data collected. The initial design involved a two-way ANOVA, with five cruises and four stations, and two replicates per cruise/station stratum. This design is identical to that used in the calculation of Tukey's one degree of freedom test for nonadditivity (see Table A8). Based on previous analyses, transformed data ($\log_{10}$ + 1) were utilized for number of species and number of individuals in each sample.

The results of the ANOVAs (Tables A33 and A34) are virtually identical for the two independent variables. In both cases, cruise is highly significant, while neither station nor the interaction term is significant. Therefore, the results of the ANOVAs indicate that some

**Table A33. Results of two-way fixed effects factorial ANOVAs for total number of individuals (untransformed and $log_{10}$ transformed) of demersal nekton collected in trawl samples during five daytime cruises at four stations at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

Variable: Number of individuals

FACTORS: CRUISE BY STATION

| SOURCE OF VARIATION | SUM OF SQUARES | DF | MEAN SQUARE | F | SIGNIF OF F |
|---|---|---|---|---|---|
| MAIN EFFECTS | 97105.250 | 7 | 13872.179 | 3.669 | 0.010 |
| Cruise | 88806.250 | 4 | 22201.563 | 5.871 | 0.003 |
| Station | 8299.000 | 3 | 2766.335 | 0.732 | 0.545 |
| 2-WAY INTERACTIONS | 38255.750 | 12 | 3187.979 | 0.843 | 0.610 |
| Cruise x Station | 38255.750 | 12 | 3187.979 | 0.843 | 0.610 |
| EXPLAINED | 135361.000 | 19 | 7124.263 | 1.884 | 0.084 |
| RESIDUAL | 75627.016 | 20 | 3781.351 | | |
| TOTAL | 210988.016 | 39 | 5409.949 | | |

Variable: $Log_{10}$ number of individuals

FACTORS: CRUISE BY STATION

| SOURCE OF VARIATION | SUM OF SQUARES | DF | MEAN SQUARE | F | SIGNIF OF F |
|---|---|---|---|---|---|
| MAIN EFFECTS | 4.869 | 7 | 0.695 | 8.896 | 0.000 |
| Cruise | 4.654 | 4 | 1.164 | 14.897 | 0.000 |
| Station | 0.210 | 3 | 0.070 | 0.894 | 0.461 |
| 2-WAY INTERACTIONS | 1.110 | 12 | 0.092 | 0.894 | 0.357 |
| Cruise x Station | 1.110 | 12 | 0.092 | 0.894 | 0.357 |
| EXPLAINED | 5.974 | 19 | 0.314 | 4.025 | 0.002 |
| RESIDUAL | 1.562 | 20 | 0.078 | | |
| TOTAL | 7.536 | 39 | 0.193 | | |

**Table A34. Results of two-way fixed effects factorial ANOVAs for total number of species (untransformed and $log_{10}$ transformed) of demersal nekton collected in trawl samples during five daytime cruises at four stations at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

Variable: Number of species

FACTORS: CRUISE BY STATION

| SOURCE OF VARIATION | SUM OF SQUARES | DF | MEAN SQUARE | F | SIGNIF OF F |
|---|---|---|---|---|---|
| MAIN EFFECTS | 85.225 | 7 | 12.175 | 10.802 | 0.000 |
| Cruise | 75.750 | 4 | 18.938 | 16.833 | 0.000 |
| Station | 9.475 | 3 | 3.158 | 2.807 | 0.066 |
| 2-WAY INTERACTIONS | 29.650 | 12 | 2.471 | 2.196 | 0.058 |
| Cruise x Station | 29.650 | 12 | 2.471 | 2.196 | 0.058 |
| EXPLAINED | 114.875 | 19 | 6.046 | 5.374 | 0.000 |
| RESIDUAL | 22.500 | 20 | 1.250 | | |
| TOTAL | 137.375 | 39 | 3.522 | | |

Variable: $Log_{10}$ number of species

FACTORS: CRUISE BY STATION

| SOURCE OF VARIATION | SUM OF SQUARES | DF | MEAN SQUARE | F | SIGNIF OF F |
|---|---|---|---|---|---|
| MAIN EFFECTS | 0.459 | 7 | 0.066 | 8.690 | 0.000 |
| Cruise | 0.420 | 4 | 0.105 | 13.916 | 0.000 |
| Station | 0.039 | 3 | 0.013 | 1.722 | 0.195 |
| 2-WAY INTERACTIONS | 0.133 | 12 | 0.009 | 1.251 | 0.318 |
| Cruise x Station | 0.133 | 12 | 0.009 | 1.251 | 0.318 |
| EXPLAINED | 0.572 | 19 | 0.030 | 3.992 | 0.002 |
| RESIDUAL | 0.151 | 20 | 0.008 | | |
| TOTAL | 0.723 | 39 | 0.019 | | |

cruise(s) is (are) significantly different from some other cruise(s), but we do not know precisely which are showing significantly different means. This is the job of multiple means testing where pairwise comparison of means among all levels of the significant main effect are made. In a two-way design, there is an important limitation to the use of multiple means testing. If the interaction term was significant in the two-way ANOVA, then simple multiple means testing for those variables cannot be employed (it would be meaningless). This is often a major problem with biological data. However, in the case of our exemplary analysis, the interaction term is not significant.

Either of two steps could now be taken. The means could be tested on the basis of the error mean square from the two-way ANOVA (0.084 in Tables A33 and A34) or a new one-way ANOVA can be run, disregarding station as a class variable (since it was not significant). In this case, the design would reduce to five cruises and eight replicates/cruise. The results of these one-way ANOVAs are presented in Table A35. Compared to the error mean square for the previous design, the error mean square for the total number of individuals is somewhat smaller in the one-way design. Since the number of degrees of freedom for the error mean square also increases, there is more likelihood of seeing significant differences with this reduced design (greater power).

Results of multiple means tests (Duncan's) are shown in Table A36 for numbers of individuals and number of species. For number of individuals, the July samples have a significantly higher mean and the December samples have a significantly lower mean than the samples from the other three months (which are not significantly different from one another). For the number of species, the July samples again showed a significantly higher mean. Only the samples with the two lowest means (significantly lower than any other mean) were not significantly different from each other.

The results of this analysis indicate that it makes no sense whatsoever to identify specific stations within the immediate area of the brine diffuser. The area should be treated as one homogeneous area and random

Table A35. **One-way ANOVAs for untransformed and $log_{10}$ transformed total number of individuals and number of species for demersal nekton collected in trawl samples during five daytime cruises at the Bryan Mound offshore diffuser site from July 1978 to April 1979.**

VARIABLE: Total number of individuals

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 4 | 88806.2500 | 22201.5625 | 6.360 | 0.0006 |
| WITHIN GROUPS | 35 | 122181.7500 | 3490.9072 | | |
| TOTAL | 39 | 210988.0000 | | | |

VARIABLE: $Log_{10}$ total number of individuals

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 4 | 4.6543 | 1.1636 | 14.133 | 0.0000 |
| WITHIN GROUPS | 35 | 2.8816 | 0.0823 | | |
| TOTAL | 39 | 7.5359 | | | |

VARIABLE: Total number of species

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 4 | 75.7500 | 18.9375 | 10.756 | 0.0000 |
| WITHIN GROUPS | 35 | 61.6250 | 1.7607 | | |
| TOTAL | 39 | 137.3750 | | | |

VARIABLE: $Log_{10}$ total number of species

ANALYSIS OF VARIANCE

| SOURCE | D.F. | SUM OF SQUARES | MEAN SQUARES | F RATIO | F PROB. |
|---|---|---|---|---|---|
| BETWEEN GROUPS | 4 | 0.4202 | 0.1050 | 12.122 | 0.0000 |
| WITHIN GROUPS | 35 | 0.3033 | 0.0087 | | |
| TOTAL | 39 | 0.3033 | | | |

Table A36. **Results of Duncan's Multiple Range Test for selected nekton variables collected in trawl samples at the Bryan Mound offshore diffuser site during five daytime cruises from July 1978 to April 1979.**

Duncan's multiple means test*

Variable: $Log_{10}$ number of individuals

| Date | 4/20 | 12/13 | 9/15 | 2/24 | 7/15 |
|---|---|---|---|---|---|
| Mean | 2.09 | 1.98 | 1.89 | 1.37 | 1.20 |

Variable: $Log_{10}$ number of species

| Date | 9/15 | 12/13 | 4/20 | 2/24 | 7/15 |
|---|---|---|---|---|---|
| Mean | 0.87 | 0.77 | 0.70 | 0.62 | 0.58 |

* Groups of means which are underlined by a dashed line are not significantly different at alpha=0.05.

sampling within the area should be conducted. This would greatly decrease the number of samples required to characterize the diffuser area.

In this exemplary analysis, we have conducted a posteriori means testing. That is, we did not specify ahead of time what specific means tests we were interested in. A priori means tests can also be utilized if the investigator has a thorough knowledge of the behavior of the parameters and ecosystem he is investigating. A priori hypothesis testing, therefore, is most applicable to later stages in an impact assessment program, and the design for this hypothesis testing should result from applications of the analysis system.

One of the major concerns in a sampling program is determining the number of replicates required to see a specific difference in two means, given particular $\alpha$ and $\beta$ levels. The use of the procedure to determine adequacy of replication requires that the user know what he wants to see from the program. Is the ability to detect a 10% difference in means necessary or feasible? Detection levels will differ for different taxa groups and for the same group in different habitats, and will be determined to some degree by fiscal constraint. The goals of an impact assessment program should reflect the inherent variability of the ecosystem.

Table A37 and Figure A19 show the number of samples required to detect a given percent difference between means for a number of variables for the April 5 and April 20, 1979 nekton collections (24 replicates/collection date) at the Bryan Mound offshore diffuser site. Type I ($\alpha$) and Type II ($\beta$) error levels were set at 0.01 and 0.10. In most applications, the $\alpha$ level is set at 0.05. Therefore, the results presented here will indicate the need for more replicates than would normally be required for most applications. Consistent with previous results, the number of species shows the least variability, with 11 to 15 samples required to detect a 50 percent difference in means for the two April cruises. Comparable numbers of samples for total number of individuals and numbers of P. aztecus for the two cruises are 25 to 151 and 42 to 63, respectively. These results reemphasize that the number of samples

Table A37. **Number of samples required to detect a given percent difference in means for (a) total number of individuals, (b) number of species and (c) number of P. aztecus, for $\alpha$ = 0.01 and $\beta$ = 0.90. Data are for nekton trawl samples collected at the Bryan Mound offshore diffuser site during April 5 and April 20 1979.**

April 5, 1979

| | Number of individuals | Number of species | P. aztecus |
|---|---|---|---|
| Mean | 49.04 | 8.33 | 8.08 |
| SD | 54.77 | 2.55 | 5.73 |
| % difference | Number of samples | | |
| 10 | 3722 | 281 | 1500 |
| 20 | 930 | 73 | 376 |
| 30 | 415 | 34 | 170 |
| 40 | 234 | 20 | 77 |
| 50 | 151 | 15 | 63 |
| 60 | 105 | 12 | 45 |
| 70 | 78 | 10 | 33 |
| 80 | 61 | 9 | 26 |
| 90 | 49 | 9 | 22 |
| 100 | 40 | 8 | 18 |

April 20, 1979

| | | | |
|---|---|---|---|
| Mean | 412.5 | 9.63 | 1.21 |
| SD | 177.16 | 2.04 | 2.13 |
| % difference | Number of samples | | |
| 10 | 551 | 136 | 963 |
| 20 | 140 | 36 | 290 |
| 30 | 64 | 18 | 110 |
| 40 | 37 | 12 | 63 |
| 50 | 25 | 11 | 42 |
| 60 | 18 | 9 | 30 |
| 70 | 15 | 6 | 23 |
| 80 | 12 | 4 | 18 |
| 90 | 11 | 4 | 15 |
| 100 | 10 | 3 | 13 |

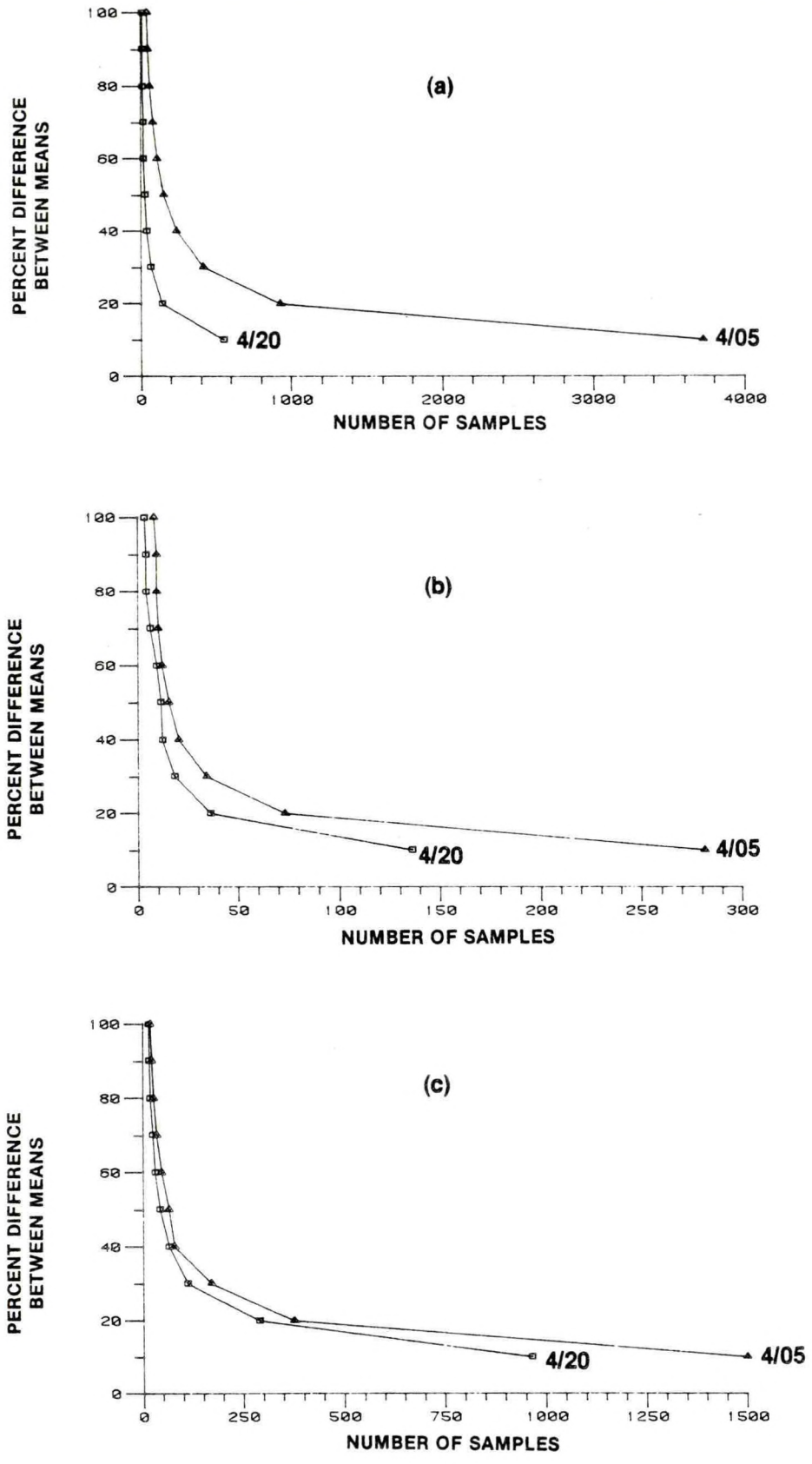


**Figure A19.** **Relationship between sample size and percent difference between means which can be detected ( $\alpha$ = .01, $\beta$ = .90) for (a) total number of individuals, (b) number of species, and (c) number of P. aztecus. Data are for nekton trawl samples collected at the Bryan Mound offshore diffuser site during April 5 and April 20 1979.**

required will vary depending on the variables in question and that the number of samples required may vary seasonally or spatially. This exercise stresses the need for adequate baseline efforts or analysis of historical data before an impact assessment sampling program is designed.

Although we have rejected the null hypothesis of no mean cruise differences at $\alpha = .05$ (or a one in twenty chance of falsely rejecting the null hypothesis) we still want to know the Type II or $\beta$ error. In other words, for the one-way ANOVAs we have conducted, what were the probabilities that we falsely accepted the null hypothesis? Table A38 presents the results of the power function calculations using the procedures in Table 16 of this report. In all cases the power of the ANOVAs is excellent and reflects the large ratio of the mean square among treatment levels to the error mean square.

**Table A38. Power of the one-way ANOVA for selected nekton variables (untransformed and $\log_{10}$ transformed) collected in trawl samples during five daytime cruises at the Bryan Mound offshore diffuser site during the period July 1978 to April 1979.**

Power of one-way ANOVA,

$\alpha = 0.05$ $\quad v_1 = 4, v_2 = 35$

| Variable | $\phi$ | Power |
|---|---|---|
| Number of individuals | 2.07 | 0.95 |
| Number of species | 2.79 | > 0.99 |
| *P. aztecus* | 2.33 | 0.99 |
| *C. nothus* | 2.19 | 0.97 |
| $\log_{10}$ number of individuals | 3.24 | > 0.99 |
| $\log_{10}$ number of species | 3.10 | > 0.99 |
| $\log_{10}$ *P. aztecus* | 3.64 | > 0.99 |
| $\log_{10}$ *C. nothus* | 3.80 | > 0.99 |

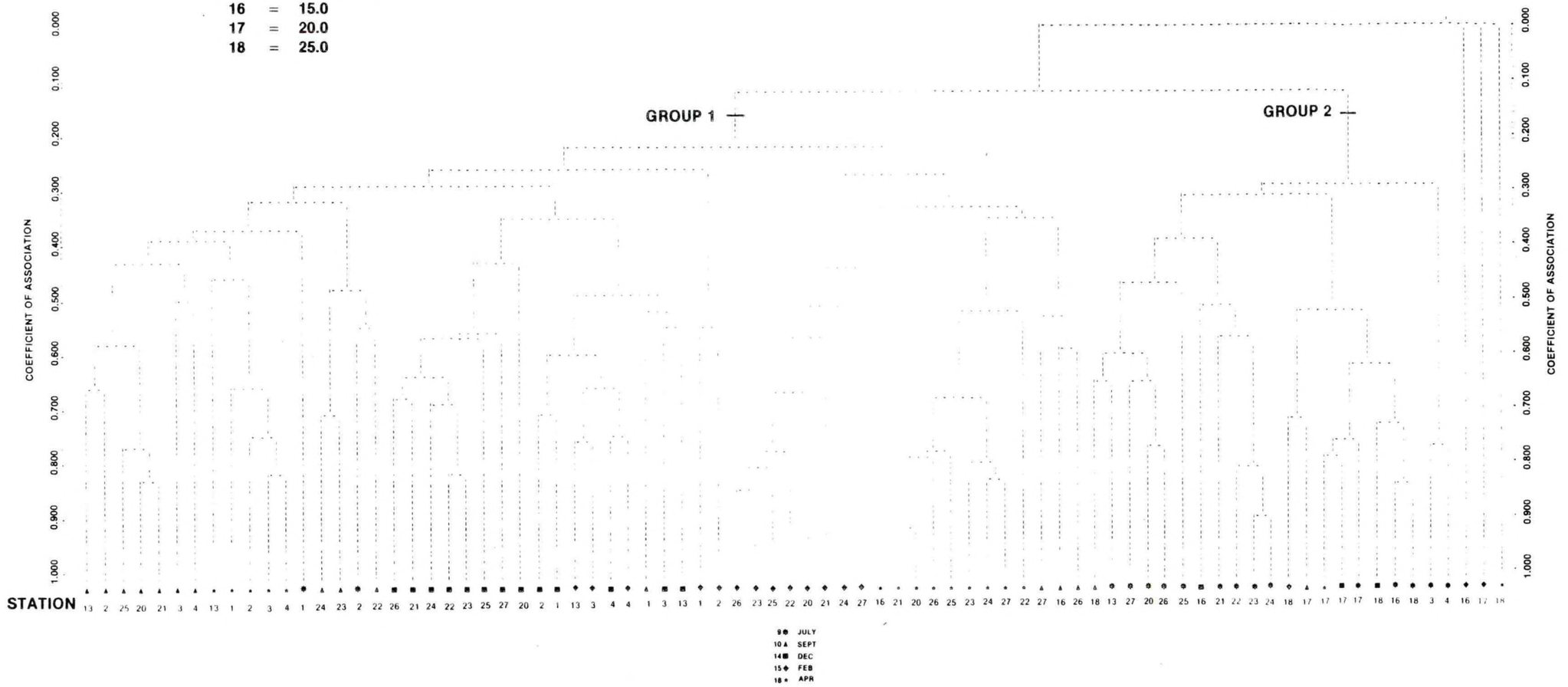


**Figure A7. Dendrogram of cluster analysis of nekton samples (selected inshore to offshore stations) from July 1978 to April 1979 in the vicinity of the Bryan Mound offshore diffuser site.**

# APPENDIX B

## STDERR - Standard error

Purpose: This subroutine calculates the standard error of the mean of a variable for a set of replicate samples.

Application: The standard error of the mean provides an index of the variability of the population mean. (see Section IV.2.2.1).

Entry: CALL STDERR (X, N, SM)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points. | one dimensional, floating-point array; input |
| N | is the number of elements in the X array | integer variable; input |
| SM | contains the standard error | floating-point variable; output |

Mathematical Method: $SM = \frac{s^2}{N}$

where $s^2$ is the variance of the X array.

STAT-PACK Subroutines Required: STDEV

```
      SUBROUTINE STDERR (X,N,SM)
      DIMENSION X(N)
C
C                          SUBROUTINE STDERR
C
C     FUNCTION: CALCULATE THE STANDARD ERROR OF THE MEAN OF AN ARRAY OF
C               OBSERVATIONS.
C
C     VARIABLE     DESCRIPTION                          TYPE
C     --------     -----------                          ----
C
C     X            ARRAY OF OBSERVATIONS                REAL; INPUT
C     N            NUMBER OF OBSERVATIONS               INTEGER; INPUT
C     SM           STANDARD ERROR OF MEAN               REAL; OUTPUT
C     XND          ARGUMENT FOR SUBROUTINE STDEV        REAL; INTERNAL
C     S            STANDARD DEVIATION                   REAL; INTERNAL
C
C     STAT-PACK SUBROUTINES CALLED: STDEV (X,N,XND,S)
C
      XND = -1.0
      CALL STDEV (X,N,XND,S)
      SM = SQRT((S ** 2) / FLOAT(N))
      RETURN
      END
```

## CUMMN - Cumulative means

Purpose: This subroutine calculates the successive cumulative means across a series of replicates.

Application: Calculating the cumulative mean across a series of replicates should demonstrate the number of replicates at which the estimate of the mean tends to stabilize (see Section IV 2.2.1). Output of this procedure should be graphically displayed for maximum interpretability.

Entry: CALL CUMMN (X, N, CX)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points | one dimensional floating point array; input |
| N | is the number of elements in the array X | integer variable; input |
| CX | is the array of cummulative means. | one dimensional floating-point array; output |

Mathematical Method: $$CX_i = \frac{\sum_{k=1}^{i} X_k}{i}$$

where $X_k$ is the kth element of X and i ranges from 1 to N

```
      SUBROUTINE CUMMN (X,N,CX)
      DIMENSION X(N), CX(N)
C
C                              SUBROUTINE CUMMN
C
C
C     FUNCTION: CALCULATE THE POOLED MEAN FOR A SERIES OF REPLICATES.
C
C     VARIABLE      DESCRIPTION                          TYPE; USE
C     --------      -----------                          ---------
C
C     X             ARRAY OF SAMPLE MEANS                REAL; INPUT
C     N             NUMBER OF SAMPLE MEANS               INTEGER; INPUT
C     CX            ARRAY OF POOLED MEANS                REAL; OUTPUT
C     TX            SUM OF SAMPLE MEANS                  REAL; INTERNAL
C
C
      TX = 0.0
      DO 100 K = 1,N
             TX = TX + X(K)
             CX(K) = TX / FLOAT(K)
100   CONTINUE
      RETURN
      END
```

## WTEST - Shapiro-Wilk W test

Purpose: This subroutine calculates the Shapiro-Wilk W statistic which is used to determine if data from a set of replicated samples are normally distributed.

Application: Because normality is an assumption of many parametric analyses, the testing of this assumption may be critical to the proper use of these analyses. (see Section IV.2.2.2).

Entry: CALL WTEST (X, N, W)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points in ascending order of value. | one dimensional floating-point array; input |
| N | is the number of elements in the array X | integer variable; input |
| W | contains the W statistic | floating-point variable; output |

Mathematical Method:

$$W = \frac{b^2}{\sum_{i=1}^{N} (X_i - \bar{X})^2}$$

where $X_i$ is the ith element of X, $\bar{X}$ is the arithmetic mean of the elements in X and b is given by:

$$b = \sum_{i=1}^{N/2} a_{n-i+1} (X_{n-i+1} - X_i)$$

Both $X_{n-i+1}$ and $X_i$ are elements of the array X and the coefficent $a_{n-i+1}$ is a table value stored in an internal array of the subroutine. The denominator used in calculating W is given by:

$$\sum_{i=1}^{N} (X_i - \bar{X})^2 = \sum_{i=1}^{N} X_i - \frac{(\sum_{i=1}^{N} (X_i)^2)}{N}$$

Constraints: The subroutine can handle samples containing up to 25 observations. Additional coefficents must be added to the internal array for samples with more than 25 observations. Coefficents for sample sizes of up to 50 can be found in Appendix 9 of Anderson and McLean (1974). The elements of array X must be in ascending order for this subroutine to function correctly.

```
      SUBROUTINE WTEST (X,N,W)
      DIMENSION X(N), COEF(625)
C
C                              SUBROUTINE WTEST
C
C     FUNCTION: CALCULATE THE SHAPIRO - WILK W STATISTIC TO TEST FOR
C               NORMAL DISTRIBUTION OF OBSERVATIONS.
C
C     VARIABLE      DESCRIPTION                              TYPE; USE
C     --------      -----------                              ---------
C     X             ARRAY OF OBSERVATIONS IN                 REAL; INPUT
C                      ASCENDING ORDER OF VALUE
C     N             NUMBER OF OBSERVATIONS                   INTEGER; INPUT
C     W             W STATISTIC                              REAL; OUTPUT
C     SUM           SUM OF OBSERVATIONS                      REAL; INTERNAL
C     SSQ           SUM OF THE OBSERVATIONS                  REAL; INTERNAL
C                      SQUARED
C     COEF          ARRAY OF SHAPIRO - WILKS                 REAL; INTERNAL
C                      COEFFICIENTS
C
      DATA COEF(1)   /0.7071/
      DATA COEF(2)   /0.7071/
      DATA COEF(3)   /0.6872/,COEF(4)   /0.1677/
      DATA COEF(5)   /0.6646/,COEF(6)   /0.2413/
      DATA COEF(7)   /0.6431/,COEF(8)   /0.2806/,COEF(9)   /0.0875/
      DATA COEF(10)  /0.6233/,COEF(11)  /0.3031/,COEF(12)  /0.1401/
      DATA COEF(13)  /0.6052/,COEF(14)  /0.3164/,COEF(15)  /0.1743/,
     &     COEF(16)  /0.0561/
      DATA COEF(17)  /0.5888/,COEF(18)  /0.3244/,COEF(19)  /0.1976/,
     &     COEF(20)  /0.0947/
      DATA COEF(21)  /0.5739/,COEF(22)  /0.3291/,COEF(23)  /0.2141/,
     &     COEF(24)  /0.1224/,COEF(25)  /0.0399/
      DATA COEF(26)  /0.5601/,COEF(27)  /0.3315/,COEF(28)  /0.2260/,
     &     COEF(29)  /0.1429/,COEF(30)  /0.0695/
      DATA COEF(31)  /0.5475/,COEF(32)  /0.3325/,COEF(33)  /0.2347/,
     &     COEF(34)  /0.1586/,COEF(35)  /0.0922/,COEF(36)  /0.0303/
      DATA COEF(37)  /0.5359/,COEF(38)  /0.3325/,COEF(39)  /0.2412/,
     &     COEF(40)  /0.1707/,COEF(41)  /0.1099/,COEF(42)  /0.0539/
      DATA COEF(43)  /0.5251/,COEF(44)  /0.3318/,COEF(45)  /0.2460/,
     &     COEF(46)  /0.1802/,COEF(47)  /0.1240/,COEF(48)  /0.0727/,
     &     COEF(49)  /0.0240/
      DATA COEF(50)  /0.5150/,COEF(51)  /0.3306/,COEF(52)  /0.2495/,
     &     COEF(53)  /0.1878/,COEF(54)  /0.1353/,COEF(55)  /0.0880/,
     &     COEF(56)  /0.0433/
      DATA COEF(57)  /0.5056/,COEF(58)  /0.3290/,COEF(59)  /0.2521/,
     &     COEF(60)  /0.1939/,COEF(61)  /0.1447/,COEF(62)  /0.1005/,
     &     COEF(63)  /0.0593/,COEF(64)  /0.0196/
      DATA COEF(65)  /0.4968/,COEF(66)  /0.3273/,COEF(67)  /0.2540/,
     &     COEF(68)  /0.1988/,COEF(69)  /0.1524/,COEF(70)  /0.1109/,
     &     COEF(71)  /0.0725/,COEF(72)  /0.0359/
      DATA COEF(73)  /0.4886/,COEF(74)  /0.3253/,COEF(75)  /0.2553/,
     &     COEF(76)  /0.2027/,COEF(77)  /0.1587/,COEF(78)  /0.1197/,
     &     COEF(79)  /0.0837/,COEF(80)  /0.0496/,COEF(81)  /0.0163/
```

```
      DATA COEF(82)  /0.4808/,COEF(83)  /0.3232/,COEF(84)  /0.2561/,
     &     COEF(85)  /0.2059/,COEF(86)  /0.1641/,COEF(87)  /0.1271/,
     &     COEF(88)  /0.0932/,COEF(89)  /0.0612/,COEF(90)  /0.0303/
      DATA COEF(91)  /0.4734/,COEF(92)  /0.3213/,COEF(93)  /0.2565/,
     &     COEF(94)  /0.2085/,COEF(95)  /0.1686/,COEF(96)  /0.1334/,
     &     COEF(97)  /0.1013/,COEF(98)  /0.0711/,COEF(99)  /0.0422/
     &     COEF(100) /0.0140/
      DATA COEF(101) /0.4643/,COEF(102) /0.3185/,COEF(103) /0.2578/,
     &     COEF(104) /0.2119/,COEF(105) /0.1736/,COEF(106) /0.1399/,
     &     COEF(107) /0.1092/,COEF(108) /0.0804/,COEF(109) /0.0530/
     &     COEF(110) /0.0263/
      DATA COEF(111) /0.4590/,COEF(112) /0.3156/,COEF(113) /0.2571/,
     &     COEF(114) /0.2131/,COEF(115) /0.1764/,COEF(116) /0.1443/,
     &     COEF(117) /0.1150/,COEF(118) /0.0878/,COEF(119) /0.0618/
     &     COEF(120) /0.0368/,COEF(121) /0.0122/
      DATA COEF(122) /0.4542/,COEF(123) /0.3126/,COEF(124) /0.2563/,
     &     COEF(125) /0.2139/,COEF(126) /0.1787/,COEF(127) /0.1480/,
     &     COEF(128) /0.1201/,COEF(129) /0.0941/,COEF(130) /0.0696/
     &     COEF(131) /0.0459/,COEF(132) /0.0228/
      DATA COEF(133) /0.4493/,COEF(134) /0.3098/,COEF(135) /0.2554/,
     &     COEF(136) /0.2145/,COEF(137) /0.1807/,COEF(138) /0.1512/,
     &     COEF(139) /0.1245/,COEF(140) /0.0997/,COEF(141) /0.0764/
     &     COEF(142) /0.0539/,COEF(143) /0.0321/,COEF(144) /0.0107/
      DATA COEF(145) /0.4450/,COEF(146) /0.3069/,COEF(147) /0.2543/,
     &     COEF(148) /0.2148/,COEF(149) /0.1822/,COEF(150) /0.1539/,
     &     COEF(151) /0.1283/,COEF(152) /0.1046/,COEF(153) /0.0823/
     &     COEF(154) /0.0610/,COEF(155) /0.0403/,COEF(156) /0.0200/
      SUM = 0.0
      SSQ = 0.0
      DO 100 K = 1,N
             SUM = SUM + X(K)
             SSQ = SSQ + (X(K) ** 2)
100   CONTINUE
      SDIFSQ = SSQ - ((SUM ** 2) / FLOAT(N))
      ILOOP = INT(N / 2)
      ISTRT = INT((N - 1) / 2) * ILOOP
      B = 0.0
      DO 200 ILO = 1,ILOOP
             IPONT = ISTRT + ILO
             IHI = N - ILO + 1
             B = B + (COEF(IPONT) * (X(IHI) - X(ILO)))
200   CONTINUE
      W = (B ** 2) / SDIFSQ
      RETURN
      END
```

## IDXDIS - Index of dispersion

Purpose: The subroutine calculates the index of dispersion of a sequence of data points (e.g., the abundance of a taxon across a number of replicate samples).

Application: The index of dispersion, I, is used to determine the relationship of the variance and the mean of a set of replicated observations (see Section IV.2.2.1).

Entry: CALL IDXDIS (X, N, DISP, CHI, DEGFRE)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points | one dimensional floating-point array; input |
| N | is the number of elements in the array X | integer variable; input |
| DISP | contains the index of dispersion | floating-point variable; output |
| CHI | contains the $\chi^2$ value associated with DISP | floating-point variable; output |
| DEGFRE | contains the degrees of freedom associated with CHI | integer variable; output |

Mathematical Method: $DISP = \frac{s^2}{\bar{x}}$

where $s^2$ is the variance and $\bar{x}$ is the mean of the X array.

STAT-PACK Subroutines Required: STDEV

```
      SUBROUTINE IDXDIS (X,N,DISP,CHI,DEGFRE)
      DIMENSION X(N)
      INTEGER DEGFRE
C
C                              SUBROUTINE IDXDIS
C
C     FUNCTION: CALCULATE THE INDEX OF DISPERSION FOR A SET OF SAMPLES.
C
C     VARIABLE      DESCRIPTION                          TYPE; USE
C     --------      -----------                          ---------
C
C     X             ARRAY OF OBSERVATIONS                REAL; INPUT
C     N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C     DISP          INDEX OF DISPERSION                  REAL; OUTPUT
C     CHI           CHI-SQUARE VALUE ASSOCIATED          REAL; OUTPUT
C                      WITH DISP
C     DEGFRE        DEGREES OF FREEDOM ASSOCIATED        INTEGER; OUTPUT
C                      WITH CHI
C     S             STANDARD DEVIATION OF X ARRAY        REAL; INTERNAL
C     VAR           VARIANCE OF X ARRAY                  REAL; INTERNAL
C
C     STAT-PACK SUBROUTINES CALLED: STDEV (X,N,XND,S)
C
      XND = -1.0
      CALL STDEV (X,N,XND,S)
      VAR = S ** 2
      DISP = VAR / XND
      DEGFRE = N - 1
      CHI = DISP * DEGFRE
      RETURN
      END
```

## MORISIT - Morisita's index

Purpose: This subroutine calculates Morisita's index of dispersion for a set of replicated samples.

Application: Morisita's index is another measure of dispersion in common use in ecological characterization studies. (see Section IV.2.2.1).

Entry: CALL MORISIT (X, N, XMOR, CHI, DEGFRE)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points | one dimensional floating-point array; input |
| N | is the number of elements in the array X | integer variable; input |
| XMOR | contains Morisita's index | floating-point variable; output |
| CHI | contains the $\chi^2$ value associated with XMOR | floating-point variable; output |
| DEGFRE | contains the degrees of freedom associated with CHI | integer variable; output |

Mathematical Method:

$$XMOR = (N)\ \frac{\sum_{i=1}^{N}(X_i)^2 - \sum_{i=1}^{N} X_i}{\left(\sum_{i=1}^{N} X_i\right)^2 - \sum_{i=1}^{N} X_i}$$

where $X_i$ is ith element of X.

```
      SUBROUTINE MORISIT (X,N,XMOR,CHI,DEGFRE)
      DIMENSION X(N)
      INTEGER DEGFRE
C
C                          SUBROUTINE MORISIT
C
C     FUNCTION: CALCULATE MORISITA'S INDEX OF DISPERSION
C
C     VARIABLE     DESCRIPTION                          TYPE; USE
C     --------     -----------                          ---------
C
C     X            ARRAY OF OBSERVATIONS                REAL; INPUT
C     N            NUMBER OF OBSERVATIONS               INTEGER; INPUT
C     XMOR         MORISITA'S INDEX                     REAL; OUTPUT
C     CHI          CHI-SQUARE VALUE ASSOCIATED          REAL; OUTPUT
C                     WITH XMOR
C     DEGFRE       DEGREES OF FREEDOM ASSOCIATED        INTEGER; OUTPUT
C                     WITH CHI
C     SUM          SUM OF OBSERVATIONS                  REAL; INTERNAL
C     SSQ          SUM OF SQUARES OF OBSERVATIONS       REAL; INTERNAL
C
      SUM = 0.0
      SSQ = 0.0
      DO 100 K = 1,N
              SUM = SUM + X(K)
              SSQ = SSQ + (X(K) ** 2)
100   CONTINUE
      XMOR = (FLOAT(N) * (SSQ - SUM)) / ((SUM ** 2) - SUM)
      CHI = XMOR * (SUM - 1) + N - SUM
      DEGFRE = N - 1
      RETURN
      END
```

## TRANS - Data transformations

Purpose: This subroutine performs a user selected transformation to an array of data.

Applications: Data transformations are often required to meet the assumptions of parametric statistical analyses (see Section IV.2.2.3).

Entry: CALL TRANS (X, XT, N, ISELCT)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of data points | one dimensional floating-point array; input |
| XT | is the array containing the transformed data | one dimensional floating-point array; output |
| N | is the number of elements in the array X | integer variable; input |
| ISELCT | is a user specified switch which determines the transformation option | integer variable; input |

Mathematical Method:

ISELCT = 1 Square root, $(X_i)^{\frac{1}{2}}$

ISELCT = 2 Arcsine, arcsine $(X_i)^{\frac{1}{2}}$

ISELCT = 3 Reciprocal, $\frac{1}{X_i}$

ISELCT = 4 Natural logarithm, ln $(X_i+1)$

ISELCT = 5 Natural logarithm, ln $(X_i)$

ISELCT = 6 $(X_{max})^{\frac{1}{2}} - (X_{max} - X_i)^{\frac{1}{2}}$

where $X_i$ is the ith element of X and $X_{max}$ is the largest element of X.

Constraints: All elements of X must be greater than or equal to zero if ISELCT = 1. If ISELCT = 2 is chosen, all elements of X must be in the range of 0 to 1. All elements of X must be nonzero if ISELCT = 3. ISELECT = 4 is chosen for logarithmic transformation if zero entries occur in the data set. ISELECT = 5 is chosen for logarithmic transformation for data with only nonzero entries.

```
      SUBROUTINE TRANS (X,XT,N,ISELCT)
      DIMENSION X(N), XT(N)
C
C                          SUBROUTINE TRANS
C
C     FUNCTION: DRIVER SUBROUTINE FOR VARIOUS DATA TRANSFORMATION OPTIONS.
C
C     VARIABLE     DESCRIPTION                          TYPE; USE
C     --------     -----------                          ---------
C     X            ARRAY OF OBSERVATIONS                REAL; INPUT
C     XT           ARRAY OF TRANSFORMED                 REAL; OUTPUT
C                     OBSERVATIONS
C     N            NUMBER OF OBSERVATIONS               INTEGER; INPUT
C     ISELCT       SWITCH USED TO SELECT THE            INTEGER; INPUT
C                     TRANSFORMATION OPTION
C
C     TRANSFORMATION OPTIONS
C
C     ISELCT       SUBROUTINE CALLED          DESCRIPTION
C     ------       -----------------          -----------
C        1             TRANS1                 SQUARE ROOT
C        2             TRANS2                 ARC SINE
C        3             TRANS3                 RECIPROCAL
C        4             TRANS4                 NATURAL LOG; LN(X(I) + 1)
C        5             TRANS5                 NATURAL LOG; LN(X(I))
C        6             TRANS6
C
      IF (ISELCT .GE. 1 .AND. ISELCT .LE. 6)          GO TO 10
            WRITE (6,6000)
6000        FORMAT (//,35H***INVALID TRANSFORMATION OPTION***,//)
            RETURN
10    GO TO (20,30,40,50,60,70),ISELCT
C
C     SQUARE ROOT TRANSFORMATION
C
20    CALL TRANS1 (X,XT,N)
      RETURN
C
C     ARC SINE TRANSFORMATION
C
30    CALL TRANS2 (X,XT,N)
      RETURN
C
C     RECIPROCAL TRANSFORMATION
C
40    CALL TRANS3 (X,XT,N)
      RETURN
C
C     NATURAL LOGARITHM TRANSFORMATION; LN(X(I) + 1)
C
50    CALL TRANS4 (X,XT,N)
      RETURN
C
```

```
C       NATURAL LOGARITHM TRANSFORMATION; LN(X(I))
C
60      CALL TRANS5 (X,XT,N)
        RETURN
C
C       (XMAX)**0.5 - (XMAX - X(I))**0.5
C
70      CALL TRANS6 (X,XT,N)
        RETURN
        END
C
C
C
        SUBROUTINE TRANS1 (X,XT,N)
        DIMENSION X(N), XT(N)
C
C                           SUBROUTINE TRANS1
C
C       FUNCTION: PERFORMS A SQUARE ROOT TRANSFORMATION ON A SET OF
C                 OBSERVATIONS.
C
C       VARIABLE      DESCRIPTION                          TYPE; USE
C       --------      -----------                          ---------
C       X             ARRAY OF OBSERVATIONS                REAL; INPUT
C       XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C       N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C
        DO 100 K = 1,N
              XT(K) = SQRT(X(K))
100     CONTINUE
        RETURN
        END
C
C
C
        SUBROUTINE TRANS2 (X,XT,N)
        DIMENSION X(N), XT(N)
C
C                           SUBROUTINE TRANS2
C
C       FUNCTION: PERFORMS AN ARC SINE TRANSFORMATION ON A SET OF
C                 OBSERVATIONS.
C
C       VARIABLE      DESCRIPTION                          TYPE; USE
C       --------      -----------                          ---------
C       X             ARRAY OF OBSERVATIONS                REAL; INPUT
C       XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C       N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C
        DO 100 K = 1,N
              XT(K) = ASIN(X(K))
100     CONTINUE
        RETURN
```

```
      END
C
C
C
      SUBROUTINE TRANS3 (X,XT,N)
      DIMENSION X(N), XT(N)
C
C                          SUBROUTINE TRANS3
C
C     FUNCTION: PERFORMS A RECIPROCAL TRANSFORMATION ON A SET OF
C               OBSERVATIONS.
C
C     VARIABLE      DESCRIPTION                          TYPE; USE
C     --------      -----------                          ---------
C     X             ARRAY OF OBSERVATIONS                REAL; INPUT
C     XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C     N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C
      DO 100 K = 1,N
             XT(K) = 1. / X(K)
100   CONTINUE
      RETURN
      END
C
C
C
      SUBROUTINE TRANS4 (X,XT,N)
      DIMENSION X(N), XT(N)
C
C                            SUBROUTINE TRANS4
C
C     FUNCTION: PERFORMS A NATURAL LOG TRANSFORMATION ON A SET OF
C               OBSERVATIONS.
C
C     VARIABLE      DESCRIPTION                          TYPE; USE
C     --------      -----------                          ---------
C     X             ARRAY OF OBSERVATIONS                REAL; INPUT
C     XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C     N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C
      DO 100 K = 1,N
             XT(K) = ALOG(X(K) + 1.0)
100   CONTINUE
      RETURN
      END
C
C
C
      SUBROUTINE TRANS5 (X,XT,N)
      DIMENSION X(N), XT(N)
C
C                            SUBROUTINE TRANS5
C
```

```
C       FUNCTION: PERFORMS A NATURAL LOG TRANSFORMATION ON A SET OF
C                 OBSERVATIONS.
C
C       VARIABLE      DESCRIPTION                          TYPE; USE
C       --------      -----------                          ---------
C       X             ARRAY OF OBSERVATIONS                REAL; INPUT
C       XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C       N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C
        DO 100 K = 1,N
               XT(K) = ALOG(X(K))
100     CONTINUE
        RETURN
        END
C
C
C
        SUBROUTINE TRANS6 (X,XT,N)
        DIMENSION X(N), XT(N)
C
C                              SUBROUTINE TRANS6
C
C       FUNCTION: PERFORMS A SQUARE ROOT FUNCTION TRANSFORMATION ON A SET
C                 OF OBSERVATIONS.
C
C       VARIABLE      DESCRIPTION                          TYPE; USE
C       --------      -----------                          ---------
C       X             ARRAY OF OBSERVATIONS                REAL; INPUT
C       XT            ARRAY OF TRANSFORMED OBSERVATIONS    REAL; OUTPUT
C       N             NUMBER OF OBSERVATIONS               INTEGER; INPUT
C       XMAX          MAXIMUM VALUE OF THE X ARRAY         REAL; INTERNAL
C
        XMAX = 0.0
        DO 100 K = 1,N
               XMAX = AMAX1(XMAX,X(K))
100     CONTINUE
        DO 200 K = 1,N
               XT(K) = SQRT(XMAX) - SQRT(XMAX - X(K))
200     CONTINUE
        RETURN
        END
```

## COCR - Cochran's test

Purpose: This subroutine performs Cochran's test for homogeneity of variance.

Application: Testing for homogeneity of variance is useful in deciding whether to transform the data to meet assumptions of parametric statistical analysis (see Section IV.2.2.2).

Entry: CALL COCR (X, Y, N, C, DEGFRE)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of sample standard deviations | one dimensional floating-point array; input |
| Y | is the array of the number of observations in each sample | one dimensional integer array; input |
| N | is the number of elements in arrays XSD and Y | integer variable; input |
| C | contains the Cochran statistic | floating-point variable; output |
| DEGFRE | contains the degrees of freedom associated with C | integer variable; output |

Mathematical Method: $$C = \frac{VMAX}{\sum_{i=1}^{N} X_i}$$

where VMAX is the largest element of array X and $X_i$ is the ith element of X.

```
      SUBROUTINE COCR (X,Y,N,C,DEGFRE)
      DIMENSION X(N), Y(N)
      INTEGER OBVTOT, Y
C
C                          SUBROUTINE COCR
C
C     FUNCTION: CALCULATES THE COCHRAN STATISTIC TO TEST FOR HOMOGENEITY
C               OF VARIANCE
C
C     VARIABLE     DESCRIPTION                          TYPE; USE
C     --------     -----------                          ---------
C
C     X            ARRAY OF SAMPLE STANDARD             REAL; INPUT
C                     DEVIATIONS
C     Y            ARRAY OF NUMBER OF OBSERVATIONS      INTEGER; INPUT
C                     IN EACH SAMPLE
C     N            NUMBER OF SAMPLES                    INTEGER; INPUT
C     C            COCHRAN'S STATISTIC                  REAL; OUTPUT
C     DEGFRE       DEGREES OF FREEDOM ASSOCIATED        INTEGER; OUTPUT
C                     WITH C
C
      VAMX = 0.0
      VSUM = 0.0
      OBVTOT = 0
      DO 100 J = 1,N
              VAR = X(J) ** 2
              OBVTOT = OBVTOT + Y(J)
              VMAX = AMAX1(VMAX,VAR)
              VSUM = VSUM + VAR
100   CONTINUE
      C = VMAX / VSUM
      DEGFRE = OBVTOT - 1
      RETURN
      END
```

## QTEST - Burr-Foster Q-test

Purpose: This subroutine performs the Burr-Foster Q-test for homogeneity of variance.

Application: Testing for homogeneity of variance is useful in deciding whether to transform the data.

Entry: CALL QTEST (X, Y, N, Q, AVGDF)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of sample standard deviations | one dimensional floating-point array; input |
| Y | is the array of the number of observations in each sample | one dimensional integer array; input |
| N | is the number of elements in arrays X and Y | integer variable; input |
| Q | contains the Q-test statistic | floating-point variable; output |
| AVGDF | contains the average degrees of freedom associated with Q | floating-point variable; output |

Mathematical Method:
$$Q = \frac{\sum_{i=1}^{N} X_i^4}{\left(\sum_{i=1}^{N} X_i^2\right)^2}$$

where $X_i$ is the ith element of the array X.

```
      SUBROUTINE QTEST (X,Y,N,Q,AVGDF)
      DIMENSION X(N), Y(N)
      INTEGER SMPDF, TOTDF, Y
C
C                          SUBROUTINE QTEST
C
C     FUNCTION: CALCULATES THE BURR-FOSTER Q-TEST STATISTIC TO TEST FOR
C               HOMOGENEITY OF VARIANCE.
C
C     VARIABLE     DESCRIPTION                          TYPE; USE
C     --------     -----------                          ---------
C     X            ARRAY OF SAMPLE STANDARD             REAL; INPUT
C                     DEVIATIONS
C     Y            NUMBER OF OBSERVATIONS IN            INTEGER; INPUT
C                     EACH SAMPLE
C     N            NUMBER OF SAMPLES                    INTEGER; INPUT
C     Q            Q-TEST STATISTICE                    REAL; OUTPUT
C     AVGDF        AVERAGE DEGREES OF FREEDOM           REAL; OUTPUT
C                     ASSOCIATED WITH Q
C
      VSUM = 0.0
      VSSQ = 0.0
      TOTDF = 0
      DO 100 J = 1,N
             VAR = X(J) ** 2
             SMPDF =  Y(J) - 1
             VSUM = VSUM + (VAR * FLOAT(SMPDF))
             VSSQ = VSSQ + (VAR * FLOAT(SMPDF) ** 2)
             TOTDF = TOTDF + SMPDF
100   CONTINUE
      AVGDF = TOTDF/ FLOAT(N)
      Q = (AVGDF * VSSQ) / (VSUM ** 2)
      RETURN
      END
```

## FMAX - Hartley's $F_{max}$ test

Purpose: This subroutine performs Hartley's $F_{max}$ test for homogeneity of variance.

Application: Testing for homogeneity of variance is useful in deciding whether to transform the data. (see Section IV.2.2.2)

Entry: CALL FMAX (X, N, F, DEGFREN, DEGFRED)

where:

| | DESCRIPTION | TYPE |
|---|---|---|
| X | is the array of sample standard deviations | one dimensional floating-point array; input |
| N | is the number of elements in the X array | integer variable; input |
| F | contains the $F_{max}$ statistic | floating-point variable; output |
| DEGFREN | contains the degrees of freedom associated with the numerator of F | integer variable; output |
| DEGFRED | contains the degrees of freedom associated with the denominator of F | integer variable; output |

Mathematical Method: $F = \frac{VMAX}{VMIN}$

where VMAX and VMIN are the maximum and minimum variances of the replicates.

```
      SUBROUTINE FMAX (X,Y,N,F,DEGFREN,DEGFRED)
      DIMENSION X(N), Y(N)
      INTEGER DEGFREN, DEGFRED, Y
C
C                              SUBROUTINE FMAX
C
C     FUNCTION: CALCULATES THE HARTLEY FMAX STATISTIC TO TEST FOR
C               HOMOGENEITY OF VARIANCE.
C
C     VARIABLE     DESCRIPTION                              TYPE: USE
C     --------     -----------                              ---------
C     X            ARRAY OF SAMPLE STANDARD                 REAL; INPUT
C                     DEVIATIONS
C     Y            NUMBER OF OBSERVATIONS IN EACH           INTEGER; INPUT
C                     SAMPLE
C     N            NUMBER OF SAMPLES                        INTEGER; INPUT
C     F            FMAX STATISTIC                           REAL; OUTPUT
C     DEGFREN      DEGREES OF FREEDOM ASSOCIATED            INTEGER; OUTPUT
C                     WITH THE NUMERATOR OF FMAX
C     DEGFRED      DEGRESS OF FREEDOM ASSOCIATED            INTEGER; OUTPUT
C                     WITH THE DENOMINATOR OF FMAX
C
      VMAX = 0.0
      VMIN = 1000000.0
      DO 100 J = 1,N
              VAR = X(J) ** 2
              VMAX = AMAX1(VMAX,VAR)
              IF (VAR .EQ. VMAX)              DEGFREN = Y(J) - 1
              VMIN = AMIN1(VMIN,VAR)
              IF (VAR .EQ. VMIN)              DEGFRED = Y(J) - 1
  100 CONTINUE
      F = VMAX / VMIN
      RETURN
      END
```

## SMPNUM - Sample size

Purpose: This interactive program calculates the sample size required to detect a given "real-world" difference between a control and experimental sample.

Application: The number of samples required to achieve a certain error level can be useful in designing an efficient sampling program from a reconnaissance survey.

Entry: SMPNUM is an interactive progrm which calculates a sample size based on user-specified criteria. The important variables are:

| | DESCRIPTION | TYPE |
|---|---|---|
| STD | the sample standard deviation | floating-point variable; input |
| DIF | the difference to be detected | floating-point variable; input |
| TALPHA | t-value for a given Type I error | floating-point variable; input |
| TBETA | t-value for a given Type II error | floating-point variable; input |
| ESTREP | the estimated sample size | floating-point variable; output |

Mathematical Method: $ESTREP = 2\left(\frac{STD}{DIF}\right)^2 (TALPHA + TBETA)$

```
C
C                                PROGRAM SMPNUM
C
C       FUNCTION: CALCULATES THE NUMBER OF SAMPLES REQUIRED TO DETECT A
C                 USER-SPECIFIED DIFFERENCE BETWEEN A CONTROL SAMPLE AND
C                 AN EXPERIMENTAL SAMPLE.
C
C       VARIABLE      DESCRIPTION                          TYPE
C       --------      -----------                          ----
C       STD           STANDARD DEVIATION                   REAL; INPUT
C       DIF           DIFFERENCE TO BE DETECTED            REAL; INPUT
C       TALPHA        T-VALUE FOR TYPE I ERROR             REAL; INPUT
C       TBETA         T-VALUE FOR TYPE II ERROR            REAL; INPUT
C       ESTREP        ESTIMATED SAMPLE SIZE                REAL; OUTPUT
C
        TYPE 9000
9000    FORMAT (15H PROGRAM SMPNUM,//,30H SMPNUM CALCULATES THE NUMBER
     &          20H OF SAMPLES REQUIRED,/,27H TO DETECT A USER-SPECIFIED
     &          12H DIFFERENCE.,//,32H RESPOND TO EACH PROMPT WITH THE
     &          19H APPROPRIATE VALUE.,//)
C
C       PROMPT FOR REQUIRED INFORMATION.
C
        TYPE 9010
9010    FORMAT (/,25H ENTER STANDARD DEVIATION)
        ACCEPT 9020, STD
9020    FORMAT (F)
        TYPE 9030
9030    FORMAT (/,26H ENTER SMALLEST DIFFERENCE)
        ACCEPT 9020, DIF
10      TYPE 9040
9040    FORMAT (/,24H ENTER T VALUE FOR ALPHA)
        ACCEPT 9020, TALPHA
        TYPE 9050
9050    FORMAT (/,23H ENTER T VALUE FOR BETA)
        ACCEPT 9020, TBETA
C
C       CALCULATE ESTIMATED SAMPLE SIZE.
C
        ESTREP = 2.0 * ((STD / DIF) ** 2) * ((TALPHA + T BETA) ** 2)
        TYPE 9060,ESTREP
9060    FORMAT (//,25H ESTIMATED SAMPLE SIZE = ,F10.1)
        TYPE 9070
9070    FORMAT (/,26H REFINE ESTIMATE (Y OR N)?)
        ACCEPT 9080, IQUEST
9080    FORMAT (A1)
        IF (IQUEST .EQ. 'Y')                                GO TO 10
        STOP
        END
```