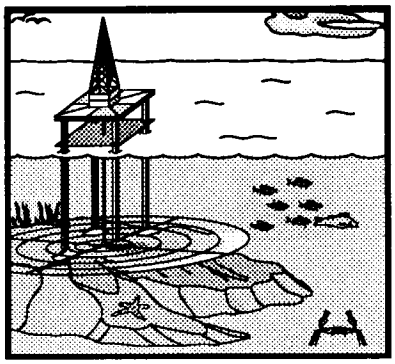
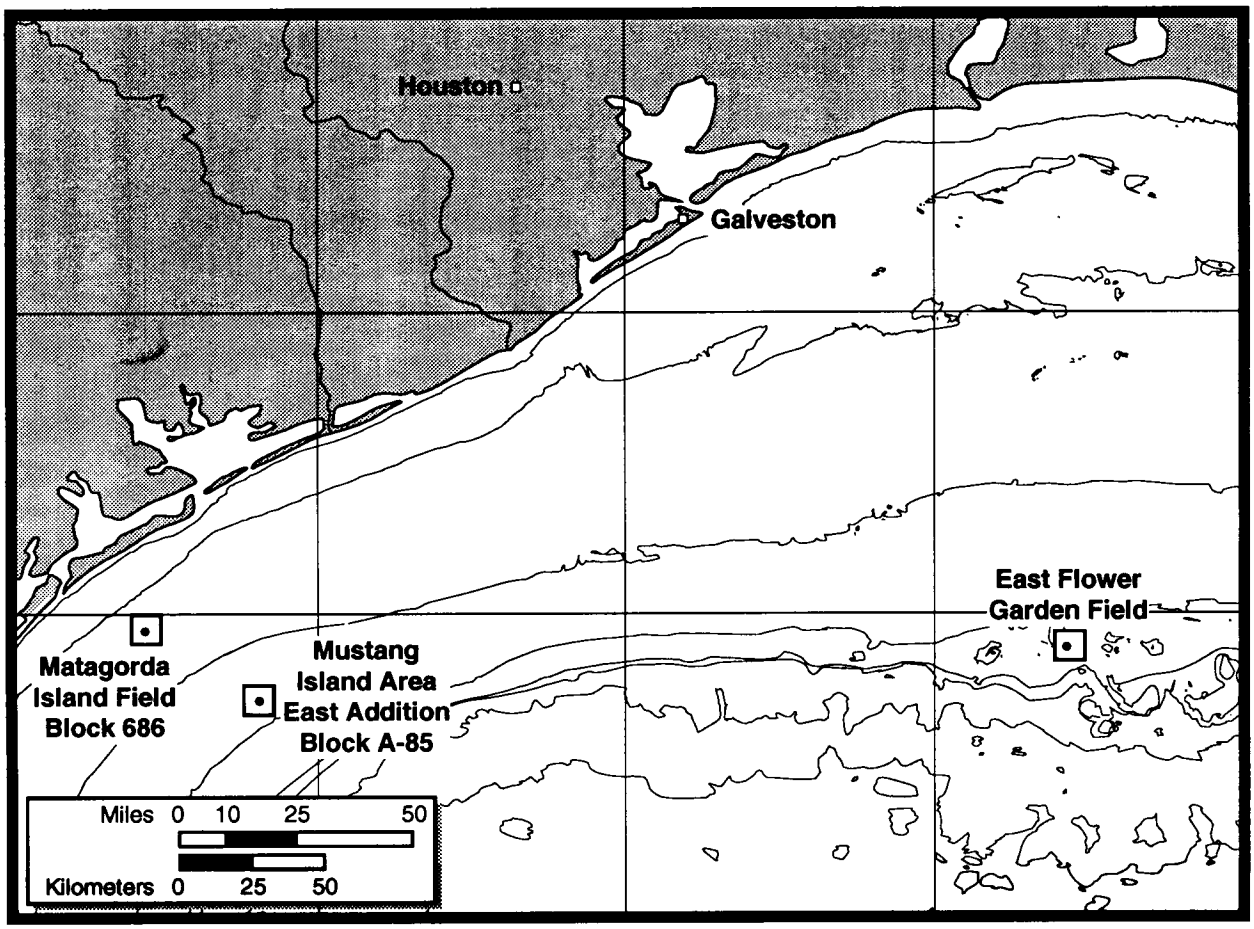
OCS Study
MMS 94-0005

Gulf of Mexico Offshore Operations Monitoring Experiment

Phase I: Sublethal Responses to Contaminant Exposure

Interim Report: Year 1





MMS
U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

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Interim Report: Year 1

Editor

Mahlon C. Kennicutt II

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PREFACE

The following GOOMEX Interim Report summarizes progress made to date in the "Gulf of Mexico Offshore Monitoring Experiment: Phase I. Sublethal Responses to Contaminant Exposure" program. As an important and purposeful part of the study design; the methodologies, interpretations, and study approach have continued to evolve during the course of the program. It should be noted that **the preliminary interpretations** presented in this volume are based on partial data sets collected during Cruises 1 and 2 and **are subject to revision as the data and additional sampling are completed**. In many instances the trends alluded to in the data need further verification and testing and should not be construed as the final product or conclusions ultimately to be produced by the GOOMEX Phase I Program.

EXECUTIVE SUMMARY

The "Gulf of Mexico Offshore Monitoring Experiment (GOOMEX): Phase I" is designed to document chronic sublethal effects associated with the exposure of marine organisms to contaminants derived from offshore oil and gas exploration and development activities. The findings of this "experiment" will be used to develop monitoring techniques and strategies to assess the importance of environmental change associated with offshore oil and gas platforms. A chronic impact is defined as an effect on the resident biota that results from long-term (> 10 years) exposure to contaminants. The most persistent contaminants that are primarily discharged at these sites are hydrocarbons and trace metals. It is also assumed that the long-term repository for these contaminants are the underlying sediments. Contaminant surveys provide information on the quantity and temporal stability of pollutants in sediments and biota. Biological studies measure life histories, reproductive success, reproductive effort and assemblage compositions for a variety of organisms from meiofauna to fish and whether detoxification systems have been induced by the contaminants.

One portion of the study is designed to analyze benthic sediment samples taken in a radial pattern around each platform. This design tests the hypothesis that biological, chemical and toxicological characteristics of the benthos vary with distance from the platform and that the observed variations are related to platform generated contaminants. Intensive sampling within the nearfield defines the spatial scale of the contaminant field. The radial sampling pattern includes the concept of comparison with control sites by extending each radii well beyond detectable sedimentary contaminants (≥ 3,000 meters). Studies include assemblage analysis of meiofauna and macroinfauna, assessment of community health based on meiofauna life history and reproduction studies, contaminant distributions (trace metals and hydrocarbons) in sediments and pore water toxicity testing by bioassay. The sampling technique of choice is the boxcore.

A second portion of the study provides near- (impacted) and far- (unimpacted) field, pairwise comparisons of potential indicators of exposure. Studies include assessment of community health based on macroinvertebrate life history and reproduction studies, histopathology of

invertebrates and fish, meiofauna genetic diversity, contaminant distributions in invertebrate and fish tissues, the presence and intensity of detoxification responses in fish, and *in vitro* toxicological assessment of contaminants by bioassay. The sampling technique of choice for fish and macroinvertebrates is the otter trawl.

The program consists of four field activities over a two year period. Initially five study sites were sampled, with a reduction to the three most suitable sites for long-term study. The final sites, selected primarily on evidence of a measurable contaminant gradient, are MU-A85, MAI-686, and HI-A389. To date, two cruises have been completed and a preliminary analysis of the data is presented in this Interim Report.

A set of replicate boxcores were taken at the near and far stations during Cruise 1 to assess the power of the study design and the appropriateness of pseudoreplicates from a single boxcore. Two replicates from a single boxcore can detect an 18-98% change in the variables measured. Sediment grain size has the greatest discrimination at 18%. Little precision is lost by using two pseudoreplicates over two replicate boxcores. For example, an 88% decrease in polycyclic aromatic hydrocarbons could be detected with two boxcores, and a 90% decrease could be detected with two subcores.

Sediments at the study sites were deposited by marine processes and are primarily of a fluvial origin. The sediments primarily consist of quartz, sands, silts, and clays with an admixture of skeletons and fragments of organisms living at the sites of deposition. Sediment texture is strongly correlated with distance from the platform with sediments under the platform highly enriched in sand. This sand appears to be related to disposal practices during drilling activities. The increase in sand parallels a proportionate decrease in organic carbon content in sediments. Inorganic carbon generally increases near the platform most likely due to deposition of calcareous debris from rig associated fauna. Spatial distributions in sediment texture are determined by the direction of the prevailing currents at the time of deposition.

Aliphatic and aromatic hydrocarbons are used to assess the presence of platform related contamination. Hydrocarbons were measured in sediments and biological tissues. In general, hydrocarbons were elevated in sediments close to the platform and rapidly decreased to background levels

at a distance of 100-200 m from the platform. The spatial distribution of hydrocarbons reflected the direction of the prevailing currents at the site and generally had a preferred directional orientation. Most hydrocarbons were highly biodegraded with the exception of several stations at MAI-686 which were rich in n-alkanes. Between cruise variations were small reflecting a stable hydrocarbon benthic contaminant field. HI-A389 and MU-A85 had strong gradients in hydrocarbon contaminants whereas MAI-686 exhibited a weak and variable gradient due to the high energy setting at the site. PAH levels in sediments are generally well below levels previously known to be associated with toxic biological effects. Data on tissue contaminant levels are too limited to draw conclusions at this point. However, PAH concentrations in fish livers were significantly higher near the HI-A389 platform than at the far station. Trends are becoming apparent in the data however a larger number of analyses are needed to confirm the significance of these preliminary indications. Further sampling will evaluate tissue contaminant distributions in more detail, i.e., by species and/or feeding type.

Barium (as barite) is a major component of drilling mud and is used to define and interpret sediment trace metal gradients at each study site. Two of the three study sites (HI-A389 and MU-A85) showed strong elemental gradients (Ba, Ag, Cd, Hg, Pb, Sb, Zn) with highly significant negative correlations with distance and positive correlations with Ba. The Ba trend at the third site (MAI-686) was significant however, fewer elements were significantly correlated with Ba (Cd, Hg, Pb, Zn). Several metals (Cu, Hg, Sb) appear to be constituents of the original barite ore used in drilling mud while others (Cd, Pb, Zn) have significant non-drilling mud, rig-related sources. The HI-A389 site clearly has the greatest spatial gradients and should be the location most likely to exhibit trace element related effects in the resident biota. Good correlations were observed between pore water bioassay results and sediment metal concentrations. Some metals, i.e., Pb and Zn, occur at levels previously shown to be associated with biological effects.

Tissue samples from near- and far-field stations at each site were analyzed for trace metals. Sample types include fish livers; soft tissues from shrimp, crabs and mollusks; whole starfish and polychaetes; and fish stomach contents. Preliminary analysis revealed few significant differences

for pairwise comparisons. Contaminants in stomach contents show a significant difference in Ba and Fe levels between near and far stations. This is probably related to the ingestion of bottom sediments during feeding. More data are needed from species specific comparison groups to further define trends in trace metal distributions in tissues.

Indices related to harpacticoid community structure are the best meiofauna indicators of platform effects. Harpacticoid abundance and diversity in sediments declined near platforms. Nematode diversity and abundance did not appear to be useful for detecting platform effects, i.e., there were no discernable trends. The nematode:copepod ratio was only an effective indicator at the HI-A389 platform, suggesting that this is not a good general index of platform affects. Within a platform site, environmental characteristics seem to have little influence on community structure. Most differences in meiofauna community structure between platforms were attributed to natural variations and gradients in abiotic conditions. Preliminary evidence indicated that there may be higher meiofaunal reproductive potentials under platforms. More gravid females were observed near the platform than away from the platform at most sites. Organic enrichment near platforms may be occurring due to the reef-like nature of the organisms associated with platforms. Differences in harpacticoid community, life history, and reproductive parameters suggest that reliable indicators of sublethal responses can be developed.

Near-field stress in macroinfauna may be indicated by an absence of ampeliscid amphipods at MAI-686. At MU-A85 and HI-A389 abundances of benthic infaunal organisms were relatively low and dominant species were generally not as dominant as at other platforms. Large populations of cirratulid polychaetes occurred at several near-field stations at HI-A389. There does not appear to be a correlation between contaminant indicators and organismal distributions. Despite the pore water toxicity indicated by bioassays at several stations at HI-A389, sediments from these stations contained large populations of cirratulid polychaetes.

The reproductive effort of invertebrate populations appear to respond to the presence of a platform. Variations in size class distribution, percent gravid females, stage of reproductive development, and prevalence of disease and parasites were noted. If a difference in size class distribution existed in a species between near- and far-field stations, the larger

individuals were typically found at the far-field station. This suggests that either there is insufficient food resources near the platforms, or mortality is higher among adult individuals nearer the platform. Far-field stations exhibited a higher proportion of gravid females than near-field stations, suggesting that proximity to the platform may induce a delay in the reproductive development of individual animals. However, other measures of reproductive development, such as histological analysis, showed no significant differences between the near- and far-field stations. In certain species (*P. aztecus* and *T. similis*), population health as defined by the presence of disease or parasites differed between the near- and far-field stations, with more evidence of pathogens and pathologies in individuals collected at the near-field station. These results are significant in that they indicate some form of physiological impact on populations living in close proximity to platforms. An immunological probe to estimate reproductive effort is in the calibration and purification stage, and inoculation experiments are awaiting processing. These data will clarify the rate and timing of reproductive development and the prevalence of disease and pathologies at the study sites.

Trawl collections yielded a large number of demersal fish of both target and non-target species. Cruise 1 produced a total catch of more than 3,500 fish consisting of 38 families and 89 species. Cruise 2 produced a total catch of more than 15,500 fish consisting of 45 families and 95 species. The overlap of target specimens between near and far stations and total catch was considerably greater during Cruise 2 as a result of improved trawling equipment and an increase in the number of trawls. Histopathological evaluations have been performed on liver and spleen samples from a total of 316 fish specimens from Cruises 1 and 2. No contaminant related liver lesions (e.g., hepatic megalocytosis, hepatocellular neoplasms) were observed in any of the tissue sections. Parasitic infections were the most common abnormalities, with microsporidians being the most prevalent. Two other prevalent lesions included inflammatory foci and granulomatous inflammation. However, these lesions were usually associated with parasitic infections. Preliminary splenic macrophage (MA) analysis from Cruise 1 indicated that the average size and the percent area occupied by MAs was greater at the near than far stations. Interpretation of splenic

MA data will require analysis of more samples and comparison with sediment and tissue contaminant data.

The detoxification work element concentrated on *in vivo* and *in vitro* indicators of organic contaminant exposure. *In vivo* assays were used to determine P4501A induction in fish and included ethoxyresorufin O-deethylase (EROD) activity, aryl hydrocarbon hydroxylase (AHH) activity and P4501A mRNA levels in livers and the concentrations of PAH metabolites in bile. For Cruise 1, AHH activity was measured in a variety of invertebrate groups. As others have demonstrated, AHH activity an ineffectual indicator of contaminant exposure in invertebrates. AHH activity was low to non-detectable in all invertebrate samples, indicating little or no induction. Consequently, *in vitro* rat hepatoma H4IIE cell bioassays were conducted to evaluate the levels of organic contaminants in extracts from invertebrate tissues. There were no statistically significant differences in catalytic enzyme activities of any species of fish for near and far station comparisons at the three sites. There was an excellent correlation between AHH and EROD activity for most fish species, which confirms that these two assays measure the same effect (i.e., P4501A induction). There were, however, a few notable exceptions. *Arius felis* and *Lagodon rhomboides* exhibited dramatically higher EROD than AHH activity. Fish that do not exhibit coordinated induction of AHH and EROD activity undoubtedly express an altered P4501A protein compared to most other fish species. This observation is unique and will be investigated further by conducting dosing experiments on Cruise 3. There is insufficient data for biliary metabolite concentrations, P4501A mRNA levels, and rat hepatoma H4IIE bioassays at this time to draw statistically valid conclusions. Further sampling is needed to provide a large enough data set to determine if P4501A induction is correlated with distance from a platform.

Sea urchin embryological development assays displayed significant toxicity to 12% of sediment pore waters tested during the first cruise. No toxicity was observed at MU-A85 and the six most toxic stations were observed at the inner stations at HI-A389. Significant toxicity was observed at 14 of 125 stations, mostly within 150 meters of a platform. The sea urchin fertilization assay displayed significant toxicity at three sites, all of which were also toxic in the embryological development assays. The sea urchin embryological development assay displayed significant toxicity to

pore waters collected in close proximity to two of the three platforms during the second cruise as well. As in the first cruise, no toxicity was observed near the platform at MU-A85. However, toxicity was observed at one of the reference stations (5C) at this site. Three of the five stations closest to the MAI-686 platform exhibited toxicity. Four of the six HI-A389 stations which were observed to be toxic from Cruise 1 were again found to be toxic. Significant toxicity was observed at eight of the 75 stations. Stations exhibiting toxicity (except the one station at MU-A85) were within ~75 meters of a platform. Preliminary correlation of data indicates a co-occurrence of toxicity and high sediment trace metal concentrations, especially zinc.

A large amount of data has been collected and a preliminary analysis and interpretation has been completed. Many work elements are beginning to be able to detect significant trends in the data that are directly related to the presence of offshore platforms. In most instances, the observed trends need to be verified by continued data collection. Some work elements do not have sufficient data to arrive at statistically significant conclusions. As more data becomes available it will be possible to analyze results at a finer scale of detail (i.e., by species). A series of new techniques continue to be developed including meiofauna genetic variability studies, an immunological probe to estimate reproductive effort in invertebrates, utilization of more appropriate organisms for bioassays (i.e., indigenous species), and evaluation of various *in vitro* toxicological assays presently utilized in mammalian and terrestrial systems.

Future program activities include two more field collections in January-February and June 1994. These additional cruises are essential to increase the overall power of the "experiment" to recognize statistically significant effects related to the presence of long-term offshore production facilities and the associated activities.

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1.0 INTRODUCTION

A mandate to conduct studies to predict, assess, and manage the effects of Outer Continental Shelf (OCS) oil and gas development activities on the marine environment is provided to the MMS under the OCS Lands Act Amendments. In response to this mandate, the MMS Environmental Studies Program has sponsored a series of ecosystem type investigations as well as rig and platform monitoring studies in the Gulf of Mexico, Pacific, and Atlantic OCS Regions. The most significant unanswered questions related to the environmental impacts of offshore oil and gas development and production are those related to chronic, low-level stresses on ecosystems that result from discharges, spills, leaks, and disruptions associated with the long-term development of energy resources (Boesch et al. 1987; Aurand 1988; Ahlfeld 1990; Kendall 1990). The Gulf of Mexico Offshore Operations Monitoring Experiment (GOOMEX) is a three-phase study to test and evaluate a range of biological, biochemical, and chemical methodologies that may indicate chronic sublethal exposure to contaminants predicted to be associated with OCS oil and gas development and production (i.e., hydrocarbons and trace metals). The interim results of Phase I of GOOMEX are reported here. Study results will be used to formulate and recommend an approach to assess the importance of environmental changes associated with long-term OCS production.

The primary objective of GOOMEX is to document fundamental responses in the resident fauna at long-term OCS production sites, which have resulted from exposure to contaminants. Biochemical responses in organisms are of primary interest since these indicators may serve as an "early warning" monitor of environmental deterioration. A secondary objective is to document any impact due to contaminant exposure at the organismal, population, or community level. GOOMEX links "state-of-the-art" determinations of chemical contaminant distributions with advanced studies of organismal response to pollutants. Study components include the analysis of contaminants (trace metals and hydrocarbons) in sediments, porewaters, and biological tissues; assemblage analysis of benthic meiofauna, macroinfauna, and macroepifauna; macroinvertebrate life history and reproduction studies; and the presence and intensity of inducible

detoxification responses in associated biota (Figure 1.1; Table 1.1). All components are linked by a common design and analytical scheme.

A "chronic" impact is defined as an effect caused by long-term exposure (> 10 years) to an accumulation of chemicals in the environment. Contaminants released over a period of years should reach a "steady state" in terms of spatial patterns of concentrations within the sediments setting the stage for possible chronic effects. Detection of impacts associated with this contaminant field requires confirmation that exposure has taken place and that the observed biological changes are not simply due to natural variations (Carney 1987). Biological response to exposure can be highly variable in mechanism and intensity. Biological changes can have a short time span (i.e., induced detoxification or reduced reproductive effort in an individual) or a long time span (i.e., compositional shifts in communities). The impact can be expected to manifest itself differently species to species and population to population, both in differences of actual levels of exposure and in life histories and population responses.

GOOMEX is designed to provide an integrated assessment of biological effects covering a range of important marine species from meiofauna to macrofauna including infauna, epifauna, mobile invertebrates and demersal fish. The biological indicators provide a breadth of potential markers of impact from traditional assemblage compositions to molecular biomarkers. Specifically, assemblage data is being collected for meiofauna and benthic infauna/epifauna. Reproduction and life history has concentrated on meiofauna and macroinvertebrates with particular emphasis on harpacticoid life histories. Macroinvertebrate reproductive effort studies will provide indicators of community health. Demersal fish are being examined extensively for chronic impacts at several levels of detail. Analyses being conducted in selected species of fish include necropsies (gross pathology), histopathology of representative tissues, stomach content food analysis, liver and stomach content contaminant analysis, and fish biliary polynuclear aromatic hydrocarbon (PAH) metabolites. All biological analyses are coordinated with concurrent measurements of contaminants in appropriate tissues. Detoxification responses are being monitored by measuring biological indicators that demonstrate that toxicants have entered organisms, have been distributed within their tissues, and are eliciting a toxicological effect. The biological indicators of contamination include

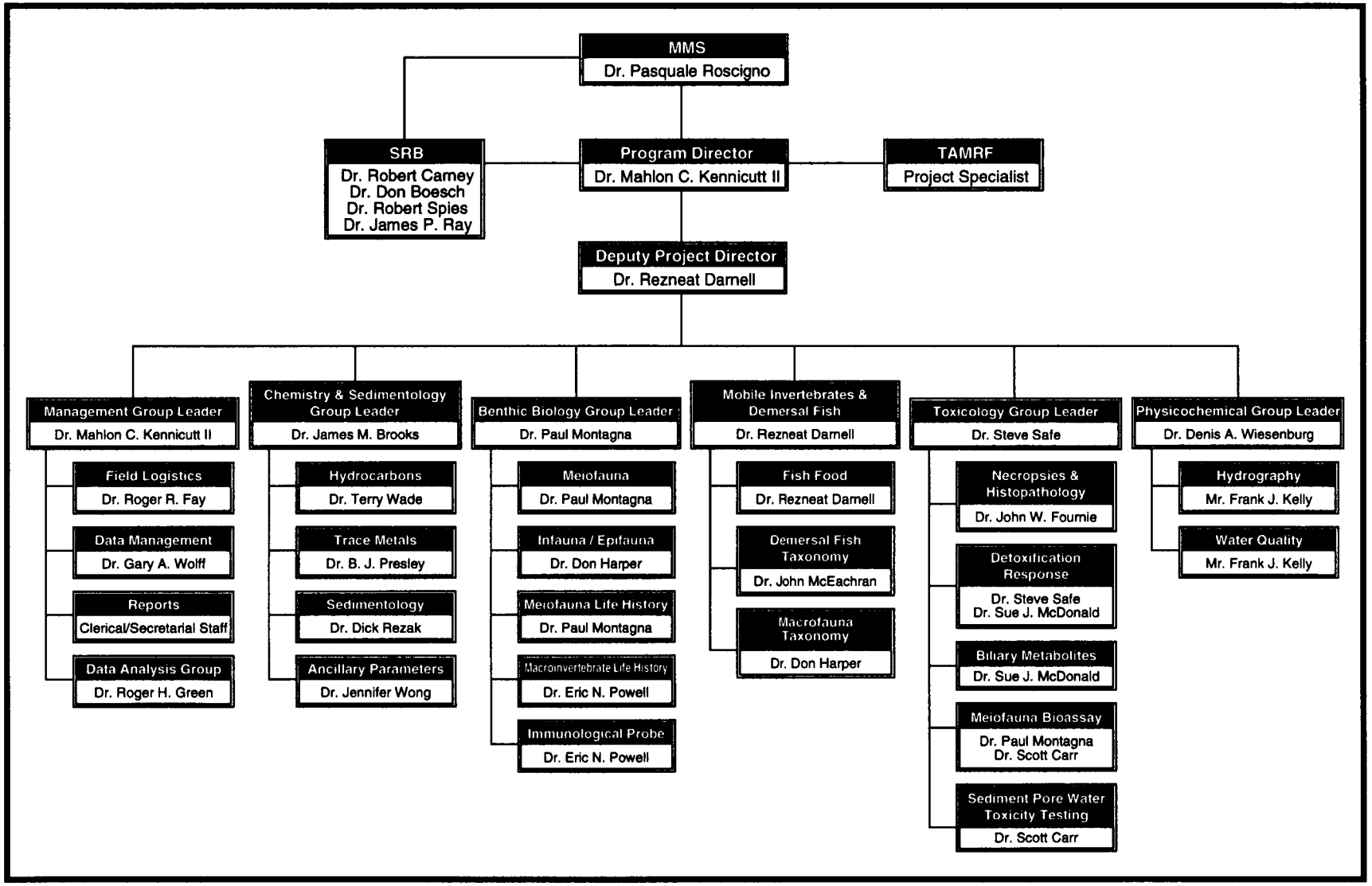


Figure 1.1. Program organizational chart.

Table 1.1. Summary of tasks, hierarchy of analyses, alternatives and optional work components of GOOMEX: Phase I.

Contaminant Gradient[1]		
Abiotic Matrices		
Sediments	Pore Water	Water Quality
•Grain Size •TOC •Redox •Mineralogy •Hydrocarbons •Trace Metals	•Hydrocarbons •Trace metals	•Temperature •Salinity •Nutrients •Oxygen
Biological Matrices		
Macrofauna	Mobile Invertebrates	Fishes
•Hydrocarbons •Trace Metals	•Hydrocarbons •Trace Metals	•Hydrocarbons •Trace Metals

Biological Response			
First Tier[2] →	Second Tier[3] →	Alternative[4]	Optional Work Components[5]
Meiofauna/Infauna •Ecology	•Meiofauna Reproductive Studies •Harpacticoid life History	•Genetic Diversity	•Meiofauna Growth and Reproductive Toxicity Assay
Macrofauna •Ecology	Community Studies	----	•Pore Water Toxicity Testing
Macroinvertebrates •Ecology	•Reproductive Effort Studies (5E)	•MFO Alternative Approaches - Rat Hematoma H-4-IIE Cell Bioassay - Antiestrogenicity bioassays (MC-7 human breast cancer cells)	•Immunological Probe
Fishes •Ecology	•Histopathology •Bile Metabolites •MFO - ^{14}C B(a)P Incubations - Cytochrome P450 Assay - EROD Activity	•MFO Alternative Approaches - CYP1A mRNA - AHH Activity - fluoremetric activity	

1-Contaminant Gradient - Provides the supporting data needed to interpret biological response data, i.e., defines the contaminant gradient (measured at all sites).

2-First Tier - Biological measurements to be made at all sites to provide basic community and individual organism information.

3-Second Tier - To be provided on a select subset of stations, primarily a near-far comparison.

4-Alternative - Suggested alternative approaches believed to be best suited to attain program goals if primary and secondary techniques are deemed inadequate or inappropriate.

5-Optional Work Components - Work elements that significantly enhance the work that have been funded by the MMS.

assays for ethoxyresorufin O-deethylase (EROD), aryl hydrocarbon hydroxylase (AHH), and P4501A mRNA levels in hepatic tissues of demersal fish. PAH metabolites are also being measured in fish bile. Toxicological analyses are coordinated with concurrent measurements of contaminants in appropriate tissues.

A number of new techniques or techniques not previously used in a marine setting are also being evaluated including: (1) an immunological probe to measure the rate of synthesis of gonadal protein in selected macroinvertebrates (reproductive effort), (2) genetic variation in meiofauna populations as indicators of stress, (3) development of bioassays utilizing indigenous meiofauna organisms to provide direct evidence of impact, (4) *in vitro* assays of contaminants extracted from a variety of matrices (including invertebrate tissues) using sensitized cell lines to indicate potential for injury, and (5) advanced methods of histopathological examination of invertebrates and fish.

The GOOMEX program was designed to provide the proper measurements and quantities of measurements to be able to produce statistically valid testing of the programmatic hypotheses. Statistical design prior to sampling and maintenance of study design throughout the program assure statistically valid conclusions. The results of GOOMEX Phase I will lead directly to a series of focused, process-oriented studies to be pursued during future phases.

1.1 Study Design

The null hypotheses to be addressed by GOOMEX are:

H_0: There are no differences in any of the chemical and/or biological parameters measured among platform sites (at distances less than 2,000 meters from the platform, or platform group) and comparison sites (located a minimum distance of 3,000 meters from any present or historic platform, or platform group).

H_0: There are no differences in assays for primary detoxification responses of the resident biota among platform sites (at distances less than 2,000 meters from the platform, or platform group) and comparison sites (located a minimum distance of 3,000 meters from any present or historic platform, or platform group).

H_0: There are no differences in the reproductive indices of the resident biota among platform sites (at distances less than 2,000 meters from the platform, or platform group) and comparison sites (located a minimum distance of 3,000 meters from any present or historic platform, or platform group).

Other null hypotheses will be advanced and tested during this study. The field investigations and laboratory analyses are performed quantitatively so that the hypotheses can be rigorously, statistically tested.

Two study designs are the basis of the GOOMEX program. Both rely on a dose-response model but differ in the details of sampling. The first design provides for an assessment of the benthos through a detailed sampling of sediments and resident organisms by quantitative boxcoring. Boxcores are taken at a series of stations determined by a combination of direction and distance from the platform, the presumed point source of contaminants. The near-field is intensively sampled based on the assumption that the concentration of contaminants decreases exponentially with distance from a point source. Control sites are provided by extension of the radii beyond the distance of measurable contaminants, generally ≥ 3000 m distance. Controls are chosen to be as similar in setting to the near-field stations as possible to minimize variation due to non-platform related processes. A total of 25 stations (5 distances x 5 radii) at 30-50 m, 100 m, 200 m, 500 m and ≥3000 m comprise the sampling grid at each site. Subaliquots of sediment are analyzed for grain size, mineralogy, redox potential, organic carbon content, carbonate content, hydrocarbons, and trace metals. Biological studies include meiofauna and macroinfauna assemblages and pore water toxicity testing.

The second study design employs a near (or impacted) versus far (or unimpacted) pairwise comparison of indicators of contaminant exposure. Due to the need for large amounts of biomass for the various work elements, boxcores were insufficient as a collection technique. Therefore, sample collection relies on otter trawls. Samples are taken as close as possible to the platform (< 100 m) and at a control as defined by the benthic sampling. The studies utilizing this study design are intensive in nature and are best evaluated at the extremes of exposure. Study elements include meiofauna genetic variability, macroinvertebrate reproduction, tissue contaminant levels (invertebrate and fish), *in vivo* and *in vitro* inducible detoxification

enzyme assays, histopathology of invertebrates and fish, contaminant metabolite production, and indigenous species bioassays.

1.2 Site Selection and Station Locations

Initially five sites were sampled during Cruise 1 (Figure 1.2). After evaluation of Cruise 1 results, three sites were selected for final study. The criteria for site selection included:

(1) The site must be located in an area with a long history of oil and/or gas development and production must have been active for no less than ten years.

(2) The sites must be located in the western and/or central Gulf of Mexico far enough to the west to be outside the perpetual, confounding influence of the Mississippi River plume.

(3) Comparison stations (controls) must be available that are located away from the suspected influences of any platform or pipeline at present or in the past and be similar to the near platform stations regarding depth, sediment characteristics, physicochemical parameters, ambient current regime, and benthic fauna.

The Mississippi River discharge can cause fluctuations in the physical and sedimentological setting that can lead to significant natural variability over time and space. It was deemed important to choose sites outside of the confounding influence of the Mississippi River Plume to enhance one's ability to recognize a perturbation. In this particular study "river" influence was primarily judged based on homogeneity of benthic substrate. However, it is recognized that the Mississippi River has substantial influence on the overlying water column throughout much of the Gulf of Mexico. Based on these criteria and the regional occurrence of major petroleum producing reservoirs, one is restricted to the western Gulf of Mexico which is primarily a gas/condensate producing region. As such "oil" platform sites per se were not included in the study. Long-term production activity and water depth were also constraining factors in site selection.

Other secondary considerations included the availability of previous studies at a site and the known presence of a strong chemical contaminant gradient. The five sites chosen were Mustang Island Block-A85, Matagorda Island Block-686, Matagorda Island Block-622, High Island Block-A389

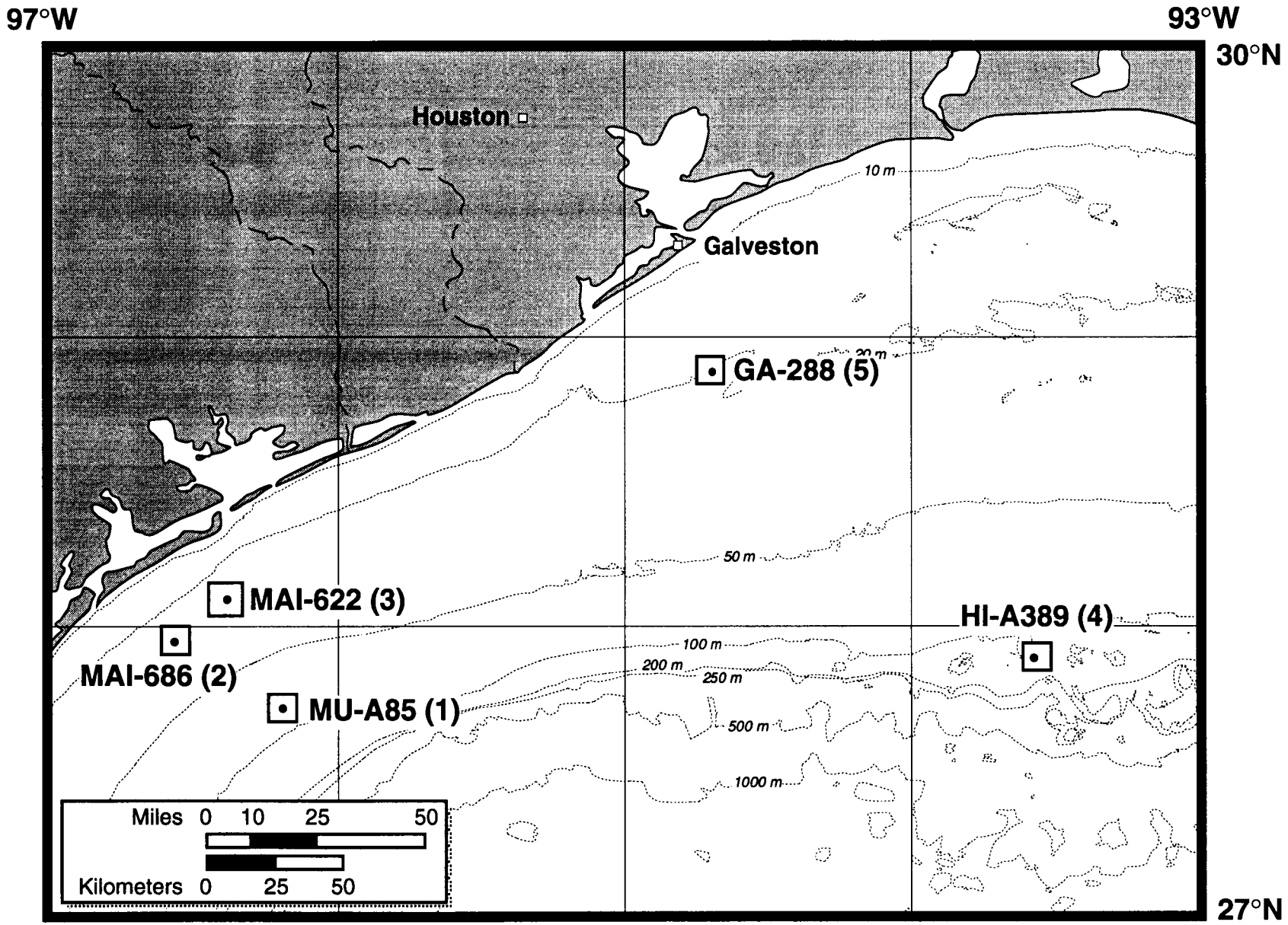


Figure 1.2. Location of study sites.

(East Flower Gardens), and Galveston Area Block-288 (Buccaneer Gas and Oil Field; Figure 1.2). Due to the short time between GOOMEX Cruises 1 and 2 only preliminary results were available to reduce the number of study sites from five to three. A primary consideration was the documentation of a gradient in chemical contaminants in sediments surrounding the platform sites. The presence of a strong spatial contaminant gradient was seen as the most important factor in selecting the final three study sites. The underlying assumption was that the stronger (steeper) the gradients the greater the likelihood of observing statistically significant biological effects between near and far field samples. A comparison of indicators of hydrocarbon contamination clearly demonstrated the highest contaminant values were at the MU-A85 and HI-A389 sites (Figure 1.3). MAI-686 and MAI-622 were similar, however MAI-686 exhibited elevated alkanes due to the leakage of condensate near the platform. GA-288 exhibited little or no gradient in hydrocarbons. Trace metal distributions, especially barium, confirmed the presence of contaminant gradients at these sites related to platform discharges (Figure 1.4). Barite (barium sulfate) is the dominant component of drilling fluids and barium is an accepted elemental tracer of the settable fraction of drilling discharges. The recommendation for final site selection based on trace metal data was determined from the magnitude of the Ba versus distance gradient and positive correlation between other metals and barium. The steepest gradients in Ba were at MU-A85 and HI-A389. The contaminants at both sites are significantly elevated and clearly related to drilling discharges. It should be noted these sites are atypical for the Gulf of Mexico in that drill muds and cuttings were shunted near bottom due to the proximity of shallow fishing banks and/or coral reefs. GA-288 shows no trend in Ba concentrations and in fact the control stations were slightly elevated, therefore eliminating this site as a study location. MAI-686 and 622 were similar however, Ba variations with distance from the MAI-686 platform were significant using an ANOVA test (Table 1.2). A more consistent contaminant field was demonstrated at MAI-686 than MAI-622 with several metals co-varying with Ba.

Most biological measurements were very preliminary at the time of final site selection and could only be used in a qualitative sense. Cluster analysis of macroinfauna data from the five sites resulted in a dendrogram having two site groups. Site Group I consisted of the three shallower sites,

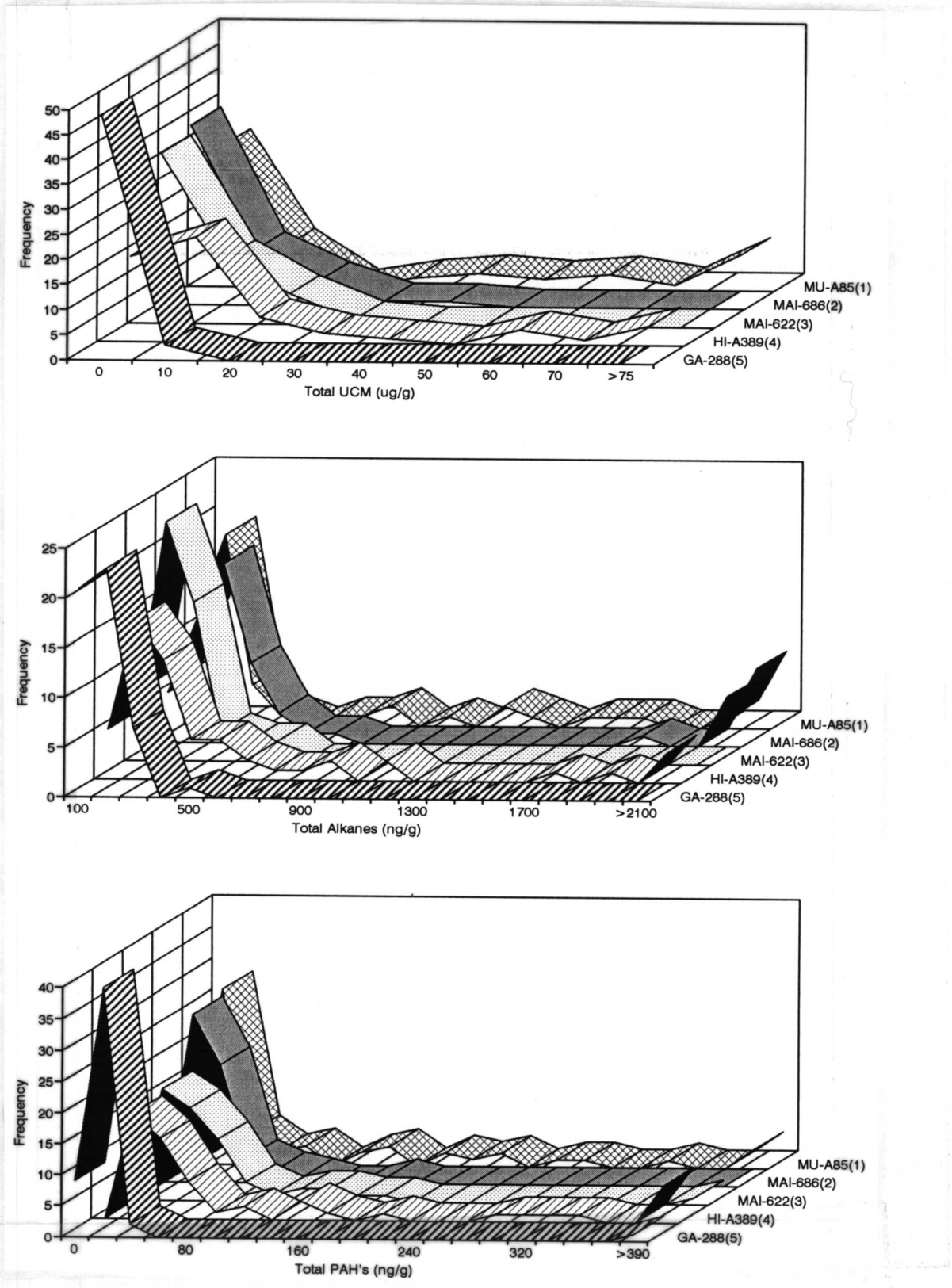


Figure 1.3 Frequency distribution of total unresolved complex mixture (UCM, ppm), total alkanes (C_{10}-C_{34}, ppb), and total polycyclic aromatics (PAH, ppb) concentrations in sediments for Cruise 1.

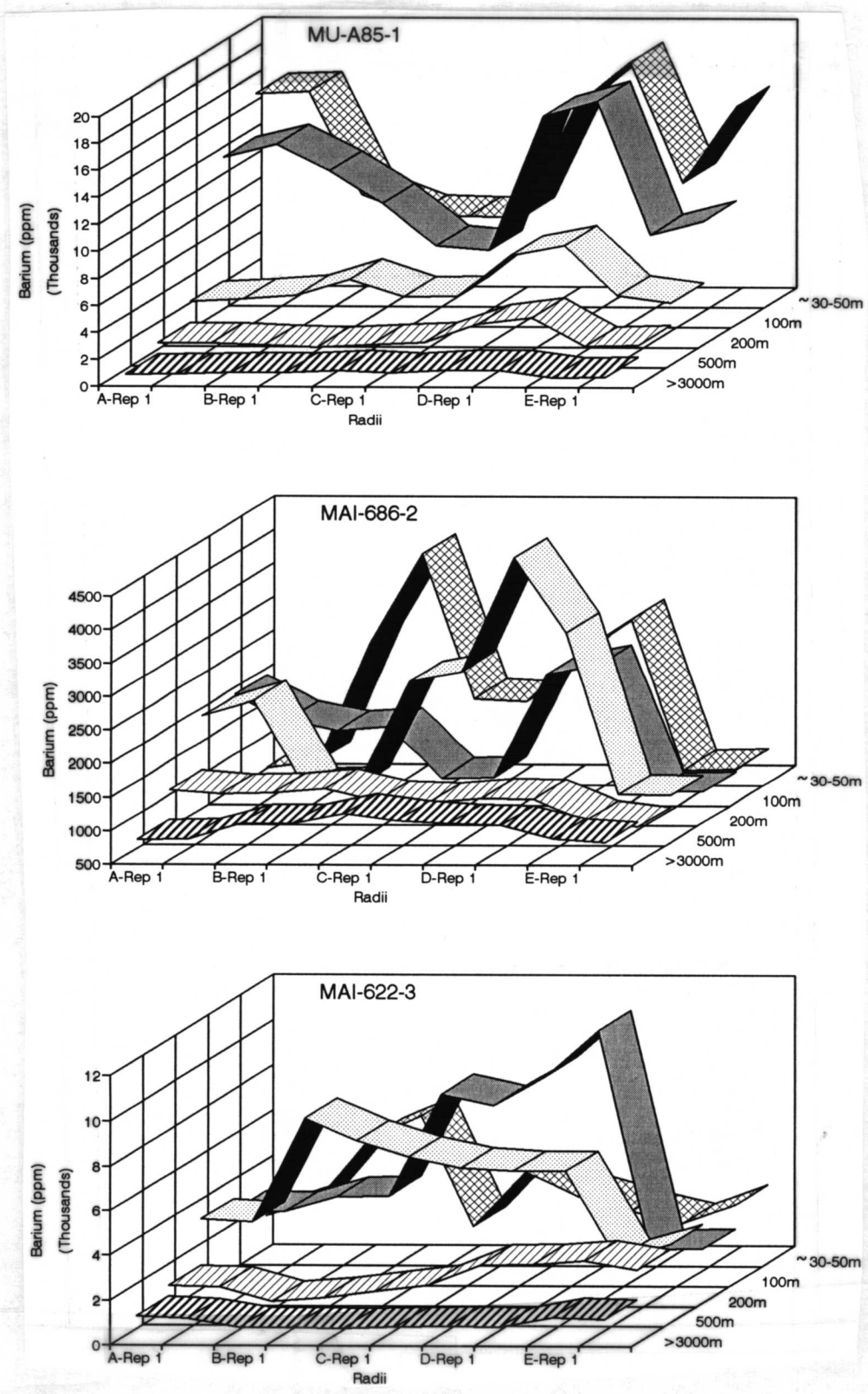


Figure 1.4 Distribution of barium concentrations in sediments from Cruise 1 by radii and distance from the platform.

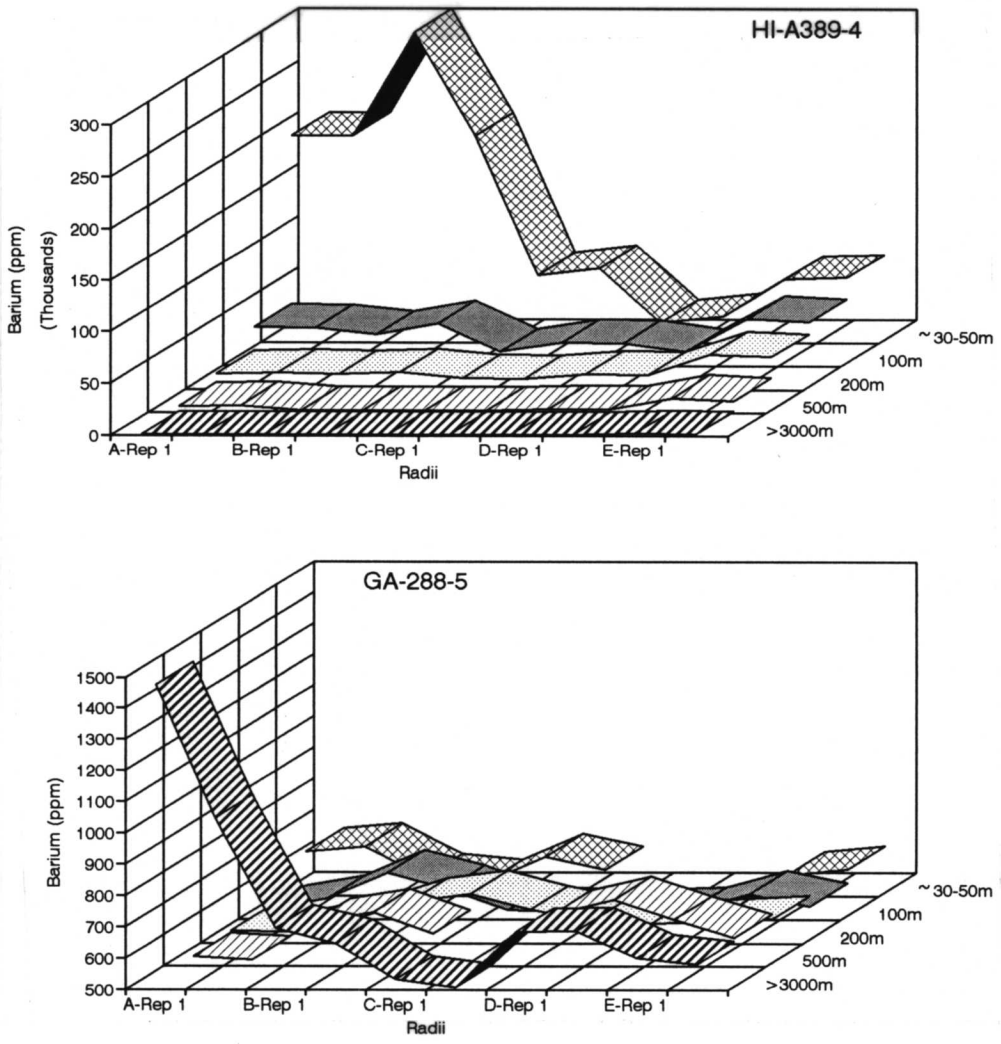


Figure 1.4 Cont.

Table 1.2. Summary of significant Spearman correlations in sediment trace element data from Cruise 1.

Site	Significant ($p < 0.01$) Negative Correlation with Distance from Site	Significant Positive Correlation with Barium
HI-A389-4	Ba, Ag, Cd, Cu, Hg, Pb, Sb, Zn	Ag, Cd, Cu, Hg, Pb, Sb, Zn
MU-A85-1	Ba, Ag, Cd, Hg, Pb, Sb, Zn	Ag, Cd, Hg, Pb, Sb, Zn
MAI-686-2		Cd, Hg, Pb, Zn
MAI-622-3	Ba	
GA-288-5	Ag, As, Cu, Pb	

GA-288, MAI-686, and MAI-622. The two deeper sites MU-A85 and HI-A389, formed Site Group II. This clustering was produced primarily by numerous species that were almost exclusively associated with a single platform. Therefore a range of setting was provided by the choice of MAI-686, MU-A85, and HI-A389. Meiofauna results were similar.

Based primarily on the existence of chemical gradients, as confirmed by Cruise 1 results; MU-A85, MAI-686, and HI-A389 were chosen as the final study sites. Station locations for boxcores and trawls for both Cruises 1 and 2 are summarized in Figures 1.5 to 1.7 at each of the three final study sites. All data were collected for all parameters for all sites and will be available through the National Oceanographic Data Center, Washington, DC (NODC) at the completion of the program. In the report that follows discussion is limited to data collected at the final three sites selected.

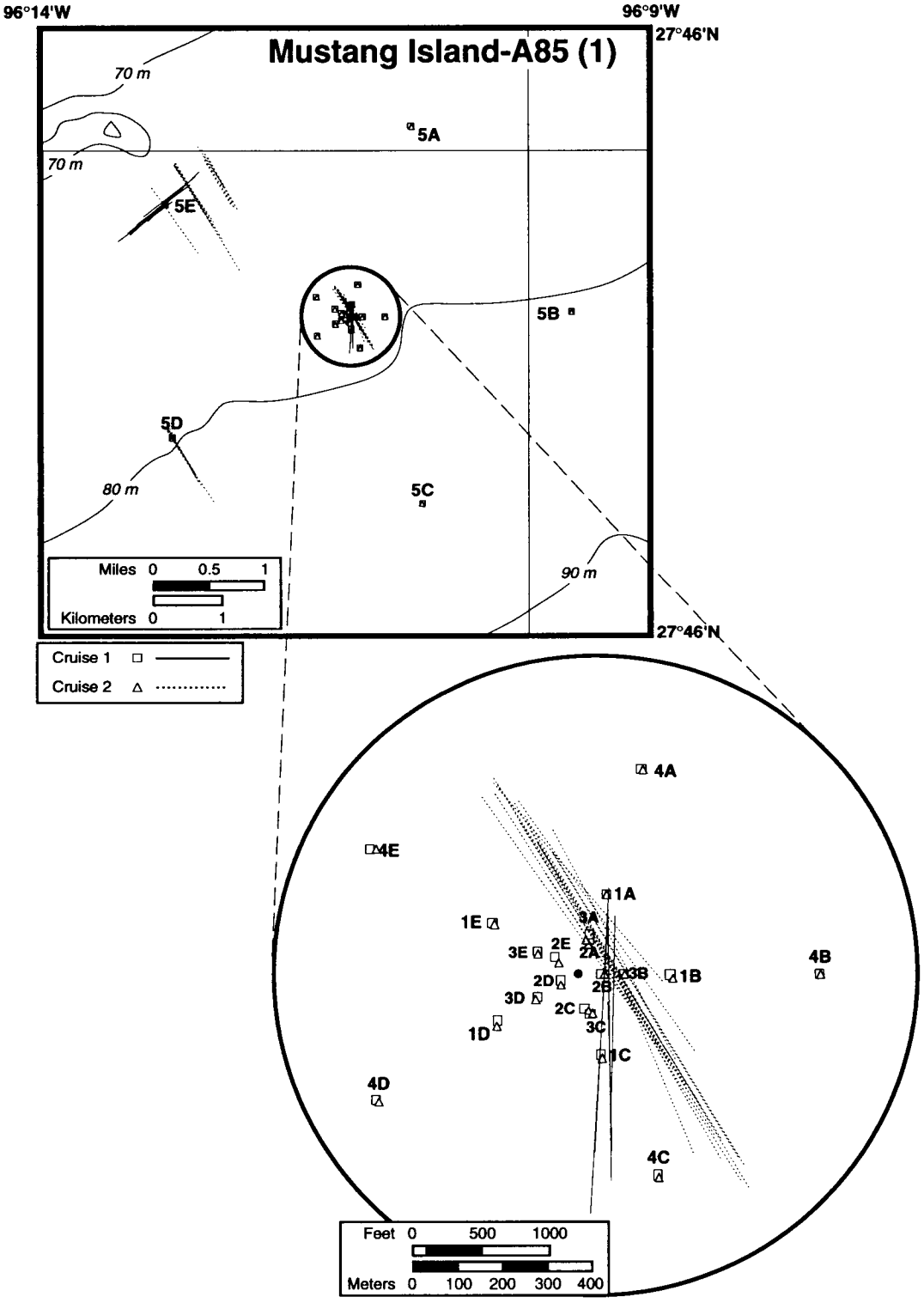


Figure 1.5. Summary of boxcore and trawl locations at MU-A85 during Cruises 1 and 2.

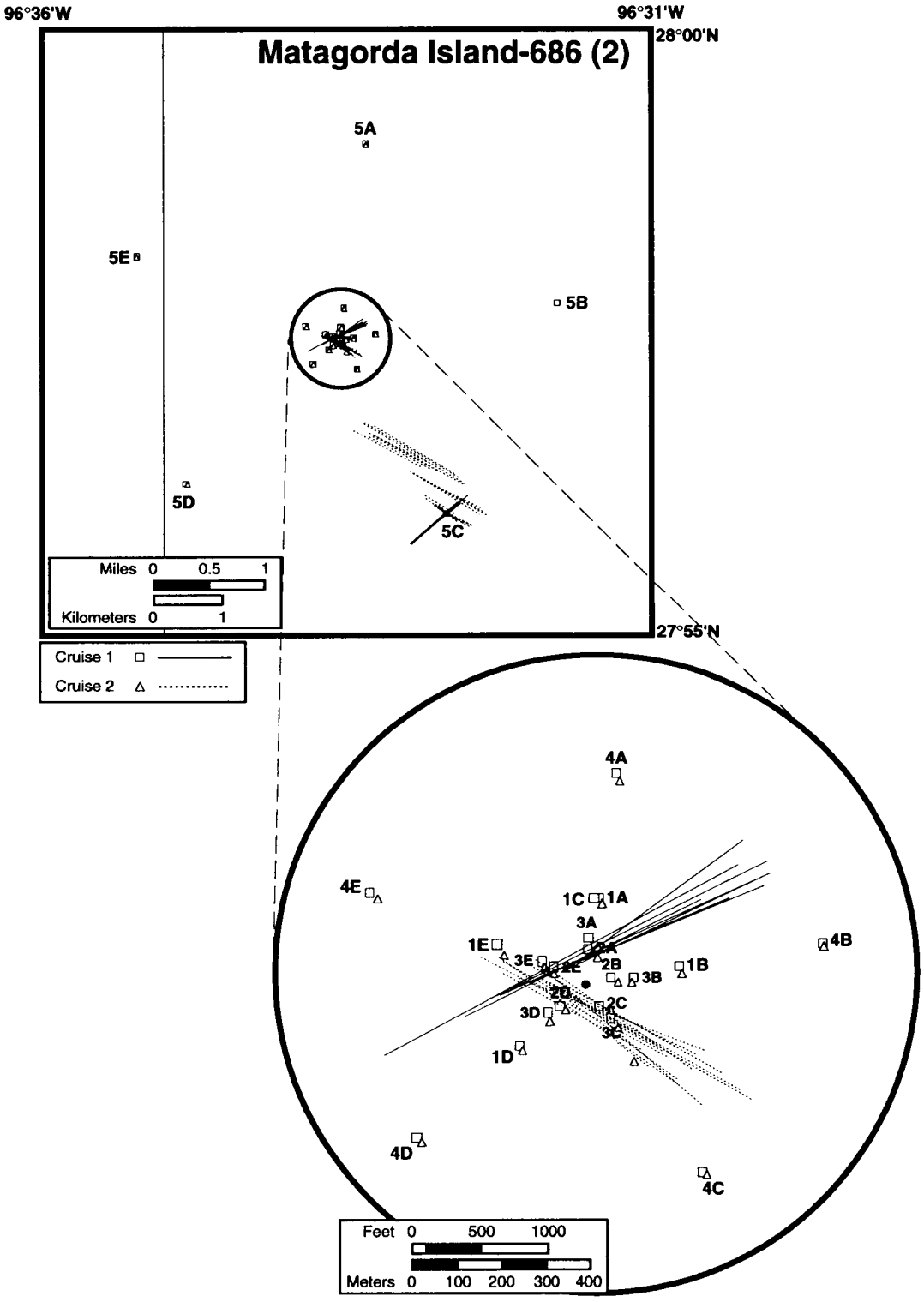


Figure 1.6. Summary of boxcore and trawl locations at MAI-686 during Cruises 1 and 2.

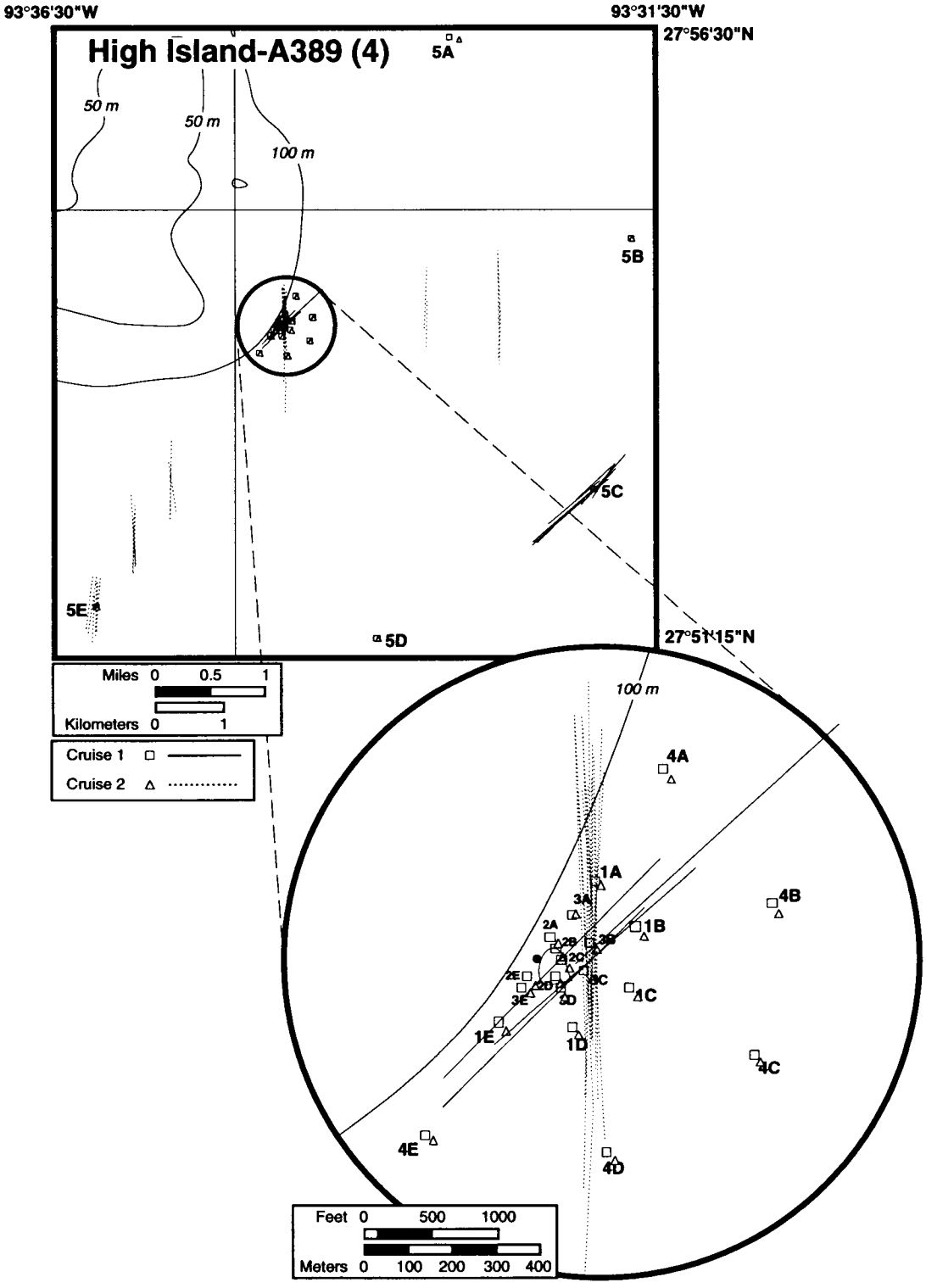


Figure 1.7. Summary of boxcore and trawl locations at HI-A389 during Cruises 1 and 2.

2.0 FIELD METHODS

2.1 Navigation

The Global Positioning System (GPS) was used for general navigation. Selective Availability (SA) reduces the accuracy of GPS to approximately 100 m. To increase the accuracy of GPS, the Skyfix Precision Positioning Service was used. Skyfix provides Differential GPS (DGPS) pseudo-range corrections via INMARSAT-A satellite links to increase positioning accuracy to ±3 meters. The DGPS system was coupled to a computerized navigation system which logged all sample locations as the samples were taken.

2.2 Hydrocasts and CTD Profiler

At each station continuous profiles of the following parameters were collected: salinity, temperature, and light transmittance (Figure 2.1). At each station, water was sampled from three depths: within the surface mixed layer, below the mixed layer and near the bottom. Discrete samples were collected using PVC Niskin bottles attached to the CTD cable. Water samples were collected for nutrient, salinity, and oxygen analysis. A complete suite of duplicate samples were drawn from three stations at each site for QA/QC purposes. Salinity samples were stored in air-tight glass bottles and nutrient samples were frozen immediately in 50 mL seasoned Nalgene bottles. The discrete salinity and nutrient samples were returned to shore and analyzed by the Marine Technical Services Group of the Department of Oceanography. Salinity was measured on an Autosal and concentrations of inorganic nutrients were determined using AutoAnalyzer techniques. The cruise chemist analyzed the oxygen samples at-sea using a modified Winkler technique. Comparison of duplicate oxygen samples indicated that the average percentage difference between duplicates was 0.13 percent with a range from 0.004 to 0.64 percent and a variance of 0.03 percent.

The primary data collection device for water column physicochemical measurements was a Sea-Bird Electronics, Inc. SBE-19 conductivity-temperature-depth (CTD) system, which outputs data to an IBM-compatible computer for data processing and storage. This system is called a SEACAT

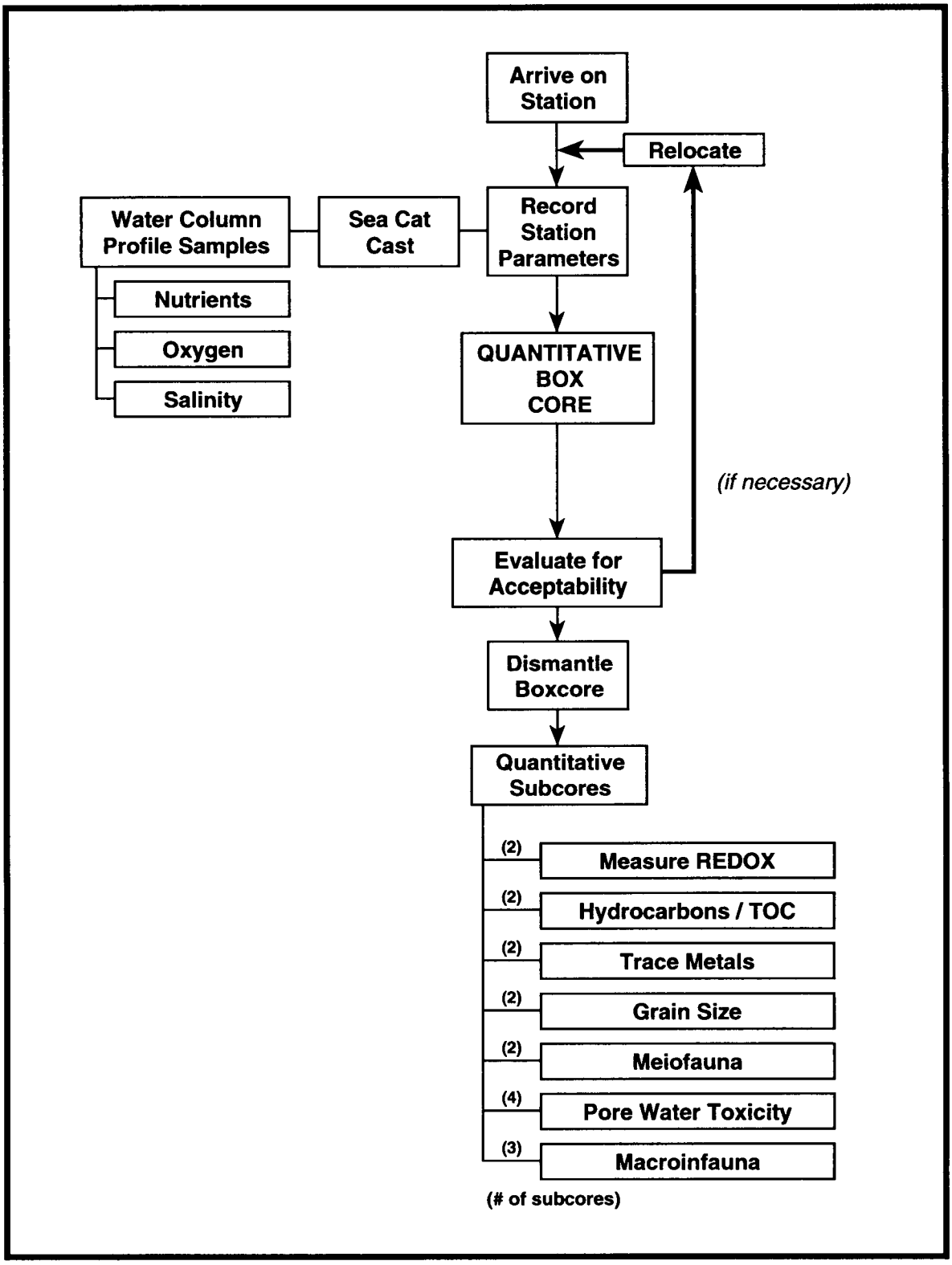


Figure 2.1. Activities at each quantitative boxcoring station including sampling protocols for water column and boxcore samples.

profiler. The SEACAT operated with a variety of externally mounted *in situ* sensors using proven hardware and software. Two SEACATs were provided for each cruise to assure that a complete backup system was available. The system collected continuous profiles of conductivity, temperature and pressure from which salinity and density were calculated. The auxiliary analog to digital channels on the SBE-19 was used to continuously measure beam attenuation coefficient light transmittance (Sea-Tech, Inc., 25 cm transmissometer).

The SEACAT used temperature and conductivity sensors which have an established record for reliability and long-term dependability. A pump supplies water to the conductivity sensor in order to match the dynamic response of the conductivity sensor to that of the temperature sensor. A strain gauge supplied pressure/depth information. All sensors output frequency signals, which were individually digitized twice per second. The SEACAT records the digitized data internally in solid state memory. When the SEACAT was brought back onboard, it was connected to a PC computer that downloaded the data to disk. Sea-Bird software combines instrument calibration data and sensor algorithms to compute temperature, conductivity, salinity, and depth. The cruise chemist processed the CTD data at sea and plots of vertical profiles of the raw data and the 1 m averaged data on an HP 7475A plotter were generated.

2.3 Boxcoring and Trawling

A boxcore was the technique of choice to quantitatively sample sediments. The boxcorer was an Ocean Instruments BX-600 Deep Ocean Corer outfitted with an extra spade; teflon coated inner core box head; stainless steel boxes fitted with stainless steel vegematic frame, rods and bolts; two sets each of 25 aluminum anodized vegematic core tubes and stainless steel screens with PVC frames to fit the core tubes; jacking dollies; vent doors; and lifting and closing cables. The boxcore provides an effective penetration of 50 cm depending on sediment texture. Two complete boxcorer systems were provided on each cruise. The 0.25 m^2 boxcore was subdivided into twenty-five 100 cm^2 compartments and used to sample sediment for each study element. To avoid bow waves, which can disrupt surficial sediments, the boxcore is deployed by stopping just above the

seabottom followed by a free fall to the sediment/water interface. The allocation of subcores to individual work elements is depicted in Figure 2.2.

The subcores around the perimeter of the boxcore were used for pore water toxicity testing. Subcores for all other work elements were randomly assigned. The nine interior subcores were distributed among the meiofauna (2), chemistry (4), macroinfauna (3), and grain size/mineralogy (2) work elements. The assignment of cores was determined by a random ordering of the nine interior subcore locations. The random number generation function used for determining the ordering of the subcores is called RAN3. RAN3 is Knuth's (1981) suggestion for a portable random number generation routine and is a subtractive method rather than a linear congruential model.

A boxcore configuration form was used to record the condition of the boxcore and provide assignment of subcores to work elements. These forms were bound and consecutively assigned to boxcores as taken at sea. The condition of the boxcore was determined on a qualitative scale:

Good (G) - Undisturbed surface in the core.

Acceptable (A) - A slight amount of disturbance of the surface of the core, with little perturbation (mixing) of the vertical structure (integrity) of the sample.

Poor (P) - Considerable vertical perturbation of the vertical structure of the core.

Boxcores determined to be poor were resampled.

Invertebrates and demersal fish were collected by otter trawl. The primary purpose of trawling was to collect specimens for chemical contaminant analysis, macroinvertebrate biological studies, detoxification, histopathology, and stomach content analyses. Trawl samples were sorted and target species selected by the onboard taxonomists (Figure 2.3). Otter trawls were of the semi-balloon type. The mesh measured 4.04 cm (1.5") and the mouth opening was 17.58 m (50'). The trawls were deployed with 67.58 m (200') bridles and were spread with 1.830 m x 0.915 m (6' x 3') wood doors. A spare set of smaller doors 1.525 m x 0.915 m (5' x 3') were carried on board as backup. Trawls were made perpendicular to the radials around the rig at a distance equal to the station distance from the rig. Trawl times on bottom varied from 10 to 15 minutes, the object being to

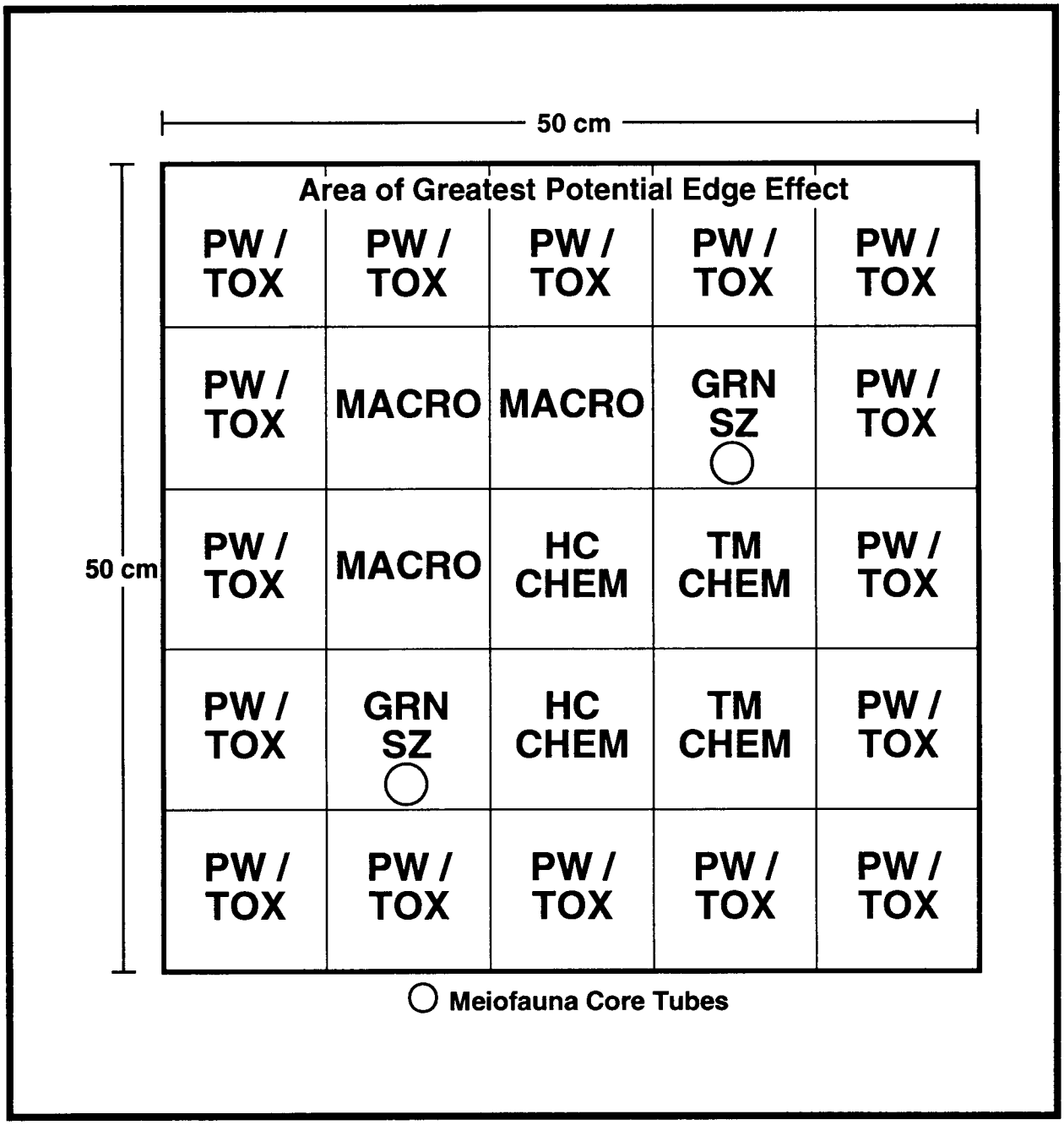


Figure 2.2. Boxcore illustrating "vegematic" partitioning. Subsamples were randomly assigned to the inner boxes. (MACRO: macroinfauna; PW/TOX: pore water toxicity testing; HC CHEM: hydrocarbon chemistry; TM CHEM: trace metal chemistry; GRN SZ: grain size).

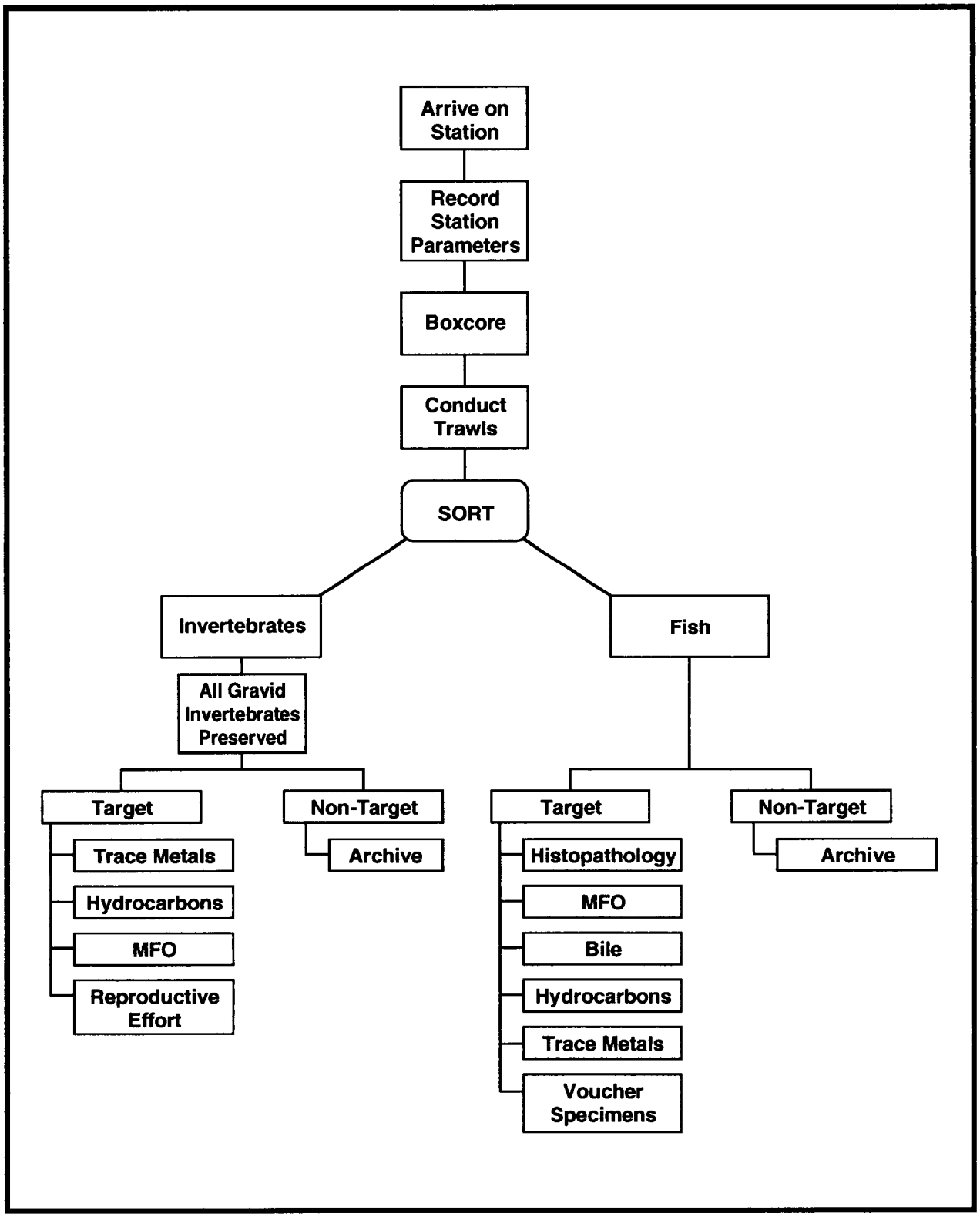


Figure 2.3. Sample processing protocol for trawl collections.

recover a sufficient number of live specimens. Shorter durations did not recover sufficient quantities and longer trawls had a high degree of mortality in the catch.

2.4 Sedimentology

Sedimentology samples were taken from two subcores at each quantitative boxcore station (Figure 2.2). The top 2 cm was sampled using a spatula or scoop. A minimum of 50 g of sample was collected in a plastic bag and stored refrigerated (4°C), not frozen. This sample was used for mineralogy and grain size analyses.

Sediment redox was measured by direct insertion of a combination platinum electrode. The electrode was calibrated with a ferrous-ferric solution every six hours and polished to expose a bright surface after each reading. The electrode was inserted to a uniform sediment depth of 2 cm. Two readings were taken per boxcore from the subcores used for sedimentology.

2.5 Sediment Samples for Contaminant Chemistry

Duplicate sediment samples for trace metals and hydrocarbons were taken from each boxcore (Figure 2.2). The subcorers for organic contaminants were thoroughly rinsed with water, dried with acetone, cleaned with methylene chloride between samplings, and covered with combusted aluminum foil after cleaning to minimize airborne contamination. Samples were taken from the upper 2 cm of the sediment with a Teflon coated scoop. The scoop was rinsed with distilled water, acetone, and methylene chloride prior to each sampling. Separate samples were taken for hydrocarbons and trace metals. Total organic and inorganic carbon were measured on a subsample of the hydrocarbon sample. Hydrocarbon subsamples were immediately placed into precleaned (combusted at 425°C) 1/2-pint glass jars with Teflon lined lids (~150 g). Trace metal samples were immediately placed into plastic bags (~150 g). All sediment samples for contaminant chemistry were stored frozen (-20°C).

2.6 Biological Tissue Sampling for Chemistry and Toxicology

The goal was to sample enough biomass of common macroinfauna, mobile invertebrates, and fish at both nearfield and farfield stations to supply all study components with the tissues needed. Mobile invertebrates and fish were collected by trawling. As far as possible, the organisms processed at the farfield and the nearfield stations were the same species. The goal for chemistry/detoxification samples was five species of mobile invertebrates and three species of demersal fish. All gravid invertebrates from trawls were either immediately frozen (LN_2) for the immunological probe work element or fixed in Bouin's for reproductive studies.

2.6.1 Macroinvertebrates

2.6.1.1 Taxonomic Identification and Sorting

The procedures followed for processing macroinvertebrates are outlined in Figure 2.4. Macroinvertebrates were exclusively collected by trawl. The specimens were rough sorted (crabs, shrimp, etc.), taxonomically identified, and the target species designated. All dissections were performed with solvent cleaned utensils in a clean environment. Once trawl collections were onboard, invertebrates were sorted from the fish and the target species identified. Collections were repeated until sufficient biomass of the target species was collected. Within practical limits, specimens of the target species that were collected were of approximately the same size class. Voucher specimens of target and non-target invertebrate species were preserved in 10 percent buffered formalin and returned to the laboratory for final identification.

2.6.1.2 Chemistry and Toxicology

Macro invertebrate species for the chemical and detoxification studies were individually tagged and kept alive until tissue removal. Various invertebrate species require the processing of different tissues for trace contaminant and detoxification analyses (Figure 2.5). All animals were rinsed with reagent water to remove extraneous materials. Large animals

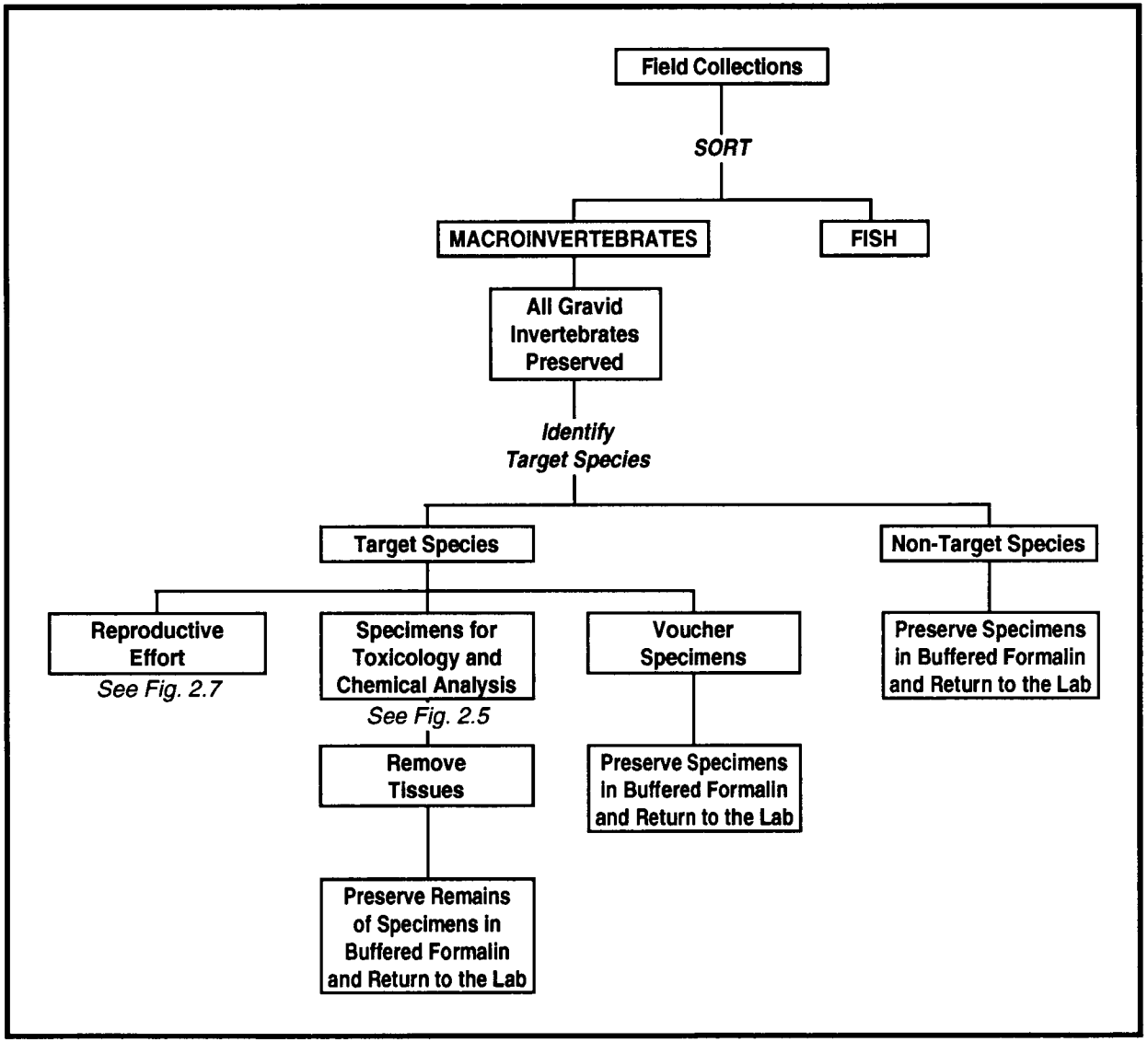


Figure 2.4. Sample processing protocol for macroinvertebrates from trawls.

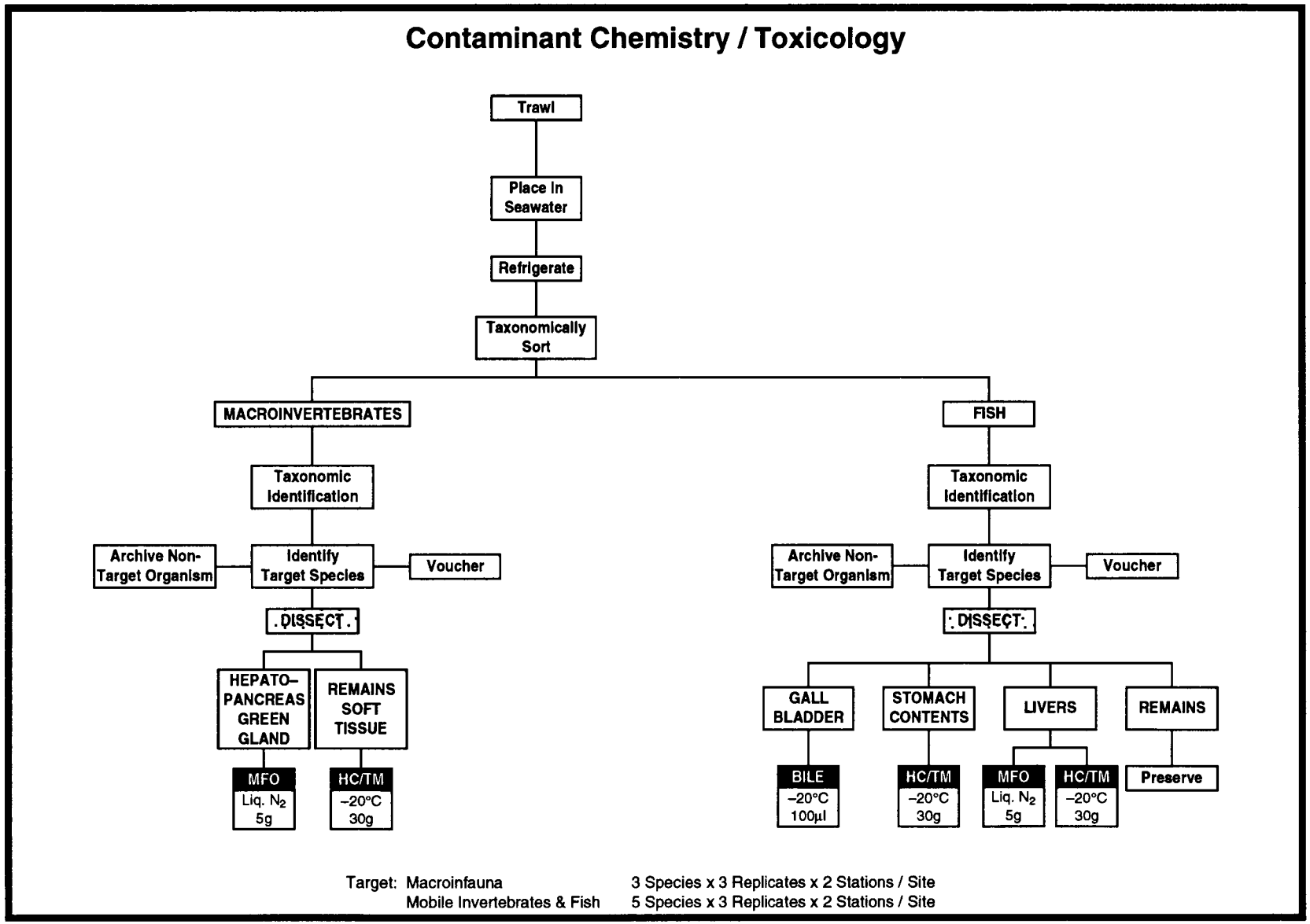


Figure 2.5. Sample processing protocol for macroinvertebrate and fish tissue for contaminant chemistry and toxicology.

were generally dissected and smaller ones were stored whole for toxicology. For crabs, scallops, and gastropods, the hepatopancreas was removed and kept on ice until all specimens had been dissected and composited. For shrimp, the green gland was sampled. A 5 g subsample for enzyme analysis was placed in a cryovial and stored in liquid nitrogen. The remaining tissue was used for trace contaminant analysis (~20 g). The trace contaminant samples were placed in clean glass jars with Teflon-lined lids and stored frozen (-20°C). Enough animals were processed to meet the biomass requirements (~25 g/replicate). Individual species were sampled in triplicate when possible. Voucher specimens were collected and preserved for all target organisms.

2.6.2 Demersal Fish

Procedures that were followed for processing demersal fish are outlined in Figure 2.6. All tissue sampling was done in a clean atmosphere and all utensils were precleaned by water, acetone, and methylene chloride rinses. Once the trawl was onboard, the fish were separated from the invertebrates. Live fish were placed in cool, aerated seawater in a large cooler. The fish were taxonomically identified and the target species designated. Trawling was repeated until sufficient biomass of the target species was collected. The specimens were tagged and separated into three parallel groups whenever possible. One species was targeted for histopathology. At each site 20 individuals of the target species were collected for histopathology. Parallel specimens were collected for stomach content analyses. For toxicology, bile was removed using vacutainers and stored at -20°C until analysis. Next, livers were removed and placed on ice and either subsampled individually or pooled depending on the size of the livers. Samples for enzyme assays and P4501A mRNA were frozen in LN_2 until analysis. Tissues for hydrocarbon and trace metal analysis were fish livers and stomach contents. Hydrocarbon samples were placed in combusted glass jars with a Teflon-lined lid. Trace metal samples were placed in clean plastic containers. All tissue samples for contaminant analysis were stored frozen (-20°C). When practical, individuals of approximately the same size were collected.

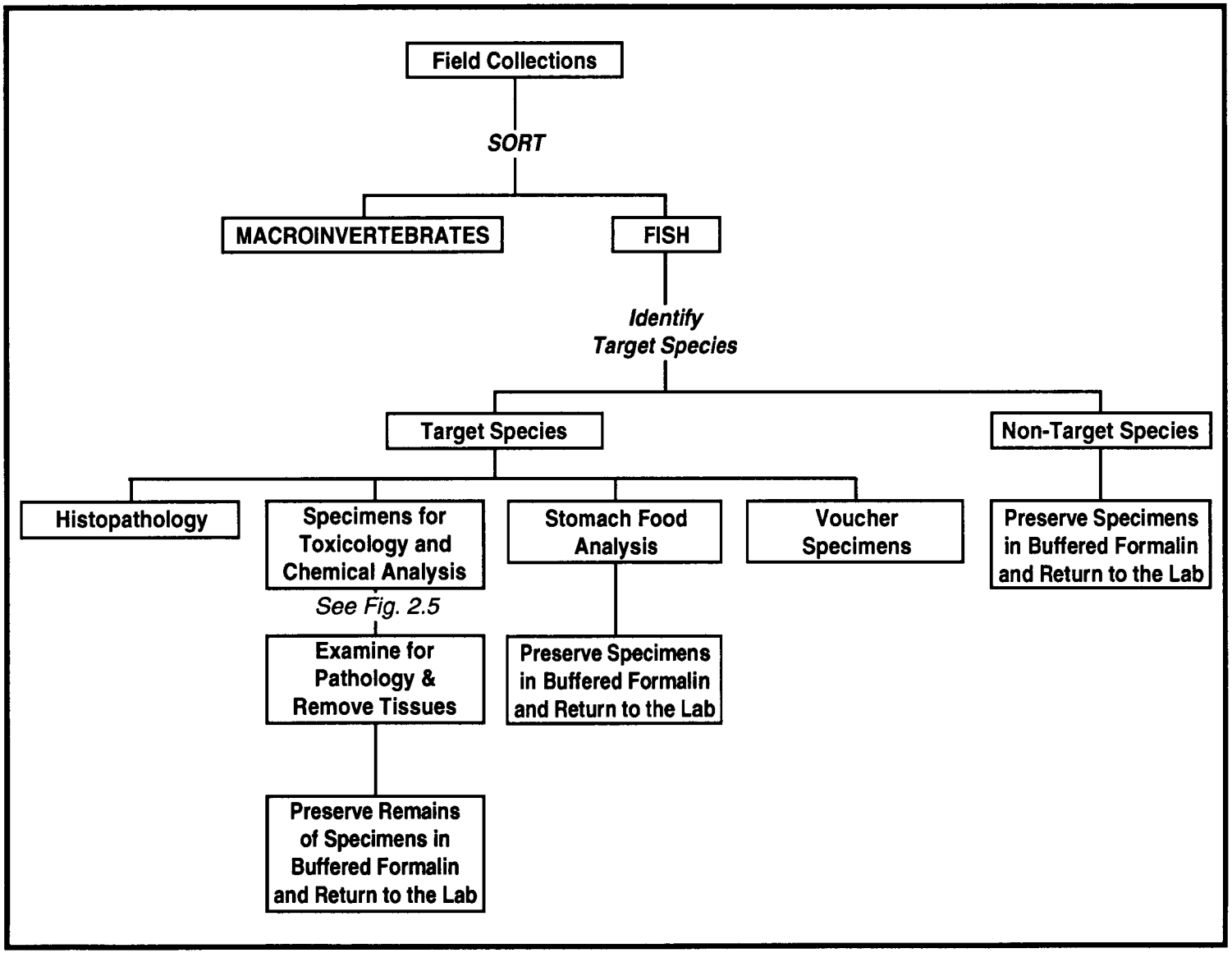


Figure 2.6. Sample processing protocol for fish from trawls.

All non-target specimens were preserved in 10% buffered formalin and returned to the laboratory for final identification. In any single collection, if the amount of non-target specimen was more than five gallons, then a subsample was taken. Specimens were retained as voucher specimens at the Texas Cooperative Wildlife Collection (TCWC).

2.7 Meiofauna

Two core samples were taken from each boxcore sample and stored for meiofaunal community analysis. Each core sample was from a different randomized subcore (Figure 2.2) to minimize autocorrelation among the replicates (Eckman 1979). Meiofauna were collected by a 1.9 cm i.d. core tube (Montagna 1991). The two core tubes were mounted inside a subcore. A mounted core tube insures that the meiofauna are collected from an undisturbed surface. Taking the sample from inner subcores reduces edge effects (Eckman and Thistle 1988). Bow waves of sampling devices in deep water can have an enormous impact on surface dwelling meiofauna (Hulings and Gray 1971). The 0-2 cm section of sediment was extruded out of the core tube and placed into a 50 cc plastic centrifuge tube. The meiofauna were anesthetized by adding 7% $MgCl_3$ and waiting about 5 minutes. The sample was then preserved with an equal volume of 10% buffered formalin (yielding a final concentration of 5% formalin). Extraction of animals for the community analyses was performed at a land-based laboratory.

For life history and reproduction analyses, three species of harpacticoids were chosen from the materials obtained in the meiobenthic community structure study. No additional sampling was required. For the meiofauna genetic variation analysis, further aliquots of sediment were collected. Since the meiofauna core tubes are about 4 cm^2, there was about 90 cm^2 remaining in each subcore. The 0-2 cm of the remaining sediment in each subcore was collected at the inner ring stations (#2) and outer ring stations (#5). The sediment was preserved with EDTA buffered alcohol and stored at 4°C (Dessauer et al. 1990). Meiofauna were extracted at a shore-based laboratory for DNA analysis.

Sediments were collected during GOOMEX Cruise 2 for meiofauna toxicity testing. The surface 2 cm of sediment remaining in the boxcorer subcores after the community samples were removed were retained and

kept fresh by aeration. Live meiofauna were extracted at a shore-based laboratory to attempt toxicity testing on indigenous species.

2.8 Macroinfauna Community Studies

Three subcores were used for analysis of macroinfauna. To minimize the effects of disturbance, only the nine central compartments were used for macroinfaunal samples. The three subcores, or samples, to be used for analysis were randomly chosen prior to dropping the boxcore. Each of the subcores was carefully extruded to a depth of 10 cm and placed in a shallow pan. The 10-cm depth was chosen because extensive prior experience in the northern Gulf of Mexico, in addition to extensive documentation in the literature, indicates that the majority of small infaunal organisms, which constitute the majority of specimens collected, are found in the upper 1-2 cm of sediment. Very few deep-burrowing organisms were missed by this process. In addition, past experience, especially in deeper water has shown that sediments below 10 cm tend to be thick clays which require a great deal of processing and yield little additional biological information. The sample was then placed on a 0.5 mm mesh sieve, submerged in a pan of seawater, and the sieve was rotated and agitated until all sediments smaller than 0.5 mm passed through the mesh. The remaining deeper sediments were examined for the presence of large, deep-burrowing organisms. Material retained on the sieve was placed in a prelabeled plastic jar and fixed with 5% buffered seawater-formalin. All jars were prelabeled. The outside label (site, station, date, replicate) was written with grease pencil. The inside label (same information) was a plastic Dymo label. These labels were used instead of paper labels because of the probability that paper labels would be abraded by shell hash, sand, or gravel.

2.9 Macroinvertebrate Reproductive Studies

The protocol for field collection for the invertebrate reproduction study is greatly revised from the original plan due to difficulties in collecting a sufficient number of individuals and species on Cruise 1. The original target for this work effort was 10 individuals from 10 different species. Five species were to be taken from boxcore samples and five species from trawls.

To fulfill this requirement, one boxcore was dedicated to this work element on Cruise 1 and animals were taken from multiple trawls. However, very few animals were obtained from the boxcore and further boxcoring did not significantly increase the number of individuals or biomass collected. Therefore, the sampling of macroinfauna from the boxcores for the reproductive effort was discontinued after Cruise 1. The number of trawls was increased to provide more individuals and to increase the likelihood of overlap of species between the near and far stations.

After retrieving each trawl, the catch was rinsed and rough sorted into four groups: echinoderms, molluscs, shrimp, and crabs. The animals were refrigerated at 4°C, if immediate fixation was not possible. The echinoderms, molluscs, and shrimp were kept in seawater and the crabs were placed in isotonic $MgCl_2$ to relax them. All individuals were placed in fixative within three hours of being collected to minimize autolysis from digestive enzymes and the effects of capture. Egg sacs from all gravid females (decopod *Crustacea*) collected in the trawl were immediately frozen in liquid nitrogen to be processed for the immunological probe study or placed in fixative for later microscopic and histological examination.

Size structure was used as a surrogate for age structure because growth curves are not generally available for most of the invertebrate species. Therefore, all specimens of the target species were counted and measured in the field (to the nearest mm). The dimensions measured are: bivalves, maximum posterior - anterior length; gastropods, from apex to the adapical tip; crabs, maximum carapace width; shrimp, tip of rostrum to the rear of the carapace; mantis shrimp, maximum length; starfish and brittle stars, central disc width; and heart urchins, largest test dimension (excluding any spines). For each species, individuals were randomly chosen and measured up to a maximum of 50. If larger numbers of shrimp or crab were collected in a single trawl and a gravid (external eggs visible) female was found, all individuals were measured regardless of the final numerical total.

During Cruise 2 several additional observations were made at the time of collection to determine the sex and to qualitatively describe the stage of reproductive development for individuals of all shrimp and crab species. For each shrimp collected, an external determination of sex was made based on the presence of the thyleca in females or the claspers in males (King 1948).

Each female was further examined and a numerical value for the stage of reproductive development assigned based on the size and color of the ovary visible through the carapace (Chamberlain and Lawrence 1983). The characteristics used to determine the stage of reproductive development based on the appearance of the ovary are summarized in Table 2.1. Crabs were sexed based on the appearance of the ventral carapace (Hill et al. 1989). The dorsal carapace of females, not preserved for histological analysis, was removed and a visual determination of reproductive stage was made based on the development and color of the ovary (Hard 1942). Definitions of the developmental stages used for female crabs are summarized in Table 2.2.

Preparation for fixation depends on the species (Figure 2.7). For crabs, a portion of the dorsal carapace is removed to allow penetration of the fixative into the tissues. For shrimp, during Cruise 1 the tail end was cut off just behind the carapace and discarded; only the head and thorax were preserved. The remaining soft tissues were processed for contaminant analysis. Based on histological analysis, a new procedure was developed for shrimp after Cruise 1. The tail of the shrimp was sliced open on the ventral surface, a piece of the carapace removed, and the head split open ventrally. This allowed for better penetration of the fixative insuring better preservation of the digestive tract and fixation of the complete reproductive system. Hermit crabs were carefully extracted from the shell and a small cut was made along the dorsal carapace. For heart urchins, a square was cut out of the lateral side of the test. For brittle stars and starfish, one arm was removed flush with the central disc. Individual bivalves and gastropods were placed in a vise and the shell cracked but not smashed. For bivalves, the adductor muscles were cut and the body mass removed from the shell. The shells were stored in 70% EtOH for species identification. For gastropods, the collumellar muscle was cut and the body carefully extracted to retain the upper whorls. Again, the shells were retained for identification. Scallops were placed in cold water until they relaxed and gaped, allowing a blade to be slipped between the valves to cut the adductor muscle.

For each species, five individuals were preserved in Helly's (Zenker-Formol) fixative (100 mL distilled water, 5.0 g mercuric chloride, 2.5 g potassium dichromate, 1.0 g sodium sulfate, immediately before use 4 mL 40% neutral formaldehyde is added) and five were placed in Bouin's fixative

Table 2.1. Characteristics used to determine the stage of reproductive development based on the appearance of the ovaries in shrimp.

Numerical Designator	Developmental Stage	Appearance of Ovary
1	Undeveloped	No ovary visible externally, no pigmentation.
2	Beginning Development	Anterior and posterior lobes of ovary faintly visible.
3	Nearly Ripe	Ovaries easily recognizable externally, anterior and posterior lobes enlarged and pigmented.
4	Ripe	Anterior and posterior lobes of ovary appear broad and dark, may even determine recent mating if it is still visible in thylecum.
5	Spent	Difficult to distinguish from Stage 2 without dissection, posterior lobe may still be pigmented and slightly enlarged.

Table 2.2. Developmental stages for female crabs.

Numerical Designator	Developmental Stage	Appearance of Ovary
1	Undeveloped	Ovary small and inconspicuous, white in color, spermathecae large and pink in color.
2	Early Development	Ovaries increased in length and diameter, orange in color, spermathecae decreased in size.
3	Mature	Ovary bright orange and large, no sponge or egg remnants on swimmeretes.
4	Spawned	Ovary bright orange and somewhat smaller than stage 3, sponge or egg remnants found on swimmerets.
5	Spent	Ovary collapsed, grey or brownish in color, sponge or egg remnants found on swimmeretes.

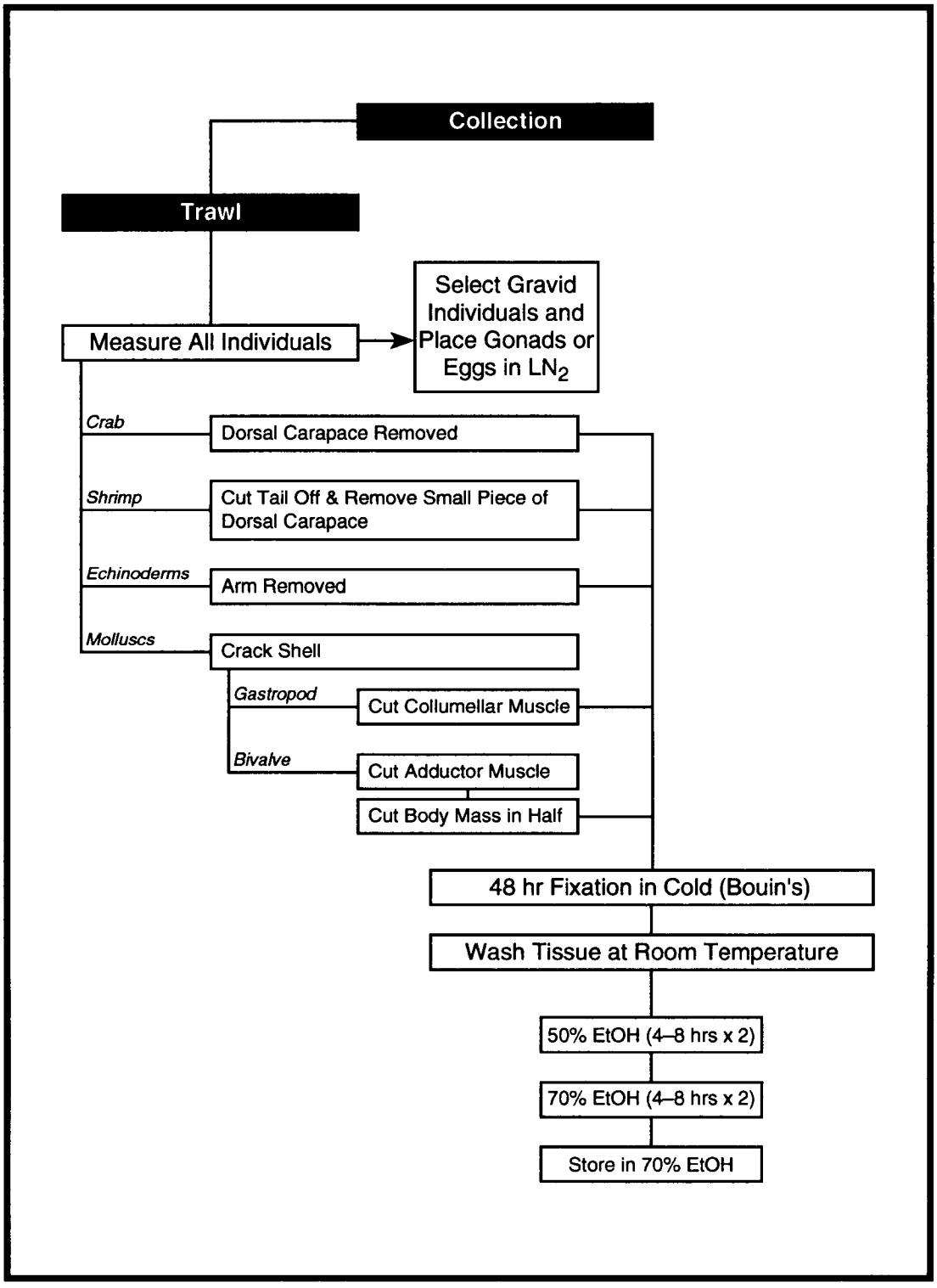


Figure 2.7. Macroinvertebrate life history sampling protocol.

(75 mL picric acid, 25 mL 40% formalin, 5 mL acetic acid). After Cruise 1, Helly's fixative was determined to be too harsh on the tissues, so for Cruise 2, 10 individuals were preserved in Bouin's fixative. The samples in fixative were stored in the refrigerator aboard ship for 48 hours, after which the jars were sealed and stored at room temperature. On Cruise 1 the largest individuals of each species were preserved. Due to the greater age of larger organisms, and therefore a greater opportunity to accumulate contaminants, the chance is greater that some form of impact related to the adjacent platform would be exhibited. On Cruise 2, an effort was made to preserve equal numbers of males and females, again retaining the largest individuals of each sex.

2.9.1 Immunological Probe Development and Incubation

The goals of this portion of the program are two-fold. First, to produce immunological probes which react to egg protein in specific species of invertebrates that can be used to determine the quantitative state of reproductive development in individuals and second, to use the probe to determine instantaneous rates of reproduction in these species by assessing the incorporation of labeled leucine into egg protein over time.

The development of immunological probes sensitive to egg and sperm protein for invertebrate species depends on the collection of sufficient eggs or sperm from the species of interest. During Cruise 1 all egg sacs from crab species were collected for the immunological probe study. Females of other species found to be gravid were also sampled. For example, the ovary and testis of scallops were removed and frozen separately in liquid nitrogen. These samples were returned to the laboratory to be used in the development of an immunological probe for quantitative reproductive effort studies. The specific details for the laboratory development of the immunological probe are presented in section 3.7.3.

Once developed, the immunological probe can be used to separate gonadal from somatic protein to determine the instantaneous rate of reproductive development. Inoculation experiments were conducted on Cruise 2 at two of the three sites to determine instantaneous reproductive effort. A portunid crab and scallop were chosen. Twenty individuals of two different species were injected with 2 mCi of ^{14}C leucine. Subsets of five

individuals of each species were incubated separately for 1, 2, 5 and 10 hours in seawater at ambient temperature, after which they were immediately frozen in liquid nitrogen. Use of the immunological probe in the treatment of these samples for determination of instantaneous reproductive rate is described in section 3.7.3.

2.10 Histopathology

At each station, all fish taken in each trawl were examined to assess for gross pathological abnormalities. While each fish was still alive or freshly dead, a thorough external inspection of the body surfaces, fins, eyes, branchial chamber, and buccal cavity was performed. All observations and measurements were recorded on a data sheet.

The following instructions were followed:

(A) Body surfaces and fins: Any discolorations of body surfaces (i.e., darkening, hemorrhaging, cloudiness), raised scales, white spots, or parasites visible to the naked eye were noted. Any lumps, bumps, or other growths, ulcerations, fin erosion, deformities of the vertebral column and/or mandibles, swelling of the anus, or any other abnormal conditions were noted as well.

(B) Eyes: The eyes were checked for cloudiness, hemorrhage, exophthalmia (i.e., pop eye), and/or depression in the orbits.

(C) Branchial chamber: The opercula were lifted and the branchial chambers examined for any perforations or deformities. The color, erosion, clubbing or other deformities, and parasitic infestations in the gills were noted. The internal surfaces of the chamber were examined for lumps, bumps or other growths, ulcerations, or any other abnormal conditions.

(D) Buccal cavity: The mouth was examined for any deformities, ulcerations, lumps, bumps, papillomas and/or growths.

(E) All specimens with gross lesions or other suspect conditions, as identified above were processed and coded individually.

(i) The entire length of the abdominal cavity was cut open using scissors or a sharp knife. The scissors were gently inserted into the abdomen near the anus and an incision to the operculum was made. Care was taken not to injure the visceral organs.

(ii) For fishes smaller than 15 cm, the entire fish was saved.

(iii) The tissue sample (visceral cavity and abnormalities excised) was placed in a perforated zip-lock bag. The perforations were large enough to permit a flow of fixative through the bag. Whole fish and heads were tagged with all pertinent information and placed directly into the fixative. Specimens were fixed in Dietrich's fixative.

(iv) The pertinent information relating to each individual sample sheet was carefully recorded on the data sheet.

(F) In addition to those specimens collected with visual abnormalities, a random, representative subset of specimens was collected from those fish that did not have visual abnormalities. All specimens of target fish species that "passed" the gross pathological inspection, up to a maximum of 20 individual fish were preserved.

Proper fixation of specimens was critical to the ultimate quality of the data obtained. Fish were examined and fixed while still alive or shortly after death.

2.11 Pore Water for Toxicity Testing

A composite of 14 subcores was used to provide a sample for pore water toxicity testing and contaminant analysis (Figure 2.2). The top 2 cm of sediment from each subcore was carefully removed with a Teflon scoop and placed in a 4-liter pre-cleaned polyethylene container. The containers were stored refrigerated at 4°C until transport to the shore-based laboratory.

3.0 LABORATORY METHODS

3.1 Physicochemical Measurements

3.1.1 Nutrients

Water samples were analyzed for phosphate, silicate, and nitrate using an Alpchem Analyzer or a Technicon AutoAnalyzer. The system was standardized by analyzing working standards of all nutrients prior to and after each set of samples. The peak height data were collected with a PC and peak heights from the AutoAnalyzer were converted to nutrient concentrations in µM by linear interpolation from absorbance relative to the working standards.

Silicate was determined by the ammonium molybdate, tartaric acid, stannous chloride method; phosphate by ammonium molybdate, hydrazine method; and nitrate by sulfanilamide, NEDA method (after reduction to nitrite with a cadmium reduction column). All of these analyses except phosphate, which was heated in a 70°C bath, were conducted at room temperature (25°C). Colorimeter interference filters in the spectrophotometers of the AutoAnalyzer were 660 nm (silicate), 880 nm (phosphate) and 550 nm (nitrate and nitrite).

For each cruise, the precision and accuracy of the analysis of each nutrient analytical technique was evaluated by analyzing at least 20 replicates of each of the highest working standards. The standard deviation in µM was 0.05 for a 6 µM nitrate standard, 0.005 for a 0.50 µM phosphate standard, 0.05 for a 6.5 µM nitrate standard, and 0.005 for a 0.50 µM nitrite standard. The coefficient of variation was 1% for each replicate group.

3.1.2 Salinity

Salinity samples were analyzed using a Guildline Model 8400 Autosal Laboratory Salinometer. The specifications of the Autosal are as follows:

Range:	0 to 40
Accuracy:	±0.003
Temperature Compensation:	±0.0007/°C

The Autosal system uses conductivity to directly to determine salinity. Each sample was analyzed three times to assure an accurate analysis. The sample was held at a constant temperature in a water bath while the conductivity was measured. The conductivity and temperature were then used to calculate salinity based on the practical salinity scale.

3.1.3 Dissolved Oxygen

Samples were collected and analyzed for dissolved oxygen by the microWinkler technique (Carpenter 1965). The microWinkler method has a precision of 0.01 mL/L oxygen at STP. Oxygen concentrations were measured at sea by the onboard chemist.

3.1.4 Quality Assurance

Quality assurance activities include proper care and maintenance of instrumentation, pre-cruise calibration of instruments, and calibration checks for parameters during the cruises. Oceanographic data is only as accurate as the calibration of the instruments used to collect it. Therefore, care was taken to assure that all instruments were in calibration before each cruise. The task is relatively simple for the Sea-Bird salinity and temperature sensors. The Sea-Bird SEACAT SBE-19 is regularly calibrated by returning it to Sea-Bird. Sea-Bird has the SEACAT calibrated by the Northwest Regional Calibration Center, which is operated under contract to NOAA.

3.2 Sedimentology

The sediment ancillary data determined includes mineralogy, grain size, total organic carbon content, carbonate content, and redox conditions. The analyses utilized were compatible with those currently being used in the NOAA National Status and Trends Program (NS&T).

3.2.1 Grain Size

Fifteen to 20 g of sample were placed in a large glass jar. This sample size minimizes the interaction of individual grains with each other during settling, avoids flocculation, and maximizes the amount of material to be weighed (i.e., small sample size increases errors in weighing). The sample was treated with ~50-100 mL of 30% hydrogen peroxide (volume varies with amount of organic matter present) for 12 hours prior to analyses to oxidize organic matter. The sample was washed with distilled water to remove soluble salts. Four hundred (400) mL of sodium hexametaphosphate solution (~5.5 g/L) was added to disperse the sample, followed by shaking for ~24 hours on a shaker table. A 62.5 μ screen was placed over a 1-liter graduated cylinder. The dispersed sediment was poured over the screen and washed with dispersant to rinse any remaining fine-grained sediment into the cylinder. This separates the gravel/sand fraction (on the screen) from the silt/clay fraction (in the cylinder). The coarse fraction was washed into a preweighed beaker with distilled water and placed in an oven (100°-130°C) to dry for 24 hours. The beaker was removed from the oven and left to cool. The beaker was allowed to equilibrate with moisture in the air. The beaker was weighed to 0.1 mg with an analytical balance. The sand fraction was dry sieved at 2 mm (-1.0 phi) and 62.5 μ (4 phi) intervals to separate gravel from sand-sized material. The weights of the gravel (>2 mm) and sand-sized (62.5 micron to 2 mm) material were recorded on data sheets.

The graduated cylinder containing the silt/clay material was filled to exactly one liter of dispersant solution. The cylinder was stirred vigorously and left to stand for one day. If the cylinder shows no sign of flocculation, analyses may proceed. If the sample flocculates, the sample was discarded and the procedure was repeated. The fine fraction was analyzed at 4 phi and 8 phi intervals. Two labelled beakers were preweighed to 0.1 mg. The cylinder was stirred vigorously starting at the bottom and working up until all the sediment was uniformly distributed throughout the cylinder. At the end of the vigorous stirring, long smooth strokes, the full length of the cylinder (from the bottom until the stirring rod breaks the surface) were used. As soon as the rod emerges for the last time, the timer was started. The pipette was inserted to a depth of 20 cm, and at the end of 20 seconds, exactly 20 mL was withdrawn (this is the 4 phi aliquot). The suspension was

pipetted into a preweighed beaker, the pipette rinsed with 20 mL of distilled water and the rinse water was added to the same beaker. At the 2:03:00 (two hours, 3 minutes) time, a 20 mL aliquot was withdrawn at a depth of 10 cm; this was the 8 phi aliquot. The suspension was pipetted into a second preweighed beaker. The pipette was rinsed with 20 mL of distilled water and the rinse water was added to the beaker. The beakers were placed in an oven and evaporated to dryness for at least 24 hours at 100-130°C. After 24 hours the samples were removed from the oven and allowed to cool to room temperature. The sample was allowed to come to equilibrium with moisture content in the air. The beakers were weighed to 0.1 mg with an analytical balance, and the weights recorded on a data sheet.

The 4 and 8 phi dry weights include the weight of the added dispersant. The dispersant weight (g/L) is multiplied by the fraction of the total solution removed (20/1000) and subtracted from the aliquot weight. This total is then multiplied by 50 (1000 mL/20 mL) to yield the sample weight of the silt + clay fraction.

Three weights are needed to calculate the total dry sample weight.

wt. sand (2 mm to 62.5 micron size range) + wt. gravel (2 mm and greater size range) + wt. of 4 phi residue = total dry sample weight

% gravel = wt. gravel fraction / total dry sample wt.

% sand = wt. sand fraction / total dry sample wt.

% silt = [(wt. 8 phi residue - dispersant) x 50] / total dry sample wt.

% clay = {[(wt. 4 phi-wt. 8 phi) - dispersant] x 50} / total dry sample wt.

Duplicate samples were run every 20 samples. Results were reported to three (3) significant figures.

3.2.2 Mineralogy

X-ray diffraction bulk mineralogy analyses were conducted to characterize the mineralogy of the sediments. The preparation procedure consists of gently crushing the sample reducing skeletal and other coarse

grains to a uniform fine silt particle size, but without causing changes in carbonate or clay phases. The sample powder was packed into a "dry power type" sample holder using the back-loading method described by Moore and Reynolds (1989). This method of sample preparation results in maximum random orientation of the mineral crystallites.

Each sample was subjected to Cu Ka radiation and scanned from 2° to 60° 2q on a computer automated Rigaku X-ray diffractometer. After several preliminary analyses, the optimal scan speed, sampling interval, and slit sizes for the sediments was selected. Binary intensity data was stored on 5.25" IBM-type floppy disks. The data was converted to an ASCII format. For each sample analysis a standard plot of intensity (in counts per second) vs. degrees 2q was generated. All major mineral phases in each sample were identified and listed in order of decreasing abundance.

3.2.3 Total Organic Carbon and Total Carbon

Carbon concentrations were determined on freeze-dried (or oven-dried at 40° to 50°C) sediment using a LECO Model 523-300 induction furnace (or equivalent) to burn samples in an oxygen atmosphere. One hundred to 500 mg (to the nearest milligram) of freeze dried (or oven dried), finely ground, homogenized sediment was weighed into a tared, carbon-free combustion crucible. The amount of sample depended upon the expected carbon concentration. Ideally between 0.5 mg and 8.6 mg of carbon was combusted to fall within the range of the standard curve. One scoop of copper and iron chip accelerator was added to the crucible containing the samples. All crucibles were kept covered with aluminum foil prior to analyses. The crucible was placed on the oven pedestal, the oven closed and oxygen allowed to flow. The carbon dioxide produced was swept from the furnace's combustion chamber with the oxygen flow. The gases then pass through a dust trap and two reaction tubes. The first reaction tube was a two-stage chamber with the first stage consisting of manganese dioxide. The manganese dioxide absorbs the sulfur oxides that form during combustion. The second stage was made of anhydrone, which removes water vapor from the gas stream. The second tube, filled with platinized silica, was maintained at an elevated temperature by an external heating jacket. The contents of this tube act as a catalyst to convert carbon

monoxide to carbon dioxide. Carbon dioxide was detected and quantified with a Horiba PIR-2000 infrared detector. The output signal from the Horiba was recorded by an HP 3396A integrator which calculates the quantity of carbon dioxide based on peak area. Standard LECO pin and ring carbon standards were placed into an empty crucible with one scoop of copper accelerator. Standards were analyzed by the same procedure as a sample to calibrate the detector.

The appropriate amount of freeze dried (or oven dried) sample was acidified with small amounts of 10% HCl in methanol solution. The treated samples were dried overnight at 50°C in the drying oven. Carbonate content was determined by subtracting the total organic carbon concentration from the total carbon concentration. To express as percent calcium carbonate, the total carbonate carbon content is multiplied by 8.33.

Quality control samples were processed the same as samples. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. Blank levels were maintained at less than 3x the method detection limit (MDL). Duplicate samples were analyzed every 20 samples, or with every sample set. Duplicates were maintained at ±20% for low level (<1.0% carbon) samples and ±10% for normal/high level (>1.0% carbon) samples. Duplicates were less precise for heterogeneous samples. Reporting units were percent organic carbon (on a dry weight basis) and percent carbonate carbon (on a dry weight basis). The minimum method performance standard for the method is detection of 0.02 percent carbon in a sample. Results were reported to two (2) significant figures. All duplicate analyses were reported. Duplicate analyses were analyzed with every 20 samples as a minimum requirement. LECO pin and ring carbon standards were analyzed as reference materials and standards.

3.2.4 Redox

An estimate of the redox condition of the sediments was obtained by Eh (redox potential, mV) measurements (Whitfield 1969). Eh measurements were performed by direct insertion of a combination platinum electrode into the top 2 cm of the sediment and reading a pH/mV meter. The Eh measurements were made directly in the subcore before boxcore disassembly. The overlying water was retained in-place to minimize

exposure to air and sediment disturbance. Readings were converted to values on the hydrogen scale (Whitfield 1969). The platinum electrode was periodically calibrated with a ferrous-ferric solution (Light 1972) and was polished to expose a bright surface before each reading.

3.3 Contaminant Analyses

The primary contaminants expected to be released from offshore platforms are hydrocarbons and trace metals. Hydrocarbons in the form of aliphatic and aromatics are released during production as well as drilling in the form of cuttings, additives, and spills. Trace metals would be expected to be released during drilling and production since metals are mud additives and platforms are metal structures.

3.3.1 Hydrocarbons

Aliphatic and aromatic hydrocarbons are important constituents of petroleum. As such they were quantitatively measured in sediments, pore waters, and biological tissues as tracers of pollutant exposure. Aromatic hydrocarbons are the constituents of oil directly responsible for a majority of petroleum's toxicological properties. All measurements are quantitative and highly quality assured allowing for rigorous and statistically valid testing of hypotheses. The protocols and procedures are identical to those presently used in the NOAA National Status and Trends Program (Figure 3.1).

3.3.1.1 Clean Procedures

All glassware was cleaned by detergent (micro cleaning solution) washing with water and rinsing with tap water. The glassware was then combusted in a muffle furnace at 400°C for at least 4 hours. Solvent rinses with acetone to dry followed by methylene chloride were substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, glassware was sealed and stored in a clean environment to prevent the accumulation of dust or other contaminants. Stored glassware was maintained capped with combusted aluminum foil.

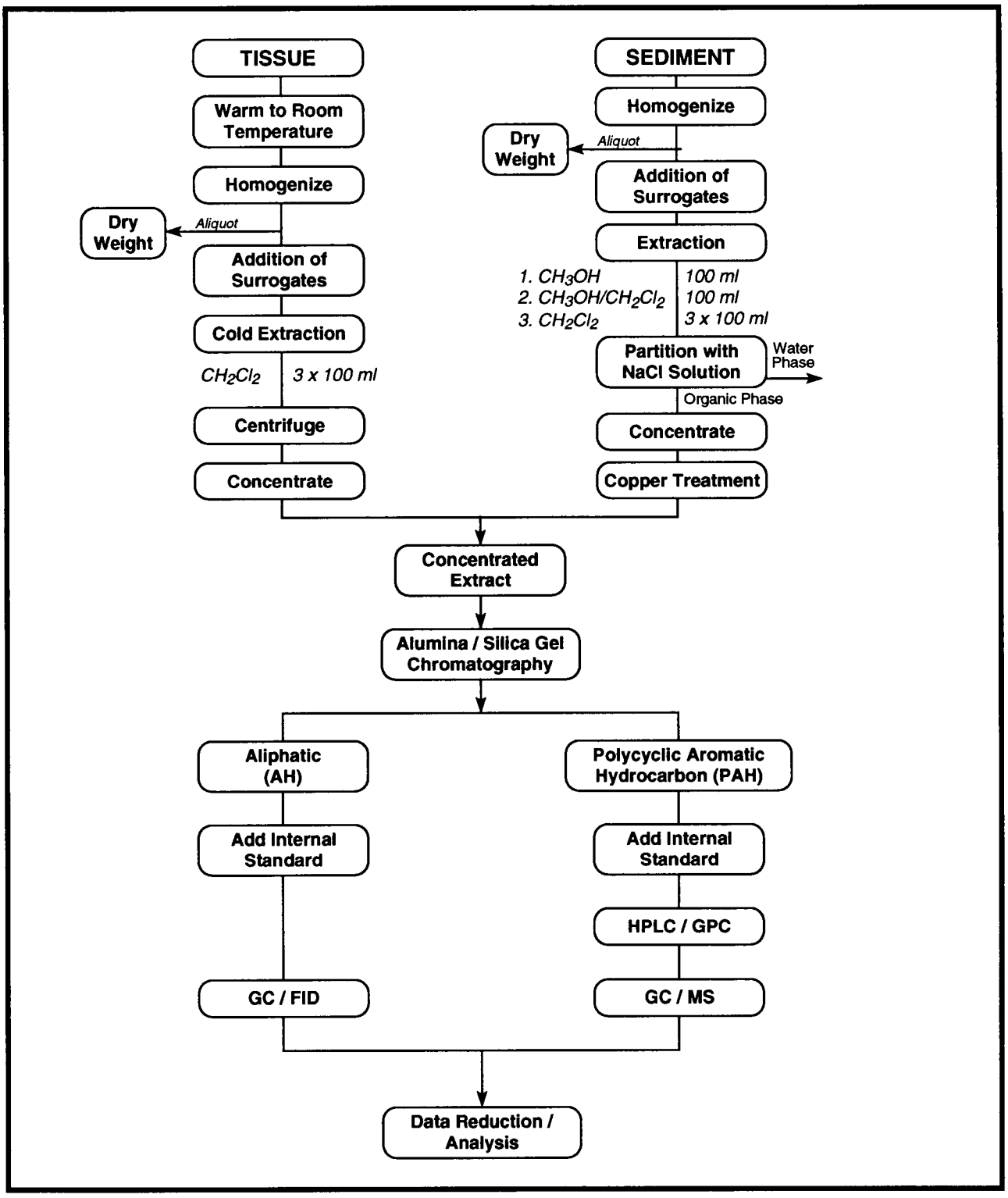


Figure 3.1. Summary of hydrocarbon analytical protocols.

3.3.1.2 Sediment Extraction

The sediment was freeze-dried before being homogenized by mortar and pestle. A subsample was weighed, freeze-dried, and reweighed to obtain percent moisture. Ten (10) g (dry weight) of sediment was added to an extraction thimble. One hundred and fifty (150) mL of methylene chloride was added to the extraction flask containing one or two boiling chips and surrogates were added. The sample was extracted for 4 to 8 hours, recycling every 4 minutes. The extract was concentrated by Kuderna-Danish techniques to 4-5 mL and transferred to a 25 mL concentrator tube to 1.0 mL.

3.3.1.3 Tissue Extraction

All tissue samples were mechanically macerated prior to extraction. If necessary, organisms were rinsed with reagent water to remove extraneous material. The macerated tissue was weighed into a centrifuge tube (2-15 g wet weight). A separate 5 g aliquot of macerated tissue was removed, placed in a tared weighing pan, and weighed. The tissue was dried at 50°C to a constant weight, allowed to cool and then reweighed to obtain percent moisture. One hundred (100) mL CH_2Cl_2, 50 g Na_2SO_4 and the appropriate amount of surrogate was added to each sample. The tissue was macerated for 3 minutes with the Tissumizer. The CH_2Cl_2 was decanted into a 500 mL flat bottom flask (centrifuge at ~2000 rpm for 5 minutes, if necessary). The extraction was repeated two more times with 100 mL aliquots of CH_2Cl_2. The CH_2Cl_2 aliquots were combined in a 500 mL flat bottom flask. The extract was concentrated as described for sediments above.

3.3.1.4 Extract Purification

A plug of glass wool and 2 cm of combusted sand were placed in a glass chromatographic column and the column was filled with hexane. Ten (10) g of alumina (deactivated 1% with water) in CH_2Cl_2 was slurry packed into the column and allowed to settle. Twenty (20) g of silica gel (deactivated 5% with water) in CH_2Cl_2 was slurry packed into the column and allowed to settle. One (1) cm of combusted sand was added on top of

the packed column and the CH_2Cl_2 was allowed to drain to the top of the sand. Fifty (50) mL of pentane was added to the column and drained to the top of the sand. A 500 mL flat bottom flask was placed under the column. The sample extract was transferred to the column in hexane. The column was drained to the top of the sand. The concentrator tube was rinsed twice with 1 mL of 50:50 pentane:methylene chloride that was added to the column. The column was drained to the sand layer. Two hundred (200) mL of 50:50 (pentane:CH_2Cl_2) was added to the column, eluted at 1 mL/min, and collected. This fraction contains the hydrocarbons. The extract was concentrated as described above.

If necessary, the fraction was further purified (especially tissue extracts) by high performance liquid chromatography using a Spectra-Physics SP8000 ternary HPLC pump, two size exclusion columns connected in series (22.5 x 250 mm Phenogel 100 Å columns), and a precolumn (8 x 50 mm Phenogel 100 A). Filtered (0.45 µm) dichloromethane was used as the mobile phase. The sample was injected onto the columns with an autosampler (Gilson Model 321). The HPLC unit was equipped with a UV absorbance detector (Model Water 440-Millipore). The fractions containing the compounds of interest were collected in 50-mL vials using the LKB Bromma 2211 fraction collector. The time interval in which the desired fraction was collected was based on the retention times of (4,4')dibromooctafluro-biphenyl (DBOFB) and perylene. These retention time markers were analyzed three times. Collection of the sample fraction began 1.5 min before the elution of DBOFB and ended 2 min after the elution of perylene. Assuming a constant isocratic flow of the mobile phase of 7 mL/min, the total time needed to collect the fraction was ~7 minutes. At the end of every batch of ten samples, the marker standard mixture was analyzed again to check retention time. After running a batch of samples (20), the columns were flushed and the precolumn was backflushed to remove sample matrix contamination from the system. On average, time for purification was ~35 minutes. The extract was then concentrated to 1 mL.

3.3.1.5 Quantitative Determination of Aliphatic Hydrocarbons and the Unresolved Complex Mixture (UCM)

This method quantitatively determines compounds from n-C_{10} to n-C_{34} and is based on high resolution, capillary gas chromatography using

flame ionization detection (GC/FID). A gas chromatograph with a split/splitless injection system, capillary column capability, and a flame ionization detector (FID) was utilized. The output from the detector was collected and processed by an automated HP-LAS 3357 data acquisition software package. A 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific or equivalent) was used. For the analysis of AH, the analytical system is set as summarized in Table 3.1.

Calibration solutions were comprised of the n-alkanes and isoprenoids (commercially available) listed in Table 3.2. Calibration standards were prepared in the concentration range of 1.25 to 50 mg/mL (at five concentrations). The surrogate compounds for all sample types was deuterated n-alkanes with 12, 20, 24 and 30 carbons. A surrogate solution is made by weighing an appropriate amount of pure material into a volumetric flask and diluting to volume with methylene chloride. The internal standard for this analysis was deuterated n-C_{16}. The matrix spiking solution consists of alkanes from n-C_{10} to n-C_{34} and pristane (Table 3.1). The matrix spike was added to samples at a concentration ~10x the MDL. The calibration mixture was also used as a retention index solution.

3.3.1.6 Quantitative Determination of Polynuclear Aromatic Hydrocarbons (PAH)

The quantitative method described determines the concentration of polynuclear aromatic hydrocarbons (PAH) and their alkylated homologues in extracts of water, tissues, and sediments. Quantitation was performed by gas chromatography-mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). Target analytes and confirmation criteria are listed in Table 3.3. The analytical systems included a temperature programmable gas chromatograph (Hewlett-Packard 5890A, or equivalent) and all accessories including syringes, analytical columns, and gases. The injection port was designed for split or splitless injection, though routine analyses were operated in a splitless mode. A 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific) was used. The autosampler was capable of making 1-4 mL injections. The mass spectrometer (HP/MSD) operated at 70 eV electron energy in the electron impact ionization mode and was tuned to maximize the sensitivity of the

Table 3.1. Analytical conditions for aliphatic hydrocarbon analysis.

Instrument:	Hewlett-Packard 5880A or HP 5890 Gas Chromatograph
Features:	Split/splitless capillary inlet system, HP-1000 LAS 3357 data acquisition system
Inlet:	Splitless
Detector:	Flame ionization
Column:	0.32-mm I.D. x 30-m DB-5 fused silica capillary column (J&W Scientific)
Gases:	
Carrier:	Helium 2 mL/min.
Make-Up:	Helium 33 mL/min.
Detector:	Air 360 mL/min. Hydrogen 33 mL/min.
Temperatures:	
Injection port:	300°C
Detector:	300°C
Oven Program:	60°C for 1 min. then 6°C/min. to 300°C, hold 5 min.
Daily Calibration:	Mid-level calibration solution; Retention index solution (20 mg/mL)
Quantification:	Internal standard/calibration

Table 3.2. Aliphatic hydrocarbons (AH) of interest.

Compounds of Interest
N-C_{11}
N-C_{12}
N-C_{13}
N-C_{14}
N-C_{15}
N-C_{16}
N-C_{17}
Pristane
N-C_{18}
Phytane
N-C_{19}
N-C_{20}
N-C_{21}
N-C_{22}
N-C_{23}
N-C_{24}
N-C_{25}
N-C_{26}
N-C_{27}
N-C_{28}
N-C_{29}
N-C_{30}
N-C_{32}
N-C_{34}

Table 3.3. Target PAH analytes.

Analyte	Quant. Ion	Conf. Ions	% Rel. Abund. of Conf. Ions
d_8-Naphthalene	136	134	15
Naphthalene	128	127	15
C_1-Naphthalenes (including isomers)	142	141	80
C_2-Naphthalenes	156	141	ND
C_3-Naphthalenes	170	155	ND
C_4-Naphthalenes	184	169,141	ND
d_{10}-Acenaphthene	164	162	95
Acenaphthylene	152	153	15
Biphenyl	154	152	30
Acenaphthene	154	153	98
d_{10}-Fluorene	176	174	85
Fluorene	166	165	95
C_1Fluorenes	180	165	100
C_2-Fluorenes	194	179	25
C_3-Fluorenes	208	193	ND
d_{10}-Phenanthrene	188	184	ND
Phenanthrene	178	176	20
Anthracene	178	176	20
C_1-Phenanthrenes/anthracenes	192	191	60
C_2-Phenanthrenes/anthracenes	206	191	ND
C_3-Phenanthrenes/anthracenes	220	205	ND
C_4-Phenanthrenes/anthracenes	234	219,191	ND
Dibenzothiophene	184	152,139	15
C_1-Dibenzothiophenes	198	184,197	25
C_2-Dibenzothiophenes	212	197	ND
C_3-Dibenzothiophenes	226	211	ND
Fluoranthene	202	101	15
d_{12}-Chrysene	240	236	ND
Pyrene	202	101	15
C_1-Fluoranthenes/pyrenes	216	215	60
Benzo [a] anthracene	228	226	20
Chrysene	228	226	30
C_1-Chrysenes	242	241	ND
C_2-Chrysenes	256	241	ND
C_3-Chrysenes	270	255	ND
C_4-Chrysenes	284	269,241	ND
d_{12}-Benz (a)pyrene	264	260	20
Benzo [b] fluoranthene	252	253,125	30, 10
Benzo [k] fluoranthene	252	253, 125	30, 10
Benzo (e) pyrene	252	253	30, 10
Perylene	264	253	20
d_{12}-Perylene	264	260	ND
Benzo [a] pyrene	252	253, 125	30, 10
Indeno[1,2,3-c,d]pyrene	276	277, 138	25,30
Dibenzo [a,h] anthracene	278	279, 139	25,20
Benzo [g,h,i]perylene	276	277, 138	25,20

ND = Not determined

instrument based on manufacturer specifications. The GC capillary column was directly inserted into the ion source of the mass spectrometer. The mass spectrometer computer system allowed for continuous acquisition and storage of all data during the chromatographic analyses. Computer software allowed display of any GC/MS data file for ions of a specific mass and plotting ion abundances versus time or scan number. Extracts were injected onto the capillary column of the gas chromatograph using the following conditions:

Injector Temp:	300°C
Transfer Line Temp:	280°C
Initial Oven Temp:	40°C
Initial Hold Time:	0 min.
Ramp Rate:	10°C
Final Temperature:	300°C
Final Time:	4 min.

Qualitative identification of target compounds was based on relative retention time (RRT) criteria. RRT windows for alkyl homologues were based on analysis of National Institute of Standards and Technology (NIST) SRM 1582 or other suitable reference oil. Just prior to analysis, an aliquot of internal standard solution was added to the sample.

The compounds in the surrogate solution were naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. All sample analyte concentrations were corrected for surrogate recoveries. The internal standards were resolved from all, but elute in close proximity to, the analytes of interest. The internal standards were fluorene-d_{10}, and benzo(a)pyrene-d_{12}. A solution containing 2 to 5-ring PAH compounds was used to fortify matrix spike samples.

A five-point response factor calibration curve was established demonstrating the linear range of the detector. The standard concentrations were 20, 100, 250, 500, 1000 ng/mL. The percent relative standard deviation for all calibrated analytes did not exceed ±15 percent with an $R > 0.99$ with a 1st degree fit of the data. After every 6-8 samples, the mass spectrometer response for each PAH relative to the internal standard was determined using check standards at concentrations of 250 ng/mL. Daily response factors for each compound was compared to the initial calibration curve. If the average daily response factors for all analytes was within ±15% of the calibration value, analyses proceeded. If, for any

analyte, the daily response factor exceeded ±35 percent of calibration value, a five-point calibration was repeated for that compound prior to the analysis of samples.

3.3.1.7 Quality Control

These methods are equivalent to NOAA National Status and Trends methodologies and quality assurance is the same as for the NOAA method. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. Blank levels were no more than 3x the method detection limit (MDL). If blank levels for any component are above 3x MDL, samples analyzed in that sample set were re-extracted and reanalyzed. If insufficient sample was available for re-extraction, the data was reported and appropriately qualified. Matrix spike/matrix spike duplicate (MS/MSD) samples were run with every 20 samples, or with every sample set, whichever was more frequent. The appropriate spiking level was 10x the MDL. Surrogate materials were spiked into every sample and QC sample. The appropriate spiking level was 10x the MDL. Surrogate and matrix spike recovery acceptance criteria are described in the instrumental section.

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives during instrumental analysis. All materials used in this method are routinely demonstrated to be free from interferences by processing procedural blanks identical to samples (one blank per 20 samples or each batch whichever was more frequent). Matrix interferences result from co-extraction of compounds other than the analytes of interest.

3.3.2 Trace Metals

Total trace element concentrations were determined in sediment, biological tissue and pore water from each study site. For most elements in sediments and tissues, NOAA Status and Trends Mussel Watch methodologies including a closed teflon bomb acid digestion are used. Table 3.4 summarizes the analytical methods used for each matrix.

Table 3.4. Trace element analytical methodologies by matrix[1].

Element	Sediment	Tissue
Al	FAAS	--
Ag	GFAAS	GFAAS
As	GFAAS	GFAAS
Ba	INAA	DCP-AES
Cd	GFAAS	GFAAS
Cr	INAA	GFAAS
Cu	GFAAS	GFAAS
Fe	INAA	FAAS
Hg	CVAAS	CVAAS
Mn	FAAS	--
Ni	GFAAS	GFAAS
Pb	GFAAS	GFAAS
Se	GFAAS	GFAAS
Sn	GFAAS	GFAAS
V	DCP-AES	DCP-AES
Zn	FAAS	FAAS

[1]Analytical methods are flame atomic absorption spectrophotometry (FAAS), graphite furnace or flameless AAS (GFAAS), instrumental neutron activation analysis (INAA), direct current plasma atomic emission spectroscopy (DCP-AES), cold vapor AAS (CVAAS), atomic florescence spectroscopy (AFS), sample iron-hydroxide precipitation pre-concentration followed by GFAAS (PC-GFAAS).

3.3.2.1 Sediment Analyses

All samples were received frozen. The samples were thawed, homogenized and a representative aliquot taken for freeze-drying. Barium, Cr, Fe and Sb were determined directly in freeze-dried samples by instrumental neutron activation analysis. Mercury was determined by cold vapor atomic absorption spectrophotometry (CVAAS) after a separate sulfuric/ nitric acid and permanganate/persulfate digestion. Samples were also totally digested in closed teflon bombs using a mixture of nitric and hydrofluoric acids. The resulting digest was analyzed for Al, Mn and Zn by flame (AAS) and V by direct current plasma atomic emission spectroscopy (DCP/AES). Manganese was not in the original scope of work and is being included at no charge to the project as a potential indicator of redox differences among individual samples and sites. All remaining elements (Ag, As, Cd, Cu, Ni, Pb, Se, Sn) were determined in the bomb digests using

graphite furnace (flameless) AAS. No significant analytical problems were encountered in the sediment analyses.

3.3.2.2 Tissue Analyses

All samples were received frozen. Where necessary the tissues (e.g. shrimp, crabs, gastropods) were thawed and the soft tissues separated prior to freeze-drying. All samples were freeze-dried and then homogenized by grinding to a powder. Mercury was determined by CVAAS after a separate sulfuric/ nitric acid and permanganate/persulfate digestion. All samples were also digested in closed teflon bombs using nitric acid. The resulting digest was analyzed for Fe and Zn by flame (AAS) and Ba and V by direct current plasma atomic emission spectroscopy (DCP/AES). All remaining elements (Ag, As, Cd, Cr, Cu, Ni, Pb, Se, Sn) were determined using graphite furnace (flameless) AAS. No significant analytical problems were encountered in the tissue analyses.

3.3.2.3 Quality Control/Assurance Procedures

All sample processing and analysis procedures were performed in such a manner as to minimize contamination and maximize data quality (accuracy and precision). A summary of these QA-related laboratory practices are given below:

1. All procedures were conducted according to approved laboratory standard operating procedures (SOP's) and only by personnel properly trained.

2. All refrigerator/freezer were checked for proper temperature daily. All balances were calibrated daily and all volume measuring devices (pipettes, etc.) were checked daily for proper operation and calibrated monthly.

3. All sample handling was done using new or acid-cleaned metal-free containers and implements. Cleaning procedures and sample processing were performed in a clean room when necessary to avoid sample contamination. Also all containers were kept closed or covered except when material was being added or removed. Distilled-deionized high purity water was used to prepare all detergent and acid cleaning solutions and for all rinses during cleaning procedures. Double-distilled, ultra-pure water was used

for all dilutions and to prepare all sample digestion/ processing reagents. Ultra-pure reagents were used whenever necessary to insure the procedural blank for a given analytical procedure was below the method detection limit for that procedure.

4. A detailed log was prepared for each digestion specifying all aspects of the procedure (e.g. SOP to be used, matrix spike levels, QA samples, etc.). As the digestion was performed, all information was recorded in a bound, pre-printed logbook for the specific digestion procedure being used. A full suite of quality assurance (QA) samples was run with each set of 30-35 samples digested. These include certified reference materials (5%), laboratory control samples (blank spikes, 5%), matrix spikes (5%), laboratory duplicates (5%) and procedural blanks (5%).

5. All standards used were traceable to National Institute of Standards and Technology standards and within expiration dates. The preparation of all standard solutions (including lot numbers, measuring devices and amounts used, etc.) was recorded in a single log book and all solutions were clearly labeled and traceable to the logbook entry.

6. During each analytical procedure, the instrument was calibrated at the beginning of the run and the calibration was checked (or re-calibrated) frequently during the run. Full re-calibrations were performed as necessary if the calibration changes more than 5% between any two checks. All data entered was verified independently by a second person.

7. Each analytical run was also evaluated based on the results of the QA samples. For example, Table 3.5 summarizes the QA data for all matrices from the analyses of the cruise 1 sample set. The acceptance criteria were also given in the Table for each QA parameter. When one or more QA parameters fell outside the acceptance criteria for a given digestion set and element, the samples were re-analyzed. If re-analysis did not bring the QA parameter(s) back to the acceptable range, the samples were re-digested and analyzed. No re-digestions were required for Cruise 1 samples.

3.4 Meiofauna

Extraction of meiofauna from sediment was performed using the decantation technique (Pfannkuche and Thiel, 1988). The sediment was dispersed and allowed to settle for a few seconds before meiofauna were

Table 3.5. Summary of QA data for GOOMEX Cruise 1 trace element data.

QA Type	QA Parameter	Acceptance Criteria	No. of QA Samples (INAA)	No. of QA Samples (AA)	AA Al	AA Ag	AA As	NAA Ba	AA Cd	NAA Cr	AA Cu	NAA Fe
SEDIMENT												
Method Detection Limit (MDL)	ppm dry wt.	--	--	--	450	0.03	0.29	16	0.01	2.5	0.44	125
Certified Reference Material	% Recovery	± 20 %	36	28	99.0	104.5	102.7	100.9	93.3	99.4	95.0	99.8
Blank Spikes (LCS)	% Recovery	± 20 %	10	17	90.8	81.4	101.5	106.5	104.0	102.4	96.0	105.2
Matrix Spikes	% Recovery	± 20 %	24	23	93.5	91.1	100.9	98.0	105.0	93.0	94.1	99.6
Laboratory Duplicates	Relative % Diff.	± 20 %	24	26	3.1	10.1	5.6	3.9	12.6	4.6	11.3	2.4
Blanks	Concentration (ppm)	Less than MDL	33	27	40	0.00	0.04	< 1.2	0.001	< 0.10	0.15	< 5.0
TISSUE						AA	AA	DCP	AA	AA	AA	AA
Method Detection Limit (MDL)	ppm dry wt.	--				0.035	0.17	0.50	0.01	0.12	0.25	12.80
Certified Reference Material	% Recovery	± 20 %		19-36		90.9	84.1	NCV	93.3	109	89.9	91.4
Blank Spikes (LCS)	% Recovery	± 20 %		20		98.1	94.5	91.8	100	97.6	87.3	94.8
Matrix Spikes	% Recovery	± 20 %		24		92.2	96.8	116	106	104	91.1	87.4
Laboratory Duplicates	Relative % Diff.	± 20 %		25		27.1	10.5	37.6	15.4	28.9	14.6	31.0
Blanks	Concentration (ppm)	Less than MDL		38		0.001	0.024	0.018	0.006	0.010	0.006	0.059

QA Type	QA Parameter	Acceptance Criteria	No. of QA Samples (INAA)	No. of QA Samples (AA)	AA Hg	AA Mn	AA Ni	AA Pb	NAA Sb	AA Se	AA Sn	AA V	AA Zn
SEDIMENT			--	--	0.008	2	0.72	0.35	0.14	0.17	0.11	2.0	2.2
Method Detection Limit (MDL)	ppm dry wt.	--	36	28	91.2	97.8	95.7	91.5	100.6	108.9	86.8	90.9	91.7
Certified Reference Material	% Recovery	± 20 %	10	17	101.2	101.8	83.9	89.4	96.1	101.2	99.2	96.8	97.0
Blank Spikes (LCS)	% Recovery	± 20 %	24	23	98.8	104.2	100.4	92.3	81.5	101.0	90.4	106.3	95.3
Matrix Spikes	% Recovery	± 20 %	24	26	16.3	5.4	5.3	15.5	21.0	51.8	6.1	6.0	11.7
Laboratory Duplicates	Relative % Diff.	± 20 %	33	27	0.000	0.00	0.05	0.02	0.010	0.01	0.03	< 0.73	0.34
Blanks	Concentration (ppm)	Less than MDL											
TISSUE					AA		AA	AA		AA	AA	DCP	AA
Method Detection Limit (MDL)	ppm dry wt.	--			0.034		0.17	0.12		0.49	0.19	0.50	1.90
Certified Reference Material	% Recovery	± 20 %	19-36		90.49		105	103		87.4	107	89.545	90.7
Blank Spikes (LCS)	% Recovery	± 20 %	20		90.24		92.7	93.4		93.8	87.3	87.1	92.5
Matrix Spikes	% Recovery	± 20 %	24		91.9		96.5	101		99.4	89.4	88.1	90.6
Laboratory Duplicates	Relative % Diff.	± 20 %	25		16.11		35.9	25.5		19.1	42.4	32.8	16.2
Blanks	Concentration (ppm)	Less than MDL	38		0.000		0.006	0.017		0.006	0.005	0.075	0.226

rinsed onto a sieve. This process was repeated several times until all animals were removed. Meiofauna were retained on a 0.063 mm sieve.

3.4.1 Taxonomic Identifications

Samples were counted to major metazoan taxonomic category and all harpacticoid copepods sorted using the subsampling techniques described in Sherman et al. (1984). All harpacticoid copepods were identified to the species level. A subset of nematodes was identified to the species level. The first 50 nematodes encountered were mounted in a drop of glycerin on a slide for taxonomic analyses. The formalin-ethanol-glycerol transfer technique was used to protect specimens from collapse (Seinhorst 1959). On rare occasions, we encountered less than 50 nematodes in a sample, in these cases all nematodes were identified to the species level. After nematodes and harpacticoids, the remaining taxa (probably 1-5% of the total fauna) were identified to the major taxonomic level. The major meiofauna taxonomic categories are listed in Table 3.6.

3.4.2 Harpacticoid Life History and Reproduction Study

Three species of harpacticoida were chosen for the meiofauna life history and reproduction studies. The harpacticoids come from the material obtained in the meiobenthic community structure studies. The three species chosen are different in terms of their suspected food requirements or in potential niche and habitat choices based on body morphology (Coull 1977; Montagna 1981). The body shape nomenclature, as recently revised by Warwick and Gee (1984), was used for these purposes. We have designated two species for these studies so far. *Cletodes dissimilis* is a semi-cylindrical depressed form from the family Cletodidae. It is a typical burrower. *Diarthrodes pusillus*, family Thalestridae, has a pyriform body, which is more typical of an epibenthic life style. The third organism is a species of *Longipedia*. *Longipedia* is an epibenthic form. A congener of this species is being used for the meiofauna toxicity tests. All three of the species used in the life history study are semi-cylindrical in shape. For every individual of the three species the copepodite stage class to determine the structure of population and the number of sexually mature males and

Table 3.6. Major meiofauna taxonomic categories.

Phylum	Class	Order
Kinorhyncha		
Gastrotricha		
Rhyncocoela		
Nematoda		
Arthropoda	Crustacea	Copepoda (Harpacticoida)
		Ostracoda
		Amphipoda
		Isopoda
		Tanaida
		Cumacea
Annelida	Polychaeta	
Mollusca		

females is determined. For all mature females and males the size of individuals and the number of gravid females is determined. For each gravid female, the number of eggs per gravid female and the size of eggs in clutches is determined to calculate clutch volume.

The life history and reproductive measurements were made by placing individuals in drops of glycerine and examining them with phase-contrast microscopy. Body size was determined using a calibrated ocular micrometer with resolution of 5 μm at 100x. Egg number, diameter and volume were measured with a resolution of 1 μm at 400x. Clutch volume was determined by assuming the eggs were spherical, calculating volume using the diameter and summing the volumes in each clutch. Life history stages were determined by counting the number of body segments in the abdomen of harpacticoid copepodites. One segment is added with each molt. Meiofauna were preserved in 70% alcohol and 10% glycerin. Voucher specimens were retained. New species that were described were sent to the Smithsonian National Museum of Natural History.

3.4.3 Toxicity Testing

Between 3000-4000 copepods were maintained in culture for toxicity testing experiments. Meiofauna can go through a life cycle in one to two

weeks, and can be cultured in dense assemblages. Harpacticoids were kept by the high density culture technique (Chandler 1986). The culture media was made from sterilized detrital mud where most dissolved organic matter was washed away. The detrital-mud contained an artificial flocculent layer which sustained high densities of harpacticoids.

3.4.3.1 High Density Culture Technique

Mud was collected from a pristine coastal site and put in the refrigerator until used. The mud was washed with distilled water through 0.5 mm and 0.125 mm sieves and left to settle for one hour. The suspended lighter clay size fraction was discarded by decantation and the remaining heavy fraction was condensed to 50% volume by drying for 24 hours at 120-140°C. The mud was stirred every 6-8 hours during the drying step. The dried medium was blended with a mixer, and 600 mL portions were added to 1 L beakers and autoclaved (120°C 16 psi 40 min). The medium was rehydrated by blending with filtered seawater in 1:1 proportions to make a wet medium. A flocculent media was prepared by suspending 20 g of the dried media into a 150 mL beaker into 130 mL 5% $Na(PO_3)_6$ in filtered seawater. This mixture was stirred and sonicated for 15-20 min, after which it was kept in a refrigerator for 4 hours to settle.

A 3-cm base layer of wet medium was put into the culture vessels and covered by a 1 cm thick flocculent layer and covered with 100 mL filtered seawater. The culture media was allowed to settle for 2 hours. A bent capillary tube was placed on the culture vessel edge to maintain the water level within 3 cm of the top and prevent overflowing. The system was then flushed for 24 hours at a drip rate of 1.0-2.0 mL/min before adding the fauna.

The culture was started with 30 gravid copepods. Inoculating copepods were pipetted into the culture vessels soon after collection as possible, washed gently with filtered seawater to remove most adhering ciliates and debris. A feeding mixture consisted of the algae *Isochrysis galbana, Duneliella tertiolecta, Thallassiosira weissflogii* and spinach fragments. The latter was prepared by blending 10 g of frozen spinach at high speed in 500 mL of filtered seawater for 3 min. This was washed with 43 μm screen, and put in 1 L beaker. The copepods were fed a four part

suspension of each of the three algal types and the spinach solution. If food accumulated on the sediment surface, cultures were not fed until it disappeared to avoid fouling.

3.4.3.2 Toxicity Testing

The pore water toxicity test on meiofauna followed a procedure similar to that used for sea urchin embryos (section 3.10). Ten mL of pore water is added to test dishes that will be kept in constant temperature and controlled light incubators. Each dish received 3 drops of a heat-killed feed mixture (as described above) at the start. Each test dish contained a freshly hatched nauplii. At the end of the incubation period acute effects were determined by counting living and dead adults, copepodites and nauplii. Sublethal effects were determined by measuring growth rates, body sizes and counting the total number of eggs produced and the number of eggs per clutch. Appropriate positive and negative controls were used.

3.5 Meiofauna Genetic Variability

In order to detect genetic differences in population structure as a function of proximity to a platform, the variability in DNA sequence of populations of animals that are near a platform, and those that are farther away have been examined. The hypothesis is that organisms near a platform, and the associated contamination, will have different haplotype frequencies than those removed from the platform, and that organisms near a platform will have less haplotype variability than those removed from the platform. We proposed to perform this analysis on the three dominant species of harpacticoid at each study site. The populations chosen were those that are closest to the platform, along the inner ring, and those which were farthest away, at the outer ring. We collected at least 10 replicate animals from the inner and outer ring at each of the three platforms, for a total of six stations and sixty samples. Since the platforms are in different biogeographical zones, it was necessary to use different species at each site.

The organisms collected from each station were preserved in ethanol, buffered with disodium ethylenediamine tetraacetate (EDTA), and refrigerated until use (Dessauer et al. 1990). DNA was extracted from individual copepods using the standard technique (Bucklin et al. 1992). This

involves digestion with a detergent and a proteinase, and a series of extractions using phenol and chloroform to produce a pure DNA sample.

Three specific regions, or loci, were chosen from the mitochondrial DNA of the copepod. These were the D-loop, or control region, the Cytochrome Oxidase I region, and the large sub-unit of ribosomal RNA. To determine intraspecific genetic variation at the molecular level, it was necessary to find a region of the genome that exhibited a high amount of sequence variation per genetic distance. The regions of DNA that code for a

step at 55°C, and a 4 minute extension step, at 72°C, during which the polymerase replicates each single strand of DNA that lies between the appropriate primer sequences (Bucklin et al. 1992). This reaction was conducted 36 times, producing greater than 1 billion fold increase in of the DNA regions situated between the two primers.

The amplification products were then separated by agarose gel electrophoresis. The gel was stained with ethidium bromide, and viewed under ultraviolet light to insure that the amplified regions were uncontaminated and were of the correct size. The PCR products were then subjected to a restriction digest. Because all restriction enzymes will cut a nucleic acid only at a very specific sequence, a change in even a single nucleotide between individuals is sufficient to produce bands of a different number or length. Statistical analyses were then performed. The restriction maps of individuals at one station are compared to those at another station. Statistically different variances between stations are tested as a measure of natural selection operating upon the animal related to the environment at that particular station.

A chronological summary of the steps in the genetic analysis procedure is outlined below:

1) DNA extraction
2) agarose gel to assess the effectiveness of the extraction
3) PCR amplification of the mtDNA D-loop
4) agarose gel to assess success of the amplification
5) second PCR amplification to purify the target DNA sample
6) restriction digest
7) agarose gel to analyze size of restriction fragments
8) statistical analysis of variation in restriction fragments

3.6 Macroinfauna

3.6.1 Processing and Identification

Each stage in the processing of samples was tracked. Each person working on a sample noted the date of completion and initialed the entry. All organisms collected were stored in 70% ethanol for the duration of the study. Voucher specimens were deposited in the U.S. National Museum, Smithsonian Institution, Washington, DC.

In the laboratory, each sample was washed with freshwater on a 0.5 mm mesh sieve. This removed formalin and any remaining sediments. Each sample was then replaced in its prelabeled jar and preserved in 70% rose bengal stained ethanol. After the elapse of at least 24 hours, to allow stain to penetrate organisms with hard exoskeletons, samples were examined using a dissecting microscope or other lens system. All stained material was removed and placed in clean 70% ethanol. This allows some destaining prior to identification. The specimens in each sample were then identified to lowest possible taxon and counted. All specimens of each species were placed in a vial with a label bearing species name, station number and date. Notes were made regarding the presence of ovigerous females, sex, and numbers of young specimens (based on general knowledge of sizes of adult organisms) in the population. The data were recorded on printed data sheets.

3.7 Macroinvertebrates

3.7.1 Taxonomic Identification

At least five (10 when possible) of the most abundant species of macroinvertebrates common to the platform and comparison sites were targeted. The sampling scheme uses a near and far approach placing station locations at the extremes of the chemical contaminant gradient. Most of the mobile invertebrates collected were fairly large, easily identifiable species. Therefore, most specimens of interest in the present study were identified on shipboard with existing taxonomic keys immediately after capture.

3.7.2 Measures of Reproductive Effort

The histological method employed is the same as for Status and Trends. Upon return to the laboratory all preserved animals were rinsed in fresh water at room temperature for 6 to 12 hrs to remove the Bouin's fixative. The samples were then dehydrated by rinsing twice each in 50 and 70% ethyl alcohol for 4 to 8 hrs and were then stored in 70% alcohol until further processing.

Methods used and tissues selected and prepared for histological embedding were species specific. Shrimp and crabs were cut in half

longitudinally. The exoskeleton and all exterior appendages were removed. Starfish were cut so that all arms were removed from the central body disc which remains intact. Starfish were decalcified at this point if the spicules were not completely dissolved by the Bouin's fixative. For scallops, the stomach, gonad, gills, and a portion of the mantle tissue were excised from the adductor muscle and other viscera.

All animal tissues were dehydrated before being embedded in paraffin for histological analysis. Samples were processed using an MX-200 TissueTek automated histological preparation machine. A standard embedding procedure is summarized in Table 3.7.

Once the samples were processed by the TissueTek, they were placed in paraffin in a vacuum oven overnight. The next day, individual tissues were placed into a mold, cast in fresh paraffin and allowed to harden. Orientation of the tissues in the mold was species specific. Shrimp were placed with the sagittal cut facing the bottom of the mold. Crabs were oriented upside-down in the mold so that the gonad/digestive tissue was next to the bottom of the mold. Scallops were placed so that the entire section was oriented horizontally in the mold. Starfish legs were positioned in the mold so that the oral surfaces were next to the bottom of the mold.

The paraffin blocks were sliced at 10 μm on an AO microtome. The sections were affixed to microscope slides using albumin adhesive by standard methods, and placed on a warming tray for 6 hrs to smooth the sections. Two or three sections, depending on size, were placed on each slide and triplicate slides were made of each sample. The slides were then exposed to formalin fumes overnight to harden the albumin adhesive. The slides were stained using a standard hematoxylin-eosin (H&E) procedure following Preece (1972; Table 3.8)

After staining, tissue sections were analyzed for sex and stage of reproduction. No scales for stages of reproductive development are available for most crab and shrimp species. We developed such scales based on literature information and our observations. The stages of reproduction were based on the size and development of eggs and the overall density of gametes. The reproductive scale for crabs was based on Johnson (1980) is summarized in Table 3.9.

Table 3.7. A standard embedding procedure for histological analysis.

Procedure	Treatment	Time
Dehydration	80% EtOH	1 hr
	95% EtOH	2 hr
	95% EtOH	2 hr
	100% EtOH	2 hr
	100% EtOH	2 hr
	100% EtOH	2 hr
	100% EtOH	1 hr
Clearing	xylene	1 hr
	xylene	2 hr
	xylene	2 hr
Infiltration	50:50, xylene and paraffin	2 hr
	paraffin	2 hr

Table 3.8. Standard hematoxylin-eosin procedure to stain slides (after Preece 1972).

Procedure	Treatment	Time
Deparaffinization	xylene	5 min
	xylene	5 min
	100% EtOH	5 min
	100% EtOH	5 min
Colloidionization	celloidin solution	quick dip
Hydration	80% EtOH	2 min
	50% EtOH	2 min
	30% EtOH	2 min
	running water	5 min
Staining	Harris' hematoxylin	8 min
Rinsing	running water	5 min
Decolorizing	acid alcohol	3 quick dips
Rinsing	running water	1 min
Bluing	lithium carbonate	.5 min
Washing	running water	2 min
Counterstaining	Picro-eosin/Navy eosin	1 min
Dehydration	95% EtOH	2 dips
	95% EtOH	3 dips
	100% EtOH	1 min
	100% EtOH	1 min
	100% EtOH	1 min
Clearing	xylene	5 min
	xylene	5 min
Mounting	Permount	24 hr to dry

Table 3.9. Reproductive development scale for crabs (after Johnson 1980).

Numerical Designator	Reproductive Stage	Characteristics of Stage
FEMALE		
1	early	eggs present, but few and very small
2	mid	ova do not fill cyst, loosely arranged
3	mature	ova fill space, closely packed, nucleated
4	fertilized	spermatophore present
MALE		
1	early	small loosely packed spermatids
2	mid	mixed ages of sperm
3	mature	closely packed sperm, sperm in ducts
4	spawning	spermatophores in anterior vas deferens

Table 3.10. Reproductive development scale for shrimp (after Bell and Lightner 1988).

Numerical Designator	Reproductive Stage	Characteristics of Stage
FEMALE		
1	undeveloped	ovary barely noticeable
2	early	ova do not fill cyst, loosely arranged
3	late	ova fill space, closely packed, no rod-shaped peripheral bodies visible
4	ripe	peripheral bodies visible in ova
5	spawning	peripheral bodies visible in remaining ova, spaces left by spawned ova visible
6	spent	few ripe ova left, remnants of follicles
7	resorbing	disorganized zone of resorption present in core of cyst, some ripe ova visible
MALE		
1	undeveloped	vas deferens visible but no sperm found
2	early	few sperm, loose packed
3	late	dense sperm
4	mature	very dense sperm, sperm in ducts

Table 3.11. Reproductive development stages for molluscs.

Numerical Designator	Reproductive Stage	Characteristics of Stage
1	undifferentiated	little or no gonadal tissue visible
2	early	follicles beginning to expand
3	mid	follicles expanded and beginning coalesce; no mature gametes present
4	late	follicles greatly expanded, coalesced, but considerable connective tissue remaining; some mature gametes present
5	fully developed	most gametes mature; little connective tissue remaining
6	spawning	gametes visible in gonoducts
7	spawned	reduced number of gametes; some mature gametes still remaining; evidence of renewed reproductive activity
8	spawned	few or no gametes visible, gonadal tissue atrophying

The scale for reproductive development was similar for shrimp species and is based on Bell and Lightner (1988). More information was available for the stages of reproductive development in penaeid shrimp, especially for females, so the scale for shrimp was more detailed than that for crabs (Table 3.10).

Stages of reproduction in molluscs was similar among species (Table 3.11). Therefore, the scale used for scallops and other molluscs analyzed was that used for oysters in the Status and Trends program and more fully described in Craig et al. (1989) and used by Wilson et al. (1992).

Little information is available on the reproductive cycles of starfish and brittle star species so the sex was determined and an approximate stage of reproductive maturity assigned based on the size of eggs in females and the overall density of gametes present (Freeman and Bracegirdle 1971).

Recent information indicates that parasites and pathogens, as well as reproductive development are important in determining the pollutant body

burden of oysters. In many cases 75% of the variation in pollutant body burden in oysters between bay systems around the Gulf of Mexico can be explained by variations in health and parasite prevalence and infection intensity. Accordingly, and because some parasites destroy gonadal tissue preferentially, a histopathological analysis was also conducted. Fortunately, the sections for gonadal analysis can be histopathologically examined. Each slide was examined for significant parasites and pathogens, as well as pathological abnormalities. In cases where tissues from large animals were subsampled (e.g., large crustaceans) for gonadal development, additional tissues were fixed for histopathology. These tissues always included the digestive gland, liver or hepatopancreas (depending on the nomenclature used) and gill when present.

3.7.3 Immunological Probe

The protocol for developing the antibody to egg and sperm protein is outlined in general form as follows. New Zealand white rabbits were used as the host animal to raise antibodies against egg or sperm protein for each species selected. Freund's adjuvant complete, which contains a water-in-oil emulsion and a mycobacteria cell suspension as an antibody production stimulant, was used for the initial injection. Subsequent injections used Freund's adjuvant incomplete, which lacks mycobacteria. For injection, the antigen (i.e., egg or sperm protein) was well-mixed with Freund's adjuvant and injected into the rabbit subcutaneously at the hind legs. The total volume of injected material was adjusted to 1 mL. This 1 mL was injected in 200 mL aliquots at several different sites around the hind legs. The injection schedule and protocol used in the production of antiserum is summarized in Table 3.12.

At the 4th and 8th week after injection, 10 mL of test blood was withdrawn from the rabbit and the antiserum isolated from the red blood cells. To do this, the blood was collected in a clotting vial, which was then stored at 4°C overnight or at room temperature for 10 hrs. After clotting, the blood was centrifuged at 700 X g for 15 min, the antiserum pipetted into a centrifuge tube, and stored at 4°C.

The immune response of the rabbit to the egg or sperm protein was identified using passive hemagglutination and ring immuno-diffusion

techniques for examining the strength of the antibody according to Garvey et al. (1977). These test techniques are described below. The ring immuno-diffusion test is the simplest method to detect the antibody-antigen reaction. A 300 mL-aliquot of the antiserum to be tested was placed in a test tube. A 300 mL-aliquot of antigen was carefully layered on top of the antiserum. The test tube was incubated at room temperature for at least 2 hrs. A positive reaction results in the precipitation at the antiserum-antigen inter-layer. The degree of precipitation varies depending on the strength of the antiserum.

Table 3.12. Protocol for producing antiserum for an immunological probe.

Time	Activity	Treatment
1st Week	Initial injection	1 mg Antigen in 500 mL + 500 mL FAC
2nd Week	Booster	500 mg Antigen in 500 mL + 500 mL FAI
3rd Week	None	None
4th Week	Booster and test bleeding	500 mg Antigen in 500 mL + 500 mL FAI
5th Week	Booster	500 mg Antigen in 500 mL + 500 mL FAI
6th Week	Booster	500 mg Antigen in 500 mL + 500 mL FAI
7th Week	None	None
8th Week	Test bleeding	None

FAC: Freund's adjuvant complete. FAI: Freund's adjuvant incomplete.

The second test procedure was the passive hemagglutination test. Freshly-drawn sheep blood was mixed in an equal volume of Alsever's solution to prevent coagulation, and centrifuged at 700 X g for 10 min. The erythrocytes were rinsed four times with 7-10 volumes of PBS(I). After the final washing, the erythrocytes were brought to a 10% concentration in PBS(I). An equal volume of 3.7% formaldehyde was added to the erythrocyte preparation. The suspension was allowed to stand, with occasional stirring, at room temperature for 4 to 6 hrs. After the initial incubation, the suspension was continuously stirred at either 30°C or 37°C for 14 to 18 hrs. These formalinized sheep erythrocytes were washed four times, each time with 10 volumes of PBS(I) and then stored as 10% concentration in PBS(I) at 4°C.

The formalinized sheep red blood cells (RBC) were collected and washed once with PBS(II). A 0.5 mL aliquot of packed RBC were suspended in 25 mL 0.05% tannic acid and incubated for 45 min at 37°C, with intermittent mixing. The RBC in tannic acid were washed three times, with

20 volumes of PBS(II), centrifuging at 700 X g for 10 min after each wash. The final wash was done using McIlvain Buffer and the RBC was resuspended in 12 mL of McIlvain Buffer. The tanned RBC remained usable for periods up to 18 hrs if kept at 4°C. Antigen was added to the tanned RBC in the proportion 3.5 mg antigen: 0.5 mL tanned RBC. The mixture of antigen and RBC was incubated at 37°C for 1 hr. After incubation, the antigen-coated RBC were rinsed three times with three volumes each of PBS(II). The antigen-coated RBC were resuspended in PBS(II) with 0.07% bovine serum albumin at 1% concentration to avoid agglutination of the RBC.

The test procedure was as follows. Freshly-harvested antiserum to be tested was serially diluted 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048 in PBS(II). A 50-mL aliquot of the serially-diluted antiserum was added to each well of a 12 x 8 well microplate. A 50-mL aliquot of the antigen-coated RBC was added to each well containing serially-diluted antiserum and the plate shaken using a microplate shaker to mix the RBC and the antiserum. The plate was incubated for 2 to 4 hrs at room temperature. After incubation, the pattern formed by the RBC at the bottom of each well was examined according to the following criteria.

Positive: diffuse compact granular film of agglutinated cells covering the bottom of the well; edges of film either folded or somewhat ragged.

Neutral: narrow ring of cells surrounding a diffuse film of agglutinated cells.

Negative: heavy ring of cells or a discrete smooth button of cells in the center of well.

The titre of the antiserum, which is the greatest dilution of a given antiserum that still elicits a positive reaction to antigens, increases as the immunization procedure progresses. However, the titre attains a stable level and will not increase with further booster injections at 2 to 3 months following the initial injection. Once a host animal shows a maximum immune response the animal is either exsanguinated or maintained at the same high level of antiserum by booster once each 4- to 6-month period.

Instantaneous rates of reproduction were measured using a radioimmunoassay based on the gonadal probes described by Choi et al. (1989). Labeled leucine was injected and incorporation followed. Labeled egg and sperm protein were separated from labeled somatic protein using

the gonadal probes and each fraction counted by liquid scintillation counter. Our polyclonal probe for oysters is active against 90% of the gonadal protein component; consequently, accurate estimates of rates of reproductive growth can be made. Free leucine will be measured by amino acid analysis as we have described (White et al. 1988) to estimate specific activity. Knowing the amount of gonadal protein (in g), the amount of leucine incorporated, and the specific activity permits the calculation of the instantaneous rate of reproductive growth and the division of energy between somatic and reproductive growth. Normalization to total gonadal protein were done by ELISA also using our probes.

The specific protocol for the radioimmunoassay used for oysters is as follows. The labeled leucine was injected into the oyster adductor muscle (leucine was chosen based on amino acid analysis of egg protein content). The oyster was sacrificed after an appropriate period and the tissue homogenized. The oyster tissue was reacted with rabbit anti-oyster egg (or sperm) IgG for 2 hr. A Protein A cell suspension was added. The Protein A cell suspension (*Staphylococcus aureus*) reacted with IgG and aided in precipitating the antigen-antibody complex. The sample was centrifuged at 7000 rpm. The pellet was resuspended, dissolved using Solusol protein digester, scintillation cocktail added and the sample counted.

3.8 Demersal Fish

Most of the demersal fishes collected were easily identifiable species. Most specimens of interest in the present study could be identified on shipboard with existing taxonomic keys immediately after they had been captured. Following appropriate necropsies and removal of stomachs, bile, and tissue samples, these specimens were immediately preserved in 10% buffered formalin and returned to the laboratory for taxonomic verification. Each specimen identified on shipboard was individually tagged, numbered, and re-identified in the laboratory. Since each tissue sample and stomach removed bears the same number as the fish from which it was removed, subsequent taxonomic changes can be made without ambiguity.

3.8.1 Necropsies

Gross pathological disorders have a scientific base; severely polluted habitats have a higher frequency of gross pathological disorders than similar,

less polluted habitats (Sinderman 1979; O'Connor et al. 1987; Buhler and Williams 1988; Malins et al. 1984; 1988). Laboratory exposures to contaminants such as PCBs, petroleum products, and pesticides suggest that many gross pathological disorders are associated with long-term contaminant exposure (Sinderman 1979; Capuzzo et al. 1988). All individuals of each target species were examined externally for gross pathological disorders including skin ulcers, fin erosion, gill abnormalities, visible tumors, cataracts, or spinal abnormalities. Fish found to have pathological defects were preserved for detailed histopathological examination. Results of the detailed examination are used to identify possible causes of aberrations and to ensure that the defects were not ones that could have resulted from abrasion and physical damage during collection.

3.8.2 Histopathology and Splenic Macrophage Aggregate Analysis Methods

Samples of liver and spleen were taken from all specimens and processed for histopathological analysis. Tissue samples were dehydrated in an ethanol gradient, cleared in xylene, and infiltrated and embedded in paraffin. Sections were cut at 6 μm on a rotary microtome and slides were stained with Harris' hematoxylin and eosin. Stained slides were examined microscopically by experienced fish pathologists. Results were tabulated to identify any pathological abnormalities and to create a baseline of histopathological data.

Splenic MA analysis was performed on tissue sections of spleen prepared for histological evaluation as described above. Data are generated using a true color (HSI imaging) Particle Analysis package (Microcomp Image Analysis System with Sony 3CCD color video camera input). The system was calibrated and data collected at 10x magnification. Three fields of view (screens) were randomly selected and analyzed from each spleen sample. After a screen was randomly selected minor adjustments of the microscope stage position were made so that no MAs are lying on the edge of the counting field and then the full color image was captured as a digital image. Analysis was performed on images generated by computer produced masks of the MAs in each screen with the number of MAs per screen and the area in μm^2 of each MA recorded and stored under an appropriate file

name for future statistical analysis. A size discriminator was used to eliminate objects < 50 μm^2 (~size of three aggregated macrophages). Total screen area counted was also determined for calculation of percent area occupied by MAs.

3.8.3 Analysis of Fish Food

Fish food analyses were conducted on fish species whose food was also being analyzed for chemical contaminants. On shipboard specimens for food analysis were identified, labeled and preserved in 10% buffered formalin. In the laboratory these specimens were re-identified, and the stomachs were removed, rinsed in water overnight, and transferred to 70% ethanol for storage. Food analysis procedures followed those of Darnell (1958) and Rogers (1977). Food volumes were determined by volume displacement. Stomach contents were then transferred to a petri dish containing a bottom grid so that the percentage composition of each food group can be visually estimated. Identification of food items was made to the lowest reasonable taxonomic level. Results were reported in terms of pooled data, i.e., all individuals examined from a given station and site (near or far) were lumped together.

3.9 Detoxification Systems

All assays were optimized with respect to pH, temperature, protein content, substrate concentration and length of time for linearity for fish. Microsomal preparations were made from all samples as noted below. Microsomal protein content was analyzed by the method of Bradford (1976).

3.9.1 Microsomal Preparatory Steps Common to All Samples

After removal from liquid nitrogen, samples were kept on ice during all processing steps. The samples were homogenized using a Polytron in an appropriate buffer.

3.9.2 Crustaceans

The green gland or hepatopancreas was homogenized in a buffer containing 0.1 M potassium phosphate, pH 7.4, 0.1 M KCl, 1 mM EDTA, 0.1 mM phenanthroline, 1.0 mM dithiothreitol, 1.0 mg/mL trypsin inhibitor. The samples were then centrifuged at 600 X g for 20 min, 10,000 X g for 20 min and finally 100,000 X g for 60 min. The microsomal pellets were resuspended in a buffer containing 100 mM potassium phosphate, pH 7.4, 1.0 mM EDTA, 1.0 mM dithiothreitol, 0.1 mM phenanthroline, 1.0 mg/mL trypsin inhibitor and 15% glycerol. Microsomes were immediately frozen in liquid nitrogen until enzyme assays were conducted.

3.9.3 Worms

Whole worms were homogenized in a buffer containing 0.05 M Tris, pH 7.5, 0.15 M KCl, 0.25 M sucrose. Following homogenization samples were centrifuged at 700 X g for 10 min, 8000 X g for 10 min and 100,000 X g for 60 min. Microsomal pellets were resuspended in a buffer containing 0.05 M Tris, pH 7.5, 1 mM EDTA in 20% glycerol and immediately frozen in liquid nitrogen until analyses.

3.9.4 Molluscs

The hepatopancreas was homogenized in a buffer containing 20 mM Tris, pH 7.6, 0.5 M sucrose, 0.15 M KCl, and 1 mM EDTA. Samples were centrifuged at 500 X g for 1 hr, 10,000 X g for 45 min and 100,000 X g for 90 min. The microsomal pellets were resuspended in a buffer containing 20 mM Tris, pH 7.6, 1 mM EDTA and 20% glycerol. Samples were immediately frozen and stored in liquid nitrogen until analyses.

3.9.5 Fish

Livers were homogenized in a 0.1M Tris, pH 7.4 buffer containing 0.25 M sucrose and centrifuged at 600 X g for 10 min, 5000 X g, for 10 min, 13,000 X g for 10 min and 100,000 X g for 60 min. The microsomal pellet was resuspended in a 50 mM Tris, pH 7.5 buffer containing 1 mM EDTA, 1

mM DTT in 20% glycerol. Samples were immediately frozen in liquid nitrogen until enzyme assays were conducted.

3.9.6 Ethoxyresorufin O-deethylase (EROD) Assay for Fish

EROD activity was measured in fish hepatic samples fluorometrically. The assays were conducted in 1.15 mL incubation mixtures consisting of 0.1 M HEPES, pH 8.0, 0.1 mg NADPH, 0.1 mg NADH, 0.7 mg BSA, 0.7 mg of $MgCl_2$, and 50 µL of microsomes (200-250 µg microsomal protein). The samples were preincubated for 2 min at 30°C. The reactions were initiated by the addition of 50 µL of 100 mM ethoxyresorufin. The samples were allowed to incubate for 10 or 15 minutes and stopped with the addition of 2.5 mL of methanol. The samples were allowed to sit in the incubator for 5 min, after which they were centrifuged for 15 min to pellet the flocculated protein. Samples were read on a spectrofluorometer at 550 nm excitation and 585 nm emission wavelength settings.

3.9.7 Aryl Hydrocarbon Hydroxylase (AHH) Assay for Fish and Invertebrates

AHH activity was assayed in both fish and invertebrates. For fish the assays were conducted in 1.0 mL of reaction mixture containing 0.1 M HEPES, pH 8.0, 0.4 mM NADPH, and 500 µg microsomal protein. The samples were preincubated at 30°C. The reaction was initiated with the addition of 80 µM benzo[a]pyrene (BaP) in 40 µL methanol. The samples were incubated for 10 or 15 min and stopped with the addition of 1 mL cold acetone, after which 3.25 mL of hexane was added. The samples were shaken, 2 mL of the organic layer was withdrawn and extracted with 5 mL of NaOH. The samples were shaken again and a NaOH aliquot was read on a spectrofluorometer at 396 excitation and 522 emission wavelength settings. The spectrofluorometer was calibrated using authentic 3-OH BaP standard. This assay was slightly modified for the invertebrate assays. The reaction mixture consisted of 50 mM Tris, pH 7.6, 0.1 mM NADPH and 500 µg protein. The reaction was initiated by the addition of 60 µM BaP in methanol. The samples were incubated for 15 min at 30°C and stopped with the addition of 1 mL of cold acetone. The extraction procedure and instrument analysis were as described for fish.

For cruise I EROD and AHH assay conditions were optimized for one species (*Pogonias cromis*) because of sample availability. However, assay conditions have been optimized for additional species collected on Cruise 2 and no significant differences in assays conditions were noted for any of the species.

3.9.8 Cytochrome P450 Assay for Fish and Invertebrates

Cytochrome P450 was determined on all sample times from Cruise 1. Cytochrome P450 content was determined by the carbon monoxide difference spectrum of sodium dithionite-reduced samples using a method similar to that described by Omura and Sato (1964). Microsomes were added to a buffer containing 50 mM Tris, pH 7.6. Carbon monoxide was bubbled into the samples prior to the addition of sodium dithionite. Cytochrome P450 was measured spectrophotometrically and the levels were quantified using an extinction coefficient of 91 $mM^{-1}cm^{-1}$ (Estabrook and Werringloer 1978).

3.9.9 Rat Hepatoma H-4-II E Assay

Invertebrate samples were first extracted by a method described in detail elsewhere (Wade et al. 1988). Briefly, samples were extracted using a Tissumizer with 50 g of $NaSO_4$ and 100 mL of CH_2Cl_2 in 200 mL centrifuge tubes. The extraction was repeated two more times with 100 mL of CH_2Cl_2. The extracts were combined and concentrated. The concentrate was purified using a silica gel/alumina column to remove matrix interferences. Further purification was performed by HPLC. The purified extracts were then evaporated to near dryness and picked up in a known volume of DMSO.

Rat hepatoma H-4-II E cells were grown as a continuous cell line in an α-minimum essential medium without ribonucleosides, deoxyribonucleosides, and sodium bicarbonate, but with L-glutamine. The medium was supplemented with 10% fetal calf serum, 10% calf serum, gentamycine sulfate (50 mg/mL), and Fungizone (2.5 mg/mL). Stock culture cells were grown in 150 cm^2 culture flasks in a humidified air/carbon dioxide (95%/5%) atmosphere at 37°C. After reaching confluency, the cultures were trypsinized and seeded in 25-cm^2 culture flasks 10^6

cells/plate in 5 mL of medium. The cells were allowed to grow for 24 h. The test extracts in DMSO were added to the cell culture system so that the final concentration of DMSO in the culture medium was 0.5%. After incubation of the extract for 24 h, the medium was removed and the cultures were washed thoroughly with phosphate-buffered saline (PBS), pH 7.4. The cells were then harvested in a Tris/sucrose (0.05 to 0.2 M) buffer, pH 8.0, by scraping with a rubber policeman. The cellular protein pellet was recovered after centrifugation for 5 min at 10,000 X g and resuspended in the Tris/sucrose buffer, the suspension was made up to a concentration of 1.0 mg of protein/mL for use in the EROD assay described above.

3.9.10 mRNA Method

3.9.10.1 Isolation of RNA

Total RNA was isolated from homogenized liver samples in 1 mL of solution D (4 M quanidinium thiocyanate, pH 5.5 and β-mercaptoethanol) by the method of Chirgwin et al. (1979) with modifications. RNA is extracted by adding 50 µl of 2 M sodium acetate to 125-500 µL of a sample in a microfuge tube, to which 500 µl of water-saturated phenol, 200 µl phenol water, and 200 µl of Sevag (24:1 chloroform: isoamyl alcohol) is added. The samples were then vortexed and centrifuged for 20-30 min at 10,000 X g. This extraction procedure was repeated twice more. The final aqueous layer was added to one volume of isopropyl alcohol and allowed to precipitate overnight at -20°C. The resulting precipitate was centrifuged at 10,000 X g for 20 - 30 min and the resulting supernatent was decanted and the pellet was washed with 100% ethyl alcohol followed by two washings with 70% ethyl alcohol. The pellet was dried at room temperature and then resuspended in 15 mL CCS (1 mM sodium citrate, 1 mM CDTA and 1% SDS at pH 6.8) by heating at 37-42°C. The RNA was quantitated on a spectrophotometer at 260 nm.

3.9.10.2 Northern Blot

A sample containing 10-15 µg RNA in 2 X sample loading buffer (73% formamide, 25% formaldehyde, 1.65% 1 M Na_2HPO_4, pH 6.8 and 1 X

northern dye) was loaded onto a 1.2% agarose gel and run at 50 - 75 V for 3 - 4 h. The gel was stained in ethydium bromide, washed and transferred to an Hybond^{TM}-N+nucleic acid transfer membrane for a minimum of 12 h. The membrane was washed in 0.1 X SCC, cross-linked by UV exposure and baked for 1-2 hours at 80°C. The membrane was placed in pre-hybridization solution (NEN) for 12 hours at 65°C and randomly labeled with freshwater rainbow trout cDNA (courtesy of John Lech) for P4501A.

3.9.11 PAH Metabolites in Bile

Biliary PAH metabolites were analyzed by HPLC/fluorescence detection, which has been described in detail elsewhere (Krahn et al. 1984; 1986). Briefly bile was injected into an HPLC, metabolites were resolved using a C-18 reverse-phase column and detected fluorometrically. Aromatic compounds fluorescing at naphthalene (290/335), phenanthrene (260/380) and BaP (380/430) excitation/emission wavelengths were analyzed. Metabolites eluting from the column within specific retention times were summed to yield total fluorescence based on equivalents of known amounts of naphthalene, phenanthrene, and BaP standards.

3.10 Pore Water Toxicity Testing

As soon as possible after the sediment samples arrived in the lab, pore waters were pressure extracted, centrifuged to remove fines, and frozen (NFCR SOP F10.9). Recent studies in our laboratory have demonstrated that if the suspended particulate material is removed prior to freezing, there is no difference in toxicity between fresh and frozen/thawed samples (Carr 1992; Carr and Chapman in review). A separate aliquot of each pore water sample was frozen for later chemical analysis. Pore water was stored frozen until two days before the start of the toxicity test, at which time samples were removed from the freezer and placed in a refrigerator to thaw slowly overnight. Samples which remained partially frozen in the morning were allowed to thaw at room temperature or in a tepid water bath. Water quality measurements (dissolved oxygen, pH, temperature, hydrogen sulfide, and ammonia) were then made, and the salinity of the samples adjusted to 35 ±1‰, if necessary. Hypersaline brine (~100‰) and bottled purified water

(Baker water for HPLC) were used to adjust sample salinity. If the dissolved oxygen concentration was below 80% saturation, samples were gently aerated until this concentration was achieved. Samples were then stored refrigerated overnight and returned to 20°C before the start of the tests.

The toxicity of the sediment pore waters was determined using two different tests with the sea urchin *Arbacia punctulata*; the fertilization test (Horning and Weber 1987; NFCR SOP F10.6) and the embryological development test (Oshida et al. 1981; NFCR SOP F10.7). The fertilization test consists of incubation of a known quantity of sea urchin sperm in a 5 mL pore water sample in a scintillation vial for 30 minutes, followed by the addition of a predetermined number of eggs and an additional 30 minute incubation. After the incubation, the test is terminated with the addition of 10% buffered formalin and the percentage of fertilized eggs is determined. Five replicates of each treatment are performed. Treatments which produce statistically significant reduced fertilization, compared to a control, are considered toxic. The embryological development test is similar, except that the eggs are added first, followed immediately by the sperm. The embryos are allowed to develop for 48 hours before the test was fixed with formalin. Treatments that significantly reduce development to the pluteus stage, compared to a control, are considered toxic. Both tests were performed on samples from the first cruise. Only the embryological development test was performed on samples from the second cruise. A reference pore water collected in Redfish Bay near Port Aransas, Texas, was included with each toxicity test. This site is far removed from any known sources of contamination and has been used previously as a reference site (Carr 1992; Carr and Chapman 1992; USFWS 1992).

In addition, a dilution series test with sodium dodecyl sulphate (SDS) was also conducted with each test as a positive control and the EC_{50} determined in order to maintain a record of gamete viability and test acceptability. Filtered (0.45 μm) seawater and seawater reconstituted from brine and bottled water were also included as controls with each test. These methods have been used in several sediment quality assessment surveys conducted at this laboratory (i.e., Tampa Bay, Florida 1991 and 1992; Galveston Bay, Texas, 1991; Pensacola and St. Andrew Bay, Florida, 1993).

Dunnett's one tailed t-test, α=0.01, was used to determine significant difference in fertilization (fertilization test) and development to the pluteus stage (embryological development test) between samples and the reference pore water. The Spearman-Karber method, with Abbott's correction, (Hamilton et al. 1977) was used to determine the EC_{50} in the SDS dilution series.

3.11 Statistical Analysis

A primary concern in the design of the GOOMEX Phase I study was to collect a data set that would provide a statistically rigorous test of the major hypotheses. The study design facilitates the description of patterns in space and time of the biological, toxicological, chemical, sedimentological, and oceanographic characteristics of each of the sites. The statistical approach as well as specific component approaches to data synthesis are described below. Alternative approaches are suggested in some instances but consistent study-wide statistical analysis was a guiding principle for this program.

3.11.1 Null Hypotheses for the Study

Although three null hypotheses were stated in Section 1.1, there is really only one general null hypothesis, which is used for each environmental descriptor and biological response variable. Some of the variables being measured are listed in Table 3.13. The following is a restatement of the null hypotheses in one simplified, general form, which was used to guide the development of the statistical models to analyze all the variables in Table 3.13.

H_o: There are no differences in the variable measured among platform sites and comparison sites.

Platform sites were defined as those sites within 2000 m of the platform (or platform group). Comparison sites are defined as sites that are more than 3000 m away from the platform (or platform group). Platform effects may not extend out to a distance of 2000 m. As stated, the null hypothesis does not allow for an *a priori* test to answer the question, how far

Table 3.13. Partial list of variables being measured in the GOOMEX study.

Dependent Variables	Independent Variables
Macrofaunal Abundance/Structure	Dissolved Oxygen
Meiofaunal Abundance/Structure	Nutrients
Tissue Trace Metal Content	Mineralogy
Tissue Hydrocarbon Content	Sediment Grain Size
Pathology	TOC/Carbonate
Stomach Analyses	Redox
Bile Metabolites	Pore Water/Sediment Trace Metals
MFO/P450	Pore Water/Sediment Hydrocarbons
Harpacticoid Life History Characteristics	
Nematode Copepod Ratios	
Macrofauna Reproduction Effort	
Pore Water Toxicity Tests	

away from platforms do effects extend? This would have to be answered with a *post hoc* test, e.g., a multiple comparison test where distance from a platform is treated as a categorical variable. In fact, distance from a platform is a continuous variable.

3.11.2 Rationale for the Sampling Design

The study design is based on a dose-response model. Contaminant concentrations should decline exponentially with distance away from a platform. Biological responses should be a function of the contaminant gradient, thus a detectable response would also dissipate with distance from a platform. The basic form of a dose response model is:

$$Y = e^{-KX}$$

where Y is the response, and X is the dose. This non-linear response can be transformed so that linear models can be used to analyze the data with the following formula:

$$\mathrm{Ln}Y = a + bX$$

This indicates that all variables should be logarithmically transformed. The only exception would be percentage data, e.g., the sediment grain size or

toxicity test results. Percentage data have a unique distribution, since the values all range from 0 to 100.

The sampling plan was designed to detect nearfield impacts and contaminant gradients extending out from a platform (P). The sampling design included a radial pattern to ≥3,000 meters distance to define the sites contaminant gradient. Oversampling within the nearfield is designed to define the spatial scale of the impact based on an exponential decline in contaminant concentrations.

The sampling design is radial with r radii (r=5), d distances per radius (d=5) and n pseudoreplicates (n=2 or 3) per each radius/distance combination. As many radii (r) and distances (d) per radius as possible are needed to be able to describe directional and distance patterns (assuming among-radius and among-distance effects are significant in an analysis of variance [ANOVA]). The total number of measurements (e.g., chemical analyses) is 50 (or 75, i.e., macroinfauna) per platform site.

Error in such data arose from several levels: (a) analytical error, (b) small-scale spatial variation within boxcores, (c) sampling error (among boxcores), and (d) location error (large-scale spatial variation that was not related to direction or distance). It is essential that the appropriate error be used for testing hypotheses. Parametric statistical tests with ≥ 10 error degrees of freedom provides the needed robustness to failures of assumptions (Harris 1975). The sampling strategy provided a total sampling effort of 150 (or 225) field samples per cruise or 600 (or 900) over all four sampling times. The design emphasizes description of spatial patterns (especially distance and direction from the platform) and estimation of patchiness on spatial scales covered by replicate sampling events.

3.11.3 Statistical Model Development

All data from boxcores; e.g., contaminant concentrations, grain size, meiofauna, macrofauna, and pore water toxicity tests are analyzed using the same statistical procedures. Data from trawls are a subset of the same design, since there are just two stations instead of 25. Some data elements utilized additional analyses that were unique to their particular area of science.

There are four different categories of study design within the overall GOOMEX sampling scheme. The table below summarizes these different study designs and the data generated by those designs. Any subset of the data (i.e., a platform at any individual sampling time) could be analyzed by itself (Case 1). This will be necessary if there are cruise-platform (C*P) interactions present. During the first cruise, five platforms were sampled, and two were eliminated from further study on subsequent cruises. These platforms can be compared to test for generality of trends at platforms (Case 2). Any single cruise could also be analyzed in this way. During the first cruise, replicate boxcores were taken at two stations so that the power of the design could be tested. This is a subset of Case 2. The total design can also be analyzed with all C*P*R*D interactions (Case 3). If interactions involving P are significant, then the design will be reduced to examine these interactions (Case 4).

	Platforms	
Cruises	1	> 1
1	Case 1: Unique C*P analyses	Case 2: Design analyses Unique C analyses
> 1	Case 4: Unique P analyses	Case 3: Total design analyses

The following is a description of the cases in detail and of a statistical model which is used for the analysis of the case. The notation is as follows: cruise = C, platform = P, radial = R, and distance = D. The models are given in SAS notation where C*P indicates an interaction term for C and P. Interaction terms are used in crossed models. R(P) indicates that R is nested (or is unique to) P. Nested terms are used in hierarchical models. It is clear that C, P, and R are categorical effects that can be treated as either a categorical effect or as a continuous variable, i.e., as distance from platform. Y is used to signify any variable in Table 3.13. Other SAS statements or notation are indicated in capital letters.

Case 1 - Single Cruise, Single Platform

This is probably a trivial case that most likely will not be used. It is only useful if there are specific questions about variance at a specific platform at a specific time. It implies there is no generality. It is also the simplest case. It is a simple analysis of covariance (ANCOVA), described by the model:

$$Y = R\ D\ R*D$$

This model can be used to discover directional (i.e., R) effects at specific platforms that are different during each cruise. The model is simply run "BY C P".

Case 2 - Single Cruise, Multiple Platforms

As indicated in the figure above, there are two uses for this case: to examine the power of the design where replicate boxcores were taken and the general case where data are analyzed "BY C". It is necessary to analyze each cruise separately if there are temporal interactions, for example, if the contaminant gradient changes through time.

Power and variance components analysis is a special case where C=1 (for Cruise 1 only) and there are two replicate boxcores at each station (S) for power analysis. S reduces to two levels, near and far, so it is not necessary to designate R and D. The design is fully hierarchical with S nested within P and the replicate boxcores (B) nested within S. Among versus within boxcore variations were calculated and compared to see how many boxcores are needed to detect differences between particular P, R and D combinations at a given time, or between two times for a particular P, R and D combination. The appropriate SAS model is:

$$Y = P\ S(P)\ B(P\ S)$$

Multiple platform analyses are the general case, and would have to be done on data from a single cruise. However, the importance of this analysis model is questionable, at least in the end, because more can be done with

the data by analyzing multiple cruises together (see Case 3 below). The R are unique to particular P, so R is nested within P. The D, on the other hand, are crossed with both P and R(P). The appropriate SAS model is:

$$Y = P\ R(P)\ D\ P*D\ D*R(P)$$

P is tested against R(P) which tacitly assumes R are random within a P. R(P) and D and P*D are tested against D*R(P). D*R(P) is tested against replicate error. If D is treated as a continuous variable, i.e., an ANCOVA, with Ln(Y) as the response variable, then a dose-response model is implied.

Case 3 - Multiple Cruises, Multiple Platforms

This is the first "in the end" analysis model, with all P and all (or at least >1) C included. It will be used to demonstrate that platforms are heterogeneous and the rest of the analyses will be "P-by-P" (Case 4). Again, R is nested within P. Here D and C are crossed with both P and R(P). The appropriate SAS model is:

$$Y = C\ P\ C*P\ R(P)\ C*R(P)\ D\ P*D\ D*R(P)\ D*C\ P*D*C\ D*C*R(P)$$

P is tested against R(P). R(P) is tested against a synthetic error constructed by SAS to yield a quasi-F test. D and P*D are tested against D*R(P). C and P*C are tested against C*R(P). D*R(P), C*R(P), D*C, and P*D*C are tested against D*C*R(P). D*C*R(P) is tested against replicate error. Again, D can be treated as continuous and ANCOVA performed.

Case 4 - Multiple Cruises, One Platform

This is the "in the end" P-by-P analysis model, with more than one cruise involved. It will be used to test and describe distance and direction effects at each platform, given that the model including P in it (Case 3) shows significant interactions involving P. This is a fully crossed model:

$$Y = C\ R\ C*R\ D\ C*D\ R*D\ C*R*D$$

C is tested against C*R. R is tested against a synthetic error term constructed by SAS. D is tested against R*D. C*R, C*D and R*D are tested against C*R*D, which is tested against replicate error. D can be treated as a continuous variable using ANCOVA.

3.11.4 SAS Models and ANOVA and ANCOVA Tables

This section summarizes the four analytical scenarios described above. The statistical models are given in SAS notation. ANOVA tables are also presented with a summary of the experimental designs. The first column gives the row number. The second column names the source of variation. The third column gives the degrees of freedom (df) for the main effect. The fourth column gives the source that is appropriate to use in the denominator for the F-test. This is based on D being a category variable. If D were treated as a continuous variable then there would be some changes in this column. The fifth column gives the design for an ANCOVA where D is continuous.

Case 1. Y = R D R*D, R is declared a random effect.

	Source	df	Test Against	df if D is Continuous
1	R	5-1 = 4	3	4
2	D	5-1 = 4	4	1
3	R*D	(5-1)(5-1) = 16	4	4
4	Rep. error	(5)(5)(2-1) = 25		40
	Total	(5)(5)(2)-1 = 49		49

Case 2. Y = P R(P) D P*D D*R(P), where R(P) and D*R(P) is declared random.

	Source	df	Test Against	df if D is Continuous
1	P	5-1 = 4	2	4
2	R(P)	(5-1)5 = 20	5	20
3	D	5-1 = 4	5	1
4	P*D	(5-1)(5-1) = 16	5	4
5	D*R(P)	(5-1)(5-1)5 = 80	6	20
6	Rep. Error	(5)(5)(5)(2)-1 = 125		200
	Total	(5)(5)(5)(2)-1 = 249		

Case 3. Y = C P C*P R(P) C*R(P) D P*D D*R(P) D*C P*D*C D*C*R(P), where R(P), C*R(P), D*R(P) and D*C*R(P) are declared random.

	Source	df	Test Against	df if D is Continuous
1	P	3-1 = 2	2	2
2	R(P)	(5-1)3 = 12	(7+8)=11*	12
3	D	5-1 = 4	7	1
4	C	4-1 = 3	8	3
5	P*D	(3-1)(5-1) = 8	7	2
6	P*C	(3-1)(4-1) = 6	8	6
7	D*R(P)	(5-1)(5-1)3 = 48	11	12
8	C*R(P)	(4-1)(5-1)3 = 36	11	36
9	D*C	(5-1)(4-1) = 12	11	3
10	P*D*C	(3-1)(5-1)(4-1) = 24	11	24
11	D*C*R(P)	(5-1)(4-1)(5-1)3 = 144	12	36
12	Rep. error	(3)(4)(5)(5)(2-1) = 300		462
	Total	(3)(4)(5)(5)(2)-1 = 599		599

*This is a synthetic error constructed by SAS and yi8elds a quasi-F test.

Case 4. Y = C R C*R D C*D R*D C*R*D, where R, C*R, R*D and C*R*D are declared random.

	Source	df	Test Against	df if D is Continuous
1	C	4-1 = 3	4	3
2	R	5-1 = 4	4+6-7*	4
3	D	5-1 = 4	6	1
4	C*R	(4-1)(5-1) = 12	7	12
5	C*D	(4-1)(5-1) = 12	7	3
6	R*D	(5-1)(5-1) = 16	7	4
7	C*R*D	(4-1)(5-1)(5-1) = 48	8	24
8	Rep. error	(4)(5)(5)(2-1) = 100		197
	Total	(4)(5)(5)(2)-1 = 249		249

*This is a synthetic error constructed by SAS and yi8elds a quasi-F test.

3.11.5 Variations in Benthic Faunal Assemblages

3.11.5.1 Power Analysis

The most direct approach that was compatible with the study design is to determine the detectable change in the population at a given power (1β,) and sample size (n) calculated by:

$$\Delta = \frac{(t_\alpha + t_\beta) x SD x \sqrt{\frac{2}{n}}}{\overline{X}}$$

where Δ is the percent change in the population, SD is the pooled standard deviation, t_α and t_β are tabled values for a two-tailed test assuming a pooled estimate of variance from a large sample size, and $\overline{X}$ is the sample mean. Values of α=0.05, and powers of 0.95, 0.80, 0.50 were used in the analysis.

3.11.5.2 Diversity Analysis

Species composition and diversity were analyzed by computing rarefaction curves (Ludwig and Reynolds 1988). This method compares species numbers between communities. The model calculated the proportion of potential interindividual encounters in a given sample (Hurlbert 1971). The expected number of species, $E(S_n)$, found in a sample of *n* individuals drawn from a population of *N* total individuals distributed among S species was:

$$E(S_n) = \sum_{i=1}^{s} \left[1 - \frac{\left(\frac{N-N_i}{n}\right)}{\left(\frac{N}{n}\right)} \right]$$

where N*i* is the number of individuals of the *i*th species. This sampling model computed the expected number of species in a random sample of size n as the sum of the probabilities that each species was included in the sample (Ludwig and Reynolds, 1988). A series of curves was generated, with the steepest representing the highest diversity.

Other diversity indices were also calculated for each replicate and for the station. This allows for statistical analyses, e.g., analysis of variance on the diversity indices also. One method was Hill's diversity number one (N1) (Hill 1973). It is a measure of the effective number of species in a sample, and indicates the number of abundant species. It was calculated as the exponentiated form of the Shannon diversity index:

$$N1 = eH'$$

As diversity decreases N1 tends toward 1. The Shannon index is the average uncertainty per species in an infinite community made up of species with known proportional abundances (Shannon and Weaver 1949). The Shannon index was calculated by:

$$H^1 = -\sum_{i=1}^{s} \left[\left(\frac{n_i}{n}\right)\ln\left(\frac{n_i}{n}\right)\right]$$

Where n_i is the number of individuals belonging to the ith of S species in the sample and n is the total number of individuals in the sample. This index is based on information theory and is a measure of average degree of uncertainty in predicting what species and individual will belong. Therefore it is biased toward common species, whereas the N1 indicates the degree of species dominance.

Eveness is an index that expresses that all species in a sample are equally abundant. Eveness is a component of diversity. Two eveness indices, E1 and E5, were calculated. E1 is probably the most common, it is the familiar J' of Pielou (1975). It expresses H' relative to the maximum value of H':

$$E1 = \frac{H'}{\ln(S)} = \frac{\mathrm{IN}(N1)}{\mathrm{IN}(N0)}$$

E1 is sensitive to species richness. E5 is an index that is not sensitive to species richness. E5 is a modified Hill's ratio (Alatalo 1981):

$$E5 = \frac{(1/\lambda)-1}{N1-1}$$

$$\text{where, } \lambda = \sum_{i=1}^{s} \frac{n_i(n_i-1)}{n(n-1)}$$

is the Simpson (1949) diversity index. E5 approaches zero as a single species becomes more and more dominant.

4.0 RESULTS AND DISCUSSION

4.1 Study Sites - History of Platform Operations

The final study sites for the GOOMEX Phase I field program are Mustang Island Block A85 (MU-A85-1), Matagorda Island Block 686 (MAI-686-2), and High Island A389-East Flower Garden Bank (HI-A389-4; Figure 4.1). Efforts to obtain information on the history of platform operations (from the oil companies, the New Orleans MMS Office, and the Regional EPA Office in Dallas) have met with limited success. Presented below are descriptions of production histories as known to date. More complete descriptions will be developed as information becomes available.

4.1.1 Mustang Island Area Block A85, Site 1

The Mustang Island-A85 field was first drilled by Conoco, Inc., and it is currently operated by Amerada-Hess. Drilling has occurred as recently as 1986, and a total of 18 wells have been spudded. Discharges associated with the first six wells are summarized in Table 4.1 (Gettleson and Laird 1980). Monthly production of gas, condensate and water is summarized in Figure 4.2. Exploratory wells No. 1, No. 2, and No. 3 were drilled between 1976 and 1978. In most instances drilling muds and cuttings were discharged near the seabottom due to the proximity of the drill-site to Baker Bank. Pre- and post-drilling sediment barium surveys documented a benthic barium plume extending 1,000 meters from the drill site.

4.1.2 Matagorda Island Area Block 686, Site 2

The Matagorda Island Block 686 platform is operated by Occidental Oil Company. Between 1977 and 1981 a total of 12 wells were drilled. This is a gas field which produces from depths of 9,152 to 12,260 ft. A summary of the drilling history and associated discharges until 1980 is provided in Table 4.2. Monthly production of gas, condensate, and water is summarized in Figure 4.3.

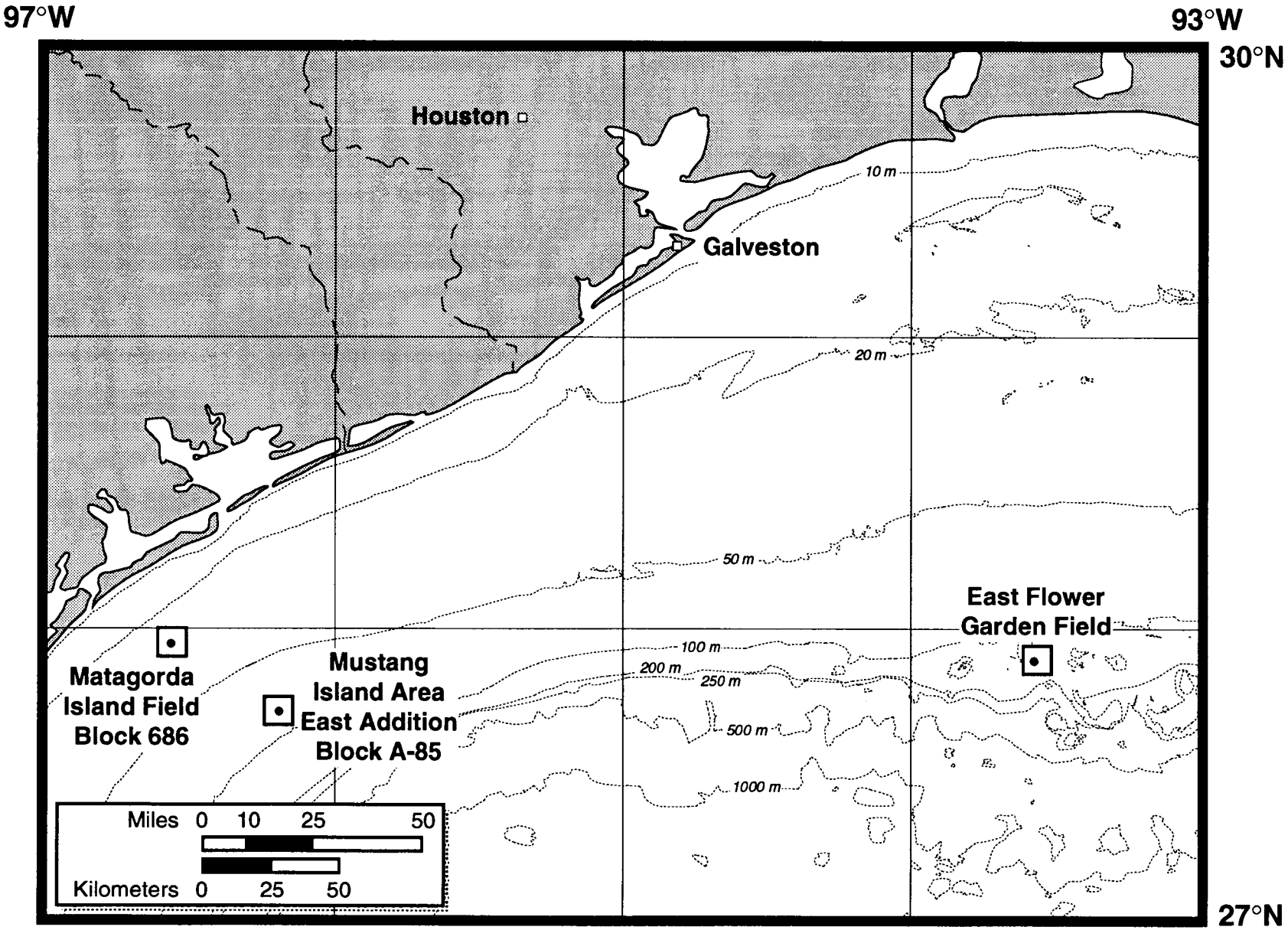


Figure 4.1. Location of the final study sites.

Table 4.1. A summary of drilling discharges from the first six wells at Mustang Island Block A-85 (from Gettleson and Laird 1980).

Well	Amount of Muds Used (lbs.)	Percent Barite by Weight of Mud Used	Weight of Cuttings Discharged[a] (lbs.)	Weight of Mud Discharged (lbs.)	Weight of Barium Discharged (lbs.)
No. 1 Block A-85	4,157,000	---	---	2,536,916[a]	317,765[b]
No. 2 Block A-85	1,875,000	---	---	1,144,267[a]	143,327[b]
Platform A No. A-3 through A-6 Block A-85	8,798,636	87	7,696,087	4,070,465	509,852[b]

[a]Estimated from mean (mud discharged/mud used) of Platform A Well No.'s A-3, 4 and 5 (A-6 judged atypical).

[b]Estimated from barium content of muds discharged from Platform A Well No.'s A-5 and 6.

--- = No Data

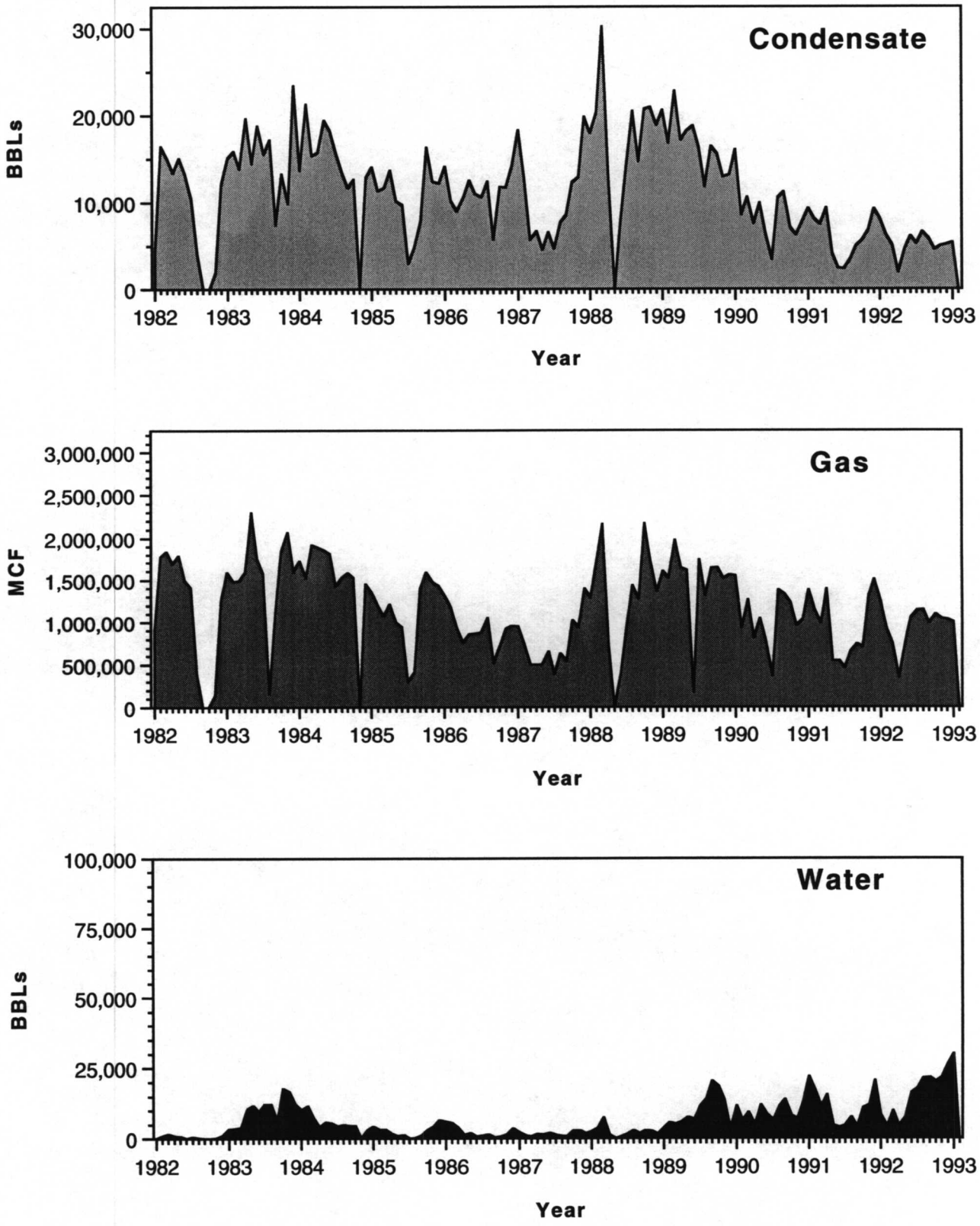


Figure 4.2. Monthly production of condensate, gas, and water at MU-A85.

Table 4.2. Summary of the drilling history and associated discharges at the Matagorda Island Block 686 site through 1980 (after Boothe and Presley 1985).

Characteristic	Matagorda 686
Type of drilling activity	Development
Type of wells	Gas
Number of wells	8 (4 additional wells drilled after 1980)
Total well depth (sum of all wells, m)	24,938
Total volume of cuttings discharged[1] (sum of all wells, m^3)	3130
Drill mud components used (total of all wells in kg x 10^3)[2]	5334
Total barite used (total of all wells in kg x 10^3, percent of total components used)	4547 (85)
Total barium used (TBU, total of all wells in kg x 10^3)[3]	2326
Discharge pipe location bearing in °T from rig (± water surface, m)	022 (-6)
Number of adjacent drilling sites (number of wells drilled within 3000 m radius of study rig	1 (1)
Nearest adjacent drilling site to study site (m, °T)	1500 (095)

[1]Estimated as 1.1 times total volume all wells

[2]Mud systems used: Seawater/gel (to 900 m), Lignite/lignosulfonate (below 900 m)

[3]Assuming the barite used was 87% $BaSO_4$ and that the percent composition of Ba in $BaSO_4$ is 58.8%.

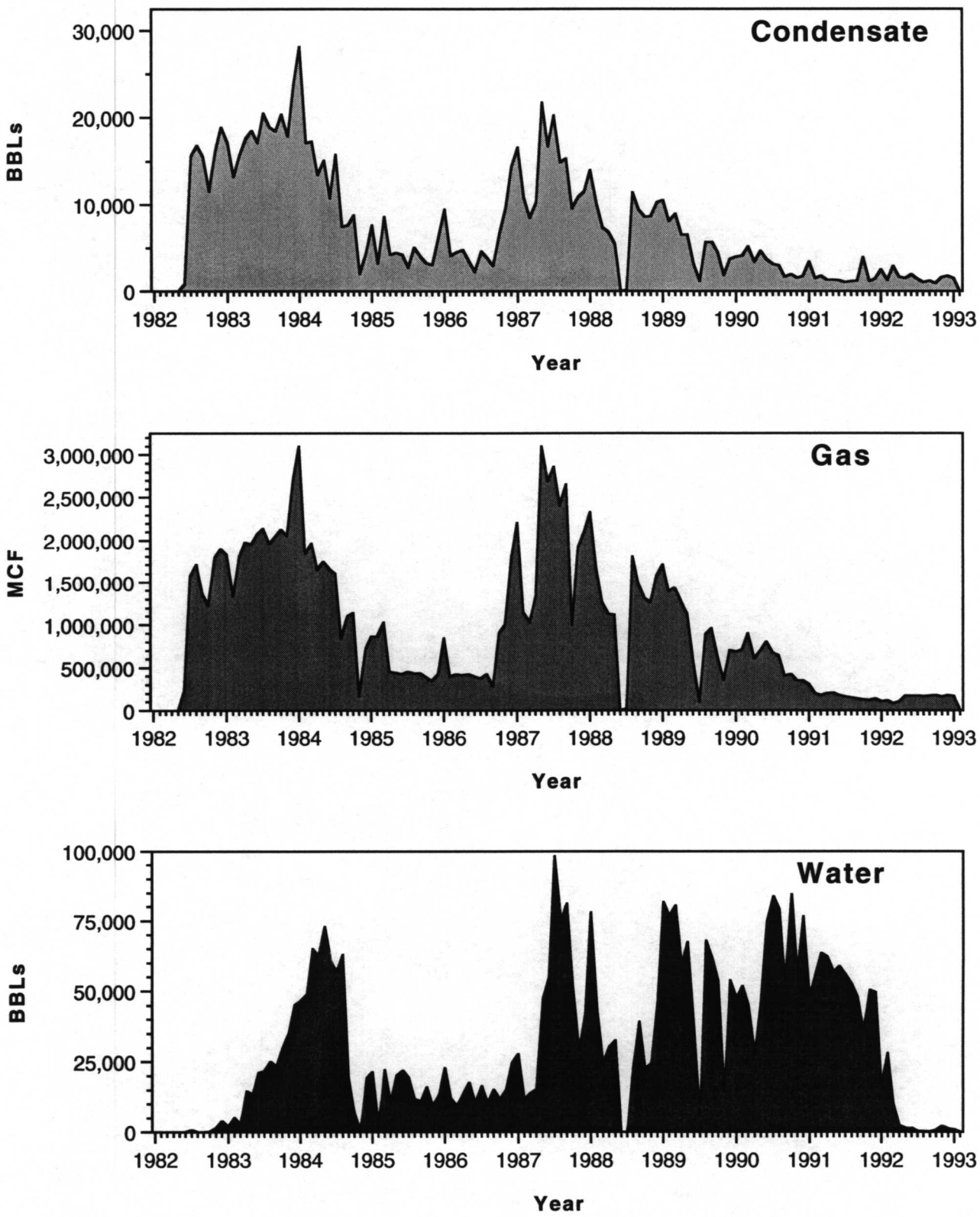


Figure 4.3. Monthly production of condensate, gas, and water at MAI-686.

4.1.3 High Island A389-East Flower Garden Bank, Site 4

The High Island A389 platform is operated by Mobil Oil Exploration and Producing U.S., Inc., and Union is a 50% partner. The platform has six wells, and current production is 22.0 MMCFD of natural gas. A comprehensive history of hydrocarbon development at the site is given by Boland et al. (1983). Preliminary exploratory drilling near the East Flower Garden Bank took place in the spring of 1975. It continued in the fall of 1977 in 129 m of water. A total of 129,000 L of drilling fluid and 1,035,000 kg of drill cuttings were discharged during the October and November 1977 drilling. Residues from the fluids and cuttings were detected up to 1000 m from the drill site by CSA (1985), but not on the reef monitoring stations 2000 m away. Results of the exploratory phases indicated the presence of commercial quantities of natural gas. The present platform was installed in October 1981, approximately midway between the two exploratory wells.

Drilling began on 26 April 1982. Drill fluid and cuttings were shunted to within 10 m of the bottom prior to release. This was required because the platform is located within the "shunting and monitoring" zone around the East Flower Garden Bank. Drilling continued into 1983, but discharges were stopped when operations required that oil-based drill fluids be used. Regulations for the use of oil-based fluids required a closed system. Cuttings, muds, and discharge water were barged for disposal elsewhere. Six production wells were drilled. The last drilling occurred on 19 April 1983. Depth of the six wells ranged from 1827 to 4313 m. Monthly production of condensate, gas, and water is summarized in Figure 4.4

4.2 Sampling Design and Power Analysis

During the first cruise, two stations at each platform were sampled twice to test the ability of the study design to detect change. One station was in the inner ring, and one station was at the outer ring. There were 40 samples (5 platforms x 2 stations x 2 boxcores x 2 subcores). This design is a fully hierarchical sampling design described by the following SAS notation:

Y = PLATFORM STATION(PLATFORM) BOX(PLATFORM STATION).

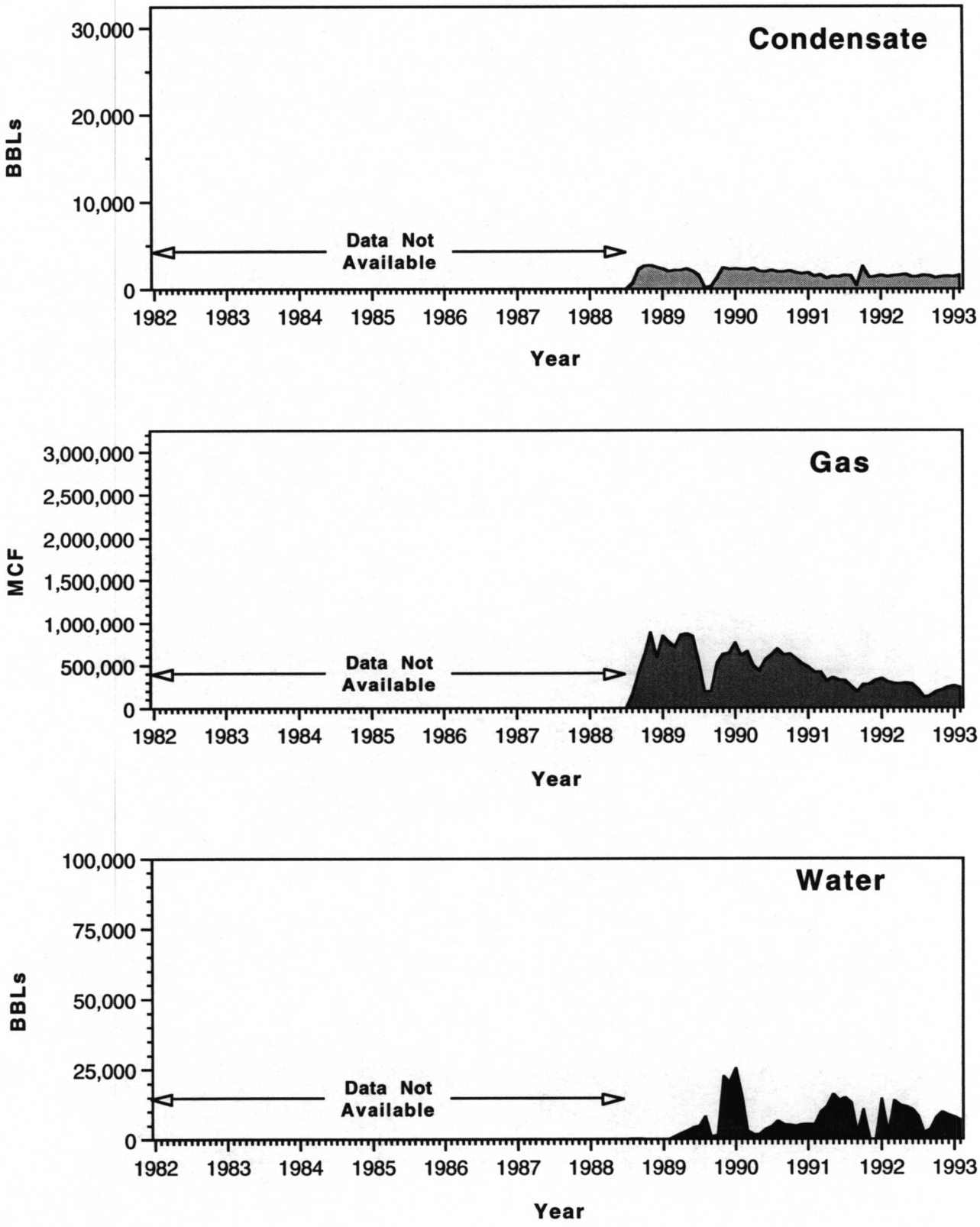


Figure 4.4. Monthly production of condensate, gas, and water at HI-A389.

Platforms are unique, stations are unique to platforms and each box was unique to a station. As in all designs, replicate (i.e., subcore) error is also fully nested. Y represents any variable measured from the boxcore. In each boxcore, environmental (e.g., sediment grain size, trace metal concentration, and hydrocarbon concentration) and biological variables (e.g., meiofauna and macrofauna populations) were measured. Using the above statistical model, the variance components of each source of variation were calculated and converted to a percentage of the variance due to that component. All variables, except for sediment grain size, were transformed using the natural logarithm.

The result of these calculations are summarized in Table 4.3. Station differences, i.e., near vs. far differences, accounted for most of the variability. In most cases, except for sediment grain size, replication error (i.e., error associated with subcores taken within boxes) accounted for more error variance than between boxcore variance. All abiotic measures had similar responses, i.e., most of the variation was among stations. However, the fauna had a different response, with most of the variation at the platform level of variance. The low variance component due to taking multiple boxcores demonstrates that little precision was lost by taking two pseudoreplicates from one boxcore.

A second issue is how much change in the variables response can be detected by the sampling design, i.e., power analysis. The detectable decrease (Δ) is calculated by:

$$\Delta = (t_\alpha + t_\beta)^2 \, s\sqrt{(2n)},$$

where t_α is the t value at $\alpha = 0.05$ (approximately 1.96), t_β is the t value for the power of the test (1-0.8) of $\beta = 0.2$, s is the root of the sample variance, and n is the sample size. The s value came from the variance components analysis. Power analysis was calculated for two cases, where we would take two boxcores (between box variation), or where we would take two subcores from within a box. To calculate the percent detectable increase (Δi) the following formula was used:

$$\Delta_i = 100(100/(100-\Delta)-1).$$

Table 4.3. Variance components analysis for boxcore data. Results based on two boxcores taken at two stations at all five platforms during the first cruise. Table finds percent of variance due to each source. Calculated using a fully hierarchical model. All variables, except sediment grain size, was transformed using natural logarithm.

Variable	Variance Source			
	Platform	Station	Boxcore	Error
Sand	0	98	1	1
Silt	5	81	13	2
Clay	0	86	12	2
Sed. Avg.	1.7	88.3	8.7	1.7
Total ALK	0	89	4	7
UCM	0	83	2	15
Total PAH	7	75	0	18
HC Avg.	2.3	82.3	2.0	13.3
Al	0	96	2	2
Ag	0	86	5	9
As	0	92	4	4
Ba	0	98	2	0
Cd	25	73	1	1
Cr	29	69	3	9
Cu	3	90	4	3
Fe	44	46	5	5
Hg	22	73	1	4
Mn	0	90	2	8
Ni	0	94	4	2
Pb	3	51	9	37
Sb	23	3	2	72
Se	48	24	8	20
Sn	14	78	0	8
V	0	95	2	3
Zn	24	71	2	3
TM Avg.	13.8	71.7	3.3	11.2
Total Meiofauna	61	29	2	8
Nematodes	60	31	1	8
Harpacticoids	63	12	7	18
Other Meiofauna	70	12	0	18
Macrofauna	46	19	5	30

Using two replicates from within one boxcore, 18-98% change in the variables is detectable within the study design followed (Table 4.4). Sediment grain size values have the greatest discrimination at 18%. Most values are much higher. As was found in the variance components analysis, there is little precision lost by using two pseudoreplicates over two replicate

Table 4.4. Power analysis for boxcore data. Results based on two replicates from within two boxcores taken at two stations at all five platforms during the first cruise. The table summarizes the % decrease detectable for either two boxcores (Between Box) or two subcores (Within Box) calculated using a fully hierarchical model. All variables except sediment grain size were transformed using natural logarithm.

Variable	% Decrease Detectable	
	Between Box	Within Box
Sand	28	18
Total ALK	87	74
UCM	98	97
Total PAH	88	90
Al	38	26
Ag	81	68
As	59	40
Ba	86	30
Cd	80	58
Cr	49	42
Cu	58	35
Fe	55	36
Hg	63	58
Mn	56	49
Ni	54	32
Pb	98	95
Sb	93	93
Se	98	96
Sn	54	58
V	49	39
Zn	69	54
Total Meiofauna	90	85
Nematodes	89	86
Harpacticoids	99	96
Other Meiofauna	93	93
Macrofauna	98	96

boxcores. For example, an 88% decrease could be detected for PAH with two boxcores, and a 90% decrease could be detected with two subcores.

4.3 Physicochemical Studies

4.3.1 Water Temperature

The spatial and temporal variability of water temperature on the Texas-Louisiana shelf is caused by advection, turbulent and convective

mixing, and air-sea exchange processes. Temperature is the most frequently measured hydrographic variable; its variability on the Texas-Louisiana shelf is relatively well documented. Temple et al. (1977) reported monthly data from the *Gus III* cruises; Robinson (1973) and Etter and Cochrane (1975) produced mean temperature maps from largely independent data sets; Ulm (1983) prepared a volumetric T-S census for the waters of the shelf; subsurface temperature time series were reported from multi-year studies at the various DOE/SPR brine disposal sites along the inner shelf and the MMS environmental studies at the Flower Gardens on the outer shelf; and analyses of thermal imagery from satellites described the synoptic scale spatial variability at the sea surface (e.g., Rezak et al. 1985).

Monthly mean sea-surface temperatures for the northwestern Gulf of Mexico (Robinson's 1973 atlas) illustrate the seasonal cycle of temperature. Shelf waters cool from summer highs near 29-30°C to inshore lows of about 14°C in January and offshore lows of about 20°C in February. Warming occurs from March through July. The eastern half of the shelf warms more rapidly and reaches higher temperatures than the western half because of upwelling that begins along the lower Texas coast in May only reaching the west Louisiana coast by July. Over the eastern shelf a warm region extends offshore in July and August. Huh et al. (1981) described the seasonal cycle of sea surface temperature in the northeastern Gulf, including the Mississippi Bight, for 1976-1977 using a time series of NOAA satellite data.

Bottom temperatures over the inner half of the shelf vary as sea-surface temperatures to the extent that the mixed-layer depth reaches the bottom. However, seaward of about 75 m bottom water temperatures reflect the annual variations in the off-shelf Gulf. Seaward of about the 120-m isobath, bottom water temperatures vary only slightly (Etter and Cochrane 1975). Mean summer temperatures near the bottom are higher nearshore than offshore, with isotherms closely paralleling isobaths. During fall and winter, mean bottom temperatures are low both nearshore and offshore with somewhat higher temperatures in between.

4.3.2 Salinity

Wind-driven currents and freshwater influx from the Mississippi-Atchafalaya River system determine much of the spatial distribution and

temporal variability of salinity on the shelf. A band of low-salinity water lies along the coast from September through June, carried by the inshore limb of the gyre of cyclonic circulation that prevails on the shelf during this time. Minimum salinities occur all along the coast in May after the spring flood of the Mississippi in April. The May distribution also shows less saline water, extending northwest, and covering the shelf from the south Texas coast to the outer edge. This pattern is attributed to convergence in the wind stress and current fields near the coast and to the offshore limb of the cyclonic gyre. The band recedes upcoast in June and disappears by August, although brackish water remains along the coast from the Mississippi delta to about 92°W and in a region extending seaward over the shelf. The absence of low-salinity water west of about 92.5°W in July is caused by upcoast currents and upwelling driven by the upcoast wind stress. In September, currents all along the coast rapidly return to prevailing downcoast flow, bringing with them a coastal band of low-salinity water.

4.3.3 Nutrients

Concentrations of nutrients; such as nitrate, nitrite, phosphate, silicate, urea, and ammonia; on the Texas-Louisiana shelf are controlled by both biological and physical processes. The interaction of these processes results in low-nutrient concentrations in the surface water of the open Gulf (Barnard and Froelich 1981) and higher concentrations nearshore, especially near river mouths (Ho and Barrett 1977). Processes affecting nutrient concentrations on the shelf include river discharge, coastal currents and winds, intrusions of open Gulf waters, upwelling, biological activity, rainfall, and proximity to coastal marshes (Ho and Barrett 1977; Barrett et al. 1978; Brooks 1980; Flint and Rabalais 1980; Dagg 1988). Nutrient concentrations in coastal waters generally decrease in a westward direction from high values found near the Mississippi River (Riley 1937).

There is a general seasonal pattern in the shelf nutrient concentrations. On the Texas shelf concentrations reach peaks in the spring and then are consumed by phytoplankton blooms, resulting in low concentrations for the remainder of the spring and summer. Nutrient replenishment occurs in the fall and winter (Flint and Rabalais 1980). A similar seasonal variation occurs on the Louisiana shelf where the spring

peak is due to high river flow (Sklar and Turner 1981) that brings the high nitrate and silicate concentrations that are strongly associated with low-salinity nearshore waters (Wiseman et al. 1986).

Coastal wind-induced upwelling has been identified as a mechanism by which surface water nutrients in the coastal boundary layer are transported to the middle shelf (Dagg 1988). In slope and deep Gulf waters, nutrient maxima occur at about 400 to 600 m (Paskausky and Nowlin 1968; Morrison et al. 1983). At the shelf-edge, bottom Ekman upwelling can raise water with high nutrient concentrations (from near the depth of the maxima) to the shelf. This is an idea developed by Cochrane (1969) for the Campeche Bank which has analogous currents along the shelf-edge.

4.3.4 Dissolved Oxygen

Dissolved oxygen concentrations on the shelf vary from well-oxygenated to hypoxic (less than 2 mg l^{-1} dissolved oxygen). Hypoxic bottom waters occur almost annually in the summer in the Mississippi Bight (Turner and Allen 1982) and frequently extend into west Louisiana (SAIC 1989) and Texas waters (Harper et al. 1981; Rabalais 1988). Hypoxia develops as early as April and continue to as late as October, covering areas of up to 10,000 km^2 (Boesch and Rabalais 1988).

Near-bottom oxygen concentrations on the inner shelf drop when strong vertical stratification develops. Such stratification may be caused by less dense coastal water overriding denser shelf water, usually in concert with winds favorable to upwelling. Boesch and Rabalais (1988) suggest that the increasing nitrate enrichment by Mississippi River discharge contributes to observed increases in frequency and intensity of shelf hypoxia since the 1960s. Nitrate increase is attributable to increased use of agricultural fertilizers, waste discharge, and atmospheric precipitation.

4.3.5 Suspended Particulate Matter

Much of the suspended sediment observed in shelf waters enters the Gulf via the Mississippi and Atchafalaya Rivers, with smaller amounts contributed by other rivers, bay discharges and coastal erosion. Most of these sediments are initially deposited close to the point where they

entered the Gulf. Shelf currents re-suspend the sediments resulting in westward transport of the fine-grained fraction by the mean flow (Adams et al. 1982). Suspended sediments can be examined using light scattering data (Plank et al. 1972). Light transmission is another technique (Pak and Zaneveld 1977) that has been used on the Texas-Louisiana shelf. Continuous measurements of light transmission (beam attenuation coefficient) show that suspended sediment is often concentrated in layers within the water column. Called nepheloid layers, these have relatively high light scattering and low light transmission. On the Texas shelf, suspended sediment is often concentrated into surface nepheloid layers associated with Texas river and bay discharge, in bottom nepheloid layers across the shelf due to sediment resuspension and advection by shelf currents, and in intermediate nepheloid layers over the slope resulting from off-shelf transport of the bottom nepheloid layer (Shideler 1981; McGrail and Carnes 1983; Sahl and Merrell 1987; Halper et al. 1988). On the Texas-Louisiana shelf several processes have been identified as important in nepheloid layer transport, which are: (1) lateral shelf water exchange processes (Shideler 1979); (2) diffusion and ebb-tide discharge from coastal inlets (Shideler 1978); (3) coastal currents associated with the passage of meteorological fronts (Holmes 1982); and (4) bottom Ekman upwelling at the shelf edge.

4.3.6 Physicochemical Characteristics of the GOOMEX Study Sites

All physiochemical measurements from GOOMEX Cruises 1 and 2 have been processed and analyzed. The quantities of the various kinds of samples collected and analyzed at each site are summarized in Table 4.5. The data include duplicates collected for quality control. Hardcopies of the vertical profiles in the form of plots and tables of one-meter, depth-averaged, CTD data have been created. The discrete bottle sample data have been manually integrated into the tables by inserting the data for each bottle at the correct depth. The data have been summarized by computing basic statistics for each parameter by site (Tables 4.6-4.11).

At most sites during the first cruise, the 25 CTD casts oversampled the 28 to 78 square kilometers of area around each platform. For example, salinity values at MU-A85 ranged between 35.75 and 36.00 PSU (Practical Salinity Units), with a mean of 35.85 PSU (Table 4.6). This small range

Table 4.5. Summary of the quantities of CTD profiles and hydrocast bottle data, including duplicates, collected during Cruises 1 and 2.

	Cruise 1 1/26/93-2/8/93	Cruise 2 6/5/93-6/17/93
CTD/ Trans. Profiles	125	75
Salinity Samples	416	251
Nutrient Samples	407	252
Diss. Oxygen Samples	417	252

Table 4.6. Cruise 1 Mustang Island-A85 basic statistics for all CTD and bottle data.

Variable*	N	Mean	Std	Minimum	Maximum
Depth (m)	1876	38.57	21.24	1.00	79.00
Temp (°C)	1801	20.57	0.27	19.36	21.07
Salinity (PSU)	1876	35.85	0.05	35.75	36.00
Sigma-t (g/cm^3)	1801	25.26	0.10	25.10	25.62
XMISS (%)	1801	86.13	2.68	47.00	89.00
SOUNDV (m/s)	1801	1524.61	0.49	1521.83	1525.76
PO_4 (µM)	75	0.04	0.03	0.00	0.11
NO_3 (µM)	75	0.27	0.46	0.00	3.01
NO_2 (µM)	75	0.08	0.08	0.00	0.25
SIO_3 (µM)	75	2.54	0.74	1.35	6.14
O_2 (mL/L)	69	5.08	0.09	4.76	5.46

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

Table 4.7. Cruise 2 Mustang Island-A85 basic statistics for all CTD and bottle data.

Variable*	N	Mean	StdDev	Minimum	Maximum
Depth (m)	1928	37.74	21.87	1.00	78.00
Temp (°C)	1853	24.24	1.90	20.44	27.13
Salinity (PSU)	1928	34.72	2.08	30.22	37.58
Sigma-t (g/cm^3)	1853	23.37	2.05	19.16	26.28
XMISS (%)	1853	77.33	3.91	55.77	82.55
SOUNDV (m/s)	1853	1532.81	2.91	1525.23	1537.32
PO_4 (µM)	75	0.11	0.11	0.00	0.55
NO_3 (µM)	75	1.13	1.46	0.02	3.93
NO_2 (µM)	75	0.19	0.25	0.00	1.14
SIO_3 (µM)	75	4.29	2.61	2.06	19.39
O_2 (mL/L)	75	4.50	0.45	3.82	5.26

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

Table 4.8. Cruise 1 Matagorda Island-686 basic statistics for all CTD and bottle data.

Variable*	N	Mean	StdDev	Minimum	Maximum
Depth (m)	637	13.44	6.86	1.00	26.00
Temp (°C)	563	16.92	0.48	15.78	17.82
Salinity (PSU)	637	33.60	0.82	31.56	34.90
Sigma-t (g/cm^3)	563	24.47	0.52	23.37	25.24
XMISS (%)	563	69.87	11.40	7.00	88.00
SOUNDV (m/s)	563	1511.20	2.46	1505.83	1515.53
PO_4 (µM)	74	0.07	0.04	0.00	0.21
NO_3 (µM)	74	0.42	0.43	0.00	2.18
NO_2 (µM)	74	0.09	0.04	0.03	0.20
SIO_3 (µM)	74	1.98	0.65	1.21	4.31
O_2 (mL/L)	74	5.54	0.09	5.37	5.82

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

Table 4.9. Cruise 2 Matagorda Island-686 basic statistics for all CTD and bottle data.

Variable*	N	Mean	StdDev	Minimum	Maximum
Depth (m)	729	13.61	7.73	1.00	29.00
Temp (°C)	655	25.61	1.32	22.44	26.89
Salinity (PSU)	728	30.95	2.18	28.33	35.50
Sigma-t (g/cm^3)	655	20.13	2.01	17.93	24.48
XMISS (%)	655	71.26	22.40	0.10	88.40
SOUNDV (m/s)	655	1531.74	1.73	1528.47	1535.00
PO_4 (µM)	74	0.09	0.13	0.01	0.63
NO_3 (µM)	74	0.65	1.32	0.00	6.03
NO_2 (µM)	74	0.15	0.20	0.00	0.89
SIO_3 (µM)	74	5.70	3.93	2.99	21.86
O_2 (mL/L)	74	4.69	0.60	2.47	5.27

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

Table 4.10. Cruise 1 High Island-A389 basic statistics for all CTD and bottle data.

Variable*	N	Mean	StdDev	Minimum	Maximum
Depth (m)	2996	62.24	35.45	1.00	157.00
Temp (°C)	2921	20.59	1.01	15.36	21.35
Salinity (PSU)	2996	36.08	0.10	35.10	36.47
Sigma-t (g/cm^3)	2921	25.43	0.32	24.88	26.63
XMISS (%)	2921	77.30	7.55	3.00	89.00
SOUNDV (m/s)	2921	1525.28	2.43	1510.53	1527.51
PO_4 (µM)	75	0.22	0.29	0.00	0.88
NO_3 (µM)	75	3.82	5.32	0.01	14.24
NO_2 (µM)	75	0.03	0.02	0.00	0.06
SIO_3 (µM)	75	2.95	1.53	1.06	6.03
O_2 (mL/L)	75	4.37	1.01	2.70	5.56

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

Table 4.11. Cruise 2 High Island-A389 basic statistics for all CTD and bottle data.

Variable*	N	Mean	StdDev	Minimum	Maximum
Depth (m)	3233	63.88	37.46	1.00	157.00
Temp (°C)	3158	21.08	3.14	15.84	27.11
Salinity (PSU)	3233	35.60	1.00	33.04	36.41
Sigma-t (g/cm^3)	3158	24.90	1.59	21.29	26.62
XMISS (%)	3158	77.12	4.28	0.60	83.30
SOUNDV (m/s)	3158	1525.83	6.73	1513.11	1537.54
PO_4 (µM)	75	0.29	0.38	0.00	0.93
NO_3 (µM)	75	4.50	6.23	0.01	14.12
NO_2 (µM)	75	0.02	0.02	0.00	0.07
SIO_3 (µM)	75	3.54	2.46	1.22	9.64
O_2 (mL/L)	75	4.27	0.96	2.87	5.15

*Temp = Temperature, PSU = Practical Salinity Units; Sigma-t = (Density-1) x 1000; XMISS = Transmissivity; SOUNDV = Sound Velocity

reflects a uniform water mass. However, at MAI-686 the salinity range was considerably greater: 31.56 to 34.90 PSU (Table 4.8). The large salinity range was caused by a combination of vertical stratification and spatial variability. The site was influenced by the brackish coastal jet that hugs the Texas coast during September through May (Cochrane and Kelly 1986). The sea surface temperature (SST) measured by the NOAA-11 satellite on February 12, 1993, just after Cruise 1, is shown in Figure 4.5. The image was provided by the Data Office of the Texas-Louisiana Shelf Circulation and Transport Processes Study, which comprises Study Unit A of the MMS sponsored Louisiana-Texas Shelf Physical Oceanography Program (LATEX). The two features of interest are the cooler band of coastal water (darker shading), which is normally associated with lower salinity, and the warm water (lightest shading) of a filament from a Loop Current eddy that extended onto the south Texas shelf during February 1993.

A priori, there is no way to know which sites will be relatively uniform in their physicochemical properties. One of the purposes of acquiring the physicochemical data was to determine if the water masses at a site changed during the period the other tasks were collecting samples. In the case of MAI-688 during the first cruise, it appears that different water masses may have been in the study areas during at least a portion of the time the site was being surveyed. The near surface and near bottom salinities in plan view at MAI-686 during Cruise 1 are illustrated in Figures 4.6 and 4.7. Note that

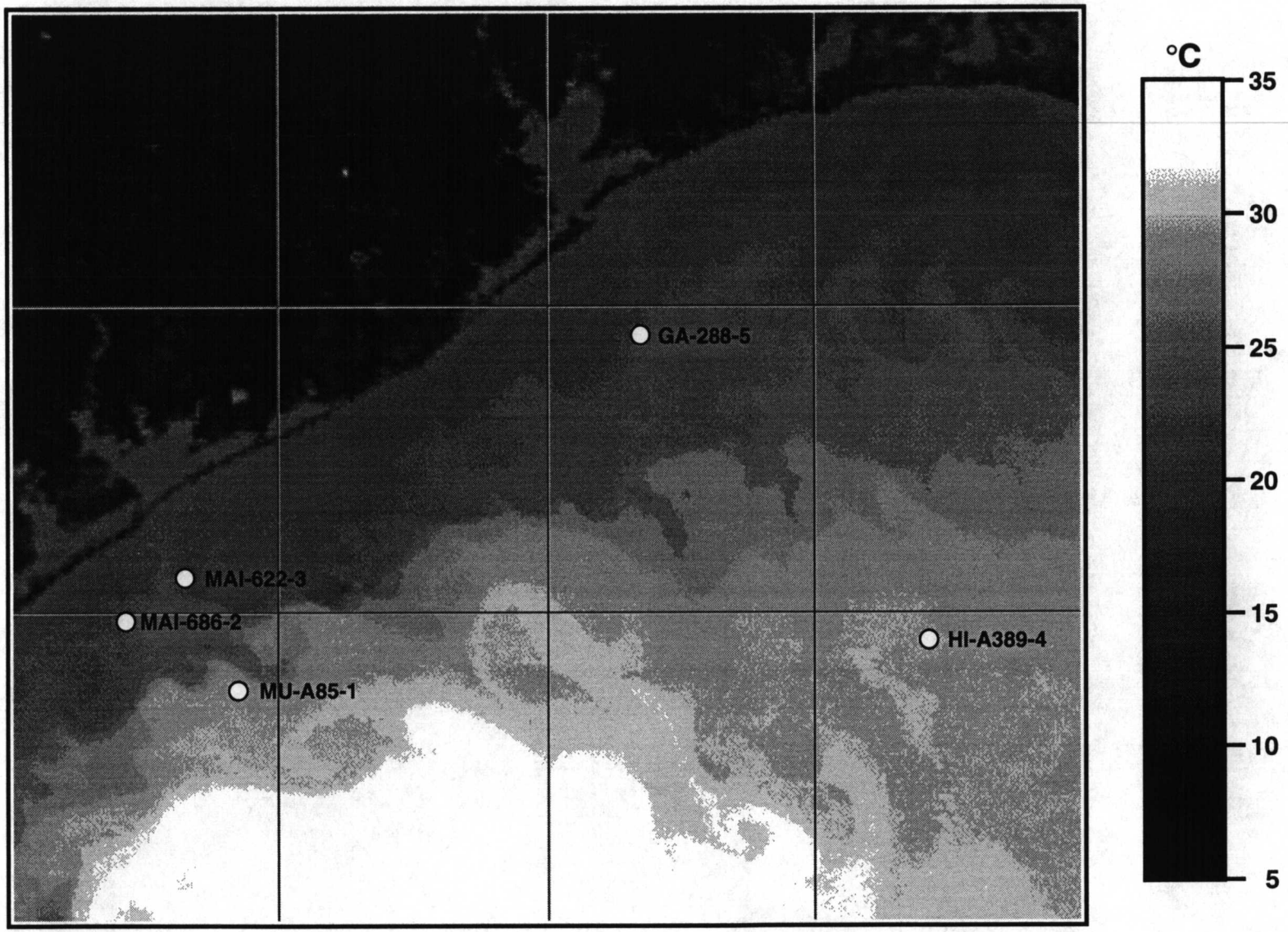


Figure 4.5. Satellite AVHRR image of sea surface temperature for February 12, 1993.

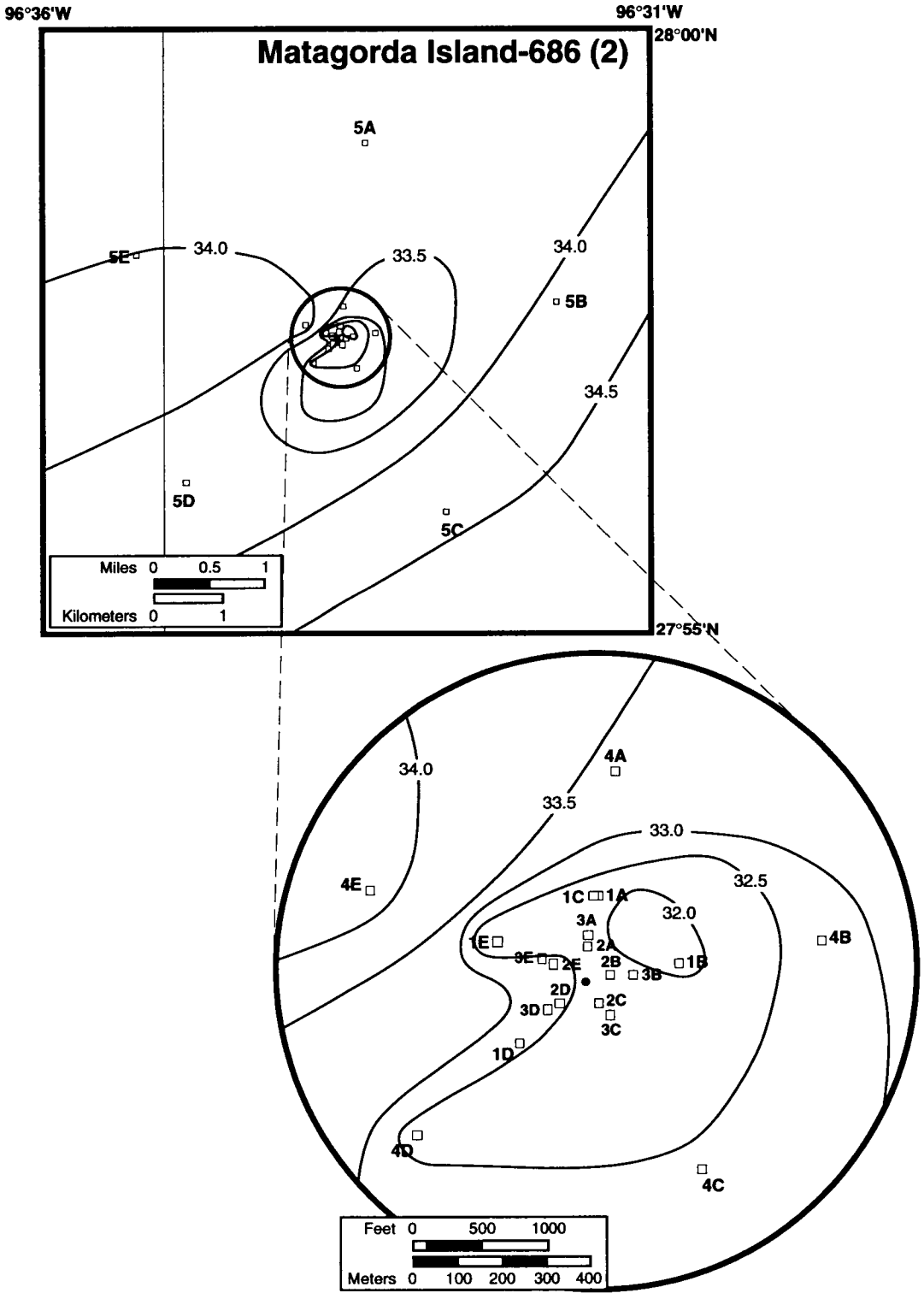


Figure 4.6. Near–surface salinities (PSU) at MAI-686 for Cruise 1.

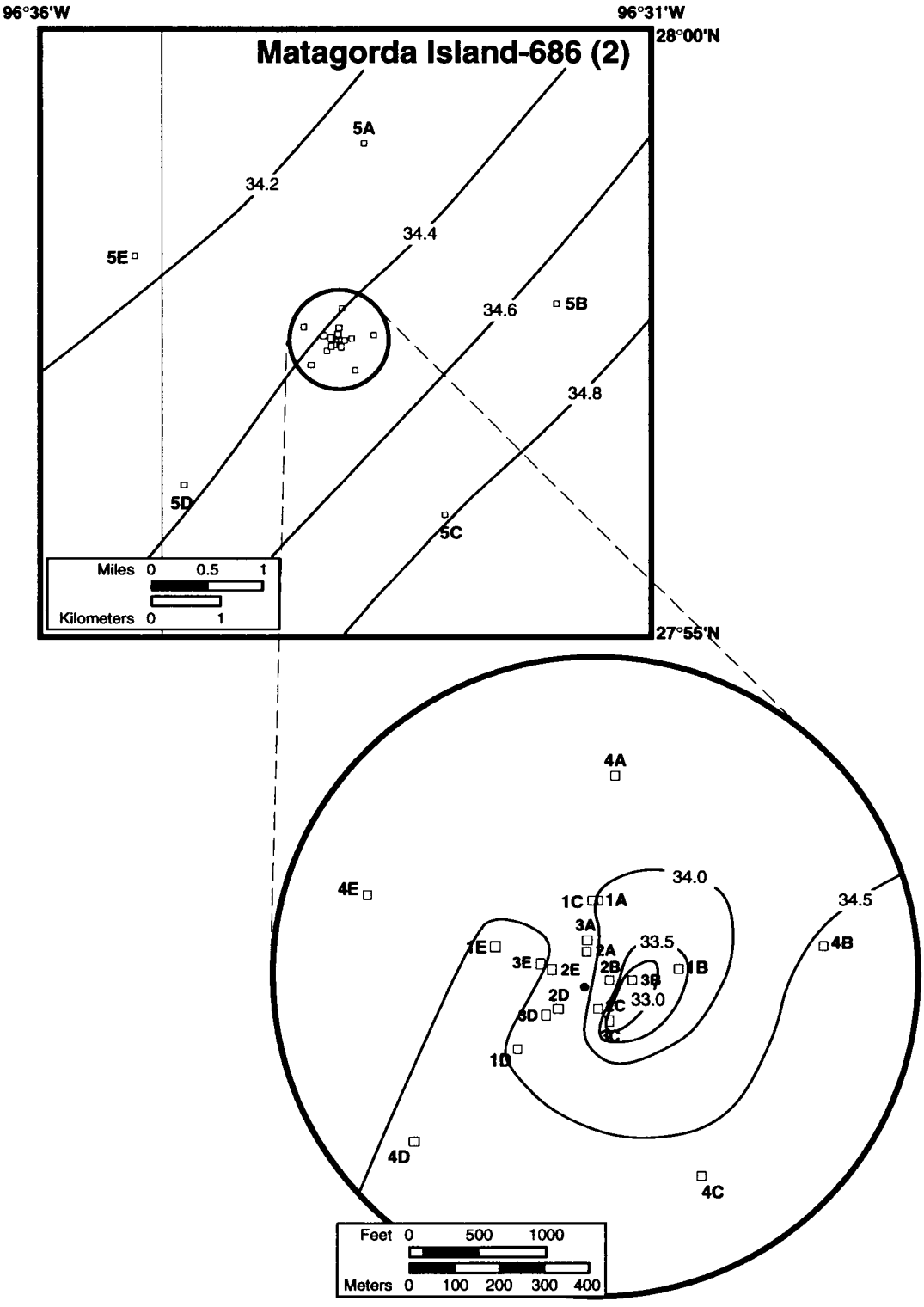


Figure 4.7. Near–bottom salinities (PSU) at MAI-686 for Cruise 1.

salinity was determined independently by both bottle samples and CTD profiles, providing confidence that the results are not an artifact. A small area of less saline water centered just southeast of the platform is evident (Figures 4.6 and 4.7). Support for the idea that this is a distinct water mass comes from the silicate values, which are generally low at the outer stations, but rise considerably in the fresher water. For example the surface-to-bottom ranges in silicate are 1.69-1.71 μM at station 5B and 1.26-1.53 μM at station 5E, which are far stations. However, in the region of fresher water, the surface-to-bottom silicate ranges are 1.69-3.96 μM at station 1B and 1.73-4.31 μM at station 2B.

Figures 4.6 and 4.7 suggest that the less saline water mass is isolated or enclosed by higher salinity water, and one possibility is that a small bolus of coastal water lay in the study area. Another, more probable scenario is that the offshore side of the fresher coastal jet extended seaward past the platform for a brief period and then retreated. Time aliasing by the sequence in which the stations were sampled created the appearance of an isolated water mass. Examination of the navigation log reveals that the outer two rings (5 and 4) were sampled first, followed by the south and east portions of the inner rings, and finally the west and north portions of the inner rings.

Site MAI-686 experienced changing water properties again during Cruise 2 (Table 4.9). On Cruise 2 the rings were sampled in the sequence 5, 2, 3, 1, 4. For a given ring the surface salinity was relatively uniform, but the mean value rose gradually from ring to ring. For ring 5, which was sampled first, the mean surface salinity was 28.6 PSU. For ring 4, sampled last, the mean surface salinity was 32.3 PSU. Vertical stratification at this site was weak during Cruise 2, except within a few meters of the bottom at some stations, where salinity rose above 34 PSU. High silicate values were found only in the bottom bottle samples for which salinity was greater than 34 PSU. The distribution of high silicate during this cruise was quite different from that found during Cruise 1, when the high silicate values were associated with less saline water, particularly near the surface.

The water properties at Site MU-A85 were not quite as uniform during Cruise 2 (Table 4.7) because of the variability introduced by normal summer stratification. As at site MAI-686, the high silicate values were found in some bottom bottle samples in association with high salinity.

The lowest values of dissolved oxygen (less than 3.0 mL/L) were found at the bottom at HI-A-389 during both cruises (Tables 4.10 and 4.11) and at the bottom at MAI-686 during Cruise 2 (Table 4.9). The low values at the High Island platform were caused by the oxygen minimum layer that impinges on the outer shelf from the deep Gulf, whereas the low values at the Matagorda Island platform were caused by summertime vertical stratification that reduces vertical mixing. As noted above, strong vertical stratification at this platform was only found at some stations and only within a few meters of the bottom.

4.4 Sedimentology

A series of abiotic measurements were made to provide a framework from which to interpret the biological and contaminant results. These measurements included grain size, mineralogy, organic and inorganic carbon content, and Eh.

Sediment texture is an important variable in evaluation of both biological assemblages and contaminant concentrations in benthic systems. Numerous studies have shown a correlation between biological and chemical changes and grain size. In benthic ecosystem studies, cross-correlations between stations are often dependent on substrate characteristics and correlations of biological changes with contaminant exposure can be confounded by the effects of variable substrates. Organic and inorganic carbon also influence sediment quality reflecting organic enrichment, drill mud and cuttings disposal, debris from the platform and other processes that contribute to a near platform effect. Redox potential as measured by Eh electrode can also be important in determining substrate quality as well as providing for the remobilization of contaminants, in particular trace metals.

4.4.1 Grain Size

Substantial heterogeneity in sediment texture were apparent at all of the sites studied. Basic statistical analysis of grain size data from Cruises 1 and 2 is summarized in Tables 4.12 and 4.13. Sediment grain size characteristics are summarized below by site.

At MU-A85, sediments at 3000 m distance (Ring 5) were sandy muds except for station 5D which contained only 8% sand and was classified as a

Table 4.12. Summary of grain size analysis of sediments from Cruise 1.

	MU-A85-1					MAI-686-2					HI-A389-4				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
% Sand															
Average	67.0	55.2	21.6	12.0	14.5	74.9	57.709	39.1	32.3	28.2	41.9	31.6	9.4	5.3	5.2
Variance	36	47	31	3	23	69	218	241	23	136	71	149	7	6	4
Std Deviation	6.0	6.9	5.6	1.7	4.8	8.3	14.8	15.5	4.8	11.7	8.4	12.2	2.6	2.5	2.1
Coef of Var (%)	9.0%	12.5%	26.0%	13.8%	33.4%	11.1%	25.6%	39.7%	14.9%	41.4%	20.0%	38.7%	28.1%	46.5%	40.4%
Minimum	56	44	16	9	8	56	28	27	24	19	30	14	5	3	3
Maximum	73	66	32	14	23	86	71	71	40	51	54	49	14	11	10
Count	12	10	10	10	12	12	10	10	10	12	12	10	10	10	12
% Silt															
Average	17.8	24.9	40.7	47.8	39.4	11.9	17.58	26.8	28.0	30.5	36.1	32.3	37.6	30.2	38.2
Variance	20	20	8	4	15	55	41	54	6	27	174	60	5	5	74
Std Deviation	4.4	4.4	2.8	2.1	3.8	7.4	6.4	7.4	2.5	5.2	13.2	7.8	2.2	2.2	8.6
Coef of Var (%)	24.9%	17.8%	6.9%	4.3%	9.8%	62.1%	36.3%	27.5%	8.9%	17.0%	36.5%	24.0%	5.8%	7.3%	22.6%
Minimum	14	19	36	45	34	6	11	12	24	21	20	21	35	26	24
Maximum	28	33	45	51	48	29	31	35	31	36	55	45	42	33	54
Count	12	10	10	10	12	12	10	10	10	12	12	10	10	10	12
% Clay															
Average	15.2	19.9	37.7	40.3	46.1	13.2	24.711	34.1	39.7	41.3	22.0	36.1	53.0	64.5	56.6
Variance	9	10	22	9	11	12	72	72	9	45	103	23	19	15	111
Std Deviation	2.9	3.2	4.6	3.1	3.2	3.5	8.5	8.5	3.1	6.7	10.2	4.8	4.4	3.9	10.5
Coef of Var (%)	19.4%	16.2%	12.3%	7.6%	7.0%	26.8%	34.3%	24.9%	7.7%	16.2%	46.3%	13.4%	8.3%	6.0%	18.6%
Minimum	12	15	27	35	41	7	16	17	36	28	5	30	44	57	36
Maximum	22	26	42	44	53	20	41	42	46	48	38	42	59	70	73
Count	12	10	10	10	12	12	10	10	10	12	12	10	10	10	12

Table 4.13. Summary of grain size analysis of sediments from Cruise 2.

	MU-A85-1					MAI-686-2					HI-A389-4				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
% Sand															
Average	65.75	55.71	18.48	11.29	13.72	66.90	54.77	43.69	31.54	24.80	39.17	29.80	10.46	4.30	3.15
Variance	25.85	113.55	8.72	6.30	12.77	363.19	192.50	296.24	20.12	114.69	168.79	47.28	48.57	4.00	1.56
Std Deviation	5.08	10.66	2.95	2.51	3.57	19.06	13.87	17.21	4.49	10.71	12.99	6.88	6.97	2.00	1.25
Coef of Var (%)	7.7%	19.1%	16.0%	22.2%	26.0%	28.5%	25.3%	39.4%	14.2%	43.2%	33.2%	23.1%	66.6%	46.6%	39.6%
Minimum	59.7	36.6	14.4	7.3	7.2	28.2	33.3	25.4	24.3	14.3	19.0	20.3	3.1	2.3	2.2
Maximum	72.6	70.8	23.1	15.4	18.9	81.9	76.3	74.8	39.6	39.0	55.8	38.2	21.5	8.3	5.8
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
% Silt															
Average	17.69	24.72	41.86	41.44	46.17	13.07	16.60	22.58	31.22	28.75	36.21	36.39	42.08	42.25	32.85
Variance	12.97	38.68	1.80	2.41	5.11	43.28	17.61	45.16	7.64	19.79	118.07	19.53	8.78	2.77	35.58
Std Deviation	3.60	6.22	1.34	1.55	2.26	6.58	4.20	6.72	2.76	4.45	10.87	4.42	2.96	1.66	5.96
Coef of Var (%)	20.4%	25.2%	3.2%	3.7%	4.9%	50.3%	25.3%	29.8%	8.9%	15.5%	30.0%	12.1%	7.0%	3.9%	18.2%
Minimum	12.5	14.9	39.1	39.0	42.7	6.0	8.7	9.5	26.4	21.6	22.8	30.9	37.9	38.8	26.0
Maximum	24.5	34.6	43.3	44.3	49.9	25.9	21.4	28.8	35.7	33.7	57.9	45.1	46.4	44.8	44.3
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
% Clay															
Average	16.56	19.57	39.66	47.27	40.12	20.03	28.63	33.70	37.24	46.46	24.62	33.80	47.46	53.46	64.00
Variance	13.09	21.15	10.56	4.03	11.07	161.10	102.65	118.92	8.56	46.98	136.29	14.72	20.08	9.73	49.30
Std Deviation	3.62	4.60	3.25	2.01	3.33	12.69	10.13	10.90	2.93	6.85	11.67	3.84	4.48	3.12	7.02
Coef of Var (%)	21.9%	23.5%	8.2%	4.2%	8.3%	63.4%	35.4%	32.4%	7.9%	14.8%	47.4%	11.3%	9.4%	5.8%	11.0%
Minimum	11.9	13.2	36.1	44.6	33.4	10.6	15.0	15.7	34.0	35.9	10.1	30.1	40.6	47.2	50.3
Maximum	23.9	28.8	45.7	50.4	45.7	46.7	45.6	45.9	43.0	54.8	45.8	42.0	53.1	57.6	71.6
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

mud. Sediments at 500 m distance (Ring 4) were also sandy mud except station 4A which was classified as a mud. All samples from the 200 m distance (Ring 1) were sandy mud. At 100 m distance four samples were muddy sand and one station, 3E, was sandy mud containing 45% sand. ediments closest to the platform were muddy sand containing between 28 and 40% mud. Cruise 2 sample analyses were generally within a few percent of the analyses from Cruise 1. Station 4C (500 m) was an exception, it was a sandy mud during Cruise 1 and a mud during Cruise 2. However, this was not a significant difference as sand content at station 4C of Cruise 1 was 13.46 and 13.94% and the equivalent sample from Cruise 2 had 9.46 and 8.88 sand for Cruise 2. The difference in sand content between the two cruise samples varied from about 4 to 5%. The boundary between sandy mud and mud classes is 10% sand. The two samples from Cruise 2 lie within the mud field by only 0.54 and 1.12% respectively. The 70% sand isopleth indicated possible bottom currents with northwest and southerly components (Figure 4.8).

During Cruise 1, all of the samples from MAI-686 at 5000 and 400 m distance were sandy muds with percent sand ranging from 19 to 50% for the 5000 m sample and 25 to 39% for the 400 m samples. All of the samples from 200 m distance were also sandy muds, with the exception of sample 1D which was a muddy sand. Sand percents ranged from 28 to 34% except for 1D which had about 70% sand. All of the remaining samples from rings $\leq$ 100 m distance are muddy sands and vary from 61 to 84% sand. On Cruise 2, samples from 5000 and 400 meters distance were all sandy mud, with sand varying from 15 to 39%. The 200 m stations were also sandy mud except for the station on the D radial which had a sand content of 74%, slightly higher than the Cruise 1 sample from the same station. The sample from the E radial at 200 m falls on the boundary between sandy mud and muddy sand. The inner two rings were much the same during both cruises except for stations 2A and 3C. At station 3C, the Cruise 1 sample was a muddy sand. On Cruise 2, the same station yielded a sample that was sandy mud. The difference is about 14% less sand in the Cruise 2 sample, from about 60% to about 46% with the boundary between the two classes at 50%. The concentration of high percent sand has an elliptical shape (Figure 4.9), oriented in a northeast-southwest direction. The pattern indicates a probable tidal current effect with the sand being transported in both

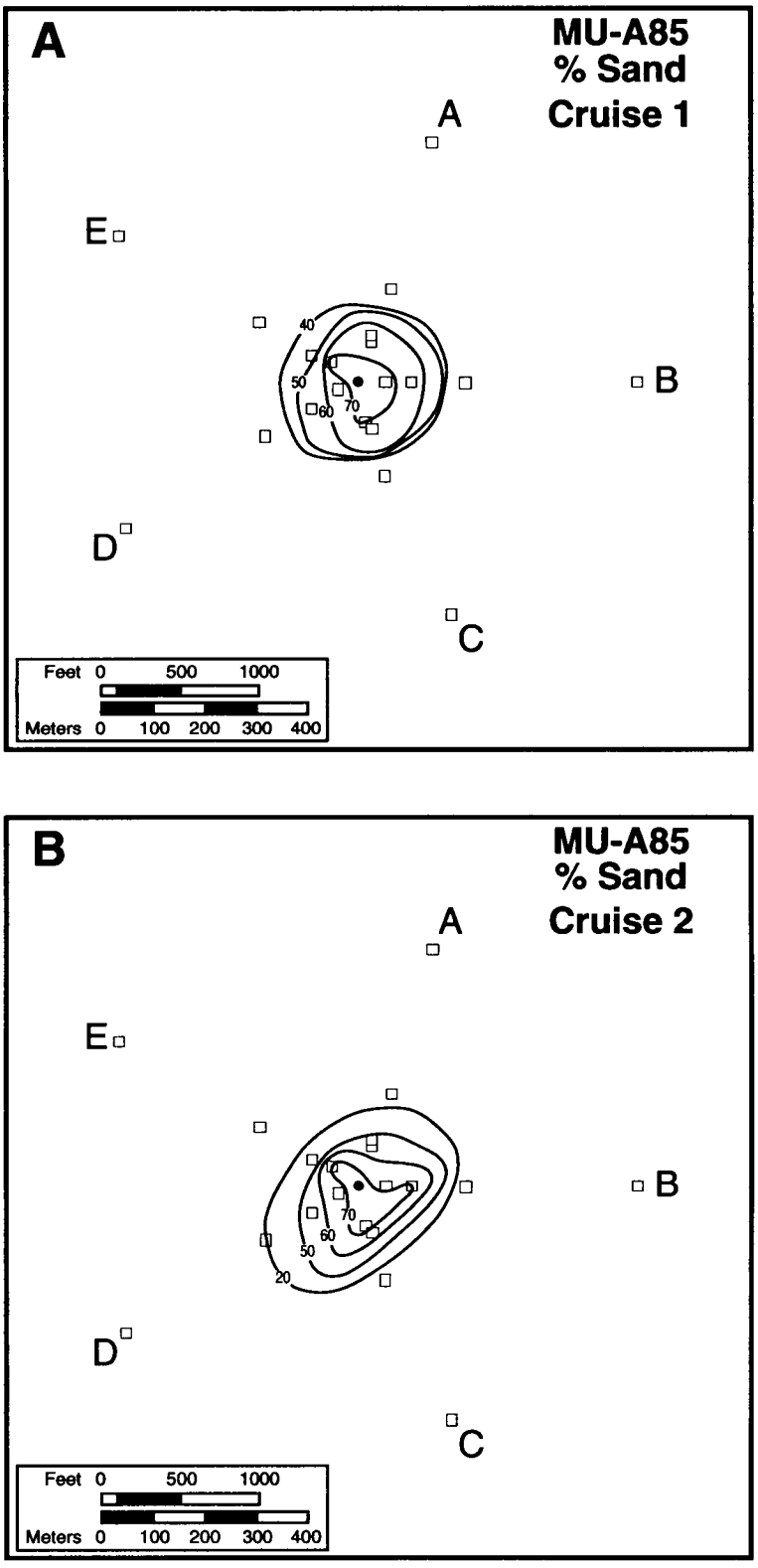


Figure 4.8. Areal distribution of sand content (%) in sediments at MU-A85 for A: Cruise 1; and B: Cruise 2.

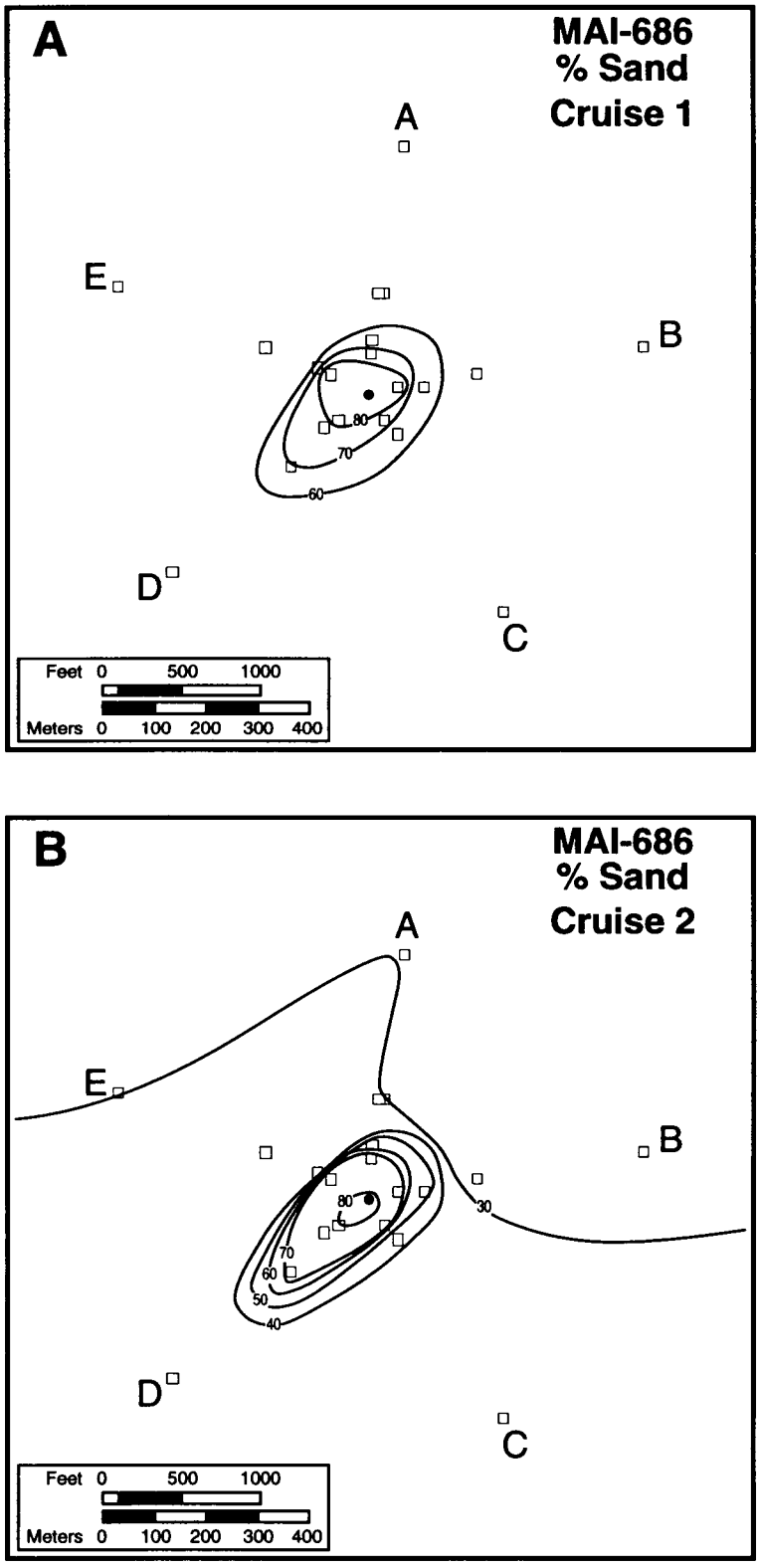


Figure 4.9. Areal distribution of sand content (%) in sediments at MAI-686 for A: Cruise 1; and B: Cruise 2.

directions away from the drillsite or a scouring of fine particles from the sediments under the platform due to tidal current acceleration around the structure.

Sediments collected at HI-A389 during Cruise 1 at 500 and 5000 meters distance are all muds except for station 4E which is borderline between mud and sandy mud. Samples 200 meters distance from the platform; stations A, B, and C; are muds while those from stations D and E are sandy muds. Samples from all five locations 100 meters distance are sandy muds. The closest samples at stations A, B, and C are sandy muds but samples D and E are muddy sands. With only one exception, the analyses of samples taken at the same station during the two cruises vary by less than 11% in sand content and most sample pairs vary by only a few percent (Figure 4.10). On Cruise 2, samples from 200 to 5000 meters distance on radials A, B, and C are all mud. The two inner stations are all sandy muds except for the sample from 50 meters distance on radial B which is a muddy sand. On radial D, samples from 5000 and 500 meters are both mud, samples from 200 and 100 meters distance are sandy mud, and from 50 meters distance a muddy sand. On radial E, the outer two stations are mud and the inner three stations are sandy mud. The distribution of sand at this site for Cruise 1 is shown in Figure 4.10.

The distribution of sands, muds, and hard bottoms on the OCS off Texas has been known in general for many years. The sediments on the continental shelf offshore Texas were deposited by marine processes of transport and deposition and are largely fluvial in origin. They consist primarily of quartz sands, silts, and clays with an admixture of the skeletons or fragments of organisms living at the site of deposition. Somewhat of a surprise is the length of time that impacts on the physical environment remain at a drillsite. After a period of more than 10 years, even in relatively shallow water where the bottom sediments are susceptible to movement by frequent winter storms, the sediments beneath and for some distance away from the platform are still coarser than the unaffected sediments of the area.

An ANOVA analysis of variance of the grain size data (i.e., % sand) shows a highly significant interaction (P=0.01) with distance, direction, and often time for sediment texture (Table 4.14). These strong interactions will be carefully analyzed and considered in the interpretation of other work element results.

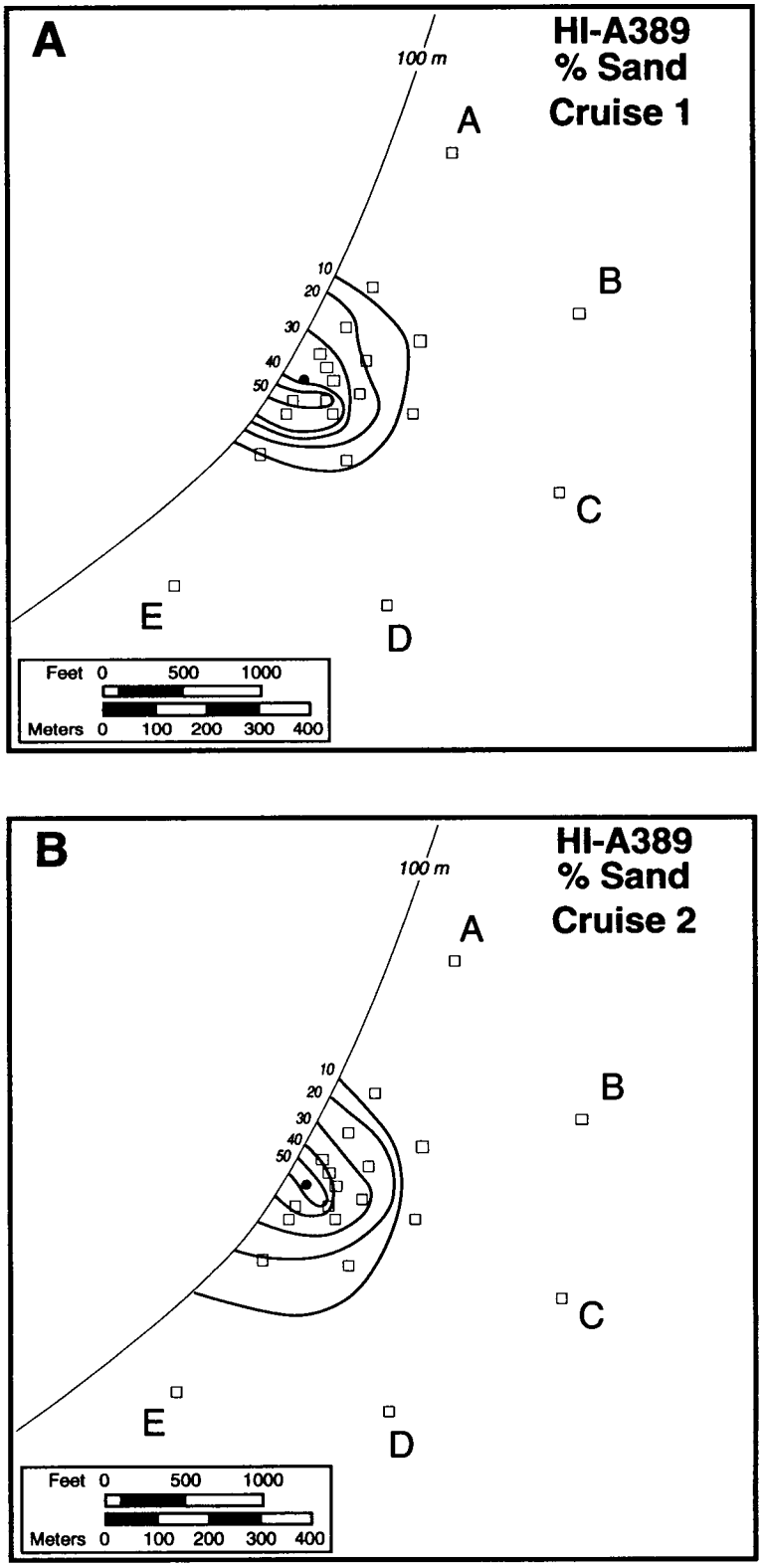


Figure 4.10. Areal distribution of sand content (%) in sediments at HI-A389 for A: Cruise 1; and B: Cruise 2.

Table 4.14. ANOVA analysis of variance for sediment sand content (%).

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85-1			
Cruise (A)	1	14.98	14.98	5.985	0.0177
Distance (B)	4	53095.55	13273.89	5304.812	**0.0001**
AB	4	37.03	9.26	3.700	**0.0098**
Radii (C)	4	585.54	146.39	58.502	**0.0001**
A•C	4	28.04	7.01	2.802	0.0347
B•C	16	2065.24	129.08	51.585	**0.0001**
A•B•C	16	394.67	24.67	9.858	**0.0001**
Error	54	135.12	2.50		
		MAI-686-2			
Cruise (A)	1	160.31	160.31	39.194	**0.0001**
Distance (B)	4	27060.21	6765.05	1653.995	**0.0001**
AB	4	484.53	121.13	29.616	**0.0001**
Radii (C)	4	2239.51	559.88	136.885	**0.0001**
A•C	4	934.51	233.63	57.120	**0.0001**
B•C	16	12573.37	785.84	192.130	**0.0001**
A•B•C	16	1687.12	105.44	25.780	**0.0001**
Error	54	220.87	4.09		
		HI-A389-4			
Cruise (A)	1	53.86	53.86	15.031	**0.0003**
Distance (B)	4	23386.48	5846.62	1631.533	**0.0001**
AB	4	65.23	16.31	4.551	**0.0031**
Radii (C)	4	1624.52	406.13	113.333	**0.0001**
A•C	4	202.83	50.71	14.150	**0.0001**
B•C	16	2428.71	151.79	42.359	**0.0001**
A•B•C	16	871.62	54.48	15.202	**0.0001**
Error	54	193.51	3.58		

4.4.2 Mineralogy

Mineralogic analysis was only conducted for Cruise 1 samples and is summarized by site. At MU-A85, 500 and 3000 m distance from the platform, the sequence of minerals was generally clays, quartz, feldspar, and calcite, in order of abundance with the exception of samples 5A, 5B, and 5C which had about equal amounts of quartz and clays. Barite was present in samples 3D, 2D, and 2E (Figure 4.11). At MAI-686, quartz, clays, and feldspar were again the dominant minerals (Figures 4.11D, E, and F). Quartz, clays, and feldspars were the only minerals identified on the X-ray diffraction patterns of all the samples taken at MAI-686. The mineralogy of samples at HI-A389 at 500 and 5000 meters distance was typical of the sediments on this side of the East Flower Garden Bank (Figures 4.11F and G). Clays dominate the clay size fraction while quartz and calcite were distributed in varying proportions in the sand and silt size fractions. The sand size fraction consisted mostly of planktonic foraminifera and eroded debris from dead coralline algae patchreefs that are scattered over the bottom at these depths. The silt size fraction is mostly a mixture of quartz and tests of foraminifera. The presence of feldspar and dolomite at this distance from the platform is most probably because of the erosion of seafloor outcrops of Pleistocene and/or older sediments. Such outcrops have been documented in the general area (Rezak et al. 1985). Sediments 200 meters from the HI-A389 platform begin to show the effects of drilling activity with traces of barite at stations 1A, 1B, 1D, and 1E. The aragonite in sample 1D is probably a mollusc fragment derived from the nearby bank. Barite was also detected at 100 meters distance at stations 3A, 3B, and 3E in about the same relative amounts. Also, quartz becomes the most abundant mineral in this ring owing to the shunting of drill cuttings. The relative amount of barite increased in the closest stations at stations 2A, 2B, and 2C. At station 2E, there was no increase of barite over the relative amount present at station 3E. The distribution of barite around this platform supports the conclusion that the predominant bottom current-direction near this site is towards the northeast (Rezak et al. 1985).

The mineralogy of the sediments on the Texas OCS has been investigated in considerable detail. The primary role of the X-ray diffraction study was to determine if barite could be identified on the diffraction

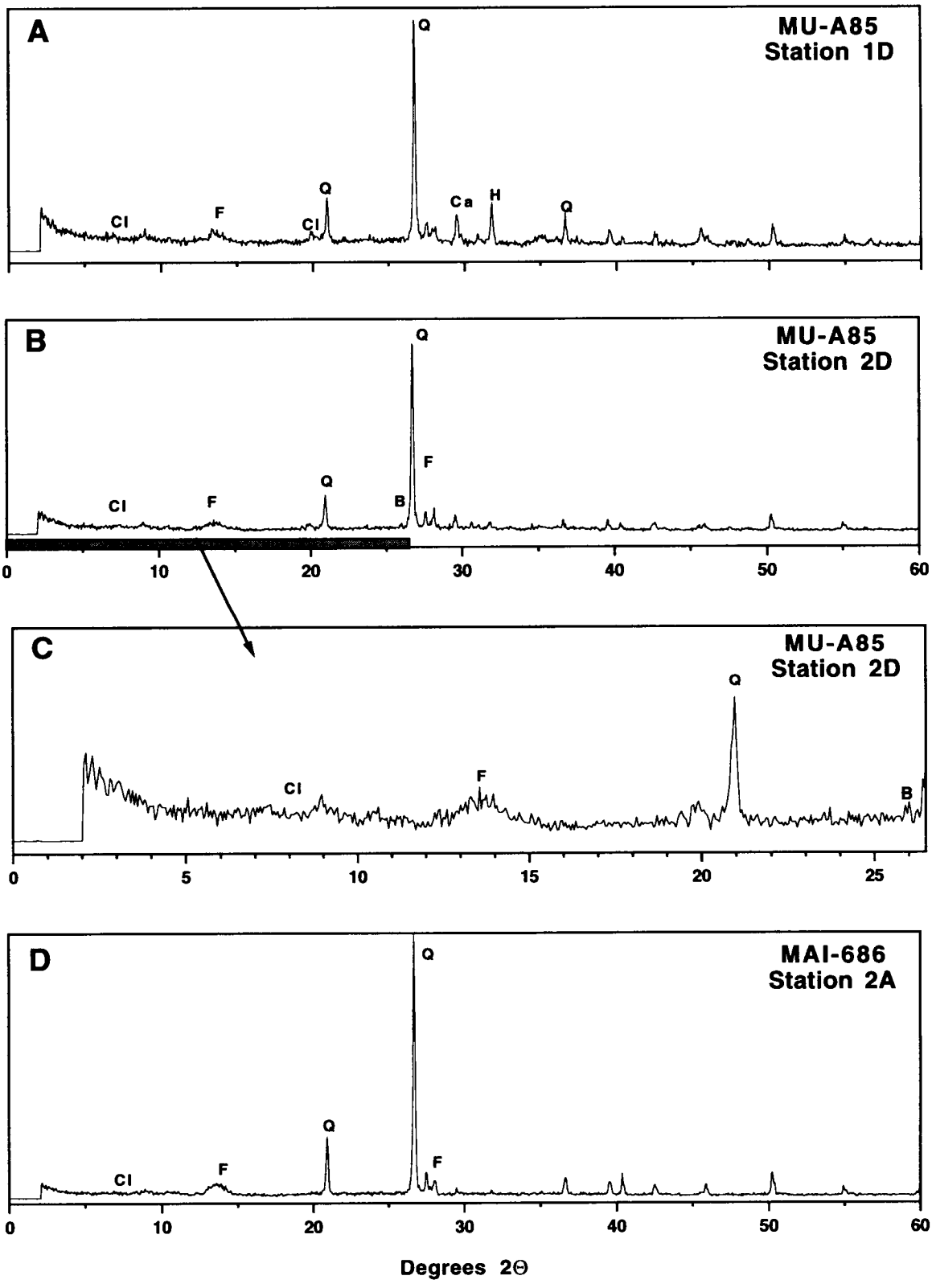


Figure 4.11. X-ray diffractograms of sediments from the study area. (B=barite, Ca=calcite, Cl=clay, F=feldspar, H=halite, I=illite, Q=quartz). **A:** MU-A85, station 1D; **B:** MU-A85, station 2D (note small main barite peak at 25.8°); **C:** Enlargement of **B** showing the barite peak; **D:** MAI-686, station 2A.

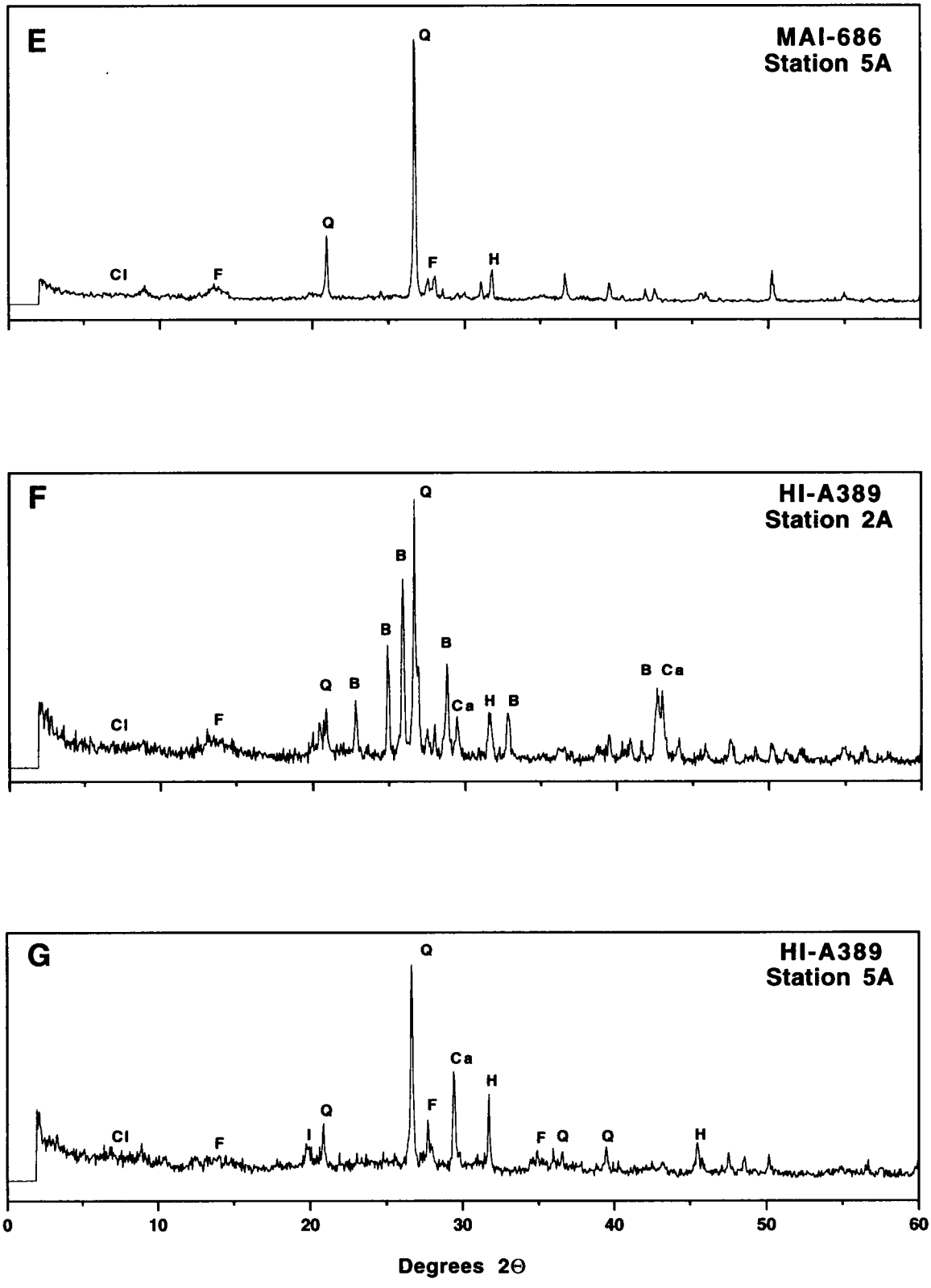


Figure 4.11 (*cont.*). **E:** MAI-686, station 5A; **F:** HI-A389, station 2A (note the main barite peak at 25.8°); **G:** HI-A389, station 5A (the halite is an artifact of the evaporation of interstitial seawater).

record. The limit of sensitivity of the technique was about 5% barite. X-ray diffraction was able to identify barite at two of the five study sites. Neutron activation analysis is a much more sensitive technique and therefore it is more effective in determining the presence and amount of barite in sediments.

4.4.3 Carbon Content

Organic and inorganic carbon content in sediments from Cruises 1 and 2 is summarized in Tables 4.15 and 4.16. Total organic carbon (TOC) content at MU-A85 from Cruise 1 ranged from 0.42% at station 2C to 1.42% at station 4A. Cruise 2 contents at MU-A85 for TIC and TOC were very similar to those of Cruise 1. TOC concentrations ranged from 0.09% at station 3D to 1.47% at station 5D. TIC concentrations ranged from 0.37% at station 1C to 2.19% also at station 1C. The next highest concentration was 1.54 at 2D. For Cruise 1, the lowest TOC concentrations at MAI-686 were generally in the area of highest percent sand. The concentrations ranged from 0.25% at station 2A to 1.26% at station 5C. The lowest TOC concentrations for Cruise 2 were at station 2D (0.21%) and there were high TOC concentrations at station 5D (1.52%) and 2A (1.35%). Percent TOC ranged from 0.17% at station 1E to 1.35% at station 2E at MAI-622. Cruise 1 distribution of TOC concentrations at HI-A389 ranged from 0.54 to 1.99%. TIC concentrations ranged from 0.04 to 4.26%. Cruise 2 concentrations at HI-A389 for TOC are similar to those from Cruise 1 and ranged from 0.59 to 2.02%.

Analysis of variance of carbon content reveals a number of significant interactions (Table 4.17). In all cases TOC has a significant interaction with distance and often with direction. These interactions reflect the significant addition of sand beneath the platform causing a dilution in organic carbon content. The areal distribution of TOC is influenced by the prevailing bottom currents. TIC enrichment near the platform most likely reflects deposition of debris from platform biota but the increases in TIC cannot account for the observed decrease in TOC.

Table 4.15. Summary of total organic (TOC) and inorganic (TIC) carbon concentrations (%) in sediments from Cruise 1.

	MU-A85-1					MAI-686-2					HI-A389-4				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
% TOC															
Average	0.57	0.67	1.16	0.97	0.93	0.30	0.39	0.86	0.97	1.00	1.13	1.06	1.15	1.16	1.19
Variance	0.02	0.03	0.04	0.10	0.06	0.00	0.01	0.07	0.05	0.10	0.15	0.04	0.11	0.04	0.06
Std Deviation	0.15	0.19	0.20	0.31	0.24	0.07	0.11	0.26	0.23	0.31	0.39	0.19	0.34	0.20	0.25
Coef of Var (%)	26.1%	27.6%	17.2%	32.3%	25.4%	21.8%	29.1%	29.9%	23.8%	31.4%	34.1%	17.7%	29.3%	17.2%	20.9%
Minimum	0.42	0.45	0.84	0.52	0.52	0.23	0.20	0.47	0.57	0.46	0.54	0.83	0.77	0.72	0.75
Maximum	0.81	1.06	1.49	1.42	1.33	0.46	0.56	1.19	1.28	1.35	1.99	1.40	1.68	1.45	1.60
Count	12	10	10	10	12	12	10	10	10	12	12	10	10	10	12
% TIC															
Average	1.01	0.87	0.60	0.87	0.77	0.32	0.29	0.26	0.22	0.18	2.07	2.83	2.00	1.93	2.17
Variance	0.05	0.04	0.03	0.14	0.06	0.08	0.03	0.06	0.04	0.03	1.44	0.40	0.17	0.08	0.12
Std Deviation	0.21	0.20	0.17	0.37	0.25	0.28	0.17	0.25	0.19	0.17	1.20	0.63	0.41	0.29	0.34
Coef of Var (%)	21.3%	22.4%	28.2%	43.0%	32.0%	86.5%	57.4%	93.2%	88.2%	89.7%	58.0%	22.2%	20.4%	15.1%	15.7%
Minimum	0.74	0.52	0.34	0.38	0.32	0.09	0.03	0.01	0.00	0.00	0.04	2.12	1.48	1.33	1.74
Maximum	1.54	1.15	0.90	1.50	1.15	0.97	0.54	0.71	0.50	0.55	3.39	4.26	2.63	2.49	2.91
Count	12	10	10	10	12	12	10	10	10	12	12	10	10	10	12

Table 4.16. Summary of total organic (TOC) and inorganic (TIC) carbon concentrations (%) in sediments from Cruise 2.

	MU-A85-1					MAI-686-2					HI-A389-4				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
% TOC															
Average	0.54	0.52	0.86	0.93	1.02	0.48	0.53	0.64	0.91	1.05	1.11	1.00	1.29	1.52	1.58
Variance	0.08	0.07	0.10	0.05	0.09	0.10	0.04	0.04	0.05	0.06	0.14	0.07	0.17	0.03	0.14
Std Deviation	0.27	0.26	0.31	0.21	0.30	0.32	0.21	0.21	0.21	0.24	0.37	0.27	0.41	0.18	0.38
Coef of Var (%)	50.5%	50.1%	35.9%	23.0%	29.2%	66.4%	39.0%	32.9%	23.6%	22.9%	33.3%	26.4%	32.1%	12.1%	23.7%
Minimum	0.3	0.1	0.4	0.6	0.4	0.2	0.2	0.3	0.6	0.5	0.7	0.7	0.6	1.1	0.9
Maximum	1.2	1.0	1.4	1.3	1.5	1.3	0.9	1.0	1.2	1.3	1.9	1.4	2.2	1.7	2.1
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
% TIC															
Average	1.09	1.09	1.08	0.89	0.88	0.37	0.34	0.49	0.29	0.13	2.12	2.89	1.91	1.45	1.67
Variance	0.08	0.10	0.22	0.05	0.08	0.06	0.08	0.18	0.03	0.01	1.05	0.95	0.23	0.09	0.08
Std Deviation	0.29	0.31	0.47	0.22	0.29	0.25	0.28	0.42	0.19	0.10	1.02	0.97	0.48	0.30	0.28
Coef of Var (%)	26.3%	28.8%	43.5%	24.3%	32.8%	67.5%	81.1%	86.6%	65.0%	76.4%	48.3%	33.6%	25.3%	20.9%	16.9%
Minimum	0.5	0.4	0.4	0.5	0.5	0.1	0.0	0.1	0.1	0.0	0.7	1.8	1.2	1.1	1.2
Maximum	1.5	1.5	2.2	1.3	1.5	0.8	0.9	1.5	0.7	0.4	3.8	4.8	2.6	2.0	2.1
Count	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table 4.17. ANOVA analysis of variance of sediment total organic carbon content (%).

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85-1			
Cruise (A)	1	0.196	0.196	3.583	0.0637
Distance (B)	4	4.136	1.034	18.925	**0.0001**
A•B	4	0.420	0.105	1.923	0.1199
Radii (C)	4	0.534	0.133	2.442	0.0577
A•C	4	0.130	0.033	0.597	0.6665
B•C	16	1.807	0.113	2.067	0.0245
A•B•C	16	1.006	0.063	1.151	0.3358
Error	54	2.950	0.055		
		MAI-686-2			
Cruise (A)	1	0.016	0.016	0.616	0.4361
Distance (B)	4	6.078	1.520	60.102	**0.0001**
A•B	4	0.527	0.132	5.214	**0.0013**
Radii (C)	4	0.393	0.098	3.883	**0.0076**
A•C	4	0.395	0.099	3.901	**0.0074**
B•C	16	2.610	0.163	6.452	**0.0001**
A•B•C	16	0.620	0.039	1.533	0.1221
Error	54	1.365	0.025		
		HIA-A389-4			
Cruise (A)	1	0.532	0.532	8.314	**0.0056**
Distance (B)	4	1.835	0.459	7.169	**0.0001**
A•B	4	0.902	0.225	3.523	0.0126
Radii (C)	4	0.308	0.077	1.203	0.3202
A•C	4	0.414	0.103	1.616	0.1836
B•C	16	2.360	0.148	2.306	**0.0116**
A•B•C	16	3.202	0.200	3.128	**0.0009**
Error	54	3.455	0.064		

4.4.4 Redox Conditions (Eh)

Redox conditions as measured by Eh electrode are summarized in Tables 4.18 and 4.19. Eh was highly variable at the sites studied. In many cases Eh showed a significant interaction with distance from the platform (P=0.01). A more detailed analysis of the data and more data will be needed to evaluate the significance of the observed variations in sediment Eh (mV).

4.5 Contaminants

4.5.1 Hydrocarbons

Hydrocarbons are an important constituent of petroleum. Aliphatic and aromatic hydrocarbons can account for more than 70% of petroleum by weight. While hydrocarbons are ubiquitous in the marine environment, petroleum-derived hydrocarbons can be recognized and are often used as tracers of petroleum contamination (Brassell et al. 1978; Philp 1985; Boehm and Requejo 1986; Kennicutt and Comet 1992). However, aliphatic hydrocarbons can be synthesized by organisms (both planktonic and terrestrial). Petroleum contains a homologous series of n-alkanes with one to more than thirty carbons with odd and even n-alkanes present in nearly equal amounts, whereas organisms preferentially produce specific suites of normal alkanes with odd numbers of carbons from fifteen to thirty-three. Petroleum also contains a complex mixture of branched and cycloalkanes not found in organisms. This complex mixture can produce a gas chromatographically unresolved complex mixture (UCM) of compounds when petroleum is extensively biodegraded. The presence and amount of the UCM can be a diagnostic indicator of petroleum contamination. Petroleum also contains an extensive suite of polycyclic aromatic hydrocarbons (PAH) which are known to be toxic to organisms. Selected PAH are synthesized by organisms but can be easily recognized from those in petroleum. The amount and composition of PAH can be effectively used as tracers of petroleum contamination. PAH are also toxic and serve as an indication of exposure in organisms. PAH are more resistant to microbial breakdown than aliphatic hydrocarbons in general and thus tend to persist in the environment longer. Based on consideration of petroleum chemistry,

Table 4.18. Summary of sediment redox (Eh, mV) measurements from Cruise 1.

Statistic	MU-A85-1 Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	MAI-686-2 Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
REDOX (mV)										
Average	117.92	115.90	255.50	141.50	317.42	220.92	231.30	133.70	126.70	126.42
Variance	377.58	376.49	16576.65	127349.65	14625.58	9527.58	8783.21	821.61	251.01	374.58
Std Deviation	19.43	19.40	128.75	356.86	120.94	97.61	93.72	28.66	15.84	19.35
Coef of Var (%)	16.5%	16.7%	50.4%	252.2%	38.1%	44.2%	40.5%	21.4%	12.5%	15.3%
Minimum	93	90	104	-862	85	117	129	97	109	87
Maximum	157	160	476	470	433	367	411	190	150	152
Count	12	10	10	10	12	12	10	10	10	12

Results expressed relative to hydrogen electrode using bottom water temperature for correction.

Statistic	HI-A389-4 Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
REDOX (mV)					
Average	61.75	119.20	171.80	117.44	75.00
Variance	2117.69	3260.76	14542.56	1874.69	7203.60
Std Deviation	46.02	57.10	120.59	43.30	84.87
Coef of Var (%)	74.5%	47.9%	70.2%	36.9%	113.2%
Minimum	0	12	30	62	-163
Maximum	148	167	418	197	166
Count	12	10	10	9	10

Results expressed relative to hydrogen electrode using bottom water temperature for correction.

Table 4.19. Summary of sediment redox (Eh, mV) measurements from Cruise 2.

	MU-A85-1					MAI-686-2				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
REDOX (mV)										
Average	61.2	21.5	133.9	65.7	56.5	37.8	29.8	72.6	75.5	67.2
Variance	2633.1	1283.5	349.9	3919.1	473.5	612.0	2025.5	815.0	105.9	749.2
Std Deviation	51.3	35.8	18.7	62.6	21.8	24.7	45.0	28.5	10.3	27.4
Coef of Var (%)	83.8%	166.6%	14.0%	95.3%	38.5%	100.0%	100.0%	100.0%	100.0%	100.0%
Minimum	18	-22	98	9	30	-20	-62	27	65	16
Maximum	168	92	166	197	111	65	88	131	95	101
Count	9	10	10	9	10	9	9	10	10	10

	HI-A389				
Statistic	Ring 2	Ring 3	Ring 1	Ring 4	Ring 5
REDOX (mV)					
Average	72.3	104.1	112.4	113.1	118.4
Variance	2357.0	13561.7	5663.2	1618.1	783.2
Std Deviation	48.5	116.5	75.3	40.2	28.0
Coef of Var (%)	100.0%	100.0%	100.0%	100.0%	100.0%
Minimum	-12	-76	-37	49	78
Maximum	164	233	206	205	178
Count	10	9	10	10	10

biological occurrences of hydrocarbons (i.e., interferences), and toxicological effects; aliphatic and aromatic hydrocarbons are chosen as the preferred organic tracers of petroleum contamination near platforms. Petroleum contamination can occur during drilling as well as production activities and is present in a variety of discharges including drilling fluids, drill cuttings, produced water, spills, deck drainage and other releases.

4.5.1.1 Sediments

Sediments are a long-term repository of the residues of petroleum released to the marine environment. Petroleum in the offshore environment can be altered by evaporation, dissolution, photooxidation, and microbial degradation and tends to adhere to particulates and become deposited in sediments. The presence and composition of petroleum contaminants in sediment are a record of the long-term, chronic accumulation of contaminants in the vicinity of platforms thus reflecting the potential for exposure of the resident biota. Based on these considerations, aliphatic and polycyclic aromatic hydrocarbons were measured at all quantitative boxcore locations.

The average, variance, standard deviation, coefficient of variation, and range of hydrocarbon concentrations in sediments at various distances from each study site are summarized in Tables 4.20 and 4.21. Strong gradients with distance from the platform are apparent in total alkane, UCM, and PAH concentrations at MU-A85 and HI-A389 sites. A less dramatic gradient is present at MAI-686. Shallow water erosional settings are unlikely to accumulate contaminants (Boesch and Rabalais 1987).

Most stations contained little or no evidence of petroleum contamination. Background concentrations for UCM and total alkanes are ~10 ppm and 500 ppb, respectively. The method detection limit for total PAH is in the 100-150 ppb range for the analytical procedure used. As demonstrated by previous studies, the concentration of contaminants decreases rapidly with distance from the platform. The decrease has a directional component, and concentrations generally reach background levels at distances greater than 100 m from the platform (Figures 4.12-4.17). Two exceptions are found for total alkanes at MAI-686 which were evident to 200 m distance and PAH which were present as

Table 4.20. Summary of the statistical analysis of hydrocarbon indicators in sediments from Cruise 1.

Statistic	MU-A85-1 Ring 2	MU-A85-1 Ring 3	MU-A85-1 Ring 1	MU-A85-1 Ring 4	MU-A85-1 Ring 5	MAI-686-2 Ring 2	MAI-686-2 Ring 3	MAI-686-2 Ring 1	MAI-686-2 Ring 4	MAI-686-2 Ring 5	HI-A389-4 Ring 2	HI-A389-4 Ring 3	HI-A389-4 Ring 1	HI-A389-4 Ring 4	HI-A389-4 Ring 5
Total Alkanes (ppb)															
											1868.5	509.1	248.0	270.2	242.9
Average	2016.2	969.1	204.6	193.3	142.9	831.0	635.5	663.7	331.8	294.8	3402848	107980	12216	21836	10514
Variance	1246331	545219	2989	759	2302	1201276	702105	716079	1833	5310	1844.7	328.6	110.5	147.8	102.5
Std Deviation	1116.4	738.4	54.7	27.5	48.0	1096.0	837.9	846.2	42.8	72.9	98.7%	64.5%	44.6%	54.7%	42.2%
Coef of Var (%)	55.4%	76.2%	26.7%	14.3%	33.6%	131.9%	131.9%	127.5%	12.9%	24.7%	208.0	206.7	135.9	118.7	89.1
Minimum	564.5	169.2	139.7	143.0	68.3	167.1	108.0	139.5	278.5	164.5	6006.4	1140.5	478.7	628.0	450.4
Maximum	4426.9	2643.4	298.4	232.3	260.1	3301.0	2663.5	2542.0	406.8	450.8	12	10	10	10	12
Count	12	10	10	10	12	12	10	10	10	12					
Total UCM (ppm)															
											87.3	21.1	14.9	6.7	1.2
Average	93.4	43.8	3.2	0.3	2.5	12.7	5.5	7.6	1.1	3.4	8914.9	550.9	64.5	11.5	6.9
Variance	8259.5	1023.0	16.3	0.0	3.4	125.9	47.6	69.4	0.2	11.6	94.4	23.5	8.0	3.4	2.6
Std Deviation	90.9	32.0	4.0	0.2	1.8	11.2	6.9	8.3	0.4	3.4	108.1%	111.3%	54.1%	50.7%	217.6%
Coef of Var (%)	97.3%	73.0%	127.4%	69.2%	73.2%	88.1%	125.2%	109.5%	36.6%	101.0%	11.6	3.5	8.8	2.7	0.0
Minimum	32.8	11.6	0.1	0.0	0.5	2.0	0.8	1.0	0.5	0.0	287.1	71.3	32.9	14.8	9.2
Maximum	381.8	112.9	13.1	0.6	6.9	36.6	23.5	24.6	1.8	11.1	12	10	10	10	12
Count	12	10	10	10	12	12	10	10	10	12					
Total PAH's (ppb)															
											378.1	224.1	47.6	29.7	24.7
Average	234.3	164.2	33.2	20.8	25.1	36.4	35.9	32.6	30.0	41.8	89535.3	99952.1	152.6	81.4	38.7
Variance	29826.9	15125.5	381.1	15.6	32.4	527.1	1855.8	148.4	24.5	300.5	299.2	316.2	12.4	9.0	6.2
Std Deviation	172.7	123.0	19.5	3.9	5.7	23.0	43.1	12.2	5.0	17.3	79.1%	141.1%	25.9%	30.4%	25.2%
Coef of Var (%)	73.7%	74.9%	58.8%	19.0%	22.7%	63.1%	120.1%	37.3%	16.5%	41.5%	90.4	30.9	22.5	21.1	14.9
Minimum	47.7	16.9	18.7	14.5	12.7	13.4	11.8	21.6	23.3	20.7	1241.3	1133.5	66.7	51.7	38.2
Maximum	672.3	432.7	81.3	26.6	33.7	93.1	157.2	58.3	40.9	83.5	12	10	10	10	12
Count	12	10	10	10	12	12	10	10	10	12					

Table 4.21. Summary of the statistical analysis of total PAH (ng/g) in sediments from Cruise 2.

Statistic	MU-A85-1 Ring 2	MU-A85-1 Ring 3	MU-A85-1 Ring 1	MU-A85-1 Ring 4	MU-A85-1 Ring 5	MAI-686-2 Ring 2	MAI-686-2 Ring 3	MAI-686-2 Ring 1	MAI-686-2 Ring 4	MAI-686-2 Ring 5	HI-A389-4 Ring 2	HI-A389-4 Ring 3	HI-A389-4 Ring 1	HI-A389-4 Ring 4	HI-A389-4 Ring 5
Total Alkanes (ppb)															
Average	2817.0	958.9	297.9	244.6	208.2	2363.1	1104.8	1459.5	378.3	442.0	1017.7	532.4	334.4	241.5	218.6
Variance	11482676	319226	3876	1705	1730	5344451	3038688	4680715	7002	12267	415016	267894	15401	734	4709
Std Deviation	3388.6	565.0	62.3	41.3	41.6	2311.8	1743.2	2163.5	83.7	110.8	644.2	517.6	124.1	27.1	68.6
Coef of Var (%)	120.3%	58.9%	20.9%	16.9%	20.0%	97.8%	157.8%	148.2%	22.1%	25.1%	63.3%	97.2%	37.1%	11.2%	31.4%
Minimum	271.4	197.6	178.4	176.3	131.1	317.1	237.0	307.3	262.8	253.7	180.0	139.3	215.6	197.0	156.4
Maximum	10852.8	1993.7	377.5	314.3	274.4	5915.0	5902.7	6175.1	568.8	611.6	2132.2	1479.5	607.6	282.7	400.2
Count	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10
Total UCM (ppm)															
Average	88.8	54.0	7.8	8.9	5.1	11.9	6.2	11.2	5.8	5.9	56.5	19.7	4.8	2.3	0.5
Variance	7391.4	1679.1	4.9	107.9	12.2	94.4	94.4	235.4	2.3	2.1	1424.0	391.2	19.7	8.8	0.1
Std Deviation	86.0	41.0	2.2	10.4	3.5	9.7	9.7	15.3	1.5	1.5	37.7	19.8	4.4	3.0	0.3
Coef of Var (%)	96.9%	75.9%	28.3%	117.2%	69.1%	81.4%	156.2%	137.6%	26.6%	24.9%	66.8%	100.3%	91.7%	129.2%	57.3%
Minimum	8.2	9.5	4.5	1.1	1.7	2.7	0.4	1.0	3.8	2.7	4.4	4.4	1.0	0.0	0.2
Maximum	313.9	158.8	12.6	30.0	14.0	30.6	32.5	43.5	9.1	7.8	116.1	61.0	15.2	10.5	1.0
Count	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10
Total PAH's (ppb)															
Average	162.8	138.1	37.6	42.8	28.9	31.5	46.6	62.2	59.1	81.9	350.3	141.7	83.8	39.3	26.1
Variance	8167.1	12207	78.3	110.5	107.5	328.7	3937.6	3022.9	646.2	1664.3	44488.6	18618.1	3585.8	603.5	37.1
Std Deviation	90.4	110.5	8.9	10.5	10.4	18.1	62.8	55.0	25.4	40.8	210.9	136.4	59.9	24.6	6.1
Coef of Var (%)	55.5%	80.0%	23.5%	24.6%	35.9%	57.5%	134.6%	88.3%	43.0%	49.8%	60.2%	96.3%	71.5%	62.5%	23.3%
Minimum	34.8	19.9	24.6	30.7	16.9	12.4	16.9	21.5	30.1	28.2	42.7	12.6	24.0	27.0	16.1
Maximum	363.6	342.1	52.3	66.0	52.8	65.0	233.1	177.9	105.9	148.5	710.7	464.5	212.4	110.9	34.8
Count	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10

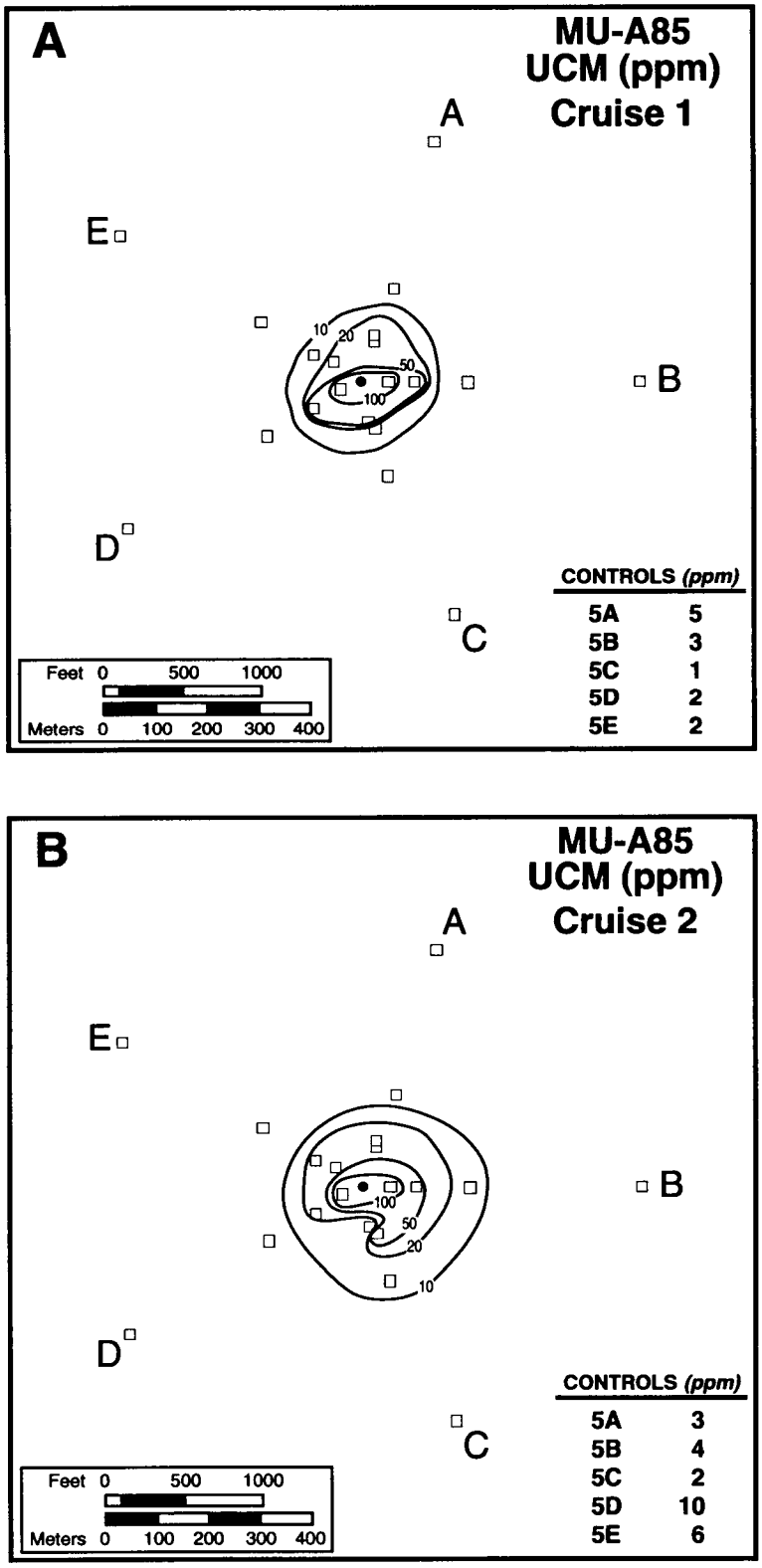


Figure 4.12. Areal distribution of the unresolved complex mixture (UCM) at MU-A85 for A: Cruise 1; and B: Cruise 2.

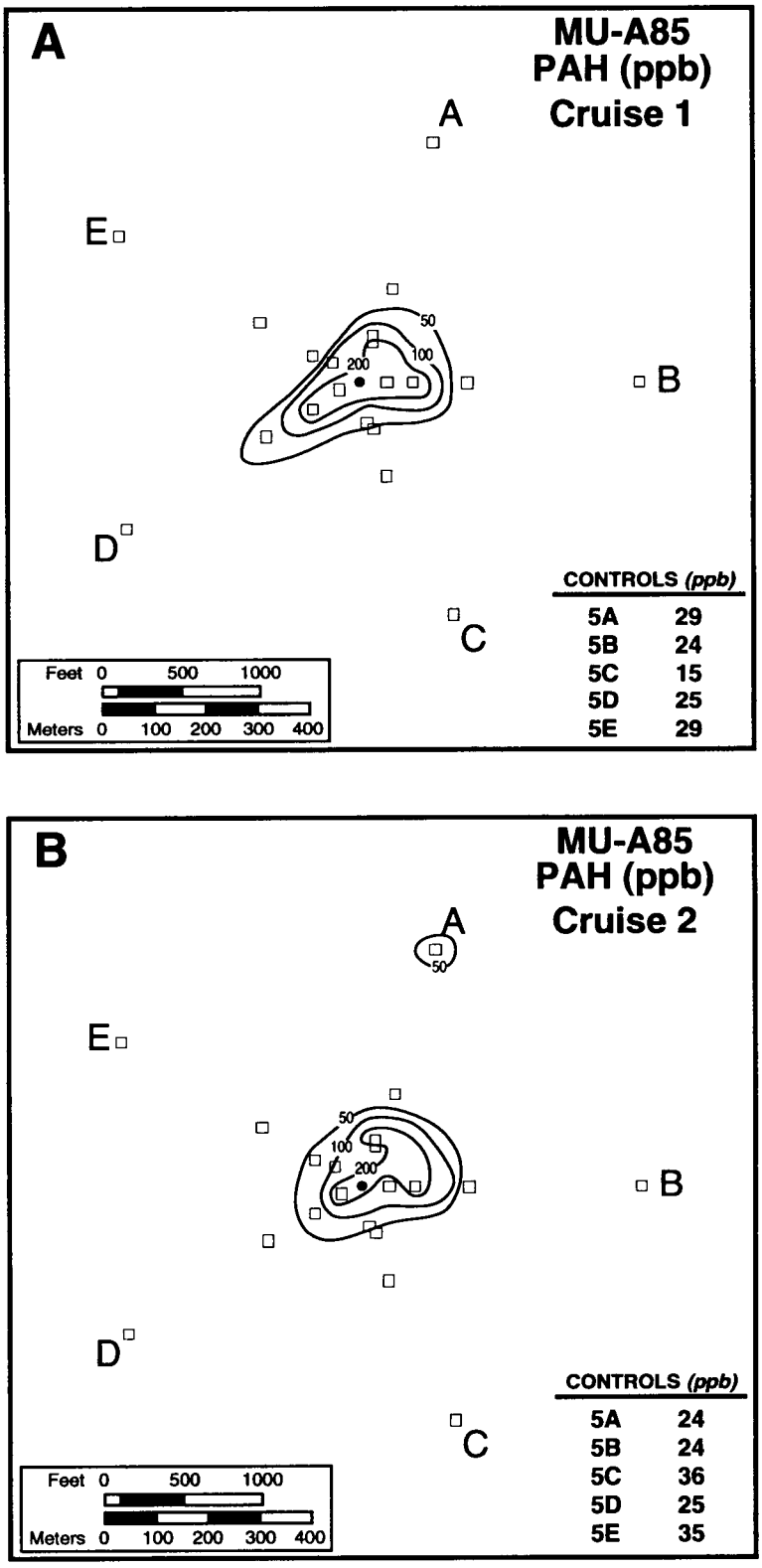


Figure 4.13. Areal distribution of total polycyclic aromatic hydrocarbons (PAH) at MU-A85 for A: Cruise 1; and B: Cruise 2.

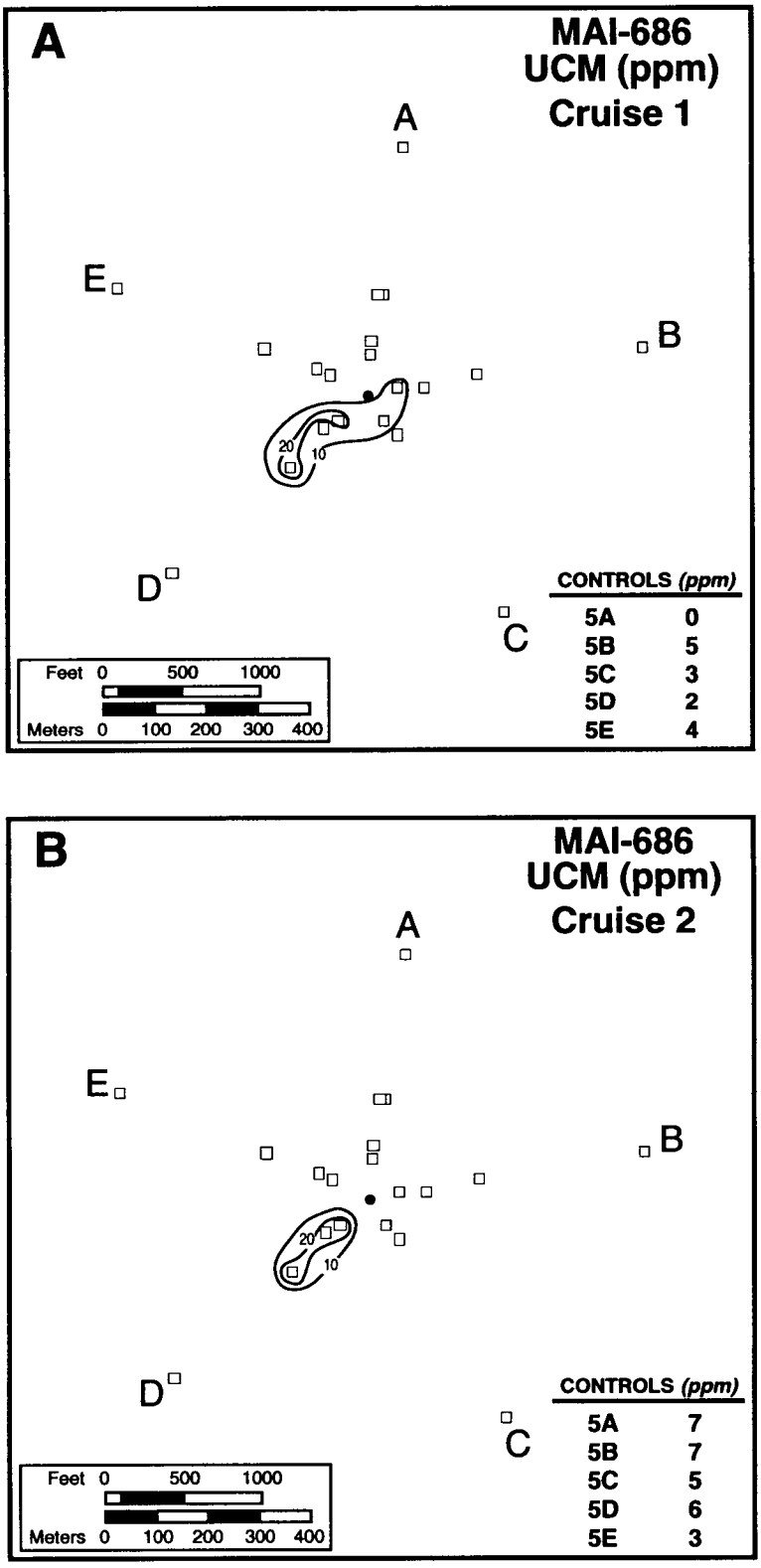


Figure 4.14. Areal distribution of the unresolved complex mixture (UCM) at MAI-686 for A: Cruise 1; and B: Cruise 2.

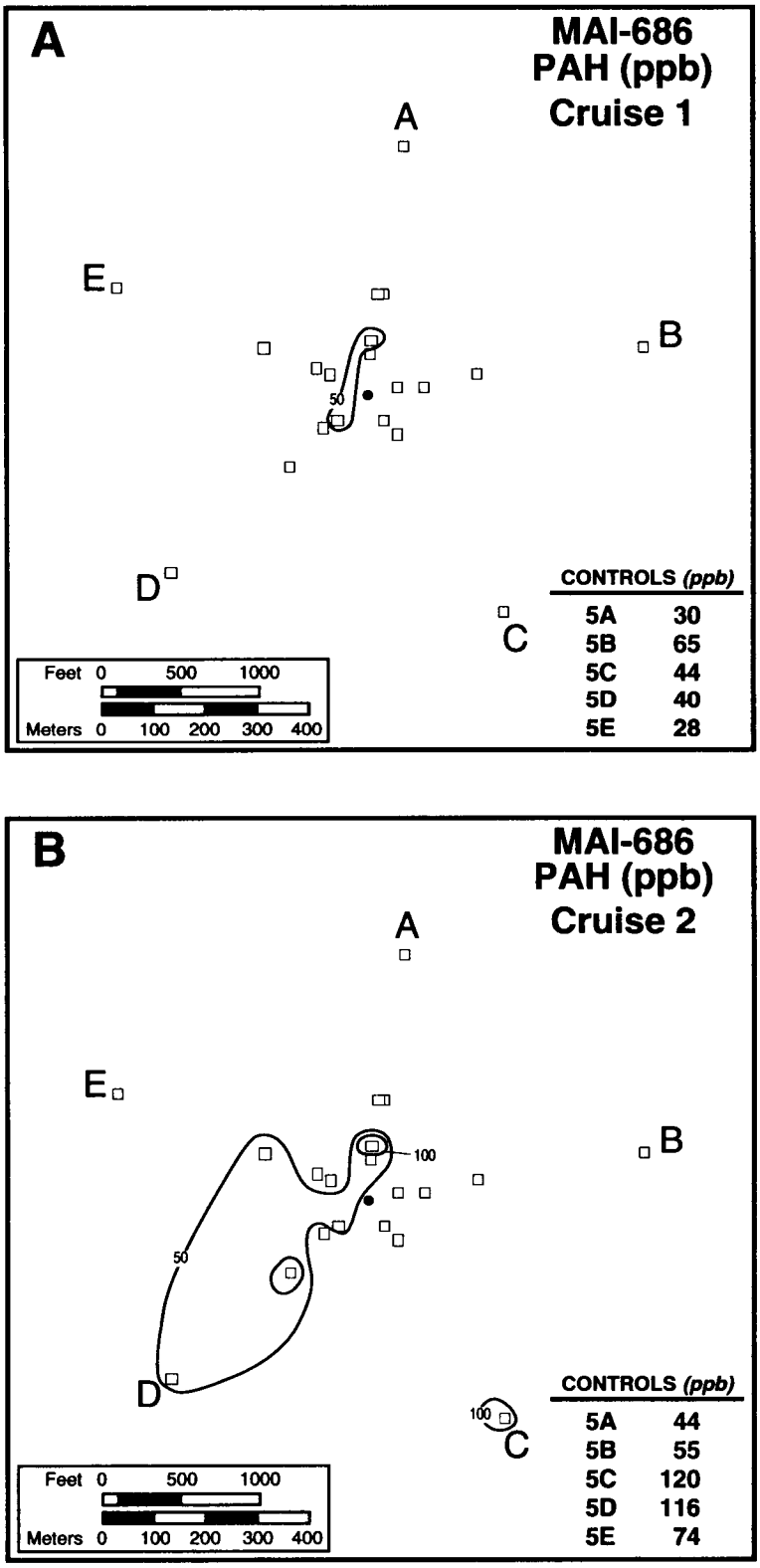


Figure 4.15. Areal distribution of total polycyclic aromatic hydrocarbons (PAH) at MAI-686 for A: Cruise 1; and B: Cruise 2.

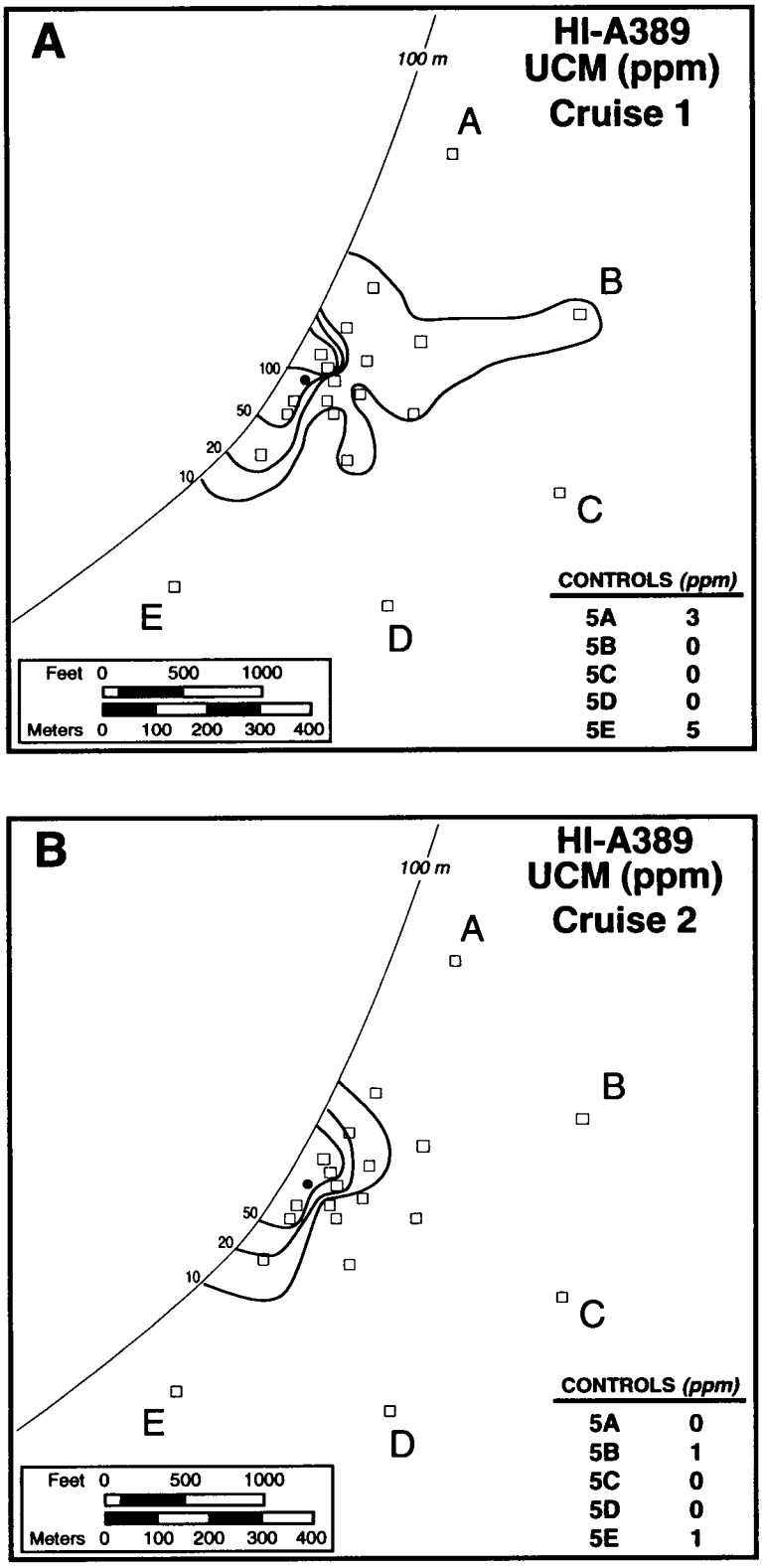


Figure 4.16. Areal distribution of the unresolved complex mixture (UCM) at HI-A389 for A: Cruise 1; and B: Cruise 2.

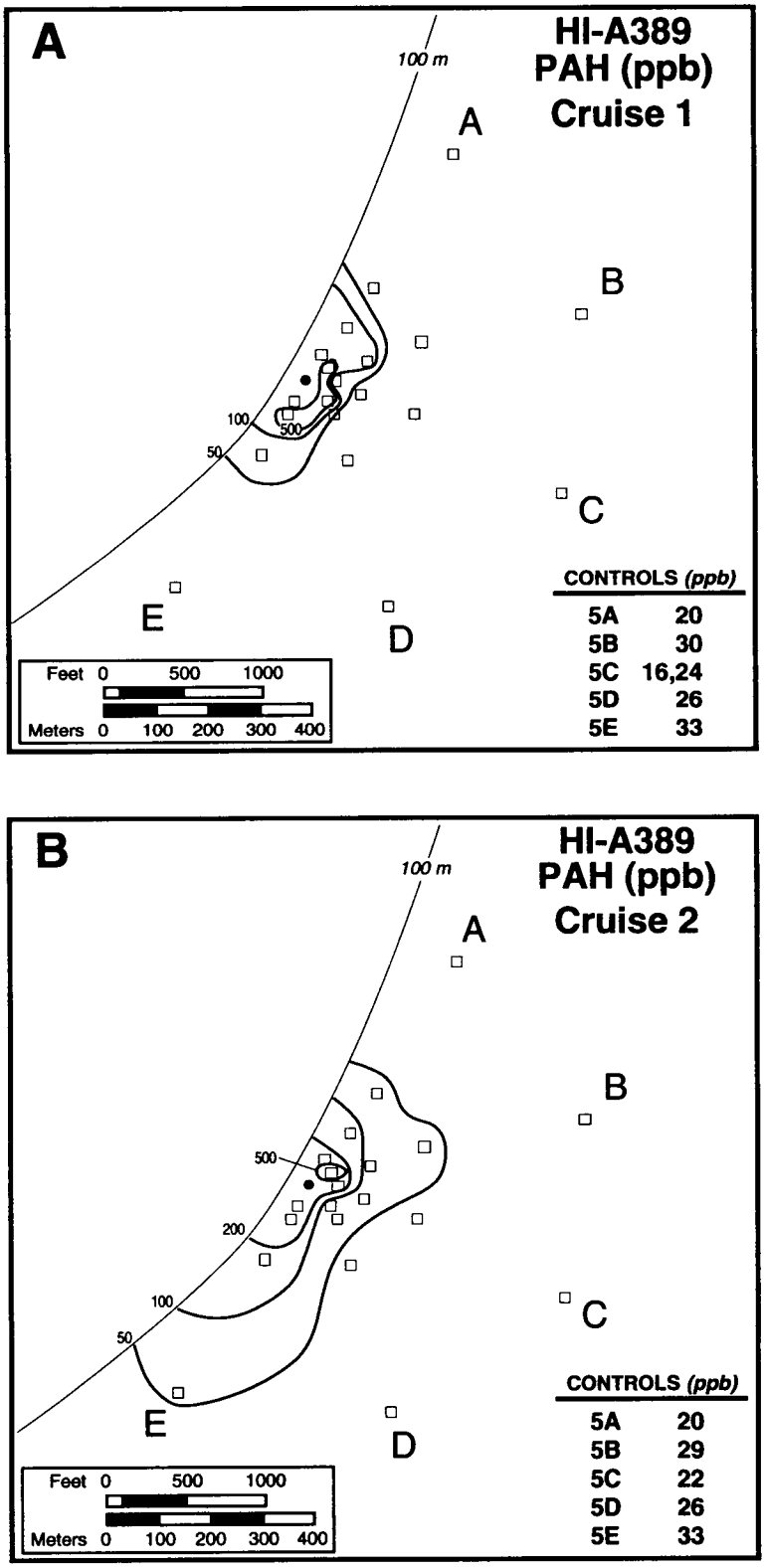


Figure 4.17. Areal distribution of total polycyclic aromatic hydrocarbons (PAH) at HI-A389 for A: Cruise 1; and B: Cruise 2.

far as 500 m from the platform at HI-A389 (Figures 4.17). The MAI-686 stations exhibit evidence of fresh condensate contamination during both Cruises 1 and 2 suggesting either a pipeline leak or a seep (Figure 4.18). In contrast, the contaminants at HI-A389 and MU-A85 were highly weathered as evidenced by the lack of n-alkanes and the presence of a large UCM (Figures 4.19 and 4.20). HI-A389 also exhibited a range of compositions suggestive of several different inputs. One area appeared to be contaminated with a low molecular weight, freshly spilled oil while other areas exhibited a bimodal UCM suggestive of two different sources of degraded petroleum (Figure 4.19).

The areal distribution of the hydrocarbon contaminants was relatively symmetrical around the platforms of HI-A389 and MU-A85 with slightly higher concentrations in the direction of the prevailing currents (Figures 4.12, 4.13, 4.16, 4.17). The hydrocarbon distribution was similar for Cruises 1 and 2 suggesting that the arcal extent of the contamination was relatively constant. The greatest variations between cruises were detected at stations nearest to the platform.

An ANOVA analysis of the sediment hydrocarbon data by site highlights the significant trends in the data. These results are illustrated for total PAH in Table 4.22. At P=0.01, Cruise 1 to Cruise 2 variations are not significant at MU-A85 and HI-A389. At site MAI-686, Cruise 1 to Cruise 2 variations are significant (P=0.0023) but the gradients observed were weak. Many of the contaminant values were close to background or the method detection limit. At MU-A85 and HI-A389, distance, radii, and distance•radii interactions are highly significant. At MAI-686 at P=0.05 radii and radii•distance interactions are less significant but still important.

4.5.1.2 Tissues

Contaminant concentrations in tissues have been widely used to assess the bioavailability of petroleum contamination. Tissue contaminants are determined by the interplay of several factors including uptake, metabolism and depuration. These confounding factors can obscure the relationship between body burden and actual exposure. Uptake, metabolism and depuration of contaminants is often species dependent. The presence of detoxification enzyme systems in fish provides an ability to metabolize

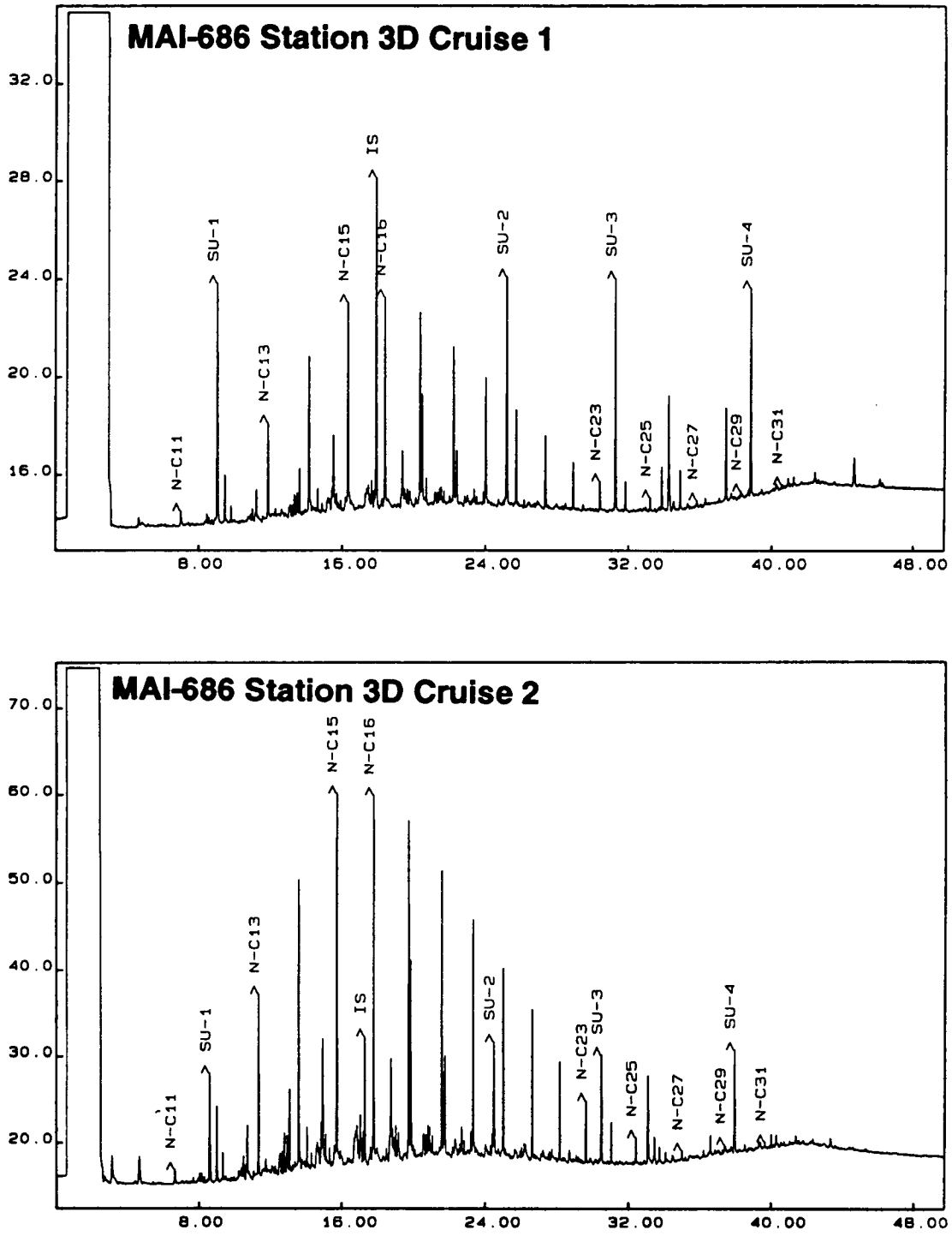


Figure 4.18 GC/FID traces of sediment extracts from MAI-686.

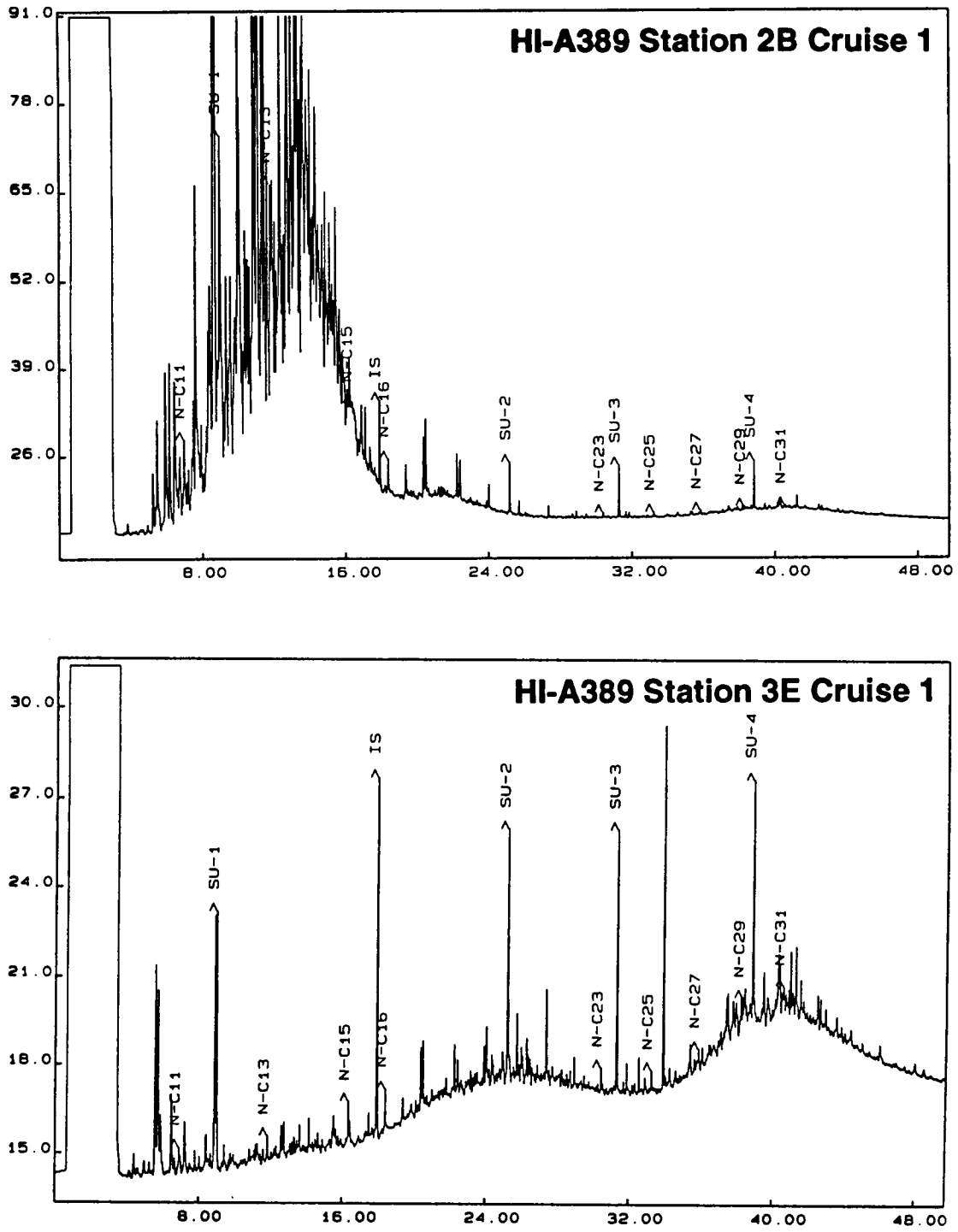


Figure 4.19 GC/FID traces of sediment extracts from HI-A389.

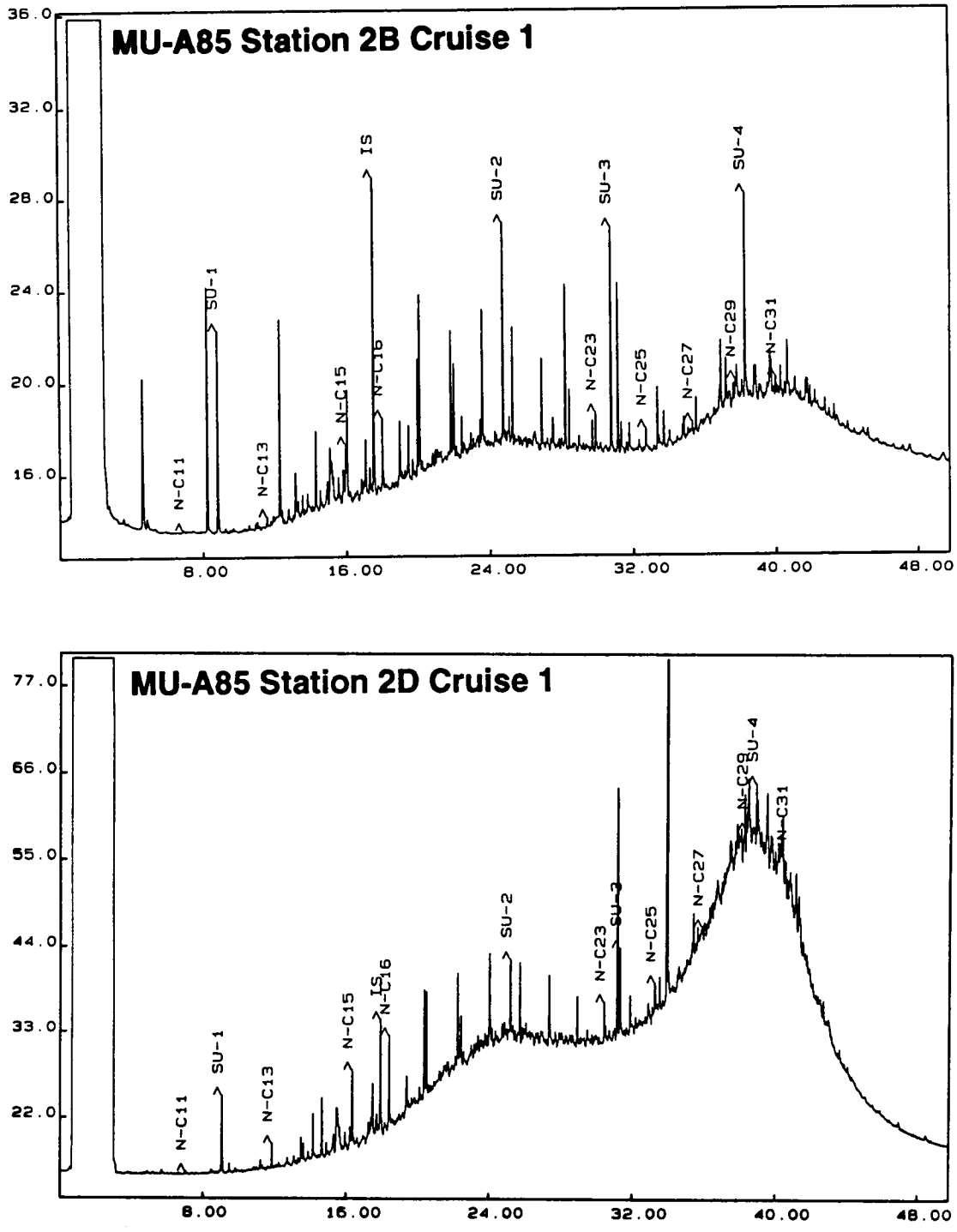


Figure 4.20 GC/FID traces of sediment extracts from MU-A85

Table 4.22. ANOVA analysis of variance of sediment PAH concentrations (ppb).

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85-1			
Cruise (A)	1	2286	2286	0.3750	0.5430
Distance (B)	4	513687	128422	21.0530	**0.0001**
A•B	4	20138	5035	0.8250	0.5148
Radii (C)	4	96731	24183	3.9640	**0.0068**
A•C	4	2676	669	0.1100	0.9786
B•C	16	196646	12290	2.0150	0.0287
A•B•C	16	82879	5180	0.8490	0.6268
Error	54	329392	6100		
		MAI-686-2			
Cruise (A)	1	10796	10796	10.3350	**0.0023**
Distance (B)	4	8434	2109	2.0190	0.1061
AB	4	6455	1614	1.5450	0.2036
Radii (C)	4	12305	3076	2.9450	0.0291
A•C	4	2145	536	0.5130	0.7263
B•C	16	41482	2593	2.4820	**0.0074**
A•B•C	16	17786	1112	1.0640	0.4119
Error	50	52229	1045		
		HI-A389-4			
Cruise (A)	1	12802	12802	0.6540	0.4224
Distance (B)	4	1873311	468328	23.9380	**0.0001**
AB	4	58413	14603	0.7460	0.5650
Radii (C)	4	349193	87298	4.4620	**0.0037**
A•C	4	76611	19153	0.9790	0.4275
B•C	16	718971	44936	2.2970	0.0130
A•B•C	16	474658	29666	1.5160	0.1313
Error	50	978225	19565		

(transform) and eliminate contaminants, such as PAH, from their tissues. However the presence of contaminants in tissues does confirm recent exposure. The materials analyzed were the soft tissues of invertebrates, liver tissues from fish, and fish stomach contents. Invertebrates may accumulate contaminants due to their often intimate relationship with the benthos. Fish livers are a primary site of PAH metabolism/storage and thus would be the most likely tissue to exhibit PAH contamination. The liver is also lipid-rich compared to muscle tissue and PAH tend to preferentially accumulate in lipid-rich organs. Stomach contents were analyzed to determine if contaminant exposure in fish is due to dietary sources or ingestion of contaminated sediments. Other routes of exposure include uptake across exposed external organs such as gills.

The averages, variances, standard deviations, coefficient of variations, and ranges by tissue type and site are summarized in Tables 4.23 and 4.24. The species analyzed for hydrocarbons from Cruises 1 and 2 are summarized in Table 4.25. Note that the sampling during Cruise 1 was very spotty and thus trends are difficult to assess. A more intensive sampling effort resulted in much better coverage and replication during Cruise 2.

ANOVA analysis of variance revealed few trends in the pairwise comparisons of near and far stations by site (Tables 4.26-4.28). The data set is limited and multiple species were grouped for this preliminary analysis of the data. As the database is expanded by further sampling, more refined statistical analyses will be possible. A significant (P=0.01) difference in PAH levels in fish liver tissues from near and far stations at HI-A389 was observed. The statistical comparisons were made on data normalized to the highest individual PAH to eliminate artifacts due to variable method detection limits. Species, ecological niche, and other factors that may control contaminant exposure and expression will need to be evaluated in order to properly evaluate the tissue contaminant data.

4.5.2 Trace Metals

The major goal of this work element is to describe the spatial and temporal distribution of trace metals in sediments around each drilling site and thereby define the inorganic exposure regime for possible chronic exposure. Trace element levels in resident biota (fish and invertebrates) are

Table 4.23. Summary of the statistical analysis of total PAH concentrations (ppb) in tissues from Cruise 1

Statistic	MU-A85-1 Soft Tissues		MU-A85-1 Liver		MU-A85-1 Stomach Content		MAI-686-2 Soft Tissues		MAI-686-2 Liver		MAI-686-2 Stomach Content	
	Near	Far	Near	Far	Near	Far	Near	Far	Near	Far	Near	Far
Average	304.03	1815.77	558.24	1107.95	364.60	237.09	67.29	77.12	208.66	n.d.	149.91	264.60
Variance	128518	7314472	468043	3664636	24094	32338	685	2908	27112	n.d.	9905	242
Std Deviation	358.49	2704.53	684.14	1914.32	155.22	179.83	26.18	53.93	164.66	n.d.	99.52	15.56
Coef of Var (%)	117.9%	148.9%	122.6%	172.8%	42.6%	75.8%	38.9%	69.9%	78.9%	n.d.	66.4%	5.9%
Minimum	17.73	20.69	99.74	61.39	105.14	26.71	37.78	30.62	100.67	n.d.	50.32	248.58
Maximum	1689.48	10424.87	2679.31	4931.08	656.92	697.31	121.06	216.68	637.53	n.d.	412.67	289.07
Count	19	17	13	5	17	10	9	16	12	0	15	4

Statistic	HI-A389-4 Soft Tissues		HI-A389-4 Liver		HI-A389-4 Stomach Content	
	Near	Far	Near	Far	Near	Far
Average	82.13	49.16	330.95	124.65	76.39	119.72
Variance	21	n.d.	259597	1224	1200	12536
Std Deviation	4.64	n.d.	509.51	34.99	34.64	111.96
Coef of Var (%)	5.6%	n.d.	154.0%	28.1%	45.3%	93.5%
Minimum	76.34	49.16	69.90	72.59	34.00	33.39
Maximum	87.69	49.16	1919.62	184.04	157.00	521.81
Count	3	1	11	8	12	17

n.d.-not determined

Table 4.24. Summary of the statistical analysis of total PAH concentrations (ppm) in tissues from Cruise 2.

Statistic	MU-A85-1						MAI-686-2					
	Soft Tissues		Liver		Stomach Content		Soft Tissues		Liver		Stomach Content	
	Near	Far	Near	Far	Near	Far	Near	Far	Near	Far	Near	Far
Average	85.99	83.03	400.91	215.06	83.57	70.44	61.70	56.64	275.37	344.38	104.14	95.04
Variance	3015.8	1274.5	166632.8	5100.6	1715.6	373.8	1659.1	292.5	4866.3	6006.5	2362.7	2501.2
Std Deviation	54.92	35.70	408.21	71.42	41.42	19.33	40.73	17.10	69.76	77.50	48.61	50.01
Coef of Var (%)	63.9%	43.0%	101.8%	33.2%	49.6%	27.4%	66.0%	30.2%	25.3%	22.5%	46.7%	52.6%
Minimum	42.17	39.50	119.00	121.45	34.82	46.57	31.03	36.88	161.53	216.84	42.64	44.34
Maximum	214.86	148.66	1476.50	384.53	170.66	95.12	178.62	89.36	406.40	423.51	234.16	206.48
Count	9	13	10	9	10	9	12	12	11	7	11	7

Statistic	HI-A389-4					
	Soft Tissues		Liver		Stomach Content	
	Near	Far	Near	Far	Near	Far
Average	147.07	171.67	306.02	210.74	92.66	83.21
Variance	6692.7	11214.3	20147.3	15082.3	1120.0	3017.9
Std Deviation	81.81	105.90	141.94	122.81	33.47	54.94
Coef of Var (%)	55.6%	61.7%	46.4%	58.3%	36.1%	66.0%
Minimum	65.56	84.18	96.02	94.79	51.37	0.00
Maximum	332.59	349.19	533.39	493.54	183.33	179.48
Count	12	4	14	10	14	10

Table 4.25. Species analyzed for contaminants from Cruises 1 and 2.

Cruise	Site	Taxonomic Name	Common Name
1	HI-A389	*Pistipomoides aquilonaris*	wenchman
1	HI-A389	*Bratula barbata*	bearded brotula
1	HI-A389	*Caulolatilus intermedius*	anchor tile fish
1	HI-A389	*Centropristis philadelphica*	rock sea bass
1	HI-A389	*Maldanidae*	spider crab
1	HI-A389	*Neomerinthe hemingwayi*	spiney cheek scorpion fish
1	HI-A389	*Ogcacephalus declivirostris*	slantbrow batfish
1	HI-A389	*Paralicthys lethostigma*	southern flounder
1	HI-A389	*Penaeus aztecus*	brown shrimp
1	HI-A389	*Pogonias cromis*	black drum
1	HI-A389	*Pontinus longispinis*	longspine scorpion fish
1	HI-A389	*Pontius longispinis*	wenchman
1	HI-A389	*Pristipomoides aquilonarius*	wenchman
1	HI-A389	*Pristipomoides aquilonarius*	squirrel fish
1	HI-A389	*U. floridana, Urophycis cirrata*	southern/gulf hake
1	HI-A389	*Urophycis cirrata*	gulf hake
1	HI-A389	*Urophycis cirrata*	gulf hake
1	HI-A389	*Urophycis cirrata, U. floridana*	southern/gulf hake
1	HI-A389	*Urophycis floridana*	southern hake
1	MAI-686	*Archosargus probatocephalus*	sheepshead
1	MAI-686	*Astropectin duplicatus*	starfish
1	MAI-686	*Busycon sp.*	whelk
1	MAI-686	*Callenectes similis*	crab
1	MAI-686	*Lutjanus campechanus*	red snapper
1	MAI-686	*Paralicthys albigutta*	gulf flounder
1	MAI-686	*Paralicthys lethostigma*	southern flounder
1	MAI-686	*Paralicthys lithostigma*	gulf flounder
1	MAI-686	*Penaeus aztecus*	brown shrimp
1	MAI-686	*Penaeus setiferus*	white shrimp
1	MAI-686	*Squilla empusa*	mantis shrimp
1	MAI-686	*Syacium gunteri*	shoal flounder
1	MAI-686	*Syacium gunteri*	shoal flounder
1	MAI-686	*Sycionia dorsalis*	rock shrimp
1	MAI-686	*Trachypenaeus similis*	shrimp
1	MU-A85	*Aequipecten sp.*	scallops
1	MU-A85	*Astropecten cingulatus*	starfish
1	MU-A85	*Brotula barbata*	bearded brotula
1	MU-A85	*Caulolatilus intermedius*	tile
1	MU-A85	*Centropristis philadephica*	rock sea bass
1	MU-A85	*Cyclopsetta chittenderi*	mexican flounder
1	MU-A85	*Cyclopsetta fimbriata*	cyclops flounder
1	MU-A85	*Epinephalus flovolimbatus*	grouper
1	MU-A85	*Equetus umbrosus*	striped drum
1	MU-A85	*Lutjanus campechanus*	red snapper
1	MU-A85	*murex fulvescens*	whelk
1	MU-A85	*Pagurus sp.*	hermit crab
1	MU-A85	*Paralicthys lethostigma*	southern flounder
1	MU-A85	*Pareques umbrosus*	striped drum
1	MU-A85	*Penaeus aztecus*	brown shrimp
1	MU-A85	*Pilumus sp.*	crab

Table 4.25. Cont.

Cruise	Site	Taxonomic Name	Common Name
1	MU-A85	*Pogonias cromis*	black drum
1	MU-A85	*polychaete*	bristle worm
1	MU-A85	*Portunus spinicarpus*	portunid crab
1	MU-A85	*Raninoides louisianensis*	crab
1	MU-A85	*Squilla empusa*	mantis shrimp
1	MU-A85	*Synodus foetens*	inshore lizard fish
1	MU-A85	*Terebra dislocata*	white turid
1	MU-A85	*Trichopsetta ventralis*	flounder
1	MU-A85	*Urophycis cirrata*	gulf hake
1	MU-A85	*Vesicomyid*	clam
2	HI-A389	*Acanthocarpus*	acanthocarpus
2	HI-A389	*Ancylopsetta dilecta*	three spotted flounder
2	HI-A389	*Epinephelus itajara*	jewfish
2	HI-A389	*Ogcocephalus declivirostris*	batfish
2	HI-A389	*Paguris*	paguris
2	HI-A389	*Paralicthys lethostigma*	so flounder
2	HI-A389	*Pontinus longispinis*	scorpion fish
2	HI-A389	*Portunus spinicarpus*	p spinicarpus
2	HI-A389	*Pristipomoides aquilonarius*	wenchman
2	HI-A389	*Squilla empusa*	mantis shrimp
2	HI-A389	*Synodus foetens*	lizard fish
2	HI-A389	*Trachypenaeus similis "A"*	shrimp "a"
2	HI-A389	*Trachypenaeus similis "B"*	shrimp "b"
2	HI-A389	*Trichopsetta ventralis*	sash flounder
2	HI-A389	*Urophycis sp.*	hake
2	MAI-686	*Archosargus probatocephalus*	sheepshead
2	MAI-686	*Callenectes similis*	blue crab
2	MAI-686	*Cynoscion arenarius*	sand seatrout
2	MAI-686	*Leopard Crab*	leopard crab
2	MAI-686	*Lutjanus campechanus*	red snapper
2	MAI-686	*Opsanus pardus*	toad fish
2	MAI-686	*Paralicthys lethostigma*	southern flounder
2	MAI-686	*Penaeus aztecus*	brown shrimp
2	MAI-686	*Rypticus maculatus*	white spotted soapfish
2	MAI-686	*Squilla empusa*	mantis shrimp
2	MAI-686	*Striped Snapper*	striped snapper
2	MAI-686	*Syacium gunteri*	syacium
2	MAI-686	*Synodus foetens*	lizard fish
2	MAI-686	*Trachpenaeus similis*	trachypenais
2	MU-A85	*Aequipectin sp.*	scallop
2	MU-A85	*Ancylopsetta dilecta*	three spotted flounder
2	MU-A85	*Caulolatilus intermedius*	tile fish
2	MU-A85	*Centropristis philadelphica*	philly fish
2	MU-A85	*Cyclopsetta chittenderi*	mexican flounder
2	MU-A85	*Lagodon rhomboides*	pinfish
2	MU-A85	*Lutjanus campechanus*	snapper
2	MU-A85	*Paralicthys lethostigma*	flounder
2	MU-A85	*Penaeus aztecus*	brown shrimp
2	MU-A85	*Pogonia cromis*	drum
2	MU-A85	*Portunus spinicarpus*	crab
2	MU-A85	*Rypticus maculatus*	white spotted soapfish

Table 4.25. Cont.

Cruise	Site	Taxonomic Name	Common Name
2	MU-A85	*Synodus foetens*	lizard fish
2	MU-A85	*Terebra dislocata*	white turid
2	MU-A85	*Trachpenaeus similis*	trachypenais
2	MU-A85	*Trachypenaeus similis "A"*	shrimp "a"
2	MU-A85	*Trichopsetta ventralis*	sash flounder
2	MU-A85	*Urophycis sp.*	hake

Table 4.26. ANOVA analysis of variance of PAH concentrations in fish liver tissues.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85			
Cruise: (A)	1	415.252	415.252	6.032	0.0195
Station: (B)	1	50.699	50.699	0.736	0.3970
A•B	1	86.685	86.685	1.259	0.2699
Error	33	2271.869	68.845		
		MAI-686			
		No Far Station Samples for Cruise 2			
		HI-A389			
Cruise: (A)	1	13.084	13.084	3.478	0.0697
Station: (B)	1	33.955	33.955	9.025	**0.0046**
A•B	1	11.579	11.579	3.077	0.0872
Error	39	146.738	3.763		

Table 4.27. ANOVA analysis of variance of PAH concentrations in fish stomach contents.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85			
Cruise: (A)	1	905.211	905.211	28.234	**0.0001**
Station: (B)	1	153.331	153.331	4.783	0.0344
A•B	1	127.354	127.354	3.972	0.0528
Error	42	1346.553	32.061		
		MAI-686			
Cruise: (A)	1	89.946	89.946	11.025	**0.0022**
Station: (B)	1	0.610	0.610	0.075	0.7863
A•B	1	1.251	1.251	0.153	0.6979
Error	33	269.237	8.159		
		HI-A389			
Cruise: (A)	1	23.946	23.946	26.333	**0.0001**
Station: (B)	1	0.481	0.481	0.529	0.4706
A•B	1	1.927	1.927	2.120	0.1518
Error	49	44.558	0.909		

Table 4.28. ANOVA analysis of variance of PAH concentrations in invertebrate soft tissues.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
		MU-A85			
Cruise: (A)	1	4321.921	4321.921	2.968	0.0907
Station: (B)	1	2005.864	2005.864	1.377	0.2457
A•B	1	2028.003	2028.003	1.393	0.2431
Error	54	78636.672	1456.235		
		MAI-686			
Cruise: (A)	1	14.575	14.575	7.402	**0.0092**
Station: (B)	1	0.641	0.641	0.326	0.5711
A•B	1	12.096	12.096	6.143	0.0170
Error	45	88.607	1.969		
		HI-A389			
Cruise: (A)	1	0.048	0.048	0.129	0.7238
Station: (B)	1	0.974	0.974	2.605	0.1261
A•B	1	1.350	1.350	3.612	0.0755
Error	16	5.980	0.374		

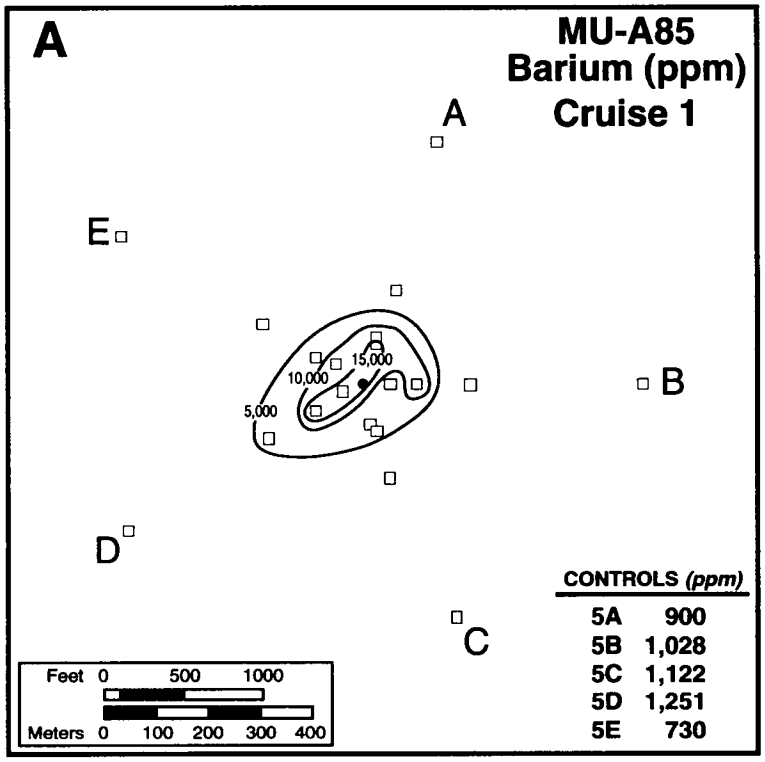
also being determined to provide potential correlates with any sub-lethal effects observed.

Barium is the dominant elemental tracer associated with discharges from rigs during drilling operations. Up to 90% of the dry weight components of drilling mud is barite (barium sulfate). Several elements including Cd, Hg, and Pb can be associated with the ore used to produce barite. Iron, a major component of the bentonite clays used in drilling mud, is a supporting elemental tracer which can be used to determine the fate of the particulate (versus dissolved) fraction of the drilling mud discharges. Once drilling operations have ceased, the discharge of particulate trace metals should become small and specific elemental tracers (e.g. Zn from sacrificial anodes, etc.) for this phase are uncertain.

This work element involves the determination of total trace element concentrations in sediments, biological tissues, and pore waters from each study site. Sixteen elements (Al, Ag, As, Ba, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sb, Se, Sn, V, Zn) were analyzed in sediments. These include elements which help characterize sediment types in the study areas (Al, Fe), those which are related to drilling mud discharges (Ba, Cr, Fe) and other metals which may be released during drilling and other operations and which have the potential for biological effects (Ag, As, Cd, Cu, Hg, Ni, Pb, Sb, Se, Sn, V, Zn). Fourteen elements were analyzed in biological samples (except Al, Sb) and seven in pore water (Ba, Cd, Cu, Hg, Pb, V, Zn).

4.5.2.1 Sediments

The preliminary interpretation of the sediment trace element data was based on correlations with barium (Ba). Barium is the major elemental tracer in drilling muds and all previous studies have shown strong trends of increasing sediment Ba concentrations with decreasing distance from the drilling site. Sediment Ba concentrations versus distance from the rig site are presented in Figures 4.21-4.23. Because of the exponential sampling distances used (30-50, 100, 200, 500, 3000 m) non-parametric statistical tests (Kruskal-Wallis k-sample test and Spearmann rank correlation) were used to evaluate elemental relationships. Preliminary results of these statistical tests on the Cruise 1 sediment data set are summarized in Table 4.29. Two of the three study sites (High Island Block A389 and Mustang



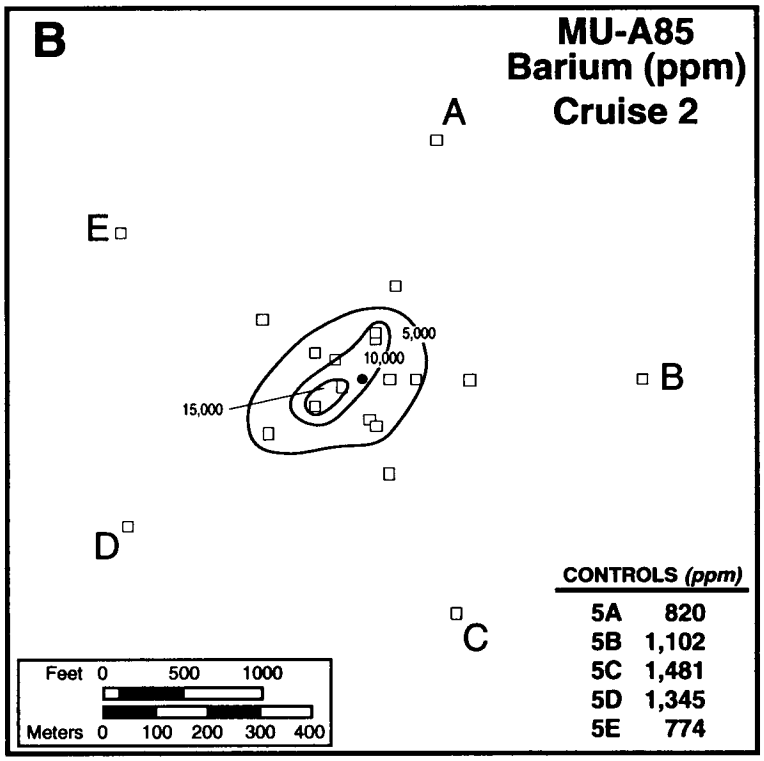


Figure 4.21. Areal distribution of barium (ppm) at MU-A85 for A: Cruise 1; and B: Cruise 2.

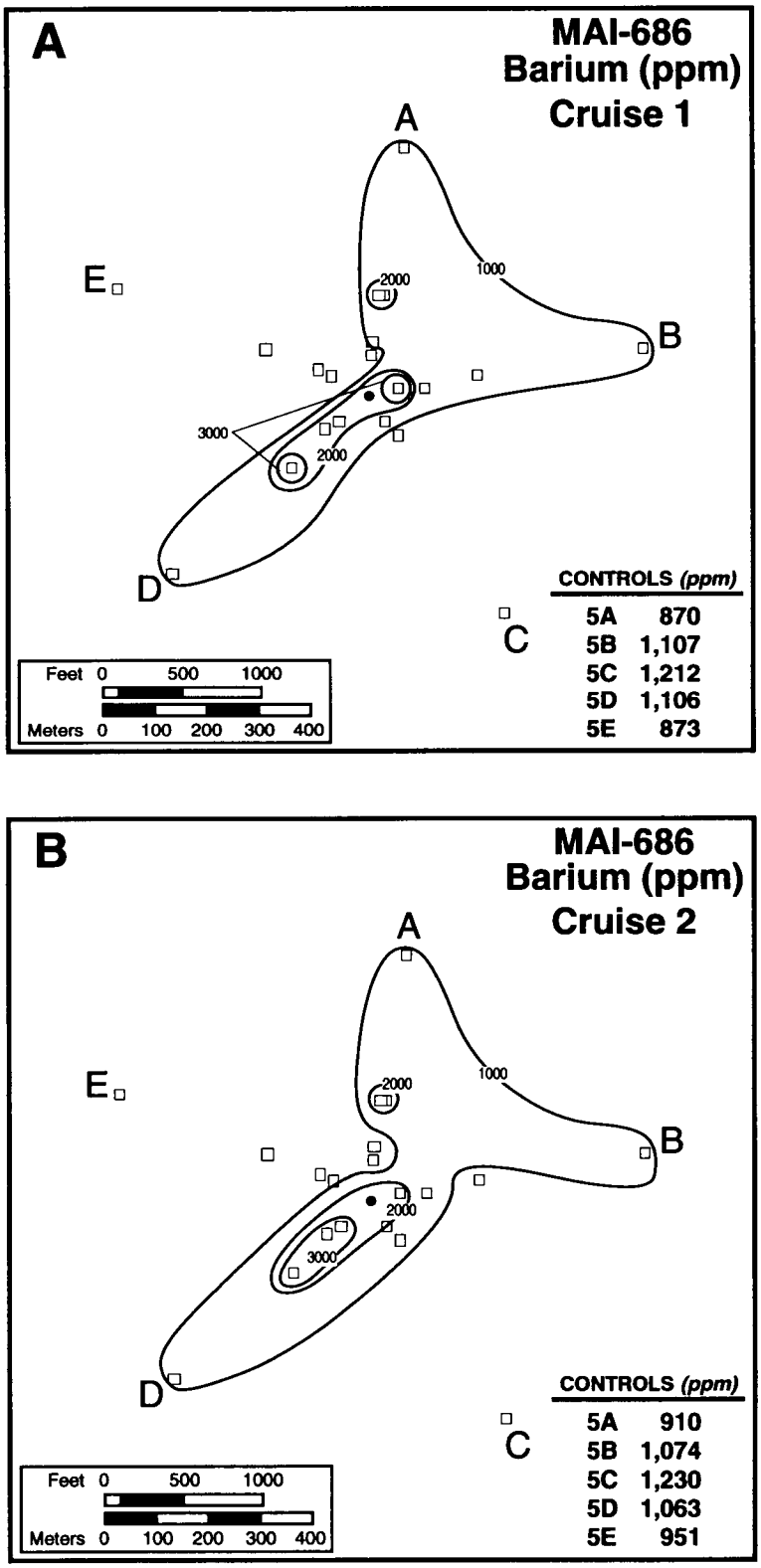
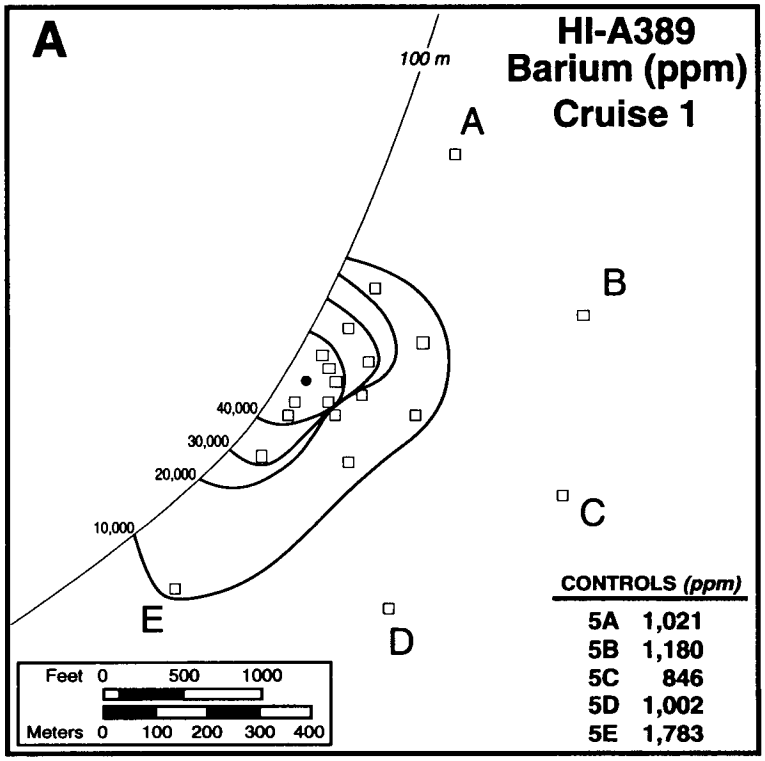


Figure 4.22. Areal distribution of barium (ppm) at MAI-686 for A: Cruise 1; and B: Cruise 2.



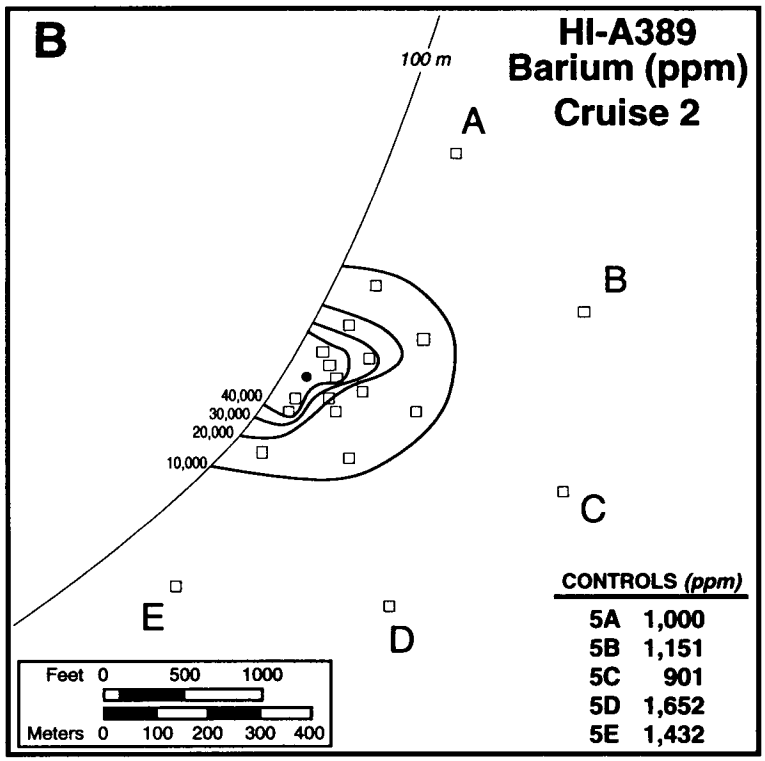


Figure 4.23. Areal distribution of barium (ppm) at HI-A389 for A: Cruise 1; and B: Cruise 2.

Table 4.29. Summary of the statistical analysis of sediment trace element data from Cruise 1.

Site	Significant ($p < 0.01$) Negative Correlation with Distance from Site	Significant Positive Correlation with Barium
MU-A85-1	Ba, Ag, Cd, Hg, Pb, Sb, Zn	Ag, Cd, Hg, Pb, Sb, Zn
MAI-686-2		Cd, Hg Pb, Zn
HI-A389-4	Ba, Ag, Cd, Cu, Hg, Pb, Sb, Zn	Ag, Cd, Cu, Hg, Pb, Sb, Zn

Island Block A85) showed highly significant negative correlations between Ba and distance (Table 4.29). The Ba versus distance trend at Matagorda Island 686 was apparent for both cruises (Figure 4.23) but not significant however the Ba concentrations were lower and similar at the inner three sampling distances (30-50, 100, 200 m). At all sites the Ba values at the 3000 m control stations were much higher than the typical 500 ppm background level observed in Texas-Louisiana Shelf sediments. This situation is most likely due to Ba input from other drilling sites in the vicinity of the 3000 m stations. At both HI-A389 and MU-A85, several metals (see Table 4.29) showed similar negative correlations with distance as well as significant correlations with sediment Ba. For most of the metals (including Hg see Figure 4.24), the correlation with Ba was so strong that it suggests the metals were constituents of the barite itself. However, the greater scatter for Pb (see Figure 4.25) and Zn suggest an independent, rig-related source was also important. Pb and Zn have been detected in produced waters and are thought to be derived from the corrosion of galvanized structures on the platform or the oil-water separator system (Neff et al. 1981). The HI-A389 site clearly has the strongest spatial gradients and should be the location most likely to exhibit trace element related effects in the local biota.

An ANOVA analysis of Ba concentrations confirms the highly significant (P=0.01) interactions with distance and direction as for the organic contaminants (Table 4.30). Barium concentrations were similar between cruises as reflected in the less significant interactions with cruise (time).

4.5.2.2 Tissues

Trace element tissue samples included fish livers (56 samples), soft tissues from shrimp (54), crabs (17), molluscs (18), echinoderms (starfish, 8), polychaetes (5), and fish stomach contents (90). A listing of species analyzed for trace metals from Cruises 1 and 2 is summarized in Table 4.25. Interpretation of the tissue data is based on near versus far-field comparisons. Preliminary comparisons were made for those tissue groups with sufficient numbers. This comparison involves combining several different species into a single group which may confound the analysis. The comparisons of the near and far field means are summarized in Table 4.31.

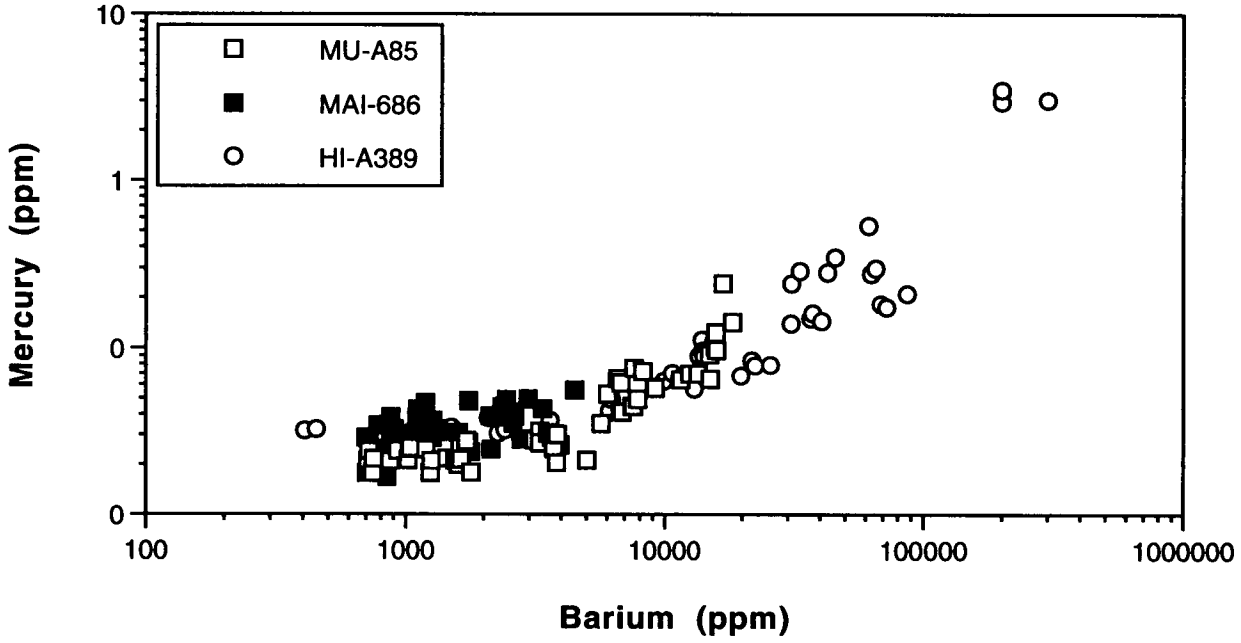


Figure 4.24. Relationship between Hg and Ba concentrations in sediments at the sites.

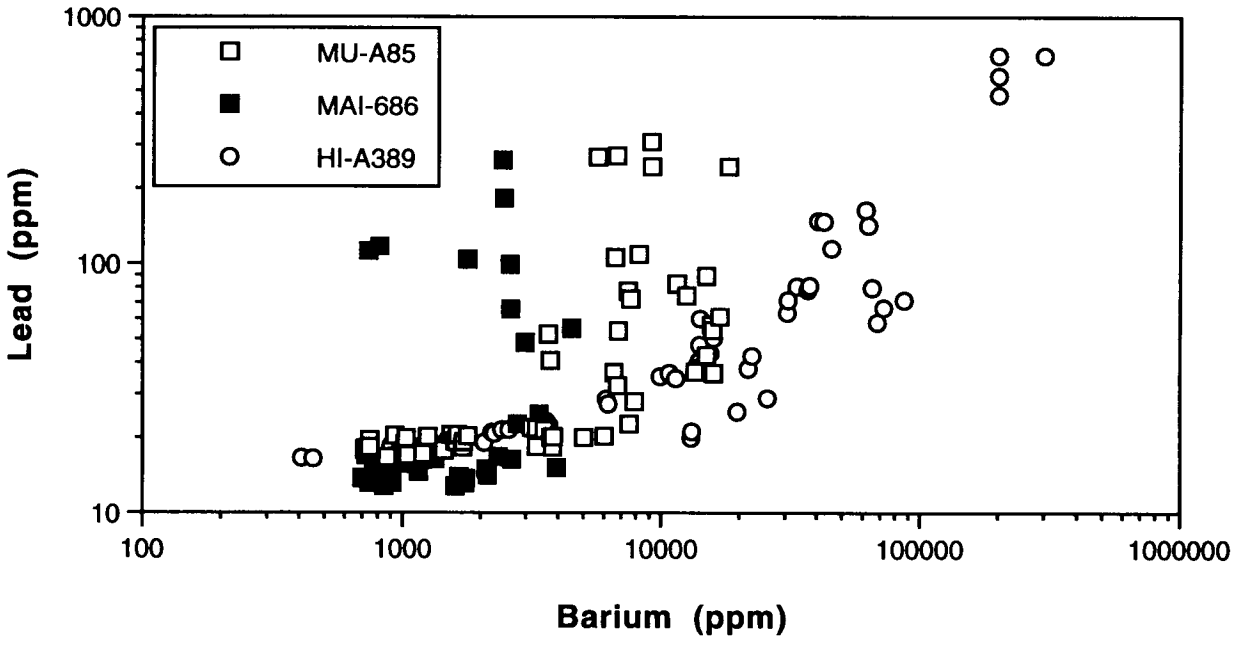


Figure 4.25. Relationship between Pb and Ba concentrations in sediments at the sites.

Table 4.30. ANOVA analysis of sediment Ba concentrations (ppm).

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-test	P value
MU-A85					
Cruise (A)	1	1211902	1211902	0.609	0.4386
Distance (B)	4	2010100000	502530000	252.455	**0.0001**
A•B	4	2323871	580968	0.292	0.8820
Radii (C)	4	352210000	88053003	44.236	**0.0001**
A•C	4	4993933	1248483	0.627	0.6452
B•C	16	357780000	22361160	11.234	**0.0001**
A•B•C	16	25917239	1619827	0.814	0.6646
Error	54	107489820	1990552		
MAI-686					
Cruise (A)	1	100833	100833	2.476	0.1214
Distance (B)	4	16647858	4161964	102.211	**0.0001**
A•B	4	117573	29393	0.722	0.5808
Radii (C)	4	28293278	7073320	173.709	**0.0001**
A•C	4	1606353	401588	9.862	**0.0001**
B•C	16	34607386	2162962	53.119	**0.0001**
A•B•C	16	5783955	361497	8.878	**0.0001**
Error	54	2198850	40719		
HI-A389					
Cruise (A)	1	804430000	804430000	7.055	0.0105
Distance (B)	4	176930000000	44233000000	387.930	**0.0001**
A•B	4	994350000	248586668	2.180	0.0841
Radii (C)	4	33257000000	8314300000	72.917	**0.0001**
A•C	4	1155100000	288790000	2.533	0.0512
B•C	16	117280000000	7329800000	64.283	**0.0001**
A•B•C	16	5703000000	356430000	3.126	**0.0010**
Error	52	5929238575	114023819		

Table 4.31. Comparison of near- and far-field data for GOOMEX Cruise 1 tissue trace element concentrations.

Sample Description		Sample No.	Ag (ppm)	As (ppm)	Ba (ppm)	Cd (ppm)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Hg (ppm)	Ni (ppm)	Pb (ppm)	Se (ppm)	Sn (ppm)	V (ppm)	Zn (ppm)
Fish Livers																
HI-A389-4	Mean Near Field	11	0.15	110.83	0.96	2.08	0.19	15.85	537.42	1.14	0.20	0.13	9.42	1.95	0.24	177.08
	Std. Dev. Near Field		0.11	113.81	0.97	3.52	0.21	7.70	363.88	1.21	0.14	0.08	5.97	3.58	0.28	158.73
	Mean Far Field	8	0.24	124.85	1.30	5.82	0.40	20.59	977.97	1.66	0.53	0.24	20.69	0.39	0.60	112.67
	Std. Dev. Far Field		0.20	119.01	2.43	6.30	0.59	12.43	559.64	1.78	0.31	0.25	10.28	0.43	0.69	40.48
MU-A85-1	Mean Near Field	12	0.05	11.30	0.21	2.04	0.12	20.57	498.76	0.46	0.18	0.11	6.74	0.26	0.43	76.62
	Std. Dev. Near Field		0.08	8.75	0.14	1.36	0.06	43.50	726.15	0.22	0.16	0.05	2.27	0.28	0.28	82.78
	Mean Far Field	4	0.30	8.74	0.09	1.11	0.16	44.19	129.67	0.54	0.02	0.11	3.71	0.28	0.00	79.65
	Std. Dev. Far Field		0.37	3.37	0.03	0.58	0.18	41.32	119.76	0.32	0.03	0.04	1.07	0.12	0.00	24.22
Shrimp Soft Tissue																
MAI-686-2	Mean Near Field	7	0.13	14.84	1.19	0.27	0.17	31.73	45.65	0.13	0.29	0.10	2.40	0.05	0.38	55.92
	Std. Dev. Near Field		0.31	5.37	0.62	0.60	0.08	24.52	19.97	0.11	0.25	0.05	0.65	0.04	0.69	14.30
	Mean Far Field	11	0.15	16.92	4.93	0.30	0.33	34.76	115.70	0.16	0.46	0.19	2.15	0.27	0.65	55.57
	Std. Dev. Far Field		0.27	7.63	5.41	0.60	0.29	22.50	121.62	0.26	0.31	0.14	0.71	0.60	0.78	9.72
MU-A85-1	Mean Near Field	4	0.59	33.98	2.41	1.83	0.07	20.86	6.42	0.44	0.27	0.02	0.45	0.00	0.35	13.75
	Std. Dev. Near Field		0.31	94.63	2.57	1.00	0.18	28.29	22.44	0.56	0.34	0.07	2.37	0.00	0.18	58.93
	Mean Far Field	5	0.61	62.83	2.29	1.00	0.30	39.12	67.34	0.45	0.34	0.26	2.98	0.00	0.70	63.41
	Std. Dev. Far Field		1.32	20.95	2.63	2.14	0.17	35.45	67.79	0.35	0.29	0.37	1.99	0.01	0.56	29.42
Fish Stomach Contents																
HI-A389-4	Mean Near Field	12	0.21	17.79	140.60	1.29	1.85	8.54	2170.78	0.48	0.50	9.93	5.32	0.03	1.44	85.55
	Std. Dev. Near Field		0.42	15.87	269.64	2.03	4.62	6.02	6733.26	0.33	1.07	32.53	2.33	0.04	2.66	37.74
	Mean Far Field	17	0.09	28.41	10.15	0.66	1.26	6.66	527.24	0.41	1.43	0.35	6.95	0.27	0.70	84.99
	Std. Dev. Far Field		0.10	24.93	12.07	0.61	0.88	4.42	363.52	0.19	1.58	0.31	2.83	0.62	0.88	23.06
MAI-686-2	Mean Near Field	15	0.08	6.12	58.65	0.31	1.40	11.72	718.59	0.19	0.66	0.56	4.17	0.10	1.91	82.23
	Std. Dev. Near Field		0.05	3.54	131.81	0.26	1.46	4.76	740.89	0.11	0.69	0.52	1.58	0.05	2.30	43.34
	Mean Far Field	4	0.10	9.40	111.74	0.28	5.23	22.28	2706.31	0.12	2.35	2.22	3.03	0.18	7.90	82.92
	Std. Dev. Far Field		0.05	1.11	48.71	0.05	2.17	12.27	1188.85	0.01	0.90	0.54	0.54	0.09	3.48	10.30
MU-A85-1	Mean Near Field	17	0.02	7.61	63.80	1.16	0.95	6.31	428.02	0.26	0.43	0.42	8.23	0.19	1.30	70.87
	Std. Dev. Near Field		0.02	6.68	139.92	1.43	1.28	4.10	555.78	0.11	0.48	0.45	10.72	0.20	2.54	31.63
	Mean Far Field	9	0.04	8.31	9.72	5.37	1.09	9.65	479.93	0.32	0.38	0.37	9.08	0.15	1.36	94.12
	Std. Dev. Far Field		0.05	5.68	14.12	7.59	1.30	7.50	605.25	0.16	0.45	0.39	7.88	0.10	1.69	46.26

The between fields means for most elements are similar. Where differences occur, they are not significant because of the considerable variability in the data. The stomach contents data do show possibly significant differences in Ba and Fe levels reflecting the ingestion of bottom sediments during feeding. More comparisons are needed using smaller comparison groups composed of the same or similar species. These tests may reveal additional significant differences in trace element levels between fields as the database increases.

4.5.3 Comparative Evaluation of Contaminant Levels

At this preliminary stage it is of interest to compare the contaminants measured in sediments with other Gulf Coast studies and with sediment levels known to elicit biological effects. While biological effects are being directly measured, comparison with literature values facilitates an early assessment of potential for effect or injury.

Total PAH concentrations were generally less than 1,000 ppb with only a few exceptions closest to the platform at HI-A389 (Figure 4.26). The range in values are comparable to nearly 90% of the PAH values measured in coastal bay sediments of the Gulf Coast during the Environmental Protection Agency's Environmental Monitoring and Assessment Program-Near Coastal (Summers et al. 1993). EMAP-NC represents 183 sites along the Gulf Coast collected on a probability based sampling design. The PAH values observed are also significantly below the Long and Morgan (1990) 10% biological effects criteria. These criteria are based on a compilation of chemical concentrations observed or predicted to be associated with biological effects (broadly defined). Note that the definition of total PAH is highly variable and these values only serve as a semi-quantitative guide. The 10% criteria is defined as the level at which biological effects were observed 10% of time. Long and Morgan (1990) also note that compounds such as PAH, which may be mutagenic or teratogenic, may not be toxic in acute tests of mortality.

In contrast, some trace metal concentrations in sediments far exceeded EPA-EMAP-NC values detected in the coastal environments of the Gulf Coast (Figures 4.27). In comparison with the 10% and 50% biological effects criteria of Long and Morgan (1990) a significant percent of sediments at the study sites would be predicted to elicit biological responses (Figures 4.28 and 4.29). While these comparisons are informative

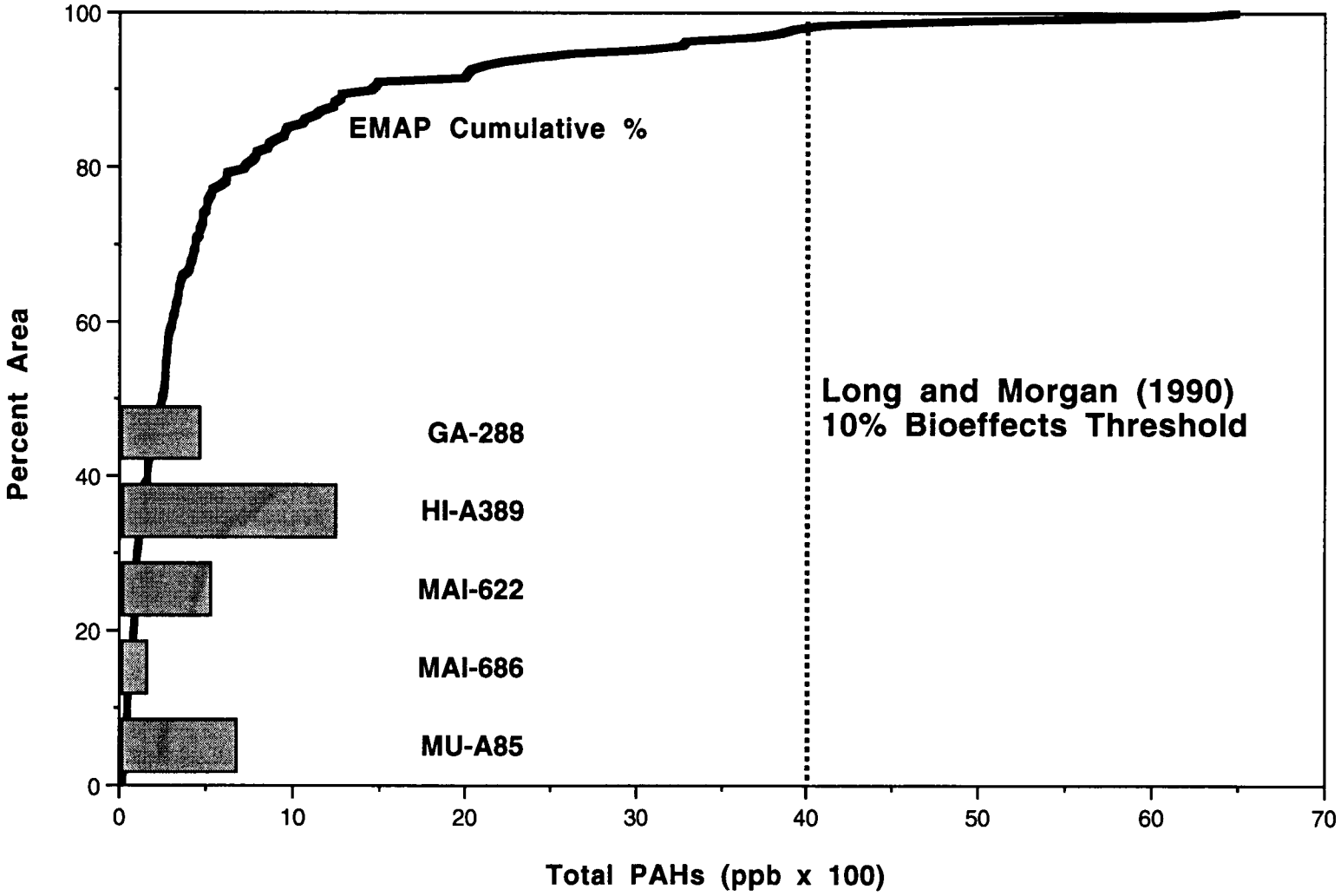


Figure 4.26. Comparison of the ranges of sediment PAH concentrations from GOOMEX study sites, cumulative percentage for EMAP-NC PAH data, and Long and Morgan (1990) 10% bioeffects threshold.

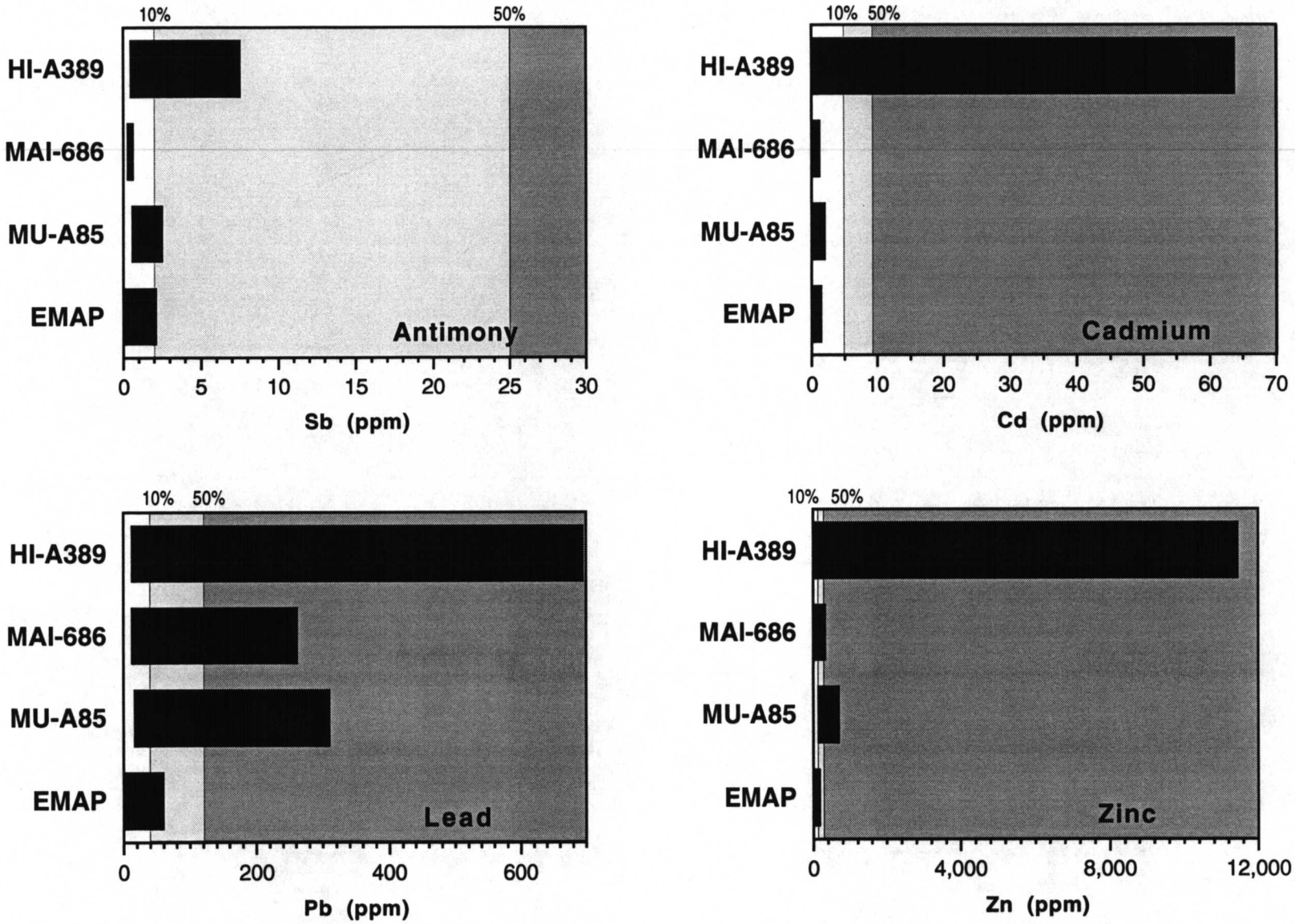


Figure 4.27. Comparison of the ranges of selected trace metal concentrations in sediments from the GOOMEX study sites, EMAP-NC data, and Long and Morgan (1990) 10% and 50% biological effects threshold.

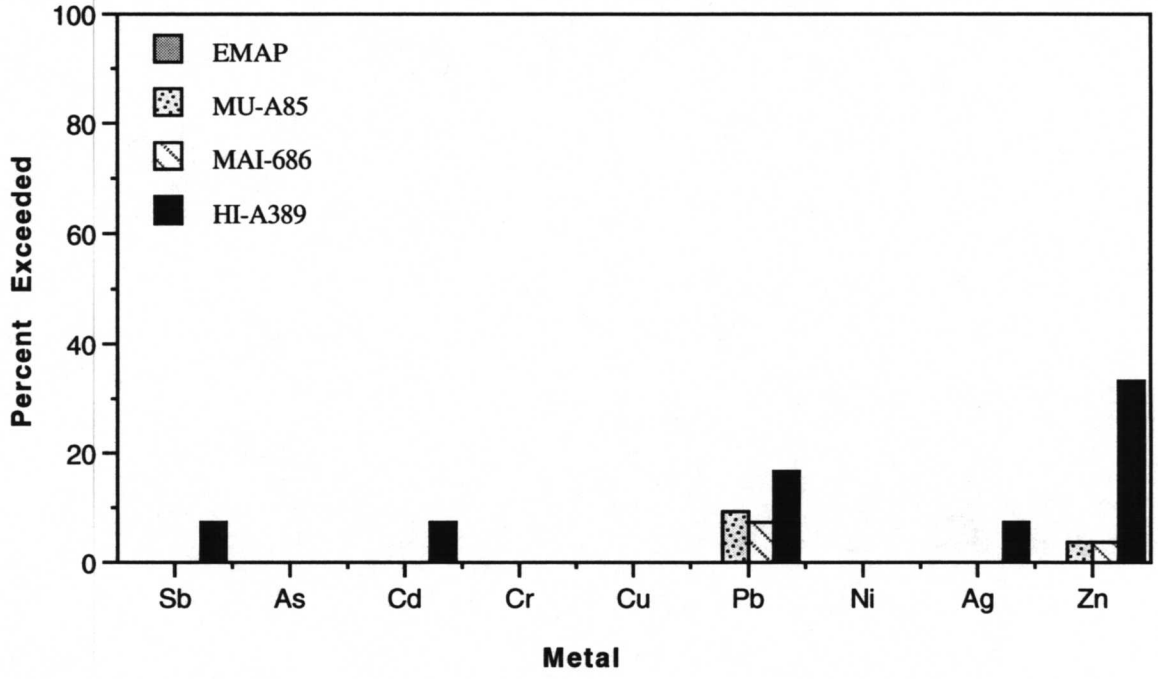


Figure 4.28. The percent of samples from the GOOMEX sites that exceeded the Long and Morgan (1990) 50% bioeffects criteria.

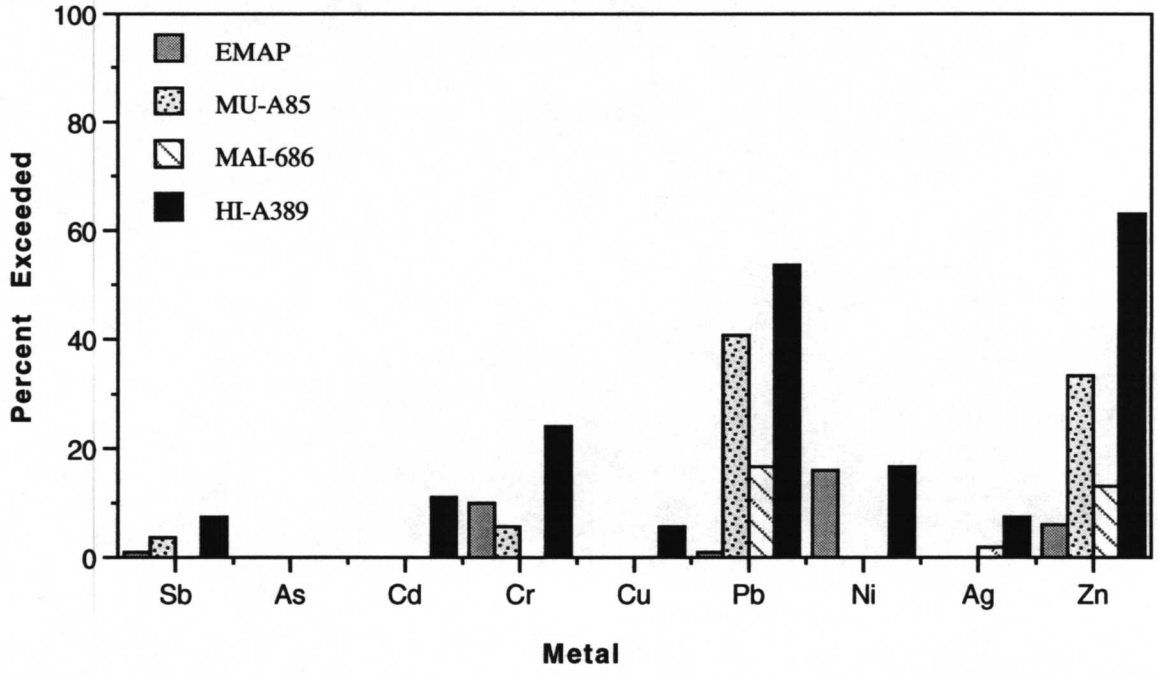


Figure 4.29. The percent of samples from the GOOMEX sites that exceeded the Long and Morgan (1990) 10% bioeffects criteria.

they are not definitive in defining a biological effect. Sediment metals may or may not be bioavailable and a more detailed evaluation of redox potential and metal speciation will be needed to determine a causual link between sediment metal concentrations and observed biological effects.

4.5.4 Abiotic Indicators of Contamination

Selected PAH and trace metals were measured in sediments at each study site. While sums of values may be useful as indicators of exposure, a statistical approach was taken to define the most accurate indicators of contamination. Principal component analysis (PCA) was performed on the contaminant data to define the principal components that most accurately describe the contaminant field. The hydrocarbons and trace metals were first considered as independent agents and then together in an effort to recognize the synergistic effects of multiple contaminants. Based on these analyses indicators of contamination will be defined to interpret concurrently measured biological effects parameters.

Initial analysis of the contaminant data has revealed several important trends. The first observation is that contaminant fields are unique at each site. This suggests that several processes are contributing to contaminant distributions and these processes are of variable importance at different sites. This also suggests that a platform by platform evaluation is most effective in understanding trends in biological effects. An analysis of sediment PAH data defines several potential inputs including fresh diesel or condensate material, background PAH from non-platform related processes, and a general biodegraded petroleum material possibly associated with cuttings at the site. The background non-platform related PAH are composed of predominantly high molecular weight material of a presumed pyrogenic origin (four and five ring PAH). These PAH also tend to correlate with suspected terrestrial sourced metals such as Al, V, Ni, Se, and Mn which are associated with the clay/silt fraction of the sediment. The PAH materials most directly related to distance from the platform include two and three ring aromatics (i.e., naphthalenes, phenanthrenes). Again these may represent PAH indigenous to cuttings or sand disposal at the site. Sand positively correlates very strongly with these PAH. However a source in additives cannot be ruled out. It is also observed that the PAH do not

correlate with Ba distributions, however as was pointed out previously a series of metals strongly co-vary with Ba (i.e., Zn, Cd, and Pb). These correlations and covariances will be used to further refine a model of contaminant sources and distributions and ultimately define a set of abiotic indicators of various contaminants at each site.

4.6 Meiofauna Communities

The term "meiobenthos" was first coined by Mare (1942) to describe those benthic organisms that are of intermediate size. These are metazoans that are smaller than macrofauna but larger than the single-celled microbenthos (e.g., bacteria, microalgae, and protozoans). By convention, the definition of meiofauna is those animals that pass through a 0.5 mm sieve but are retained on a 0.063 mm sieve (Coull and Bell 1979). Meiofaunal communities can be further subdivided into two groups. Temporary meiofauna are those juveniles of the macrofauna that will eventually grow into organisms larger than 0.5 mm. Permanent meiofauna are those groups where adults are less than 0.5 mm, e.g., Nematoda, Copepoda, Gastrotricha, Turbellaria, Acarina, Gnathostomulida, Kinoryncha, Tardigrada, Ostracoda, and some Rhyncocoela, Oligochaeta and Polychaeta. In 1971, Thiel suggested that the grouping of benthic organisms according to size differences had little taxonomic or ecological justification, except convenience of sample processing (quoted from Thiel 1975). However, the explosive growth of ecological literature since 1971, has shown that meiofauna are different from macrofauna, and have different roles in marine ecosystems (for reviews see: Coull and Bell 1979; Coull and Palmer 1984). Even where meiofauna share ecological properties with macrofauna, the processes operate on much smaller spatial and temporal scales (Bell 1980). Meiofauna are good for indicating contaminant exposure because they have short generation times, conferring them with the potential for a rapid response. Meiofauna also live and grow in the benthos with no planktonic larvae thus assuring life long exposure to sediment contaminants (Warwick, 1988). Therefore, meiofauna are ideal organisms for studying long-term processes relevant to impacts near platforms.

Most of what is known about meiofauna is from studies of shallow systems. Many studies have also been done in the deep sea, but there have

been few studies of shelf or slope meiofaunal communities (Coull et al. 1982). Studies where meiofauna have been sampled seasonally from shelf sediments are rare. Meiofauna abundances decrease with water depth (Thiel 1978; Coull et al. 1982; Montagna 1991) and average about 800 individuals per 10 cm^2 (Table 4.30). Nematodes are generally the dominant taxa comprising between 50 and 95% of the community, followed by harpacticoid copepods, which will comprise between 20 and 5% of the community (Coull et al. 1977; Coull et al. 1982; Montagna 1991).

4.6.1 Vertical Distribution

Little information was available about the vertical distribution of meiofauna in the Gulf of Mexico OCS. To determine the appropriate sampling depth, OCS sediments near Port Aransas in Matagorda Island Block 746 at a water depth of 18 m were sampled. Seventy-one percent of the fauna were in the top 2 cm and 93% were in the top 4 cm (Table 4.33). Almost all (95%) harpacticoids were in the top one cm of sediment. Since little information gained by sampling below two cm, all samples were taken from the 0-2 cm depth interval. The total density to 4 cm, 3,743 individuals•10 cm^{-2}, is much higher than values reported in the literature (Table 4.32).

Table 4.32. Continental shelf meiofauna densities reported in the literature (depth in meters, meiofaunal density in n•10 cm^{-2} to a sediment depth of 10 cm).

Ocean	Shelf Location	Depth	Density	Author
E Pacific	Santa Maria Basin	90-410	1900	Montagna 1991
E Pacific	Santa Maria Basin	565	97	Montagna 1991
E Pacific	Santa Barbara Channel	18	1750	Montagna et al. 1989
Gulf of Mexico	Louisiana	8-13	1810	Murrell and Fleeger 1989
Gulf of Mexico	Louisiana	355	580	Pequegnat et al. 1990
Gulf of Mexico	E Flower Gardens	72	*98	Powell et al. 1983
Gulf of Mexico	S Texas	10-82	*200	Pequegnat and Sikora 1979
Gulf of Mexico	S Texas	91-134	*50	Pequegnat and Sikora 1979

*Indicates sediment depth of 10 cm.

Table 4.33. Vertical distribution of meiofauna in the Gulf of Mexico (18 m water depth). The average of 3 replicates, density in $n \bullet 10\ cm^{-2}$, and $n \bullet core^{-1}$. (NEMA = Nematoda, HARP = Harpacticoida, NAUP = harpacticoid nauplii, and OTHER = other taxa).

Depth (cm)	Total ($n \bullet 10\ cm^{-2}$)	Total	NEMA	HARP	NAUP	OTHER
		($n \bullet core^{-1}$)				
0-1	2088	592	216	116	80	180
1-2	778	221	186	3	0	32
2-3	476	135	121	1	0	13
3-4	401	114	101	1	0	12
4-5	150	43	38	0	0	5
5-6	69	20	18	1	0	1
6-7	32	9	4	0	0	5
7-8	6	2	1	0	0	1
8-9	6	2	1	0	0	1
9-10	4	1	0	0	0	1
0-10	4010	1139	686	122	80	251

4.6.2 Sampling Analysis

Two assumptions that formed the basis of the sampling protocol were verified during Cruise 1: (1) that sampling the top two cm of sediment was sufficient and (2) that two replicate cores per boxcore were sufficient for the study design. Two stations at each platform, designated "near" and "far", were oversampled to test these assumptions. Cores were split into two sections at 0-2 and 2-4 cm to verify the adequacy of sampling the top two cm of sediment. A second boxcore was taken at a select subset of stations to determine the power of the sampling design. The second boxcore was used to document between boxcore replication.

The majority of organisms were concentrated in the top 2 cm of sediment (Figure 4.30). Eighty-two percent of the meiofauna were in the top 2 cm and 18% in the 2-4 cm section. Little new information is provided by analyzing the 2-4 cm section. Thus, it was decided to only process the top 2 cm of sediment. There were differences in the vertical distribution among taxa. Seventy-eight percent of the nematodes and 98% of the harpacticoids were found in the top 2 cm. An additional reason to restrict

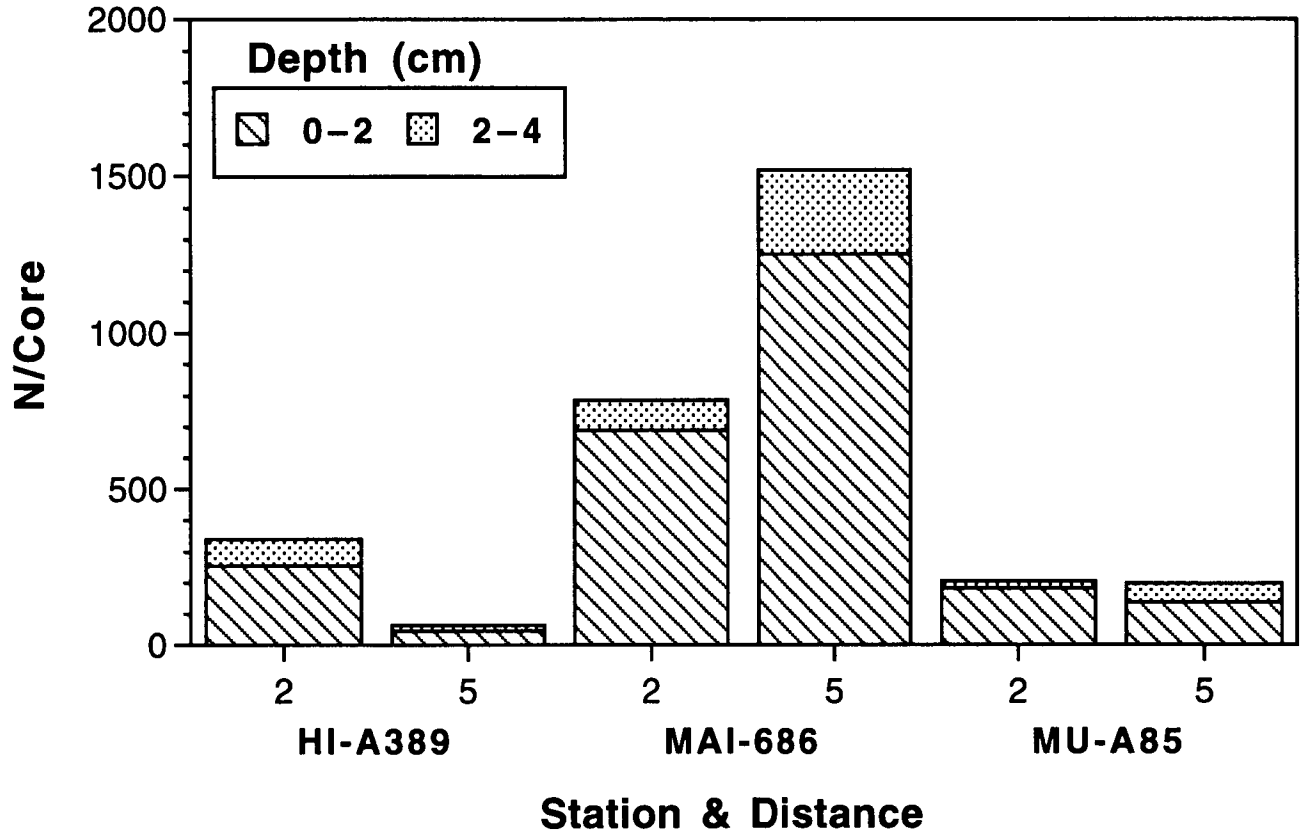


Figure 4.30. Distribution of meiofauna abundance with depth in the sediment.

the analysis to the top 2 cm was that the harpacticoids were the focus of the reproductive studies.

An analysis on this same data set was performed to determine the power of our sampling design. The overall average density was 722 individuals•core^{-1}, and the mean square error was 62,983. The mean population size was converted to 1.00 so that the percentage change of population size could be calculated (Figure 4.31). The change of population density increases with decreasing numbers of samples. At any given number of samples (*n*), certainty about change increases with increasing differences. There is a break in the curve at about 4 samples, meaning more than 4 samples does little to increase resolving power. Most tests might be based on 20 samples (=2 replicates x 5 radii x 2 distances), and we could detect only a 40% change with this design with 0.80 power (=0.20). With just 2 samples, i.e. 2 replicates per boxcore, we can detect a 100% change (which is a doubling) among stations (Figure 4.32).

A preliminary analysis of variance is summarized in Table 4.34. The purpose of this analysis was to determine if using subcores as replicates from one box core was sufficient. Most of the variability in the study is among platforms (54%) and the platform*station interaction (33%). Practically, none of the variability is due to replicate boxcores (0.1%). Therefore, one boxcore per station and replicate subcores was a sufficient sampling protocol.

4.6.3 Community Structure and Abundance

Meiofauna average density at all study sites was 656 individuals•core^{-1} (which is equivalent to 2,320•10 cm^{-2}; Figure 4.33). As noted earlier, these densities are much higher than previously reported (Table 4.32). Overall, nematodes comprised 70% of the organisms, harpacticoids are 14%, and other taxa represented the remaining 15%. There were a total of 11 taxa comprising the other category. None of these taxa occurred in large numbers. There were slight differences in the community structure among the platform sites (Table 4.35).

The community composition significantly varied within platform stations. At HI-A389, nematodes were abundant at the D and E radii (Figure 4.34). At MAI-686 (Figure 4.35) there were increases of harpacticoids and

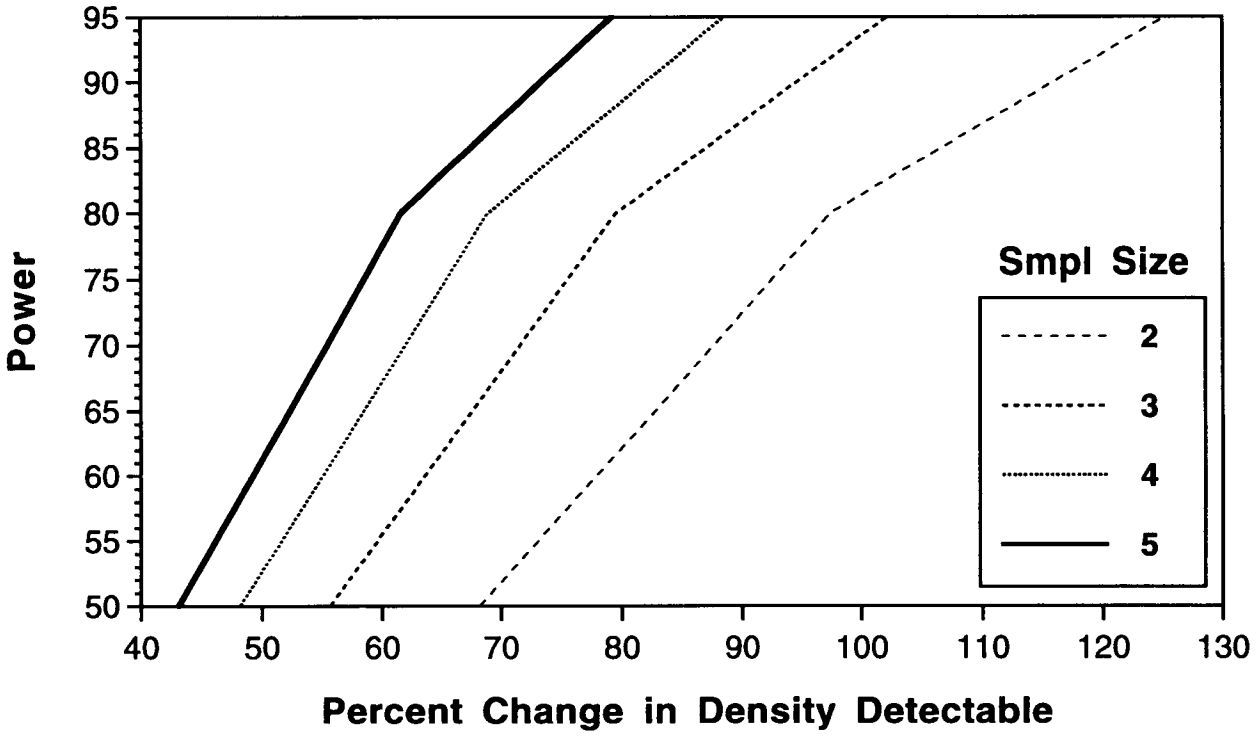


Figure 4.31. The power of the sampling design.

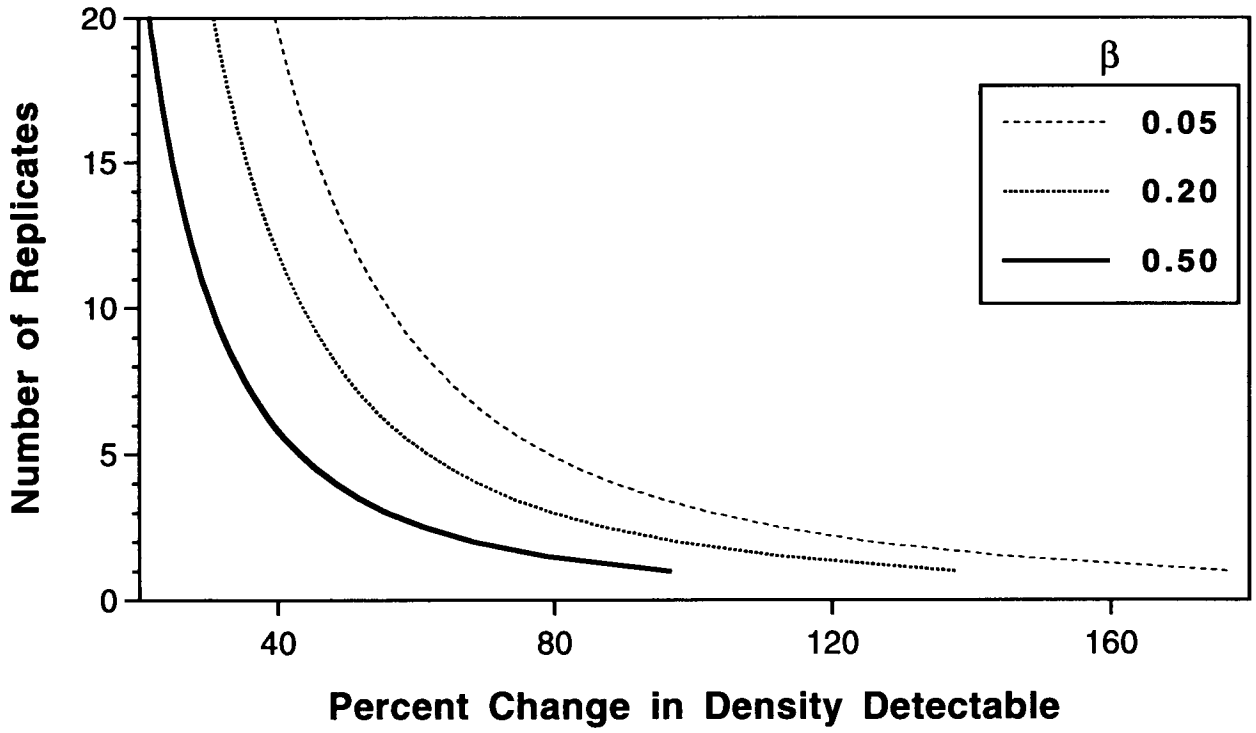


Figure 4.32. Detectable changes in density as a function of the number of replicates.

Table 4.34. Analysis of variance of meiofauna at the near and far stations. There were 5 platforms, 2 stations, 2 boxcores, and 2 replicates for a total of 40 samples. (DF=degrees of freedom, MS=type I mean squares, Estimate=variance component estimate, %=percent of component estimate of total variance).

Variance Component	DF	MS	Estimate	%
Platform	4	1.557	0.1466	53.9
Station	1	0.159	0.0113	4.2
Platform*Station	4	0.385	0.0994	33.2
Boxcore	1	0.030	0.0003	0.1
Error	29	0.023	0.0233	8.6
Total	39		0.2718	100.0

Table 4.35. Meiofauna community structure among platforms. Average n individuals•core^{-1} for all stations around the platform.

Platform	Total	Nematodes		Harpacticoids		Others	
	n	*n*	%	*n*	%	*n*	%
GA-288	794	541	68%	120	15%	133	17%
HI-A389	258	203	79%	24	9%	31	12%
MAI-622	1024	733	72%	133	13%	158	15%
MAI-686	1043	736	71%	157	15%	150	14%
MU-A85	213	122	57%	48	23%	43	20%

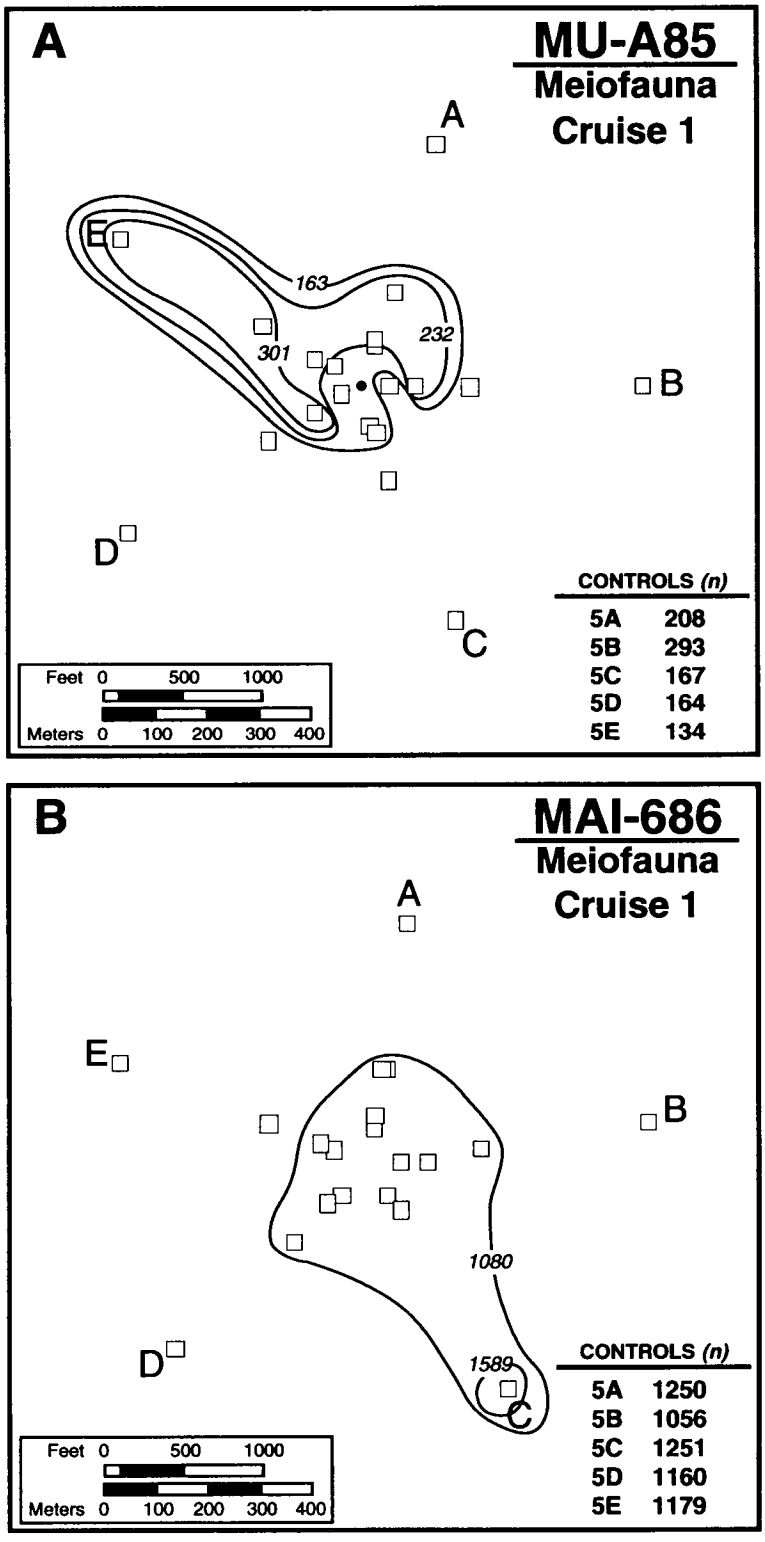


Figure 4.33. Abundance of meiofauna in the 0–2 cm sediment section during Cruise 1 for A: MU-A85; B: MAI-686; and C: HI-A389. (n=number of individuals).

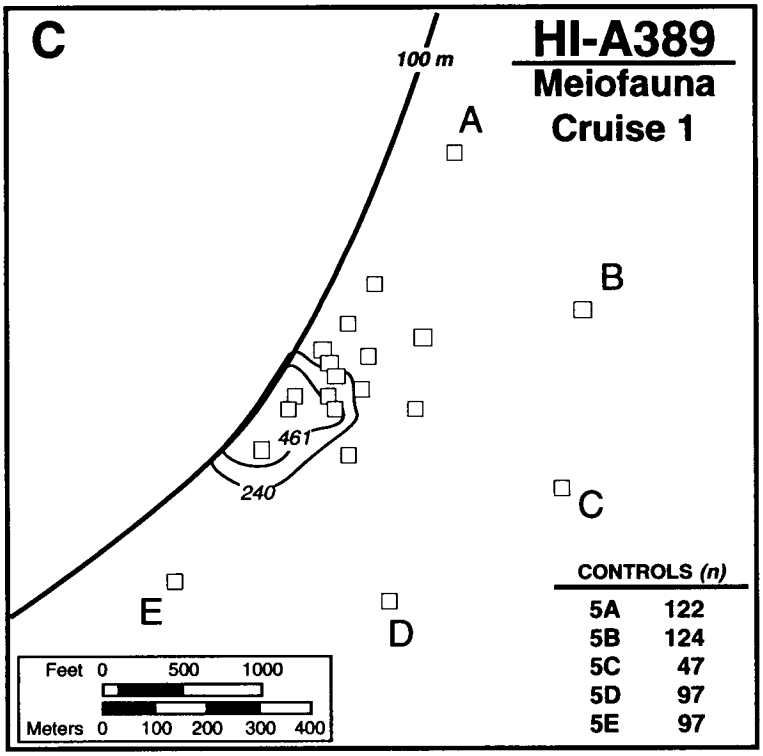


Figure 4.33. (continued).

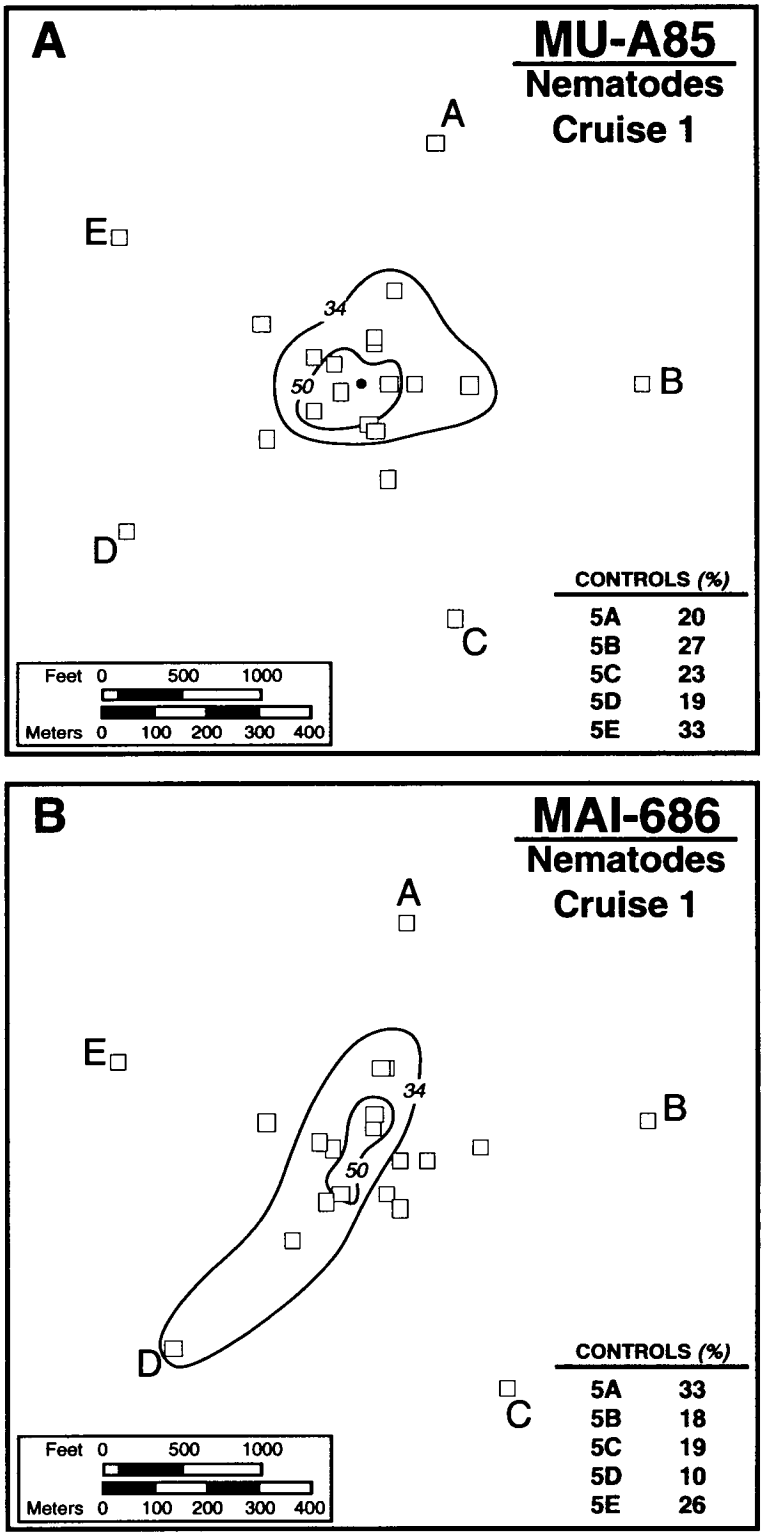


Figure 4.34. Abundance of nematodes in the 0–2 cm sediment section during Cruise 1 for A: MU-A85; B: MAI-686; and C: HI-A389.

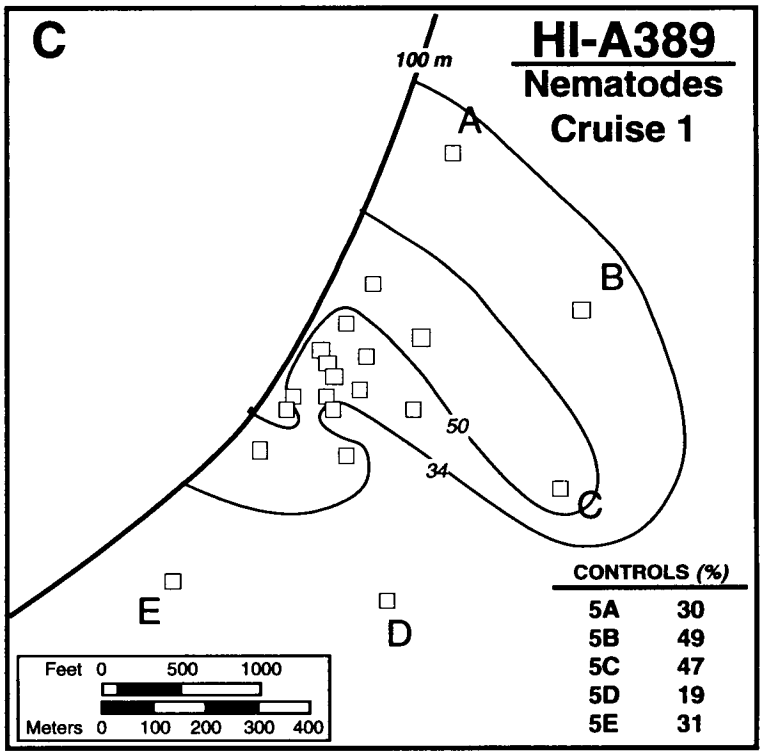


Figure 4.34. (continued).

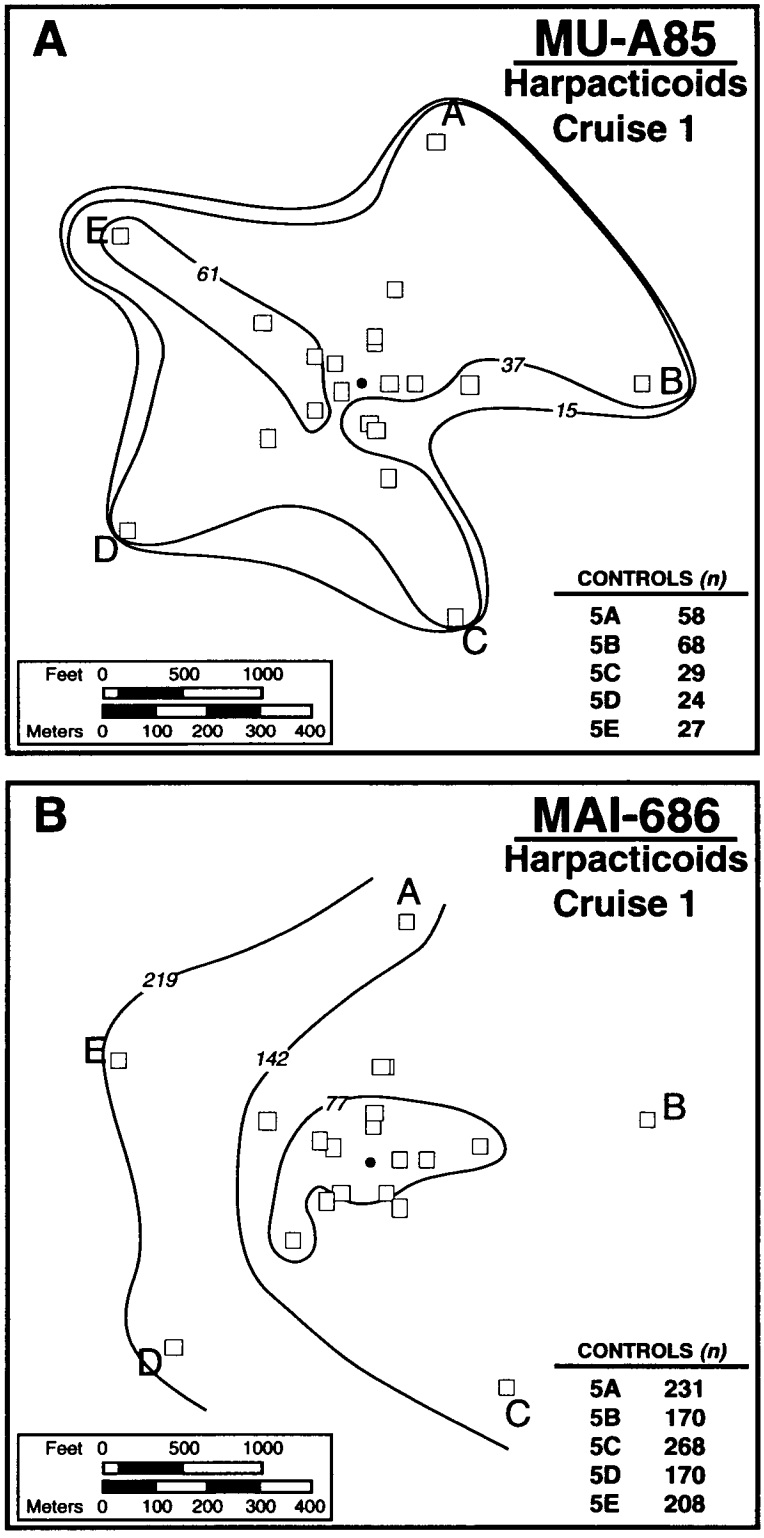


Figure 4.35. Abundance of harpacticoids in the 0–2 cm sediment section during Cruise 1 for A: MU-A85; B: MAI-686; and C: HI-A389. (n=number of individuals).

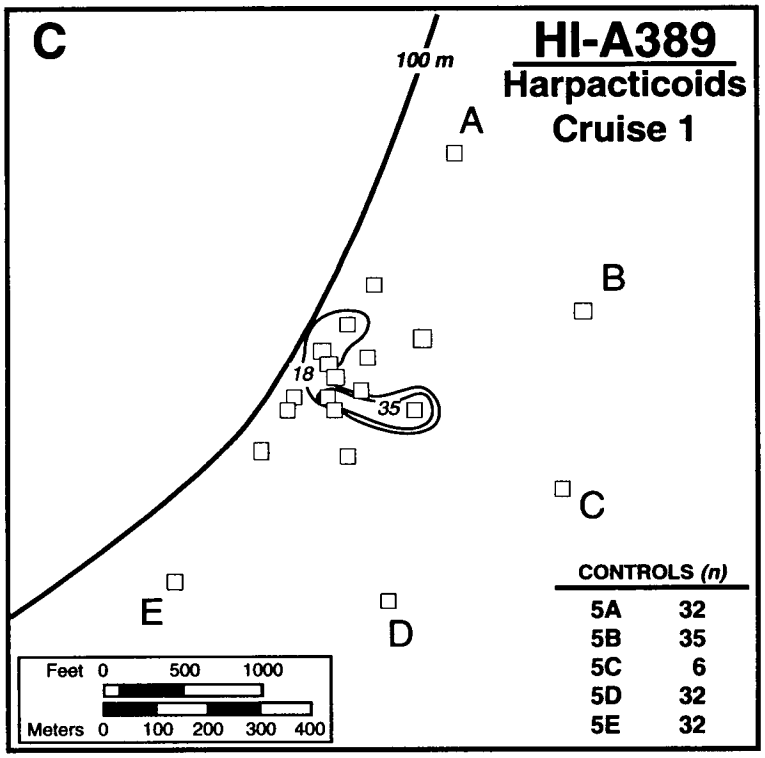


Figure 4.35. (continued).

total density with increasing distance from the platforms. Both sites also had relatively high Eh (redox) in the inner 2 or 3 rings during the first cruise and high (highly hypoxic) redox is better for nematodes than for harpacticoids. A similar trend was found at MU-A85 (Figures 4.32 and 4.33), but harpacticoids were very abundant at this platform at most distances. A statistical analysis reveals that there were no significant differences among radii. However, there were differences with distance from platforms in all meiofaunal components (Table 4.34). We also computed the nematode to harpacticoid ratio to determine if this index could predict platform effects. It was also significantly different with different distances. One important result was that there were generally no radial effects. This means that all the samples from radii can be pooled to form a sample with 10 replicates (=5 radii x 2 replicates) for statistical analysis. As described earlier, we can detect very small changes with 10 replicates (Figure 4.31). Depending on the power chosen, we can detect between 30% and 60% change in abundance.

Effects related to distance from the platform were clearly evident (Table 4.36). For all variables, either the 50 or 100 m distance had the lowest density estimates. Harpacticoid abundance was the best indicator of a platform effect. Density increased steadily to about 1000 m, where the density was indistinguishable from background levels at 3000 m. Even though both nematodes and harpacticoids had low densities near platforms, the nematode:harpacticoid ratio did predict platform effects. There were more nematodes relative to harpacticoids near the platform (within 200 m) than at background levels (1000-3000 m).

There seems to be some differences among the platforms (Figures 4.36-4.39). The significance of these relationships was driven by a strong trend at MAI-686. For total density, there was a slight enrichment at HI-A389, and only a slight platform effect at MU-A85 (Figure 4.36). This trend was driven by nematode abundance (Figure 4.37). Harpacticoid abundance was the cleanest indicator of proximity to the platform. Harpacticoid density increased with distance at all platforms (Figure 4.36). While the trend in harpacticoid density was weakest at HI-A389, the nematode:harpacticoid ratio was the highest at this site (Figure 4.39).

Abiotic factors, e.g., sediment texture, depth, salinity and oxygen concentration were correlated with water depth, and therefore confounded

Table 4.36 Comparison of average meiofauna abundances at all platforms using Tukey multiple comparison Tests. Means ($n \cdot core^{-1}$) connected by a line are not different at the 0.05 level.

Taxa	Distance /Mean				
TOTAL	50	100	3000	200	1000
	502	612	663	684	843
Nematodes	50	3000	100	200	1000
	351	434	456	493	588
Harpacticoids	50	100	200	3000	1000
	65	71	92	119	127
Others	100	50	200	3000	1000
	85	86	99	111	127
Nem:Harp	3000	1000	200	50	100
	3.5	4.3	7.0	7.9	8.0

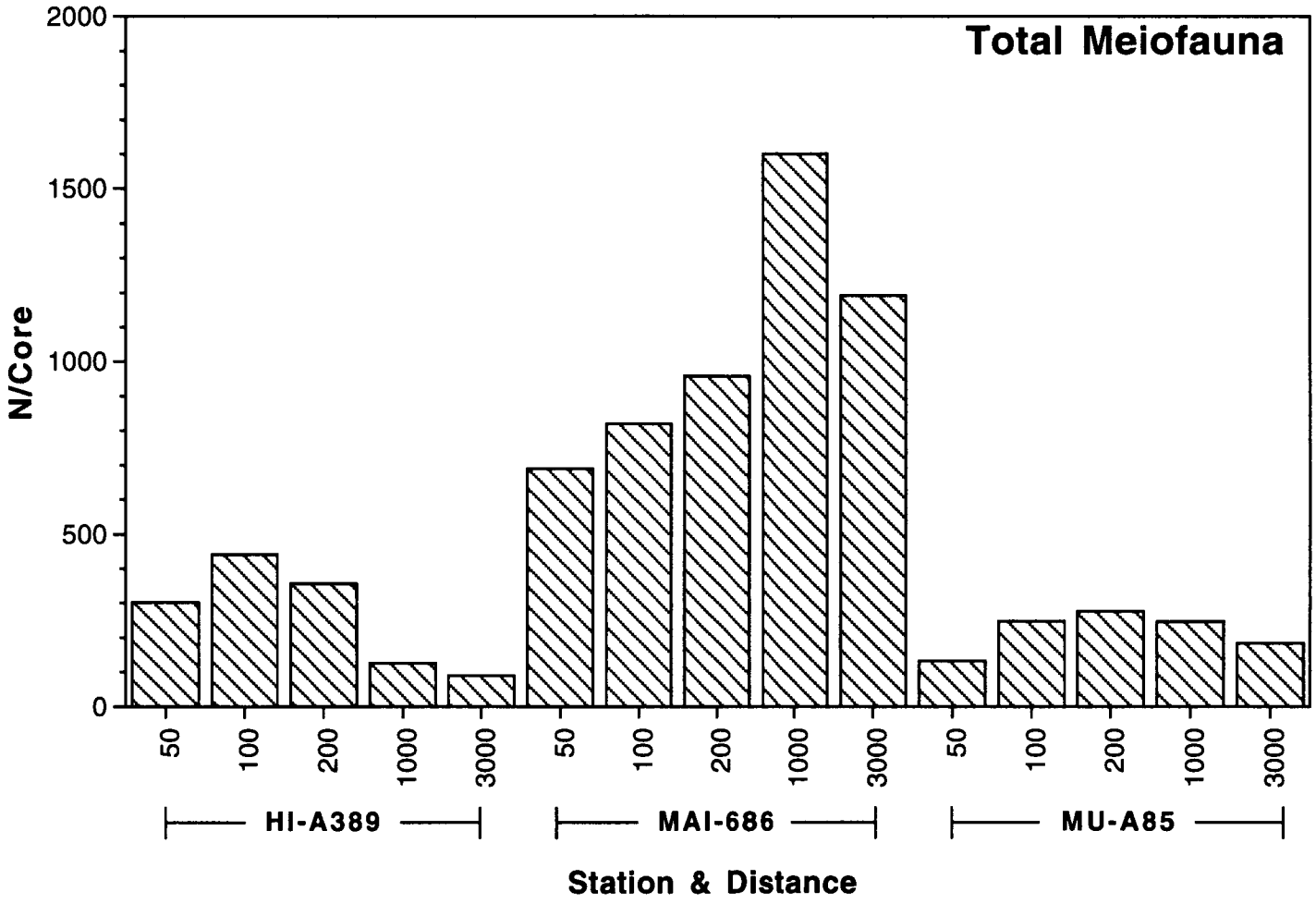


Figure 4.36. Meiofauna abundance at the study sites with distance from the platform.

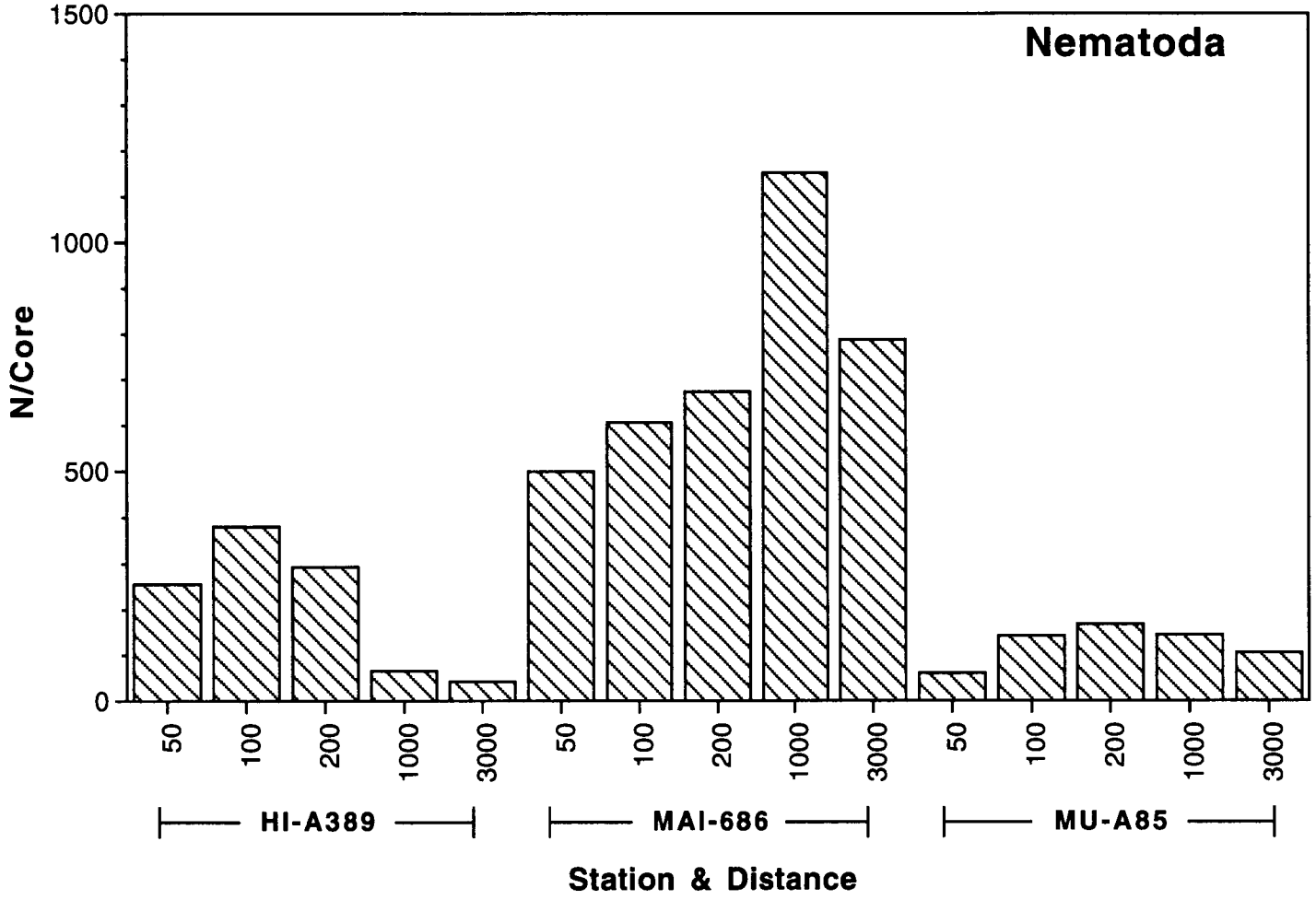


Figure 4.37. Nematode abundance at the study sites with distance from the platform.

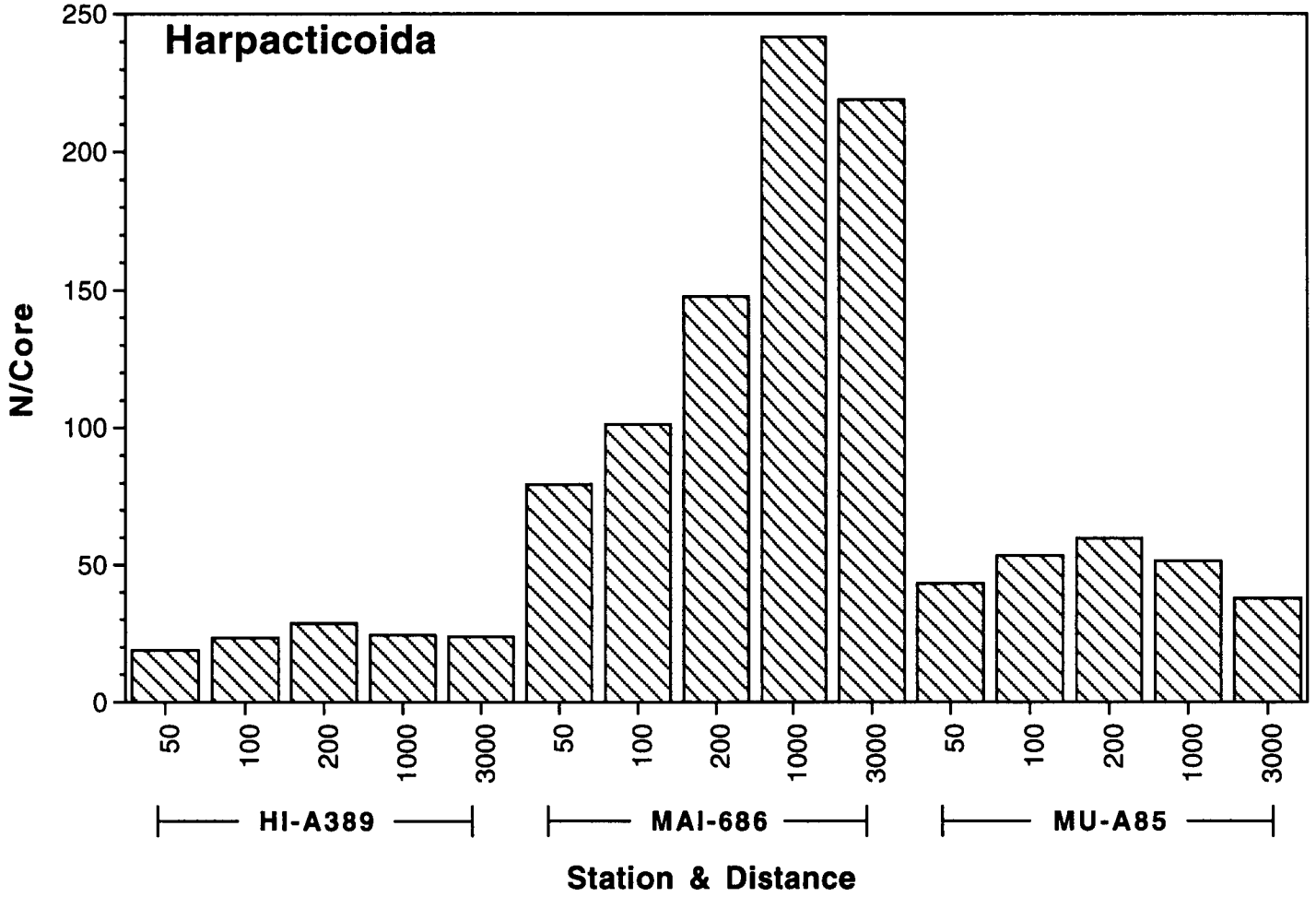


Figure 4.38. Harpacticoid abundance at the study sites with distance from the platform.

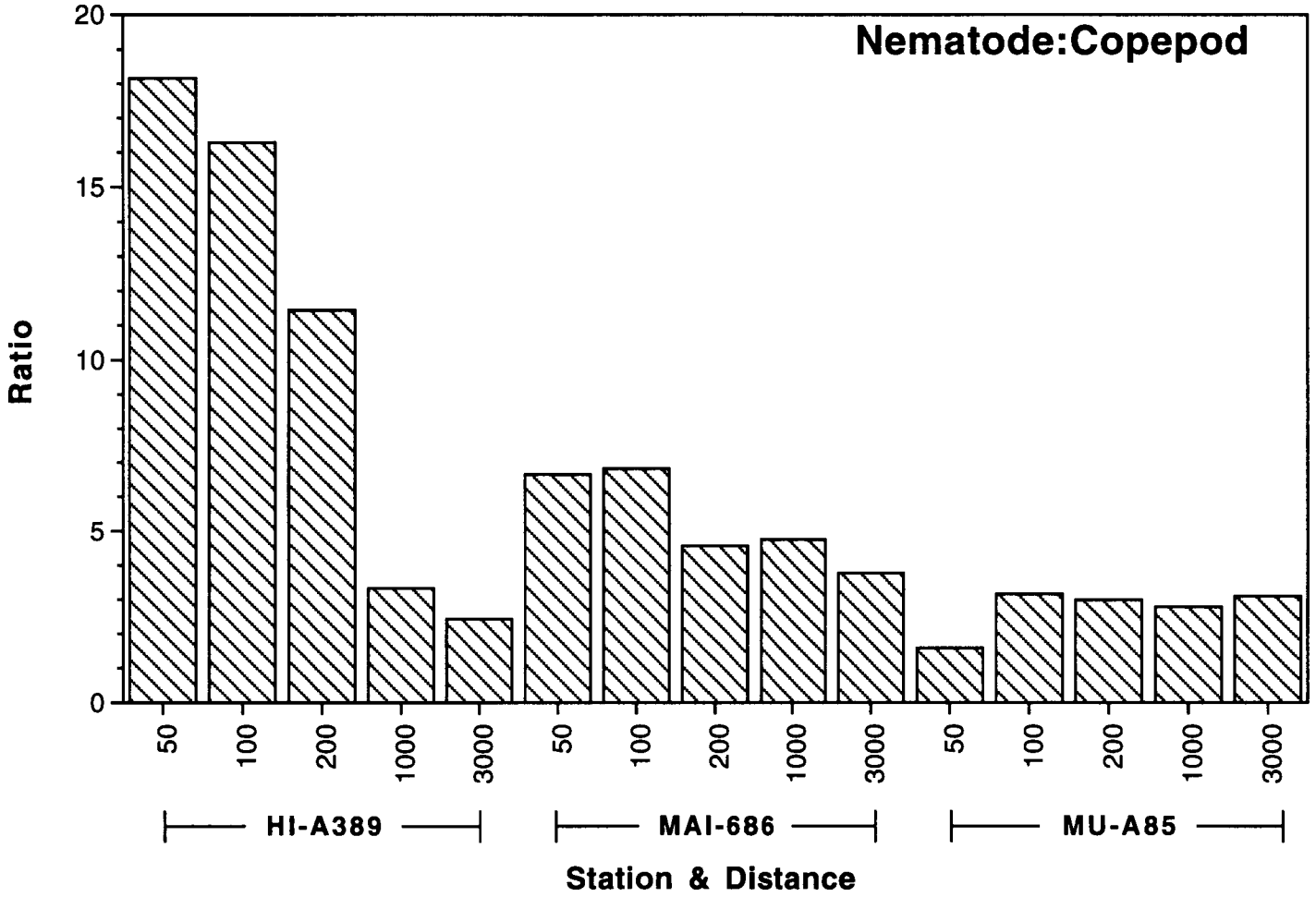


Figure 4.39. Nematode:copepod ratios at the study sites with distance from the platform.

with platforms (Tables 4.37 and 4.38). The highest meiofaunal densities were found at platforms that are the shallowest, lowest salinity, highest oxygen content and most coarse grained.

Within platforms there were few trends that can be attributed to these abiotic factors (Table 4.38). The only exception would be the distribution of harpacticoids. Harpacticoids are affected by sediment composition. Sediment grain size increased near the platforms. Harpacticoids were negatively correlated with the sand content and positively correlated with fine sediment content. When factor analysis was performed, it confirmed this observation. Variables in the analysis included water depth, salinity, oxygen, sand, silt, clay, and nematode, harpacticoid, and other meiofauna abundances. The first factor contained 92% of the variability of the data, and was related to differences among platforms. The second factor explained 7% of the variability and related to distance from the platform.

All nematodes from subsamples of the first cruise have been identified. From diversity indices (e.g., H' and Hill's N1) no statistically significant differences among the platforms and distances from platforms (Figure 4.40) were found. There may be a gradient of decreasing diversity at HI-A389.

Harpacticoid species distributions have been analyzed from all the near and far stations (Figure 4.41). Diversity was higher away from a platform than near it. Only at HI-A389 was the trend not noticeable. This was related to the lack of organisms at this station in general.

Meiofauna community studies have shown that harpacticoids exhibit a change in various population measures as a function of distance from the platform. This was true for total abundance and species diversity. Nematode diversity did not appear to vary with distance from the platform.

4.6.4 Meiofauna Toxicity Testing

The most useful toxicity bioassays would incorporate a short time period, measure a biologically meaningful response, and be independnt of environmental conditions. As such a test of indigenous species bioassays based on meiofauna is being developed. Indigenous meiofauna was collected during the second cruise in June 1993 and maintained in cultures. After one week the cultures collapsed. In cultures that did not collapse, population growth was not detected. As an alternative approach, a species of

Table 4.37. Average values of biotic and abiotic parameters at each platform.

Variables	Platform		
	MU-A85	MAI-686	HI-A389
Depth (m)	72.55	21.15	119.82
Salinity (ppt)	35.92	34.30	36.34
Oxygen ($mL \cdot L^{-1}$)	5.01	5.46	3.12
Sand (%)	32.8 7	46.81	19.03
Silt (%)	34.57	22.83	35.04
Clay (%)	32.56	30.36	45.92
Sediment Redox (mV)	**190**	**168**	**81**
Meiofauna ($n \cdot core^{-1}$)			
Total	213.48	1043.00	257.37
Nematodes	122.14	735.74	202.80
Harpacticoids	48.3 5	157.13	23.50
Others	43.00	150.13	31.07
Nem:Har ratio	2.71	5.30	10.32

Table 4.38. Correlation analysis of abiotic factors with meiofauna data. (Cruise 1 the top 2 cm only). Pearson correlations were calculated on the average of the distances (5) from platforms so n=5. The table summarizes correlation coefficients and P value for the hypothesis that the coefficient=0.

	TOTAL	NEMA	COPE	OTHER	NC
Depth	0.2176	0.0426	0.5871	0.3880	-0.7466
	0.7251	0.9458	0.2980	0.5186	0.1472
Salinity	-0.7986	-0.8228	-0.6106	-0.5878	0.4619
	0.1052	0.0871	0.2740	0.2973	0.4335
Oxygen	0.8345	0.7689	0.7883	0.8244	-0.6193
	0.0788	0.1287	0.1132	0.0860	0.2653
%Sand	-0.8322	-0.6878	-0.9757	-0.9100	0.9192
	0.0804	0.1993	0.0045	0.0320	0.0272
%Silt	0.8201	0.6673	0.9697	0.9359	-0.9099
	0.0891	0.2185	0.0063	0.0193	0.0320
%Clay	0.8273	0.6893	0.9658	0.8844	-0.9118
	0.0839	0.1980	0.0075	0.0463	0.0310

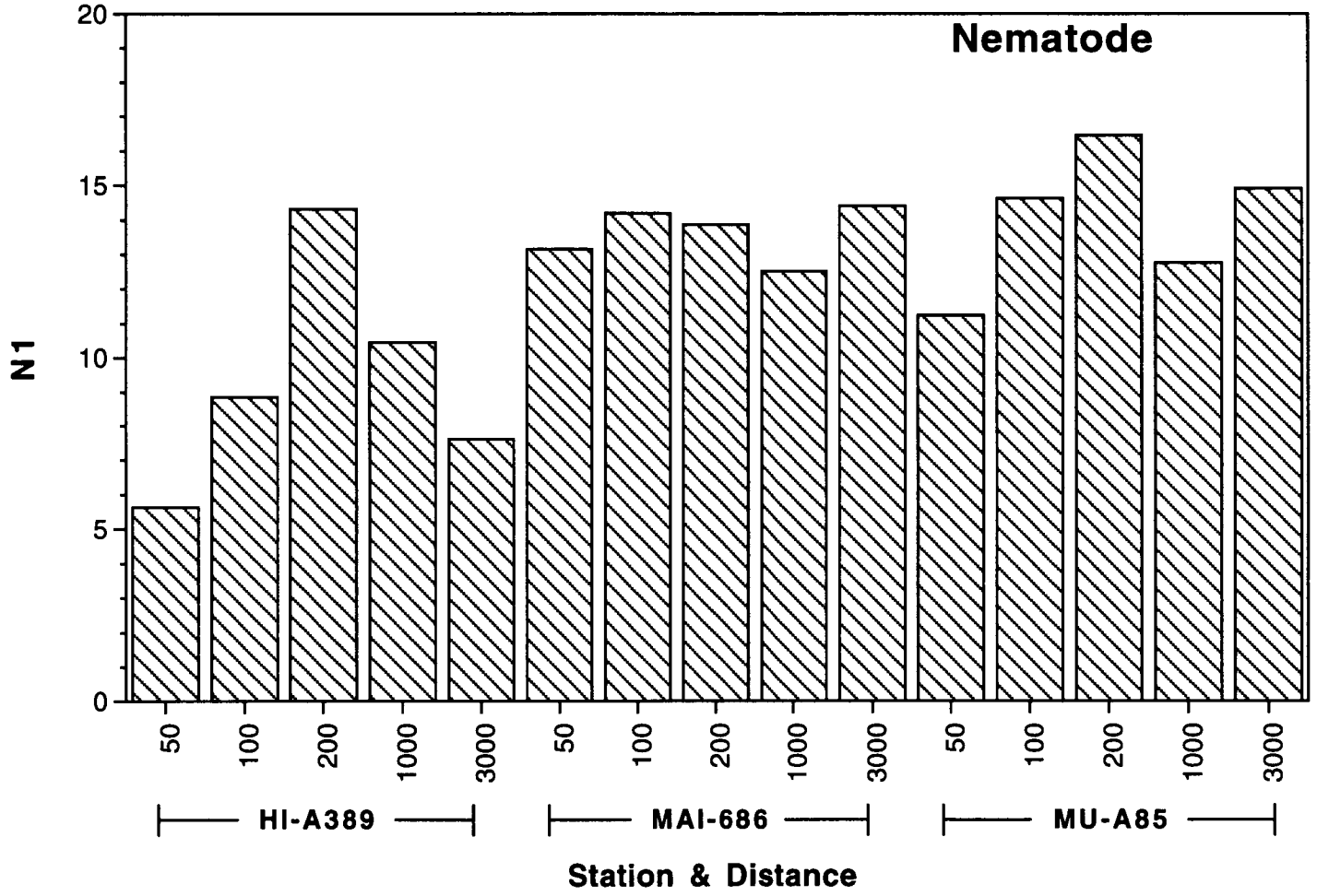


Figure 4.40. Nematode species diversity at the study sites with distance from the platform. N1 is the Hill's diversity index, which represents the number of dominant species.

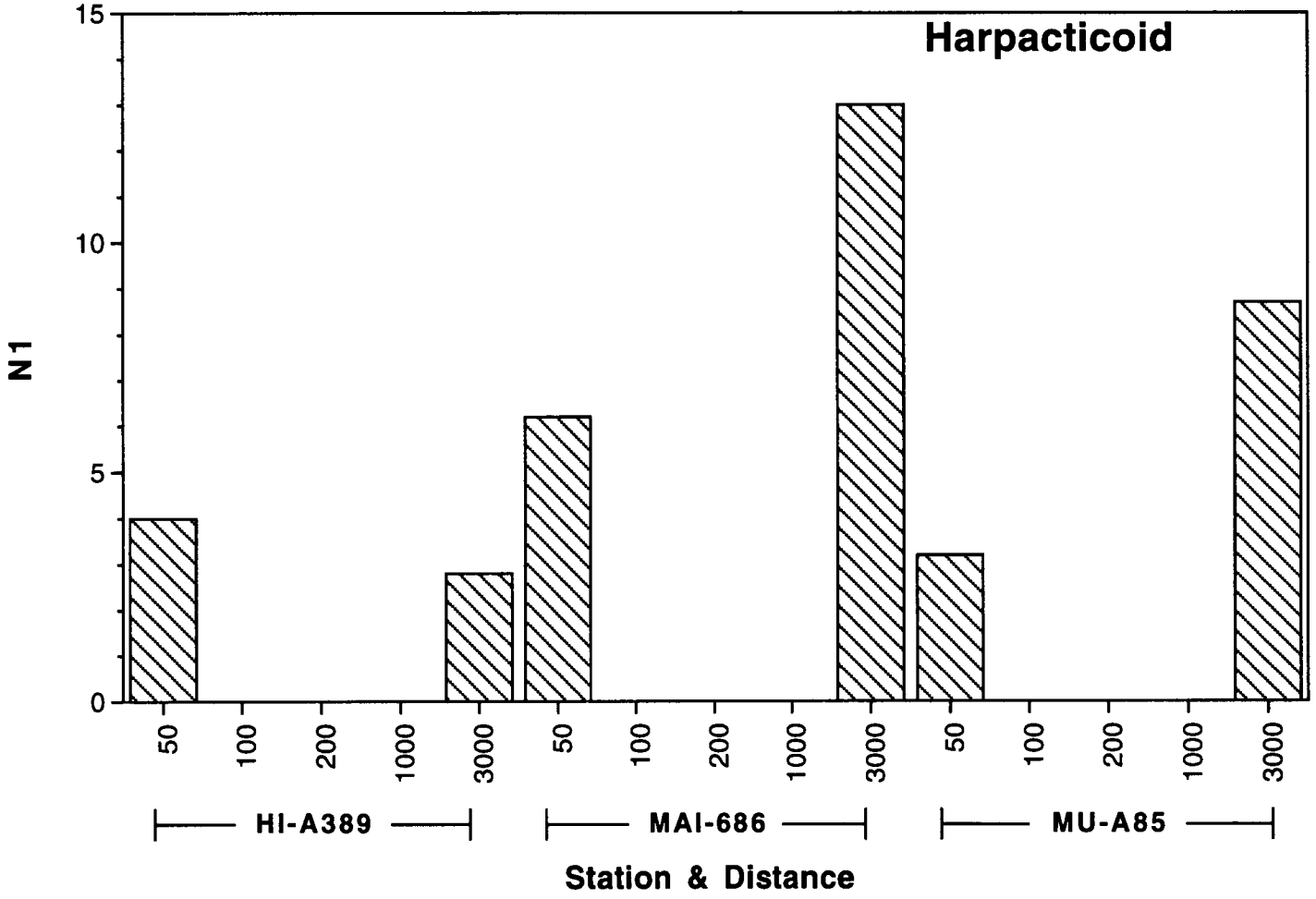


Figure 4.41. Harpacticoid species diversity at the study sites with distance from the platform. N1 is the Hill's diversity index, which represents the number of dominant species.

Longipedia was isolated from Aransas Pass. A species of *Longipedia* was present in the communities at the study sites, so this congeneric species is used as a substitute for the indigenous species. So far, these cultures have been maintained for more than three weeks. The animals have reproduced and reached a density of 300-400 individuals/vessel. Studies are continuing. Approximately, 3000-4000 animals are needed for toxicity tests.

4.7 Meiofauna Life History and Reproduction

Harpacticoid copepods are ideal animals for a reproductive study. Although, nematodes are the dominant taxon, there are at least five reasons why harpacticoid copepods are suitable for meiobenthic reproductive studies at the platform sites:

1) Harpacticoids have reproductive characteristics that are easy to measure. Harpacticoids are crustaceans that brood eggs externally, have five copepodite (or juvenile) stages that are easily identifiable, and have an adult stage that is sexually dimorphic.

2) Harpacticoid diversity increases with depth (Coull 1972), so that the likelihood of detecting changes in community structure is very high since we know we will be dealing with a diverse group of organisms.

3) Harpacticoids are usually more sensitive to hydrocarbon exposure than nematodes (Fricke et al. 1981; Bodin and Boucher 1983; Hennig et al. 1983). It has been suggested that nematode:copepod ratios (i.e., decreasing numbers of harpacticoids) are a good indicator of exposure to hydrocarbons (Hennig et al. 1983) and organic waste (Raffaelli and Mason 1981; Raffaelli 1982; Amjad and Gray 1983; Shiells and Anderson 1985). However, one must be careful when applying such an index (e.g., the nematode:copepod ratio) for comparing exposure at different habitats or different times, because meiofauna abundances and community structure are dependent on granulometry and seasonal fluctuations (Coull et al. 1981). All the necessary variables have been connected (i.e., chemical, geological, spatial, and temporal parameters) to evaluate the usefulness of the nematode:copepod ratio.

4) Harpacticoids are more sensitive than nematodes to the addition of barite and sand (Cantelmo et al. 1979). This phenomenon may be difficult to separate from 3. But, granulometry data should be useful in separating out effects due to toxicity and effects due to changes in surface sediment composition.

5) Harpacticoids disperse at faster rates than nematodes, thus are the first colonizers of drilling muds and cuttings. After an intertidal mud flat was disturbed by turning it over, all meiofauna recovered to predisturbance levels within one tidal cycle (Sherman and Coull 1980). This is explained by the fact that meiofauna act like passive particles of sediment, and are resuspended in a direct relationship to erosion velocities of surface sediments (Palmer and Gust 1985). Behavior does play a very important role. Since harpacticoids are more often found in surface sediments, they are much more common in the drifting meiofauna than nematodes (Bell and Sherman 1980; Palmer 1984). Therefore, it is no surprise that harpacticoid populations recovered within one week during a colonization experiment of oiled sediments, but nematodes took up to 90 days to recover fully (Alongi et al. 1983). Harpacticoids also recolonized azoic trays faster than nematodes in natural hydrocarbon seep sediments of OCS areas in California (Palmer et al. 1988).

Harpacticoid life history is complex, but reproductive parameters are easy to measure (Webb and Montagna 1993). Harpacticoids undergo five naupliar stages and six copepodite stages before they reach maturity, cohorts are easy to identify. The sixth copepodite stage is the mature adult, and the adults are sexually dimorphic. Whereas sex ratios of mature adults are sometimes 1:1, more often females predominate. Copepods brood their eggs and have multiple broods, thus embryos can easily be counted and measured for size differences at the platform and comparison sites, and through time. Harpacticoids generally have between four to twenty broods per individual, with between four to thirty eggs in each clutch (Hicks and Coull 1983). Egg diameters are in the range of 20-150 μm. Adult harpacticoid body lengths will be between 0.3-1.5 mm, the smaller sizes being interstitial, the larger sizes being epibenthic.

Two breeding patterns exist: (1) continuous breeders, where eggs are produced throughout the year and (2) discontinuous, where breeding is restricted to seasonal events. Subpopulations of one species has been shown to have different reproductive patterns, although separated by only 8 m (Palmer and Coull 1980). It is not known why harpacticoids exhibit such variable life history patterns, but one hypothesis suggests food availability and differential capability of various species to exploit these resources are important (Hicks 1979).

Development rates are influenced by temperature, food supply and salinity. Development times will increase with lower temperatures (Feller 1980; Palmer and Coull 1980). A complete life cycle (Hatch to Hatch) can take from 11 days at 20°C to 36 days at 8°C (Muus 1967). Hicks and Coull (1983) reviewed 31 studies and found all development times less than 42 days except one study that took 167 days (Feller 1980). Development times development rates of estuarine harpacticoids are modified by changes in salinity (Hicks and Coull 1983).

All the studies cited above were performed in the laboratory with estuarine species. Development rates are unknown for shelf species. Salinity is constant, temperatures vary within a narrow range, and the food supply decreases along a gradient away from Mississippi River influence. In very deep water (2000 m) it is suspected that life cycles may take up to 1 year (B. C. Coull Pers. Comm.). On the continental shelf (between 80-300 m) life cycles are between two and six months. Thus, semi-annual sampling is short enough to detect reproductive differences between platform and comparison sites.

Preliminary data indicates that near and far comparisons show no platform effect on the number of gravid females present (Figure 4.42). Body lengths of mature individuals indicate an enrichment effect under the platform.

4.8 Meiofauna Genetic Variability

These studies are intended to determine if genetic differences in invertebrate populations can be used to detect and monitor chronic, sub-lethal effects of offshore oil and natural gas production platforms. There are known and definable contamination gradients around each offshore platform. These are likely to cause differential natural selection pressures on populations of organisms that are adjacent to a platform, and those that are removed from one. This would result in the creation of genetically distinct populations. This distinction should be detectable by comparing the variation in genetic structure between these populations, focusing specifically on three locii of the mitochondrial genome.

Our null model is that contaminants are not having an effect upon the genetic diversity meiofaunal populations. If this is the case, then a near-field

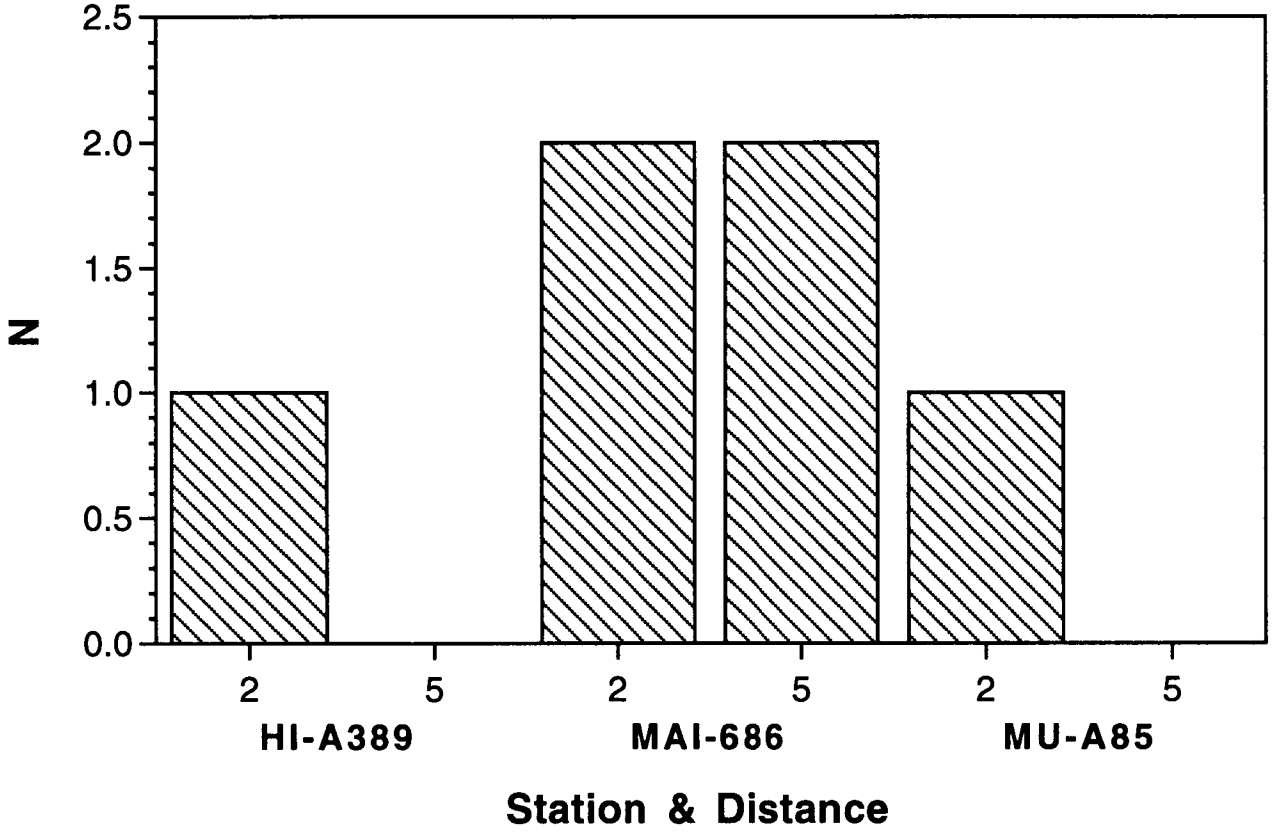


Figure 4.42. Total number of gravid copepods at the study sites.

population should have the same haplotype frequency and diversity as a far-field population. The null model is not accepted if there are (1) changes in haplotype frequencies between populations, or (2) a loss of haplotype diversity in animals closer to the platform, and presumably more susceptible to contaminant exposure, relative to those farther away. This is not an attempt to identify a specific gene locus that is under existence of any xenobiotic pressures, selective pressure, but an attempt to assess the existence of any xenobiotic pressures which can be attributed to the proximity of a platform.

While there are several other mechanisms which can drive genetic change in a population, the role of natural selection can be elucidated by employing replication of individuals, species, distances from a platform, and platform sites. Because the populations of harpacticoid copepods are not small (a mean of 2000 m^{-2}), it is thought that genetic drift and immigration will not be significant factors. The stochastic expression of haplotypes at contaminated sites, if it occurs, will only help to magnify changes between impacted and non-impacted sites by increasing the founder effect. It is unlikely that PAH mediated mutagenesis will alter the population genetics of these organism, since mutants are most often selected against in a stressful environment, thereby intensifying the differences between stations. Furthermore, preliminary findings to indicate that metals, not PAHs, may be responsible for most of the observed toxicological effects.

Harpacticoid copepods have short generation times (approximately 2-6 weeks), have benthic larval development, and are sensitive to a variety of contamination gradients. In addition life history, reproduction, and toxicity studies on harpacticoids are already being studied. Furthermore, arthropod DNA has been extensively characterized. For these reasons, harpacticoid copepods are excellent model organisms for this study.

Processing of samples from Cruise 2 has begun. Before this could be done, it was necessary to optimize the parameters in the PCR protocol. This was done by pooling 10 individual harpacticoids of the genus *Longipedia*. Once the amplification is perfected, only individual copepods will be used for restriction digests. These animals were originally collected from traps set in the Corpus Christi ship channel, although future organisms can be harvested from laboratory cultures currently being established.

The initial DNA extraction technique has been perfected or these copepods, achieving a high yield of pure DNA. The best results when the organisms were placed in a digestion buffer composed of Tris-disodium ethylene-diamine tetra-acetate (EDTA), N-Lauroylsarcosine (SDS), a detergent, and Proteinase-K, and ground with a micro pestle tip. By allowing the extraction to proceed for two hours, and then extracting once with freshly made, pH buffered phenol, once with phenol and chloroform, and then twice with chloroform, the yields were maximized. Although the photographs of an ethidium bromide stain of an agarose gel do not reproduce well, a computer graphic of one of these gels has been included (Figure 4.43).

The mitochondrial genome encodes for 13 proteins, 22 tRNAs (designated by single letter amino acid symbol), and 2 RNAs, the 12 and 16 subunits (Figure 4.44). Black coloration indicates upon which of the two strands the gene is actually situated (Stryer 1988; Clary and Wolstenholme 1985).

Currently primers are being developed for the actual amplification. Three sets of primers are being developed, one for the D-loop or control region, one for the Cytochrome Oxidase I sub-unit, and one for the 16s rRNA sub-unit. The most success has been with the 16s rRNA region, which is a highly conserved region of the molecule. The D-loop is the most variable region of the molecule, and will probably provide the most information once the PCR protocol can be optimized. Amplication of DNA in the proper size range has been achieved (Figure 4.42), but the procedure needs to be repeated a few more times in order to gain confidence in the consistency of the technique. Little success has occurred with the Cytochrome Oxidase I sub-unit, which is intermediate in conservation between the other two loci. The restriction enzymes which provide the most useful restriction maps are being established. So far, two enzymes, N1a III and Hae III, have been successfully utilized. Four others, Alu I, Rsa I, BstU I, and Mse I are currently being optimized. Successful amplification of two species has been achieved and the processing of the remaining samples is occurring at a rate of 16 per day.

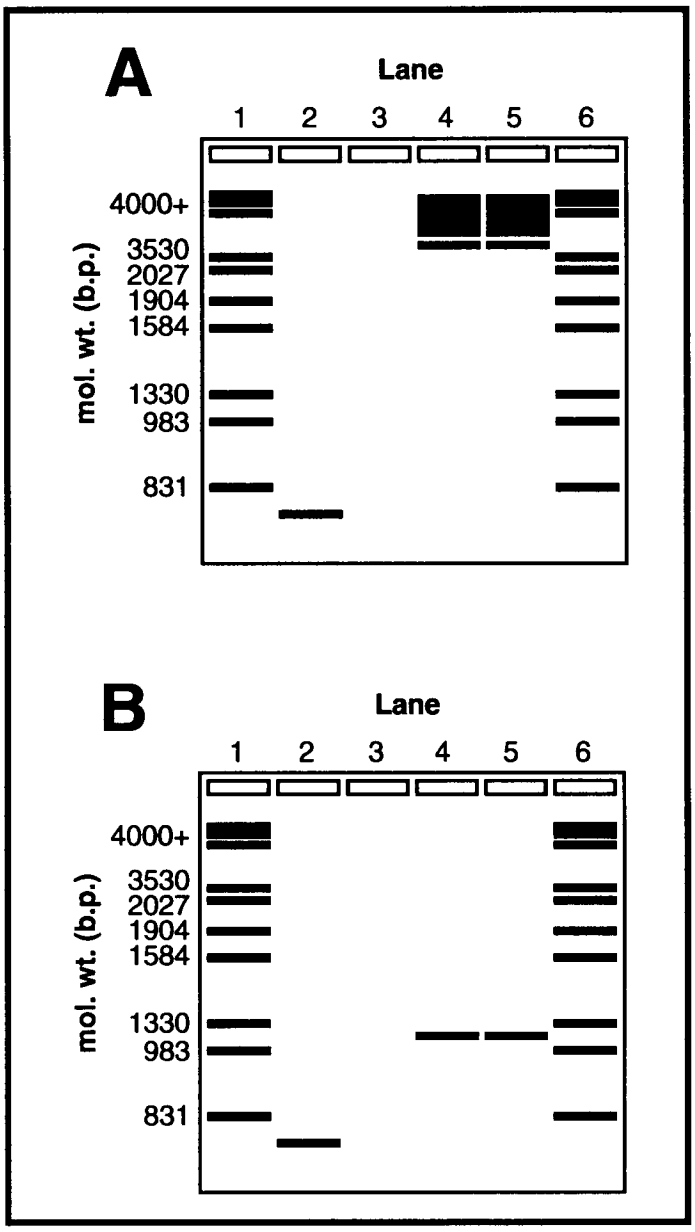


Figure 4.43. Computer graphic representation of 2% agarose gels stained with ethidium bromide for viewing DNA under ultraviolet light. The photographs taken of these gels do not reproduce well. A: Template DNA. Lane 1=molecular weight marker (EcoRI/HindIII digest of lambda DNA), lane 2=positive PCR control, lane 3=negative PCR control, lane 4-5=template DNA (the complete genome isolated from a pool of 10 harpacticoids), lane 6=molecular weight marker; B: PCR fragment. Lane 1=molecular weight marker, lane 2=positive PCR control, lane 3=negative PCR control, lane 4-5=1000 bp fragment of mtDNA D-loop, lane 6=molecular weight marker.

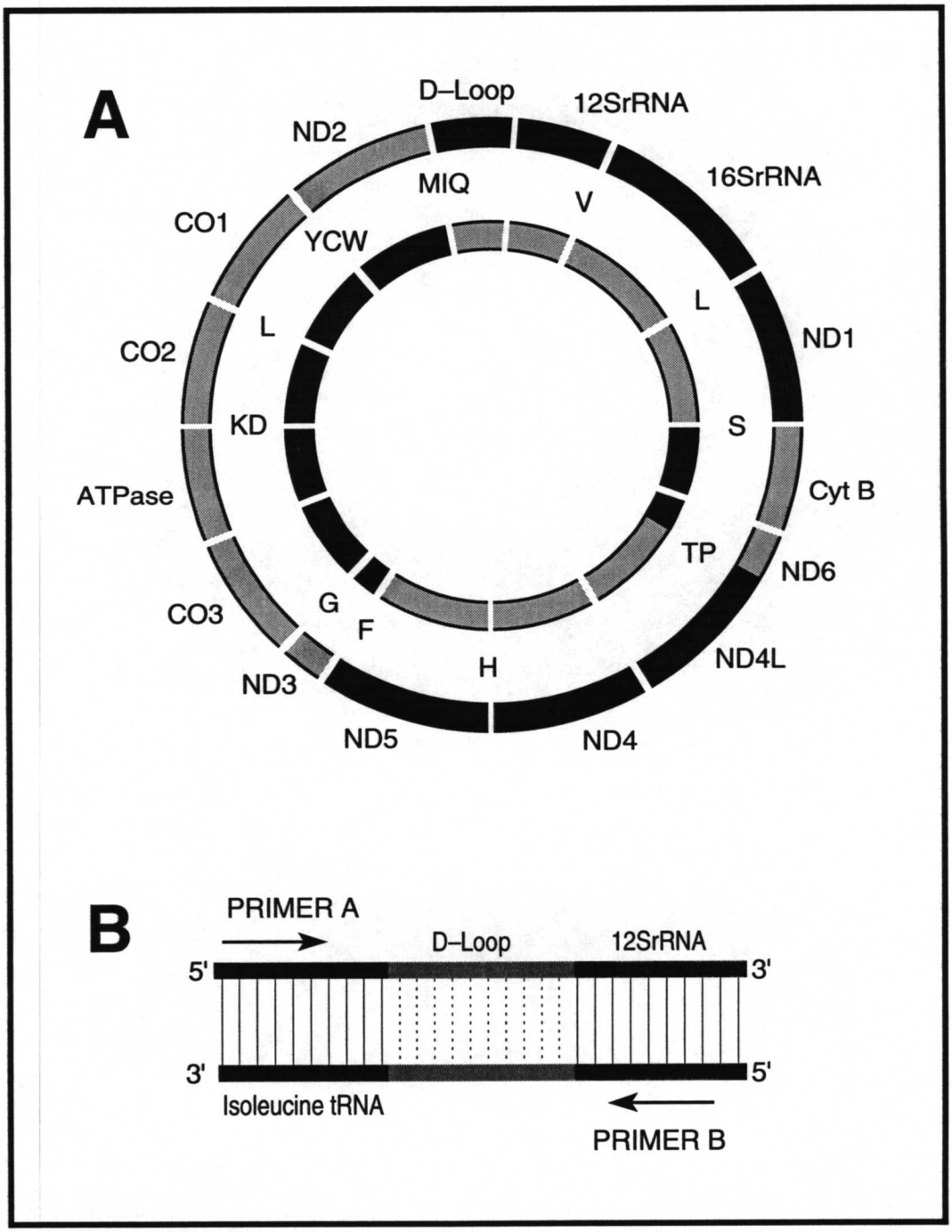


Figure 4.44. The crustacean mitochondrial genome. A: gene organization; B: close–up of amplified region, including primers.

4.9 Macroinfauna

Benthic infaunal organisms make ideal subjects for studying both the general ecology of a region or area, and the acute and chronic effects associated with human perturbations such as discharges of organic and toxic substances. Most contaminants eventually settle to the bottom due to density relative to sea water or by adsorbing to sediment particles which then settle. These substances then affect the organisms associated with the bottom - the infauna and epifauna. The benthic infauna are primarily non-motile or slow moving, small organisms, and tend to be associated with specific sediment types. This is caused, in many cases, by the larval stages being induced to metamorphose by specific sediment types, or by adults already established. Infaunal organisms that cannot easily escape an environmental stress, and those that cannot tolerate the stress, perish. Larvae of these organisms may be unable to settle and metamorphose if the stress persists, and the stressed area thus remains devoid of intolerant species. Changes in species composition and abundance as one approaches a discharge site is one indicator of stress.

Studies of benthic organism assemblages inhabiting soft bottoms have been conducted in much of the northern and northwestern Gulf of Mexico since the 1950's. These studies have tended to cover wide areas with infrequent sampling, or limited areas with frequent sampling. The earliest studies were conducted by Hildebrand (1954) and Parker (1960), who reported on macroepifauna and infauna, respectively, over broad areas of the shelf. More regional studies involving infauna have been done on the Louisiana shelf (Southwest Research Institute 1981; Fitzhugh 1984; McKinney et al. 1984, Gaston 1985, Rabalais et al. 1991). Macroinfaunal studies on the Texas continental shelf include work done along the upper Texas coast (Harper and Case, 1975), at the Buccaneer Oil/Gas Field, (Harper et al, 1981), and off Freeport, Texas (Harper et al. 1981, 1991) and in the vicinity of Matagorda Island Area Block 622 field (Continental Shelf Associates 1989). The South Texas Outer Continental Shelf Study (Berryhill 1977) was a shelfwide study in the western Gulf of Mexico which had an infaunal component.

Prior studies indicates several trends in distributional patterns of infauna. The principal abiotic factors governing distribution appears to be

sediment type and water depth, the latter being a manifestation of increasing stability of other abiotic factors such as temperature, salinity, dissolved oxygen, etc. Shallower water, in contrast, is much less stable, being subjected to seasonal variations in abiotic factors. Abundances of infaunal organisms generally decrease with increasing depth across the continental shelf, and seasonal variability in species composition decreases with increasing depth. Abundances of infaunal organisms also decrease toward the southwest. Infaunal assemblages are usually dominated by polychaetous annelids in soft muddy bottoms. Mollusks or crustaceans may dominate in sandy or shelly bottoms.

Studies near oil field discharges in estuaries have indicated that polychaetous annelids may be better able to tolerate hydrocarbons in the sediments because they occurred in larger numbers close to the platform that other groups (Mackin 1971, Armstrong et al. 1979). Crustaceans may be least able to tolerate discharges. In particular, ampeliscid amphipod populations were depressed in the vicinity of both platforms in the Buccaneer Field Study (Harper et al. 1981). Also, ampeliscid amphipod populations were reduced to virtually zero in the northwestern Gulf of Mexico coincident with the occurrence of hypoxia (Harper et al. 1991). These data suggest that crustaceans in general and ampeliscid amphipods may be useful as indicators of environmental stress.

4.9.1 Co-Occurrence of Species

Many samples yielded small numbers of individuals and species (Table 4.39). Platforms MU-A85 and HI-A389, both deep-water sites, had seven of twenty-five stations (28%) with no species common to either pairs of, or all three, replicates. MAI-686, in shallower water, had only one such station. Furthermore, platforms MU-A85 and HI-A389 each had twenty one stations in which there was no overlap in species between all three replicates, compared with eight such stations at MAI-686. The uniqueness in terms of species composition of many samples collected at MU-A85 and HI-A389 suggests that the assemblages are being undersampled, either because the individual sample size is too small or because an insufficient number of samples is being collected.

Table 4.39. Comparison of number of species co-occurring in replicate boxcore samples, MU-A85, Cruise 1.

		Total spp in reps	Species common to replicates				Total spp in reps	Species common to replicates			
Distance	Station	1, 2, 3	1 & 2	2 & 3	1 & 3	1, 2, 3	4, 5, 6	4 & 5	4 & 6	5 & 6	4,5,6
50-m	1A	20	1	3	2	1					
	1B	19	0	0	0	0					
	1C	17	1	2	1	0					
	1D	35	2	1	1	0					
	1E	19	0	0	2	0					
100-m	2A	27	2	0	0	0					
	2B	19	4	0	1	0	14	3	0	0	0
	2C	21	2	3	1	1					
	2D	14	0	0	0	0					
	2E	12	1	0	0	0					
200-m	3A	15	0	0	0	0					
	3B	12	0	0	0	0					
	3C	41	1	2	2	1					
	3D	22	3	3	1	0					
	3E	24	0	1	1	0					
599-m	4A	20	0	0	0	0					
	4B	8	0	0	0	0					
	4C	7	0	0	0	0					
	4D	25	1	0	0	0					
	4E	19	0	1	3	0					
3000-m	5A	17	2	0	1	0					
	5B	17	1	1	1	1					
	5C	24	0	0	0	0					
	5D	16	0	1	0	0					
	5E	14	3	0	0	0	16	3	0	0	0

Table 4.39. Cont.

		Tot spp in reps	Species common to replicates				Tot spp in reps	Species common to replicates			
Distance	Station	1, 2, 3	1 & 2	2 & 3	1 & 3	1, 2, 3	4, 5, 6	4 & 5	4 & 6	5 & 6	4,5,6
50-m	1A	26	2	2	1	1					
	1B	12	1	1	2	1					
	1C	30	5	4	4	3					
	1D	25	1	2	2	1					
	1E	8	0	1	0	0					
100-m	2A	14	3	3	3	2	5	0	0	0	0
	2B	12	3	0	0	0					
	2C	18	3	0	0	0					
	2D	24	3	1	0	0					
	2E	23	3	1	3	1					
200-m	3A	17	2	3	2	2					
	3B	12	1	0	0	0					
	3C	15	4	2	2	2					
	3D	22	3	0	0	0					
	3E	7	0	0	0	0					
599-m	4A	6	2	1	1	1					
	4B	7	1	2	1	1					
	4C	8	3	1	2	1					
	4D	15	5	5	4	4					
	4E	14	1	1	1	1					
3000-m	5A	10	3	3	1	1					
	5B	14	3	2	3	2					
	5C	21	3	4	4	2	15	0	1	1	0
	5D	13	1	3	1	1					
	5E	18	2	3	0	0					

Table 4.39. Cont.

		Tot spp in reps	Species common to replicates				Tot spp in reps	Species common to replicates			
Distance	Station	1, 2, 3	1 & 2	2 & 3	1 & 3	1, 2, 3	4, 5, 6	4 & 5	4 & 6	5 & 6	4,5,6
50-m	1A	14	2	0	0	0					
	1B	9	0	0	0	0					
	1C	8	0	0	0	0					
	1D	7	0	0	0	0					
	1E	19	6	0	0	0					
100-m	2A	10	4	4	4	3					
	2B	8	3	2	2	2					
	2C	24	0	2	0	0	17	3	4	3	2
	2D	23	5	5	5	4					
	2E	15	1	2	0	0					
200-m	3A	18	0	0	0	0					
	3B	19	2	1	2	0					
	3C	21	2	3	1	0					
	3D	22	0	1	0	0					
	3E	22	6	8	6	5					
599-m	4A	7	0	0	0	0					
	4B	11	0	2	0	0					
	4C	11	0	0	0	0					
	4D	8	0	0	0	0					
	4E	20	2	1	0	0					
3000-m	5A	18	0	0	1	0					
	5B	12	1	0	2	0					
	5C	7	0	1	0	0	11	0	0	0	0
	5D	13	0	0	1	0					
	5E	7	1	0	1	0					

4.9.2 Dominant Species and Infaunal Abundances

The total numbers of species and individuals, and the dominant species, at each station were determined (Table 4.40). Note that at the deep-water platforms, MU-A85 and HI-A389, there were seven and eight stations, respectively, at which no species was numerically dominant. Numerical dominants at most stations in the MU-A85 and HI-A389 study areas were present in relatively low abundances (Table 4.40). The exception to this generality occurred at four of the near-field stations, 2A, 2B, 2E, and 3E, all of which had large populations of the cirratulid polychaetes *Cirratulus cirratus*, *Cirriformia* sp. A, and *Tharyx annulosus*. Numerical dominants occurred at all MAI-686 stations, and, in general, occurred in greater numbers on the 4th and 5th rings (Table 4.40). MAI-686 stations frequently contained the polychaetes *Mediomastus californiensis*, *Paraprionospio pinnata* and *Neanthes micromma* in the near-field to intermediate-field regions, and ampeliscid amphipods in the far-field region (Table 4.40). If ampeliscid amphipods are indeed sensitive to environmental stress, their absence near the platform may indicate a platform effect.

At both MU-A85 and HI-A389, the number of species appears to be quite variable on each ring (Table 4.40). However, when the data are averaged, both sites display similar trends (Figure 4.45). The average number of species increased from ring 2 to ring 3, then decreased. The smallest numbers occurred in the far-field region. Numbers of species at MAI-686 displayed no regular pattern; the number of species alternately decreased and increased with increasing distance from the platform (Figure 4.45).

At MU-A85, abundances of infauna tended to be larger in an elliptical near-field area that appeared to extend southeast through northwest than at greater distances from the platforms (Figure 4.46). On average, however, the trend of mean number of individuals was similar to that of the number of species; the number increased from ring 2 to ring 3, and the smallest numbers were in the far-field. At HI-A389 the numbers of individuals were much larger in the near-field region; the isopleth forms an ellipse that appears to tend toward the southwest (Figure 4.47). Averaged ring data show a marked decrease in numbers of individuals with increasing distance from the platform, a result of the large number of cirratulid polychaetes

Table 4.40. Number of species, number of individuals/3 replicates, percent sand and TOC in the sediments, and dominant species at each station at MU-A85, Cruise 1. Blank indicates no dominant species. Numbers in parentheses are the total number of indivdiduals of the species.

Distance	Station	# spp	# ind	% sand	% TOC	Dominant species
50-m	2A	28	38	57.1	0.43	*Paramphinome* sp. (8)
	2B	19	32	72.2	0.63	*Neopanope texana* (9)
	2C	21	33	70.1	0.44	*Goneplacidae* (4)
	2D	14	18	60.7	0.56	*Goneplacidae* (4)
	2E	12	16	70.8	0.74	
100-m	3A	15	17	59.9	0.61	
	3B	12	14	64.5	0.51	
	3C	41	65	51.7	0.59	
	3D	21	31	54.9	0.87	
	3E	24	30	44.7	0.78	*Paramphinome jeffreysii* (4)
200-m	1A	19	27	17.1	1.40	*Paralacydonia paradoxa* (4)
	1B	18	21	31.6	1.00	
	1C	17	24	17.3	1.17	*Levensenia gracilis* (3)
	1D	35	48	23.8	0.87	*T. annulosus/Levensenia gracilis/Paramphinome* sp. (3 ea)
	1E	20	38	18.2	1.29	*Chaetozone sp. A* (11)
500-m	4A	21	26	9.4	1.40	*Goneplacidae* (3)
	4B	8	11	12.9	0.95	*Paralacydonia paradoxa* (4)
	4C	7	7	13.5	0.75	
	4D	25	28	12.3	0.96	*Prionospio cirrifera* (3)
	4E	19	45	10.8	0.77	*Foraminifera A* (21)
3000-m	5A	17	24	13.4	1.20	*Nephtys incisa* (5)
	5B	17	21	10.2	1.29	*Nephtys incisa* (4)
	5C	24	26	21.5	0.54	
	5D	16	18	7.7	0.91	
	5E	14	18	19.2	0.99	*Paralacydonia paradoxa* (3)

Table 4.40. Cont.

Distance	Station	# spp	# ind	% sand	% TOC	*Dominant species*
50-m	2A	12	182	34.4	1.05	*Cirratulus cirratus* (79)/*Cirriformia* sp A (50)
	2B	8	112	39.2	1.30	*Cirratulus cirratus* (75)/*Cirriformia* sp. A (28)
	2C	25	31	31.0	0.69	*Aricidea suecica* (3)/ *Oligochaeta* (3)
	2D	23	43	53.7	1.81	*Spiophanes wigleyi* (6)
	2E	15	73	51.3	1.28	*Cirratulus cirratus* (52)
100-m	3A	18	21	26.7	1.08	
	3B	19	37	14.3	0.91	*Cirrophorus lyra* (8)
	3C	21	44	26.1	1.09	*Levensenia reducta* (9)
	3D	23	39	48.4	1.00	*Paramphinome jeffreysii* (5)
	3E	22	88	42.4	1.16	*Cirriformia sp B* (37)/*Tharyx annulosus* (18)
200-m	1A	14	20	9.7	1.01	*Levensenia reducta* (3)
	1B	9	9	5.5	1.46	*Notomastus americanus* (2)
	1C	8	8	7.9	0.82	
	1D	7	7	13.4	0.86	
	1E	19	41	10.2	1.61	*Tharyx annulosus* (7)
500-m	4A	8	8	4.1	1.16	
	4B	12	21	4.6	1.26	*Foraminifera B (4)/ Aglaophamus circinata* (3)
	4C	11	12	4.1	1.19	*Nephtys incisa* (2)
	4D	8	9	3.3	1.44	
	4E	20	23	9.7	0.99	
3000-m	5A	19	22	4.4	1.26	*Foraminifera B* (3)
	5B	12	16	3.2	1.42	*Nephtys incisa* (3)
	5C	9	11	4.1	0.75	
	5D	13	17	4.7	1.24	*Mediomastus californiensis* (4)
	5E	8	9	5.3	1.25	

Table 4.40. Cont.

Distance	Station	# spp	# ind	% sand	% TOC	*Dominant species*
50-m	2A	15	32	72.5	0.26	*Neanthes micromma* (8)
	2B	12	19	81.7	0.31	*Neanthes micromma (4)*
	2C	18	32	76.9	0.36	*Mediomastus californiensis* (8)
	2D	24	39	84.5	0.26	*Cerebratulus lacteus* (7)
	2E	23	44	60.1	0.39	*Mediomastus californiensis* (8)/*Cerebratulus lacteus* (6)
100-m	3A	18	30	65.9	0.30	*Cerebratulus lacteus* (6)/*Neanthes succinea* (5)
	3B	13	16	62.3	0.52	*M. californiensis*/*Paraprionospio pinnata* (3 each)
	3C	15	33	61.0	0.35	*Prionospio* sp. (6)
	3D	23	45	70.1	0.21	*Mediomastus californiensis* (7)/*Cerebratulus lacteus* (6)
	3E	7	10	29.0	0.47	*Cerebratulus lacteus* (3)
200-m	1A	26	42	27.7	1.08	*Mediomastus californiensis* (7)
	1B	12	26	30.7	1.02	*Paraprionospio pinnata* (7)
	1C	30	79	33.8	1.05	*Ampelisca agassizi* (21)
	1D	25	51	68.7	0.62	*Paraprionospio pinnata* (7)/*Lumbrineris verrilli* (6)
	1E	8	14	33.4	0.52	*Spionidae* (6)
500-m	4A	6	33	30.7	1.27	*Ampelisca agassizi* (18)
	4B	8	30	33.6	1.02	*Ampelisca agassizi* (13)
	4C	9	40	39.3	0.76	*Paraprionospio pinnata* (27)
	4D	15	40	25.3	0.82	*Ampelisca agassizi* (17)
	4E	14	28	32.3	0.86	*Ampelisca agassizi* (11)
3000-m	5A	10	30	49.6	0.49	*Paraprionospio pinnata* (11)
	5B	14	32	20.7	1.07	*Ampelisca verrilli* (10)
	5C	23	90	20.0	1.24	*Ampelisca agassizi* (30)
	5D	12	24	21.0	1.15	*Ampelisca agassizi* (8)
	5E	18	37	38.2	0.73	*Paraprionospio pinnata* (12)

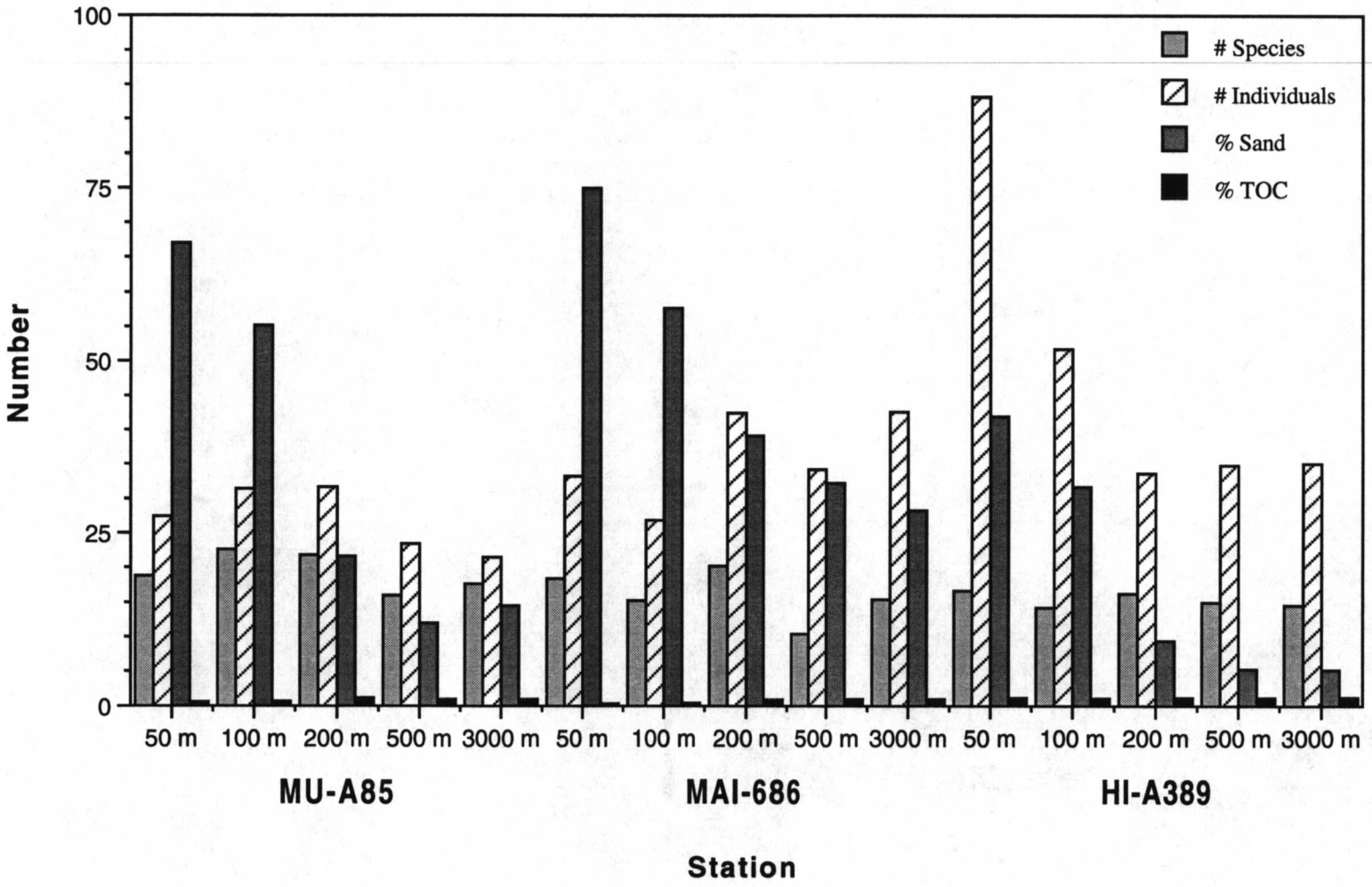


Figure 4.45. Comparison of mean numbers of infaunal species and individuals with mean percent sand in the sediment, and mean percent TOC for each ring during Cruise 1.

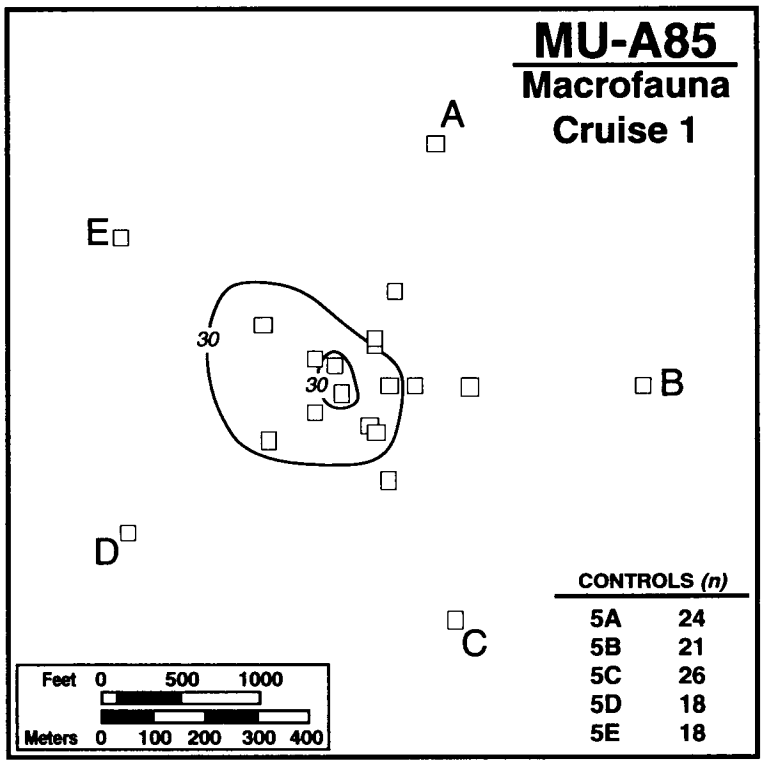


Figure 4.46. Numbers of individual macroinfauna in the 0–10 cm section of sediments at each station along transects at MU-A85 during Cruise 1.

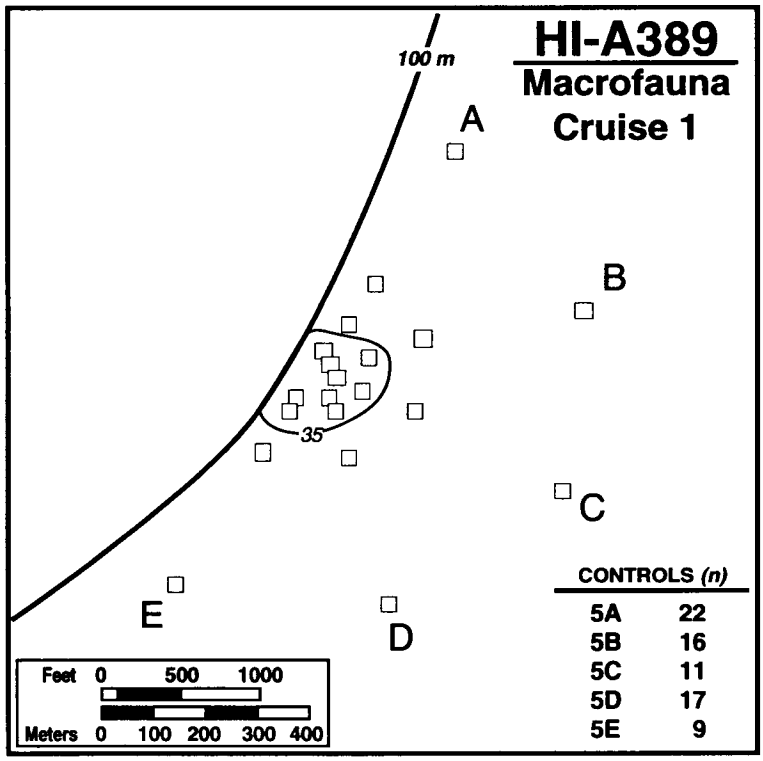


Figure 4.47. Numbers of individual macroinfauna in the 0–10 cm section of sediments at each station along transects at HI-A389 during Cruise 1.

mentioned above (Figure 4.45). At MAI-686, no particular abundance trend is evident. Both low and high numbers occur in the near-field area. Averaged ring data showed no regular pattern. As with the species, the numbers of individuals alternately increased and decreased with increasing distance from the platform (Figure 4.45).

In summary, near-field stress at MAI-686 may be indicated by the absence of ampeliscid amphipods, or the absence of amphipods may be a result of high sand content or predation near the platform. At platforms MU-A85 and HI-A389, abundances of benthic infaunal organisms were relatively low and dominant species were generally not as dominant as at other platforms. Large populations of cirratulid polychaetes occurred at several near-field stations at HI-A389. The silt-clay content of the sediments was higher in this study area than at the other two sites.

4.9.3 Assemblage Indices

Shannon-Weaver diversity index (H'), Gleason's richness index (D) and eveness (J) were calculated for each station (Table 4.41). At MU-A85, the diversity index was, with few exceptions between 2.0 and 3.0 (Table 4.41). Lower indices occurred at one 100-m stations and two 500- stations. The richness index was quite variable, with high and low values occurring on each ring. The eveness index was mostly in the 0.9 to 1.0 range. The lowest diversity indices were found at HI-A389, where large populations of cirruatulid polychaetes at stations on the 50-m ring depressed the index values to below 1.3 (Table 4.41). These populations also resulted in low richness and eveness values. The richness index values at HI-A389, in general, were much more similar to each other than at MU-A85. The eveness values, with the exceptions noted above, were in the 0.9-1.0 range. MAI-686 diversity indices were mostly in the 2.0-3.0 range (Table 4.41). The exceptions occurred at four stations on the 200-m and 500-m rings. These same four stations also had lower richness and eveness indices. When the data are averaged for each ring, most stations have similar diversity indices (Figure 4.48). The 50-m ring at HI-A389 and the 500-m ring of MAI-686 were slightly lower than most other stations. Species richness was slightly higher at MU-A85 than at the other two sites. The richness index was lowest at the 500-m ring of MAI-686. Average eveness was slightly

Table 4.41. Comparison of Shannon-Weaver diversity (H'), Gleason Richness Index (D) and Eveness (J) at MU-A85 stations, Cruise 1.

Distance	Station	H'	D	J
50-m	2A	3.130	7.477	0.939
	2B	2.909	6.459	0.882
	2C	2.790	5.434	0.931
	2D	2.505	4.497	0.949
	2E	2.790	4.235	0.977
100-m	3A	1.609	2.485	1.000
	3B	2.441	4.168	0.982
	3C	3.517	9.582	0.947
	3D	2.889	5.824	0.948
	3E	3.128	7.830	0.984
200-m	1A	2.704	4.910	0.954
	1B	2.846	5.583	0.974
	1C	2.751	5.034	0.971
	1D	3.452	8.782	0.997
	1E	2.348	4.253	0.939
500 m	4A	2.918	5.831	0.974
	4B	1.814	2.817	0.872
	4C	1.945	3.083	1.000
	4D	3.164	7.202	0.983
	4E	2.189	4.728	0.743
3000-m	5A	2.669	5.034	0.942
	5B	2.714	5.255	0.958
	5C	3.183	7.202	0.989
	5D	2.714	4.926	0.979
	5E	3.283	7.758	0.965

Table 4.41. Cont.

Distance	Station	H'	D	J
50-m	2A	1.078	1.543	0.490
	2B	0.969	1.483	0.466
	2C	3.412	9.005	0.931
	2D	2.917	5.813	0.930
	2E	1.335	3.263	0.493
100-m	3A	2.821	5.583	0.976
	3B	2.653	4.984	0.901
	3C	2.739	5.253	0.899
	3D	2.938	5.815	0.950
	3E	2.226	4.771	0.731
200-m	1A	2.626	4.529	0.969
	1B	2.271	3.753	0.986
	1C	2.079	3.366	1.000
	1D	1.945	3.083	1.000
	1E	2.742	4.847	0.931
500 m	4A	2.079	3.366	1.000
	4B	2.345	3.671	0.943
	4C	2.271	3.753	0.986
	4D	2.043	3.185	0.982
	4E	3.014	6.378	0.990
3000-m	5A	2.761	5.340	0.974
	5B	2.304	3.789	0.961
	5C	2.889	5.663	0.981
	5D	2.438	4.151	0.946
	5E	1.732	2.404	0.967

Table 4.41. Cont.

Distance	Station	H'	D	J
50-m	2A	2.257	4.640	0.891
	2B	2.305	3.735	0.927
	2C	2.562	4.537	0.904
	2D	2.813	6.115	0.885
	2E	2.854	5.849	0.910
100-m	3A	2.572	4.704	0.907
	3B	2.445	4.235	0.953
	3C	2.493	4.004	0.920
	3D	2.702	5.341	0.874
	3E	1.834	2.605	0.942
200-m	1A	3.045	6.688	0.934
	1B	2.137	3.069	0.891
	1C	2.785	6.617	0.818
	1D	2.862	5.652	0.912
	1E	1.772	2.652	0.852
500 m	4A	1.180	1.430	0.658
	4B	1.520	2.058	0.731
	4C	1.290	2.168	0.587
	4D	2.129	3.656	0.786
	4E	2.237	4.116	0.839
3000-m	5A	2.035	2.860	0.848
	5B	2.211	3.718	0.838
	5C	2.327	6.523	0.665
	5D	2.091	3.461	0.841
	5E	2.476	4.707	0.856

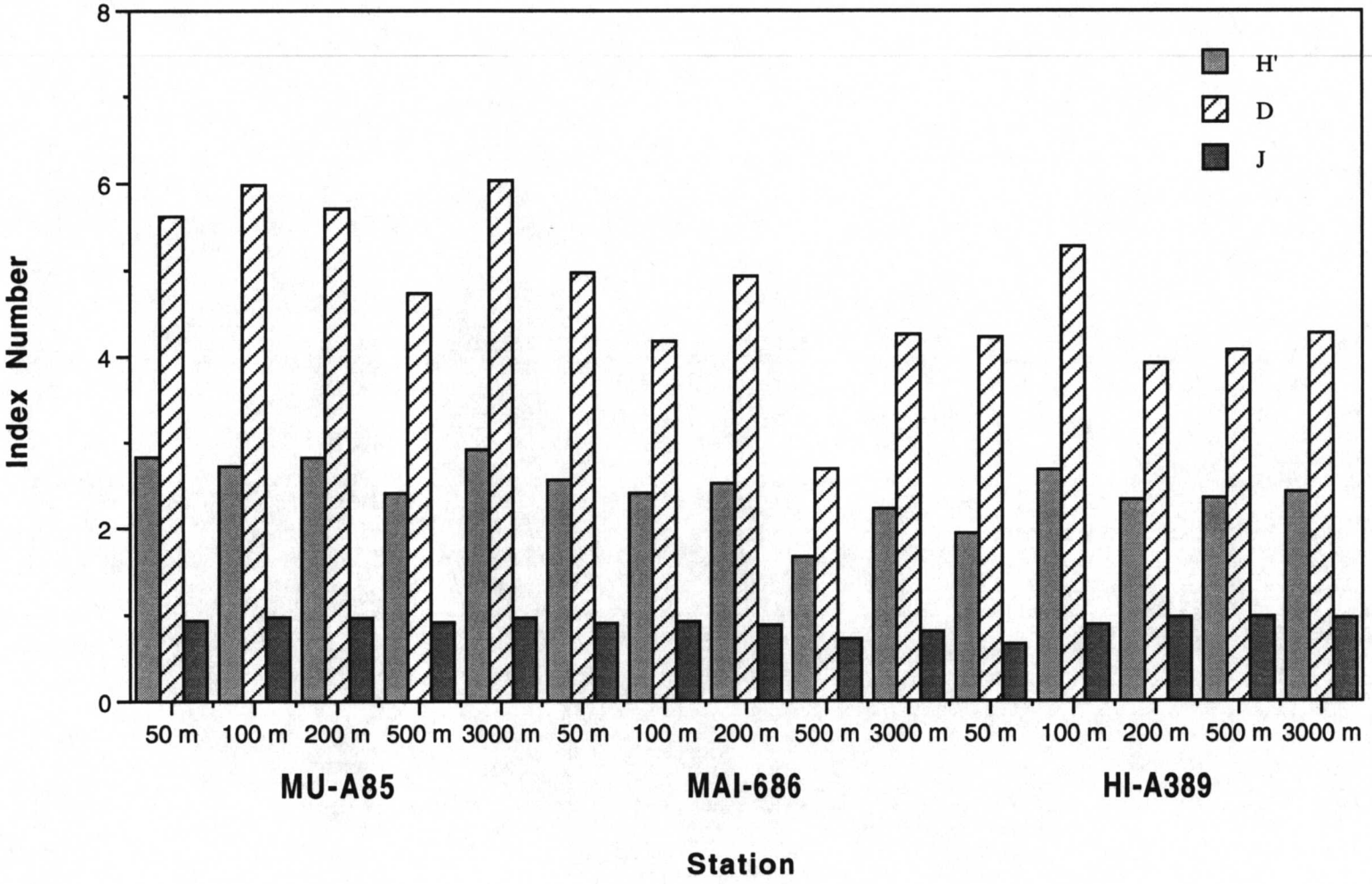


Figure 4.48. Comparison of mean values of Shannon-Weaver diversity index (H'), Gleason's richness index (D) and evenness index (J) for each ring during Cruise 1.

depressed on the HI-A389 50-m ring, but was otherwise similar at all stations. In summary, while there is a great deal of variability in the various indices at individual stations, the averaged indices appear to be relatively similar. The two exceptions occurred at the 50-m ring of HI-A389, where large populations of polychaetes occurred, and on the 500-m ring at MAI-686, where fewer than average species were collected.

4.9.4 Cluster Analysis

Cluster analysis was performed using the Bray-Curtis dissimilarity coefficient, flexible sorting, and square root transformation. Only those species which occurred at four or more stations (about 85% of all species at any given site) were retained for the analysis.

Clustering at MU-A85 resulted in two major site groups (Figure 4.49). All but 4 stations located within 100 m of the platform were included in Group I. Two major species groups were also produced (Figure 4.50). Species in Group I occurred more frequently at Site Group II stations (two-way table not shown). Site Group I was delineated by the fewer species which constituted Species Group II.

At MAI-686, two major Site Groups were also identified (Figure 4.51). Site Group II consisted of stations within 100 m of the platform on transects A, C, D and E. Note that station 1C, which because of navigation error, was located on top of station 1A, were in the same cluster, but were not clustered together. The species group dendrogram contained two major species groups (Figure 4.52). Species in Species Group II defined Site Group II; these species were generally more abundant in, and showed greater fidelity to, Site Group II (two-way table not shown).

HI-A389 also had two major site groups (Figure 4.53). Site Group I consisted of 5 inner ring stations and 2 outer ring stations. The species group dendrogram consisted of two groups of approximately equal size (Figure 4.52), and the two way table indicates that Site Group II was characterized by infrequent occurrences of species (Figure 4.54)

In summary, cluster analysis suggests that near-field assemblages are different from far field assemblages at MU-A85 and MAI-686. The differences between groups appears to be due to the occurrence of different species rather than absence of species at both of these platforms. Again

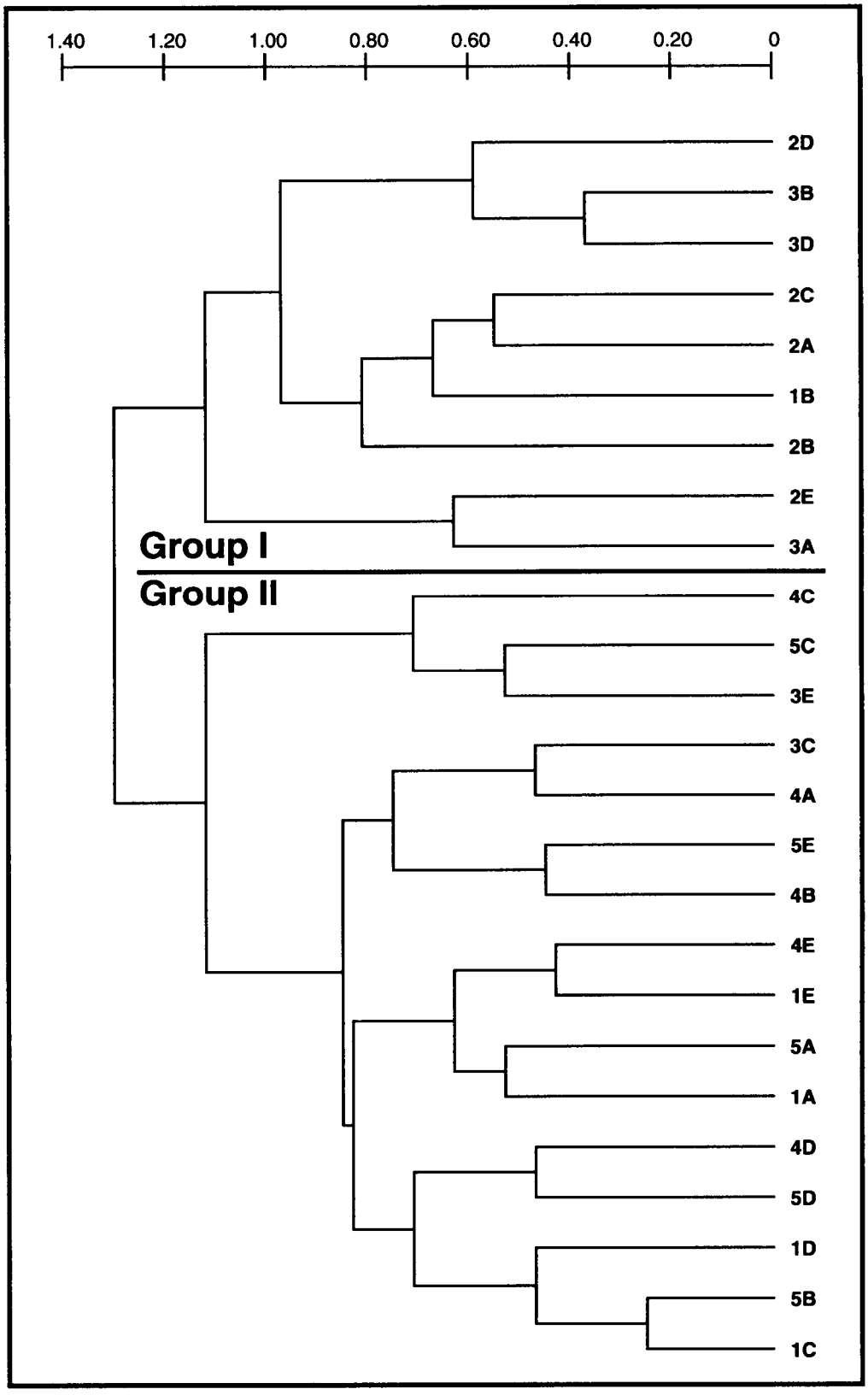


Figure 4.49. Site group dendrogram for MU-A85 stations sampled during Cruise 1.

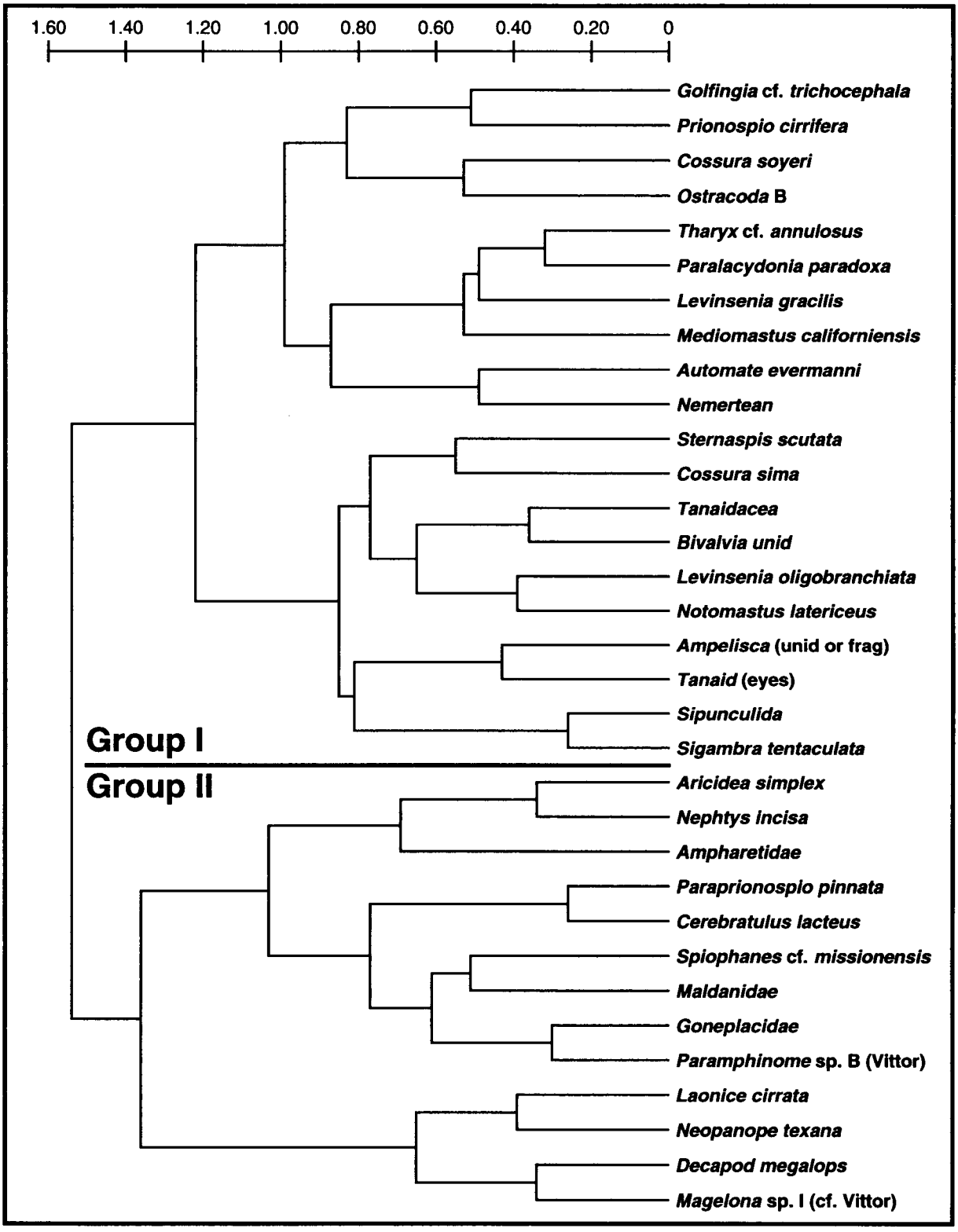


Figure 4.50. Species group dendrogram for MU-A85 stations from Cruise 1.

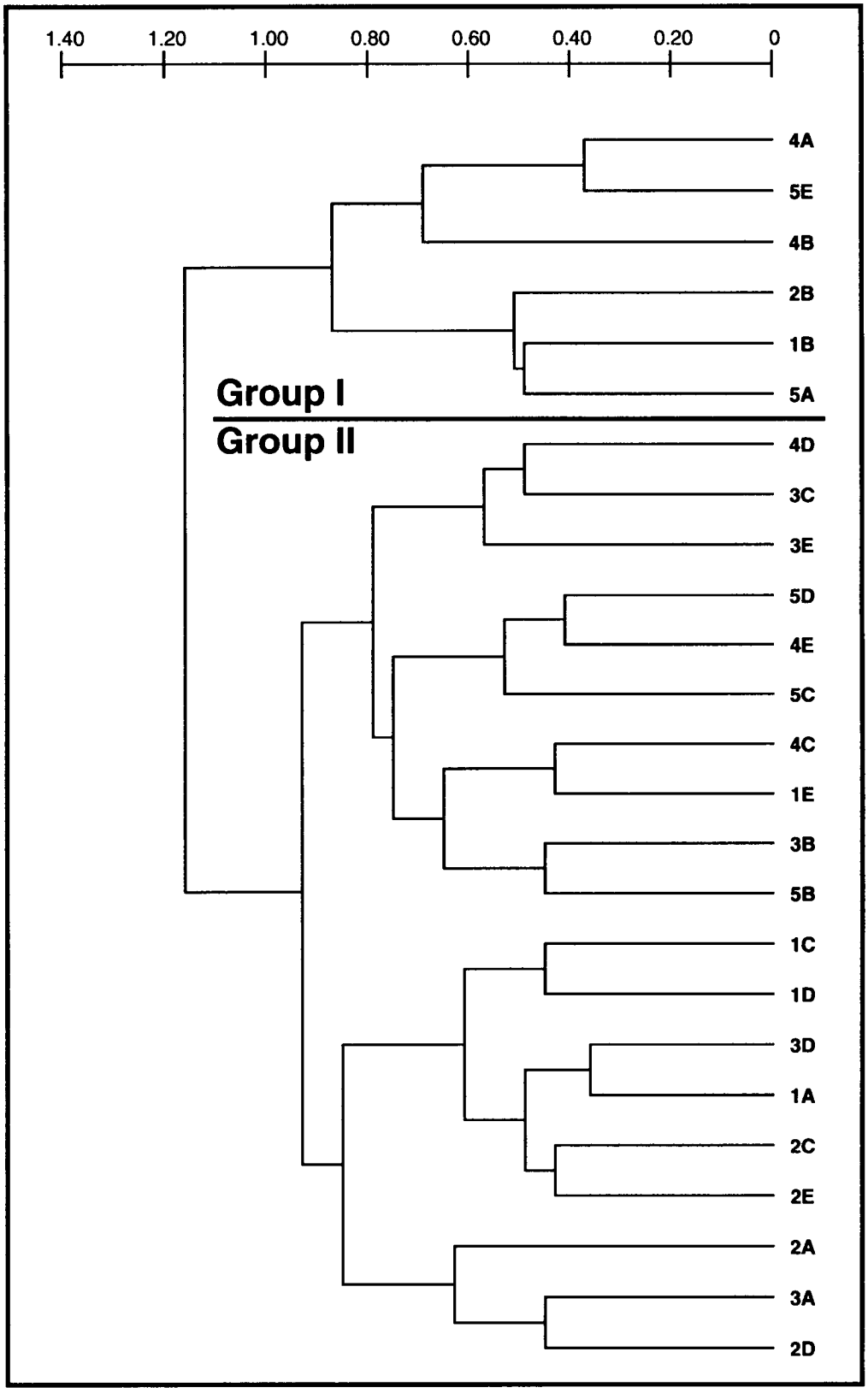


Figure 4.51. Site group dendrogram for MAI-686 stations sampled during Cruise 1.

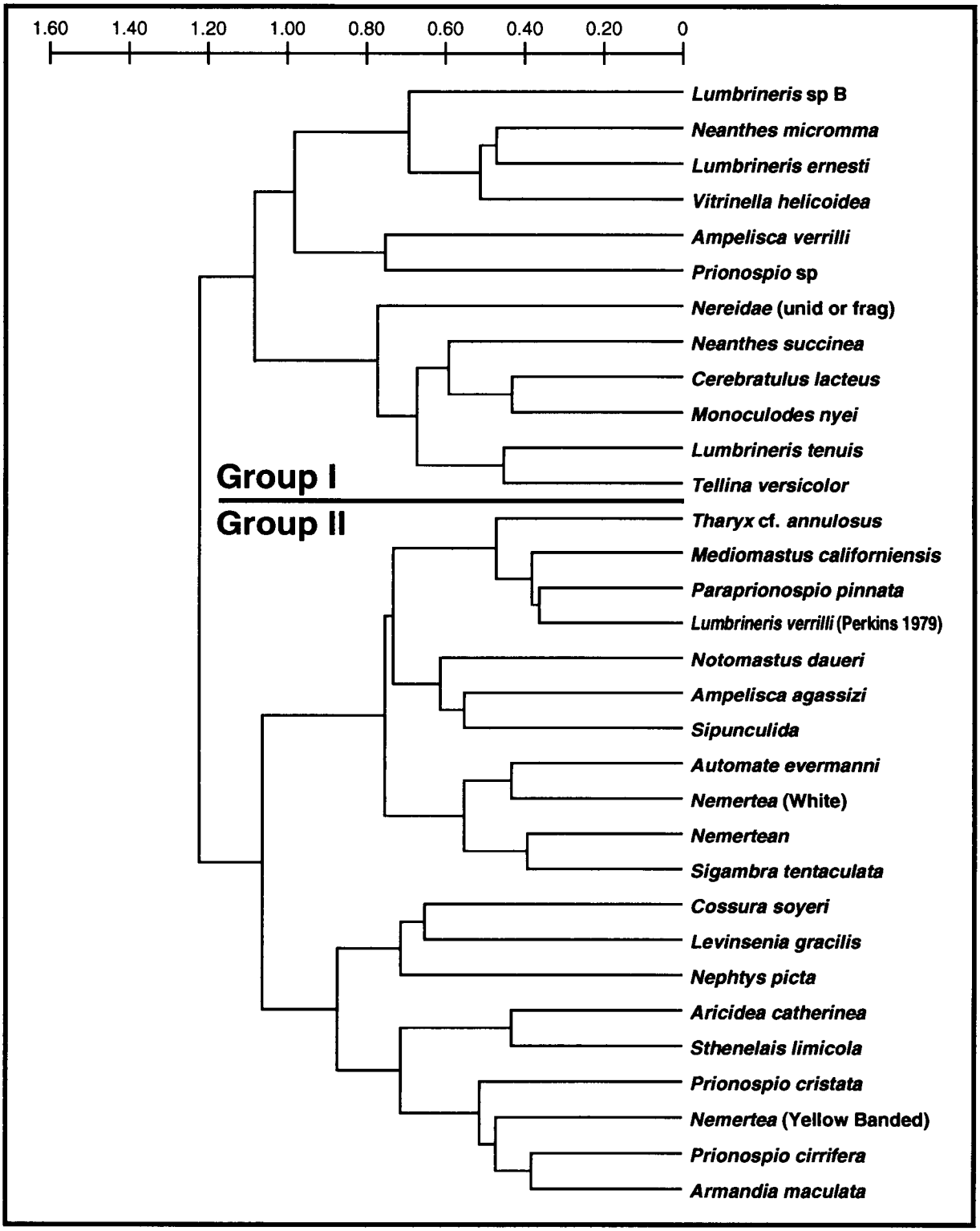


Figure 4.52. Species group dendrogram for MAI-686 stations from Cruise 1.

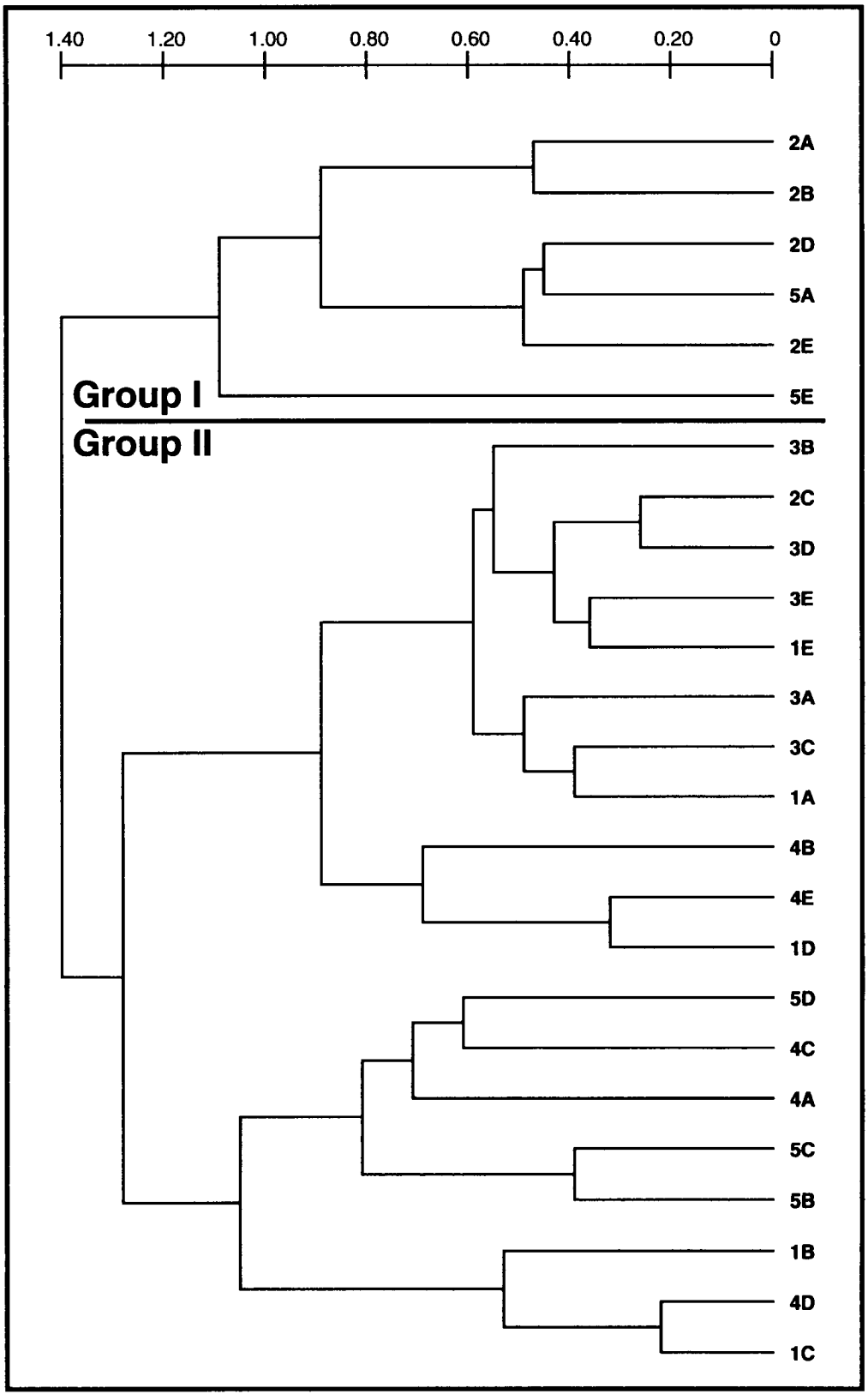


Figure 4.53. Site group dendrogram for HI-A389 stations sampled during Cruise 1.

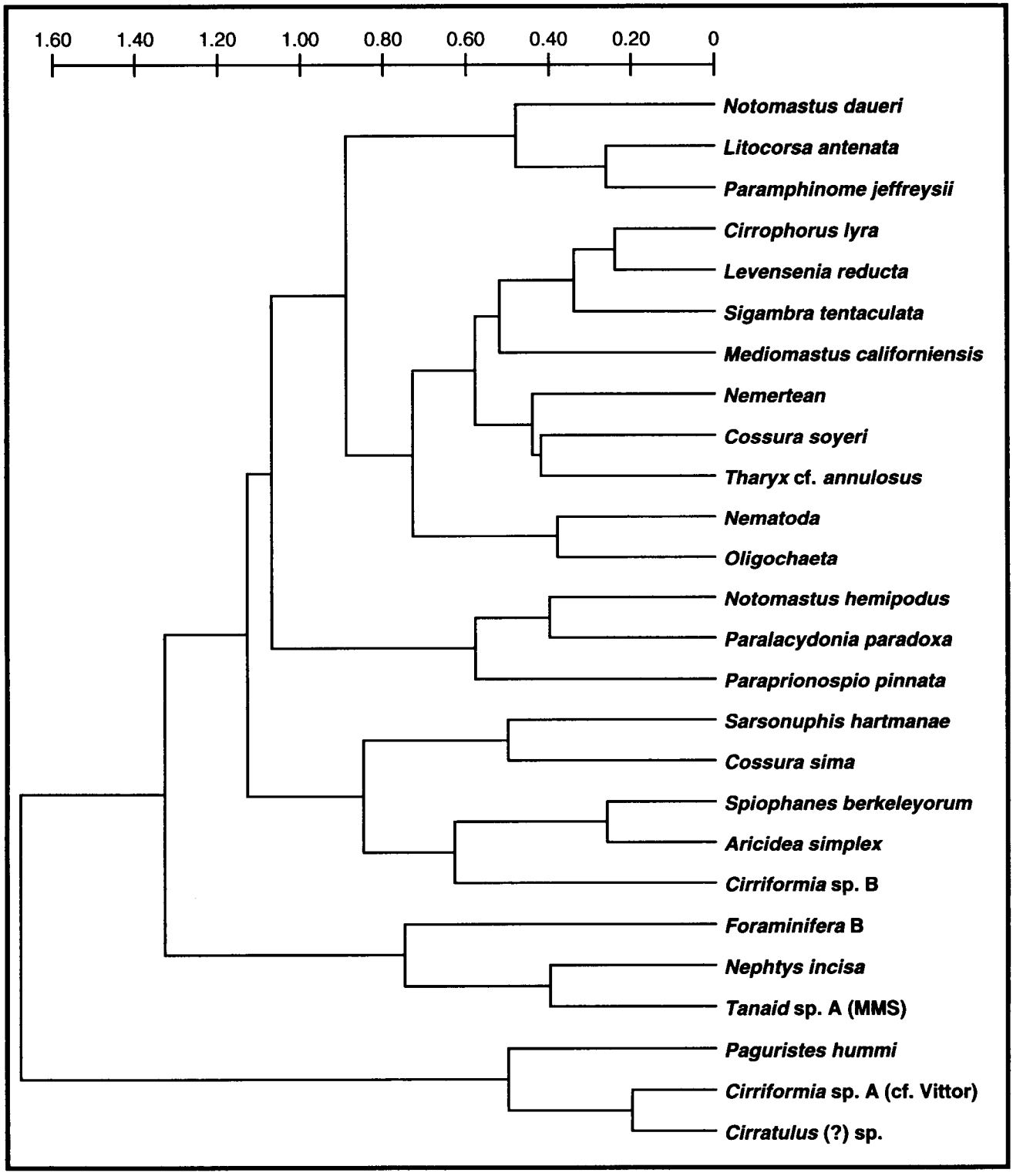


Figure 4.54. Species group dendrogram for HI-A389 stations from Cruise 1.

there does not appear to be an abundance difference between groups. HI-A389 had isolated stations grouped in clusters, and showed no trends.

4.9.5 Comparison with Other Studies.

As reported in section 4.3, all three study areas had larger amounts of sand in the sediments nearest the platforms. At MU-A85, nearfield sediments contained 60-80% sand. The percentage of sand decreased rapidly with distance from the platform to between 15 and 20%. Sediments around HI-A389 contained the smallest percentages of sand of all three sites - about 44% - and the sand percentage decreased to 12% or less in the farfield region. Sediments at MAI-686 contained the largest percentages of sand, about 75% in Ring 2, decreasing to 30% in the farfield region. The largest mean number of individuals was correlated with the HI-A389 sediments having the smallest percentage of sand, or conversely the largest percentages of silt-clay (Figure 4.45). HI-A389 sediments also contained, on the average, larger percentages of total organic carbon than at the other sites.

Hydrocarbon contaminants occurred in the sediments at all sites (Section 4.4). Both deep-water sites had high amounts of UCM close to the platform indicating degradation of hydrocarbons. MAI-686 had much smaller quantities of UCM as well as other forms of hydrocarbons. There does not appear to be any correlation between UCM and organismal distribution.

Pore water toxicity tests, defined in terms of success of fertilization of sea urchin eggs and in terms of normal larval development, revealed no stations at MU-A85 and only two stations at MAI-686 that showed positive toxicity results. At HI-A389, however, 6 stations, all northeast and southwest of the platform, on rings 2 and 3, showed toxic effects. All of these stations but one (3A) coincide with the large populations of cirratulid polychaetes reported above. The sediments may be toxic to sea urchin embryos, but they do not appear to inhibit settlement and survival of infauna.

4.10 Macroinvertebrates

Many physiological and environmental parameters affect the health of marine invertebrates. Among these are salinity, temperature, food availability, disease, parasitism and exposure to environmental contaminants.

One important measure of a population's health is its ability to reproduce the next generation. In the invertebrate reproduction portion of this study, we use measures of reproductive development and the presence of parasites and disease to assess the impact of offshore oil production on the health of natural populations of invertebrate species. Results obtained from this work effort describe the size and sex distributions of the invertebrate populations, the stages of reproductive development represented in the population, the health of the population based on the presence or absence of parasites, disease and abnormal development, and the instantaneous reproductive rate of individual species collected at sites near and far from offshore platforms.

Based on data collected during Cruise 1, the macroinvertebrate work element was reorganized to accommodate our ability to collect organisms in the field yielding pair-wise comparisons between near and far sites. As discussed previously, the paucity of individuals collected in the boxcore samples led us to drop the macroinfauna portion of the study. A large number of species including several polychaetes, nemerteans, and amphipods, were present at most platforms and at both the nearfield and farfield locations, but the number of individuals of any one species was inadequate for statistical analysis.

Target species collected from the trawls are chosen based on the most abundant invertebrates at each site collected on both cruises. The target species selected are chosen to provide comparisons between near and far-field and a diversity of taxa. Species overlap between the near and far-field sites was poor for Cruise 1, particularly at HI-A389. This situation was greatly improved during Cruise 2 through more intensive trawling efforts. Concentration on trawling increased the number of individuals collected and also resulted in more species overlap between the near and far stations.

Tables 4.42 and 4.43 list the species collected at each site and demonstrate the amount of overlap achieved during Cruises 1 and 2. Similar species were collected at each platform during both cruises, but some differences in species composition exist. On Cruise 2 one crab species (*Portunus spinimanus*) and 3 shrimp species (*Solonocera atlantidis* and 2 unidentified species at this time) were collected that had not been collected previously. On the other hand several species, including *Echinocardium flavescens* (heart urchin), 3 molluscs (*Murex fulvescens, Anadara transversa* and *Pitar cordatus*), 3 crabs (*Raninoides louisianensis, Dromida antillensis,*

Table 4.42. Animals collected from each study site at near- (N) and far-field (F) stations during Cruise 1 (if the full complement of histological samples (10) were not collected, the number of animals collected is indicated).

Species	MU-A85 N	MU-A85 F	MAI-686 N	MAI-686 F	HI-A389 N	HI-A389 F
Shrimp						
Penaeus aztecus	X	X	X	X		
Trachypenaeus similis			X	X	9	
Sicyonia brevirostris						
Sicyonia dorsalis				X		
'broken back'						
Crabs						
Callinectes similis		7	5	X		
Portunus gibbesii			X	X		
Portunus spinicarpus	X	X			X	
Acanthocarpus alexandria						X
Myropsis quinquespinosa					X	X
Dromidia antillensis						9
Raninoides louisianensis		9				
Munida irrasa					X	
hermit crab					8	
Molluscs						
Amusium papyraceum	X	X				
Anadara transversa		X				
Pitar cordatus		X				
Murex fulvescens					X	
Polystira albida		X				
Echinoderms						
Astropecten cingulatus	X	X			6	
Astropecten duplicatus		X		X		
brittle star						
Echinocardium flavescens					X	
Stomatopod						
Squilla empusa				X		

Table 4.43. Animals collected from each platform at both the near- and far-field stations during Cruise 2 (if the full complement of histological samples (10) were not collected, the number of animals collected is indicated).

Species	MU-A85 N	MU-A85 F	MAI-686 N	MAI-686 F	HI-A389 N	HI-A389 F
Shrimp						
Penaeus aztecus	X	X	X	X		
Trachypenaeus similis	3	X	X	X		
Solenocera atlantidis	X	X			X	6
Shrimp 'B'					X	
Shrimp 'C'					4	
Crabs						
Callinectes similis	3	9	X	X		
Portunus gibbesii			X			
Portunus spinicarpus	X	X			X	2
Portunus spinimanus			X			
Acanthocarpus alexandria					5	X
Myropsis quinquespinosa					3	X
hermit crab					X	X
Molluscs						
Amusium papyraceum	X	X				
Polystira albida	X	X				
Echinoderms						
Astropecten cingulatus	7	9			5	5
Stomatopod						
Squilla empusa	X	X	X	X	X	7

and *Munida irrasa*), and 3 shrimp species (*Sicyonia dorsalis*, *Sicyonia brevirostris* and 'broken-back') collected during Cruise 1 were not collected on Cruise 2. The target species which we were able to collect during both cruises include a starfish (*Astropecten cingulatus*), 3 portunid crabs (*Portunus gibbesi*, *Portunus spinicarpus*, and *Callinectes similis*), two species of shrimp (*Penaeus aztecus*, and *Trachypenaeus similis*) and a scallop (*Amusium papyraceum*), representing each of the four major groups of organisms.

4.10.1 Size Frequency Distributions

Size-frequency distributions were constructed for all species captured in the trawls at all platforms. Chi square analyses were conducted on the size frequency distributions for species collected at both the near and far-stations for each platform. However, only those which show significant differences between the near and far are presented.

For Cruise 1, the size frequency distributions for *Penaeus aztecus* and *Astropecten cingulatus* at GA-288; *Sicyonia dorsalis* and *Squilla empusa* at MAI-622; and *Portunus spinicarpus* and *Astropecten cingulatus* at MU-A85 are significantly different between the near and far-field stations (Table 4.44). For each of these species except the distributions are significantly different because the individuals collected at the far station are larger than those individuals collected at the near station (Figure 4.55). Six significant results out of a possible 24 tries (Table 4.44) are more significant results than would be expected by chance (Binomial test, $\alpha = 0.10$; Daniel 1978). Two of the species which showed significantly different size class distributions are shrimp. If only shrimp species are considered, 2 significant results out of 9 possible tries (Table 4.42) are fewer than would be expected by chance (Binomial test, $a = 0.05$), so shrimp, in general, are neither larger or smaller at the near or far-field sites as compared to the other. Starfish (*Astropecten cingulatus*) have significantly different size-frequency distributions at MU-A85. Starfish are generally larger far from the platform, so no pattern emerges. These results suggest that, overall, the individuals collected from populations at the far stations are larger than those found at the near stations. The other overlapping species collected

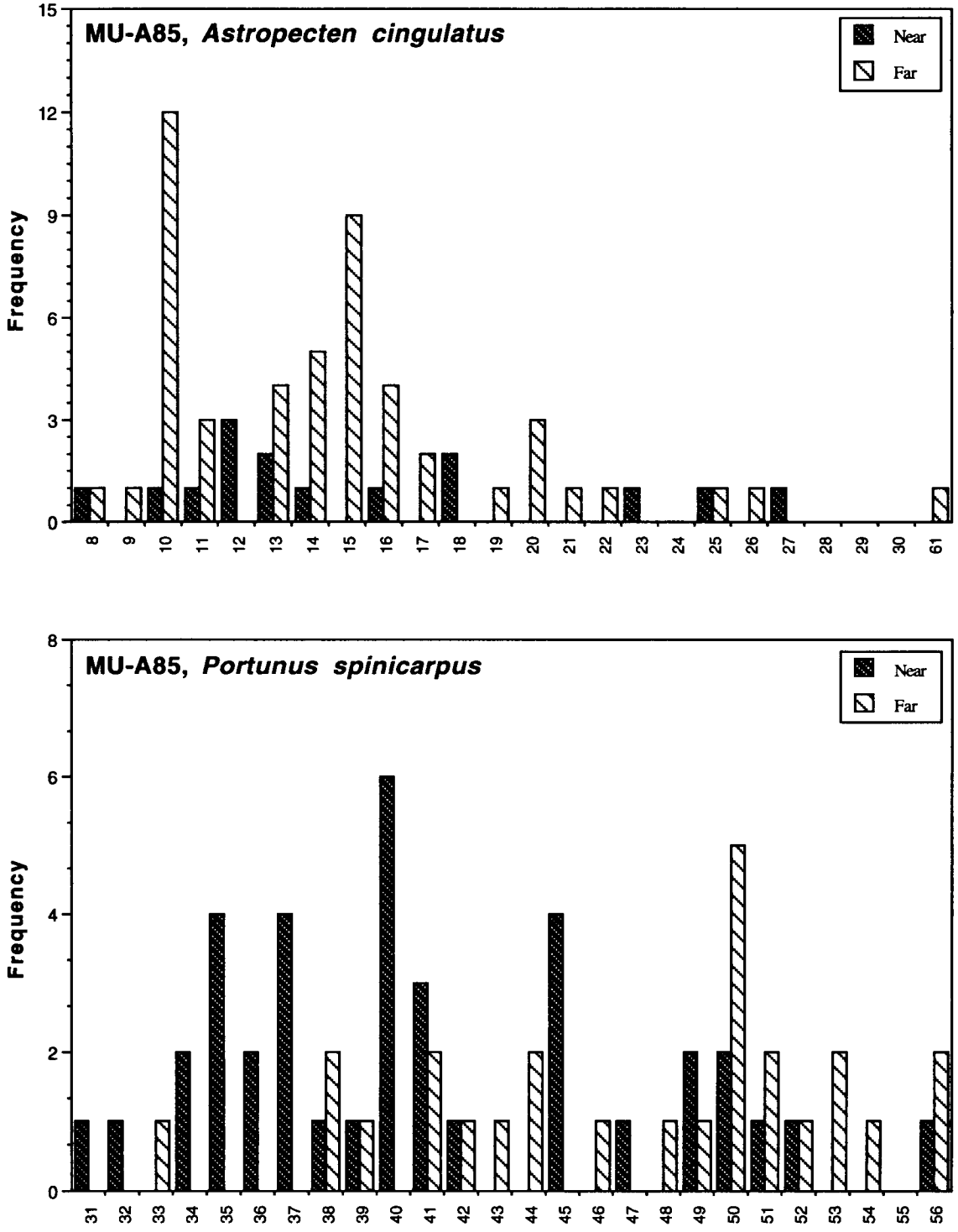


Figure 4.55. Size frequency distributions for species from Cruise 1.

Table 4.44. Results of chi square analyses for overlapping species comparing size-frequency distributions between the near- and far-field stations for Cruise 1 (significant results (P=0.100) are shown in bold type).

Platform	Species	Probability
HI-A389	*M. quinquespinosa*	0.232
MAI-686	*T. similis*	0.837
	P. aztecus	0.120
	A. duplicatus	0.110
	P. gibbesii	0.446
	C. similis	0.273
MU-A85	*P. aztecus*	0.351
	A. cingulatus	**0.023**
	P. spinicarpus	**0.057**
	A. papyraceum	0.575

Table 4.45. Results of chi square analyses for overlapping species comparing size-frequency distributions between the near and far-field for Cruise 2 (Significant results (P=0.100) are shown in bold type).

Site	Species	Probability
MU-A85	*P. aztecus*	0.359
	S. atlantidis	0.286
	P. spinicarpus	0.972
	C. similis	0.294
	S. empusa	0.528
	A. cingulatus	0.518
	A. papyraceum	**0.009**
	P. albida	0.756
MAI-686	*T. similis*	**0.100**
	P. aztecus	0.242
	C. similis	0.569
	S. empusa	0.251
HI-A389	*A. alexandria*	0.572
	S. empusa	0.623
	hermit crab	0.928
	S. atlantidis	0.441
	A. cingulatus	0.333

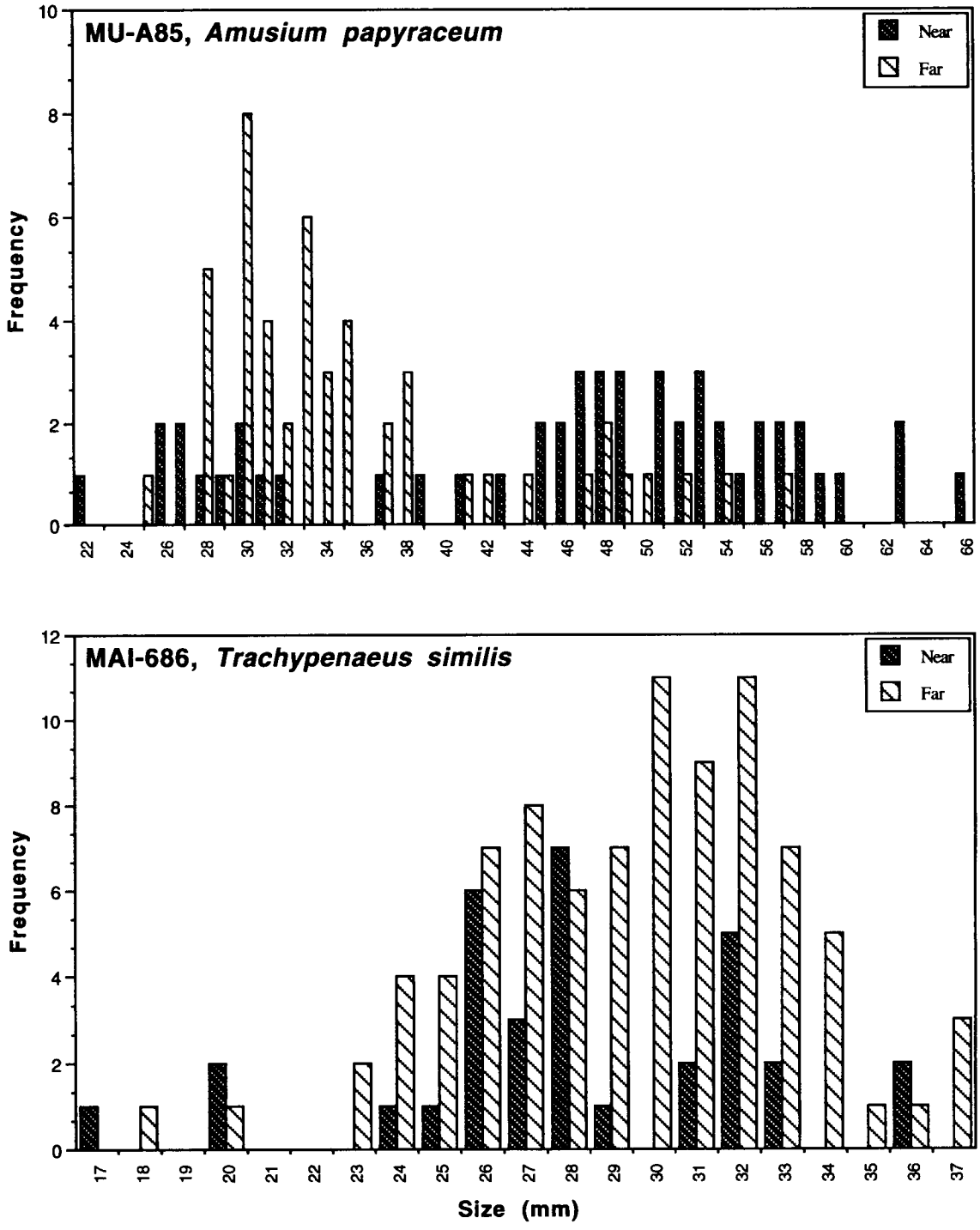


Figure 4.56. Size frequency distributions for species from Cruise 2.

during Cruise 1 showed no significant differences between the size frequency distributions from the near and far stations.

For Cruise 2, species overlap was more extensive as were the number of individuals collected (Table 4.43). Size-frequency distributions were constructed for these sites and again only the distributions that are significantly different by chi square analysis are shown (Table 4.45 and Figure 4.56). There are no significant differences in size frequency distribution between species collected at the near and far stations at HI-A389. At MAI-686, the size frequency distribution of *Trachypenaeus similis* is significantly different (P = 0.100), with the individuals at the far station during Cruise 1 showed no significant differences between the size frequency distributions from the near and far stations.

For Cruise 2, species overlap was more extensive as were the number of individuals collected (Table 4.43). Size-frequency distributions were constructed for these sites and again only the distributions that are significantly different by chi square analysis are shown (Table 4.45 and Figure 4.56). There are no significant differences in size frequency distribution between species collected at the near and far stations at HI-A389. At MAI-686, the size frequency distribution of *Trachypenaeus similis* is significantly different (P = 0.100), with the individuals at the far station being larger than those collected at the near. The scallop, *Amusium papyraceum*, at MU-A85 has a size frequency distribution significantly different (P = 0.009) at the near and far stations, with the larger individuals collected from the near station. These (2 out of 17 tries) are fewer significant results than would be expected by chance (Binomial test, α = 0.10; Table 4.45). These results indicate that the individuals collected during Cruise 2 show no pattern in size-frequency distributions between the near and far stations.

Because more extensive data was collected concerning the sex of individuals during Cruise 2, some species were analyzed as to differences in size class distributions between the near and far sites based on sex. At MU-A85, *P. aztecus*, and *S. atlantidis* were analyzed in this way and the size-frequency distributions of neither the males nor females was significantly different. *P. aztecus* and *C. similis* at MAI-686 were also analyzed as to differences in size based on sex, again with no significant results (Table

4.46). Therefore, the size-frequency distributions of both males and females for these species are similar at the near and far stations.

Several similar species were collected during both Cruise 1 and 2, so some between cruise comparisons can be made. No species at HI-A389 or MAI-686 had significantly different size class distributions between the two cruises (Table 4.47). At MU-A85, *A. papyraceum* and *P. spinicarpus* have significantly different size class distributions at the far station (P = 0.000 and P=0.051, respectively; Table 4.47). In both cases, the larger individuals were collected during Cruise 1 (Figure 4.57). Similarly, at MAI-686, *T. similis* at the near station and *C. similis* at the far station also have significantly different size-frequency distributions when comparing Cruise 1 and Cruise 2 (P=0.084 and P = 0.000 respectively; Table 4.47). In the case of *T. similis* it is clear that larger individuals were collected on Cruise 1 (Figure 4.57), but the trend is not as clear for *C. similis*. The results for *C. similis* are highly significant, but it is difficult to determine whether the individuals collected on Cruise 1 are larger or smaller than those for Cruise 2. The individuals collected during Cruise 2 had a very large number in the 30 to 40 mm size range, but also had the largest individuals collected, with several between 80 and 100 mm. This suggests that the differences in the distributions between Cruise 1 and Cruise 2 may be biased by the high number of small individuals collected during Cruise 2 when compared to the small number of total individuals collected during Cruise 1. The significant differences mostly occur because the individuals collected during Cruise 2 are smaller than those collected during Cruise 1. These results suggest that there were either more young individuals, possibly the young-of-the-year, collected during the spring compared to the winter cruise indicating a spawning event sometime between the cruises, or that the improved trawling was more efficient at collecting the smaller individuals.

4.10.2 Reproductive Development

Information concerning reproduction in crab species was collected during Cruise 1 and 2 by determining the percentage of the females collected in the trawls that were gravid. Percent gravid then, is the ratio of gravid female crabs, identified by the presence of the egg sac or 'sponge' on the ventral carapace, to the total number of female crabs collected. Percent

Table 4.46. Results of chi square analyses for overlapping species comparing the size-frequency distributions of each sex between the near- and far-field stations for Cruise 2 (significant results (P=0.100) are shown in bold type).

Platform	Species - Sex	Probability
MU-A85	*P. aztecus* - female	0.415
	P. aztecus - male	0.628
	S. atlantidis - female	**0.168**
	S. atlantidis - male	0.735
MAI-686	*P. aztecus* - female	0.274
	P. aztecus - male	0.347
	C. similis - female	0.268
	C. similis - male	0.582

Table 4.47. Results of chi square analyses comparing size-frequency distributions of species collected during both Cruise 1 and Cruise 2 (significant results (P=0.100) are shown in bold type).

		Probability	
Platform	Species	Near	Far
MU-A85	*P. aztecus*	0.880	0.797
	A. cingulatus	0.582	0.505
	A. papyraceum	0.433	**0.000**
	P. spinicarpus	0.665	**0.051**
MAI-686	*T. similis*	**0.084**	0.124
	P. aztecus	0.258	0.607
	C. similis	0.110	**0.000**
HI-A389	*M. quinquespinosa*	0.252	0.585
	A. alexandria	--	0.237
	A. cingulatus	0.710	--
	hermit crab	0.747	--
	P. spinicarpus	0.256	--

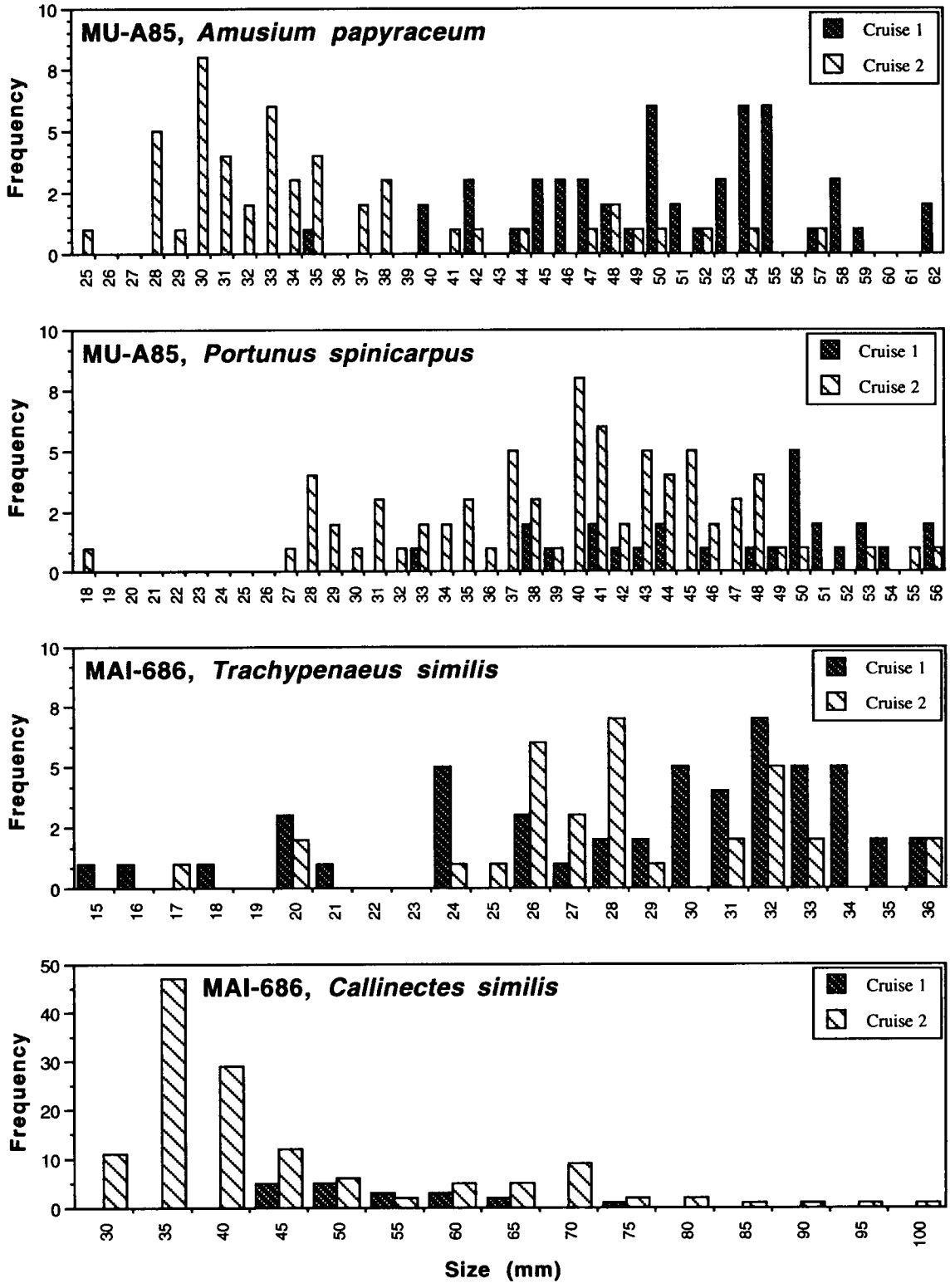


Figure 4.57. Size frequency distributions comparing species from Cruises 1 and 2.

gravid for the crab species is shown in Table 4.46 for Cruises 1 and 2. This table also provides some information as to the timing of the reproductive cycle of these species. At MAI-686 during Cruise 1, *P. gibbesii* had a very high percentage of gravid females; up to 60% of the females at the near station were in sponge and 38% at the far station. *P. gibbesii* was only collected at the near station of MAI-686 during Cruise 2 and were 12% gravid at that time. This would suggest that the majority of the gravid *P. gibbesii* at the far-field site may have spawned sometime between the two cruises, but some individuals had yet to spawn by the time of the second cruise. *C. similis* increased in percent gravid between the two cruises at MAI-686, but whether the population had spawned or was still preparing to spawn is difficult to determine. The data for *P. spinicarpus* at MU-A85 is also difficult to interpret because the percent gravid increases at the near and decreases at the far stations between the two cruises.

In general, Table 4.48 shows that more species increased in percent gravid between Cruise 1 and Cruise 2 than decreased, and that the species are gravid at the far station more frequently than at the near station. This is a common problem in analyzing data describing the percent gravid females with respect to an environmental stress. Environmental and physiological stresses tend to suppress reproduction in invertebrates either by delaying spawning or by decreasing the amount of spawn produced. This delayed reproductive development with proximity to the rig may explain the observation that the far stations had more gravid females than the near stations in most cases, and also the more frequent increase in percent gravid females at the near stations between the two cruises, when the far stations were more frequently decreasing in percent gravid. The timing of reproductive development and spawning may become clearer as more data are collected but a more time intensive survey will probably be required.

The average weight of the egg sacs can serve as a measure of the number of eggs produced and the stage of maturity of the eggs. The average weight of egg sacs is highly variable among the stations and between the near and far stations at the same station (Table 4.48). *C. similis* collected at MAI-686 far stations have fewer gravid females, but the sacs found are heavier. This may suggest that the eggs were more mature and that most of the population had spawned at MAI-686. Data of this kind may provide

Table 4.48. Percent female crabs collected that were gravid at each site for Cruises 1 and 2. For Cruise 1 columns, the bottom number indicates the average wet weight in mg of the egg sacs collected (NC = no individuals collected).

	MU-A85		HI-A389		MAI-686	
	Near	Far	Near	Far	Near	Far
Cruise 1						
C. similis	NC	22% 4484			0% 0	4% 2051
P. gibbesii	--- ---	--- ---			60% 876	38% 596
P. spinicarpus	0% 0	13% 776			---	---
Cruise 2						
C. similis	0%	0%	---	---	13%	13%
P. gibbesii	---	---	---	---	12%	NC
P. spinicarpus	7.1%	1.4%	0%	0%	10%	NC

more information as to whether the population is preparing to spawn or had recently spawned.

For Cruise 2 we added several analyses to improve our ability to assess reproductive development through visual inspection and gross dissection. These analyses were conducted on shrimp and crab species and provide a much larger, more complete data set for sex ratios and reproductive development. These analyses provide more information on sex and reproductive development than could realistically be obtained through histological analysis, because a larger number of individuals can be examined. Results of these analyses for shrimp and crab species are shown in Tables 4.49 and 4.50.

For several species (*T. similis*, *S. atlantidis*, *P. spinicarpus* and *P. aztecus*), the individuals collected were overwhelmingly female at both near and far stations regardless of the collection site (Table 4.49). The *C. similis* collected were mostly male. The remaining overlapping species which could be sexed by external observation were collected in an approximately 50:50 male to female ratio.

Table 4.49. The ratio of sexes for species collected during Cruise 2. Results were by external visual inspection. See Table 4.51 for the sex ratio information for animals histologically analyzed.

Platform	Species	Male : Female Near	Far
MU-A85	*T. similis*	0 : 3	2 : 41
	P. aztecus	23 : 27	16 : 34
	S. atlantidis	18 : 29	14 : 39
	C. similis	10 : 0	9 : 0
	P. spinicarpus	8 : 42	1 : 73
MAI-686	*T. similis*	2 : 31	4 : 84
	P. aztecus	6 : 15	21 : 29
	C. similis	127 : 22	119 : 15
HI-A389	*S. atlantidis*	22 : 10	3 : 3
	M. quinquespinosa	1 : 2	7 : 7
	A. alexandria	2 : 3	13 : 8
	P. spinicarpus	15 : 17	0 : 2

Table 4.50. Results of the visual inspection and gross dissection for the determination of sex and reproductive stage in shrimp and crab species collected during Cruise 2. Numerical rankings for reproductive stage are described in the text.

Site	Species	Reproductive Stage	Near Frequency	Far Frequency	Chi Square Probability
MU-A85	*P. spinicarpus*	1	27	18	0.406
		2	7	3	
		3	0	0	
		4	0	0	
	T. similis	1	8	1	0.377
		2	14	2	
		3	11	0	
		4	0	0	
	S. atlantidis	1	3	26	0.504
		2	2	8	
		3	0	2	
		4	0	0	
	P. aztecus	1	9	18	0.377
		2	11	8	
		3	1	1	
		4	1	0	
MAI-686	*C. similis*	1	1	2	0.228
		2	3	1	
		3	0	1	
		4	0	0	
		5	0	2	
	P. aztecus	1	4	16	0.406
		2	3	7	
		3	0	0	
		4	0	0	
	T. similis	1	15	47	0.423
		2	1	11	
		3	3	12	
		4	2	3	

Each female shrimp and crab was dissected and a numerical value assigned to it depending on its stage of reproductive development (Table 4.50). For the majority of the species, the individuals were about midway in their reproductive development, although some individuals of certain species were more advanced. Chi square analyses were conducted on overlapping species from each platform to determine if there was a difference in the distribution of stages of reproductive development between the two sites at each platform. No significant results were obtained, suggesting that there are no differences in the stages of reproductive development exhibited by individuals collected from the near and far stations.

4.10.3 Histology and Histopathology

A total of five individuals per species were examined at both far and near stations from Cruise 1. Histological sections were stained and examined microscopically for sex, stage of reproductive development and the presence of parasites and pathogens or other disease conditions. The results of these analyses is presented in Table 4.51 for Cruise 1 only. Cruise 2 samples are still being analyzed.

Histological analyses were concentrated on those species which have overlap between the near and far stations at each platform. We also analyzed some samples from HI-A389 regardless of whether we had overlap because either we collected that species at another platform so that other information exists, or because we collected it on Cruise 2 at HI-A389. Other samples are being held in reserve in 70% alcohol and will be analyzed in the future if that species is collected again to provide between cruise comparisons. For some species, *T. similis* in particular, the females are much larger than the males. The sampling protocol called for preservation of the larger individuals, males and may have been inadvertently excluded. A larger data set for some species on Cruise 2 obtained by external inspection, will provide a better data set because equal number of males and females were preserved for histological analysis.

The sex ratios of the 5 individuals analyzed histologically range from 100% female to nearly 100% male (Table 4.51). No significant differences are observed in sex ratio between the distribution of stages of sexual

Table 4.51. Results of histological analysis of Cruise 1 samples (M=male, F=female). Numerical values for reproductive stage are defined in the text. For parasites, A=absent, P=present (more details provided in text).

		NEAR			FAR		
Site	Species	Sex	Reproductive Stage	Parasites	Sex	Reproductive Stage	Parasites
MU-A85	*P. aztecus*	M	3	P	M	2	A
	P. aztecus	M	3	P	M	3	P
	P. aztecus	M	3	P	M	3	P
	P. aztecus	F	3	P	M	2	P
	P. aztecus	F	2	P	F	4	P
MU-A85	*P. spinicarpus*	F	2	A	M	3	A
	P. spinicarpus	M	2	A	F	2	A
	P. spinicarpus	M	2	A	F	3	A
	P. spinicarpus	M	3	A	F	3	A
	P. spinicarpus	F	3	A	F	3	A
MU-A85	*A. papyraceum*	M,F	5	A	M,F	5	A
	A. papyraceum	M,F	5	A	M,F	5	A
	A. papyraceum	M,F	5	A	M,F	4	A
	A. papyraceum	M,F	5	A	M,F	4	A
	A. papyraceum	-	-	-	M,F	4	A
MU-A85	*A. cingulatus*	M	late	A	F	early	A
	A. cingulatus	M	late	A	F	early	A
	A. cingulatus	F	early	A	F	early	A
	A. cingulatus	F	early	A	F	early	A
	A. cingulatus	F	early	A	-	-	-
MU-A85	*A. duplicatus*	-	-	-	M	late	A
	A. duplicatus	-	-	-	F	late	A
	A. duplicatus	-	-	-	M	late	A
	A. duplicatus	-	-	-	M	late	A
	A. duplicatus	-	-	-	F	late	A

Table 4.51. Cont'd.

Site	Species	NEAR Sex	NEAR Reproductive Stage	NEAR Parasites	FAR Sex	FAR Reproductive Stage	FAR Parasites
MAI-686	*P. aztecus*	F	3	P	F	2	P
	P. aztecus	F	2	P	F	2	P
	P. aztecus	M	3	P	F	3	P
	P. aztecus	F	2	P	M	3	P
	P. aztecus	F	2	A	F	3	A
MAI-686	*T. similis*	F	3	P	F	3	A
	T. similis	F	3	P	F	2	A
	T. similis	F	3	P	F	2	P
	T. similis	F	2	A	F	3	A
	T. similis	F	2	P	F	3	A
MAI-686	*C. similis*	F	1	A	M	3	A
	C. similis	M	3	A	F	3	A
	C. similis	M	4	A	F	3	A
	C. similis	-	-	-	M	3	A
	C. similis	-	-	-	M	4	A
MAI-686	*P. gibbesii*	F	2	A	M	3	A
	P. gibbesii	F	2	A	F	1	A
	P. gibbesii	M	3	A	M	3	A
	P. gibbesii	M	3	A	F	2	A
	P. gibbesii	F	2	A	M	3	A
MAI-686	*A. duplicatus*	M	mid	A	F	late	A
	A. duplicatus	F	late	A	F	late	A
	A. duplicatus	F	late	A	M	mid	A
	A. duplicatus	F	late	A	F	late	A
	A. duplicatus	-	-	-	M	late	A

Table 4.51. Cont'd.

Site	Species	NEAR Sex	NEAR Reproductive Stage	NEAR Parasites	FAR Sex	FAR Reproductive Stage	FAR Parasites
HI-A389	*T. similis*	F	3	A			
	T. similis	F	3	P			
	T. similis	F	3	A			
	T. similis	F	3	A			
HI-A389	*P. spinicarpus*	M	3	A			
	P. spinicarpus	M	3	A			
	P. spinicarpus	M	3	A			
	P. spinicarpus	M	3	A			
	P. spinicarpus	F	2	A			
HI-A389	*A. cingulatus*	F	early	A			
	A. cingulatus	F	early	A			

development between the near and far stations at any platform. It is interesting to note how advanced in reproduction the species are in general for a winter sampling period. Results corroborate observations of the number of individual females found in sponge among the crab species at these platforms (Table 4.48).

Each histological sample was also scanned for the presence of parasites, pathogens and other evidence of disease. Unfortunately, this analysis was hampered by poor preservation of the more interior tissues of the larger organisms. Fixative did not penetrate completely into the tissues of these larger individuals, so that some disintegration of the digestive gland tissues by autolysis occurred during fixation. This problem has been solved for Cruise 2. Furthermore, little information on the normal histology or the histopathology of the species is available in the literature, *P. aztecus* being an important exception so that comparable histopathological analysis will require more detailed study of the normal histology than is presently available.

Even so, the slides have been scanned for parasites and pathologies, and occurrence of invasive organisms has been noted, while identifications have not yet been made. Some generalities can be drawn from this preliminary analysis. In all cases more parasites were observed in the tissues of *P. aztecus* than any other species at any site. The parasites found in *P. aztecus* were concentrated in the gonad area and the digestive diverticula. Most appear to be parasitic cestode worms. *P. aztecus* was also noted to be infected with what is believed to be *Baculovirus* sp. (Murchelano and MacLean 1990). This virus is responsible for mass mortalities in artificial mariculture situations and its presence in the native stock is interesting. *T. similis* was also frequently found to be infected with parasitic worms. Particularly interesting for this species is MAI-686, where parasites were identified in all but one of the samples from near the platform, and in only one individual from the far station. Chi square analyses were conducted on the parasite presence/absence results for both *P. aztecus* and *T. similis*. Neither species showed a significant difference in the presence of parasites in their tissues between the near and far stations (P=0.162 for *T. similis*; P=0.256 for *P. aztecus*). Further analyses may change some results in regard to parasite prevalence though the general trends should remain the same. Almost all individuals analyzed had parasites present in the tissues

regardless of whether they were collected from the near or far station. Infection intensity is currently being analyzed to help discern differences between the near and far field stations.

Other conditions that have been noted are evidence of wound repair and inflammation in the digestive regions of some of the shrimp. At this point the crab species appear to be relatively parasite-free, but several individuals appear to have abnormalities in the lamallae of the gills. The starfish and scallops analyzed histologically also appear to be free of parasites. These samples will be re-analyzed as more information on common parasites and disease conditions becomes available.

4.10.4 Reproductive Immunological Probes

The rate of instantaneous reproductive development can be measured using species specific immunological probes. Work on these probes is progressing for the scallop and 3 portunid crabs. The eggs collected from these species during Cruise 1 were injected into rabbits and all have produced a high antibody titre. Purification and calibration of the probe is underway.

The goal of this portion of the program is to assess the effects of offshore oil and gas exploration and production on population health as described by variations in the reproductive effort of specific invertebrates species. However, very little is known about the reproductive effort of most continental shelf invertebrates, particularly as it relates to environmental contamination from petroleum hydrocarbons or heavy metals. Accordingly, the way in which reproductive effort is affected in these species is also largely unknown. Several measures of reproductive development have been used to assess any differences in the reproductive effort of invertebrates living near and far from oil producing platforms. The platforms may exert an influence on reproductive development by affecting food supply, increasing disease prevalence and/or exposure to contaminants.

4.10.5 Summary

Influence of proximity to the platforms can be seen in certain portions of the data collected regarding the reproductive effort of the resident

populations, including size class distributions, percent gravid females; stage of reproductive development; and prevalence of disease, parasites and pathologies. Environmental stresses tend to decrease growth rates in invertebrate species (Sindermann 1983; Peterson and Black 1988). Although few populations sampled in this study were significantly different in size at the near and far stations, some significant differences were present did occur. If a difference in size class distribution existed at a platform between the individuals collected at the near and far-field stations, the organisms collected from the far-field station were typically larger. Differences in size-frequency distributions, with the smaller individuals being found in closer proximity to these platforms, could be explained by (1) the platforms may have an adverse affect on growth in these species; (2) larger individuals may actively move away from the platforms, or (3) mortality of adults may be higher nearer the platform. These three explanations could be caused by decreased food resources near the platforms or increased exposure to contaminants that detrimentally affect growth and health, including vulnerability to predators. Food resources for most of these species was not directly sampled, although the paucity of macroinfauna in the boxcores may suggest an overgrazing of the benthic populations and therefore reduced food availability.

Evidence for an impact of proximity to the rig on reproductive development is less clear from the data. Environmental stresses such as exposure to contaminants can affect reproductive activity by delaying reproductive development or by decreasing the quantity of gametes produced. The percent gravid data provided in Table 4.46 is a good example of environmental stress. In this table it is obvious that in some cases the far station has a higher proportion of gravid females than the near station. One explanation is that exposure to contaminants has slowed reproductive development in those organisms near the platform, so that the population at the far station is nearer to being ready to spawn. Alternatively, higher food resources further from the platforms may have increased reproductive capacity. Histological analysis follows the trend in gravid females, with more reproductively advanced individuals found far from the platforms. Significant differences in reproductive development must be followed by more time intensive studies to identify the reasons for the differences observed.

While exposure to contaminants can delay or reduce the reproductive development of individuals, the reproductive cycle also serves, at least in oysters, to depurate certain contaminants, particularly PAHs, from the tissues. In oysters from Confederate Reef (Galveston Bay, Texas), individual oysters with the highest PAH body burden also were more reproductively advanced, and males had lower body burdens than females (Ellis et al. in press). For PAH's at least, a mechanism for uptake and depuration seems to be clearly related to the reproductive cycle. First, PAH's are lipid soluble and are therefore found in the eggs of females which are high in lipid content, and as these individuals spawn, the PAH's are depurated through release of the gametes. Therefore, in species collected at the platforms, variations in the concentration of certain contaminants may follow the reproductive cycle by increasing and decreasing with spawn events.

Other environmental parameters such as temperature, salinity and food availability affect the concentration of contaminant body burden and also determine disease levels at least in oysters, because of their control over the reproductive cycle (Wilson et al. 1990; Hofmann et al. 1992). Therefore, it is clear that determining the effects of proximity to a platform and the chemical contaminants measured on the reproductive cycle of these organisms is a complex issue involving the interaction of several parameters. The difference between reproductive development noted between the winter and summer cruises is fairly distinct. For most species, more individuals advanced in reproductive development were taken during the winter. More data from future cruises will help determine the actual reproductive season for these species. However, the presence of reproductively advanced individuals at both the near and far stations says nothing about the quantity of spawn produced by those populations, or the viability of the larval stages.

For oysters, a relationship exists between disease, contaminant body burden, and reproduction (Wilson et al. 1992). In certain species collected during this study, particularly *P. aztecus* and *T. similis* population health as defined by the presence of parasites and disease, differed between the near station and far station. More evidence of parasites and disease in shrimp taken at the near station may be the result of several factors. First, the largest individuals collected in the trawls were preserved. If size is used as a surrogate for age in these species, these individuals should have been the

oldest collected. Older individuals should have a greater probability of being parasitized or diseased by virtue of their longer life and extended exposure to the chemical contaminants present in their environment. However, near-field shrimp tended to be smaller, so age seems not to be the explanation. Second, final hosts (probably fish) may be more common around rigs, thus a source of infectious stages may be higher. Third, the species may be more susceptible to parasites and diseases. However, we saw no correlation of reproduction and parasites was apparent. However, these correlations are inherently difficult to identify.

Improved sampling by concentrating on the trawl to insure better overlap of species and collection of more individuals will provide more extensive data sets for future comparison. The results obtained to this point suggest that there is some form of impact on populations living in close proximity to a platform. The individuals tend to be smaller, have a delayed reproductive development (at least in crab species) and have greater incidence of parasitism and pathologies. Further statistical analysis, particularly correlations between body burdens of chemical contaminants and stages of reproductive development will help clarify the relationship and impact of offshore oil production on these species. In the final analysis, a more intensive time series may be necessary to explain the origin of these trends.

4.11 Demersal Fish

The collection of demersal fishes has yielded a large number of both target and non-target specimens with a total of 51 families and 199 species. The first cruise (January and February, 1993), producing a total catch of over 3500 fish consisting of 38 families and 89 species, was collected at the near and far stations of five different oil platforms in the northern Gulf of Mexico. The HI-A389 near station yielded the largest number of families (19) and species (33) with a total catch of 583. The MU-A85 near station had the second highest yield with 18 families and 30 species and a total catch of 627. The HI-A389 stations had the best results for duplicate target specimens at both near and far stations, with *Urophycis cirrata* and *Pristipomoides aquilonaris* supplying enough biomass for chemistry, toxicology, and stomach contents.

With the exception of one species of serranid, all specimens used for analysis were identified. Samples of the unidentified serranid were sent to Dr. Carol Baldwin of the Smithsonian Institution who identified this specimen as *Hemanthias leptus*. A species of holocentrid, *Corniger spinosus*, that was caught in the final trawl at the MU-A85 far station may be a new record for the Gulf of Mexico. The reported range for *Corniger spinosus* was from South Carolina to Cuba and Brazil and was considered to be exclusively distributed in the tropical sector of the Western Atlantic Ocean. Recently, this species has been reported from the Canary Islands and off of Benin from the West Coast of Africa. A literature search has revealed no other record of this species in the Gulf of Mexico.

The second cruise (June, 1993) produced a total catch of 15,500 fish consisting of 45 families and 95 species from the near and far stations at three different oil platforms: MAI-686, MU-A85, and HI-A389 (Table 4.52). This was considerably more than the total catch (3500+) from GOOMEX Cruise 1 and was the result of improved trawling operations. The near station at MAI-686 yielded the largest number of families (30) and species (48), and the far station had the largest overall catch (4861). This large catch was the result of five species caught in large quantities: *Saurida brasiliensis* (300+), *Orthopristis chrysoptera* (600+), *Upeneus parvus* (1000+), *Peprilus burti* (800+), and *Syacium gunteri* (600+). Some of the fish collected at the near station at HI-A389 had visible tumors and discoloration. This was not evident at any of the other stations.

4.11.1 Fish Food Analysis

Stomach analyses were carried out on fish specimens taken from Cruises 1 and 2 to provide information concerning the food items analyzed for chemical contamination. For Cruise, 1 a total of 212 specimens were analyzed belonging to 19 fish species. These represented specimens taken at both near and far sites at the five preliminary stations. For Cruise 2, a total of 260 specimens were analyzed representing 9 species taken at near and far sites at the final three stations. Since larger numbers of specimens were collected during Cruise 2, the near/far comparison for this cruise rests upon a much firmer numerical database.

Table 4.52 Summary of Cruise 2 fish catches.

Site Station	Number of Trawls	Total Catch	Number of Families/ Species	Most Common Species (#'s)
MU-A85-1				
Near	20	1597	24/42	*Halieutichthys aculeatus* pancake batfish - 136 *Serranus atrobranchus* blackear bass - 245 *Pristipomoides aquilonaris* wenchman - 204
Far	18	1399	24/35	*Halieutichthys aculeatus* pancake batfish - 188 *Stenotomus caprinus* longspine porgy - 161 *Lagodon rhomboides* pinfish - 176
Total Catch		2996		
MAI-686-2				
Near	16	2848	30/48	*Anchoa hepsetus* striped anchovies - 600+ *Lutjanus campechanus* red snapper - 344 *Upeneus parvus* dwarf goatfish - 310
Far	22	4861	26/33	*Saurida brasiliensis* largescale lizardfish - 300+ *Orthopristis chrysoptera* pigfish - 600+ *Upeneus parvus* dwarf goatfish - 1000+ *Peprilus burti* gulf butterfish - 800+ *Syacium gunteri* shoal flounder - 600+
Total Catch		7709		
HI-A389-4				
Near	18	1740	24/42	*Ogcocephalus declivirostris* slantbrow batfish - 125 *Serranus atrobranchus* blackear bass - 368 *Pristipomoides aquilonaris* wenchman - 485
Far	20	2966	20/35	*Pontinus longispinis* longspine scorpionfish - 800+ *Pristipomoides aquilonaris* wenchman - 1000+
Total Catch		4706		

During Cruise 1, shrimp constituted over half the total food (55.8%) and taken together the several crustacean groups made-up almost two-thirds of the total food (64.8%). Basically the same food items appeared in the food of a given species at the near and far stations, but often they were in different proportions. Noteworthy was the fact that at site MAI-622 the shoal flounder (*Syacium gunteri*) showed 95% shrimp at the near station and 95% polychaetes at the far station. For this cruise the near/far comparisons were based upon small numbers of specimens. Detailed

information concerning specimens analyzed and food percentages is given in Tables 4.53 and 4.54.

For Cruise 2 sufficiently large samples were available for comparisons of at least two species at each station except at MU-A85 where the numbers were less than 20 individuals per station (Table 4.55). Food volumes ranged from less than 0.5 mL to 52.0 mL, but for a given species comparable volumes are shown at the near and far stations of a given site. Particularly low food volumes were found in species whose stomachs evert at the deep water stations (*Pontinus longispinis* and particularly *Pristipomoides aquilonaris*). In overview, fish made up over half the food (55.0%), crustaceans over one-third (37.7%), and squids and *Sargassum* leaves made-up the measurable remainder, with trace amounts of polychaetes, amphipods, and mantis shrimp.

At site MAI-686 near and far comparisons are based on two species, the sand seatrout (*Cynoscion arenarius*) and the shoal flounder (*Syacium gunteri*; Table 4.56). In both instances there is virtually no difference between the food at the near and far sites. Although polychaetes, shrimp, and crabs were encountered, *C. arenarius* fed almost entirely on fish including young lizardfish (*Synodus* sp.) and other forms. *S. gunteri* contained 100% shrimp at both near and far sites, with traces of polychaetes and amphipods.

At site MU-A85 near and far comparisons are based on two species, the three-eyed flounder (*Ancylopsetta dilecta*) and the inshore lizardfish (*Synodus foetens*). For *A. dilecta* at the near station fish made up 99% and crabs 1% of the food, with a trace amount of shrimp. At the far station fish constituted 60% and shrimp 40% of the food. Among the food items were young lizard fish (synodontids) as well as larvae and small juveniles of other fish species. At the near station *S. foetens* contained 100% squid (*Loligo* sp.) with trace amounts of mantis shrimp (*Squillidae*). At the far station *S. foetens* contained 95% fish (synodontids and others) and 5% squid (*Loligo* sp.).

At site HI-A389 near/far comparisons are based on three fish species, the three-eye flounder (*Ancylopsetta dilecta*), longspine scorpionfish (*Pontinus longispinis*), and wenchman (*Pristipomoides aquilonaris*). At the near station *A. dilecta* consumed fish (85%; including *Saurida brasilensis* and others), shrimp (13%), and crabs (2%). At the far station this species

Table 4.53. Results of stomach content analysis for Cruise 1 fish. The values represent the percent composition for each food category. The letter "e" indicates that all stomachs were empty.

Station Number	Fish Species	Number of Individuals	Silt, Sand	Tubes (Polych., amphip.)	Polychaete Worms	Squids	Crustaceans					Fishes	Flesh (undet.)
							Copepods	Amphipods	Shrimp	Crabs	Undet.		
1-N	*Centropristis philadelphica*	2							90	10			
	Equetus umbrosus	28							100				
	Pogonias cromis	3		50						10	40		
	Cyclopsetta chittendeni	2-e											
1-F	*Synodus foetens*	2-e											
	Urophycis cirrata	3	t	95							5		
	Centropristis philadelphica	7							90			10	
	Trichopsetta ventralis	5						10	20	20			50
2-N	*Lutjanus campechanus*	10							100				
	Archosargus probatocephalus	9		10				t	86	4	t		
	Cynoscion arenarius	3							100				
	Paralichthys albigutta	2							99			1	
	Syacium gunteri	11			27			6	67				
2-F	*Syacium gunteri*	6	t		t			2	98				
4-N	*Ogcocephalus declivirostris*	10			80							20	
	Urophycis cirrata	10-e											
	Pontinus longispinis	4-e											
	Caulolatilus intermedius	3			95			1	4				
	Hemanthias leptus	7							100				
	Pristipomoides aquilonaris	10							70			30	
4-F	*Urophycis cirrata*	7-e											
	Pontinus longispinis	10			1				9			90	
	Pristipomoides aquilonaris	10-e											
	Percent Composition			5.7	12.0	3.7	.1	.9	55.8	6.3	1.7	9.3	4.6

Table 4.54 Comparison of the food of fish species taken at both near and far stations for the five sites sampled during Cruise 1. The values in parenthesis represent the number of stomachs examined at each site.

Site	Fish Species	Near		Far		Food Category
MU-A85-1	*Centropristis philadelphica*	(2)	90%	(7)	90%	shrimp
			10%			crabs
					10%	fishes
MAI-686-2	*Syacium gunteri*	(11)	27%	(8)	t	polychaetes
			6%		2%	amphipods
			67%		98%	shrimp
HI-A389-4	*Urophycis cirrata*	(10)	empty	(7)	empty	
	Pontinus longispinis	(4)	empty	(7)	1%	polychaetes
					9%	shrimp
					90%	fishes
	Pristipomoides aquilonaris	(10)	70%	(10)	empty	shrimp
			30%			fishes
GA-288-5	*Orthopristis chrysoptera*	(1)	empty	(1)	21%	polychaetes
					1%	copepods
					3%	amphipods
					75%	"flesh" (undet.)

Table 4.55. The results of stomach content analysis for Cruise 2 fish. The values represent the percent composition for each food category. Trace amounts are denoted as "t".

Site and Species	No. of stomachs Examined	With Food	Food Volume (mL)	Sargassum Leaf	Polychaetes	Squids	Crustaceans Amphipods	Shrimp	Crabs	Mantis Shrimp	Fish
MU-A85											
A. dilecta - near	5	4	2.4					t	1		99
A. dilecta - far	2	2	1.3					40			60
S. foetens - near	16	6	16.0			100				t	
S. foetens - far	17	7	23.5			5					95
P. aquilonaris - near	20	6	<0.5	18				t	2		80
C. chittendeni - far	1	0	---								
L. campenchanus - far	12	6.	2.5			2		50			48
MAI-686											
C. arenarius - near	20	13	9.0		t			2			98
C. arenarius - far	20	14	5.5						t		100
S. gunteri - near	20	13	1.3		t		t	100			
S. gunteri - far	20	19	3.0		t		t	100			
HI-A389											
A. dilecta - near	20	13	2.5					13	2		85
A. dilecta - far	20	15	4.5	t	t			70	10		20
P. longispinis - near	14	10	2.0						40		60
P. longispinis - far	20	11	4.0						95		5
P. aquilonaris - near	20	9	1.5					60	t		40
P. aquilonaris - far	20	6	0.5					5	50		45
P. lethostigma - near	2	1	52.0								100
Percent composition				1.1	---	6.3	---	25.9	11.8	---	55.0

Table 4.56. Comparison of the food of fish species taken at both near and far stations for Cruise 2. The values in parenthesis represent the number of stomachs examined at each site.

Site and Species	Near		Far		Food Category
MU-A85					
Ancyclopsetta dilecta	(5)	t	(2)	40	Shrimp
		1		---	Crabs
		99		60	Fish
Synodus foetens	(16)	100	(17)	5	Squid
		t		---	Mantis Shrimp
				95	Fish
MAI-686					
Cynoscion arenarius	(20)	t	(20)	---	Polychaetes
		2		---	Shrimp
		---		t	Crabs
		98		100	Fish
Syacium gunteri	(20)	t	(20)	t	Polychaetes
		t		t	Amphipods
		100		100	Shrimp
HI-A389					
Ancylopsetta dilecta	(20)	---	(20)	t	*Sargassum* leaf
		---		t	Polychaetes
		13		70	Shrimp
		2		10	Crabs
		85		20	Fish
Pontinus longispinis	(14)	40	(20)	95	Crabs
		60		5	Fish
Pristipomoides aquilonaris	(20)	60	(20)	5	Shrimp
		t		50	Crab
		40		40	Fish

ate shrimp (70%), fish (20%; including Gobiidae, Bothidae, and others), crabs (10%), and trace amounts of polychaetes, and a *Sargassum* leaf. *P. longispinis* fed upon fish and crab at both the near and far stations. At the near stations the percentages were 60% fish and 40% crabs. At the far station crabs made up to 95% of the food and fish (including Gobiidae) only 5%. The crabs included a wide diversity of sizes and families. *P. aquilonaris* fed upon fish, shrimp, and crabs. At the near station fish made up 40%,

shrimp 60%, and crabs only a trace. At the far station fish were 45%, crabs 50%, and shrimp only 5%.

All the fish examined are carnivorous and feed largely upon fish, shrimp, crabs, and occasionally on squids. The proportions vary with location and season. In the present study there was little difference in the food of two fish species at the near and far sites of the shallow water station (MAI-686). However, considerable near/far differences were noted in the food of all the other fish species at the two deeper stations, and these differences may aid in explaining differences in chemical contaminants body burdens, at these stations.

4.11.2 Histopathology

While gross fish pathology is a potential response indicator of environmental status that is easy and economical to measure, it will not allow the identification of important contaminant-associated pathological abnormalities. Histopathological examination of selected tissues is widely recognized as a reliable method for assessing the adverse effects of exposure to anthropogenic contaminants in marine fishes. For example, certain pathological conditions in the liver of wild fishes morphologically resemble lesions induced in fishes by experimental exposure to a variety of toxicants including carcinogens. Furthermore, some of these lesions have also been shown to be positively associated with exposure to xenobiotic chemical contaminants in field studies.

Pigment-bearing macrophages are prominent features in fish spleen, kidney, and sometimes liver (Agius 1980). In advanced teleosts, they form discrete aggregations called macrophage aggregates (MAs) (Wolke et al. 1985a). Occurrence of MAs may vary depending on the size, nutritional status, or health of a particular fish species (Agius 1979, 1980; Agius and Roberts 1981; Wolke et al. 1985a, b). In addition, the number and size of MAs increase with age in some species (Brown and George 1985; Blazer et al. 1987). Suggested functions for these aggregates include the centralization of foreign material and cellular debris for destruction, detoxification or reuse (Ellis et al. 1976; Ferguson 1976).

Changes in splenic MA parameters (e.g., number, size) in relation to environmental contamination have been noted by several investigators

(Kranz and Peters 1984; Wolke et al. 1985b; Benyi et al. 1989; Spazier et al. 1992). Hence, it has been suggested that these structures may be sensitive but non-specific indicators of stress. Preliminary studies from the Environmental Monitoring and Assessment Program in the Gulf of Mexico demonstrated an increase in splenic MA number and percent of tissue replaced in most species from contaminated sites. Some species were better indicators of elevated tissue contaminants while others were good indicators of sediment contaminants (Blazer et al. submitted).

Histopathological evaluations have been performed on liver and spleen samples taken from 189 fish specimens examined from Cruise 1. No contaminant-related liver lesions (e.g., hydropic vacuolation, hepatic megalocytosis, hepatocellular neoplasms) were observed in any of the tissue sections. However, a number of other pathological findings were seen and these are summarized in Tables 4.57 and 4.58. Parasitic infections were the most common abnormalities, with microsporidians being the most prevalent. Two other prevalent lesions included inflammatory foci and granulomatous inflammation. These lesions, however, were usually associated with parasitic infections (e.g., nematodes).

Splenic MA analysis was performed on fishes from both near and far stations from two Cruise 1 sites, MAI-686 and MU-A85. Analysis was performed on specimens from these sites because flatfishes (*Syacium gunteri* or *Trichopsetta ventralis*) were collected at all stations and these sites were selected as final study sites. No analyses were performed on the scorpaenids (*Pontinus longispinis*) collected from site HI-A389 because the specimens were small and few spleens survived processing.

Preliminary analysis of the MA data collected for sites MAI-686 and MU-A85 indicates a statistically greater (P=0.0001) number of MAs per mm^2 at site MAI-686 (Figure 4.58A) with no difference between the near and far stations at either site. When the data are analyzed by MA size class (Figure 4.58A) the site difference is noted as a preponderance of small MAs (50-500 and 500-1000 μm^2 size classes) at site MAI-686 with no significant difference among stations for most other size classes.

Table 4.57. Histopathologic findings by species for GOOMEX Cruise 1.

Species	Tissue*	n	Lesion	% Prevalence
Pontinus longispinis	LV	40	Coccidians	12.5
			Granulomatous inflammation	17.5
			Inflammatory focus (foci)	5.0
			Nematodes	2.5
	SP	18	Granulomatous inflammation	5.6
Syacium gunteri	LV	108	Encysted helminths	0.9
			Granulomatous inflammation	1.9
			Inflammatory focus (foci)	13.9
			Microsporidians	22.2
			Nematodes	2.8
	SP	104	Sporozoan (?)[1]	3.8
Trichopsetta ventralis	LV	39	Granulomatous inflammation	30.8
			Inflammatory focus (foci)	23.1
			Microsporidians	15.4
			Nematodes	15.4
	SP	36	Sporozoan (?)[1]	5.6

* LV = Liver; SP = Spleen 1 Unidentified parasite, probable sporozoan

Table 4.58. Histopathologic findings by site for GOOMEX Cruise 1.

Site	Station	Species	Tissue	n	Lesion	% Prevalence
MU-A85	F	*Trichopsetta ventralis*	LV	19	Granulomatous inflammation	31.6
					Inflammatory focus (foci)	47.4
					Microsporidians	10.5
					Nematodes	5.3
	N	*Trichopsetta ventralis*	LV	20	Granulomatous inflammation	30.0
					Microsporidians	20.0
					Nematodes	25.0
			SP	19	Sporozoan (?)[1]	10.5
MAI-686	F	*Syacium gunteri*	LV	19	Inflammatory focus (foci)	15.8
	N	*Syacium gunteri*	LV	20	Granulomatous inflammation	5.0
					Inflammatory focus (foci)	35.0
					Microsporidians	35.0
					Nematodes	5.0
			SP	20	Sporozoan (?)[1]	10.0
HI-A389	F	*Pontinus longispinis*	LV	20	Coccidians	25.0
					Granulomatous inflammation	20.0
					Inflammatory focus (foci)	10.0
					Nematodes	5.0
			SP	15	Granulomatous inflammation	6.7

*LV = Liver; SP = Spleen [1] Unidentified parasite, probable sporozoan

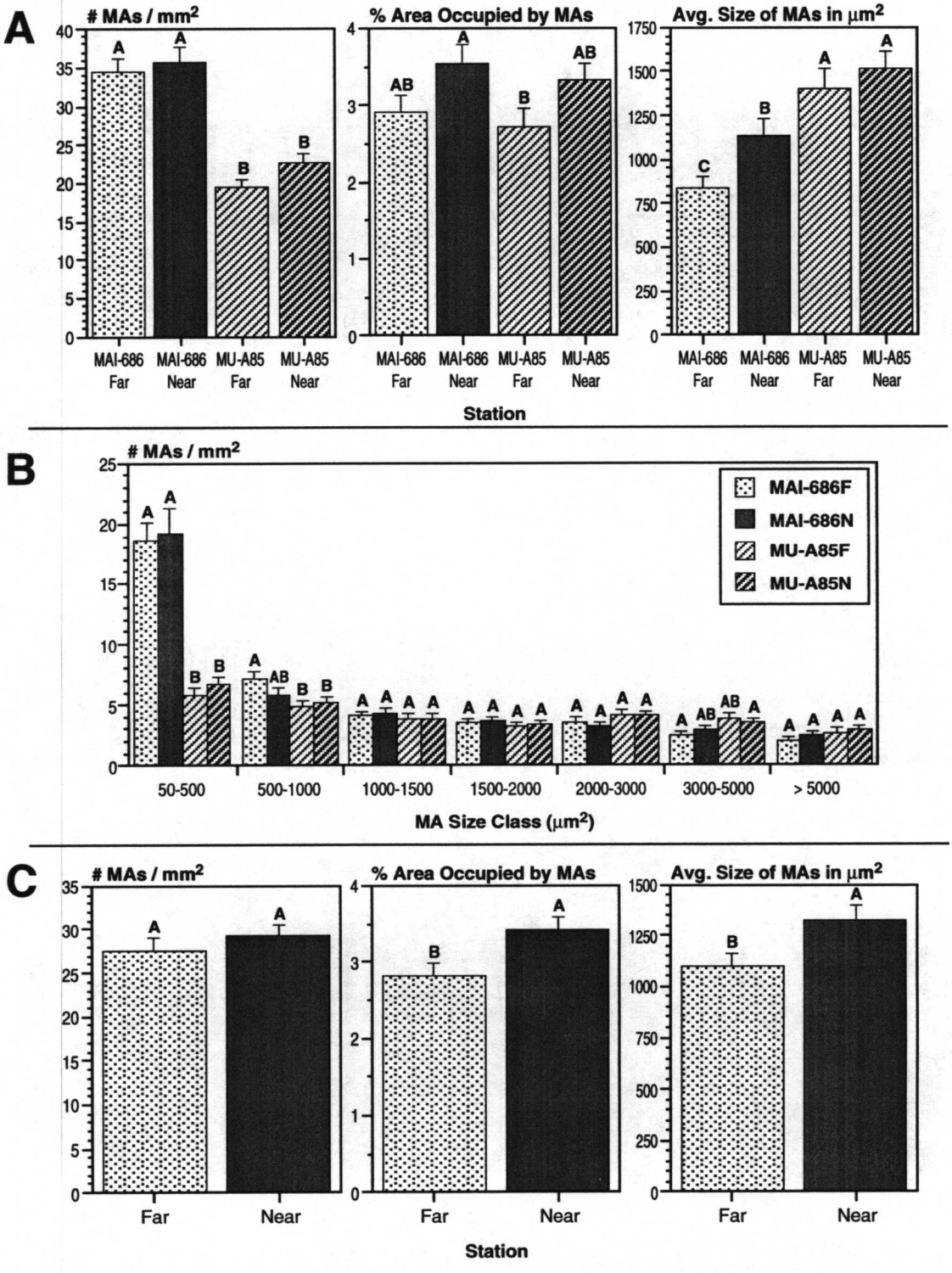


Figure 4.58. Splenic macrophage aggregate (MA) analyses at MU-A85 and MAI-686. A: splenic MA's by station; B: MA size distributions; and C: near/far MA comparison.

Size (μm^2) of MAs was not statistically different between stations at site MU-A85 (Figure 4.58A), however, the MAs at site MAI-686 were statistically different (smaller) from site MU-A85 further demonstrating the preponderance of small MAs at MAI-686. MAs were also statistically different ($P > 0.0207$) between stations at site MAI-686 with those at the near site significantly larger. The only statistical difference among stations for the percent area occupied by MAs (Figure 4.58A) is between stations MAI-686 near and MU-A85 far (Pr=0.0171). All other contrasts of percent area occupied show no statistical differences at the 0.05 level although near/far comparisons at each site do demonstrate greater area replaced by MAs at the near station in both instances (MAI-686 at P=0.0553 and MU-A85 at P=0.0778).

When the data are combined for all fishes collected at near or far stations and comparisons are made (Figure 4.58C), no difference is noted for the number of MAs per mm^2. However, the average size of MAs is greater (P=0.0184) at near stations and percent area occupied demonstrates a statistically greater (P=0.0103) replacement of splenic tissue by MAs at near stations. It is probable that the statistical difference demonstrated for percent area replaced when all near/far data are combined but which falls short of statistical significance (MAI-686 at P=0.0553 and MU-A85 at P=0.0778) when station near/far comparisons are made is a result of the larger n value for the overall near/far comparison. Larger n values could possibly demonstrate statistical differences for percent area occupied for the near/far station comparisons.

Interpretation of these preliminary MA data is difficult at this time. Cruise 2 and subsequent sampling data are required to identify any possible trends, and contaminant data are required to establish correlations. While limited, these data seem to demonstrate a greater replacement of splenic tissue by MAs at near stations. Based on previous studies this could indicate greater environmental stress at near sites. Data showing a preponderance of smaller MAs at site MAI-686 are more problematic. These data could indicate that more MAs are developing at this site, however, we have not identified this situation previously and further study is warranted.

Preliminary histopathological evaluations have also been performed on liver and spleen samples taken from 127 fish specimens examined from Cruise 2. No contaminant related lesions were observed in any of the tissue

sections; however, other pathological findings similar to those from Cruise 1 were identified (Tables 4.59 and 4.60). Again, parasitic infections were the most common abnormalities, with microsporidians being the most prevalent.

4.12 Detoxification

One hundred and twenty-two fish and 80 invertebrates collected on Cruise 1 were analyzed for biomarkers of PAH exposure. Both hepatic EROD and AHH activities were measured in all fish samples. However, only AHH activity was assayed in invertebrate samples. Cytochrome P450 levels were measured in all invertebrate and hepatic fish samples. Biliary PAH metabolite concentrations were measured in the bile of 81 fish. Thirty-five fish and invertebrate extracts were assayed using the rat hepatoma H-4-II E cell bioassay protocol. One hundred and forty-seven fish obtained during Cruise 2 were analyzed for hepatic EROD and AHH activity. Thirty-two extracts from invertebrate tissues have been assayed using rat hepatoma H-4-II E cell bioassays. Northern Blot analyses have been completed on 41 fish liver samples for CYP1A mRNA levels.

The species of fish and number of replicates that were collected on Cruise 1 and 2 are given in Tables 4.61 and 4.62. The species of fish that were collected on both cruises and therefore are the target species for the duration of the study are summarized in Table 4.63. Significantly more time was invested in trawling during Cruise 2 which resulted in more replicates as well as greater near/far station overlap in the species collected.

4.12.1 AHH Activity in Invertebrates

The invertebrate AHH activity from Cruise 1 was low to nondetectable and as such it was concluded that this assay is not a useful indicator of PAH exposure. Previous studies have also shown that constitutive inducible AHH activity is low to nondetectable in invertebrates. The presence of monoxygenase enzymes has been confirmed in a number of invertebrate species (Lee 1982; James 1989; Livingstone 1991); however, there is no conclusive evidence showing that they are inducible with exposure to aromatic hydrocarbons. Recently, Hahn et al. (1992) conducted a study to

Table 4.59. Histopathologic findings by species for GOOMEX Cruise 2.

Species	Tissue*	n	Lesion	% Prevalence
Syacium gunteri	LV	41	Digeneans	2.4
			Microsporidians	58.5
			Nematodes	9.8
	SP	40	Sporozoan (?)[1]	7.5
Trichopsetta ventralis	LV	85	Digeneans	1.2
			Fatty degeneration, focal	1.2
			Granulomatous inflammation	18.8
			Inflammatory focus (foci)	16.5
			Microsporidians	22.4
			Nematodes	7.1
	SP	83	Sporozoan (?)[1]	6.0

* LV = Liver; SP = Spleen [1] Unidentified parasite, probable sporozoan

Table 4.60. Histopathologic findings by site for GOOMEX Cruise 2.

Site	Station	Species	Tissue*	n	Lesion	% Prevalence
HI-A389	F	*Trichopsetta ventralis*	LV	20	Fatty degeneration, focal	5.0
					Granulomatous inflammation	5.0
					Inflammatory focus (foci)	5.0
					Microsporidians	25.0
					Nematodes	15.0
	N	*Trichopsetta ventralis*	LV	20	Digeneans	5.0
					Granulomatous inflammation	15.0
					Inflammatory focus (foci)	10.0
					Microsporidians	10.0
MAI-686	F	*Syacium gunteri*	LV	24	Microsproidians	54.2
					Nematodes	4.2
			SP	24	Sporozoan (?)[1]	12.5
	N	*Syacium gunteri*	LV	17	Digeneans	5.9
					Microsporidians	64.7
					Nematodes	17.6
MU-A85	F	*Trichopsetta ventralis*	LV	23	Granulomatous inflammation	21.7
					Inflammatory focus (foci)	34.8
					Microsporidians	26.1
					Nematodes	8.7
			SP	23	Sporozoan (?)[1]	4.3
	N	*Trichopsetta ventralis*	LV	22	Granulomatous inflammation	31.8
					Inflammatory focus (foci)	13.6
					Microsporidians	27.3
					Nematodes	5.5
			SP	20	Sporozoan (?)[1]	10.0

* LV = Liver; SP = Spleen [1] Unidentified parasite, probable sporozoan

Table 4.61. Fish species and number of replicates collected during Cruise 1.

Site	Species	Replicates
MU-A85 NEAR	*Centropristis philadelphica*	2
	Cyclopsetta chittendeni	1
	Equetus umbrosus	3
	Lutjanus camprechanus	4
	Paralichthys lethostigma	2
	Pogonias cromis	2
	Synodus foetens	2
MU-A85 FAR	*Brotula barbata*	1
	Caulolatilus intermedius	3
	Centropristis philadelphica	1
	Cyclopsetta chittendeni	1
	Epinephalus flavolimbatus	1
	Paralichthys lethostigma	1
	Synodus foetens	2
	Trichopsetta ventralis	1
	Urophycis cirrata	1
MAI-686 NEAR	*Archosargus probatocephalus*	4
	Cynoscion arenaris	2
	Lutjanus campechanus	2
	Parlichthys albigutta	2
	Paralichthys lethostigma	2
	Pogonias cromis	4
	Syacium gunteri	3
MAI-686 FAR	*Syacium gunteri*	4
HI-A389 NEAR	*Caulolatilus intermedius*	1
	Centropristis philadelphica	2
	Ogcocephalus declivirostris	1
	Paralichthys lethostigma	3
	Pogonias cromis	2
	Pristipomoides aquilonaris	1
	Urophycis cirrata and *floridana*	3
HI-A389 FAR	*Pontinus longispinus*	3
	Pristipomoides aquilonaris	5
	Urophycis cirrata and *floridana*	4

Table 4.62. Fish species and number of replicates collected during Cruise 2.

Site	Species	Replicates
MU-A85 NEAR	*Ancyclopsetta dilecta*	2
	Centropristis philadelphica	1
	Cyclopsetta chittendeni	3
	Equetus sp.	1
	Lagodon rhomboides	4
	Lutjanus camprechanus	3
	Paralichthys lethostigma	1
	Pristipomoides aquilonaris	2
	Rypticus maculatus	2
	Synodus foetens	4
	Trichopsetta ventralis	1
	Urophycis floridana	2
MU-A85 FAR	*Ancyclopsetta dilecta*	3
	Caulolatilus intermedius	1
	Cyclopsetta chittendeni	2
	Lagodon rhomboides	3
	Lutjanus campechanus	5
	Paralichthys lethostigma	1
	Synodus foetens	4
	Trichopsetta ventralis	2
	Urophycis floridana	3
MAI-686 NEAR	*Archosargus probatocephalus*	2
	Cynoscion arenaris	7
	Lutjanus campechanus	5
	Lutjanus synagmis	1
	Opsanus pardus	4
	Paralichthys lethostigma	3
	Rypticus maculatus	3
	Syacium gunteri	4
	Synodus foetens	1
	Cynoscion arenaris	6
	Syacium gunteri	9
	Synodus foetens	8
HI-A389 NEAR	*Ancyclopsetta dilecta*	3
	Ogcocephalus declivirostris	4
	Paralichthys lethostigma	5
	Pontinus longispinus	4
	Pristipomoides aquilonaris	3
	Synodus foetens	3
	Trichopsetta ventralis	3
	Urophycis cirrata and floridana	3
HI-A389 FAR	*Ancyclopsetta dilecta*	3
	Ogcocephalus declivirostris	2
	Paralichthys lethostigma	1
	Pontinus longispinus	3
	Pristipomoides aquilonaris	4
	Synodus foetens	3
	Trichopsetta ventralis	3
	Urophycis cirrata and floridana	2

Table 4.63. Species that were common to Cruises 1 and 2.

Site	Species
MU-A85 NEAR	*Centropristis philadelphica*
	Cyclopsetta chittendeni
	Equetus sp.
	Lutjanus campechanus
	Paralichthys lethostigma
	Synodus foetens
MU-A85 FAR	*Caulolatilus intermedius*
	Cyclopsetta chittendeni
	Paralichthys lethostigma
	Synodus foetens
	Trichcopsetta ventralis
	Urophycis sp.
MAI-686 NEAR	*Archosargus probatocephalus*
	Cynoscion arenaries
	Lutjanus campechanus
	Paralichthys lethostigma
	Syacium gunteri
MAI-686 FAR	*Syacium gunteri*
HI-A389 NEAR	*Ogcocephalus declivirostris*
	Paralichthys lethostigma
	Pristipomoides aquilonaris
	Urophycis sp.
HI-1389 FAR	*Pontinus longispinus*
	Pristipomoides aquilonaris
	Urophycis sp.

evaluate the Ah receptor in marine organisms. In mammals, the induction of cytochrome P4501A1 is regulated by the Ah receptor. Hahn et al. (1992) were able to confirm the presence of the Ah receptor in several teleost and elasmobranch fish species. However, the presence of the Ah receptor was not detected in any agnathan fish or 9 invertebrate species. This suggests, that unlike most vertebrates, invertebrates lack a functional Ah receptor which is consistent with the failure to observe induced P4501A-dependent activity.

4.12.2 EROD and AHH Activity in Fish

EROD and AHH activities for Cruise 1 ranged from non-detectable to 133 pmol/min/mg and non-detectable to 111 pmol/min/mg, respectively. The highest EROD activity was measured in *Caulolatilus intermedius* collected at HI-A389 near station on Cruise 1. However, the number of

replicates and the species overlap was insufficient to demonstrate any statistically significant differences within individual species at the near and far stations for any of the sites sampled during Cruise 1.

A wide range of EROD activities were measured for Cruise 2 samples, ranging from non-detectable to 344 pmol/min/mg and AHH activities varied from non-detectable to 98 pmol/min/mg. The highest enzyme activities were measured in *Lagodon rhomboides* (MU-A85) and *Syacium gunteri* (MAI-686). *Lagodon rhomboides* had consistently higher EROD activities than any other species collected during either cruise. Replication and species overlap were significantly improved on Cruise 2. For example, *Synodus foetens* was collected at all stations and *Paralichthys lethostigma* was collected at all stations except at the MAI-686 far station, and five species (*Paralichthys lethostigma, Ancyclopsetta dilecta, Urophycis* sp., *Synodus foetens* and *Trichopsetta ventralis*) were collected at both near and far stations in lease blocks HI-A389 and MU-A85. In spite of the improvement in species overlap, there were still no statistically significant differences between near and far stations for any individual species at the three sites. The data from Cruise 1 and 2 were combined and statistically evaluated. Again, there were no significant differences between the near and far stations by species at each site.

Both EROD and AHH activities were measured for all fish collected during Cruises 1 and 2 to evaluate the relative sensitivities of these enzyme assays. There was an excellent correlation between AHH and EROD activity (r^2= 0.65) for virtually all fish species. Thus the two assays apparently measure the same effect (i.e., P4501A induction). The only exceptions were *Arius felis* (collected at GA-288) and *Lagodon rhomboides* (collected at MU-A85) whose EROD activities were significantly higher than the corresponding AHH activities. The fish that are outliers for the coordinated induction of AHH and EROD activity (i.e., EROD >> AHH) undoubtedly express an altered CYP1A protein compared to most other fish species and this observation is unique.

EROD and AHH activities were generally low in fish and may, in some cases, represent constitutive levels. However, the variable EROD and AHH activities observed indicate that there may be species differences in the expression of response. Results from subsequent cruises will be needed to confirm this observation. It is also proposed to inject several species of fish

with a known P4501A1 inducer and maintain them for several days in a flow-through tank on board the ship. These experiments will determine the maximum induction response in these species and thereby facilitate the interpretation of the fractional activities observed.

Based on the data from the first two cruises it appears that the differences in fish EROD activity at the near and far stations exhibit only minimal differences. Future cruises will provide additional data on differences in PAH exposure and induction response as a function of distance from the platforms.

4.12.3 Cytochrome P450 for Fish and Invertebrates

Cytochrome P450 was measured in both invertebrates and fish for Cruise 1 only. The data was inconsistent with no discernable patterns and therefore is not considered further.

4.12.4 Biliary PAH Metabolites in Fish

Evidence of sample degradation was observed in the bile of some fish, particularly those collected at the deep sites (i.e., HI-A389 and MU-A85). Bile apparently degrades in fish that are dead or moribund and as a result is not useful for PAH metabolite determination. Two factors may contribute to the degradation of bile from some samples. At the deeper sites trawl recovery takes longer and the effects of differential pressure was observed in that many fish had their stomachs everted. Also, in trawls where a large number of fish were captured, too much time may elapse between capture and dissection. Samples that were judged compromised were not included in data analyses.

Qualitatively, the highest mean concentrations of naphthalene and phenanthrene equivalent metabolites were measured in *L. rhomboides* collected at MU-A85 near (Figure 4.59) and *P. longispinis* and *C. philadelphica* collected at HI-A389 (Figure 4.60). The highest mean concentration of benzo[a]pyrene metabolites was measured in *Urophycis* (Figure 4.61). Although significantly more trawling occurred during Cruise 2 the species overlap for near/far station comparisons are poor due to sampling problems. With the limited data set, it is not possible to ascertain

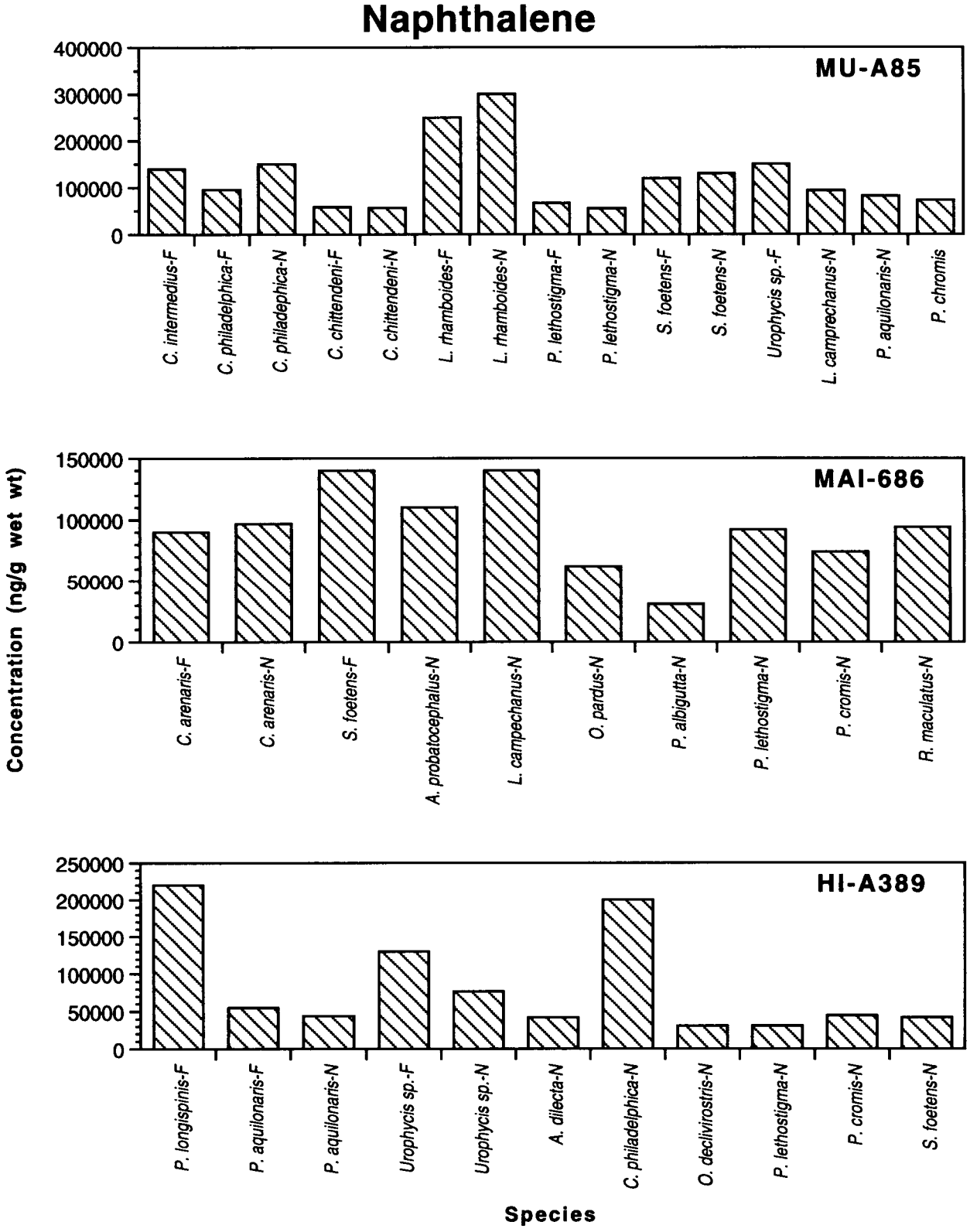


Figure 4.59. The concentration of naphthalene metabolites in the bile of fish captured at the near (N) and far (F) stations for MU-A85, MAI-686, and HI-A389.

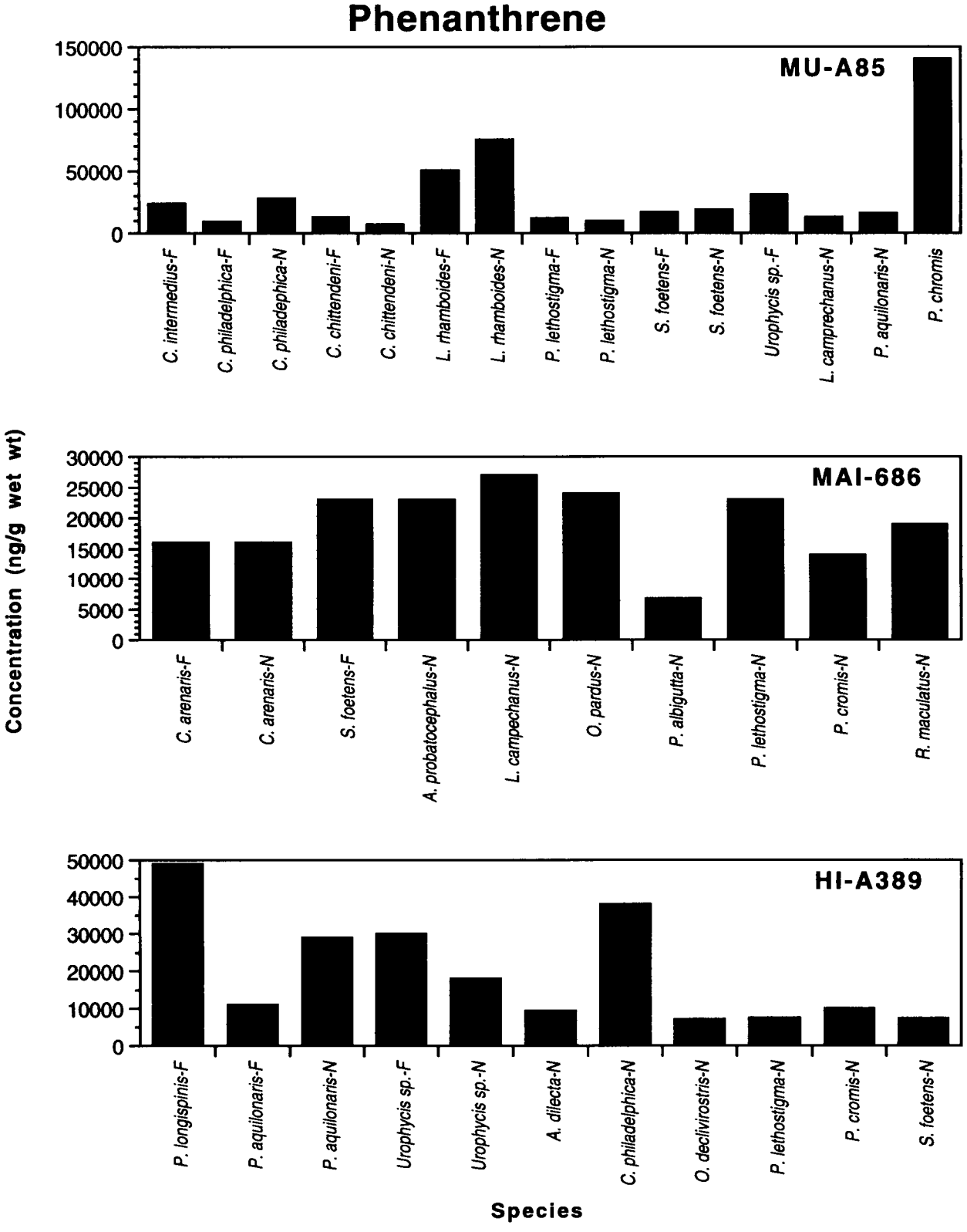


Figure 4.60. The concentration of phenanthrene metabolites in the bile of fish captured at the near (N) and far (F) stations for MU-A85, MAI-686, and HI-A389.

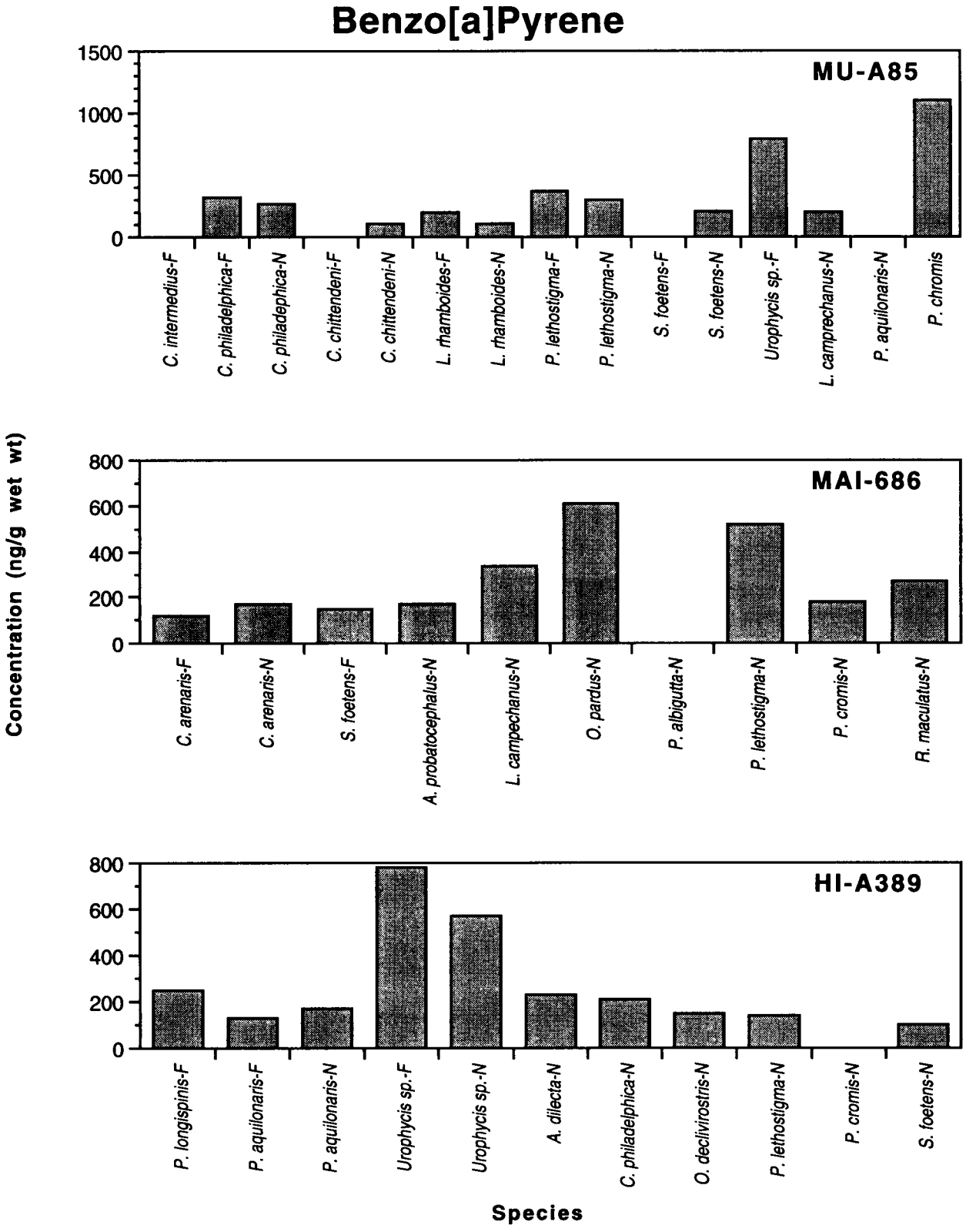


Figure 4.61. The concentration of benzo[a]pyrene metabolites in the bile of fish captured at the near (N) and far (F) stations for MU-A85, MAI-686, and HI-A389.

whether the observed differences in mean metabolite concentration are due to exposure or to intrinsic species variation. Additionally, there is no correlation between EROD and AHH activity and naphthalene, phenanthrene, and BaP metabolite equivalents. Naphthalenes and phenanthrenes are not strong inducers of P4501A and no correlation is expected, but their presence does indicate exposure to lower molecular weight PAHs. BaP is a potent inducer of P4501A and levels of BaP equivalent metabolites have been correlated with elevated hepatic AHH activity in fish (Collier and Varanasi 1991). The low concentrations of BaP equivalents in fish collected is not an unexpected result since most oils contain low levels of BaP. High concentrations of BaP metabolites are typically correlated with exposure combustion-derived PAH.

4.12.5 Rat Hepatoma H-4-II E Bioassay

Rat hepatoma H-4-II E cells are highly Ah-responsive and these cells are utilized as a bioassay for determining the induction (AHH and EROD) potency of extracts derived from invertebrates. Bradlaw and Casterline (1979) and Bradlaw et al. (1980) first reported that halogenated aromatic hydrocarbons readily induce AHH activity in rat hepatoma H-4-II E cells in culture. Other studies have also correlated the induction of EROD activity in this cell line with exposure to known levels of PAHs and PCBs in marine fish and invertebrates (unpublished data). The method involves extracting invertebrate tissues for aromatic hydrocarbons and PCBs by using conventional techniques. Subsequently, rat hepatoma 4-II-E cells are exposed to this extract and after an incubation period EROD activity is measured to assess P4501A1 induction in the cell line. The advantage of this technique is that the bioassay-derived induction response is an integrated assessment of the potential biological effects of a complex mixture. The results obtained from the rat hepatoma cell bioassay data from Cruise 1 were difficult to interpret primarily due to the small sample size and small amounts of extract from these samples.

Larger samples were extracted for Cruise 2 and the data are presented in Table 4.64. All extracts induced CYP1A1 activity in the rat hepatoma H-4-II-E cells. The data set is too small to evaluate near/far station comparisons. However, qualitatively, higher EROD activities were measured in cells

Table 4.64. EROD activities of rat hepatoma H-4-II E cells dosed with extracts of invertebrate tissues collected on Cruise 2.

Station	*Species*	EROD Activity pmol min^{-1} mg^{-1} g^{-1} tissues
MU-A85 near	*Penaeus aztecus*	22.1 ± 14.6
	Portunus spinicarpus	10.8 ± 3.3
MU-A85 far	*Penaeus aztecus*	20.8 ± 4.5
	Amusium papyraceum	50.5 ± 7.6
	Shrimp "A"	10.2 ± 6.1
MAI-686 near	*Calinectes similis*	35.4 ± 2.7
	Trachypenaeus sp.	18.9 ± 7.5
	Squilla empusa	25.1 ± 2.2
MAI-686 far	*Calinectes similis*	74.2 ± 93.9
	Squilla empusa	36.9 ± 6.5
	Trachypenaeus sp.	18.6 ± 3.0
	Penaeus aztecus	34.3 ± 2.9
HI-A389 near	*Squilla empusa*	20.0 ± 4.9

exposed to extracts from scallops and crabs compared to shrimp extracts. This pattern is consistent with expected levels of organic contaminant bioaccumulation.

4.12.6 P4501A mRNA

The levels of P4501A mRNA were determined by Northern Blot analysis in 45 fish livers collected during Cruise 2 (Figure 4.62). P4501A mRNA was detected in all samples but the levels suggest only minimal induction. However, P4501A mRNA transcripts differed in molecular weights for various species, suggesting genetic differences in this gene between species.

4.13 Sediment Pore Water Toxicity Testing

Sediment samples were received for processing in two groups from each cruise. All of the pore water samples were extracted within 10 days of

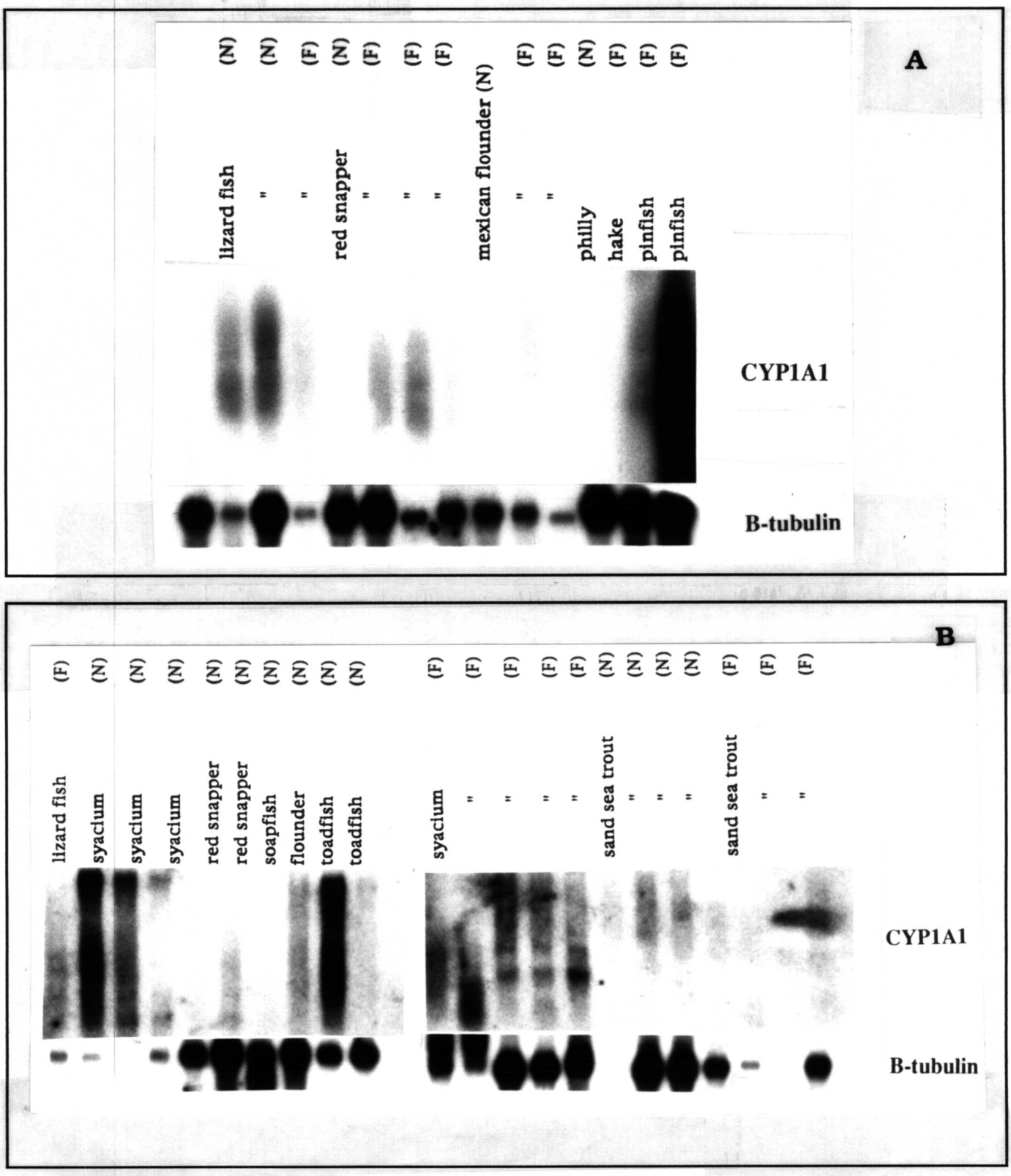


Figure 4.62. Northern Blot of CYP1A1 mRNA in fish liver at A: MU-A85; B: MAI-686; and C: HI-A389.

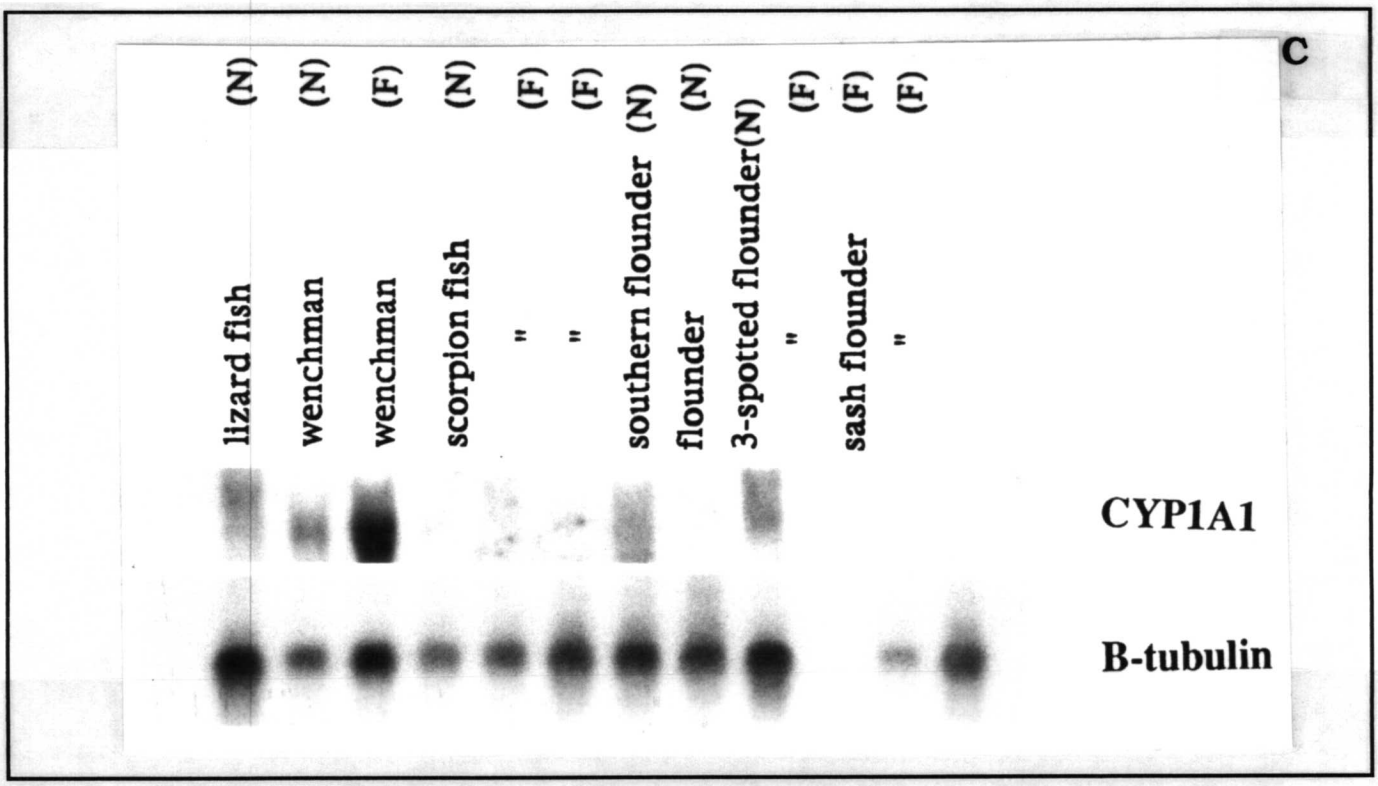


Figure 4.62. (continued).

the time of collection. The water quality parameters for the pore waters were very similar among all stations from both cruises. All Cruise 1 sample salinities were between 34 and 37‰, therefore no sample dilution by more than 4% was needed to reach the target salinity (35 ± 1‰) for the tests. No dilution of samples was required for Cruise 2 samples, since the salinities of all samples were between 34 and 36‰. Hydrogen sulfide concentrations were below the detection limit (0.01 µg/L) in all pore water samples.

Significant toxicity was observed at four of the five platforms sampled during the first cruise. No toxicity was observed at Mustang Island-A85-1 and the six most toxic stations were observed at High Island-A389-4. Significant toxicity was observed at 14 stations overall with the sea urchin embryological development assay but at only three stations with the sea urchin fertilization test. All stations showing reduced fertilization also exhibited impaired development in the embryological development assay. All but three of the toxic stations were within 150 meters of a platform. The results of the sea urchin pore water fertilization and embryological development tests for the first cruise are summarized in Figures 4.63 and 4.64. Significant toxicity was observed in the vicinity of two of the three platforms in the second cruise. As in the first cruise, no toxicity was observed near the Mustang Island-A85-1 platform, but toxicity was observed at one of the reference stations (5C). Three of the five stations closest to the platform were observed to be toxic at Matagorda Island-686. Four stations near the platform exhibited significant toxicity at High Island-A389 and corresponded with four of the six stations observed to be toxic from the first cruise. Significant toxicity was observed at 8 stations overall with the sea urchin embryological development assay for the 75 samples from the second cruise. All of the toxic stations (except the one reference station at Mustang Island-A85) were within ~75 meters of a platform. The results of the sea urchin pore water embryological development tests for Cruise 2 are summarized in Figures 4.65 and 4.66.

In the positive control test with a dilution series of SDS, the EC_{50} was 6.29 mg/L in the fertilization test (Cruise 1). The EC_{50}s for SDS in the embryological development tests were 3.91 mg/L (Cruise 1) and 4.65 mg/L (Cruise 2). These are normal values for these tests and indicate normal gamete viability. Filtered seawater and reconstituted seawater controls were not statistically different from reference pore water in any test.

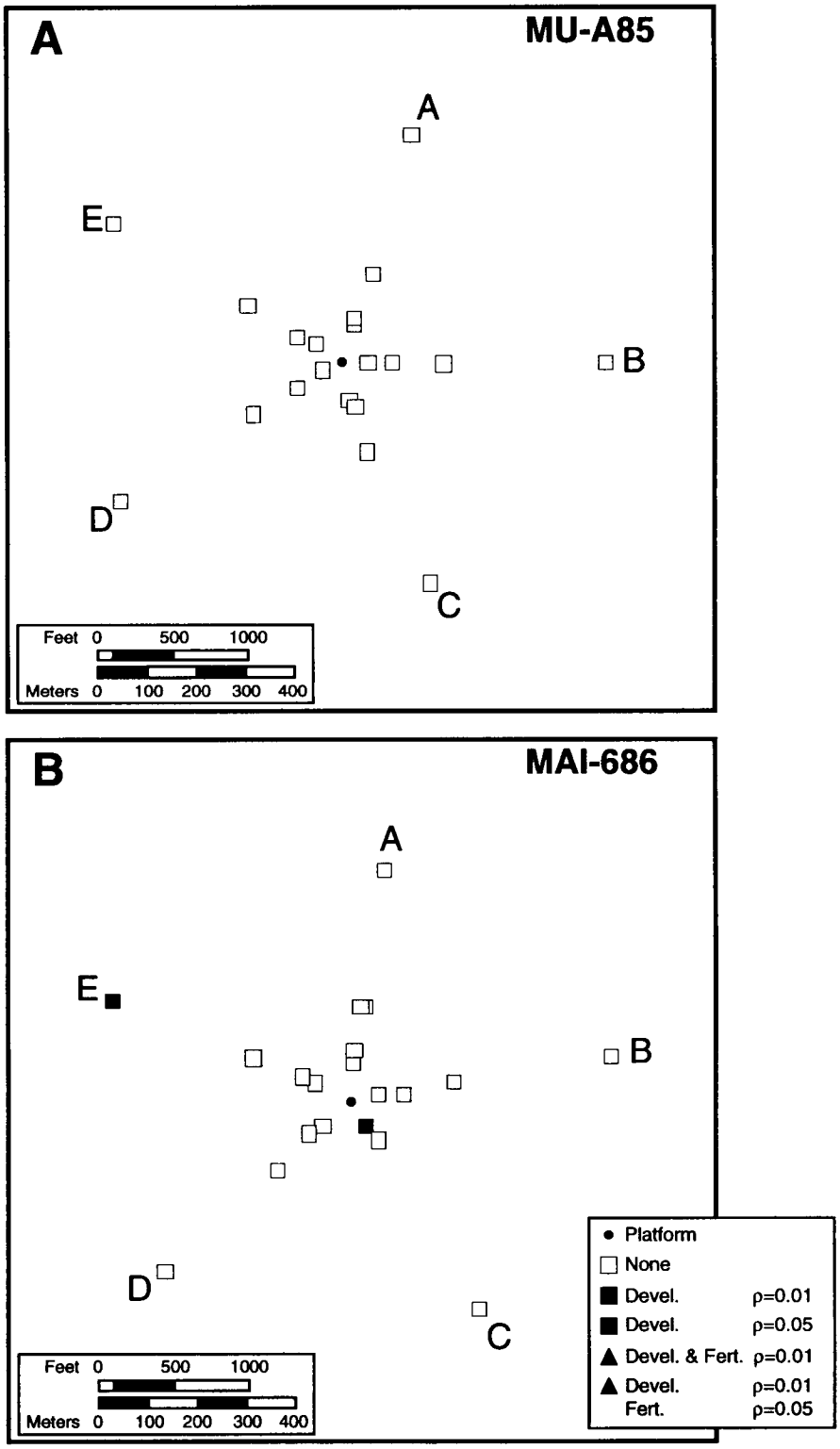


Figure 4.63. Summary of porewater toxicity test results at A: MU-A85; and B: MAI-686 (Devel.=standard sea urchin developmental assay; Fert.=standard sea urchin embryo fertilization assay).

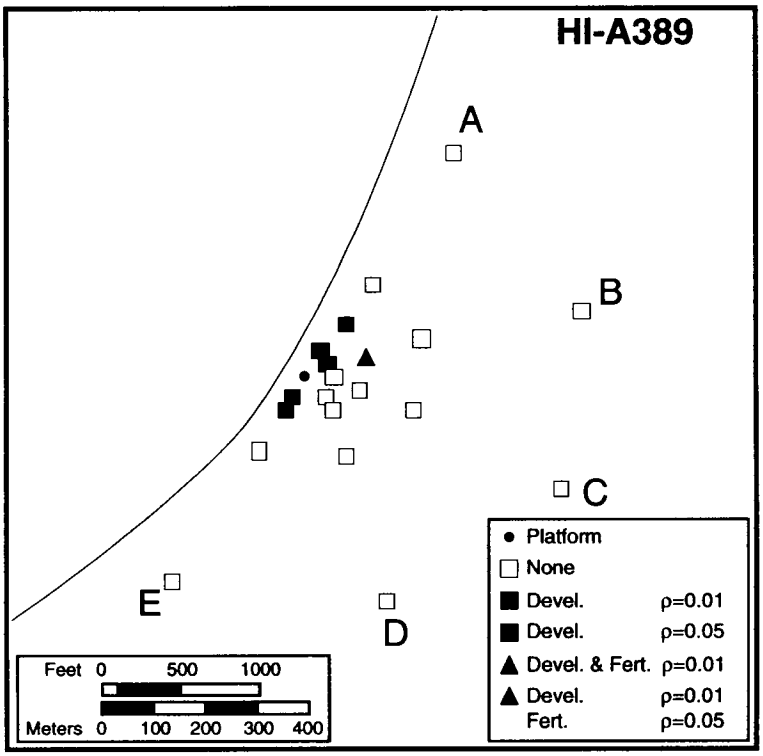


Figure 4.64. Summary of porewater toxicity test results at HI-A389 (Devel.=standard sea urchin developmental assay; Fert.=standard sea urchin embryo fertilization assay).

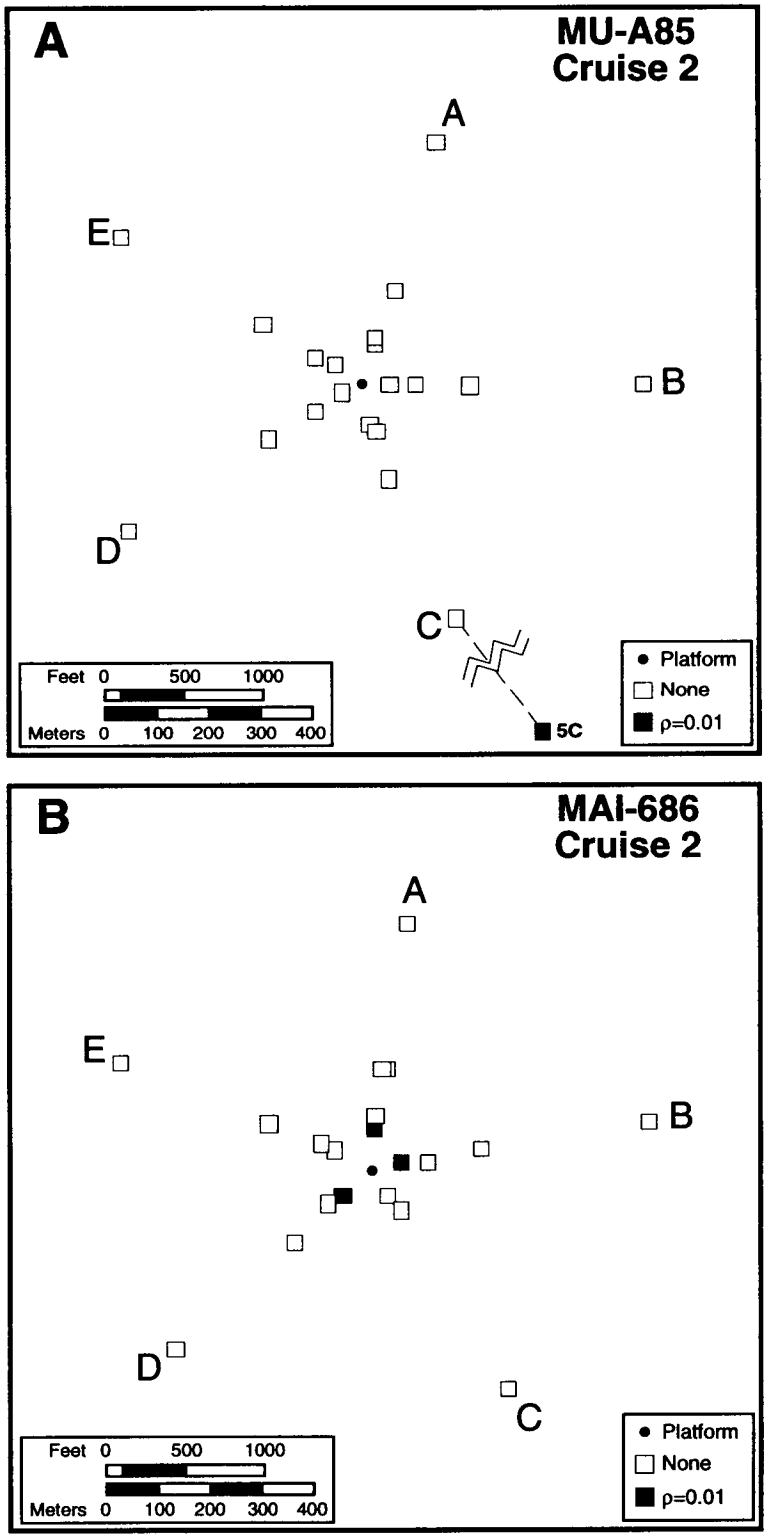


Figure 4.65. Summary of sea urchin embryological development toxicity test results for Cruise 2 at A: MU-A85; and B: MAI-686.

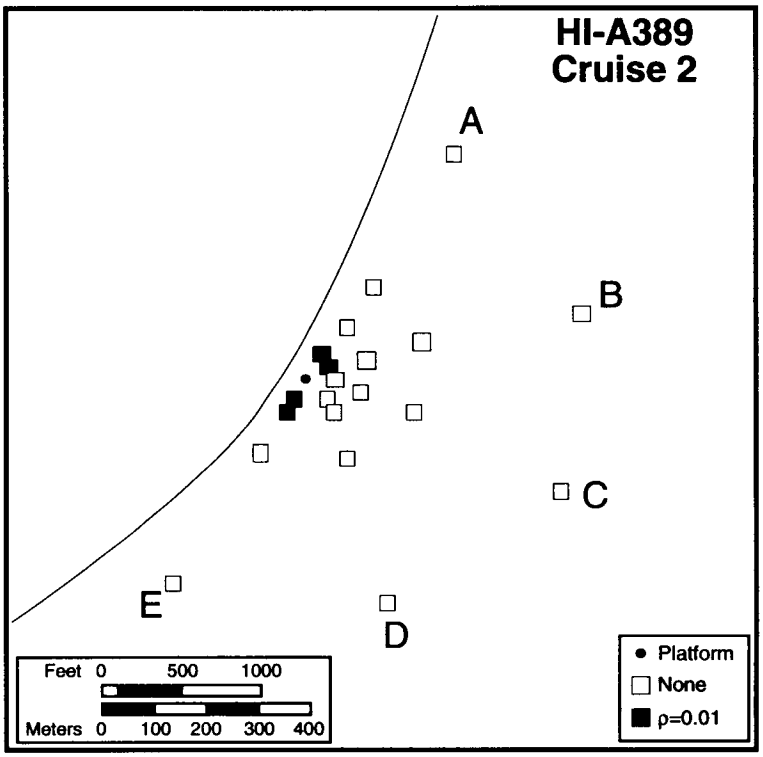


Figure 4.66. Summary of sea urchin embryological development toxicity test results for Cruise 2 at HI-A389.

The toxicity of sediment pore water has not yet been examined statistically in relation to sediment chemistry, but a relationship with sediment trace metal concentrations is immediately evident, especially at the High Island site (Figures 4.67 and 4.68). MacDonald (1993) determined a "probable effects level" (PEL) for zinc in whole sediments to be 300 mg/Kg. All of the toxic High Island sites were near or exceeded this concentration (Figure 4.67). Other metals which appear to be elevated at sites exhibiting toxicity were lead (Figure 4.67), cadmium (Figure 4.69), and barium (Figure 4.68). The PELs for lead (160 mg/Kg) and for cadmium (7.5 mg/Kg) are also exceeded at some sites, and the PEL of silver (2.5 mg/Kg) was exceeded at one site (2B). However, since the PEL of zinc is far exceeded at all of the sites where the PEL of other elements are exceeded, it is possible that the toxicity of the High Island sediments is largely controlled by the zinc concentration.

Examination of hydrocarbon concentrations in whole sediment revealed no immediately clear pattern, although some of the same sites which had elevated trace element concentrations also had elevated whole sediment hydrocarbon concentrations. The data has not been completely evaluated, but PAH do not appear at this point to be the major source of toxicity in our tests. Concentrations of total PAH, low molecular weight PAH, and high molecular weight PAH in whole sediments do not approach their respective PELs (MacDonald 1993). Also, the High Island site which had the highest sediment total PAH concentration (2D) was not toxic (Figure 4.69).

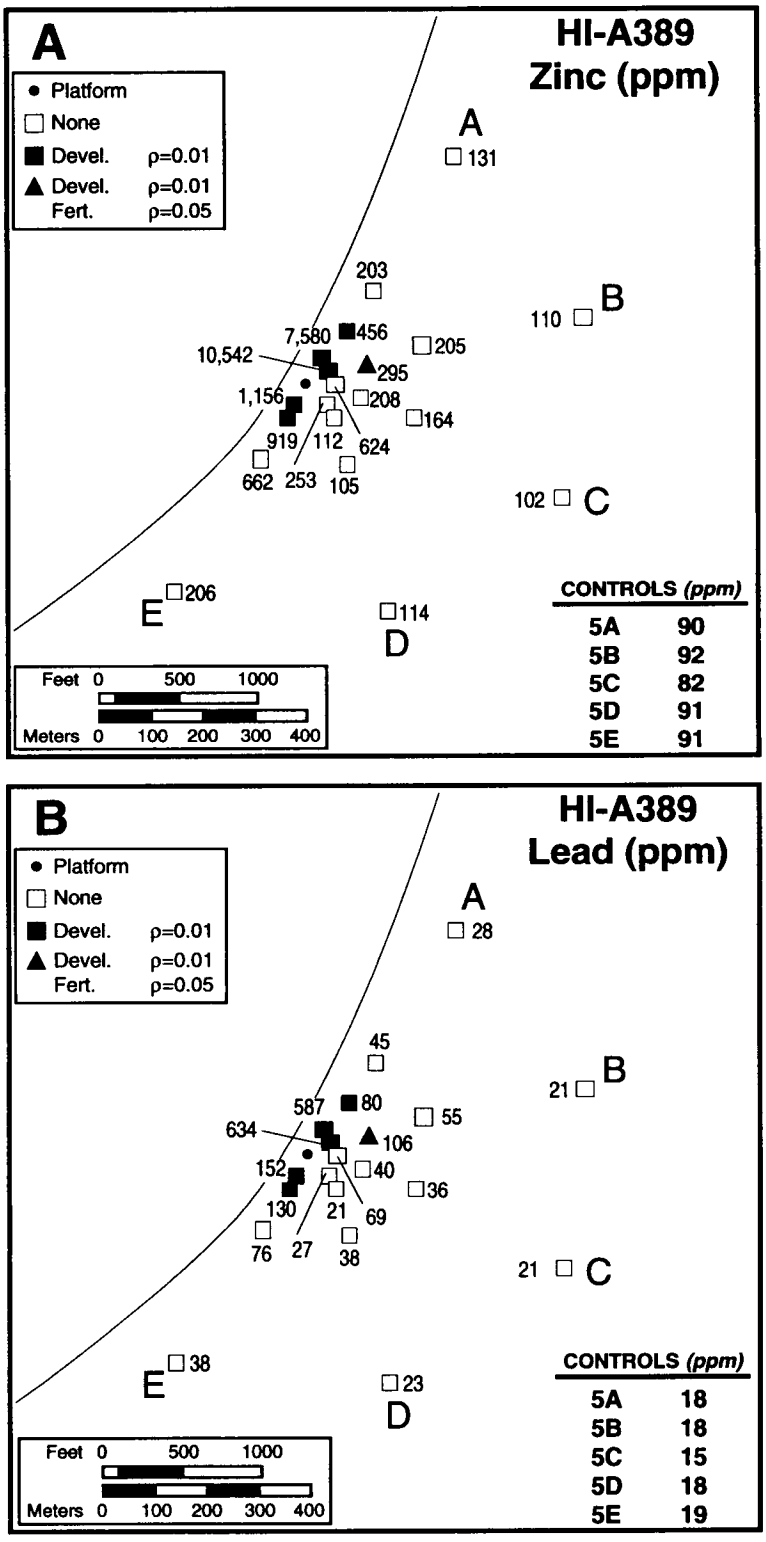


Figure 4.67. Whole sediment A: zinc; and B: lead concentrations and pore water toxicity at HI-A389. (Devel.=standard sea urchin developmental assay; Fert.=standard sea urchin embryo fertilization assay).

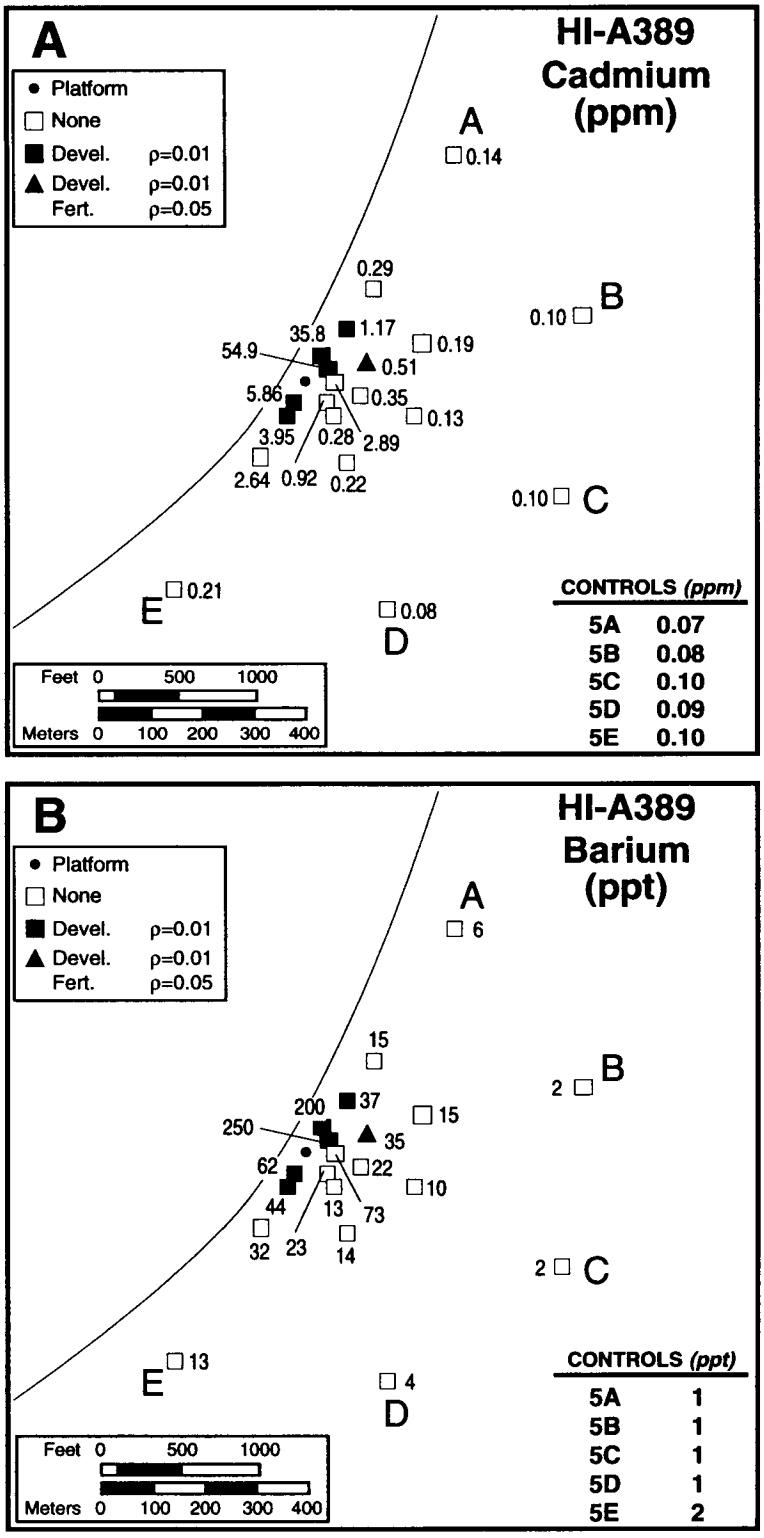


Figure 4.68. Whole sediment A: cadmium; and B: barium concentrations and pore water toxicity at HI-A389. (Devel.=standard sea urchin developmental assay; Fert.=standard sea urchin embryo fertilization assay).

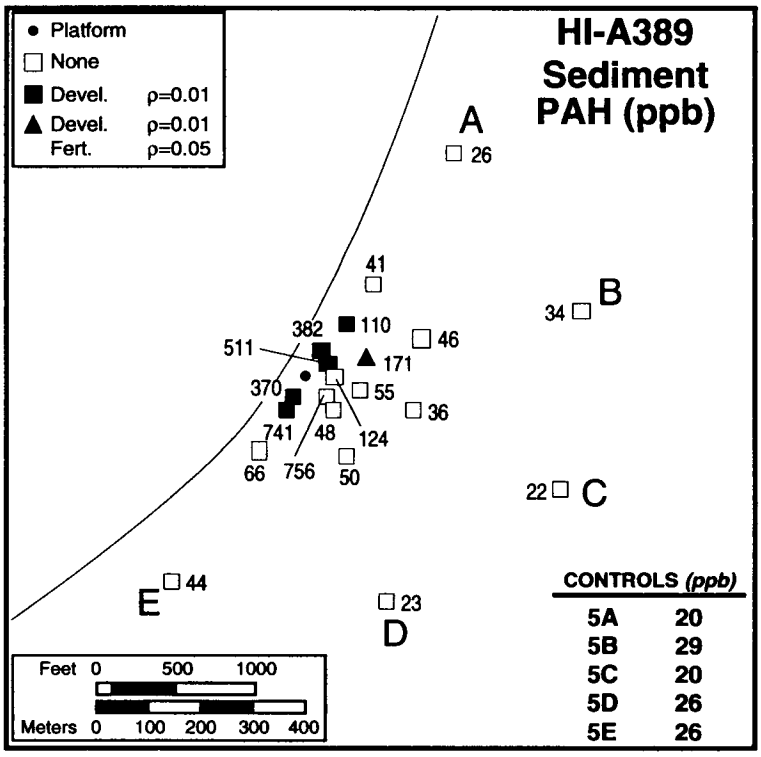


Figure 4.69. Whole sediment total PAH concentrations and pore water toxicity at HI-A389. (Devel.=standard sea urchin developmental assay; Fert.=standard sea urchin embryo fertilization assay).

5.0 INTERIM SUMMARY

A large amount of data has been collected and a preliminary analysis and interpretation has been completed. Many work elements are beginning to be able to detect significant trends in the data that are directly related to the presence of offshore platforms. In most instances the observed trends need to be verified by continued data collection. Some work elements do not have a sufficiently large enough sample to draw statistically significant conclusions. These data are often confounded by the grouping of data to create large enough data sets to be analyzed. As more data becomes available, we will be able to analyze these results at a finer scale of detail (i.e., by species). The following provides a brief summary overview of results to date.

A set of replicate boxcores were taken at the near and far stations during Cruise 1 to assess the power of the study design and the appropriateness of pseudoreplicates from a single boxcore. Two replicates from a single boxcore can detect an 18-98% change in the variables measured. Sediment grain size has the greatest discrimination at 18%. Little precision is lost by using two pseudoreplicates over two replicate boxcores. For example, an 88% decrease in PAH could be detected with two boxcores, and a 90% decrease could be detected with two subcores.

Sediments at the study sites were deposited by marine processes and are primarily of a fluvial origin. The sediments consist of quartz, sands, silts, and clays with an admixture of skeletons and fragments of organisms living near the sites of deposition. Sediment texture is strongly correlated with distance from the platform with sediments under the platform being highly enriched in sand. This sand appears to be related to disposal practices during drilling activities but scouring by bottom currents cannot be ruled out. The addition of sand has proportionately diluted the organic carbon content of the sediments. Inorganic carbon generally increases near the platform most likely due to deposition of calcareous debris fromthe rig associated fauna. Spatial patterns of sediment texture are oriented along the direction of the dominant currents.

Aliphatic and aromatic hydrocarbons were used to assess the presence of platform related contamination. Hydrocarbons were measured in sediments and biological tissues. In general, hydrocarbons were elevated in

sediments close to the platform and rapidly decreased to background levels at a distance of 100-200 m from the platform. Spatial patterns of contaminants are aligned along the direction of the prevailing currents at the site generating a significant directional orientation. Most sediment hydrocarbons were highly biodegraded with the exception of a few stations at MAI-686 which were rich in n-alkanes. Between cruise variations were small reflecting a stable, chronic hydrocarbon benthic contaminant field. HI-A389 and MU-A85 had strong gradients in hydrocarbon contaminants whereas MAI-686 exhibited a weak and variable gradient due to the high energy setting at the site. Data on tissue contaminant levels is too limited to draw many conclusions at this point. However, PAH concentrations in fish livers were significantly higher near the HI-A389 platform than at the far-field station. Trends are becoming apparent in the data however a larger number of analyses will be needed to confirm the significance of these preliminary indications.

Barium (as barite) is the dominant component of drilling mud and is used to define sediment trace metal gradients at each study site. Two of the three study sites (HI-A389 and MU-A85) showed strong elemental gradients (Ba, Ag, Cd, Hg, Pb, Sb, and Zn) with highly significant negative correlations with distance and positive correlations with Ba. The Ba trend at the third site (MAI-686) is significant by an ANOVA analysis but fewer elements correlated with Ba (Cd, Hg, Pb, Zn) than at other sites. Several of the correlate metals (Cu, Hg, Sb) appear to be constituents of the barite ore used in drilling mud while others (Cd, Pb, Zn) have significant non-drilling mud, rig-related sources. The HI-A389 site clearly has the strongest spatial gradients and should be the location most likely to exhibit trace element related effects in the resident biota. Good correlations were observed between the pore water bioassay results and solid phase (bulk) metal concentrations.

Tissue samples from near- and far-field at each site were analyzed for trace metals. Fish livers; soft tissues from shrimp, crabs and mollusks; whole starfish and polychaetes; and fish stomach contents were analyzed. Preliminary analysis revealed few significant differences for pairwise comparisons. The stomach contents data do show significant differences in Ba and Fe levels. This is probably caused by the ingestion of bottom sediments during feeding. More comparisons are needed using smaller

comparison groups composed of the same or similar species. Additional significant differences in trace element levels may be detected as the number of analyses increases.

Harpacticoids are the best meiofauna indicator of platform affects. Harpacticoid abundance and diversity declined in sediment near the platform. Nematode diversity and abundance did not appear to be useful for detecting platform effects, since there were no discernable trends. The nematode:copepod ratio was only an effective indicator at the HI-A389 platform, suggesting that this is not a good general index of platform affects. Within a platform site, community structure was not significantly effected by environmental conditions. Most differences between platforms were attributed to natural variations and gradients in abiotic conditions. Preliminary evidence indicates that there may be higher reproductive potentials under platforms. More gravid females generally occurred near rather than far from the platform. There may be an organic enrichment affect near platforms due to the reef-like nature of the organisms associated with the platform legs. The ability to detect differences in harpacticoid community, life history, and reproductive parameters demonstrated that reliable indicators of sublethal responses can be developed.

Near-field stress in macroinfauna may be indicated by an absence of ampeliscid amphipods at MAI-686. at MU-A85 and HI-A389 abundances of benthic infaunal organisms were relatively low and dominant species were generally not as dominant as at other platform sites. Large populations of cirratulid polychaetes occurred at several near-field stations at HI-A389. There did not appear to be a correlation between contaminant indicators and organismal distributions. Despite pore water toxicity indicated by bioassays at several stations at HI-A389, sediments from these stations contained large populations of cirratulid polychaetes.

The reproductive effort of invertebrate populations appeared to respond to the presence of a platform. Variations in size class distribution, percent gravid females, stage of reproductive development and prevalence of disease and parasites were noted. If a difference in size class distribution existed in a species between the near and far-field stations, the larger individuals were typically found at the far-field station. This suggests that either there is insufficient food resources near the platforms, or mortality is higher among adult individuals nearer the platform. Far-field stations

exhibited a higher proportion of gravid females than near-field stations, suggesting that exposure to contaminants may be delaying the reproductive development of individuals living in close proximity to the platforms. However, other measures of reproductive development, such as histological analysis, showed no significant differences between the near- and far-field stations. In certain species (*P. aztecus* and *T. similis*) population health, as defined by the presence of disease or parasites, differed between the near and far-field station, with more evidence of pathogens and pathologies in individuals collected from the near-field station. These results are significant in that they indicate that some form of physiological impact on populations living in close proximity to a platform. The immunological probe to estimate reproductive effort is in the calibration and purification stage, and the inoculation experiments are awaiting processing. These data, as they become available, will help clarify the rate and timing of reproductive development and the prevalence of disease and pathologies in these species.

Trawl collections have yielded a large number of demersal fish of both target and non-target specimens with a total of 51 families and 119 species. Cruise 1 produced a total catch of over 3,500 fish consisting of 38 families and 89 species. Cruise 2 produced a total catch of over 15,500 fish consisting of 45 families and 95 species. The overlap of target specimens and total catch was considerably higher for the second cruise and was the result of improved trawling equipment and an increase in the number of trawls. Histopathological evaluations have been performed on liver and spleen samples from a total of 316 fish specimens from Cruises 1 and 2. No contaminant related liver lesions (e.g., hepatic megalocytosis, hepatocellular neoplasms) were observed in any of the tissue sections. Parasitic infections were the most common abnormalities, with microsporidians being the most prevalent. Two other prevalent lesions included inflammatory focii and granulomatous inflammation; however, these lesions were usually associated with parasitic infections. Preliminary splenic macrophage (MA) analysis from Cruise 1 indicated that the average size of MAs is greater at the near stations and the percent area occupied by MAs at these stations was statistically larger. Accurate interpretation of splenic MA data will require analysis of more samples as well as a comparison with sediment and tissue contaminant data.

The detoxification work element concentrated on in vivo and *in vitro* indicators of organic contaminant exposure. The *in vivo* assays were used to determine P4501A induction in fish and included ethoxyresorufin O-deethylase (EROD) activity, aryl hydrocarbon hydroxylase (AHH) activity and P4501A mRNA levels in livers and the concentrations of PAH metabolites in bile. For Cruise 1 AHH activity was measured in a variety of invertebrate groups. As others have demonstated, AHH activity was shown to be an ineffectual indicator of contaminant exposure in invertebrates. AHH activity was low to non-detectable in all invertebrate samples, indicating little or no induction. Consequently, *in vitro* rat hepatoma H4IIE cell bioassays were conducted to evaluate the levels of organic contaminants in extracts from invertebrate tissues. There were no statistically significant differences in the catalytic enzyme activity of any species of fish for near and far station comparisons at the three platforms. There was an excellent correlation between AHH and EROD activity for most fish species, which confirms that these two assays measure the same effect (i.e., P4501A induction). There were; however, a few notable exceptions. *Arius felis* and *Lagodon rhomboides* exhibited dramatically higher EROD than AHH activity. The fish that are outliers for the corrdinated induction of AHH and EROD activity undoubltedly express an altered P4501A protein compared to most other fish species and this observation is unique. This observation will be further investigated by conducting dosing experiments on Cruise 3. There is insufficient data for biliary metabolite concentrations, P450IA mRNA levels, and rat hepatoma H4IIE bioassays to draw statistically valid conclusions. Future samples will be needed to provide a data set large enough to be able to determine if P4501A induction can be correlated with distance from a platform.

Sea urchin embryological development assays displayed significant toxicity to sediment pore waters at several of the sites during the first cruise. No toxicity was observed at MU-A85 and the six most toxic stations were observed at the inner stations at HI-A389. Significant toxicity was observed at 14 of 125 stations, mostly within 150 meters of a platform. The sea urchin fertilization assay displayed significant toxicity at three sites, all of which were also toxic in the embryological development assay. The sea urchin embryological development assay displayed significant toxicity in the vicinity of two of the three platforms during the second cruise. As in the

first cruise, no toxicity was observed near the MU-A85 platform, but toxicity was observed at one of the reference stations (5C). Three of the five stations closest to the MAI-686 platform exhibited toxicity. Four of the six HI-A389 stations which were observed to be toxic from Cruise 1 were again found to be toxic. Significant toxicity was observed for eight of the 75 samples. All of the toxic stations (except the one station at MU-A85) were within ~75 meters of a platform. Preliminary analysis of Cruise 1 data indicates a link between toxicity and bulk sediment trace metal concentrations, especially zinc.

A number of issues are actively being addressed in an effort to improve and fine-tune the overall study. These include technical as well as data analysis issues. On the technical side a series of new techniques continue to be developed for possible application including meiofauna genetic diversity studies, an immunological probe to estimate reproductive effort in invertebrates, utilization of more appropriate (i.e., indigenous species) organisms for bioassays, and evaluation of various *in vitro* toxicological assays presently utilized in mammalian and terrestrial systems.

Future program activities include two more field collections in January-February and June, 1994. These additional cruises are essential to increase the overall power of the experiment to recognize statistically significant effects related to the presence of long-term offshore production facilities and the associated activities.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.