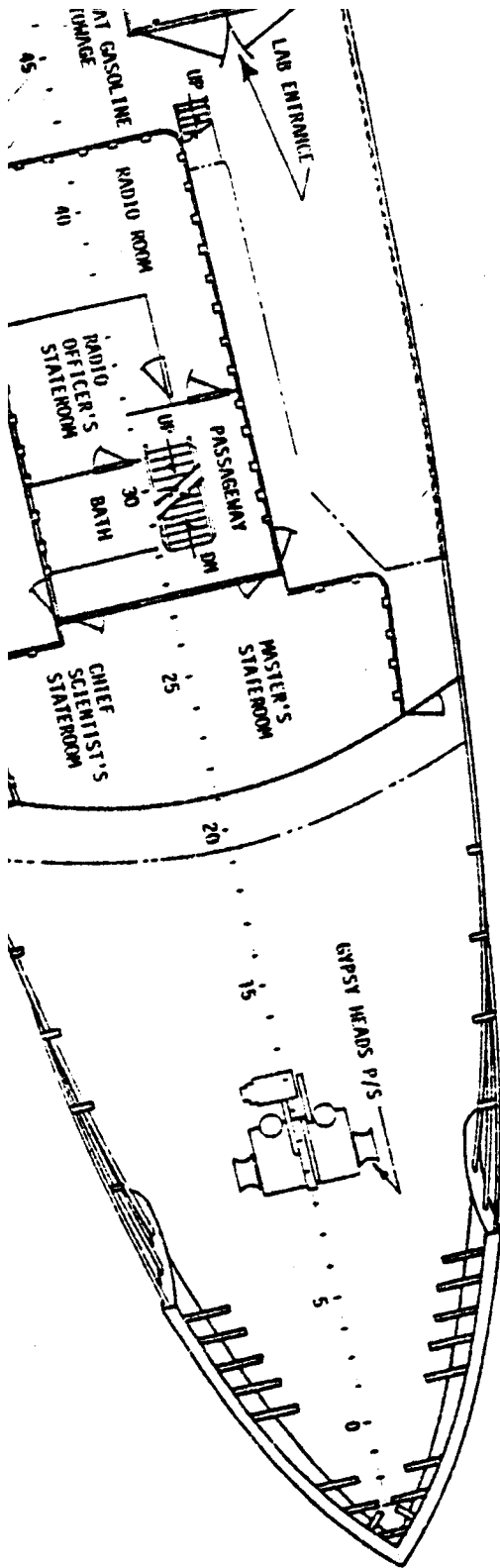


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**NEW ENGLAND OCS
ENVIRONMENTAL BENCHMARK
DRAFT FINAL REPORT VOLUME I
MAY 19, 1978**

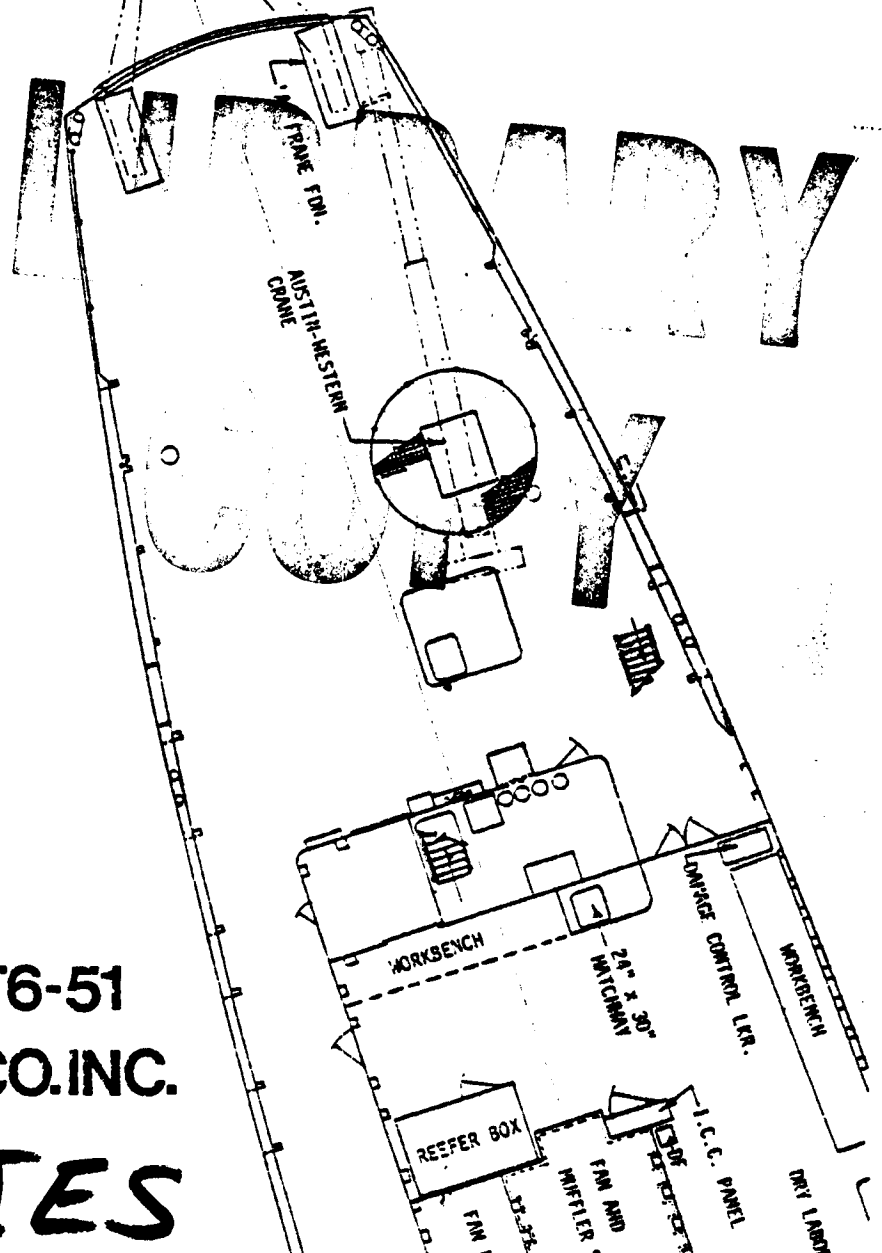


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STUDIES



NEW ENGLAND OCS ENVIRONMENTAL BENCHMARK

Draft Final Report

Contract No. AA550-CT6-51

**DEPARTMENT OF
ATL. OCS REG.**
Submitted to:

**Bureau of Land Management
Department of the Interior
Room 2451
Washington, D.C.**

Submitted by:

**Energy Resources Company Inc.
185 Alewife Brook Parkway
Cambridge, Massachusetts**

**Joel E. Alpert, Ph.D.
Program Manager**

May 19, 1978

PRELIMINARY DRAFT

FOREWORD

This draft document presents the initial findings of the first year's sampling effort on the North Atlantic OCS program. The production of this report was somewhat hampered by time and monetary problems. Because of these problems there was no substantive input by either of the two benthic infauna Principal Investigators or the histopathology Principal Investigator. Despite these shortcomings, it is felt that this document answers many questions regarding processes on Georges Bank and raises an equal number of questions which should be pursued before active oil development commences in the North Atlantic OCS area.

The reader should keep in mind that this is a preliminary draft report. The rapid turnaround time required that editorial changes be made almost simultaneously with the scientific input. Because of this it was impossible for the authors to review the final manuscript in its entirety before submission to the BLM. While we feel that this process in no way affects the individual results presented by the authors, we believe that the release of any of the data contained in this report should be carefully controlled so that the recipient is well aware of the constraints.

PRELIMINARY
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ACKNOWLEDGEMENTS

The program manager would like to acknowledge the excellent cooperation and aid given him by all the numerous scientific personnel involved in this program. It was an extreme pleasure to be associated with such an outstanding scientific team.

Likewise, the cooperation of the two BLM COAR's, Eiji Imamura and Ken Berger, deserves special mention. The success of the program was in large measure a direct result of their active support.

Finally, special mention should be made of the excellent support given to the program manager by the Energy Resources production department and, in particular, Charline Lake. Without their untiring efforts this manuscript would not have been produced in such a timely fashion.

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CHAPTER SIX QUALITY CONTROL

CHAPTER ONE

BACKGROUND

1.1 Introduction

This report presents the findings of the first year New England Outer Continental Shelf (OCS) environmental benchmark study. This contract was awarded to Energy Resources Co. Inc. by the Bureau of Land Management (BLM) on September 1, 1976. The report is a compilation and integration of data obtained by the 10 Principal Investigators and over 100 other scientists who worked on the program. The results must be considered preliminary since they are based on data from only one sampling cruise per season, a base that is in most cases too limited to allow definitive statements regarding oceanographic processes.

1.2 The Marine Environmental Studies of the New England Outer Continental Shelf

In addition to the New England OCS environmental benchmark study being performed by the Energy Resources team and reported in this document, two other major field programs in the same region are being funded by the BLM. The U.S. Geological Service (USGS) has been conducting a program designed to investigate sediment mobility, seston flux, sediment characteristics and distribution, and geologic hazards. Some of the results of this USGS study are appended to this final report. Simultaneously, Raytheon and EG&G are

conducting a program of study designed to describe the current circulation (surface, middle, and bottom), and mixing processes of the waters of the Georges Bank region. The results of this physical oceanography study will be reported in separate documents.

Other studies in the general Georges Bank region during the year were performed by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service; Dalhousie University; and the Universities of New Hampshire and Rhode Island. In addition, a considerable amount of site-specific work was done during and after the Argo Merchant oil spill on December 15, 1976 (see, for example, Grose and Mattson, 1977; Center for Ocean Management Studies, University of Rhode Island, 1978).

1.2.1 Objectives and Purpose

The benchmark studies have been defined (Truesdell, 1976) as "a broad area, multi-year survey program intended to provide a statistically, scientifically sound characterization of key environmental aspects including physical, biological, geological, and chemical. The objective is to establish the range of variation of critical parameters that will reflect the impact of Outer Continental Shelf (OCS) oil and gas exploration and development activity. This benchmark will be used as the framework for comparison of measurements made on site specific surveys to determine whether the site is representative or atypical of that geographic area and will be used to determine which sites should be monitored or studied more closely. Benchmark data will also be useful in defining the general type of environment with which we are

dealing, and in evaluating the longer-term natural variability of environmental parameters.

"Benchmark studies will help us address critical questions that the decisionmaker will need to have answered.

"1. What is the biological, chemical, geological, and physical nature of the environment being considered for leasing?

"2. Are there any unique or sensitive aspects to that environment that will be upset if exploration and production take place? (This assumes we know something about sensitivity of various species, populations, or ecosystems to the effects related to oil and gas development.)

"3. If development proceeds what organisms are present that might be impacted?

"The benchmark study generally consists of four types of information. That used to establish a chemical benchmark of ambient levels of high molecular weight hydrocarbons and selected trace metals; that used to establish the nature and status of biologic communities especially resident species; that used to identify possible indicator organisms or processes; and that information used to support the interpretations of the other data sets."

The specific long-range objectives for the North Atlantic OCS benchmark study and first-year goals as outlined in the contract are listed below.

Long-Range Objectives

The long-range objectives of the study are to:

1. Determine the ranges of high molecular weight (HMW) hydrocarbons and selected trace metal concentrations in the sediments and selected macrofauna species preceding oil and gas development against which possible man-induced chemical change can be assessed in the future.
2. Delimit the major chemotopes, lithotopes, and biotopes for the study area, and characterize each with respect to natural seasonal variability and interrelationships.
3. Characterize the existing health of selected benthic macrofauna preceding oil and gas development, and establish a histological data base that can serve as a reference for later comparisons.
4. Describe dominant microbes in sediments and in the upper water layers and evaluate their "potential" and possible importance in the degradation of oil.
5. Describe the HMW hydrocarbon and selected trace metal concentrations in the water column.
6. Identify and describe unique or fragile/endangered areas.
7. Collect other data supportive of the above objectives.

First-Year Goals

Within the first year, the geochemical objective is to initiate seasonal studies to determine the range in concentrations of HMW hydrocarbons and selected trace metals in the sediments preceding oil and gas development.

The objective with respect to macrofaunal chemistry is to initiate seasonal studies to determine the existing range of HMW hydrocarbon and trace metal concentrations in selected benthic macrofauna species preceding oil and gas development.

The analysis of the benthic infaunal community has the objective of initiating seasonal studies of benthic infaunal (>0.5 mm) communities and foraminiferal populations to determine the natural ranges of selected parameters.

The objective of the histopathology effort is to initiate histological studies to determine the health of selected benthic macrofaunal species preceding oil and gas development.

The objective with regard to descriptive chemistry is to describe seasonal variation in the concentrations of HMW hydrocarbons and selected trace metals in the water column.

The microbiological objective is to enumerate all microbes and identify dominant microbes in the surface film, near-surface water, and sediments; to evaluate the oil-degrading "potential" of these organisms; and to examine the relationship between oil concentration and the ratio of hydrocarbonoclastic microbes to aerobic heterotrophic microbes.

In addition, the data support effort has the objective of collecting samples and conducting analyses supportive of (1) benchmark data interpretation, (2) the program conducted by the contractors for physical oceanography (Raytheon and EG&G), and (3) the program conducted by USGS.

1.2.2 Program Organization

The study involved more than 100 scientists from nine participating organizations. The final organizational structure for the program and the names of the Principal Investigators together with their scientific responsibilities and affiliations are listed in Table 1-1.

1.3 The New England OCS: A Description of the Region

1.3.1 Geography and Physiography

The area of study lies roughly between longitudes 65°30' W and 71°00' W and latitudes 40°00' N and 43°00' N (Figure 1-1). It encompasses all of Georges Bank proper, Georges Basin, part of the Gulf of Maine southwest of the Franklin Swell, the Great South Channel, Nantucket Shoals, and the area due south of Martha's Vineyard and Nantucket. The relatively shallow Georges Bank acts as a barrier between the Gulf of Maine waters to the north and the Atlantic deep waters and Gulf Stream waters to the south.

During the last ice age the southern front of the ice sheet in this region more or less covered the northern part of Georges Bank across what is now Great South Channel,

TABLE 1-1

PARTICIPANTS IN THE NORTH ATLANTIC ENVIRONMENTAL BENCHMARK STUDY

NAME	SCIENTIFIC RESPONSIBILITY	ORGANIZATION
Joel Alpert	Program Manager	Energy Resources Co. Inc.
Roy McDonald	Data Manager	Energy Resources Co. Inc.
Chris Hydeman	Program Secretary	Energy Resources Co. Inc.
Reinier Courant	Field Manager	Energy Resources Co. Inc.
Steve Piotrowicz	Inorganic chemistry	Energy Resources Co. Inc.
Paul Boehm	Hydrocarbon chemistry	Energy Resources Co. Inc.
Dave Strimaitis	Physical oceanography	Energy Resources Co. Inc.
Tom Novitsky	Microbiology	Energy Resources Co. Inc.
Mort Jangorbhani	Neutron activation analysis	Mass. Inst. of Technology
Richard Toner	Macrobenthos, foraminifera, zooplankton	Marine Research Inc.
Allan Michael	Benthic infauna taxonomy	Taxon, Inc.
Leslie Watling	Amphipods	Darling Center
Peter Larsen	Benthic infauna taxonomy	Bigelow Laboratory
Scott Warner	Hydrocarbon chemistry	Battelle Columbus Laboratory
Chris Wethe	Micronutrients, grain size	University of Delaware
Jeffrey Tinsman	Histopathology	University of Delaware
Don Maurer	Polychaetes	University of Delaware
Lowell Sick	Trace metals	University of Delaware

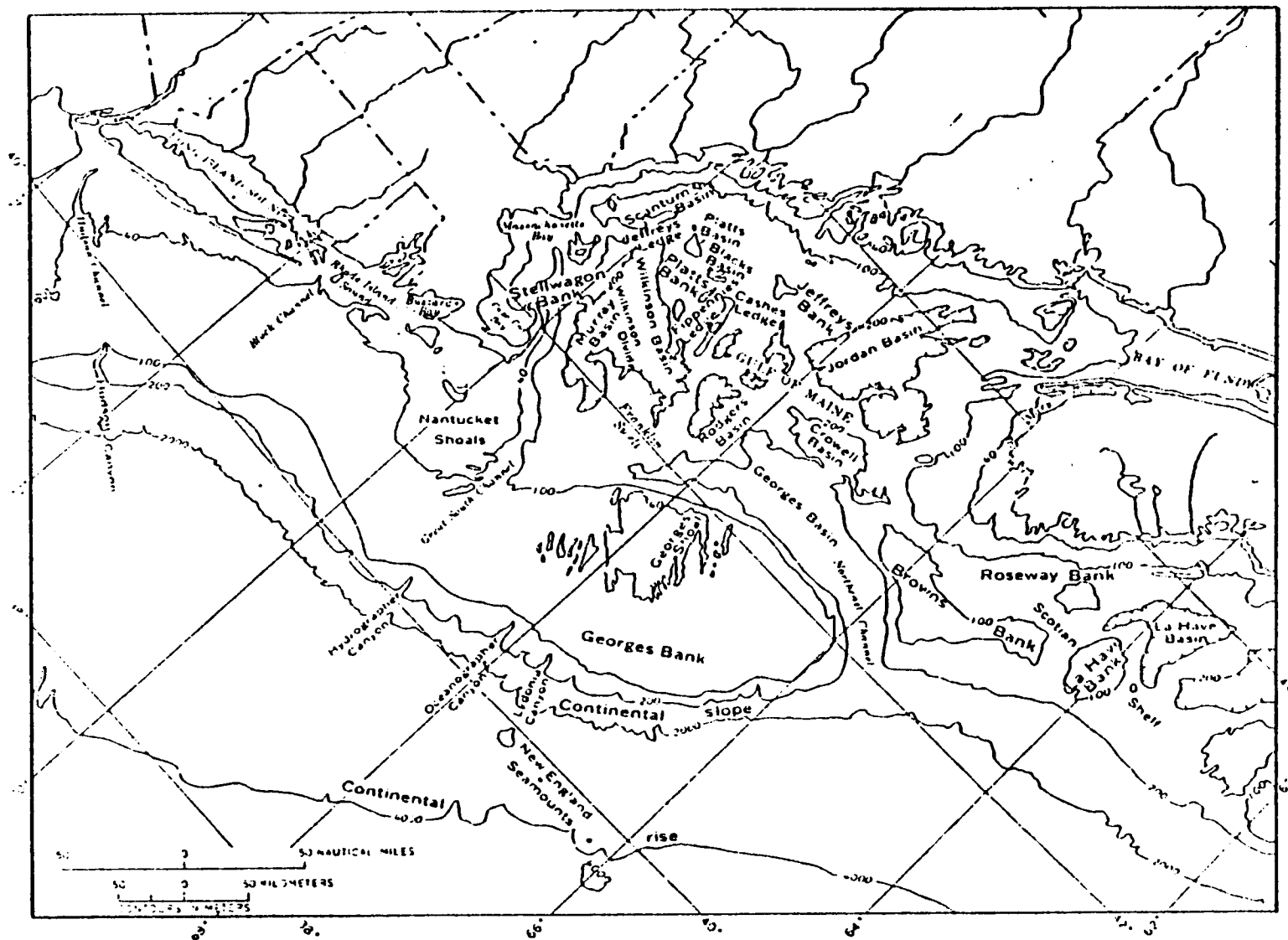


Figure 1-1. Bathymetric map of the continental margin off the Northeastern United States. (Schlee, 1973.)

stretching westward across Nantucket and Martha's Vineyard. Together with present depositional agents, this glacial history determines the topography as well as the type of sediments on Georges Bank (Schlee, 1973).

The main topographical features of the region are:

1. The extensive areas of sand waves and tidal shoals southeast of Nantucket and on the northern half of Georges Bank.
2. Great South Channel and Northeast Channel.
3. The canyons on the southern flank of Georges Bank.

Tidal ridges on Georges Bank align approximately northeast-southwest and can stretch for several kilometers. Superimposed on these ridges, which tend to be broad, are smaller amplitude sand waves. The axes of the latter are normal to the axial trends of the tidal ridges (Stewart and Jordan, 1964; Uchupi, 1968). Except for the canyons on the flanks, the southern part of Georges Bank is relatively featureless (Schlee, 1973).

1.3.2 Currents and Hydrography

The general hydrographic features of Georges Bank have been discussed several times (e.g., Bumpus, 1976; Colton et al., 1968). The major factors include strong mixing over the shallower sections of the Bank due to wind and semi-diurnal tides; winter overturning, spring runoff, and the summertime mixed layer in the Gulf of Maine; and the intermittent intrusion of Slope water along the southern

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edge of the Bank as the Shelf-Slope water front responds to large-scale forcing.

Summer data consistently reveal exceptional mixing over the Bank after the heated layer that dominates the water structure in the Gulf of Maine has been weakened. This mixing, along with some degree of upwelling at the Bank's boundaries, has been cited as one of the underlying processes supporting the high productivity of Georges Bank (Yentsch, 1977).

A second feature thought to enhance the region's productivity is the presence of the Slope water front near the southern boundary of the Bank (Horne, 1977; Fournier et al., 1977). This water mass, lying between Shelf and Gulf Stream waters, frequently interweaves with the Shelf water along the front and encourages lateral exchange across the front (Voorhis et al., 1976; Horne, 1977). At times the front also becomes extremely convoluted under the influence of anticyclonic eddies that are formed from shoreward Gulf Stream meanders. These eddies drift southwest between the Continental Shelf and the Gulf Stream, frequently pulling Shelf water into their circulation and pushing Slope water up the southern slope of the Bank. Such eddies have been studied by Saunders (1971) and Gotthardt et al. (1974), among others.

General nontidal circulation features have been described by Bigelow (1927) and Bumpus and Lauzier (1965) and reviewed by Bumpus (1976). These indirect circulation measurements as well as more recent current water studies (Brown et al., 1977; Butman et al., 1977) show the surface circulation to be a counterclockwise gyre in the Gulf of Maine accompanied by a seasonal clockwise gyre over Georges

Bank. This double gyre system is strongest in spring, breaking down into a weaker Gulf of Maine gyre with a southerly and westerly flow across Georges Bank by autumn. Near-bottom drifts over the shallower parts of the Bank tend to follow a clockwise course with a net flow to the west across Great South Channel. In deeper water, the bottom drift has not been well characterized, but there is evidence of upwelling along much of the Bank's boundary.

1.3.3 Sedimentology

Most of the surficial sediments in the study area were deposited during the time of the Pleistocene glaciation. The glacial terminal moraine running from Martha's Vineyard and Nantucket, through Nantucket Shoals and Great South Channel, and along the northern edge of Georges Bank (Schlee, 1973) divides the study area into two distinctive sedimentary regions. Fine sands and silty sands predominate in the smooth, gently sloping region south of the moraine; coarse sands and gravels are found in the irregular, shoally northern area of the Bank (Emery and Uchupi, 1965).

The fine sands and silty sands of the southern half of Georges Bank are remnants of a Pleistocene glacial outwash plain. These sediments were deposited through glaciation and incised by rain and meltwaters. As sea level rose after the glacier receded, the resultant channels were filled with stratified sediments. Continuous seismic profiles of George Bank have revealed these buried channels formed by the glacial meltwater. The surface sediments have been smoothed and reworked by intermittent transgressions and regressions of sea level since Pleistocene time (Knott and Hoskins, 1968). The only fine-grained sediments found

in this region of the Outer Shelf are located south of Rhode Island and Martha's Vineyard. These silty sand and sandy silt deposits lie over older sandy sediments and probably were deposited on a shallow part of the Shelf during the last sea level rise. As a veneer structure, they represent only the most recent depositional environment found in the area, one of low energy.

The sands and gravels of northern Georges Bank are remnants of the glacial moraine. North of the Bank, the irregular topography and the complex nature of the sediments are indicative of a glaciated region. Such poorly sorted, variable sediments show characteristics of a ground moraine with a minimum of reworking. The Pleistocene glaciers overrode this area and dropped unstratified deposits ranging from clay to boulders. The seaward shoals have protected these sediments from major marine reworking (Emery and Uchupi, 1965).

Sediment types observed during the current study corresponded to the findings of Schlee (1973), Wigley (1961), and Stetson (1939). The repeated sampling of the same stations and the number of replicates taken at each station for this study provided a unique look at the local variability of the sediments. In general, sediments from along the remnant moraine and from the glaciated basin areas exhibited high local variability. Sediments from south of the moraine tended to be more homogeneous within a station.

1.3.4 Biology

1.3.4.1 Microbiology

Most of our knowledge of the microbiology of the northwestern Atlantic comes from studies on the Gulf of Maine and the Scotian Shelf; Georges Bank has received little attention from microbiologists until the present study. Generally, information from microbiological studies on similar areas (e.g., Grand Banks) is relevant to Georges Bank. An article by Galen E. Jones entitled "Marine Microbiology on the Continental Shelf off the Eastern United States from the Bay of Fundy to Cape Hatteras" (in preparation for the BLM) provides a comprehensive review of such parallel microbiological studies.

Reuszer (1933) and Sieburth et al. (1976) conducted investigations that included stations on Georges Bank. Reuszer (1933) found that during the summer of 1932, bacterial (heterotroph) concentrations in seawater off Cape Cod Light were low, ranging from 0 to 12 bacteria per ml when determined from plate counts. Bacterial concentration in bottom sediments from this area ranged from 23,100 bacteria per gram of dry mud at Station 8 (furthest from shore) to 5 million bacteria per gram of dry mud at Station 6 (closest to shore). Reuszer (1933) also compared the organic content of the mud to the bacterial concentration and found a strong positive correlation. Waksman et al. (1933) obtained similar findings on the Gulf of Maine.

Sieburth et al. (1976) have recently shown that bacterial concentration in the subsurface water of Georges Bank (Station 2) ranges from 50 bacteria per ml (monosaccharide medium) to 1,000 bacteria per ml (peptone yeast

medium). Values obviously vary with the investigator and with the plating medium used. Generally, current techniques produce higher values than those used before 1950. Sieburth et al. (1976) also examined the surface microlayer at Station 2 and found between 50 and 500 bacteria per ml. Although several of the stations in the northwestern Atlantic had surface microlayers with high bacterial counts, Station 2 (Georges Bank) did not.

Three other studies not conducted on Georges Bank are also relevant. The first concerns the distribution of heterotrophic and hydrocarbon-utilizing bacteria in the northwestern Atlantic. Using a most probable number (MPN) technique, Mulkins-Phillips and Stewart (1974) found between 140 and 800 heterotrophic bacteria per ml and between 6.6 and 74 hydrocarbon-utilizing bacteria per ml in water of the Scotian Shelf. They found that numbers of bacteria (both heterotrophic and hydrocarbon-utilizing) decreased with distance from shore. Their work confirmed the earlier findings of Gunkel (1973), Atlas and Bartha (1973), and Kinney et al. (1969) with respect to both numbers of bacteria found and the relative proportions of heterotrophs and hydrocarbon utilizers.

In a second study, Anthony (1963) found that Scotian Shelf sediments contain 13,000 bacteria per gram sediment (dry weight). Anthony also found that bacterial concentrations in sediments as determined from plate counts were subject to a standard error of ± 36 percent and furthermore that bacterial concentrations determined by the fluorescence (direct count) technique of Strugger (1944) were higher than concentrations determined from plate counts by a factor of 10,000.

Finally, using a direct counting method similar to that used by Batoosingh and Anthony (1971), Dale (1974) found high bacterial counts in Nova Scotian intertidal sediments (117 million to 9.97 billion bacteria per gram dry sediment). In addition, Dale found a high negative correlation between bacterial number and sediment grain size. Organic carbon and nitrogen in the sediment also varied inversely with grain size. Organic carbon and nitrogen, on the other hand, showed a positive correlation with bacterial number. Dale was unable to distinguish a factor controlling bacterial number in the sediment.

1.3.4.2 Benthic Infauna

Benthic infauna in aquatic ecosystems have been widely studied in the past several decades. Following the early work of Petersen and Jensen (1911) and Petersen (1913, 1914, 1915), several investigations appeared which immediately underscored the dependence of finfish stocks on benthic resources (Blegvad, 1928, 1930; Jensen, 1919; Petersen, 1918). More recently, Jones (1952) demonstrated the importance of benthos in the diet of flatfish. Wigley (1965) showed the general correlation between the density distribution of benthic organisms and that of groundfish on Georges Bank.

Recent work has demonstrated the importance of benthic infauna in the recycling of nutrients and other materials incorporated into marine sediments and in the determination of bottom sediment characteristics. Renfro (1973) found that burrowing activity greatly accelerates the release of heavy metals from contaminated sediments. Rhoads (1973) demonstrated an increase in suspended seston due to biogenic

reworking. Young and Rhoads (1971) described the importance of polychaetes in stabilization of soft sediments in Cape Cod Bay, and Rhoads and Young (1971) found that fecal cones of holothurians provide a stable surface for certain suspension feeders that are otherwise excluded from very soft sediments. Similar modifications of the benthic environment resulting in changes in community structure have been documented in other areas (Rhoads, 1967; Rhoads and Young, 1970).

In addition to their importance to community trophic structure, benthic infauna also serve as excellent indicators of environmental perturbation. Most species are effectively sessile within the spatial scope of monitoring programs and may serve as early indicators of stress via bioaccumulation potential (Bryan and Hummerstone, 1971, 1973a, 1973b), precipitous decline of individual species (Blumer et al., 1970; Sanders et al., 1972), or subtle changes in community composition (Michael et al., 1975).

Although Georges Bank is commonly regarded as one of the most productive fishing grounds in the world (Woods Hole Oceanographic Institution, 1976), relatively little is known about its benthic populations. The first description of them appeared as recently as 1961 (Wigley, 1961); subsequent articles (Wigley and McIntyre, 1964; Wigley and Theroux, 1970) have provided little new information. The present study will be the first to establish quantitative distribution of the benthos at a species level over the Bank.

1.3.4.3 Polychaetes

Deepwater polychaetes from the western Atlantic were described by Hartman (1965) and Hartman and Fauchald (1971). The most comprehensive treatment of polychaetes from the northeastern coast of the United States was performed by Pettibone (1963a). Pettibone described 183 species from 29 families; cited records of depth, sediment preference, and reproductive condition; and collated the research of such earlier workers. Since then Pettibone has published research on paraonids, spionids, sigalionids, pilargids, nereids, polynoids, trochochaetids, and ampharetids (Pettibone, 1962, 1963b, 1965, 1966, 1970a, 1970b, 1971, 1975, 1976, 1977).

Other taxonomic studies concerning families or species of Georges Bank are Day (1967, 1973), Fauvel (1923, 1927), Gardner (1976), and Hartmann-Schroder (1971). Pratt (1973) reviewed the literature on polychaetes from Nantucket to Cape Hatteras. Kinner and Maurer (1978) described a total of 136 species representing 34 families from the Delaware Bay region. Kinner (1978) identified and analyzed a series of quantitative samples taken along the Continental Shelf from Cape Cod to Cape Hatteras. The samples were collected by the National Marine Fisheries Service and generously loaned to us for the present study.

Several general documents on polychaetes that have some bearing on Georges Bank are issues of an informal newsletter of polychaete research and a bibliography of polychaetes by Charlene Long. The former presents reports of research in progress, titles of published work, changes in addresses of polychaete specialists, and research requests. Finally, a

key for polychaetes dealing with orders, families, and genera may become very useful in stabilizing taxonomic usage at these levels (Fauchald, 1977).

1.3.4.4 Ichthyoplankton, Zooplankton, Benthos, and Foraminifera

Early work on the ichthyoplankton of Georges Bank addressed the distribution and survival of haddock (Melanogrammus aeglefinus) eggs and larvae (Walford, 1938; Colton and Temple, 1961; Colton and Marak, 1962; Colton, 1965). Colton (1959) reported a high incidence of dead fish larvae in the Slope water off Georges Bank following a shift in the current in 1956. Colton (1961) discussed the distribution of Bothus ocellatus and myctophid larvae in the Bank area. The Bureau of Commercial Fisheries, Woods Hole, Massachusetts began an ichthyoplankton study of Georges Bank, Browns Bank, and the Gulf of Maine in 1953 (Marak and Colton, 1961). The main object of this survey was to study the early life history of the haddock, but data are presented on other commonly collected larval species (Marak et al., 1962a, 1962b). In 1971 the International Commission for the Northwest Atlantic Fisheries began a series of cooperative cruises to survey larval Atlantic herring (Clupea harengus harengus) in the Gulf of Maine-Georges Bank area. Colton and Byron (1977) summarized the abundance, length, and distribution of the more common larval fish taken on these cruises between 1971 and 1975.

In studies of the zooplankton on and around Georges Bank, several authors have described the seasonal fluctuation and quantitative distribution of crustaceans and other pelagic organisms from the area (Bigelow, 1926; Fish and Johnson,

1937; Riley et al., 1948) and used the distribution and abundance of selected species of copepods to show the intrusion of oceanic water across the Bank (Colton et al., 1962). Detailed information on the abundance and variability of copepods and certain species of Sagitta in relation to the hydrographic conditions on the Bank was obtained from 11 surveys of the area from 1939 to 1940 (Clarke et al., 1943). The effects of physical factors on the distribution of larger forms of crustacea, such as amphipods, euphausiids, mysids, and decapods, were also studied (Whitely, 1948).

Because of their role in the nutrition of herring-like fish, the various copepods belonging to the genus Calanus have been studied with respect to their taxonomy (Frost, 1971); distribution (Jaschnov, 1970); and molting cycle, development of sexual characteristics, and sex ratio (Conover, 1965).

In 1872 a qualitative survey of the benthic organisms was performed (Smith and Harger, 1874). It was not until 1961 that the first quantitative inventory was made and certain relationships between abundance of benthos and sediment defined (Wigley, 1961). More detailed benthic studies of the Bank followed (Wigley, 1968), as well as studies evaluating the abundance of commercial molluscs (Wigley and Emery, 1968; Wigley and Theroux, 1970).

There has been no study of Georges Bank foraminifera per se. Some studies have been done on near-shore and Shelf areas all along the North Atlantic coast. Cushman (1918-31) published a monograph of the foraminifera of the Atlantic Ocean, but it was not until 1939-40 that cores were collected from the edges of the Bank (Phleger, 1942; Sen

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Grupta, 1971). Parker (1948) surveyed the Continental Shelf from the Gulf of Maine to Maryland. There followed near-shore studies of the area around Martha's Vineyard (Todd and Low, 1961), around Nantucket (Lidz, 1965), in Vineyard Sound (Murray, 1969), and off Massachusetts (Kafescioglu, 1975).

1.3.5 Chemistry

1.3.5.1 Hydrocarbon Chemistry

Background hydrocarbon biogeochemical data on the immediate study area are lacking. However, there is a fair amount of information on northwestern Atlantic Continental Shelf and estuarine sediment hydrocarbon chemistry from studies of Farrington and Tripp (1975) on the New York Bight-Hudson Channel region, Farrington et al. (1978) on the Gulf of Maine and Buzzards Bay, Boehm and Quinn (1978) on Rhode Island Sound, and Farrington and Quinn (1973) on Narragansett Bay. Virtually no published data are available concerning OCS macrofaunal, zooplankton, dissolved seawater, and particulate hydrocarbon chemistry in the area. Teal and Farrington (1978) studied the relation of macrofaunal hydrocarbons to the sediment hydrocarbon chemistry in which they live, as did Boehm and Quinn (1978). The above-mentioned studies all aim toward a description of the sources and diagenesis of hydrocarbons in sedimentary environments. Certain trends and similarities within the region studied have become apparent. In a recent study of the sediment chemistry on the Scotian shelf, Kerzer et al. (1978) determined the hydrocarbon compositions of surface sediments near shore and out on a transect to Sable Island. Concentrations and possible sources of the observed distributions were described.

Furthermore, the literature includes several hydrocarbon geochemical and biogeochemical studies on the North American OCS and the world's OCS's in general. Farrington and Meyers (1975) have summarized these studies, many of which offer sound comparative information on the sources and transport of hydrocarbons in the marine environment. In addition, much information is available on hydrocarbon distributions on the OCS from other BLM programs, although much of it has not appeared yet in the open literature with the exceptions of Reed et al. (1977) and Gearing et al. (1976).

Much of the recent work on polynuclear aromatic hydrocarbons (PAH) in marine sediments and urban air in industrial regions has been performed by Hites and coworkers at MIT and by Blumer and coworkers at Woods Hole Oceanographic Institution (WHOI), among others. Information on sources of PAH's, discrimination between petroleum-derived PAH's and those from combustion processes, and the fate of PAH's in sediments is found in Hites, La Flamme, and Farrington (1977); Muller, Grimmer, and Boehnke (1977); Hites and Biemann (1975); Hase and Hites (1976); Blumer and Youngblood (1975); and Lee et al. (1977).

1.3.5.2 Trace Metal Chemistry

Sedimentary Trace Metal Chemistry

Continental Shelf sediments on Georges Bank proper are mostly sand (62 to 2,000 μ m in diameter), with high gravel concentrations at some sites (Milliman, 1972). Carbonates are minor components of these sediments, averaging less than 5 percent of the total mass. Sediments in the Gulf of Maine

and on the Continental Slope, on the other hand, are dominated by silt and clay-sized materials with occasional biogenic sand present. These sediments tend to have a high organic content (0.1 percent or greater) compared to the sands (approximately 0.01 percent). The fine-grained silts and clays are anoxic just below the oxygenated sediment-water interface.

The sources of suspended matter in the study area are resuspended bottom sediments, biogenic material, and, possibly, atmospherically deposited material; freshwater runoff is a negligible factor. Waters on the Bank tend to have lower suspended loads than those to the west of Nantucket Shoals (Milliman, personal communication). The waters of the Bank proper are generally well mixed throughout the year. The waters of the Slope and Gulf of Maine regions tend to be well mixed in winter and partially stratified in summer, with this stratification breaking down in autumn. Resuspended bottom material, in general, is distributed throughout the water column through all seasons directly onto the Bank. During the seasons when Slope, Nantucket Shoals, and Gulf of Maine regions are well mixed, there is evidence of mixing of bottom material throughout the water column. During summer, however, stratification creates two different distributions of suspended matter. The suspended material in these waters, below the thermocline, has relatively large quantities of resuspended bottom material in it. Suspended matter in surface waters off the Bank, especially during periods when a thermocline is present, is composed of biogenic and possibly atmospherically derived material. There is evidence that intrusions of water from off the Shelf can significantly alter this pattern.

No direct studies of the extent of atmospheric deposition of particulates have been made in the region,

although Duce et al. (1976) investigated the atmospheric contributions of trace metals to New York Bight. They estimated the atmospheric lead flux at Nantucket, Massachusetts to be approximately 85 MT/1,000 km² per year and the zinc flux to be approximately 76 MT/1,000 km² per year. There is evidence in this work of a large atmospheric source of Pb to the Georges Bank region.

The trace metal geochemistry of the Georges Bank region has not been extensively investigated. This study, together with the work of the USGS, is the first concentrated effort to understand Georges Bank as a dynamic, biogeochemical system.

Benthic Trace Metal Chemistry

Although marine zooplankton constitute a major component of the total biomass of the marine environment and thereby play a major role in controlling trace metal cycling, only minimal information exists regarding trace metal composition and flux. Much of the available information concerning concentrations of metals in marine plankton was gathered prior to the development of present technology for high-precision trace metal analysis within low detection limits (e.g., Vinogradov, 1953; Nicholls et al. 1959). Several studies concerning the trace metal composition of marine plankton did not distinguish between phytoplankton and zooplankton (Martin, 1969; Windom et al., 1973). Although Knauer and Martin (1973) reported seasonal variation in trace metal composition of phytoplankton in one area off the California coast, neither seasonal nor geographic variation in trace metal composition among zooplankton has been monitored.

Specifically, concentrations and distributions of trace metals among plankton communities of Georges Bank have not been thoroughly investigated. Trace metal concentrations in phytoplankton populations of the North Atlantic region have been measured on a random basis by several investigators (Brewer and Spencer, 1975; Windom et al., 1973). Similarly, a survey for trace metals was conducted among specific species of zooplankton collected only randomly on the New England Continental Shelf and Georges Bank.

Furthermore, interrelations between physical, chemical, and biological processes in the water column on Georges Bank have not been adequately investigated. Redfield (1941) observed relatively high zooplankton concentrations in the northeastern region of Georges Bank and specifically related such concentrations to a convergence zone between the Gulf of Maine and Georges Bank gyres. However, further investigation of possible physical and chemical oceanic processes and their relation to the distribution and bio-availability of trace elements has not been conducted. The only suggestion that water column processes may have significant effects on the bio-availability of trace elements in the Georges Bank region has resulted from a correlation between flux and distribution of nutrients and primary productivity (Yentsch, 1977; Fournier, 1977). A correlation between convergence zones of water (i.e., vertical fronts) and enhanced trace metal concentrations in entrained phytoplankton and zooplankton has also been recently reported (Horowitz and Presley, 1977; Sick et al., 1978).

Similarly, trace metal composition among macrobenthic invertebrates has not been thoroughly investigated with regard to species variation, seasonal trends, or geographic ranges. Although Bryan and Hummerstone (1973) studied

seasonal variation for a large array of trace metals among two species of scallops collected in the English Channel, no attempt was made to discern geographic patterns. Windom (1974) has analyzed several species of macrobenthic organisms but did not attempt to monitor seasonal or geographic trends. Although selected elements, most notably zinc, have been studied in regard to seasonal fluctuations (Galtsoff, 1953, 1964) and geographic variation in and among estuaries (Wolfe, 1970), no attempt has been made to examine such variations among a large variety of metals or several species of organisms, specifically on the Continental Shelf. Furthermore, many of the benthic studies regarding trace metal accumulations have concerned only estuarine and coastal populations and not Continental Shelf communities.

The correlation of the trace element composition in benthic invertebrates with the trace element composition of the ambient environment has been studied predominantly in laboratory and estuarine situations. Geographic distribution patterns and nutritional phenomena of benthic organisms, as well as the chemical composition of macrobenthic invertebrate tissues (Korringa, 1952), have been correlated with sediment and near-bottom suspended sediment composition. Dehlinger et al. (1974) reported that most macrobenthic organisms (particularly bivalves) reflect the trace metal compositions of any industrial and domestic runoff with which they come in contact. Although the effect of water column dynamics on the benthic community is not well understood, general hydrographic dynamics and the shallowness (<100 m) of Georges Bank support the assumption that chemical phenomena in the water column integrally affect benthic populations.

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CHAPTER TWO

SAMPLING

PREPARED BY
DATE

2.1 General Cruise Information

2.1.1 Introduction

Water column and sediment samples were collected from 12 stations during the four cruises. Stations 1, 7, and 42 (Figure 2-1) were selected as representative of water masses associated with inshore phenomena such as terrestrial runoff and anthropogenic effluents. Stations 36, 37, and 39, located on the northwestern margin of Georges Bank, were selected to be representative of conditions resulting from the confluence of the Gulf of Maine counterclockwise circulating surface gyre and the Georges Bank clockwise circulating surface gyre. Several stations (13, 18, and 26) were positioned on the southwestern margin of the Bank to facilitate a general investigation of effects of the Georges Bank gyre and possible effects that the large Shelf-Slope vertical front might have on chemical and biological distributions in the water column. Effects attributable to the vortex phenomenon of a gyre and the supposed relatively static physical condition in the central portion of Georges Bank were investigated at central Stations 18, 32, and 37. Stations 1, 4, and 7 were established to investigate possible effects from relatively abundant ship traffic and for chemical and biological reasons. The broad distribution of stations on the Bank was also thought to cover areas having a high probability of becoming oil lease sites. Likewise

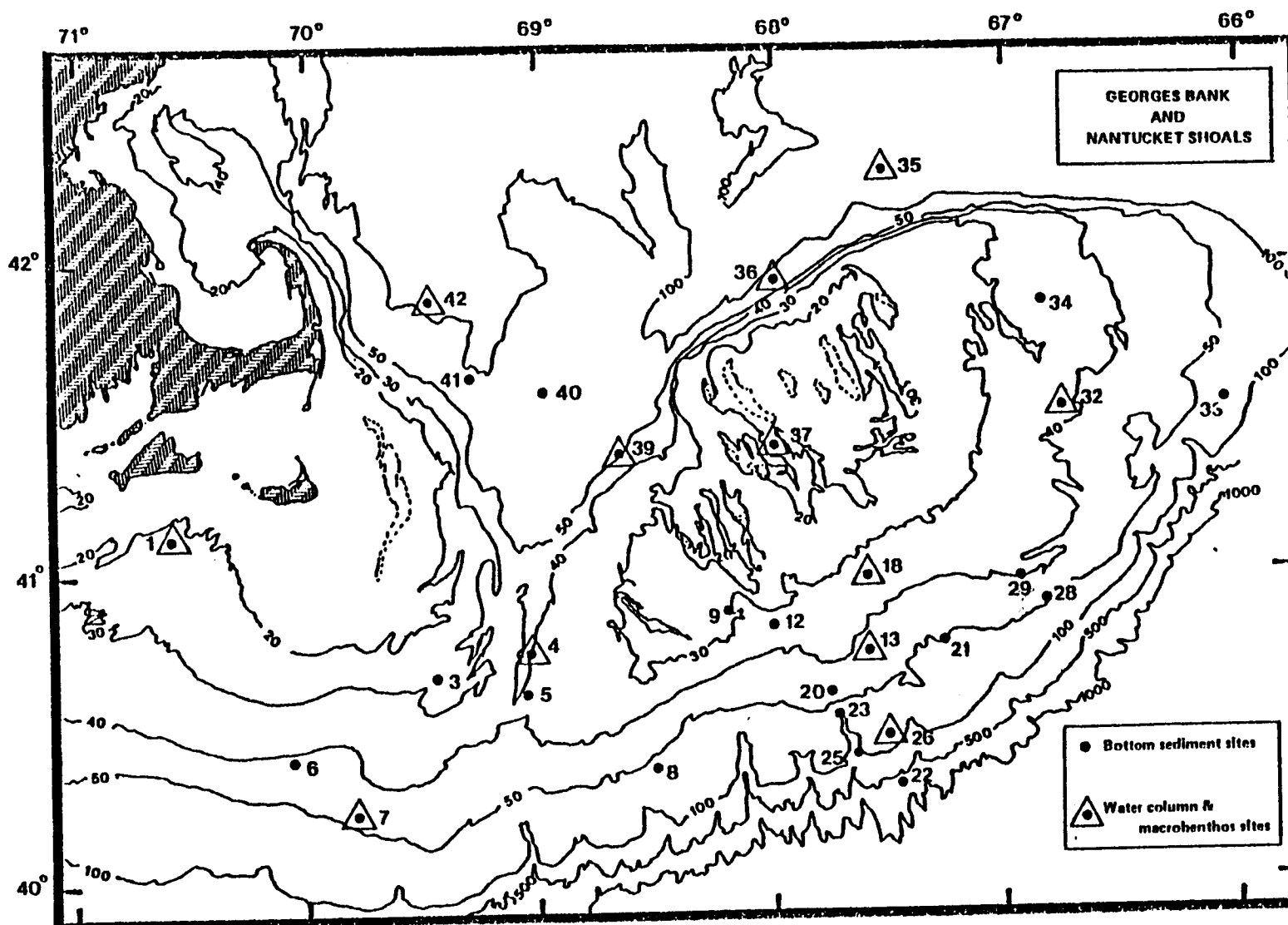


Figure 2-1. Station locations and coordinates

the 30 sediment-only stations sampled over the four seasons were chosen to meet the criteria that they be:

1. Proposed oil and gas lease sites.
2. Areas of high fishing intensity.
3. Areas important as spawning grounds.
4. Areas representing a range of sediment types.
5. Areas representing a range of depths.
6. Areas representing a wide range of physiographic provinces, current, and sediment movement.
7. Areas representing both sides of the zoogeographical boundary.
8. Areas of possible pollutant accumulation.
9. Areas of previous benthic studies.
10. Areas such that adequate control sites be available.

Table 2-1 details the sampling performed for each station type. The station locations are given in Figure 2-1 and their coordinates in Table 2-2.

2.1.2 Sampling Plan

Early in the sampling program, it became clear that, because of the size of the available vessels, the most

TABLE 2-1
SAMPLING ACTIVITY AND STATION TYPE

SAMPLING ACTIVITY	STATION TYPE	
	BOTTOM SEDIMENT	WATER COLUMN
Water column		
Rosette sampler		
CTD/O	x	x
Transmissometry	x	x
Nephelometry	x	x
Salinity/dissolved oxygen	x	x
Micronutrients	x	x
Dissolved organic carbon		x
Particulate organic carbon		x
Particulate trace metals		x
Suspended sediments (USGS)	x	x
Bodman bottle: dissolved and particulate hydrocarbons		x
Brown-McGowan bongo system: zooplankton for trace metals, hydrocarbon, taxonomy		x
Sediment		
Smith-McIntyre grab sampler: sediments for		
Microbiology	x	x
Trace metals	x	x
Hydrocarbons	x	x
Total organic carbon	x	x
Total organic nitrogen	x	x
Sediment analysis	x	x
Foraminifera	x	x
Infaunal taxonomy	x	x
Dredges/trawls		
Epibenthic macrofauna for trace metals, hydrocarbons, histopathology, taxonomy		x
Macroinfauna for trace metals, hydro- carbons, histopathology, taxonomy		x
Benthos for trace metals, hydrocarbons, histopathology, taxonomy,		x
Underwater photography	x	x

TABLE 2-2
STATION LATITUDES AND LONGITUDES

STATION NO.	LOCATION	
1	41°08' N	70°33' W
2	40°44' N	69°53' W
3	40°40' N	69°27' W
4	40°42' N	69°03' W
5	40°37' N	69°02' W
6	40°25' N	70°03' W
7	40°13' N	69°47' W
8	40°22' N	68°30' W
9	40°49' N	68°11' W
10	40°59' N	67°50' W
11	40°51' N	68°00' W
12	40°53' N	68°00' W
13	40°41' N	67°35' W
14	40°43' N	67°36' W
15	40°44' N	67°34' W
16	40°42' N	67°35' W
17	40°57' N	67°33' W
18	40°57' N	67°35' W
19	40°34' N	67°45' W
20	40°36' N	67°45' W
21	40°45' N	67°18' W
22	40°24' N	67°29' W
23	40°29' N	67°43' W
24	40°35' N	67°12' W
25	40°27' N	67°37' W
26	40°27' N	67°30' W
27	40°27' N	67°31' W
28	40°55' N	66°48' W
29	41°59' N	66°56' W
30	41°10' N	66°41' W
31	41°35' N	66°36' W
32	41°30' N	66°44' W
33	41°32' N	66°02' W
34	41°50' N	66°50' W
35	42°13' N	67°35' W
36	41°55' N	68°00' W
37	41°27' N	68°00' W
38	41°20' N	68°12' W
39	41°20' N	68°43' W
40	41°34' N	68°59' W
41	41°36' N	69°16' W
42	41°50' N	69°30' W

practical plan was to divide each cruise into two legs. The first leg would visit only (but not all) bottom sediment stations; the second would visit all water column stations as well as those bottom sediment stations not visited on the first leg. The plan was judged to involve no sacrifice in synopticity.

2.1.3 Field Sampling Cruises

Table 2-3 details the shakedown cruise and all the field sampling cruises to Georges Bank. All cruises were highly successful; close to 100 percent of planned samples were collected.

2.1.4 Field Sampling Equipment

The various pieces of equipment used in the sampling program are listed in Table 2-4. By the spring cruise most equipment performed satisfactorily and needed no further modification.

2.2 Water Column Sampling

A General Oceanics rosette sampler on a plastic-coated conducting wire was used as the basic module to collect water samples, with 10-liter Go-Flo bottles as the collecting vessels. The bottles enter the water closed; a pressure release opens them at prescribed depths for collection. The Go-Flo bottles used for trace metal and organic carbon samples were coated on the inside with Teflon.

TABLE 2-3
FIELD SAMPLING CRUISES

CRUISE	DATES	RESEARCH VESSEL	HOME PORT	NO. OF LEGS	SHIP TIME (DAYS)			
					TRAN- SIT	CRUISE	PORT	TOTAL
Shakedown	12/4/76 through 12/7/76	<u>Fay</u>	Miami, Fla.	1	10	3	2	15
Winter	2/7/77 through 3/8/77	<u>Gyre</u>	Galveston, Tex.	2	20	22	8	50
Spring	5/2/77 through 6/2/77	<u>Gyre</u>	Galveston, Tex.	2	4	16	15	35
Summer	8/15/77 through 9/4/77	<u>Gilliss</u>	Miami, Fla.	2	10	13	7	30
Fall	11/8/77 through 11/20/77	<u>Knorr</u>	Woods Hole, Mass.	1	0	13	0	13

TABLE 2-4
SAMPLING EQUIPMENT USED^a

EQUIPMENT	CRUISE			
	WINTER	SPRING	SUMMER	FALL
Water column				
Rosette: CTD/O	(+)	+	+	+
Nephelometer	+	+	+	+
Transmissometer	+	+	+	+
10-l Go-Flos	+	+	+	+
Bodman bottles	(+)	+	+	+
Brown-McGowan bongo system	(+)	(+)	+	+
Rubber raft	+	+	+	+
Sediment				
Smith-McIntyre grab sampler	(+)	+	+	+
Dredges/trawls				
Rocking chair dredge	(+)	(+)	(+)	(+)
Epibenthic sled	(+)	+	-	-
Clam dredge	(+)	-	-	-
Anchor dredge	+	-	-	-
Otter trawl	(+)	+	-	-
Blake trawl	-	(+)	+	+
Underwater camera	(+)	+	+	+

^a+ = used, no further modifications needed; (+) = used, further modifications needed; - = not used.

All samples were taken at two depths: approximately 5 m below the surface and approximately 5 m above the bottom. An additional mid-depth sample was collected for suspended sediment analysis.

Table 2-5 shows the number of samples taken during each cruise.

TABLE 2-5
WATER COLUMN SAMPLES COLLECTED^a

SAMPLE TYPE	CRUISE			
	WINTER	SPRING	SUMMER	FALL
Salinity/dissolved oxygen	42(42)	42(42)	40(40)	28(28)
Micronutrients	42(42)	41(42)	40(40)	28(28)
Suspended sediments (USGS)	42(42)	41(42)	40(40)	28(28)
Particulate trace metals	12(12)	12(12)	10(10)	12(12)
Dissolved organic carbon	12(12)	12(12)	10(10)	12(12)
Particulate organic carbon	12(12)	12(12)	10(10)	12(12)

^aThe first number indicates the actual number of samples collected, the second the number of samples that should have been collected.

2.2.1 Water Column Sampling: Zooplankton and Ichthyoplankton

Zooplankton and ichthyoplankton samples were collected concurrently using two Brown-McGowan bongo net systems; one system was fitted with a pair of 202 μ mesh Nynetex nets, the other with a pair of 505 μ Nynetex nets (Table 2-6). Taxonomic samples were collected in one net; trace metal and hydrocarbon samples for the chemists were collected in the adjacent net. This system permits the mouths of the nets to be closed upon

HULL 100-1000
200-1000
200-1000

TABLE 2-6
NUMBER OF SAMPLES OBTAINED, STATIONS SAMPLED,
AND GEAR USED FOR COLLECTING ZOOPLANKTON AND
ICHTHYOPLANKTON SAMPLES DURING CRUISES 1 THROUGH 4

CRUISE	NUMBER OF SAMPLES		NETS USED		STATIONS SAMPLED
	EXPECTED	OBTAINED	202 μ MESH	505 μ MESH	
1	24	17	7	10	1, 7, 13, 18, 32, 35, 36, 37, 39, 42
2	24	24	12	12	1, 4, 7, 13, 18, 26, 32, 35, 36, 37, 39, 42
3	20	20	10	10	1, 4, 13, 18, 26, 32, 36, 37, 39, 42
4	24	24	12	12	1, 4, 7, 13, 18, 26, 32, 35, 36, 37, 39, 42

immersion and opened and closed by messenger once under water. The bottom starting depth was indicated by a Benthos model 2216 bottom-finding pinger that showed (on a deck readout) the height of the net above bottom to the nearest meter. At the starting depth, which was approximately 2 m to 3 m below the surface, the net mouth was opened. The net was lowered to about 5 m off the bottom and then hauled to the surface. Before breaking the surface the net was closed by a messenger-operated chokeline to isolate the net

contents from contact with surface contaminants. The cod ends for each net were 1-liter glass jars and, in the case of trace metal and hydrocarbon collections, the jars were seawater aged and acid washed. A calibrated General Oceanics model 2030 flowmeter (one for each size mesh net) was mounted within one hoop of the bongo net sampling apparatus for each tow.

During Cruises 1 and 2 the messenger satisfactorily removed the canvas doors in front of the nets and thus permitted the flow of water through the nets, but it frequently failed to disengage the arresting arm from the flowmeter. During Cruise 1, the arresting arm at times also failed to stop rotation of the flowmeter after the nets were released from the frame. When the flowmeter malfunctioned, the volume of water that filtered through the nets was estimated on the basis of the sampling duration. To remedy the problem, after Cruise 2 the flowmeter was suspended in a bridle within the canvas throat of the net. Therefore, when the canvas doors were removed, the meter was activated by the flow of water; when the tow was completed and the net released from the frame, it collapsed around the flowmeter, stopping the propellor and ensuring reliable measurement. During Cruise 3 an "outside" meter was suspended from the 202 μ mesh system, and during Cruise 4 an outside meter was suspended from both the 202 μ and 505 μ mesh systems for corroborating the inside meter readings. This method gave a good check on the reliability of the system.

All samples used for metal and hydrocarbon analyses were taken from the net adjacent to the net having the flowmeter.

Following retrieval, each net was carefully removed from the frame. The net containing the taxonomic samples

was thoroughly rinsed from the outside with seawater from the ship's saltwater washing system. To prevent surface film or shipboard contamination the net containing the metal and hydrocarbon samples was washed with seawater collected below the ship's hull and stored in a 90-liter Bodman bottle.

For taxonomic studies and biomass determination, the biomass samples from the 202 μ and 505 μ mesh nets were preserved in 1-liter glass jars charged with 100 ml of 40 percent formalin. Samples were scheduled for collection at 12 stations on each cruise except Cruise 3, when 10 stations were sampled due to a mixup in sampling instructions prior to the cruise.

2.2.2 Water Column Sampling: Marine Microbiology

Seawater samples were collected with a sterile bag sampler (General Oceanics), a screen sampler, or a 10-liter Go-Flo bottle (contained in the rosette sampler).

Subsamples were taken from each bag sampler and the screen sampler for the following analyses: chlorophyll a, adenosine 5'-triphosphate (ATP), bacterial direct count, phytoplankton direct count, and heterotrophic and hydrocarbonoclastic viable count. Subsamples taken from the Go-Flo bottles were used only for ^{14}C tracer studies of heterotrophic and hydrocarbonoclastic activity. Subsurface water collected in the bag sampler was obtained from either the inflatable raft or the mother ship. Surface water was collected only from the inflatable raft. About 1.5 liters of surface water for microbiological analyses were collected in the manner used by the chemists with the following exceptions: (1) the screen sampler was first used by the chemists to obtain their samples (this ensured thorough

rinsing of the screen to remove all solvent used in the screen preparation process) and (2) the sample water was collected in a sterile brown bottle (1-gal) via a sterile funnel.

Subsurface water was obtained from the Go-Flo bottles for the ^{14}C experiments since it has been shown (Watson, unpublished) that ^{14}C experiments performed on water collected with the sterile bag sampler usually yield depressed results.

The following depths were sampled:

1. On Cruises 1 and 2, the surface microlayer (weather permitting), 1 m subsurface at all stations visited, and water column profiles at all stations where the inflatable was not launched.
2. On Cruises 3 and 4, the surface microlayer (weather permitting), 1 m subsurface, and near bottom.

2.2.3 Water Column Sampling: Particulate HMW Hydrocarbons

A total of 90 liters of seawater (see Section 2.2.4) was filtered to provide a particulate hydrocarbon sample corresponding to a dissolved hydrocarbon seawater sample (filtrate). The filters used were 142-mm Gelman A-E glass fiber filters, which were precombusted for 4 hours at 450° C in a muffle furnace. The filters were kept in aluminum foil until use and were handled only with stainless steel tweezers. All filters were loaded and unloaded in a clean van aboard ship. A stainless steel in-line Millipore filter holder was

used for all filtrations. Generally, only one filter was required for the 90 liters of water although occasionally the filter clogged due to heavy particulate loadings and had to be changed before filtration continued.

The spent filter(s) for any given sample were wrapped in solvent-rinsed aluminum foil and were frozen immediately at -20° C.

On one occasion two filters were placed in series to examine the possibility of adsorption of dissolved hydrocarbons by the filters (Section 4.2.4).

2.2.4 Water Column Sampling: Dissolved and Surface Film HMW Hydrocarbons

2.2.4.1 Dissolved HMW Hydrocarbons in the Water Column

Because low concentrations of hydrocarbons were expected in the dissolved fraction and because the background levels of hydrocarbons normally occurring in the ocean surface film and hydrocarbons from the research vessel were potentially of the same magnitude, it was necessary to obtain large-volume samples so as to yield enough material to analyze in comparison to the analytical blank (see Section 3.2.4).

The sampling device chosen was a Bodman bottle manufactured by Benthos Company of Falmouth, Massachusetts. The sampler was constructed of anodized aluminum; a Teflon-impregnated version was used as well. Each bottle was closed and evacuated (10 mm Hg) onboard. As it was deployed, water entered through a one-way valve because of the pressure differential. At 10 m to 15 m, the descent was halted and

the bottle slowly raised to approximately 3 m below the surface, where the expanding air left in the bottle caused it to open. It was then lowered to depth and closed by messenger. The opening and closing could be monitored by an attached pinger that changed ping rate as the bottle opened or closed.

Once onboard and secured, the bottle was pressurized with water-pumped, filtered nitrogen to 10 psig and a stainless steel line attached at the outlet through quick-connect fittings. This line was connected to a stainless steel Millipore in-line filter holder (Section 2.2.3), and the water was collected in pre-washed, solvent-rinsed 30-gal stainless steel drums or 12-gal glass carboys. After filtration, 25 ml distilled chloroform were added to the water (filtrate) to retard bacterial activity. The samples were then stored in the dark until analyses began in the laboratory. The average time from the moment of sampling through completion of the filtration step was about 30 minutes.

Near-surface (3 m to 5 m) and near-bottom (3 m off) samples were taken at a maximum of 12 stations each season. Attempts were made to include in the near-bottom samples any turbid layer detected by nephelometry.

2.2.4.2 Surface Film HMW Hydrocarbons in the Water Column

The surface film (microlayer) was sampled with a 16-mesh stainless steel screen by personnel on a rubber raft at least 100 yards upwind and out of the research vessel's slick shadow. The screen was placed through the surface vertically

and brought up horizontally. The frame was allowed to drain again in a vertical position for approximately 10 seconds before collection began again. Approximately 30 ml of water in the screen mesh were collected per dip; a total of 7.5 to 8.0 liters of water were obtained in two amber glass 1-gal jugs. The collected samples were poisoned with 10 ml chloroform/gal sample. Between 26 m² and 78 m² of surface were sampled, the uncertainty being attributable to the range in the estimated film thickness (100 μm to 300 μm).

2.2.5 Water Column Sampling: Particulate and Dissolved Organic Carbon (POC and DOC)

Subsamples for POC and DOC were collected with equipment free of organic matter; no use was made of fittings, hoses, or other implements made of plastic or other organic material.

Whatman glass fiber filters type RA-934-AH, with an effective pore size of 0.3 μm, were used for POC sampling. The filters, aluminum foil containers for the filters, and test tubes used for sample storage were all heated in a muffle furnace at 450° C for 2 hours to combust all the organic matter present. The filters, foil, and test tubes were stored in a vacuum desiccator until use.

Samples were taken with 10-liter Teflon-lined Go-Flo bottles. The bottles were put overboard closed to avoid contamination by the surface layer. Samples were taken in the water column approximately 5 m from the bottom and 5 m from the surface. The water collected was passed through the glass fiber filters to collect material for the particulate carbon and nitrogen analyses, and the filtrate was used for the DOC analysis.

At each station the filters were loaded in a clean area into six stainless steel in-line filter holders with silicon O-rings substituted for the normal Viton O-rings. The in-line filter was connected to the outlet port of the Go-Flo bottle by a length of silicon tubing, and 500 ml of water were filtered for each of the three replicates run at each station for each sampling depth. After sampling, the filters were removed from the holders with stainless steel forceps, folded, placed into the precombusted aluminum tubes, and set into properly labeled test tubes. The test tubes were stored in a vacuum desiccator until the samples were ready for analysis.

A 150-ml glass bottle was acid-washed and triple-rinsed with distilled water in preparation for the DOC sampling. Aluminum foil was used inside the bottle caps to prevent the contact of the sample with the cap liners. The foil was preheated at 450° C for 2 hours to remove organics, kept clean in a vacuum desiccator, and handled only with stainless steel forceps prior to use.

A 100-ml aliquot from the POC sampling filtrate was used for the sample. Samples were stored frozen at -10° C until ready for analysis.

2.2.6 Water Column Sampling: Trace Metals

2.2.6.1 Trace Metals in Suspended Particulates in the Water Column

Immediately before removal of a suspended particulate sample from a Go-Flo bottle, the bottle was shaken, an acid-cleaned polyethylene tube was attached from the spigot to a

pressure filtration vessel, and 4 to 10 liters of sample were withdrawn. The pressure filtration system used was one designed and constructed by ERCO to minimize contamination during shipboard processing of the sample and losses due to settling. The system is constructed entirely of polyvinyl chloride (PVC) and is shown in Figure 2-2. It allows filtration of the sample at a controlled pressure (≤ 10 psi) to minimize possible lysing of cells, is closed to the atmosphere and therefore to contamination, and is so compact that four units fit into the relatively tight space available on the research vessel. Purified, water-pumped, filtered N_2 gas provided the pressure. The filter is mounted at the lowest point in the system, and there are no corners or edges where particulates can settle.

The entire 4- to 10-liter sample was filtered through acid-cleaned $0.4 \mu m$ Nuclepore filters. The filtration pressure was gradually increased (to a maximum of 10 psi) as the filter clogged. The filter holders were then transferred to a clean filtration vessel containing ultra-pure water and rinsed with approximately 25 ml of water. The holders were then sealed in ultra-clean polyethylene bags (Clean Room Products Corp., Bay Shore, New York) and frozen for transport to the laboratory.

Despite the specially designed filtration system, one area of potential loss of particulates could not be eliminated: settling within the Go-Flo bottle itself. The time elapsed from the moment a bottle was tripped until the sample was drawn was as long as 30 minutes, during which particulate matter probably settled below the spigot, which is located about 2 cm above the bottom of the Go-Flo bottle. Once particles settle out, shaking only partially redistributes them, and those that are resuspended may settle out in the

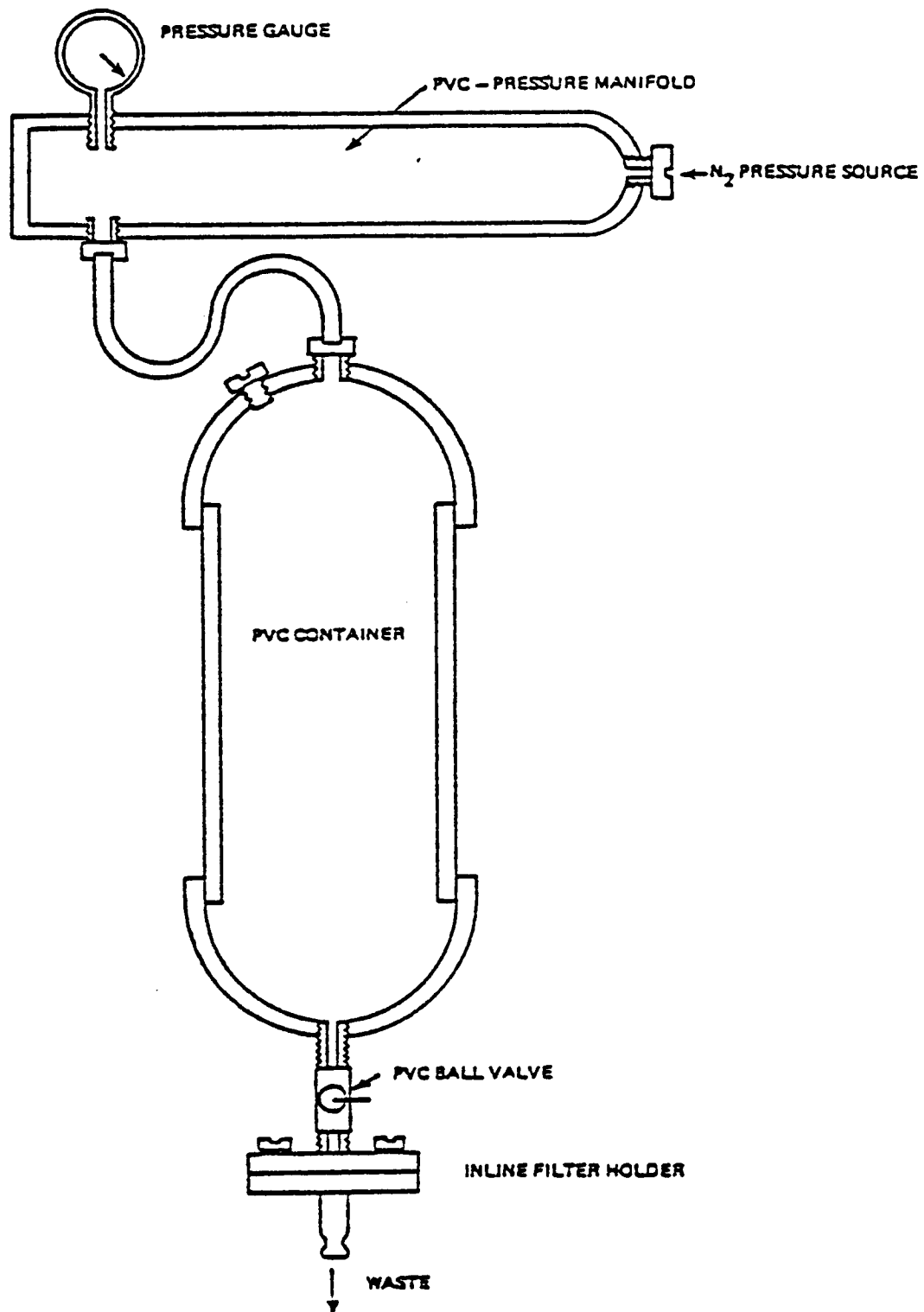


Figure 2-2. Pressure filtration system (PVC).

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11-11-11

5 minutes it takes to draw a sample. Gardner (1977) has concluded that nearly 50 percent of open ocean particulates could be lost in the time we required to draw a sample. We have indirect evidence that such loss has occurred in this work (Section 4.2.6). It appears that the qualitative interpretations of suspended matter data are quite consistent with data provided by the transmissometer; however, quantitative interpretation can be made only with great caution.

2.2.6.2 Trace Metals in Zooplankton in the Water Column

All zooplankton samples were collected with 70 mm diameter bongo frames fitted with double trip mechanisms. A steel ball approximately 90 kg in weight and a pinger were attached to the noncontaminating tow wire at points 3 m and 5 m below the bongo frame, respectively. The double trip mechanism was used to open the bongo nets 3 m below the water's surface and correspondingly to close the system at the 3-m depth following each cast. Tow speed on all cruises was approximately 2 to 3 knots. Water depth was monitored according to the pinger traces recorded on the CTD display module or the ship's precision depth recorder (PDR) system. All tows were oblique through the water column from 3 m to 5 m off the bottom to 3 m from the surface. In addition to noncontaminating, coated tow wire, the side of the bongo frame used to collect trace metal samples was Teflon coated. Samples were collected in acid-washed glass cod end jars. Bongo nets were towed from the stern on Cruises 1 and 2 and from a side hydrowinch on Cruises 3 and 4. An attempt was made on all cruises to tow in large concentric circles to avoid prop wash if the tow was from the stern and influence of the ship's hull if the tow was from the side. Tows averaged 60 minutes during Cruises 1 and 4 and 30 minutes

on Cruises 2 and 3. Winch speed was adjusted to sample each segment of the water column equally regardless of sample time or depth. Two zooplankton fractions were collected with two bongo systems fitted with 202 μ and 505 μ mesh nets, respectively.

Following each tow, cod ends were removed, nets cleaned, and zooplankton samples processed. Net washdown on Cruises 1 and 2 consisted of reloading the frames and towing nets without cod ends, taking care to open and close nets 3 m below the surface. On Cruises 3 and 4, the net used to collect zooplankton for trace metal analyses was washed down with nitrogen-pressurized seawater collected with a 90-liter Benthos water sampler at 5 m. The connecting hose for washing nets was Teflon. Samples were processed in the clean lab modular van. Each zooplankton fraction (i.e., 202 μ or 505 μ) was split using a solid Teflon folsom splitter mounted on an epoxy-coated, gimbaled stand. The half of the slit to be used for trace metal analyses was concentrated in an acid-washed, polyethylene collector fitted with a 1 μ mesh net. Concentrated zooplankton were removed with a Teflon spatula, placed in acid-washed plastic bags, heat-sealed, and frozen at -10° C. Approximately 10 g to 20 g of zooplankton biomass was collected on each tow.

2.2.7 Water Column Sampling: Dissolved Micronutrients, Dissolved Oxygen, and Salinity

Samples for dissolved oxygen, micronutrients, and salinity were taken with 10-liter Go-Flo bottles at approximately 5 m from the bottom and 5 m from the surface at each station. The sequence of sampling from each Go-Flo bottle was dissolved oxygen, then micronutrients, and finally salinity.

Dissolved oxygen samples were collected in narrow-mouthed glass-stoppered 300-ml biochemical oxygen demand (BOD) bottles with tapered ground glass stoppers and flared mouths. One end of a length of bubble tubing was inserted into the outlet of the Go-Flo bottle and the other into the BOD bottle. The BOD bottle was flushed with sample water and allowed to drain. With the bottle upright, sample water was allowed to flow in slowly to minimize turbulence. When the bottle was filled to overflowing, the tube was withdrawn. Two milliliters of MnCl_2 and then 2 ml of NaOH-NaI were added below the surface of the liquid. The bottle was carefully stoppered so as to avoid entrainment of air, shaken, and set aside for several minutes to allow the floc to settle. The bottle was then shaken and allowed to settle again. After the floc had settled enough to produce at least 100 ml of clear supernate, 2.0 ml of concentrated H_2SO_4 were added by allowing the acid to run down the neck of the bottle. The bottle was restoppered, mixed by gentle inversion until the floc had dissolved, and stored with a water seal until analysis. All samples were analyzed onboard ship within 24 hours of collection.

Micronutrient samples were collected in 500-ml polyethylene screw cap bottles. (New bottles were preconditioned by being allowed to stand 1 month with seawater in them.) The bottles were acid-washed and triple-rinsed with distilled water prior to use.

Samples for dissolved phosphate, nitrite, and nitrate were passed through an in-line glass fiber filter to remove particulates as they were extracted from the Go-Flo bottle. Duplicate samples were taken from each Go-Flo. Sample bottles and their caps were rinsed 3 times and then filled to the shoulder of the bottle. Samples were immediately frozen at -10°C and were kept frozen until analysis.

Samples for dissolved silicate analysis were removed from the Go-Flo bottle with a plastic syringe. The sample was expelled from the syringe into the sample bottle through a membrane filter in a stainless steel filter holder. The syringe and sample bottle were rinsed 3 times with sample water and then a 50-ml sample was taken. The sample was kept at 4° C until analyzed (samples were not frozen so as to prevent precipitation of the silicates [T. Church, personal communication]).

Salinity samples were taken, in duplicate, in 300-ml glass "citrate" bottles. The sample bottle and cap were flushed 3 times before the bottle was filled and stoppered. The samples were stored upright until analyzed.

2.2.8 Water Column Sampling: Conductivity, Temperature, and Dissolved Oxygen (CTD/O) Profiling

Water column profiling of salinity, temperature, and dissolved oxygen was accomplished with a completely integrated rosette sampling platform. This platform consisted of a General Oceanics multibottle rosette sampler (sized for 30-liter bottles) provided by the USGS, which was equipped with as many as nine 10-liter Go-Flo bottles, one Benthos pinger, one Neil Brown Mark III conductivity-temperature-depth/oxygen (CTD/O) profiler, and one Montedoro-Whitney CTD/transmissometer/nephelometer, also provided by the USGS. The transmissometer was mounted horizontally across the base of the rosette frame, the pinger was either located in a bottle position or mounted on a brace between the upper and lower protective rings, and the CTD/O was mounted in place of a bottle.

Data from the Mark III CTD/O system were digitized and sent up the cable 30 times a second in frequency-shift keyed (FSK) format. In the ship laboratory, this signal was recorded with a high-quality tape recorder while a real-time display of all measured parameters was provided by the CTD deck unit. In addition to signals sensed by the CTD/O instrument, analogue signals for transmission and nephelometry were patched into the CTD/O system for digitization underwater, so the deck unit also displayed the transmission and nephelometry data. While the instrument system was in the water, the temperature and transmission levels were monitored graphically as a function of depth on an Esterline-Angus X-Y-Y plotter.

The sampling routine included the following precautionary measures:

1. The system was held between 5 m and 10 m beneath the surface long enough for the system temperature, as measured by the internal oxygen probe thermistor, to approach that of the water. This equilibration step facilitated interpretation of the oxygen probe data.
2. Drop rate of the system was approximately 45 m/min. This rate was necessary to ensure proper oxygen probe flushing.
3. The downcast was one continuous drop, with all water bottle sampling performed on the upcast. If a sample had been taken on the way down, the CTD/O would have been shut off for about 40 seconds for each bottle closed. This would have caused the oxygen probe to go off scale, and the next cast segment would have been valueless.

4. Prior to water sampling at a particular level, the deck display was frozen and all data were recorded in the log. These values were the basis for comparing the water sample analyses to the profiles and had therefore to represent steady-state values of the water column at sampling time.

As the system neared the bottom, the Benthos pinger return displayed on the ship's PDR provided an excellent measure of distance off the bottom. An attempt was made to terminate all casts at 5 m above the bottom. By monitoring the true depth echo, the CTD/O pressure signal, and the Benthos pinger, this target was usually attained. The weakest links in the system were the verbal communication between the winch operator and the CTD monitor, and human error. Table 2-7 shows all CTD/O samples obtained during the 1977 North Atlantic benchmark cruises.

TABLE 2-7
CTD/O SAMPLES OBTAINED

SEASON	SAMPLES OBTAINED	COMMENTS
Winter	41	No sample for Station 8 ^a
Spring	41	No sample for Station 31 ^a
Summer	39	No sample for Station 1 ^a
Fall	29	

^aDue to instrument malfunction.

2.3 Benthic Sampling

2.3.1 Benthic Sampling: Core/Grab Sediment Samples

Although a box corer was acquired for the field sampling program, it turned out to be so cumbersome in any limit of sea that it was decided to collect sediments with the much more reliable and easier to handle Smith-McIntyre grab sampler. The Smith-McIntyre could not penetrate as deeply as the box corer but was adequate since only surface sediments had to be collected.

Table 2-8 shows the number of samples collected during each cruise.

TABLE 2-8
SURFACE SEDIMENT SAMPLES COLLECTED^a

SEASON	NUMBER OF STATIONS SAMPLED
Winter	41(42) ^b
Spring	42(42)
Summer	40(40)
Fall	28(28)

^aThe number in parentheses indicates the total number of stations visited.

^bOne station not visited due to bad weather.

2.3.1.1 Core/Grab Benthic Sampling for Grain Size Analysis

Samples for grain size analysis were removed from each taxonomic grab sample and from each geochemical grab sample on which chemical analysis of individual replicates was performed. Samples were obtained while the sediment remained in the Smith-McIntyre grab. Grain size samples were removed from the geochemical grabs after all geochemical sampling had taken place. Samples from the taxonomic grabs were removed after microbiology or total organic carbon/total organic nitrogen (TOC/TON) sampling had taken place.

The subsamples for grain size analysis were obtained randomly from the central portion of the grab with a stainless steel scoop. Presealed, sterile whirl-pac bags were used for sample storage. Each 200-cm³ grain size subsample was placed in a properly labeled whirl-pac bag, sealed, and kept frozen at -10° C until the size analysis was performed.

A 200-cm³ subsample for grain size analysis was also taken from each pooled geochemical sample. These subsamples were obtained randomly from the pooled samples, placed in whirl-pac bags, sealed, and frozen. These samples were shipped and stored frozen at -10° C until analysis.

2.3.1.2 Core/Grab Benthic Sampling for Biological Analysis: Sediments

Microbiology

Sediment samples for microbiological analysis were collected using a modified Smith-McIntyre grab sampler.

Sterile glass disposable syringes (2.5 ml) that had delivery ends cut off just above the barrel constriction were used to transfer 1-cm³ subsamples to sterile glass scintillation vials, flasks, or serum bottles for transfer to the laboratory. Only the surface layer (approximately 1.5 cm) of the sediment contained in the grab sampler was sampled. Subsamples, 1 cm³ each, were removed for LPS determination (1), direct count (1), ATP (1), dry weight (1), heterotrophic and hydrocarbonoclastic viable count (2), and heterotrophic and hydrocarbonoclastic activity (10). In addition, a scintillation vial was three-quarters filled with sediment to serve as an ATP control.

Macrofauna

The sample size for benthic infauna and sampling efficiency of the grab depended on the character of the substratum, hard bottoms somewhat limiting the sample size. Rocks, large bivalves, or shell fragments occasionally prevented the jaws from closing fully so that a portion of the sample was lost. When partial drainage had occurred the decision to accept or reject a sample was at the discretion of the scientist in charge of benthic sampling (in most cases, a biologist).

One subsample for grain-size analysis and 16 cores for microbial analysis were removed from the first biological replicate at each station. A grain-size subsample and a subsample for TOC/TON analysis were removed from all subsequent biological replicates.

The remainder of each sample was sieved through a 0.5 mm mesh Nitex screen. Time, space, and manpower limitations in the field prevented a thorough sieving at this stage; sieving

was completed after return to the laboratory. After sieving, the material retained on the sieve and the sieve cloth itself were placed in a muslin bag and immersed in a 6 percent $MgCl_2$ solution for approximately 30 minutes before fixing. The muslin bag was then placed in 10 percent buffered seawater formalin and sealed in 30-gal metal drums for shipment back to the laboratory.

Following the arrival of the 30-gal sample drums at the Taxon, Inc. facility in Salem, Massachusetts, all samples were transferred to 70 percent isopropanol for storage until processing. Because of the large number of samples this procedure required several weeks for each cruise. The extended period of time in formalin for the final sample transfer produced undesirable fragility in some of the crustacean groups, particularly the amphipods. To correct this problem for the second and all subsequent cruises, the formalin in the drums was immediately changed to isopropanol in bulk upon receipt of the drums. No further fragility problems were noted. ✓

During the transfer procedure the samples were resieved through the original 0.5 mm Nytex screen cloth in an attempt to minimize sample volume. At this point each sample was logged onto a record sheet which was used to trace all phases of the analyses of each sample.

Microfauna

Samples for microfaunal analysis were also taken from the grab, by removing two subcores from the main sample. Of these two subsamples, one sample was archived and one sample was analyzed. The size of the subsample depended on the substratum sampled.

2.3.1.3 Core/Grab Benthic Sampling for Chemical Analysis

HMW Hydrocarbons

A Smith-McIntyre grab was used to obtain all sediment samples. The grab was placed on a wooden sediment stand and the doors opened, exposing the two sides of the grab. Since the same sample was to be used for hydrocarbon and trace metal chemistries, TOC, and grain size determinations, special precautions were used in subsampling the grab. A Teflon-coated aluminum trowel was used to obtain approximately 250 grams of wet sediment from the top 5 cm of each side of the grab. A total of between 400 g and 500 g was obtained from each grab and combined with five other replicates to yield the pooled sediment sample. The six combined replicate subsamples were stored in two Teflon canisters totaling 3,000 ml in volume. The canisters were frozen at -10° C immediately after the sampling operation.

During the spring cruise, in addition to the pooled samples, subsamples (3,000 g) from all six replicates were separately stored in Teflon canisters and the seven samples - pooled plus replicates - were analyzed as part of the sediment pooling experiment. As each station was defined as having a 0.5-mile radius from station center, the pooling experiment was designed to examine small-scale spatial heterogeneity as well as to determine the adequacy of our pooling procedure.

Upon return to ERCO laboratories, the sediment samples were thawed and mixed thoroughly with Teflon utensils in a Teflon basin, all in a laminar flow clean bench. Two 1,000-g samples were placed in solvent-rinsed glass jars for hydrocarbon analysis and quality control/backup sample

respectively. The jars were frozen at -10° C until analysis was performed. From this mixed sample, subsamples for trace metal analyses, TOC, and grain size determinations were also obtained.

Trace Metals

Upon arrival of the Smith-McIntyre grab samples for chemical analysis on deck one-half of the grab was opened at a time for sampling. Samples were taken using Teflon-coated aluminum scoops that had been rinsed with acid, solvent, and ultra-pure water immediately prior to use. Samples were collected by personnel wearing plastic gloves who were careful to keep the samples out of contact with the sides of the grab and to minimize exposure time of the sample to ambient air and other sources of contamination.

Approximately 400 g to 500 g of sediment were taken from each of six grabs, placed in acid-cleaned and solvent-rinsed Teflon jars, and frozen at -10° C for storage and transport. Dry fallout samples and combination samples of wet and dry fallout were collected on each cruise. These samples consisted of Teflon jars exposed to the atmosphere for periods of 12 to 24 hours, acidified, and frozen at -10° C for storage and transport. They were exposed in the vicinity of the grab sampling operation to assess the rate of atmospheric deposition that could introduce contaminants to the sample.

TOC/TON

Samples for total organic carbon and nitrogen were removed from each replicate bottom sample collected for

taxonomic analysis except those from which microbiologic samples were removed. A 50-cm³ subsample was removed using a stainless steel scoopula from an otherwise undisturbed area in the central portion of the grab. Care was taken to prevent contact with organic material other than that which was to be measured in the sample. Each subsample was placed in a properly labelled, acid-washed glass container and sealed. The glass jars were chosen large enough that the subsamples did not completely fill them, in order to prevent cracking during storage. The samples were stored frozen at -10° C until ready for analysis.

A 50-cm³ subsample was also removed from each pooled geochemical sample. These subsamples were obtained randomly from the pooled samples and placed in acid-washed glass containers. These TOC/TON samples were shipped frozen at -10° C and stored in that condition until the analyses were performed.

2.3.2 Benthic Sampling: Dredge/Trawl Samples

Dredge and trawl casts to collect macrobenthic epifauna and infauna were made only at the 12 water column stations.

A rocking chair dredge, epibenthic sled, clam dredge, and anchor dredge were acquired and used during the winter cruise. None appeared to collect epibenthic macrofauna efficiently; therefore, a Blake trawl was borrowed from the Oceanography Department of Texas A&M University for the spring cruise. The Blake trawl showed itself to be superior in sampling efficiency and two frames were purchased for and used on all subsequent cruises.

An otter trawl was also acquired and after some minor modifications successfully collected large numbers of demersal fish.

Table 2-9 shows the number of stations sampled for epibenthic macrofauna and macrobenthic infauna.

TABLE 2-9
DREDGE/TRAWL SAMPLES OBTAINED^a

SEASON	NO. OF STATIONS VISITED	ROCKING CHAIR DREDGE	BLAKE TRAWL
Winter	12	8(12)	-
Spring	12	12(12)	12(12)
Summer	10	10(10)	10(10)
Fall	12	12(12)	11(12)

^aNumbers in parentheses represent number of samples planned.

2.3.2.1 Dredge/Trawl Benthic Sampling for Chemical Analysis

Trace Metals

Six species of macrobenthic invertebrates, selected for their ubiquitous geographic and seasonal distribution as well as their possible ecological relevance to chemical distribution, were collected at each station. Station sites were selected on the basis of their ecological significance,

including sediment regime and possible significance to potential petroleum lease sites. Only four species were collected per station on Cruises 3 and 4. Most samples were collected using a Blake trawl. At stations having high proportions of large clams (e.g., Arctica islandica), the rocking chair dredge was used. Trawl time at most stations was 60 minutes.

After a tow was completed, the contents were immediately dumped into a plastic-lined tray and sorted on deck in an area sheltered from stack emissions. Samples were withdrawn by hand or with Teflon tweezers, by personnel wearing plastic, dust-free smocks, gloves, and head coverings. If time and weather conditions permitted, dissection into individual organs was performed on organisms of the larger genera (i.e., >100 g in total weight). This dissection was carried out with plastic knives on Teflon surfaces in the clean lab modular van.

Samples of the same species were placed in 6-mil thick plastic bags made for clean room use (Clean Room Products, Inc., Bay Shore, New York). The bags were heat-sealed, placed inside a second plastic bag, heat-sealed, and frozen at -10° C for storage and transport.

HMW Hydrocarbons

Dredge/trawl samples for hydrocarbon analyses were obtained at a maximum of 12 stations during the four sampling cruises. When the sampling gear was brought onboard its contents were emptied directly into a stainless steel tray (3 ft x 5 ft). The sample was then sorted with stainless steel tongs and tweezers; animals were identified and

placed in solvent-rinsed glass jars. Four to six species were obtained at each station and where possible the same species were sampled over several seasons. After several dredge/trauls using the rocking chair dredge, the epibenthic sled (winter and spring), and the Blake trawl (summer and fall), only those species obtained in sufficient quantities for analysis - 12 individuals for larger organisms (bivalves), 20 g wet tissue weight for smaller organisms - were labeled and immediately frozen at -20° C.

Although there was close coordination in sampling between the hydrocarbon and trace metal chemists, identical species distributions were not obtained because of the different sample size requirements.

Special consideration was given to Arctica islandica distributions. This species was always sought at those stations where normal abundances permitted a statistically valid sample size to be obtained (see Section 4.3.2.1). The most widely distributed species included the sea urchin (Strongylocentrotus droebachiensis), the hermit crab (Pagurus acadianus), the sand dollar (Echinarachnius parma), and the starfish (Asterias vulgarus).

During the shakedown cruise (November 1976) large quantities of Arctica were obtained from Station 1 for use in laboratory quality control and precision measurements, as well as for a pooling experiment (see Section 4.3.2.1).

2.3.2.2 Dredge/Trawl Benthic Sampling for Histopathological Analysis

At each station specimens were removed from the macrobenthic samples for histopathological analyses. Up to six species were collected for Cruises 1 and 2 and up to four species were collected for Cruises 3 and 4. For each cruise an attempt was made to select species which were from different phyla and which had different feeding habits. The same species were utilized for chemical analyses.

2.3.2.3 Dredge/Trawl Benthic Sampling for Taxonomic Analysis: Macrobenthos and Foraminifera

Originally, five types of sampling gear, i.e., rocking chair dredge, Blake trawl, crab dredge, epibenthic sled, and otter trawl, were scheduled for benthic sampling. During Cruise 1, samples were collected with the rocking chair dredge, crab dredge, and epibenthic sled. The anchor dredge was tried unsuccessfully at one station; the otter trawl proved large and cumbersome and required nearly 4 hours from beginning to end of the sampling. After Cruise 1, a re-evaluation was made of the sampling gear in reference to the purpose and needs of the survey and in consideration of the time constraints imposed on all investigations. On the basis of the Cruise 1 experience, it was decided that two pieces of gear would successfully sample and characterize the benthic communities: the rocking chair dredge and the Blake trawl. These two pieces of gear were used on Cruises 2, 3, and 4 (Table 2-10).

Each sampler was put over the side and towed along the bottom for one-quarter to one-eighth mile depending on

TABLE 2-10

NUMBER OF SAMPLES OBTAINED, STATIONS SAMPLED, AND GEAR USED FOR
COLLECTING MACROBENTHIC AND FORAMINIFERA SAMPLES DURING CRUISES 1 THROUGH 4

CRUISE NO.	DESCRIPTION	NUMBER OF SAMPLES		MACROBENTHOS				FORAMINIFERA	STATIONS SAMPLED
		EXPECTED	OBTAINED	ROCKING CHAIR DREDGE	BLAKE TRAWL	CRAB DREDGE	EPIBEN- THIC SLED	SMITH- McINTYRE GRAB	
1	Macrobenthos	72	37	14		14	9		1, 7, 18, 32, 37, 39, 42
	Foraminifera	42	41					41	1-34, 36-42
2	Macrobenthos	48	47	24	23				1, 4, 7, 13, 18, 26, 32, 35, 36, 37, 39, 42
	Foraminifera	42	42					42	1-42
3	Macrobenthos	40	38	16	22 ^a				1, 4, 13, 18, 26, 32, 36, 37, 39, 42
	Foraminifera	40	40					40	1-6, 8-34, 36-42
4	Macrobenthos ^b	48	44	22	22				1, 4, 7, 13, 18, 26, 32, 35, 36, 37, 39, 42

^aTwo of 22 samples collected with modified Blake trawl: teeth added to crossbar.

^bForaminifera sampling terminated after Cruise 3.

the density of the first tow. Replicate samples were collected with each sampler at each station. Retrieved nets were rinsed through 4-mm sieves with seawater and placed in labeled jars with 10 percent formalin. In some cases the large size of the sample made splitting necessary.

Foraminifera samples were obtained from bottom sediment samples that were collected with a Smith-McIntyre grab (and taken concurrently with the benthic sampling). Once the samples were aboard ship, a 36-mm i.d. plastic cylinder was inserted into the surface of the sediment to a depth of exactly 3 cm, and a core of this dimension was extracted. The core was then divided into three equal segments and labeled top cm, middle cm, and bottom cm; each segment was stored in glass jars charged with 95 percent ethyl alcohol. Samples were scheduled for collection at 42 stations (Table 2-10); due to monetary considerations, sampling was terminated after Cruise 3.

2.3.3 Benthic Sampling: Dissolved Micronutrients, Dissolved Oxygen, and Salinity

Sampling procedures for dissolved micronutrients, dissolved oxygen, and salinity at benthic stations were the same as those at water column stations (Section 2.2.7).

2.3.4 Benthic Sampling: Conductivity, Temperature, and Dissolved Oxygen Profiling

Benthic station procedures for CTD/O profiling were the same as water column procedures. Refer to Section 2.2.8 for a brief account.

2.3.5 Benthic Sampling: Suspended Sediments

Water samples for determination of suspended particulate matter were collected in 10-liter Go-Flo bottles at three depths in the water column: near-surface (about 5 m), mid-depth, and near-bottom (about 5 m above the bottom). Samples were collected at 42 stations during each of the four sampling seasons.

After the rosette arrived on deck, 4 liters of water were transferred to a 4-liter graduated cylinder supplied by USGS. An in-line Millipore filter holder containing two pre-weighed 0.45 μm Millipore filters was connected to the outlet fitting at the bottom of the cylinder. The water was then vacuum-filtered until all the water had passed through the filter or the filter clogged. The volume of water that had been filtered was recorded.

After completion of the filtration, the holder was dismantled and the filters were washed 5 times with distilled water. The filters were transferred to plastic petri dishes and frozen at -10°C until analysis.

2.3.6 Benthic Sampling: Transmissometer/Nephelometer

Section 2.2.8 presents a general description of the instrumentation used to obtain transmission and nephelometry data. The major difference between this system and others previously used on the benchmark programs is that the transmissometer/nephelometer data are digitized and meshed with the CTD/O data within the Neil Brown Mark III system in addition to being recorded internally within the Montedoro-Whitney unit. This allows a much higher acquisition rate

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(30 samples per second) and a simpler array of deck hardware (just the CTD/O unit).

During data acquisition, a digital-to-analogue converter within the CTD/O deck unit allowed the plotting of transmission and temperature versus depth. Thus a graphic characterization of the water column could be obtained to determine the level for mid-layer sampling of suspended particulates. A summary of the transmissometer/nephelometer profiles obtained is presented in Table 2-11.

TABLE 2-11
SUMMARY OF TRANSMISSOMETER/NEPHELOMETER PROFILES

SEASON	SAMPLES	
	OBTAINED	EXPECTED
Winter	30 ^a	42 ^a
Spring	41	42
Summer	39	40
Fall	28	28

^aInstrument malfunction caused difference between number expected and obtained.

CHAPTER THREE

ANALYTIC METHODS AND MATERIALS

3.1 Introduction

Methods of analysis used during the course of the project included those initially specified by the BLM and detailed in the original ERCO technical proposal as well as in the modified and best and final versions. In several instances the analytical methods originally specified by the BLM produced results of dubious quality; in these cases ERCO was frequently able to suggest other methods that improved the quality of the data obtained. Alternative methods were used only with the explicit permission of the BLM.

Methodology for microbiological analysis was either suggested to BLM by ERCO or was developed in the ERCO microbiology laboratory.

3.2 Water Column Analysis

3.2.1 Water Column Analysis: Zooplankton and Ichthyoplankton

A total of 75 zooplankton samples designated for taxonomy and enumeration were analyzed in the laboratory. These samples, taken by both 202 μ and 505 μ mesh nets, contained fish eggs and larvae (designated as ichthyoplankton) as well as zooplankters (microscopic animals:

typically and predominantly crustaceans, polychaetes, and bivalves). Each sample was split into two equal subsamples by a Marine Research, Inc. (MRI) plankton splitter, and the analyses were carried out by two separate groups in MRI, one concerned with ichthyoplankton and the other with zooplankters.

3.2.1.1 Taxonomy of Zooplankton and Ichthyoplankton in the Water Column

Ichthyoplankton

The subsamples for ichthyoplankton analysis were placed in a 202 μ or 333 μ mesh sieve and washed with tap water to remove excess formalin. Each sample was placed in a beaker of seawater; enough residual formalin remained in the plankton to prevent deterioration for several days.

Samples in which the numbers of ichthyoplankton were low were counted entirely. Samples containing abundant forms were split into fractions. Every attempt was made to ensure that the splits were performed so that representative and reproducible samples were obtained.

Small portions of the sample were poured into special trays of MRI design, which are divided by baffles and ruled markings so that the entire portion can be systematically examined under a dissecting microscope without any organisms being missed or counted twice. Numbers were recorded on multiple tally counters and then transferred together with station data, including flowmeter readings, to specially designed data forms for subsequent coding on computer cards.

Representative specimens, as well as those difficult to identify, were removed to a reference collection. About 17 percent of the samples, chosen at random from each series, were recounted, the technician carrying out the recount in no case having made the first count. Any significant errors detected in this way were corrected appropriately.

After being counted, the samples were returned to a 10 percent buffered solution of formalin in seawater, labeled with all station labels and pertinent notes (made on herbarium paper to prevent deterioration), sealed with tape, and stored for future reference.

Previous ichthyoplankton analyses that MRI has carried out show that combined subsampling and operator error in the laboratory is about 10 percent and is small relative to the variability between replicate samples taken in the field at a single station.

Zooplankton

MRI's standard method of counting zooplankton was modified for this study because of the duration (as long as 1 hour) that the bongo net system was "fished:" samples for taxonomic analysis were some 10 times as dense as anticipated. In addition, because the extended sampling time increased the probability of capture, the samples occasionally contained rare and scarce species. As a result, subsampling studies were conducted under the guidance of Dr. Woollcott Smith, Statistician at Woods Hole Oceanographic Institution, with the object of modifying the original counting scheme.

Specimens varied in size from less than 1 mm to over 25 mm in length. To test the possible irregular splitting of large forms, the Chaetognath Sagitta elegans was counted in four one-quarter splits. Four investigators, each counting a separate split under a dissecting microscope, found 1,386, 1,653, 1,369, and 1,382. The conclusion is that S. elegans, the largest species observed to date and in some cases the dominant species in the winter samples, did in fact split satisfactorily for counting purposes. The other larger species found were the following arthropods, which numbered fewer than 100 per split: Rhincalanus nasutus, barnacle nauplii, mysis stage shrimp, mysid shrimp, amphipods, and the Euphausiids, Thysanoessa longicaudata, and meganyctiphanes norvegica.

For the relatively smaller forms, consisting mostly of arthropods, the four investigators counted three 1-ml subsamples from each of their respective splits. There was no statistical difference among the counters; it appears that the smaller forms split rather evenly. Over 28 species or groups of zooplankters were enumerated, ranging from 1 to over 200 for a particular species or group per Sedgwick-Rafter cell, totaling between 300 to 500 zooplankters per cell. In addition, as a measure of quality control, each investigator was tested against the others for species identification by independently counting and identifying zooplankters on the same slide.

A second series of counts was made to determine if one large subsample, i.e., 10 ml (representing 1 percent of the total sample volume), would provide a better estimate of the sample population than the method described above. The four splits were reunited and shaken vigorously to redistribute the zooplankters evenly. Exactly 10 ml were removed with a

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Hensen-Stempel pipette. All zooplankters in the 10 ml were counted. The counts using the first method were compared with those from the second method, with large variation at times. In addition, the second method clearly underestimated the S. elegans population (3,900 vs. the total population of 5,981 established by the first method). Also, the number of rare or scarce species found by the first method was grossly over- or underestimated by the second method. These findings suggested that a modified sampling scheme should incorporate a total count of the large species from an appropriately sized split.

Therefore, three 1-ml subsamples were counted as was originally planned, but a total count was also made of the large forms and the relatively rare species in a split of an appropriate size. When insufficient numbers of specimens were present on a single Sedgwick-Rafter, subsampling continued until at least 1,500 organisms were counted.

Aliquots of the sample were used to determine the wet weight and dry weight of the zooplankton and ichthyoplankton by the following steps:

1. 47-mm Millipore filters were prewashed with distilled water to remove detergent from the filter papers.
2. The filters were dried for 30 minutes at 75° C and stored in a laboratory desiccator until cooled.
3. The filter papers were weighed to the nearest 0.01 mg.

4. Zooplankton were filtered onto the paper with Millipore filtering apparatus and excess water was exhausted.
5. The sample was weighed, the difference between the original weight of the dried filter paper and this weight being the wet weight of the zooplankton biomass.
6. The samples were then dried at 60° C for 24 hours or until a constant weight was obtained. The dry weight of the zooplankton biomass was the difference between the weight of the dried filter paper and this weight.

Recounts were made on 20 percent of our samples.

Tallying, recording data, collecting representative species, and preserving and storing samples were accomplished in the manner described for ichthyoplankton.

3.2.1.2 Hydrocarbon Chemistry of Zooplankton and Ichthyoplankton in the Water Column

The zooplankton samples, which were frozen onboard ship at -10° C, were thawed in the laboratory. Each sample was carefully searched for foreign materials (paint chips, tar particles, etc.) with a magnifying glass, and these materials were removed. A 10 g to 100 g wet weight subsample was taken from the thoroughly mixed sample and a small aliquot taken from this subsample for a dry weight determination.

The subsample was combined with equal volumes of 0.5 N KOH (aqueous, pre-extracted) and distilled methanol. Aliphatic and aromatic hydrocarbon internal standards (tridecylcyclohexane and hexaethyl benzene) were added to the reactants. The mixture was refluxed for 4 hours. After cooling, the digested saponified mixture was diluted with saturated NaCl and the entire mixture back-extracted with three 500-ml portions of distilled hexane. The hexane extracts were combined and concentrated to a small volume with a rotary evaporator. This concentration caused the formation of a large quantity of gel, which was removed by taking up the entire extract in chloroform, charging it to an alumina (5 percent deactivated) column, and eluting with a great excess of chloroform. Concentration of the chloroform eluate by rotary evaporation resulted in no further gel formation.

Total extractable lipids (nonsaponifiable) were quantified by weighing an aliquot of this concentrated extract. The extract was then further concentrated under a stream of water-pumped, purified nitrogen to a small volume (20 μ l to 50 μ l). The concentrate was taken up in 0.5 ml of hexane and charged to a preparatory column as described below.

Duplicate analyses were performed during each season as a check on analytical precision. The results are presented in Section 4.2.1.

Column Chromatography

In the laboratory, the column chromatographic procedure was thoroughly studied before it was applied to samples. A standard mixture of 50 μ g each of the following compounds was used:

1. Decalin - naphthenic hydrocarbon.
2. n-C₃₀ - n-alkane.
3. Cholestane - naphthenic.
4. Nonadecylbenzene - alkyl substituted aromatic.
5. Phenanthrene - 3-ring aromatic.
6. Chrysene - 4-ring aromatic.
7. Methyl oleate - fatty acid methyl ester.

This mixture was charged to the test columns in 0.5 ml hexane to examine the elution patterns of different column configurations as shown in Table 3-1. The columns were packed in a Kontes chromaflex glass column of 1.0 cm i.d. The criteria for adequate separation of the hydrocarbon components into two fractions f₁ and f₂ were:

1. All cholestane, decalin, and n-C₃₀ eluted in f₁ with no nonadecyl benzene in f₁.
2. All nonadecyl benzene in f₂.
3. All phenanthrene and chrysene elute in f₂.
4. No methyl esters in f₁ or f₂.

The following column configuration, which meets all of these criteria, was selected: 2.5 g of 5 percent deactivated alumina, 80 to 200 mesh, packed over 7.5 g of fully activated silica, 100 to 200 mesh. The elution pattern selected was:

TABLE 3-1
COLUMN CHROMATOGRAPHY

PROCEDURE	SILICA (g) ^a	ALUMINA (g)	f ₁	f ₂	COMPOUND ^b										
					1	2	3	4	5	6	7	8	9	10	11
1	4(B)	2(A)	30 ml hexane	30 ml hexane benzene (1:1)	f ₁	f ₁	f ₂	-	f ₁	-	-	f ₂	-	-	-
2	4(B)	2(A)	30 ml hexane	30 ml benzene	f ₁	f ₁	f ₂	-	f ₁	-	-	f ₂	-	-	-
3	6(B)	1(A)	30 ml hexane	30 ml hexane: benzene (1:1)	f ₁	f ₁	f ₂	-	f ₁	-	-	f ₂	-	-	-
4	6(B)	1(A)	30 ml hexane	30 ml benzene	f ₁	f ₁	f ₂	-	f ₁	-	-	f ₂	-	-	-
5	7.5(A)	-	18 ml hexane	30 ml hexane: benzene (60:40)	f ₁	f ₁	f ₂	f ₂	f ₁	-	f ₁	nd	-	-	-
6	10(A)	-	25 ml hexane	25 ml hexane: benzene (1:1)	f ₁	f ₁	f ₂	f ₂	f ₁	-	f ₁	nd	-	-	-
7	6(A)	3(A)	30 ml hexane	30 ml benzene	f ₁	f ₁	f ₂	f ₂	f ₁	-	f ₁	nd	-	-	-
8	7.5(A)	2.5(A)	18 ml hexane	30 ml hexane: benzene (60:40)	f ₁	f ₁	f ₂	nd	f ₂	-	f ₁	nd	-	-	-
9	10(A)	3(A)	25 ml hexane	25 ml hexane: benzene (1:1)	f ₁	f ₁	f ₂	nd	f ₁	-	f ₁	nd	-	-	-
10	6(A)	3(A)	26 ml hexane	30 ml benzene	f ₁	f ₁	f ₂	f ₂	f ₂	-	f ₁	f ₂	-	-	-
11	7.5(A)	2.5(A)	18 ml hexane	f ₂₁ = 15 ml benzene f ₂₂ = 5 ml benzene f ₂₃ = 5 ml benzene	f ₁	f ₁	f ₂₁	f ₂₁ f ₂₂	f ₂₁	-	f ₁	nd	-	-	-
12	7.5(A)	2.5(A)	18 ml hexane	25 ml benzene	f ₁	f ₁	f ₂	f ₂	f ₂	-	f ₁	nd	-	-	-
13	7.5(A)	2.5(B)	f ₁₁ = 15 ml hexane f ₁₂ = 3 ml hexane	f ₂₁ = 5 ml benzene f ₂₂ = 15 ml benzene f ₂₃ = 5 ml benzene	-	-	-	f ₂₂	f ₂₁ f ₂₂	f ₂₂	-	-	f ₂₃	f ₂₂ f ₂₃	nd

^a(A) = fully activated (175° C for 24 hours); (B) = 5 percent deactivated.

^b1 = decalin; 2 = n-C₃₀; 3 = phenanthrene; 4 = chrysene; 5 = nonadecylbenzene; 6 = squalene; 7 = cholestane; 8 = methyloleate; 9 = methyl stearate; 10 = dodecyl palmitate; 11 = cholesteryl palmitate; nd = none detected; - = not in original standard mixture.

18 ml hexane (= 1.2 column volumes) for f_1 and 20 ml benzene (= 1.3 column volumes) for f_2 .

In a related experiment designed to specifically address the problems related to possible incomplete saponification of the macrofaunal samples, and hence carryover of cholesteryl, methyl, and wax esters into the total lipid fraction, another standard mixture was charged to the column, consisting of 50 μ g each of:

1. Squalene - polyunsaturated hydrocarbon.
2. Chrysene - 4-ring aromatic.
3. Nonadecylbenzene - alkyl benzene.
4. Dodecyl palmitate - wax ester.
5. Cholesteryl palmitate - cholesteryl ester.
6. Methyl stearate - saturated methyl ester.

This experiment appeared to show that there was no need to use more than 20 ml benzene in the f_2 fraction. Methyl ester breakthrough began in the last 5 ml (f_{23} , procedure 13). The only source of concern was the apparent breakthrough of the wax ester in the f_{22} fraction (procedure 13). This experiment indicated that wax esters could break through the column if complete saponification was not achieved. However, no problems of this nature were observed.

A series of silica gel thin layer chromatographic (TLC) experiments was performed to verify the absence of wax esters or other esters in chromatographed samples having

large lipid contents (i.e., the zooplankton samples). Several f_2 fractions from zooplankton samples were re-chromatographed by TLC. The absence of wax, methyl, and cholesteryl esters in the f_2 fraction was verified in this way, as well as the large quantity of polyolefinic hydrocarbons present in these samples.

Later in the study (during the analysis of the fall samples), methylene chloride (CHCl_2) was substituted for benzene as the f_2 elutant as part of an effort to remove benzene from the laboratory. A similar study to that described above verified that 15 ml CHCl_2 could be used in place of 20 ml benzene with equivalent separations and recoveries.

The main precaution taken in the procedure was not to overload the column. Therefore, no more than 50 mg of lipid material in 0.5 ml of hexane were charged to the column. All columns were packed wet in benzene or methylene chloride and pre-rinsed with two column volumes each of benzene/methyl chloride and hexane.

Gas Chromatography

The f_1 (aliphatic) and f_2 (aromatic/olefinic) fractions were analyzed by gas chromatography. All samples were run on glass capillary (wall-coated open tubular) columns. The instrumental conditions were:

1. Column: 15 m, SE-30 glass capillary, wall-coated open tubular (WCOT), 0.25 mm i.d., 50,000 to 70,000 theoretical plates - N_{eff} , J&W Scientific, baseline separations of pristane/ $n\text{-C}_{17}$ and phytane/

n-C₁₈ pairs; column connections - Teflon shrink tubing.

2. Injection mode: spitless Hewlett Packard 18835A inlet system.
3. Gas chromatograph: Hewlett Packard 5840A.
4. Carrier gas: helium, 1.0 ml/min.
5. Temperatures: injector, 250° C; detector, 300° C; oven, 60° C to 100° C at 8°/min and 110° C to 260° C at 3°/min.
6. Average time of run: n-C₁₄ to n-C₃₄, 75 minutes.

The output from the gas chromatograph was transmitted through the Hewlett Packard 18846A digital communications interface through a terminal to a PDP-10 computer. Quantities of all resolved components were automatically determined relative to the internal standard, retention indices computed on the basis of standard n-alkane runs repeated every day, and selected data stored for future reference. The integration algorithm of the 5840A allows for a changing baseline, and this baseline can be reset at all peak valleys. Thus the peaks may be integrated above the unresolved complex mixture (UCM).

The UCM was quantified by use of a planimeter: the total area of the UCM was related to the area of the internal standard and thus quantified.

Gas Chromatography/Mass Spectrometry (GC/MS)

Combined GC/MS was performed on a Hewlett Packard system (a Hewlett Packard 5710A gas chromatograph equipped with an SE-30 glass capillary column interfaced with the Hewlett Packard 5840A quadrupole mass spectrometer). The mass spectrometer conditions were as follows: ionization voltage, 70 electron volts; electron multiplier voltage, 2,200 volts; and scan conditions, 50 amu to 500 amu at 225 amu/second.

The mass spectrometer was routinely calibrated using perfluoro-tri-n-butylamine (PFTBA). Sample volumes were generally in the range of 1.0 μ l to 1.5 μ l.

Data were collected on the Hewlett Packard 5934A data system. The mass spectral analyses focused on the search for 2- to 5-ring aromatic compounds and their alkyl homologs. Selected mass searches were used to obtain ion chromatograms for these compounds. The Textronix 4012 terminal was used to integrate the area under the peaks of the ion chromatograms. To correct for the parent ion (m^+) response factors, calibration curves were determined by the analysis of three amounts of selected compounds and the integration of ion chromatograms. The concentrations of individual components were determined by relating the integrated areas under the ion chromatograms to that of the internal standard and applying the appropriate response factors.

3.2.1.3 Trace Metal Chemistry of Zooplankton and Ichthyoplankton in the Water Column

Zooplankton samples processed onboard ship were transported frozen to the trace metals laboratory at the University of Delaware for further processing and trace metal analyses. All samples were rinsed in distilled isotonic ammonium formate (to remove extraneous salt and surface absorbed trace metals) before being frozen and were freeze-dried prior to further analyses.

Approximately 0.5 g of freeze-dried sample was weighed on a Cahn microbalance and placed in an LFE low-temperature oxygen plasma asher. Combustion by plasma ashing, as discussed previously (Third Quarterly Report, New England OCS Environmental Benchmark, October 1977), was found to be most effective if conducted at a power setting of 100 Watts and for a digestion time of 36 hours. Ashed samples were dissolved in 70 percent double-distilled nitric acid and filtered through 0.45 μ Nuclepore membrane filters. After filtration, samples were placed in the Teflon liner of a Parr 125-ml bomb, sealed, and further digested at 90° C for 2 hours. Samples were then rinsed in a 50-ml acid-cleaned polyethylene graduated flask and brought to a final volume of 50 ml. All processing and sample transfers were conducted on Teflon-covered laboratory bench surfaces.

The processing of all samples, including the freeze-drying, was accomplished in a two-room ultra-clean laboratory complex with a double door, an air-lock entranceway, an epoxy-coated interior, a laminar-flow air system, a particle filter, an electrostatic precipitator, and a system that maintains positive air pressure.

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Zooplankton samples were analyzed with a Perkin-Elmer atomic absorption spectrophotometer housed in the clean laboratory complex and equipped with a simultaneous background deuterium arc corrector, an electrodeless discharge lamp (EDL) power supply and lamps, an automatic sample injection system, and a computer phone patch coupling. Analyses for zinc were conducted with flame atomization; all other metals were analyzed by carbon rod atomization. Although no dilutions beyond the initial 50-ml sample volume were required to analyze for chromium, nickel, and lead, samples analyzed for cadmium and copper required typical dilutions of 2x, while analyses for iron and zinc required dilutions of 3x to 6x. All values were expressed as $\mu\text{g (g dry wt)}^{-1}$.

Quality control procedures and background corrections consisted of analyzing both procedural and reagent blanks for selected trace metals. The shipboard procedural blanks taken on each cruise were National Bureau of Standards (NBS) standard bovine liver passed through each size (505 μ or 202 μ mesh) plankton net with seawater and through the Teflon folsom plankton splitter with distilled water. In the laboratory at the University of Delaware, procedural blanks with NBS bovine liver were taken periodically during the freeze-drying and oxygen plasma ashing steps of laboratory processing. Other filter blanks were taken with Nuclepore filter pads processed as described for a typical sample. In addition, reagent blanks for both 70 and 6 percent dilutions of double quartz distilled nitric acid and distilled water were taken for reagents used on shipboard during each cruise and associated with each batch of samples processed in the laboratory.

Aliquots of zooplankton and macrobenthic samples were shipped to ERCO for neutron activation analysis (NAA) for V and Ba. Irradiations were performed with the same operating parameters for the reactor and detector system described in Section 3.2.6. The pre-irradiation chemical processing of the samples differed for the two analyses.

Samples for Ba analysis were evaporated to dryness in acid-cleaned polyethylene vials, the vials were sealed, the outside surface was cleaned, and the samples were irradiated. Because of the high Na content of the tissues, the samples for V analysis had to be treated to eliminate the interferences high Na levels would produce. The samples were treated with the hydrated antimony pentoxide chromatography procedure of Girardi and Sabbioni (1968). As in the short irradiations of suspended matter, backgrounds due to sulfur and Cl rose when the H_2SO_4 oxidation step was eliminated. In addition, a problem with chemical yield of the hydrated antimony pentoxide (HAP) procedure was encountered, probably because of formation of a VO_5 species and its exchange with the SbO_5 of the column at the high pE of the HNO_3 elutant. The procedure of Girardi and Sabbioni (1968) called for the use of HCl to wash the column, but this would have caused further problems in the short NAA procedure prescribed. Vanadium, therefore, was not observed in any biologicals. Barium, in general, was not observed due to the lack of sensitivity of the long irradiation technique for Ba.

3.2.2 Water Column Analysis: Marine Microbiology

Chlorophyll a and Phytoplankton Biomass

Chlorophyll a was determined by the fluorometric method of Holm-Hansen et al. (1965) after extraction by the method of Lorenzen (1967). A 100-ml aliquot of each sample was filtered through a glass fiber filter (Gelman type A-E) that had previously been soaked with saturated $MgCO_3$ solution. The filter then was placed in a vial and stored in a desiccator at $-5^{\circ} C$ in the dark until extraction ashore. A fluorometer (Turner model 111), which had been calibrated with standardized chlorophyll a (Sigma), was used for the analysis. The fluorometer was equipped with a blue light source (T-5 envelope), a 5-6 primary filter (Corning), and a 2-64 secondary filter (Corning). Samples were read before and after acidification and the values obtained were used to calculate the chlorophyll a content. Phytoplankton biomass was calculated from [chlorophyll a] x 50 (Lorenzen, 1967).

Bacterial Direct Count

Bacterial direct counts were performed according to Watson et al. (1977) with acridine orange-stained preparations. Bacteria were viewed and counted on a Zeiss standard research microscope equipped with epifluorescence optics. Portions of the sample (10 ml), to which 10 percent glutaraldehyde (0.1 ml) had been added as preservative, were stored at $4^{\circ} C$ in tightly capped scintillation vials. Usually 2 ml of subsample provided sufficient bacterial density for counting (approximately 100 bacteria per grid field). The number of nonbacterial ultraplankton was

determined in a similar manner. A 50-ml portion of glutaraldehyde-preserved sample (1 ml 10 percent glutaraldehyde per 100 ml) was stained with 0.5 ml 0.1 percent acridine orange for 3 minutes. The stained subsample was then filtered through a 0.2 μm pore size polycarbonate membrane (Nuclepore) that had been stained with Irgalan black according to Watson et al. (1977). After filtration the membrane was allowed to air dry and then was transferred to a microscope slide. A drop of Cargylles type A immersion oil and then a coverslip were placed on the membrane. Counts were performed with epifluorescence microscopy with the oil immersion x 100 objective (Zeiss, Neofluar). Only distinctly nonbacterial ultraplankton cells (2 μm to 20 μm in diameter) were enumerated, and at least 100 cells or 50 fields were counted per sample.

Ultraplankton and Microplankton Direct Count

Fluorescing ultra- and microplankton were also determined by epifluorescence microscopy. The light source and filter arrangement used were those described by Watson et al. (1977). A 50-ml portion of glutaraldehyde-preserved sample (with no acridine orange added) was filtered through a 0.2 μm pore size polycarbonate membrane (Nuclepore) stained with Irgalan black. After filtration the membrane was mounted on a slide and red fluorescing ultraplankton were enumerated using the 16x objective. At least 50 fields were counted. The 40x objective was used when the presence of detrital material interfered with recognition of ultraplankton. With the same preparation, all fluorescing microplankton on the membrane were counted. A differential count of only red fluorescing microplankton was taken simultaneously. The number of microplankton was also

determined from iodine-preserved samples with the membrane filter technique (Standard Methods, 1974). This method was modified slightly by allowing the membrane filter to dry overnight before it was mounted in oil. The plankton biomass was estimated from measurements of representative acridine orange-stained and total fluorescing plankton by the method of Mullin, Sloan, and Eppley (1966).

Lipopolysaccharides (LPS) and Bacterial Biomass

LPS were determined according to Watson et al. (1977) using limulus amebocyte lysate (Associates of Cape Cod, Inc.). Subsamples for LPS determination were stored in vials made pyrogen-free by heating at 180° C for 3 hours. Before the subsampling, 10 ml of 3 percent (wt/vol) NaCl solution (pyrogen-free) were added to the vial containing the subsample. Vials were tightly capped and stored at 4° C until analysis ashore. Sediment subsamples treated in this manner can be stored for up to 2 weeks without affecting the bacterial-associated LPS (Watson, unpublished). Bacterial biomass was calculated from [LPS] x 6.35 (Watson et al., 1977).

Adenosine Triphosphate and Total Biomass

Seawater subsamples (100 ml) were filtered through Millipore membrane filters (0.45 μ m pore size). The still moist membrane filters were then placed in boiling 0.02 M Tris buffer (Sigma), pH 7.8, at 5° C. The solution was boiled for 5 minutes in a tightly capped scintillation vial, rapidly cooled on ice, and stored frozen (-20° C) until onshore analysis. Controls consisted of autoclaved seawater and spiked samples that had been filtered and extracted as

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described above. ATP was determined on a DuPont Luminescence Biometer (peak height) by the method of Holm-Hansen and Booth (1966) as modified by Cheer, Gentile, and Hegre (1974). Luciferin and luciferase (type II) were obtained from Sigma Chemical Co. Total (microbial) biomass was calculated from $[ATP] \times 250$ (Strickland et al., 1969).

Heterotrophic Viable Count

Heterotrophic viable titers were determined by the membrane filter microbiology technique (Jannasch and Jones, 1959). Appropriate quantities of seawater to yield 30 to 150 colonies were filtered through 0.2 μ m pore size 45-mm Nuclepore membranes. Two dilutions were incubated on plates at 5° C and 20° C for approximately 4 and 2 weeks respectively. Colonies were counted with a stereo microscope. Randomly selected colonies were selected for taxonomic analysis. The medium used for heterotrophic viable counts was marine agar (Difco).

Hydrocarbonoclastic Viable Count

Sterile velveteen pads were used to replicate colonies from heterotrophic plates onto a hydrocarbon agar that consisted of the basal medium of Makula and Finnerty (1968) supplemented with 1.5 percent Noble agar (Difco) and 5 percent silica gel-hydrocarbon mix. The silica gel-hydrocarbon mix was made by adsorbing a mixture of hydrocarbons onto 100 to 200 mesh silica gel to a final concentration of 10 percent wt/wt. Replicates were also made onto agar medium containing no hydrocarbon mix to determine agar degraders. All colonies that grew on the hydrocarbon agar

but not on the agar alone were picked and transferred to hydrocarbon broth (Makula and Finnerty's basal medium containing 0.5 percent vol/vol hydrocarbon mix). Isolates that grew in this liquid medium were termed hydrocarbon degraders. Incubation at either 5° C or 20° C was allowed to proceed for at least 1 month. Isolates were maintained in hydrocarbon-agar slants.

Heterotrophic Activity

Heterotrophic activity was determined by the method of Wright and Hobbie (1966) as modified by Hobbie and Crawford (1969). Seawater (50 ml) taken from a Go-Flo bottle was added to a 100-ml serum bottle. ^{14}C -glutamate or ^{14}C -acetate (0.5 ml of 0.5 $\mu\text{Ci/ml}$ solution) was then added to the seawater. The serum bottle was quickly capped with a rubber serum stopper to which a Kontes plastic cup had been attached. The cup contained a fluted filter paper strip (Whatman no. 1). Before the filter paper was incubated at either 5° C or 20° C, 0.2 ml β -phenethylamine was added by syringe to the filter paper. A control bottle had 0.5 ml HgCl_2 solution added as well. Bottles were incubated for 2 and 4 weeks at 20° C and 5° C respectively. At the end of this time 0.5 ml of 50 percent trichloroacetic acid was added to stop the reaction and liberate $^{14}\text{CO}_2$. A trapping efficiency control was also run. This control consisted of ^{14}C sodium bicarbonate in sterile seawater. $^{14}\text{CO}_2$ was liberated as described above. Values obtained from the trapping efficiency control were used to correct experimental results. Small amounts (0.1 ml) were also extracted periodically from each experimental bottle to check actual substrate (glutamate or acetate) concentrations.

Saturated filter paper strips were removed from the bottles after CO₂ was completely evolved (about 2 hours). The strips were placed in Aquasol (New England Nuclear) and counted in a Packard Tri Carb 3255. Counts per minute were converted to disintegrations per minute; background poisoned controls and trapping efficiency controls were used to convert the data to mg C/m³ per day.

Hydrocarbon Activity

Hydrocarbon activity was determined in a manner similar to that used to determine heterotrophic activity. ¹⁴C-dodecane (Amersham-Searle, 125 μCi/ml) was added to 50 ml of seawater in a serum bottle to give a final concentration of 0.25 μCi ¹⁴C-dodecane/ml. Incubation was allowed to proceed for 14 days. Reaction vessels, controls, and workup were similar to those described above for heterotrophic activity.

Laboratory Experiments on Bacterial and Fungal Isolates

Bacteria and fungi were isolated from either plate counts or hydrocarbon enrichments. Enrichments consisted of 10 ml to 100 ml seawater or 1 cm³ sediment added to basal medium containing various concentrations of hydrocarbon mix, South Louisiana crude oil, or individual hydrocarbons. Selected isolates were used for experiments on the effect of oil on growth and on the effect of nutrients on hydrocarbon degradation.

To study the effect of oil on the growth of pure cultures, marine broth (Difco) and South Louisiana crude oil were employed. Growth of pure cultures of hydrocarbon- and non-hydrocarbon-degrading bacteria was followed by plate count and direct count in the presence of various concentrations of oil.

To study the effect of nutrients on hydrocarbon degradation, several methods were employed. The first was to use the gas chromatograph to examine the residual hydrocarbon mix after 4 weeks of growth of a pure culture. The second involved the use of the gas chromatograph to examine the residual hydrocarbon mix after 4 weeks of growth of a seawater or sediment enrichment (mixed culture). The third was the use of ^{14}C dodecane to study the hydrocarbon oxidation after 1 to 2 weeks of growth of a pure culture.

All methods except enrichment used the basal medium of Makula and Finnerty (1968). All methods except that employed in the growth experiment used the hydrocarbon mix (described above) at a final concentration of 0.5 percent vol/vol. GC experiments employed an extraction of the culture with methylene chloride and separation into f_1 and f_2 fractions on silica gel/alumina columns as described in Section 3.2.1.2. All gas chromatography was performed as described in Section 3.2.1.2.

3.2.3 Water Column Analysis: Particulate Hydrocarbons

The frozen glass fiber filters were thawed and carefully cut into strips with prerinsed stainless steel scissors. The filter pieces were introduced into hexane, internal standards (androstane and hexaethyl benzene) were

added, and the mixture was refluxed for 2 hours. The hexane was decanted, chloroform was added, and reflux was begun again. After another 2 hours the mixture was cooled and the hexane and chloroform were combined. The extract was then concentrated to about 50 μ l and diluted to 0.5 ml with hexane. An aliquot was taken and used for the total extractable lipid determination. The extract was then fractionated into f_1 and f_2 by column chromatography (Section 3.2.1.2). The two fractions were analyzed by gas chromatography (Section 3.2.1.2).

3.2.4 Water Column Analysis: Dissolved and Surface Film Hydrocarbons

3.2.4.1 Dissolved Hydrocarbons in the Water Column

Samples brought back from the field consisted of either 45 or 90 liters of seawater. Extractions were performed in a stainless steel-glass-Teflon liquid-liquid extractor designed by A. Himmelblau of ERCO. The extractor (Figure 3-1) consisted of a reservoir sitting on top of the extracting column. The water samples were pumped by a magnetic pump directly out of the stainless steel or glass containers through all stainless tubing into the extractor. The drained barrel was rinsed with 2.5 liters distilled chloroform and the chloroform was pumped into the extractor as well.

A countercurrent flow of seawater and chloroform was established with turbulent mixing occurring at the three diffuser plates within the column. That greater than 98 percent extraction was achieved in 6 hours' operation of

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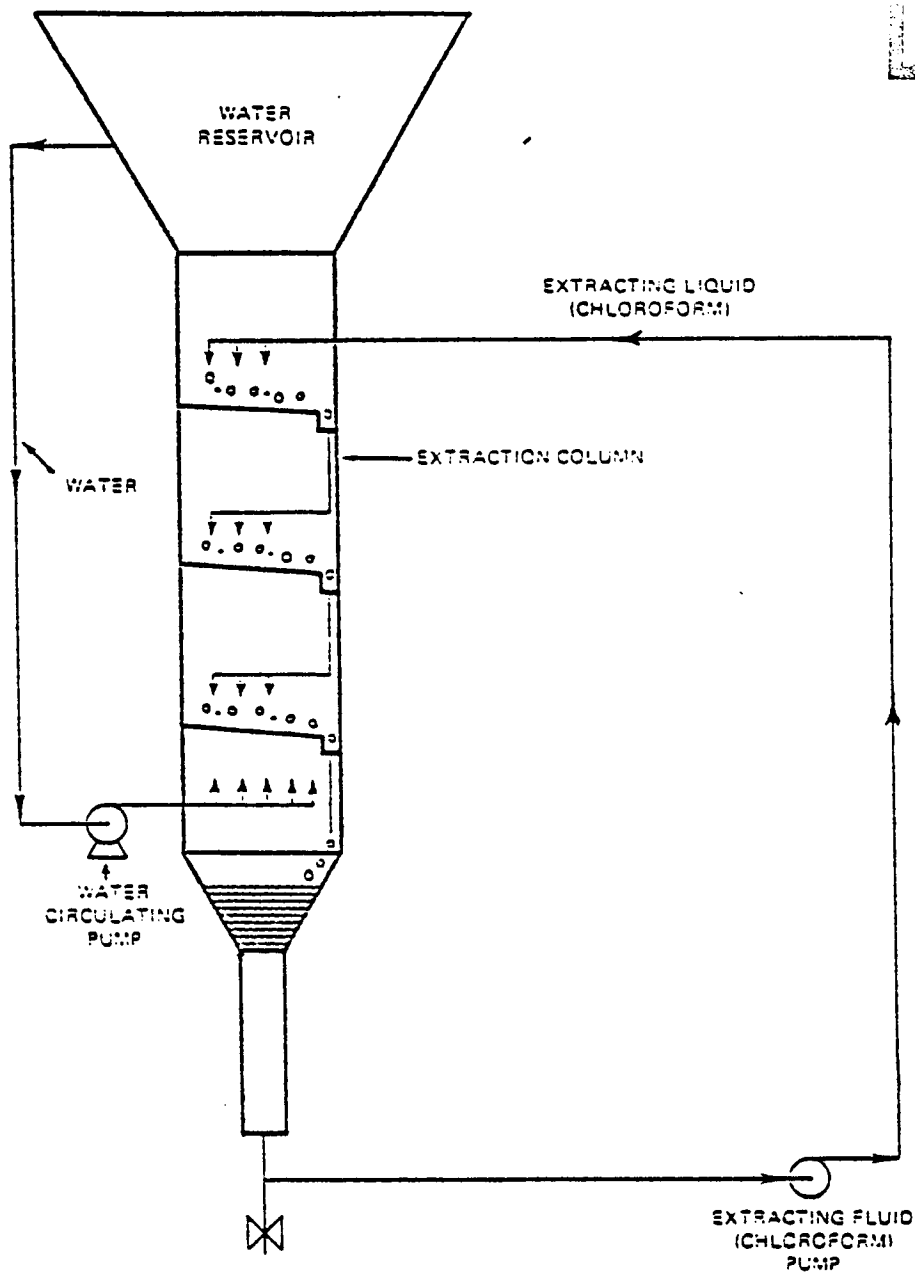


Figure 3-1. Large volume continuous liquid/liquid extractor.

this system was determined by successive 2-hour extractions of the same water. After 6 hours no further significant quantities of hydrocarbons were recovered. The system's pumps were shut down after the 6-hour extraction, at which time the chloroform was drained to the bottom of the extractor. The chloroform extract was spiked with internal standards (hexaethyl benzene and cholestane) and was concentrated on a rotary evaporator to approximately 10 ml. Further concentration to approximately 100 μ l was achieved under a stream of purified nitrogen. The extract was taken up in 0.5 ml hexane, an aliquot was taken for total extractable lipid determination, and the remainder was charged to the alumina/silica gel column for fractionation.

This extraction technique was extremely useful for large-volume samples. Between each sample run the system was rinsed with 90 liters distilled water/2 liters chloroform. This cleaned the system thoroughly and precluded salt buildup and hence corrosion of the 304 stainless steel.

A discussion of analytical recoveries, precision of duplicate and replicate analyses, and procedural blank levels is presented in Section 4.2.4.

3.2.4.2 Surface Film Hydrocarbons in the Water Column

Samples obtained generally consisted of 7.5 to 8 liters of surface film water from the stainless steel 16 mesh screen. The samples had already been poisoned with chloroform onboard.

In the laboratory each sample was added to four 2-liter separatory funnels and spiked with internal hydrocarbon

standards (hexaethyl benzene and androstane). The samples were extracted 3 times with chloroform and the extracts were combined and taken to a small volume by rotary evaporation and nitrogen flushing. Extractable lipid weights were determined on an aliquot by the Cahn electrobalance. The remaining extract was taken up in hexane and charged to the alumina/silica gel column (Section 3.2.1.2).

Recoveries of 95 to 98 percent of the internal standards, relative to external standards added to the extract, were achieved by this extraction procedure.

3.2.5 Water Column Analysis: POC and DOC

Particulate organic carbon and nitrogen were measured using a model 185B Hewlett-Packard carbon-hydrogen-nitrogen analyzer (CHN). Prior to analysis, standard curves for carbon and nitrogen were obtained with y Cystine as the standard. The standardization was rechecked at regular intervals during sample analysis. Filter blanks were run to account for any traces of carbon or nitrogen present on the glass fiber filter. In addition, blanks were run at regular intervals with only aluminum boats and catalyst to ensure the proper operation of the CHN.

Samples were combusted at from 1,000° C to 1,100° C in a sealed furnace for 20 sec in the presence of an oxidizing catalyst. With helium as the carrier gas, the combustion products were passed through a gas chromatographic column and then by a thermocouple. The thermal conductivity was proportional to the concentration of carbon and nitrogen present. Samples were run in triplicate (Menzel and Vaccaro, 1964; Sharp, 1973).

The samples for dissolved organic carbon were first thawed to room temperature. To each of the 100 ml replicates, 0.1 ml of phosphoric acid was added to bring the pH of the sample to between 1 and 2. The samples were then bubbled with oxygen for 10 minutes to drive off inorganic carbonate. Five-milliliter aliquots of sample were placed in 10-ml ampules which contained 200 mg of potassium and were combusted at 450° C for 2 hr to remove all organic matter present. The 5-ml aliquots of sample were then rebubbled for 10 sec with oxygen, and the ampules were sealed. To oxidize the organic carbon to carbon dioxide, the sealed ampules were autoclaved at 250° C for 30 min to 45 min.

The dissolved organic carbon samples were analyzed with a Beckman model 865 infrared analyzer. A calibration curve was obtained daily with a 0.15 percent CO₂ gas calibration mixture. Determinations of blanks were made by running triplicate analyses on 1.0 ml, 3.0 ml, and 5.0 ml of a single sample. The results were then extrapolated to determine the value at zero volume. In sample analysis the end of the ampule was crushed and the CO₂ to be analyzed was passed through the infrared analyzer with oxygen as the carrier gas. A Hewlett-Packard model 3380S integrator was used to determine the area under the output curve from the infrared analyzer. The amount of carbon present in the sample was then calculated.

The results of the three determinations per sample for both particulate and dissolved organic carbon were averaged to obtain the reported values. Particulate organic carbon and nitrogen values were expressed in µg/l. Dissolved organic carbon values were expressed in mg/l.

3.2.6 Water Column Analysis: Particulate Trace Metals

Because of the extremely low levels of material collected for particulate trace metal analysis (1 mg to 3 mg on the average), caution had to be taken to control contamination during filter preparation and processing. Upon return to the laboratory, the 0.4 μm pore size 47-mm Nuclepore filters were thawed, removed from the in-line filter holders, and placed in acid-cleaned petri dishes. Samples were handled entirely in a class 100 clean bench. The filters were dried over silica gel to a constant weight, usually for 96 hours, and weighed on a Cahn model 4100 electrobalance to determine the mass of material collected. The analysis of numerous blanks indicated that in some cases the trace metal levels of the Nuclepore filters were sufficient to obscure the detection of some elements in a suspended phase. Mass change problems were encountered in weighing the filters, especially on humid days. An extensive experiment was conducted to determine the optimum conditions for the handling and analysis of suspended samples. The results of this experiment are presented in Appendix G. The results of the mass change experiment were available too late to allow a contract modification without affecting conclusions based on seasonal variations in levels of suspended material collected. All filters, however, were acid-cleaned prior to use to lower the filter contribution to the overall blank levels.

After being weighed, the filters were mounted in an acid-cleaned plastic filter holder (Nuclepore 411800) and the drain closed. Ten milliliters of a 25 percent Ultrex acetic acid solution were then placed in the filter holder and the chimney was covered. The filters were allowed to sit for 2 hours in this solution to enable assessment of the

levels of weakly sorbed metals present. After 2 hours, the weak acid solution was vacuum filtered by a Fisher Filtrator into acid-cleaned polyethylene vials for analysis and storage.

The filters were then placed in Teflon digestion bombs (Eggimann and Betzer, 1976), 750 μ l of Ultrex HCl were added, and the bombs were sealed and placed in a hot water bath (90°) for 1 hour. After cooling, 250 μ l of Ultrex HNO₃ were added and the digestion continued for 30 minutes at 90°. After cooling, 50 μ l of Ultrex HF were added and the filters digested at 90° for an additional 30 min to dissolve any silica present. When cool, the solution and filter were transferred to a clean polyethylene vial to which 9 ml of double-deionized water were added, and the analysis was performed.

The weak-acid leachable solutions were analyzed for the elements Cd, Cr, Cu, Fe, Ni, Pb, and Zn by atomic absorption. The conditions used are given in Table 3-2. Because of the low levels of Fe present, Fe was determined by flameless techniques. Zinc was present in sufficient quantities in both samples and blanks for flame analysis. The refractory suspended matter samples subjected to acid dissolution were also analyzed with the same techniques and operating conditions. In addition, the refractory samples were analyzed by neutron activation analysis for V and Ba. Vanadium was determined by irradiating an aliquot of sample for 30 seconds at a flux of 8×10^{12} N/cm² per second in one of MIT's "rabbit" facilities. The samples were allowed to cool for 5 min and were counted for 300 seconds' live time with a 16.3 percent 1.82-keV-resolution (1336-keV line of Co-60) Ge(Li) detector coupled to a Canberra model 8180 MCA. The spectra were stored on magnetic tape for

TABLE 3-2
ATOMIC ABSORPTION ANALYSIS OPERATING CONDITIONS

FLAMELESS													
PE-603							HGA-2100						
ELEMENT	λ (nm)	SLIT (nm)	LAMP/POWER	D ₂ ARC	INTEGRA-TION TIME (sec)	PE-56 FULL SCALE RANGE (mv)	RAMP 10 DRY		RAMP 10 CHAR		ATOMIZE		PURGE GAS/FLOW
							TIME (sec)	TEMP (°C)	TIME (sec)	TEMP (°C)	TIME (sec)	TEMP (°C)	
Cd	228.8	0.7	HCL/4 ma	On	6	5	15	150	17	250	6	2,100	N ₂ /25
Cr	357.9	0.7	HCL/25 ma	Off	6	10	15	150	17	1,100	6	2,700	Ar/25
Cu	324.7	0.7	HCL/15 ma	Off	6	5	15	150	17	900	6	2,700	N ₂ /25
Fe	302.1	0.7	HCL/30 ma	Off	6	10	15	150	17	1,100	6	2,700	N ₂ /25
Ni	232.0	0.2	HCL/25 ma	On	6	5	15	150	17	1,000	6	2,700	N ₂ /25
Pb	283.3	0.7	EDL/10 W	On	6	5	15	150	17	700	6	2,300	N ₂ /25
FLAME													
PE-603													
ELEMENT	λ (nm)	SLIT (nm)	LAMP/POWER	D ₂ ARC	INTEGRA-TION TIME (sec)	PE-56 FULL SCALE RANGE (mv)	FUEL FLOW		OXIDANT	OXIDANT FLOW (l/min)	OXIDANT PRESSURE (psi)		
							FUEL	(l/min)					
Fe	302.1	0.2	HCL/30 ma	Off	2	10	C ₂ H ₂	4	Air	22	30		
Zn	213.9	0.7	HCL/20 ma	On	2	10	C ₂ H ₂	4	Air	22	30		

reduction at the MIT computer laboratory. The presence of large amounts of Cl^- in the samples because of the specified dissolution procedure, therefore, precluded longer irradiations. Vanadium was not observed in any of these samples because of the high background due to Cl^- . The samples from Cruise 1 were analyzed after hydrated antimony pentoxide chromatography (Girardi and Sabbioni, 1968) in an effort to remove Na. They were then oxidized with H_2SO_4 and heat to liberate Cl_2 . The result was unsatisfactory: a higher background was present because of S present in the final solution analyzed. The chemistry specified for short neutron activation precluded V analysis of these samples.

Long irradiations for Ba were performed in a rotating rack in a long irradiation facility at the MIT Nuclear Reactor Laboratory. Samples were dried in plastic vials, sealed, and irradiated for 7 to 12 hours at a neutron flux of 10×10^{12} N/cm^2 per sec. After cooling for 2 weeks, they were counted for 2 hours (live time) using the same Ge(Li) crystal and MCA system described above. Data were transferred to magnetic tape and computer reduced. The Ba^{131} isotope was not sufficiently sensitive to detect Ba in any of these samples. The lack of sensitivity of atomic absorption for V and Ba requires an alternate technique such as neutron activation for their analysis. The most frequently used technique for these elements in low-level samples such as suspended particulates samples is nondestructive instrumental neutron activation analysis (INAA) (Spencer et al., 1972; Brewer, personal communication). Procedures are presently being reviewed; until they are revised, low levels of V and Ba will not be detected.

3.2.7 Water Column Analysis: Dissolved Micro-nutrients, Dissolved Oxygen, and Salinity

Dissolved micronutrients were analyzed with methods suggested by Strickland and Parsons (1972). Tests were run to determine the concentrations of dissolved phosphate, nitrite, nitrate, and silicate. Calibrations were run with standard solutions in concentrations that bracketed the expected sample concentrations.

For the dissolved phosphate measurement, the sample was warmed to room temperature. Ten milliliters of a mixed reagent (ammonium molybdate, sulfuric acid, ascorbic acid, and potassium antimony tartrate) were added to three 100-ml aliquots of the sample. Sample absorbances were measured with a 10-cm cell in a Beckman DU Spectrophotometer set at a wavelength of 885 nm. Calibration was accomplished with a 5-mM phosphate standard (analytical grade potassium dihydrogen orthophosphate dissolved in high-purity distilled water). A cell-to-cell blank and reagent blanks were run to correct the sample absorbances. The phosphate concentration was reported in microgram atoms phosphorus per liter.

For the dissolved nitrite measurement, 0.2 ml sulphanilamide solution was added to each 10-ml aliquot of sample. After 2 to 8 minutes, 0.2 ml of a naphthyl ethylenediamine solution was added to each sample aliquot. After sufficient time for color development, absorbances were measured by the use of a Bausch and Lomb Spectronic 70 with a 1.0-cm cell at a wavelength of 543 nm. A 5-mM nitrite standard (analytical grade sodium nitrite dissolved in high-purity distilled water) was used for calibration. Reagent blanks were run to correct the sample absorbances. The nitrite concentration was reported in microgram atoms nitrogen per liter.

For the measurement of dissolved nitrate, the combined concentration of nitrite and nitrate was measured for each sample and the concentration of nitrite (measured separately) subtracted from the result. For the combined analysis, the sample was passed through a cadmium column so that the nitrate in the sample was reduced to nitrite. Analysis then proceeded as with the regular nitrite analysis. The nitrate concentration of each sample was reported in microgram atoms nitrogen per liter.

Only nonglass laboratory ware was used in the dissolved silicate analysis. Twenty-five milliliters of sample were added to a 10-ml molybdate solution. Fifteen milliliters of reducing agent were then mixed into the sample, and the sample was allowed to stand for 3 hours to complete the reduction of the silicomolybdate complex. (The reducing agent consisted of 100 ml of metol sulfite solution, 60 ml oxalic acid solution, and 60 ml of a 50 percent sulfuric acid solution, made up to a volume of 300 ml with distilled water.) Sample absorbances were measured using a 1-cm cell against distilled water in a Beckman DU Spectrophotometer set at a wavelength of 810 nm. Calibration was accomplished using a silicate standard (sodium silicofluoride). Reagent blanks were run to correct the sample absorbances. The silicate concentration was reported in microgram atoms silicon per liter.

Dissolved oxygen measurements were made onboard ship using the Winkler titration procedure (Standard Methods, 1971). Samples were run in duplicate within 24 hours of collection. The average value of the replicates was reported as the dissolved oxygen concentration in ml/l.

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Salinity was measured with a Beckman induction salinometer. Samples were run in duplicate; the average value was reported in parts per thousand. The salinometer was standardized before each use by being calibrated with Copenhagen water (19.381 o/oo chlorinity, 35.0127 o/oo salinity).

3.2.8 Water Column Analysis: Conductivity, Temperature, and Dissolved Oxygen Profiling

Analysis of the CTD/O data consisted of calibration, transcription, editing, computation, validation, and graphic presentation of the profile data. Computed salinities have been compared with the measured salinities and found to be inherently more accurate. In the determination of dissolved oxygen profiles, however, the Winkler analysis was used extensively.

Raw Data Description

The Neil Brown Instrument Systems Mark IIIB CTD/O system used on the North Atlantic benchmark program sampled temperature, conductivity, pressure, oxygen probe current, oxygen probe temperature, transmission, and nephelometry 32 times per second. However, the oxygen probe data were updated only once per second. These data were internally digitized and formatted (FSK) before being transmitted up the single conductor cable to the deck unit. The formatted signal was recorded for processing on standard audio tape.

After the February cruise the 3-mm micro-profiling conductivity cell was replaced with a new 8-cm general-

purpose cell. The new cell had a time constant more closely matched to the temperature probe and was less sensitive to fouling because of its larger internal dimension. This modification had little impact on the analysis programs.

The formatted data were represented in a serial bit stream of all parameters in the following order:

1. Frame synch word, 8 bits.
2. Pressure, 16 bits.
3. Temperature, 16 bits.
4. Conductivity, 16 bits.
5. Utility word (sign information), 8 bits.
6. Oxygen current, 12 bits.
7. Oxygen temperature, 8 bits.
8. Nephels, 8 bits.
9. Transmission, 8 bits.
10. Frame synch word, 8 bits.

Processing began only after these data were transcribed to tape in computer-compatible binary code. This step was performed at WHOI with a generalized CTD acquisition program, CAQ76.1.

Data Transcription

Early in 1977, WHOI's Information Processing Center developed a generalized CTD acquisition program. Dr. Robert Millard, Jr. provided technical specifications for the program, and George Powers developed the coding. The coding in previous versions limited the number of parameters to five (temperature, pressure, conductivity, DO current, and DO temperature) and could not, therefore, handle transmission and nephelometry data. The new system contains the flexibility for the user to describe the data stream in detail and allows a very generous parameter string.

CAQ76.1, used on the WHOI Shore System's Hewlett Packard 2100-series computer, accepted data from the deck unit as it was played back by tape recorder. If the program did not find a particular scan complete (i.e., containing both complementary frame synch words), that scan was ignored. Each good scan was written to 9-track tape in CTD76 format (still being refined by Dr. Millard), in which each data item is represented as a 16-bit magnitude integer. A scans-missed entry indicates the number of bad scans omitted since the last accepted one, so that data can be treated as part of a time series in later processing.

Only the downcast is transcribed to tape in this way since an upcast would introduce a great deal of wake disturbance contamination to the profile. If the quality of the downcast was unacceptably poor, however, the upcast was used as a last resort, as in the case of one station in February. Once a cruise had been transcribed, the data tapes were transferred to ERCO's computer support facility.

Data Processing

The major processing components developed to treat the CTD/O/transmissometry/nephelometry data were:

1. Initial oxygen calibration.
2. Temperature lag adjustment.
3. Editor.
4. Averaging and computation.
5. Plots.
6. Final review of oxygen calibration.

Each of these steps was associated with a number of computer routines operated in time-sharing mode to retain maximum user flexibility.

The initial oxygen calibration used simultaneous data from the CTD/O and the Winkler analyses of discrete samples (near-surface and near-bottom) to assess the temperature sensitivity of the probe membrane and to develop a calibration factor for each profile. With an exponential temperature sensitivity correction assumed (Appendix F, Section F.1), the log of the ratio of values from the two determination methods yielded a nearly linear function of temperature. The slope of this function was the temperature coefficient in the argument of the exponential correction. Once this coefficient was set for a cruise, a calibration factor was calculated for each station that was merely the ratio of the two oxygen determinations. In this way the probe was used

as a means of determining the vertical structure of oxygen in the water column, while the actual magnitudes of the end points were taken from the Winkler titrations. Since two points of comparison are used in developing single-station calibration factors, questionable oxygen determinations were readily found. If both surface and bottom values were "bad" (because of, e.g., a label mixup), that particular profile was calculated on the basis of the overall cruise average calibration. The programs involved are discussed further in Appendix F, Section F.2.

The temperature lag analysis was performed on a limited segment of the raw CTD data. A station with large temperature gradients was selected, and that portion of the record containing a rapid time rate of change of temperature was isolated. The lag analysis program (Appendix F, Section F.2.2) was run with various user-specified time lag values (in units of scans), and the resulting corrected temperatures were used to compute and plot salinity versus depth. The lag value that minimized the salinity excursions (spikes) was then used in later processing calculations.

Once an initial oxygen calibration matrix had been calculated and a lag value selected, the edit and scaling programs could be applied. Because of the size of the data records, an entire downcast for one station could not be edited at one time; therefore, three editors were written, one each for the pressure, the temperature and conductivity, and the oxygen probe current and temperature. Transmissometer-nephelometer data were not edited.

All three editors operated in like fashion. The scans-lost column and the column (or columns) of data to be checked were read into core, and all values were checked

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sequentially for spikes. The criteria for anomalous jumps were user-specified in the temperature-conductivity editor but were fixed for the pressure editor, where they were based on the instrument lowering rate. For oxygen data, the 1-second update characteristic made it particularly easy to spot bad scans since the data ought to change in 32-block segments. A bad scan value was interpolated from the surrounding good values. The column of edited data was then scaled to physical units. In addition, deck pressures were subtracted from the pressure column to remove the atmospheric component in the pressure editor, the oxygen calibration factor was applied to the oxygen current column in the oxygen editor, and temperatures were lag-corrected and smoothed along with conductivities in the temperature-conductivity editor. Further details are presented in Appendix F, Section F.2.3.

The final computational program recombined all the edited data, calculated the salinity and density for each scan, and saved this as a new file. This particular file was later written to tape and was not used in the benchmark analysis. Should later micro-scale analyses be desired, these data will be useful. While this large file was being created scan by scan, the data were also being sorted by depth into 0.5-meter segments. Only those values obtained while the instrument was falling were included, and they comprised a uniform depth series (the next pressure datum was accepted when it exceeded the last accepted datum). All scans in a pressure level were averaged, and the number of scans so averaged was passed on. Dissolved oxygen was calculated, and the final aggregate files were stored. These files formed the basis of further data interpretation products. Appendix F, Section F.2.4, contains more details.

All variables were plotted versus depth from these files, and the dissolved oxygen was checked against bottle data station by station. If the calibration factor was bad, a new one could be prescribed and the oxygens recomputed (Appendix F, Section F.2.6). Temperature-salinity plots and vertical sections were also constructed by the computer. Horizontal maps were drawn by hand.

3.3 Benthic Analysis

3.3.1 Benthic Analysis: Core/Grab Sediment Samples

After the 1,500-cm³ frozen sediment sample was allowed to thaw, it was emptied into a clean, pre-rinsed Teflon basin and homogenized with a clean, pre-rinsed Teflon spatula, all within a class 100 clean station. Great care was taken at all times to avoid contamination of the sample.

After homogenization, the following subsamples for hydrocarbon and trace metal analyses were collected in clean, pre-rinsed noncontaminating vessels and sent to the appropriate laboratories:

1. 1,000 g for hydrocarbon analyses.
2. 1,000 g for hydrocarbon quality control.
3. 100 g for trace metal analyses.
4. 100 g for trace metal quality control.
5. 200 g for grain size analyses.

The subsamples were shipped frozen at -10° C and kept frozen at -10° C whenever necessary.

3.3.1.1 Core/Grab Benthic Grain Size Analysis

The samples for grain size analysis were thawed and then washed through a 62 micron sieve to separate the sand and gravel from the silt-clay fraction. The coarse fractions were oven dried, weighed, and then passed through a 2-mm sieve. A sieve analysis was performed on the gravel retained on the 2-mm sieve. The sand fraction was treated with 0.3 N acetic acid to remove carbonates (Ireland, 1971) and, when necessary, 50 percent hydrogen peroxide to remove organic material; then it was rewashed and dried. One-gram subsamples of the sand fraction were obtained with a micro sample splitter for analysis in the settling tube, which was patterned after the design of Gibbs (1974). The settling tube was calibrated with a mixture of sands from the study area. The sand particles were sieved into 0.25- ϕ fractions and their specific gravities determined. The shape factor of the sand particles was determined through the use of a petrographic microscope with an ocular micrometer. The settling velocity of each size group was determined at several known water temperatures. The results of the calibration runs matched the work of Zeigler and Gill (1959) for particles with the same shape factor.

Sand fraction subsamples were run in triplicate in the settling tube. The settling tube system was interfaced with a Hewlett Packard 9825 calculator to determine the sand fraction size distribution at 0.0625- ϕ intervals. These data, together with the results of the gravel size analysis

and the pipette analysis, gave the complete size analysis for each sample.

For the analysis of the silt-clay fraction, the pipette method was used. The portion of the sample that had been washed through the 62 micron sieve was poured into a 1-liter cylinder. A measured quantity of dispersant was added to prevent flocculation, and the water level in the cylinder was brought up to 1,000 ml with distilled water. The sample slurry was shaken vigorously and allowed to stand for 24 hours. If the sediment showed no sign of flocculation, the sample was ready for analysis. If additional shaking did not prevent flocculation, the sample had to be reprepared. The pipette analysis then proceeded according to the methods outlined by Folk (1968).

3.3.1.2 Core/Grab Benthic Biological Analysis

Macrofauna

Macrobenthos

Upon return to the laboratory, each sample was rinsed with fresh water in a 4-mm sieve to remove as much of the formalin as possible. The sieved material was placed in large enamel or glass trays and sorted to species. Each species was measured (length for most, carapace width for crabs, length for shrimp, and disk diameter for ophiuroids). The species as a group was then wet weighed after being blotted dry. If more than 25 specimens of a species were present, only 25 were measured and weighed. The remaining individuals were weighed for total weight. The number of

individuals was then extrapolated on the basis of the mean individual weight of the 25 specimens.

Voucher specimens were made for each species encountered, the remainder being discarded. Information recorded on data cards included species name, length and weight of 25 organisms, total weight, and total number of individuals for each group or species.

Only minor problems were encountered. Hermit crabs often could not be removed from their shells without the shells' being broken with a hammer. Brittle stars rarely had their arms, so measurements therefore were taken across their discs only.

Foraminifera

Each sample was gently rinsed through a 63 micron sieve until all fine material passed through. Sieve and contents were placed in a Rose Bengal bath (1 g/liter water) for 1 hour, rinsed, transferred to an evaporating dish, and dried in an oven at 60° C. When staining was insufficient, the organisms were added to either ethylene glycol or anise oil after identification to intensify the staining.

The cooled, dry sample was shaken gently through a 2-mm sieve on top of a 63 micron sieve. The contents of the 2-mm sieve were hand-picked for large forams. Contents of the 63 micron sieve were slowly poured into a funnel with rubber tubing and clamp attached. The funnel contained a bromoform-acetone mixture of approximately 2.395 density. The foraminifera, being less dense than the liquid, floated on the surface; the sand grains, being more dense, sank. After the

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sand had settled to the bottom of the tubing, the tubing was clamped off just above the bottom. When the clamp was released, the sand drained out, leaving foraminifera suspended on the surface of the fluid. (Density of the bromoform-acetone combination was checked with a hydrometer every day and appropriate adjustments were made if necessary.)

The foraminifera-bromoform-acetone mixture was then released into another funnel lined with filter paper. When the filter paper dried, the sample was placed a little at a time under a dissecting microscope. Each foram was picked out with a damp, fine-tipped paintbrush and placed on a specially designed cardboard slide that glued each organism in place. About 35 specimens were sorted in this manner.

Each foram was identified and a note was made as to whether it had reached the laboratory alive, as indicated by the uptake of Rose Bengal, or dead (colorless).

Voucher collections were made for each specimen identified, and identifications were verified by Prof. Larry Frankel of the University of Connecticut.

Microbiology

Bacterial Direct Count

Bacterial direct counts on 1-cm³ sediment subsamples were performed as described in Section 3.2.2.

Lipopolysaccharides and Bacterial Biomass

LPS were determined as described in 3.2.2, except that 1-cm³ sediment subsamples were transferred to sterile pyrogen-free scintillation vials containing 10 ml of 3 percent (wt/vol) NaCl solution (pyrogen-free) and 10 mg NaHCO₃. Samples were stored at 5° C for analysis.

Adenosine Triphosphate and Total Biomass

ATP was determined as described in Section 3.2.2, except that sediment subsamples for ATP analysis were extracted aboard ship immediately after collection using a modification of the method of Christian, Bancroft, and Weibe (1975). One cm³ of sediment was added to 8.0 ml boiling 0.1 M NaHCO₃ (pH, 8.5), vortexed 30 sec, transferred to a conical centrifuge tube, and centrifuged at 12,800 xg for 5 min. Four ml of the supernatant were transferred to a sterile scintillation vial containing 3.5 ml 0.2 M Tris buffer (pH, 7.8). Vials were stored at -10° C until analysis ashore. Controls consisted of autoclaved sediment and ATP spiked sediment subsamples similarly extracted. The factor 250 was used to convert ATP values to microbial biomass (µg C) (Strickland et al., 1969).

Heterotrophic Viable Count

Heterotrophic viable counts were determined as described in Section 3.2.2, except that sediment samples were blended in 50 ml filtered, sterilized seawater for 1 minute. After a 5-minute settling period, appropriate volumes were withdrawn

for dilution and filtration. Plates were incubated at 5° C and 20° C.

Hydrocarbonoclastic Viable Count

Hydrocarbonoclastic viable counts were determined as described in Section 3.2.2.

Dry-Weight Determination

Subsamples for dry-weight determination were frozen until returned to the laboratory ashore. There they were dried in a desiccator at 100° C until three similar consecutive weights were obtained.

Heterotrophic Activity - ^{14}C

Heterotrophic activity was determined as described in Section 3.2.2, except that 1 cm³ sediment was added to 50 ml sterile basal medium (Makula and Finnerty, 1968).

Hydrocarbonoclastic Activity - ^{14}C

Hydrocarbonoclastic activity was determined as described in Section 3.2.2, with the exception that 1 cm³ sediment was added to 50 ml sterile basal medium (Makula and Finnerty, 1968).

The composition of the hydrocarbon mix is given in Table 3-3.

TABLE 3-3
COMPOSITION OF THE HYDROCARBON MIX AFTER
WALKER AND COLWELL (1974)

FRACTION HYDROCARBON	PERCENT BY WEIGHT
f_1	
Decane	7.80
Undecane	7.80
Dodecane	7.80
Tridecane	7.80
Tetradecane	7.80
Pentadecane	7.80
Hexadecane	7.80
Heptadecane	7.80
Octadecane	7.80
Nonadecane	7.80
Eicosane	7.80
Tetramethylpentadecane	3.90
Cyclohexane	3.90
f_2	
Cumene	3.90
Naphthalene	0.66
Phenanthrene	0.66
1,2-Benzanthracene	0.39
Perylene	0.39
Pyrene	0.39

Macrofauna

The benthic infaunal samples from Georges Bank were divided 70 and 30 percent between Taxon, Inc. of Salem, Massachusetts and Bigelow Laboratory in West Boothbay, Maine, respectively.

Before being sorted, the samples were stained with rose bengal to increase the visibility of the fauna. The samples were resieved through a 0.5-mm metal sieve if it appeared that the sample volume could be reduced further.

The samples were then presorted into the basic taxonomic categories delineated in the contract (Polychaeta, Amphipoda, Mollusca, Echinodermata, other crustacea, and miscellaneous) under stereomicroscopes at a magnification of 7x.

During all phases of sample processing, emphasis was placed on correct labelling of samples.

It was found that some sample residues from Cruise 1 contained a significant number of missed animals. This problem was corrected by elutriating the residue into a fine sieve. The material caught in the sieve was then resorted.

Sample transfer to taxonomists for final identification was initiated when approximately 20 samples had been sorted. Polychaetes were sent to Don Maurer at the University of Delaware in Lewes, Delaware; amphipods to Les Watling at the Ira C. Darling Center in Walpole, Maine; and molluscs and echinoderms to Peter Larsen at Bigelow Laboratory. The "other crustacean" and "miscellaneous" specimens were identified at Taxon.

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All samples were transferred by registered mail, return receipt requested, so that no samples would be lost in transit. The transfers were logged onto the sample record sheets at the time of transfer. "

Identification followed the following references:

1. For amphipods, Bousfield (1973), McCain (1968), Shoemaker (1930, 1945a, 1945b), Sars (1895), and Stephenson (1923, 1925, 1931, 1944).
2. For molluscs, Abbott (1974); Gosner (1971); Heath (1918); Morris (1973); and Turner, Rheinhardt, and Baranowski (unpublished).
3. For echinoderms, Dearborn (unpublished), Downey (unpublished), D'yakanov (1967, 1968), Fell and Serafy (unpublished), Gosner (1971), Grainger (1966), Mortensen (1927), and Pawson (1977).
4. For other crustaceans and miscellaneous:
Cutler (1977), McCloskey (1973), Millar (1970), Miner (1950), Smith (1964), Watling (unpublished), and Williams (1974).

In addition, reference collections at the National Marine Fisheries Service and the Marine Biological Laboratory in Woods Hole were consulted concerning species that were difficult to identify.

Identification was taken to the species level whenever possible. High-power stereomicroscopes and compound microscopes were used as necessary. Wet weights were recorded for each taxonomic group in each sample.

Taxon was responsible for the compilation of the infaunal data from Bigelow Laboratory and the Ira Darling Center. The polychaetes became the responsibility of the University of Delaware and were treated separately after leaving Taxon. Data were logged onto the sample record sheets upon receipt; these sheets proved to be a valuable bookkeeping aid in minimizing transfer errors.

The transfer of samples and data between laboratories was a lengthy process, in large part because of "returns," items that were misidentified by the presorters and transferred to the wrong laboratory as a result. The time lag diminished as the project progressed, but some delay seems unavoidable when several laboratories are used for the identification of benthic infauna on a large scale.

Infaunal data were submitted to ERCO on ERCO data submittal forms for inclusion into the data base as the data were received at Taxon. Each species was assigned a 10-digit taxonomic code by the National Oceanographic Data Center (NODC) in Washington, D.C., a process that also involved considerable delay. About half the species encountered were not represented in the code listing; codes for them had to be specially requested. Coding was the single most time-consuming phase of data submittal.

On the whole, however, the analysis of the benthic infauna progressed smoothly. When minor problems arose, procedures were changed to correct them. Regular checks on all phases of sample processing uncovered minor errors before they became major.

Because the volumes of the samples from many of the benthic stations from the first and second cruises were greater than anticipated, sorting time was extended, and identification and data submittal consequently were delayed. The delay was effectively eliminated by a series of executive decisions that reduced the number of samples from 252 to 116.

3.3.1.3 Core/Grab Benthic Chemical Analysis

For a discussion of core/grab benthic chemical analysis see Section 3.3.1.1.

Hydrocarbons

Several techniques for the extraction of hydrocarbons from sediments have been discussed in the literature. These include (1) prolonged soxhlet extraction with various solvents and azeotropic mixtures, (2) solvent reflux, (3) combined saponification extraction, (4) solvent tumbling at room temperature, and (5) heating and heat space measurements. Depending on the nature of the sediment (recent vs. ancient; silt/clay vs. sand) and the aim of the analytical task (hydrocarbon geochemistry vs. recent petroleum incorporation), the efficiencies of the various extraction techniques may or may not be comparable. No statistically rigorous comparison of all the various widely-used techniques and sample types has been performed. Farrington and Tripp (1975) compared two of the more rigorous geochemical extractions (soxhlet vs. combined extraction/saponification) and found that the sampling variability was equivalent to the analytical variability in the two methods.

The BLM specified soxhlet extraction and the technique was indeed used throughout the study. The feeling of the project team, however, is that combined saponification/extraction would yield equivalent results, would cut procedural workup time, and would be less expensive.

The soxhlet extraction procedure used is illustrated in Figure 3-2. The sediment was thawed, rinsed with distilled water, placed in pre-extracted cellulose soxhlet thimbles or pre-combusted glass fiber thimbles and freeze-dried in a chamber freeze-drier. The sample was then extracted with a toluene:methanol azeotrope (spiked with internal standards, androstane and hexaethyl benzene) for 24 hours. Fresh solvent was then used to extract the sample for another 26 hours (50 hours total or 300 turnovers). The extract was concentrated by rotary evaporation and an aliquot weighed for total extractable lipid determination. The remaining extract was saponified, the saponification mixture was back-extracted with hexane, and the hexane concentrate was then charged to an alumina/silica gel column (Section 3.2.1.2) and fractionated (f_1 and f_2). Gas chromatography (Section 3.2.1.2) was used to quantify the resolved components and unresolved complex mixture in the samples.

Duplicate analyses were performed on approximately 10 percent of the samples to determine analytical precision, which was in the range of about 10 to 30 percent for total hydrocarbons and individual aliphatic compounds. Recovery of components of the BLM performance mixture spiked to an actual sediment sample was also determined.

The BLM performance standard was used as a check on the column chromatography procedure and spiked to actual

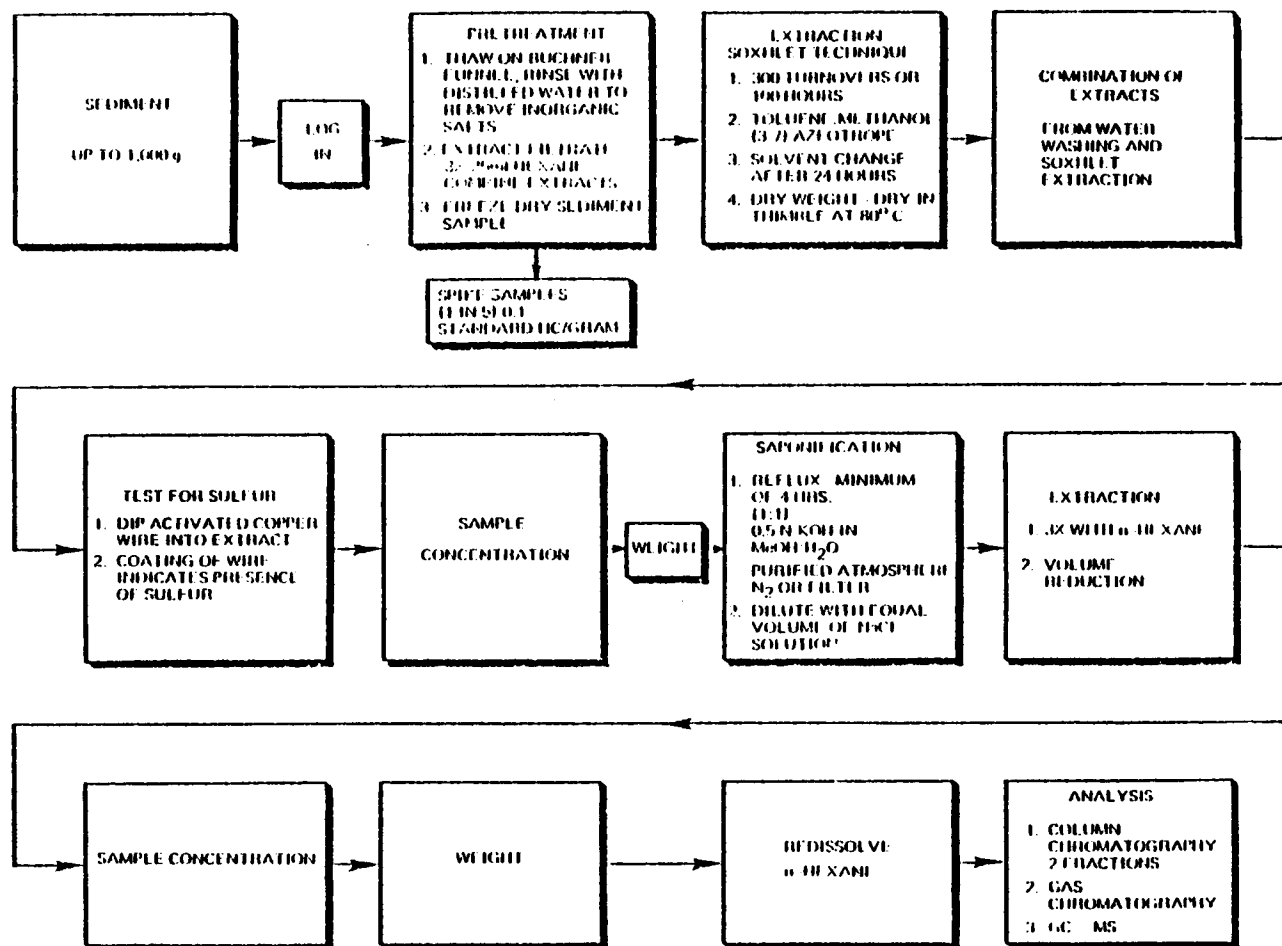


Figure 3-2. Hydrocarbon analysis of sediment samples.

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sediment samples to monitor absolute recoveries from these sample types. Relative to an internal standard of methyl hexadecane added when the mixture was charged to the column, recoveries of f_1 components ranged from 75 to 120 percent of their actual values. Recoveries of the f_2 components with retention indices 1400 and above ranged from 80 to 95 percent. Significant (>50 percent) losses of naphthalene occurred during concentration of the sample by rotary flash evaporation and nitrogen concentration, although the sample did not approach dryness.

Preliminary quantification of the spiked sediment recovery experiment indicated that component recoveries were roughly equal to those achieved in the column chromatography separations alone. Table 3-4 indicates that the only two problem components appeared to be phenanthrene and n-C₃₂. Reasons for their relatively low recoveries (approximately 65 percent) remain unclear.

Trace Metals

Sediments for trace metal analysis were dried at 60° C and the >3 mm size fraction physically removed. Approximately 10 g of sediment were then placed in clean plastic jars to which 10 ml of 25 percent Ultrex acetic acid were added. The containers were gently oscillated for 2 hours to remove the weakly associated trace metals. This weak acid leaching procedure was selected for three reasons:

1. The 25 percent HAc solution has a pH of about 2, which is near or just below the zero point of surface charge of most silicates. It was felt

TABLE 3-4
RECOVERY OF BLM PERFORMANCE STANDARD
COMPONENTS (SEDIMENT SAMPLE SPIKE)^a

COMPONENT	RETEN- TION INDEX	AMOUNT SPIKED (μg)	AMOUNT RE- COVERED (μg)	% RE- COVERED
Aromatic				
Dimethyl naphthalene	1390	5.0	4.10	82
Acenaphthene	1435	0.5	0.41	82
Fluorene	1531	0.5	0.49	98
Dihydrophenanthrene	1630	0.5	0.46	92
Phenanthrene	1710	0.5	0.31	62
Dimethylphenanthrene	1943	0.5	0.53	106
Pyrene	2018	0.5	0.50	100
Nonadecylbenzene	2590	0.5	0.52	104
Aliphatic				
n-C ₁₅	1500	1.0	0.8	80
n-C ₁₆	1600	0.1	0.1	100
n-C ₁₇	1700	0.5	0.4	80
Pristane	1710	10.0	8.5	85
18:1	1790	0.1	0.1	100
n-C ₁₈	1800	0.1	0.1	100
Phytane	1810	0.1	0.1	100
n-C ₁₉	1900	0.1	0.1	100
Androstane	1950	0.5	0.6	120
20:1	1990	0.1	0.1	100
n-C ₂₀	2000	0.1	0.1	100
n-C ₂₁	2100	0.1	0.1	100
n-C ₂₂	2200	0.1	0.1	100
n-C ₂₆	2600	0.1	0.09	90
Cholestane	2790	0.5	0.4	80
n-C ₂₈	2800	0.1	0.1	100
n-C ₃₀	3000	0.1	0.1	100
n-C ₃₂	3200	1.5	1.0	65

^aCorrected for natural occurrences.

that a more realistic estimate of the availability of trace metals to organisms by release in sediments was obtained with this solution than with a 5 N HNO_3 solution with a pH of approximately -0.6, well below the zero point of surface charge.

2. The HNO_3 solution gave a more unstable baseline in flameless analysis, especially for the more refractory elements such as Cr, probably because of the formation of CN compounds in the reducing environment of the heated graphite analyzer (HGA) analysis.
3. The HNO_3 solution appeared to destroy clay minerals, so that slightly greater quantities of Cu and Ni were leached by the HNO_3 than the HAc solution, and 20 times more Fe and Cr were removed into HNO_3 than HAc in test samples.

The sediment plus acid solutions were then transferred to clean Teflon centrifuge tubes and were centrifuged. The supernatant was decanted to clean plastic vials for storage and analysis.

Separate dried sediment samples were analyzed for total metals present. Twenty-five percent of all sediment samples were analyzed for total metals as compared with 100 percent for weak-acid leachable (WAL) metals. Samples for total metals were ground with agate utensils and sieved to separate the approximately 100- μm fraction. Approximately 0.5 g of this fraction was placed in Teflon digestion bombs (see Section 3.2.6), to which 3 ml of concentrated Ultrex HCl were added. The sample was digested for 1 hour at 90° C. After cooling, 1 ml of concentrated Ultrex HNO_3 was added

and the digestion continued for 30 minutes at 90° C. When cool, 3 ml of concentrated Ultrex HF were added and the sample was digested for an additional hour. This quantity of HF was sufficient to dissolve the silicates present and also to ensure that the solubility products of CaF_2 and/or BaF_2 were not exceeded in these low-carbonate sediments. The samples were allowed to cool and then were transferred to clean 25-ml polypropylene volumetric flasks and diluted to volume for analysis. Samples were stored in clean plastic vials.

The elements determined in both fractions were the same as those listed in Section 3.2.6. Sufficient material was present in these samples to allow for the determination of Fe with flame techniques (Table 3-2). Vanadium and Barium were determined in all of these samples by neutron activation analysis. Levels of Ba present were generally sufficient for this element to be determined in at least 50 percent of the WAL and total digestion (TD) sediments. The levels of vanadium were generally quite high; thus vanadium was observed in all samples. The presence of Cl^- , however, gave backgrounds so high that dead-times and poor counting statistics resulted. The elements determined by atomic absorption were easy to analyze in sediments. USGS G-2 standard rock was found to closely approximate the composition of these sediments and therefore was included as an unknown whenever sediments were processed.

TOC/TON

The samples for total organic carbon and nitrogen were thawed and then treated with sulfurous acid to remove the carbonates (Gibbs, 1977). After being dried in a desiccator,

the samples were ground to a fine homologous powder so that they would combust properly during analysis.

TOC and TON were measured with a Hewlett-Packard model 185B carbon, hydrogen, nitrogen analyzer (CHN). Prior to analysis, standard curves for carbon and nitrogen were obtained using γ Cystine as the standard. The standardization was rechecked at regular intervals during sample analysis. In addition, blanks were run at regular intervals to ensure the proper operation of the CHN.

Samples were combusted at 1,000° C to 2,000° C in a sealed furnace for 50 seconds in the presence of an oxidizing catalyst. With helium as the carrier gas, the combustion products were passed through a gas chromatographic column and then by a thermocouple. The thermal conductivity was proportional to the concentration of carbon dioxide and nitrogen present in the sample. A Hewlett-Packard model 3371B integrator was used to determine the quantities of carbon and nitrogen present. Samples were run in triplicate (Menzel and Vaccaro, 1964; Gross, 1971; Sharp, 1973; Sharp, personal communication).

The average concentrations of organic carbon and organic nitrogen were determined from the replicates run for each sample. The percentage of organic carbon and organic nitrogen present in each sample were then calculated and reported.

3.3.2 Benthic Analysis: Dredge/Trawl Samples

Macrobenthic organisms for chemical analysis were stored frozen in noncontaminating containers or clean bags.

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FOIA b 7
FOIA b 7

Histopathology samples were processed onboard ship.

Taxonomy samples were also processed onboard ship whenever possible, or were stored and analyzed in MRI laboratories as described below.

3.3.2.1 Dredge/Trawl Benthic Chemical Analysis

Hydrocarbons

The frozen samples were thawed in the laboratory and a minimum of 12 individuals were pooled to create a single sample (Section 4.3.2.1). When tissue could be separated easily from hard parts (e.g., in bivalve molluscs), only the tissue was homogenized. For other sample types, notably Pagarus spp., the hermit crabs, and Asterias and Leptasterias, the starfish, the soft tissues were homogenized with some exoskeletal material. When separation of tissue and the large amount of exoskeletal material was impossible (as in E. parma, the sand dollar, and Strongylocentrotus, the sea urchin), the whole organisms were homogenized in a Waring blender or a Virtis homogenizer.

Figure 3-3 shows the analytical scheme. Briefly, after a sample aliquot was obtained for dry weight determination, the sample was combined with 0.5 N KOH/distilled water/methanol, and internal standards (cholestane, hexaethyl benzene) were added. The resultant mixture was refluxed for 4 hours, during which time saponification and digestion occurred simultaneously. The digestate was added to a separatory funnel, saturated NaCl was added, and the mixture was back-extracted 3 times with hexane. Concentration of the hexane extract caused the formation of a heavy gel in

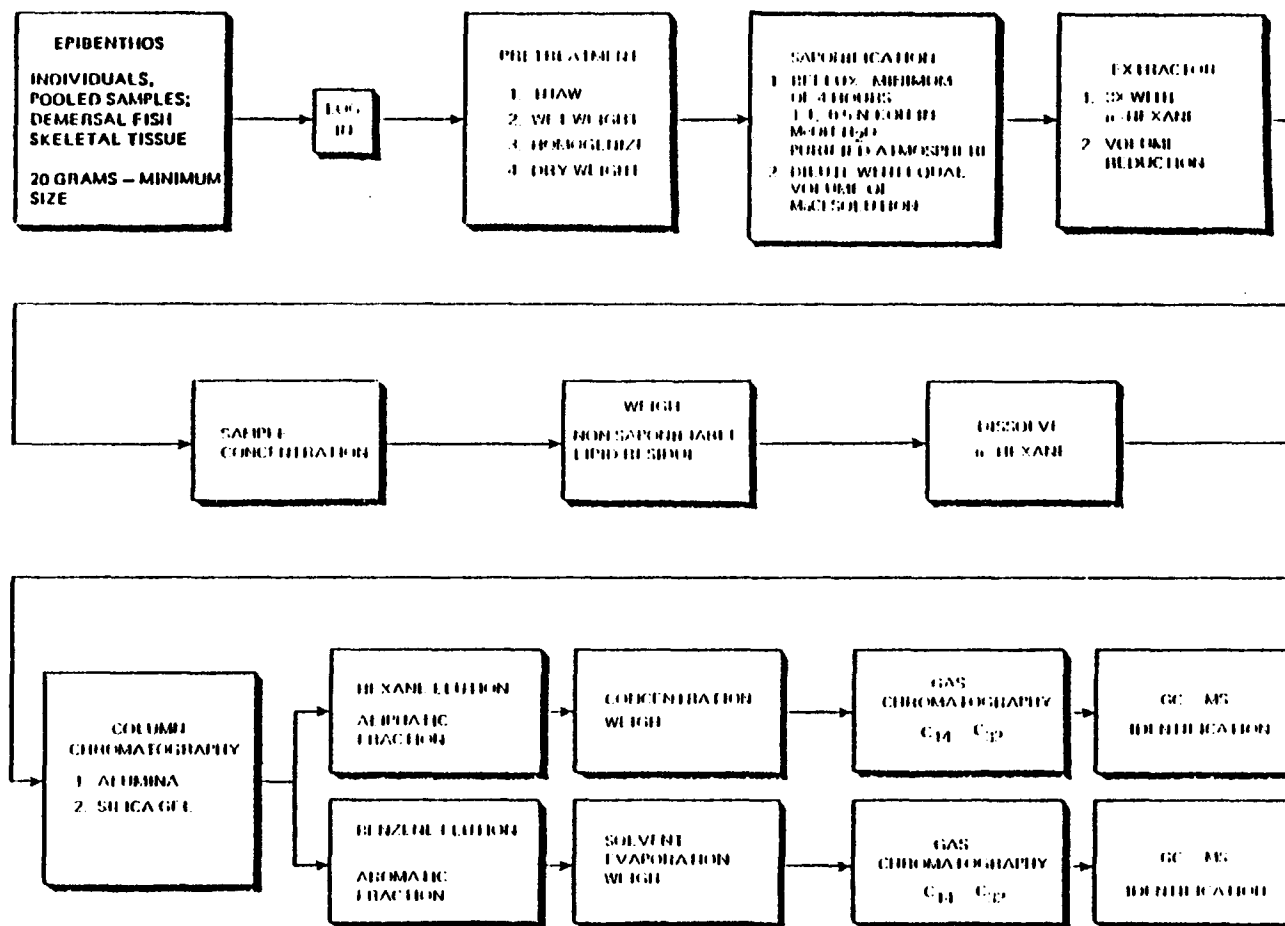


Figure 3-3. Hydrocarbon analysis of epibenthos.

most cases. This gel material was taken up in methylene chloride and charged to a 5 percent deactivated alumina column; the column was eluted with excess methylene chloride. Use of this column eliminated the gel problem.

An aliquot of the CHCl_2 eluate was weighed to determine nonsaponifiable lipids. A maximum of 50 mg of lipid material was charged to the alumina/silica gel column (Section 3.2.1.2) and fractionation and gas chromatography proceeded as before.

A significant problem encountered here was our inability to get a true tissue dry weight for all samples. When it was impossible to remove tissue from exoskeleton (e.g., E. parma), the dry weight includes skeletal material as well. This precludes a strict interspecies comparison of hydrocarbon concentration data but does not affect our ability to consider spatial and seasonal variations within a given species. If time permitted, a relatively simple study addressing the tissue weight contribution to organisms such as the sand dollar or the sea urchin could be performed.

Trace Metals

Macrobenthic invertebrates collected on Georges Bank were frozen on shipboard and transported to the University of Delaware for further processing and trace metal analyses. Prior to freezing, all whole organisms were rinsed in distilled isotonic ammonium formate. Generally, benthic invertebrates <100 g total wet weight were analyzed as whole organisms. Examples of such species collected on Georges Bank included Pagurus acadianus, Dichelopandalus sp., Asterias vulgaris, Ophiura sarsi, and visceral mass of Echinarachnius parma. All laboratory processing and AAS

analyses, including quality control procedures, were as described for zooplankton.

Individual organs were dissected out from macroinvertebrates that weighed more than about 100 g. Species typically dissected into individual organs included Arctica islandica, Placopecten magellanicus, and Buccinum undatum. Organs typically selected for trace metal analyses were gills, mantles, muscles, gonads, and digestive glands. Organs were dissected out (either onboard ship or in the trace metals laboratory), rinsed with distilled isotonic ammonium formate, and freeze-dried. They were dissected with acid-cleaned plastic ware. Plastic knives were sharpened to a razor-sharp edge. All individual organs were processed on Teflon surfaces within an ultra-clean laboratory (i.e., a clean lab modular van onboard ship and a trace metals laboratory onshore).

Dilutions for all trace metals were as indicated for zooplankton. However, dilutions for iron for most macroinvertebrate organs were frequently 11x or 12x. All other processing and trace metal analyses were as described for zooplankton analyses.

For a discussion of neutron activation analysis, see Section 3.2.1.3.

3.3.2.2 Dredge/Trawl Benthic Histopathologic Analysis

In the histopathology laboratory, the tissues were removed from the fixative and washed to remove excess fixative. The tissues were then dehydrated with a tissue-maton. The tissues were then cleaned and embedded in paraffin blocks. Each block was labelled with the reference

number of the tissue sample. The tissue blocks were then sectioned at 6 microns with a rotary microtome. After the tissues were cut, each block was sealed in paraffin and archived. Thin sections were applied to microscope slides, air-dried, and then strined with standard hematoxylin and eosin procedures. Slides were then coverslipped with Permount resin, and the slide was labelled with a reference number for the tissue sample.

3.3.2.3 Dredge/Trawl Benthic Taxonomic Analysis

The macrobenthos were presorted to the extent possible onboard ship. Those samples that could not be sorted onboard were taken to the laboratory for further analysis. All species were sorted to at least the family level, with many being sorted to the genus and species levels. Information was kept on life stage nad other physical descriptions as well. After identification all samples were properly labelled and archived in buffered preservatives appropriate to the taxonomic group.

3.3.3 Benthic Analysis: Dissolved Micronutrients, Dissolved Oxygen, and Salinity

See Section 3.2.7.

3.3.4 Benthic Analysis: CTD/O

Analysis of both benthic and water column station data followed the methods described in Section 3.2.8.

3.3.5 Benthic Analysis: Nephelometer

Data from the transmissometer-nephelometer were handled with other CTD/O data. Major analytical differences were that transmissometer/nephelometer data were not edited and were not scaled to physical units, and that no vertical sections or horizontal maps were produced. Primary responsibility for interpretation of these data lies with the USGS.

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