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First Annual Progress Report for the Period July 1 , 1984 - June 3 O, 1985

Chesapeake Bay Research and Submerged Aquatic Vegetation Initiatives, Virginia Institute of Marine Science

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CHESAPEAKE BAY RESEARCH AND SUBMERGED AQUATIC VEGETATION INITIATIVES

by the
Virginia Institute of Marine Science
of
The College of William and Mary

July 1985

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EXECUTIVE SUMMARY OF ACCOMPLISHMENTS

James River Seed Beds

Fifteen of our initiative research projects are directed toward understanding the decline of the uniquely valuable and once highly productive James River Oyster Seed Beds. These projects are distributed between biological (Projects 1A-C, 2A-G and 3) and physical (Project 4A-D) disciplines. The former are investigating the genetic structure and distribution of potential brood stocks, the effects of predation and fouling, and the effects of chlorinated sewage and subsequent dechlorination on spat settlement and survival. Physical studies are designed to improve understanding of the circulation of the lower James and to apply that knowledge to mechanisms of oyster larvae transport and settlement. The ultimate goal of these interdisciplinary efforts is provision of the knowledge required for a reasoned approach to restoration and management of this valued resource. Accomplishments to date include:

- Oysters from the up and downriver limits of the James River seed beds are genetically distinct, although there is evidence of some exchange of larvae. These local differences in genetic structure may be useful in further studies of brood stocks, mechanisms and patterns of larval transport and post settlement mortality.
- Spatfall monitoring, already reported on for 1984, is continuing in 1985 at 20 stations in the James River.
- Predation rates of the flatworm *Stylochus ellipticus* on James River oyster spat have been experimentally determined in the laboratory. Known as a predator of barnacles, *Stylochus* is shown by this study to be an effective predator of oyster spat as well.

- Predation of mud crabs, Panopeus herbstii, has been shown to vary from 0 in winter temperatures of 2.2°C to approximately 0.5 spat/crab/day in spring temperatures. Experiments are continuing to provide a complete annual cycle of temperatures. Also shown is a positive linear relationship between size of spat eaten and size of mud crab.
- Collections at 3 sites are continuing for an evaluation of the abundance of these predators on the James River Seed Beds in order to combine above-measured predation rates and prey abundance in an assessment of impact on spat populations.
- Studies of planted shell have shown a rapid progression of fouling with about 50% of initially clean shell surface covered after 4-5 weeks. In addition to detritus and sediment, fouling components include the fleshy bryozoan Bowerbankia gracilis and encrusting bryozoans. Barnacles and hydroids were unimportant as fouling organisms on shell planted during late July and August. Studies employing diver deployed and sampled plots are continuing.
- Although shells containing endolithic algae appear to obtain less initial set than algae-free shells, no statistical difference in sets can be demonstrated. Survival of spat on shells with and without algae also is similar.
- Although sewage/estuarine water mixtures variably affect attachment of oyster pediveligers (depending perhaps on particular incidence of toxics in sewage), in all laboratory experiments conducted dechlorination of chlorinated sewage resulted in dramatic improvement in settlement of larvae.
- Field studies near the Warwick River sewage treatment outfall showed no apparent effect on either oyster or barnacle settlement in 1984.

These observations are being refined and repeated for verification in 1985.

- Hydraulic model studies have been used to provide first order estimates of oyster larvae movements in the lower James River, ranking six historically important oyster rocks in terms of their probable contribution of larvae to seed beds.
- Combined use of a state-of-the-art dual frequency echo sounder and a CTD sensor (conductivity-temperature-depth) has permitted the identification of important features of circulation near Wreck Shoal and Newport News Point. These features (fronts and convergence zones) are expected to play an important role in the concentration and transport of oyster larvae.
- Newly-acquired current meters have been tested and deployed in the James River, providing data on tidal currents and net flows. Analysis of James River data showed conformity to the classical pattern for 2-layered estuaries.
- Comparisons of historical bathymetric data with those from 1984 show that oyster harvesting at the principal rocks has lowered the bottom by several feet.
- Side-scan sonar surveys designed to measure roughness (sand waves and/or ripples) of the bottom detected long furrows across oyster beds, attributed to ships straying from channels. Vertical relief of these furrows reached up to one meter.

Critical Finfish Stocks

- Climatological data necessary for development of larval finfish recruitment models and the preparation of Fishery Management Plans

(FMPs) have been added to VIMS existing data base, now embracing the years 1948 through 1984.

- Trawl survey data have been searched for errors, and corrections are underway.
- A total of 74 plankton collections, from which striped bass eggs and larvae are currently being sorted and identified, were obtained from the Pamunkey River during April-May 1985. Results will be used for estimates of total egg production and calculations of female striped bass biomass required to produce the observed egg deposition.
- Observations designed to explore the possible effects of acid rain on striped bass spawning success were completed with the use of recording pH meters and supplemented alkalinity measurements. Results are inconclusive, however, due to the lack of rainfall and drought conditions in April and May of 1985. Acidity never varied significantly from neutral (range of pH was 6.6 to 7.9), and alkalinity did not fall below 0.27 meq/liter.
- Preliminary observations on the viability of striped bass eggs showed a large percentage of non-viable eggs on the spawning grounds of the Pamunkey River. Subject to verification by examination of stained and preserved eggs, samples never contained more than 28.5% viable eggs (range 0-28.5%).

Toxics in the Environment

- Specialized instrumentation (mass spectrometers and high performance liquid chromatographs) have been installed and brought into operating condition. Tests are continuing to assure optimal procedures for examining metabolites of polynuclear aromatic hydrocarbons (PAHs).

Preliminary experiments have been undertaken using bile from grey trout and bluefish.

- Major efforts are currently directed toward improving reliability of measurements using the interface between liquid chromatograph and mass spectrometer.
- A computing system has been interfaced with analytical equipment employed in the Early Warning System for Pollutants in Seafood. Samples of bluefish, grey trout, croaker and spat have been processed since summer 1984 and are continuing to be collected. PCB concentrations in seafood appear to increase, as expected, through summer and early fall, and certain PCB congeners are more prevalent than others.

<u>Submerged Aquatic Vegetation (SAV)</u>

- Fifteen (15) acres of SAV were transplanted at 10 sites in 5 different river systems of the lower Chesapeake Bay, one-half with a slow-release fertilizer. Replicate sub-plots in each transplant site were established to assess survivorship.
- Survivorship for all sites, as of April 1985, was 74%. Excluding two York River sites (Mumfort Island and Clay Bank) with poor survivorship, this rate increases to 89%. Losses were due to early failure and ice scouring.
- In June 1985, several other sites exhibited poor survival: Healy Creek because of uprooting by ray or crab activity, Queens Creek unfertilized plots appeared to be washed out or covered by sand, and the Coan River site where oyster harvesting disrupted the beds.
- Fertilizer has had a significant effect on growth of the transplant units.

- Experimental seeding was begun in November 1984 and the first examination of test plots undertaken in April 1985. It appears that creating furrows along the bottom and use of large numbers of seeds will produce the best results. Seeds have been collected in spring 1985 for an expansion of this effort.

Early Projections of Needs Beyond the Current Biennium

Several of the Initiatives included in this annual report include either complex and difficult problems not readily solved in a two-year timeframe or monitoring programs that are designed to be long term. Cessation of funding on June 30, 1986, would preclude solution of the long-standing problems addressed and, for the most part, terminate critical monitoring of the health of Chesapeake Bay. A total of $1.3 million has been identified as necessary for these continuing efforts in the 1986-1988 biennium.

Oyster Section Studies

Introduction

The oyster fishery of the Chesapeake Bay is in a state of slow but consistent decline. One area pivotal to the maintenance and refurbishment of the fishery in the Commonwealth of Virginia is the seed oyster bed region of the James River. The program of oyster biology funded by the initiatives focussed on several, poorly documented subject areas related to maintenance of the seed oyster beds. The collective purpose of the subprojects within Projects 1 and 2 is to provide a better understanding of the biology of this important economic resource. This document reports on eleven such subprojects, describing their initial purpose, where applicable the methods employed in effecting research, and accomplishments to date. Several of these projects have been extensively modified since the work began in mid-1984. While many of these modifications have been already been reported to the Council on the Environment through a letter to Ms. J. Carter-Lovejoy, dated 3/27/85, they will be repeated here for the sake of clarity and completeness.

PROJECT 1

Composition and Distribution of Broodstocks

Introduction

There are three subprojects in this subject area. They address population genetics of oysters in the James, York and Rappahannock rivers, the monitoring of larval settlement and a comparison of sex ratios in oysters in the James River.

Project 1A

Population Genetics of Oysters in the James, York and Rappahannock Rivers

Purpose

In attempting to define which of the broodstocks of the lower James are the best producers it is necessary to investigate the possibility that there are genetically distinct populations of oysters in the James River, and to define to what extent observed differences are due to general environmental clines as observed in all estuaries through comparison with oysters in the York and Rappahannock Rivers.

Accomplishments

Using the technique of starch gel electropheresis we have conducted an examination of the genetic structure of two subpopulations of *C. virginica* within the James River, Virginia. Unlike other genetic studies of *C. virginica* within the Chesapeake Bay the two subpopulations we have examined represent extremes along an environmental/salinity gradient within the James River (Figure 1). The subpopulation at Nansemond Ridge (NAN) is located near the down river extreme and the subpopulation at Horsehead (HHD) is located near the upriver limit of *C. virginica's* range. The down river extent of *C. virginica's* range is restricted by oyster pathogens and predators common in high salinity waters. Starch gel electropheresis can be used to examine the degree of homozygosity or heterozygosity at specific enzyme loci. The enzyme loci examined were leucine aminopeptidase (Lap), phosphoglucomutase (Pgm), and phosphoglucose isomerase (Pgi). These enzymes were chosen because previous investigations have linked them with aspects of growth, metabolism, and environmental regimes.

The HHD and NAN subpopulations had differences in their heterozygosity levels and allele frequencies, especially at the Pgm and Lap loci. It appears that different alleles are preferred at each of the locations, indicating that the two subpopulations are genetically segregated. At the Pgm locus the predominant allele in the HHD subpopulation was "B" and in the NAN subpopulation it was "C". Allele "A" at the Pgm locus occurred at a much higher frequency in the HHD subpopulation than in the NAN subpopulation. At the Lap locus, allele "B" was predominant in both subpopulations; however, allele "C" occurred at a higher frequency at the NAN subpopulation.

At each of the three loci examined the HHD subpopulation was more heterozygous than the NAN subpopulation. Again, the differences in heterozygosity levels were greatest at the Lap and Pgm loci. Overall, the level of individuals heterozygous for one or more of the examined loci was 90% in the HHD subpopulation and 70% in the NAN subpopulation.

The observed differences between the NAN and HHD subpopulations in the James River are probably primarily a manifestation of different selection

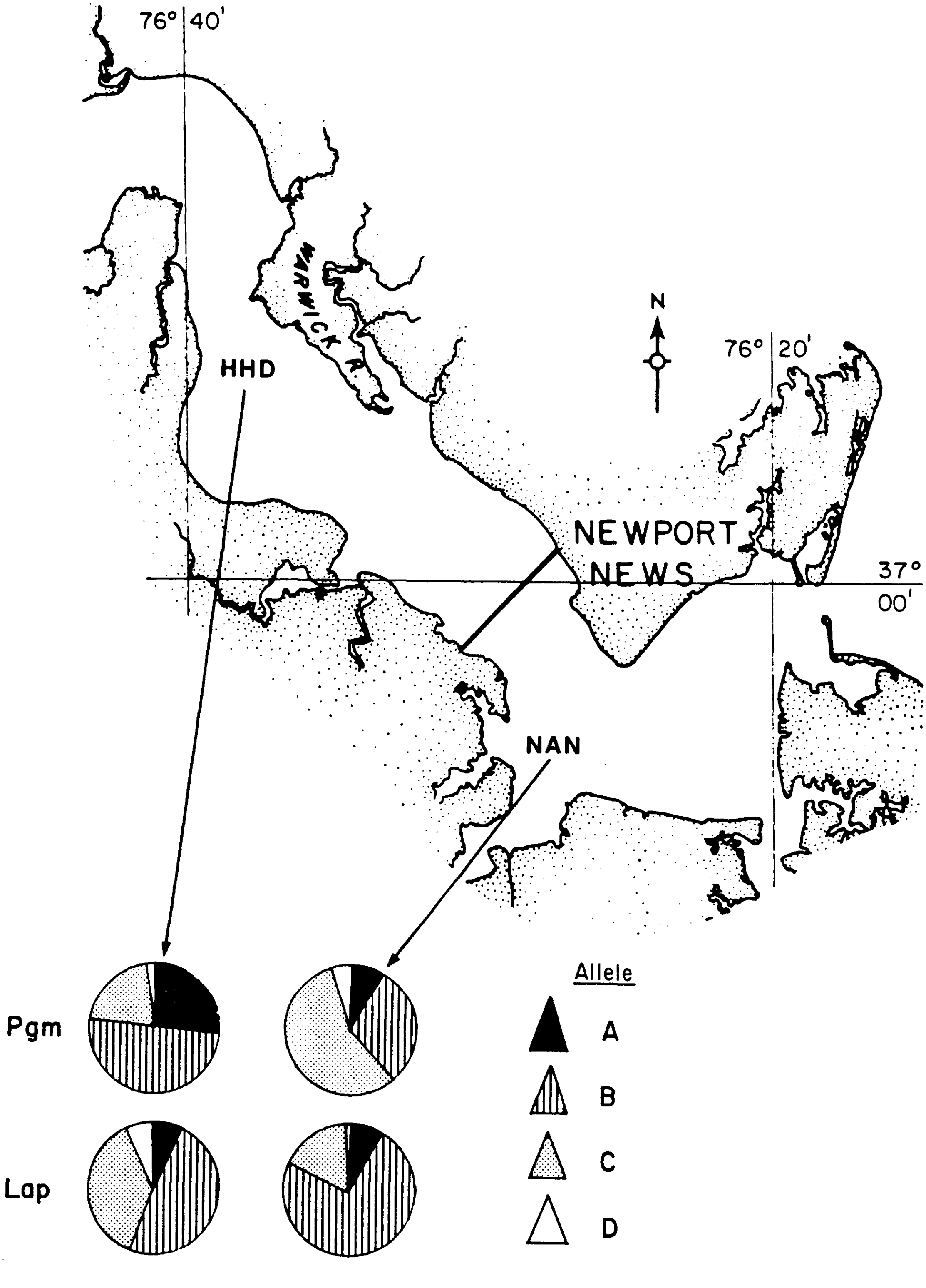


Figure 1. The lower James River, Virginia. Locations of subpopulations HHD and NAN, pie-charts illustrate the relative distribution of alleles for the Pgm and Lap loci.

pressures, buffered by minor levels of migration between the subpopulations. Though different alleles are seemingly preferred at each of the subpopulations, none of the alleles are exclusive to either subpopulation. Because C. virginica has a planktonic larval period and the studied subpopulations are geographically proximate, it is not unlikely that at least a slight exchange of larvae occurs between these two subpopulations. Genetically distinct subpopulations can be established as a combined response to selection pressures and migration levels among different subpopulations. According to E. P. Ruzecki (VIMS - see other contributions in this Annual Report), during the periods of maximum spatfall, water in the James River flows primarily upriver over the north shoals and primarily down river over the southern shoals. This pattern of water movement can create a nearly closed, cyclic water flow in which larvae may travel.

The apparent genetic fidelity of C. virginica to its local environment, as indicated by our results, can be utilized to provide insight regarding brood stocks, mechanisms and schemes of larval transport, and post settlement mortalities. Intensive genetic studies of genetically distinct subpopulations can be used to determine the subpopulation from which spat are derived. Also, the level of post-settlement mortalities caused by a winnowing of the subpopulation genome by local selection pressures can be determined. Hatchery reared stocks can be manipulated to produce genetically advantageous seed and natural brood stocks can be assessed for their ability to supply genetically compatible set to oyster rocks.

Continuing Effort

This project will be expanded to include an additional five sampling stations in the James River and three sampling stations in the York and Rappahannock Rivers. For enhanced resolution of the genetic structure of each subpopulation 12 loci will be examined for each sampling station. The resultant data will allow an evaluation of larval dispersal patterns, determination of advantageous genotypes for each of the sampling sites, and an estimate of post-settlement mortalities caused by genetic selection.

Project 1B

Monitoring of Oyster Larval Settlement

Purpose

Each year VIMS personnel effect a weekly survey of oyster spatfall at various locations throughout Virginia waters. The program begins in June and proceeds through October. Spatfall is quantified by counting numbers of newly settled oysters on previously prepared "strings" of oyster shells. The purpose of this section of the Initiative funded research was to expand the number of shellstring monitoring stations in the James River and thereby provide a more comprehensive description of temporal and spatial variability in oyster settlement in the James.

Accomplishments

Due to the obvious seasonal nature of the field component of this program, work on the 1985 field program has, at the time of writing, only just begun. Data for 1984 field collections has been reported previously in the third quarterly report for the 1984-85 funding period. A brief summary of that report is provided below.

In the James River thirteen monitoring stations are usually occupied. These are at Deepwater Shoal, Horsehead Bar, Mulberry Swash, Point of Shoal, Wreck Shoals (2 stations), Days Point, White Shoals, Miles W. H., Brown Shoals, Naseway Shoal, Ridge and Hampton Flats. Seven additional stations were occupied for the initiative funded study. These were at Long Rock, East End, Dry Shoal, Rock Wharf Shoal, Dog Shoal, Horne Brothers and Cruiser Shoal.

The stations were sited to create four transects. The transects run over important shoals, and all of the stations are either on or near important oyster shoals except the Horne Brothers station and the Hampton Flats station. The Horne Brothers station is near deep water but not adjacent to an oyster shoal. The Hampton Flats station is on an abandoned oyster ground which has been referred to as a former "brood stock area". All of the monitoring station sites were located in reference to long-term setting success, current patterns, former oyster plantings and the mixing of water masses.

For comparisons between years, the spatfall potential is reported for standard weeks in a standard calendar. The collections are made at each new quarter of the moon except on weekends. Lunar collection periods are assigned to a standard week which has four or more days in common with the lunar period by a computer program. If the collection period has ten or more days, the program splits the data into two standard weeks.

Spatfall potential is reported as spatfall per shell per week, and the rates are based upon a seven-day week. A grouping of data is seen for stations with catch rates of one spat per shell per week and higher. With the exception of the Deepwater Shoal, Ridge, Miles W. H., and Hampton Flats, the stations reach this catch rate in the seventh and eighth week and drop below this catch rate in the fourteenth and fifteenth week. Several of the stations dip below this level and recover around the first of August. The peak of setting on seventeen stations is the twelfth and thirteenth week, while at Horne Brothers, Cruiser Shoal and Hampton Flats the peak of setting is the eleventh and twelfth week. During this nine-week period extending from the last two weeks of July through the first week of September, over ninety-eight percent of the setting on shellstrings occurs.

On July 13, 1984, the temperature had reached 24.5°C on the bottom downriver and was 1-2.5° higher upriver. On September 10th the temperature had decreased to 24.0°C ± 0.2 on the bottom on those stations where it was measured, but bad weather prevented collections downriver of White Shoals. The salinities were below average during the summer, and were possibly related to the fresh water influence which had extended very late into the spring. For example, on April 26th, the surface and bottom salinities at Horsehead Bar was 1.0 ‰ and salinity at the beginning of the ebb on Wreck Shoals was 2.0 ‰ on the surface and 6.8 ‰ on the bottom. On August 20th and September 4th, the salinities at Deepwater Shoal were still too low for oysters to feed normally. August 20th fell in the middle of the twelfth week, which was the peak of setting.

Normally the mean salinity at Wreck Shoals in the spring is 7.0 ‰ and the mean salinity in the summer is 14.0 ‰. The salinity on the bottom at Wreck Shoals fell below the mean on July 20th and did not reach the mean again during the summer. The salinities upriver at Deepwater Shoal on August 20th and September 4th prevented spawning. Although 1984 was below the average for setting in the 1980's, it would have been a good year in the 1970's, except for the Deepwater Shoal station. The set on shellstrings at Deepwater Shoal was close to the typical set at this station in the 1970's. The extended period of freshets and the low salinities during August lowered the level of set in the James River in 1984.

Project 1C

Sex Ratios in Oysters

Purpose

Individual oysters have the ability to change their sex. The general dogma is that the majority of oysters mature first as males before changing to females. Clearly, an imbalance between males and females in a population will hinder the reproductive potential of that population. The purpose of this project is to examine sex ratio in oysters from the James River for the presence of an "imbalance" and further compare these data with those collected from archived specimens from the same location.

Accomplishments

Due to increased effort on field programs in Spring 1985 there has been no further effort on this project during the fourth quarter of the 1984-85 funding. The status of the project remains as described in the third quarterly report. We do not anticipate further new data collection on this project in 1985.

PROJECT 2

Effect of Predators and Fouling on Spat Settlement and Survival

Introduction

A possible cause of the decline in lower James River seed oysters is an increase in abundance of fouling organisms and/or predators. It is also possible that predators or fouling, while not the cause of the decline, could be preventing a recovery by the seed oyster. This project aims to determine the seasonal abundance of predators of oyster spat in the lower James River seedbeds and, in the laboratory, to examine their selection of prey and rates of predation; to measure the seasonal abundance of fouling organisms in the field and determine, in the laboratory, their effects on spatfall; and to determine whether blue-green algae living under the surface of oyster shells reduces success of spatfall.

PROJECT 2A

Predation on Oysters by the Flatworm *Stylochus ellipticus*

This project, as with projects 2B and 2C, is a laboratory study of predator-prey interactions.

Purpose

This study was undertaken to determine the potential magnitude of predation by *Stylochus ellipticus* (Girard) on oyster spat in the James River. *S. ellipticus* is found associated with oysters and barnacles in the Chesapeake Bay. Although its prey preference in the James River is as yet undetermined, *S. ellipticus* is known to be a predator on barnacles. The distribution of *S. ellipticus* in the James River has been correlated with the distribution of the barnacle *Balanus improvisus*; however, this barnacle is also the most abundant on oyster reefs in the James River. *S. ellipticus* has highest abundance on Wreck Shoal, the most commercially productive oyster reef in the James River.

Methods

S. ellipticus were collected from oyster shells from James River Inshore Wreck Shoal dredgings. Dredged oyster shells were placed in river water-filled buckets at room temperature (~28°C) for several days, allowing oxygen tension to fall to near zero. Flatworms migrating to the surface were collected and transferred to a tank containing laboratory-reared oyster spat in well-aerated, circulating, 50 μm-filtered York River water. Numerous boxes (gaping spat, hinges attached and meat missing) were noted after several days, indicating feeding by the flatworms prior to the start of Experiment I. The flatworms were starved for one week prior to the beginning of Experiment II.

Experiments I and II used different apparatus and protocols. Apparatus for Experiment I consisted of three cylindrical plexiglass tanks, two experimental and one control. Fifty oyster spat were collected from the James River, soaked in a saturated brine solution to remove flatworms and placed into each tank. In this experiment, barnacles were present on the shells, but were not counted or closely examined. The location of each spat on the shell substrate was noted and each was measured for maximum length. Twenty *S. ellipticus* were placed in each of the two experimental tanks, none in the control tank. One-half volume of filtered (50 μm), York River water was changed daily and circulation was maintained with an air-lift placed in the center of each tank. Ambient water temperature was maintained as it was considered desirable to investigate predation rates relative to seasonal conditions. Each tank was wrapped with dark plastic to reduce incident light and nearby windows in the laboratory were covered with black plastic to preclude solar heating.

Experiment II was conducted using flowing, 50 µm-filtered York River water. Twelve tanks (9 experimental and 3 controls) consisting of 1 gal. glass jars closed with 500 µm mesh nylon screening were used. Ambient temperature water was introduced to the bottom of each tank through plastic tubing inserted in the center of the jar mouth and sealed to the screen with silicon aquarium sealant. The screening was sealed by force-fitting plastic rings over the screen around the jar mouth. Flatworms were collected as in Experiment I, but were starved for one week prior to introducing into the experimental tanks. Spat were laboratory spawned and reared, and set on clean oyster shell. Shells were sorted into three groups according to the size of spat on them. One of each size-group was introduced into four of the tanks. Thus there were 4 tanks with each size group, 3 in which were placed 5 flatworms each with the fourth serving as a control.

The sizes of spat in each of the three groups were:

Group A - mean = 7.8 mm (± 2.95 S.D.), range = 3.0-13.4 mm

Group B - mean =13.3 mm (± 4.05 S.D.), range = 6.0-20.0 mm

Group C - mean = 5.7 mm (± 1.58 S.D.), range = 3.0-8.7 mm

Group C was discontinued because excessive mortality in the control indicated that the spat were not especially viable. Another indication that the spat in Group C were not viable was their small size in relation to the other groups which were the same age.

After the introduction of S. ellipticus into the experimental tanks the spat were counted and measured at 5-7 day intervals. Boxes were noted as was the presence of proximal flatworms when observed. Water temperature and salinity was measured and recorded approximately every other day. Upon termination of the experiment, water was removed from the experimental tanks by siphoning through a fine mesh screen, leaving just enough to cover the shells. The tanks were then moved to a warm location and the oxygen tension allowed to decrease to near zero, facilitating retrieval of flatworms.

Predation rates were computed as:

$$M_p = S/P_t/D$$

where S=the number of boxes, P_t=the number of flatworms present at day t, and D=the number of days.

P_t was extrapolated from the number of flatworms remaining in the tank at the end of the experimental period and was computed by:

$$P_t = [(D_f - D_t)/D_f](P_i - P_f) + P_f$$

where P_t = flatworms remaining at time t, i = initial, and f = final. This calculation assumes that S. elliptícus mortality was occurring at a constant rate.

Accomplishments

The results of the described experiments are summarized in Table 1.

Table 1. Predation rates and environmental conditions of S. ellipticus predation on oyster spat in the laboratory (rates are spat/Stylochus/day).

Experiment Number	Mean Prey Size (mm)	Salinity $^0/oo$	Temperature °C	Predation Rate-mean	Predation Rate-range
I	13.9	20.7	9.2	0.008	0.0-0.023
IIA	7.8	18.4	10.1	0.012	0.0-0.078
IIB	13.3	18.4	10.1	--	--

Predation occurred at a higher rate in Experiment IIA than in Experiment I and none occurred in Experiment IIB. There was no mortality of oyster spat in the controls of Experiments I, IIA, and IIB. Excessive mortality of oyster spat in Experiment IIC control indicated that these spat were not viable, so it was discontinued. In both experiments where predation occurred there was high variability in the mean predation rate (the standard deviation equalled the mean rate in both cases). Therefore, these rates cannot be judged statistically significant and these data should be considered preliminary. In Experiment I and IIA the mean size of prey eaten was approximately equal to the mean size of the prey presented (Table 2).

Table 2. Sizes of oyster spat presented to and eaten by S. *ellipticus*.

Experiment Number	Mean spat size (mm)	S. D. (mm)	Range (mm)
I			
Presented	13.9	6.73	5.1-30.6
Eaten	13.1	4.54	8.6-20.6
IIA			
Presented	7.7	2.56	3.0-13.4
Eaten	7.8	2.95	4.7-13.4
IIB			
Presented	13.3	4.05	6.1-20.0
Eaten	—	—	—

Experiment II was conducted at higher ambient water temperatures and showed a three-fold increase in the maximum rate of predation over Experiment I and a 50% increase in the mean predation rate (Table 1). During Experiment I the ambient water temperature decreased from 11.0°C at the beginning to 8.3°C at termination. Experiment II experienced a temperature increase from 8.3°C to 14.8°C. Changes in mean predation rates during the experimental periods (data not shown) were closely associated with changes in water temperature.

These experiments demonstrate that *Stylochus ellipticus* is a predator on James River oyster spat. Both starved adult and newly metamorphosed *S. ellipticus* are present during the season of setting of oyster spat. More than one *S. ellipticus* may feed on one oyster spat so experiments to determine predation rates should be conducted with no more than a single predator per treatment. It is suggested predation increases with increasing water temperature. It is still not known whether some James River S. ellipticus are ingestively conditioned to eat only barnacles or oysters or both. But, because both barnacles and oysters are present in the river at the time of metamorphosis of the flatworm larvae, it is probable that they are conditioned to eat both.

Further experiments are now underway to determine the rate of predation on oyster spat by individual S. ellipticus. These experiments are designed to determine the rates of predation relative to the density of the prey as well. This will allow the calculation of a functional response curve for the species and facilitate comparisons with other potential oyster predators.

This work was presented at the Annual General Meeting of the National Shellfisheries Association (AGM-NSA) in Norfolk in June 1985 and stirred considerable interest in both academic and industry groups. Comparable studies are now planned in Maryland by University of Maryland Researchers.

Project 2B

Predation on Oysters by the Mud Crab Paropeus herbstii.

Purpose

Mud crabs are widespread and abundant members of the James River benthic community. They are known to prey on oysters but quantitative data on predation rate and predator-prey size interactions are lacking. the purpose of this project was to quantitatively assess the potential impact of crab predation on oysters in the James River.

Accomplishments:

Three experiment have been performed to determine the rates of predation by the mud crab, Panopeus herbstii, on oyster spat. Experiments 1 and 2 where run sequentially using the same crabs. Conditions in the two experiments were identical except for the water temperatures and salinities. The crabs used in these experiments were grouped according to size (carapace width): 2 small (~14 mm); 2 medium (~16 mm); and 2 large (~25-30 mm).

During Experiment 1 (2/11-2/19 1985) the ambient water temperature averaged 2.2°C and salinity averaged 18.4 ^{o}/oo. There were no spat mortalities. It is likely that mud crabs do not feed during winter low temperature periods.

Experiment 2 was a continuation of Experiment 1 and was effected in a period of rapidly warming water conditions. Predation began occurring at ~ 9°C and the data was split at this point to reflect the differences in feeding at different water temperatures. Predation rates ranged from 0 to 0.03 spat/crab/day, and averaged 0.02 (± 0.017 S.D.) spat/crab/day from the period 25 February through 24 April, 1985. The water temperature rose during this time from 9°C to 20.6°C, and averaged 11.5°C (± 4.38 S.D.). There was a positive linear relationship between the size of spat eaten and crab carapace width (spat size (mm) = 0.72 x crab carapace width (mm) - 6.617, r = 0.95). There were no oyster mortalities in the controls (i.e. those without predators).

Experiment 3 was conducted from 15-30 May. Ambient water temperatures averaged 21.4°C (± 1.02 S.D.) and salinities, 20.8 ^{o}/oo (± 1.30 S.D.). Crabs of approximately the same size, mean carapace width 24.9 mm (± 1.91 mm

S.D.), were used because in the previous experiments major predation occurred among the larger crabs. Since the main thrust of these experiments is to quantify the potential predation on spat, the larger crabs should provide estimates of the maximum predation rates. Predation rates ranged between 0 and 0.56 spat/crab/day. The average predation rate was 0.36 spat/crab/day ($\pm$ 0.456 S.D.). No mortalities occurred in the controls.

These experiments are still continuing at the time of writing in order to complete a complete annual cycle of temperature change (as can be seen temperature influences predation considerably). These experiments have already clearly demonstrated that mud crabs can be significant predators. Completion of an annual cycle of experiments will allow us to gather a significant body of quantitative information on this subject.

Project 2C

Predation on Oysters by the Snail *Odostomia impressa*.

Purpose

The snail *Odostomia impressa* is, like the mud crab, widespread and abundant in the James, especially in areas of oyster reef. *Odostomia* is considered a predator upon oysters. The purpose of this project is to provide quantitative information on predation by *Odostomia impressa* on oysters in the James River.

Accomplishments

Due to (1) focussed efforts on *Stylochus* and *Panopeus* and (2) limited availability of wet laboratory space (now being rectified) efforts involving

Odostomia have been limited. We plan to increase efforts in this area in 1985-86 as efforts with Stylochus and Panopeus are being concluded. We are maintaining close contact with workers at Texas A&M University who are actively examining the influence of Odostomia on oysters. These data will provide much useful background and guidance in our own planned studies.

PROJECT 2D

Seasonal Changes in Abundance in the Field of Predators Noted in 1 A-C Above

Purpose

To provide a complimentary data set to projects A-C in order to subsequently provide a basis for calculation of impact of the three predators; Stylochus, Panopeus and Odostomia, on the James River seed bed area.

The methods used in this study have been described in previous reports and will not be repeated here. The original project plan was to sample three sites (Wreck Shoal, Point of Shoals, Thomas Rock) at regular intervals for a two year period. In March of 1985 it became evident that if this sampling program were to remain intact then even a full time effort by all of the personnel working on initiative studies would still be insufficient to process and sort samples. The program was subsequently revised to examination of one site (Wreck Shoal) for one year (July 84 - July 85).

Accomplishments

Wreck Shoal Offshore was sampled at 2-week intervals between August 9 and December 11, 1984 and at approximately monthly intervals since then; except that samples were not collected in January or June 1985.

Seventy-two samples have been collected. Each sample has been separated into three size fractions (4000 µm, 2000 µm, and 500 µm). To this date all fractions have been examined for 27 of the samples - including all samples collected in August and September. The larger two fractions have been examined in 25 other samples.

The delay in completion of these examinations is due to the extremely long time required to examine the 500 µm fraction. Between 25 and 40 hours are needed to examine one of those fractions depending on the volume of material involved. Our attempts to develop a sample splitter for that fraction have not so far been successful. Until a dependable sample splitter is developed we will continue to examine the whole 500 µm fraction. With or without the splitter, completion of the examinations is assured by December 1985.

In view of the incompleteness of our sample examinations we cannot advance much definitive information at this time. The following outstanding facts have been obtained from the data at hand:

(a) Mud crabs (*Panopeus*) and blue crabs (*Callinectes*) have been almost completely absent in our samples. This may indicate that the suction sampler used is not adequate for sampling those species.

(b) The number of snails in one square meter of bottom ranged between 89 and 4054 in August and September 1984. The number in most of the samples, however, was around 2000 snails. At least 95% of these snails are *Odostomia impressa*.

(c) The number of flatworms (Stylochus) in one square meter of bottom ranged between 89 and 911 in August and September 1984. Most of the samples, however, had between 200 and 300 flatworms.

Project 2E

Seasonal Abundance and Size Class Distribution of Oysters

Purpose

The intended purpose of this project was to relate oyster size distribution and density to predator size, numbers and density; however, the sampling schedule and design does not give an adequate measure of predation rate per se. Thus, although this data set is "free" given that it uses the same sample as in 2D it is really limited to a survey of size and abundance. The purpose, therefore, has been refocussed on the questions (i) What sets? and (ii) What survives from that set nine months later and prior to the seed transplanting season? This objective is met by examining only early settlement stages at the beginning of the study (August - September 1984) and those surviving June-July 1985.

Accomplishments

Sorting of samples from the 1984 collections is nearing completion. 1985 samples are still being collected. The number of spat 2 cm or less in height in one square meter of bottom ranged from 520 to 5108 in samples collected in August and September 1984. In most of the samples, however, the number fluctuated between 1000 and 2000 spat. In August most of the spat were 2 mm or less while in September most of them were evenly distributed in the 3-5 mm range.

Project 2F

Fouling of Shell Substrate

Purpose

To examine the rate or extent of fouling progression on planted oyster shell and the relationship between fouling and density of oyster spat settlement.

Each summer, the Virginia Marine Resources Commission (VMRC) deposits oyster shells on public grounds in the James River. The objective of this plant is to provide a large amount of suitable substrate on which oyster larvae can attach (set) and grow to commercial seed market size. Last year (1984), 263,000 bushels of oyster shells were planted in the James River seed area at a cost of approximately $140,000.

The timing of the plant is thought to be crucial in determining the success of oyster set. The conventional wisdom is that planting shells just prior to the period of maximum set minimizes fouling on the shells, and hence reduces competition for space between oyster larvae and fouling organisms and material; however, no quantitative data has ever been published concerning the rate or extent of fouling on oyster shells and the relationship between fouling and the density of spat set. It is evident that information of this sort is vital if spat set on shell planting is to be maximized.

Accomplishments

Experiment #1

The results of a fouling study conducted during the summer of 1984 are herein presented. A group of shells (approx. 1500 shells) were deposited each week, beginning on July 26, through the summer. Each shell group was sampled at successive weekly intervals and preserved for later examination. Shells were examined under a dissecting scope using a point sampling technique utilized by other researchers studying fouling on artificial substrates. The examination yielded a coverage value for each component (both biotic and abiotic). Fouling organisms, decaying organic debris, earthy material, and any component that might inhabit spat set were recorded as contributing to fouling.

Fouling progresses rapidly in that after four to five weeks on the bottom, an initially clean shell becomes fouled over 50% of its surface (Figure 2). Note that the fouling depicted in Figure 2 is artificially low since it assumes that both shell surfaces are exposed. In actuality, because the shells lay on the bottom, less than 100% of the shell surfae area is exposed to fouling.

Within about three weeks after planting, the most important fouling components found on the shells are abiotic (decaying organic debris and earthy material), and within 4-5 weeks 20-30% of the shell surface is covered by these abiotic components. Important biotic fouling components include the fleshy bryozoan *Bowerbankia gracilis* (covers 10-15% within 4-5 weeks) and the encrusting bryozoans *Electra crustulenta* and *Membranipora tenuis* (covers 10-15% within 4-5 weeks). Barnacles and hydroids, generally considered to be major foulers on oyster shells, are unimportant on shells planted during late July and August, when this study was conducted.

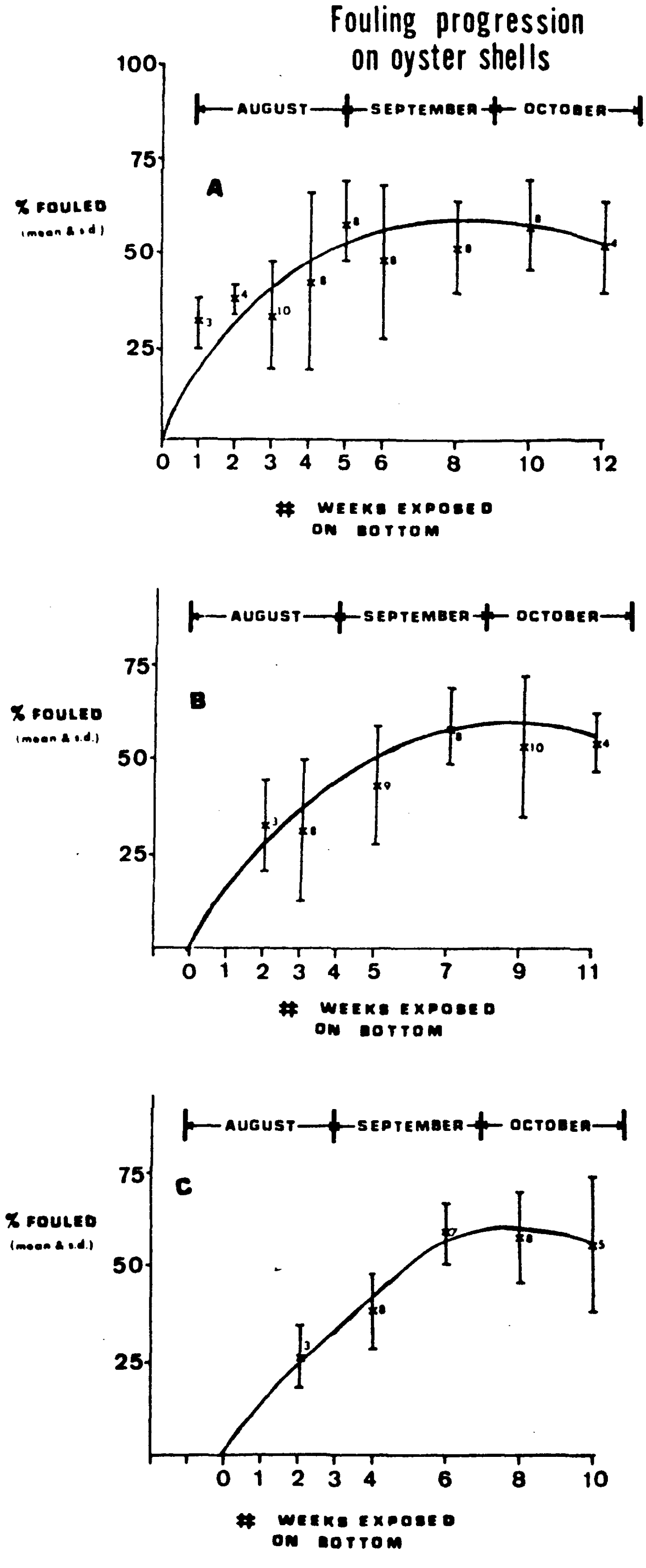


Figure 2. Fouling progression on oyster shells planted at one week intervals. Bars depict mean and 1 standard deviation of shell samples. Coverages on both sides of shells are combined. The curve is fit by eye. A) Shows fouling progression on shells planted on July 26, B) shows fouling progression on shells planted on August 2, and C) shows fouling progression on shells planted on August 9.

Sedimentation may also be important with respect to inhabiting spat set, but its effect could not be determined.

The results of this study indicate that fouling on oyster shells progresses rapidly in August and probably covers most of a shell's exposed surface within 4-5 weeks; however, the relationship between fouling and the inhibition of set is not yet clear. Perhaps, there is no significant difference in set between shells fouled on 50% of their surface and those fouled on 20%, since oyster larvae are capable of searching for an acceptable place to set and metamorphose.

This work was presented at the A.G.M. of the National Shellfisheries Association in Norfolk in June 1985 and was very well received.

Experiment #2

In an attempt to overcome difficulties in operation and inadequacies in experimental design encountered in experiment #1 a second study has been initiated. Basically, this uses diver deployed and serviced plots on an area of Wreck Shoal marked with a underwater grid system of stakes and line. The plots were filled with clear, marked shells in a specific concentration on dates in May, June and July '85. On a continuing time sequence shells were reclaimed and will continue to be so until the field component of the study finishes in September 1985. Shells are collected by divers in such a manner as to minimize disturbance of the fouling community. Once a plot is sampled it is not sampled again because, by definition, it has already been disturbed (and disturbance is a considerable complicating problem in data analysis). Laboratory examination of the collected material will be as for material in experiment #1. The anticipated results from this study should give a clear quantitative description of the sequence and magnitude of fouling for temporally separate shell planting and thereby provide valuable guidelines for future shell plantings as practiced on an annual basis, by the Virginia Marine Resources Commission.

Project 2G

Influence of Endolithic Algae on Oyster Settlement and Early Post Settlement Survival

Purpose

To quantify the influence (positive or negative) of endolithic algae on oyster settlement, early post settlement growth and survival.

Until recently, the distribution of James River endolithic algae has never been investigated, nor have endolithics been quantified or studied in terms of their possible impact on oyster set intensity, growth, or spat mortality. Studies conducted during 1983 and 1984 have shown the presence of endolithic algae living in oyster shells at selected sites throughout the Lower James River, Virginia. It is uncertain if endolithic algae inhibits or enhances spat set. Endolithic algae live in natural crevices of shells or bore into the carbonate structures. Boring into shells is accomplished by the dissolution of carbonate with carbonic acid secretions. Eventually, the algae spread horizontally within the shell, forming a dense mat or network of intertwined algal filaments. This network of borings weakens the structure of shells and enhances the natural dissolution of shell carbonate by seawater. This project is examining the relation between endolithic algae and oyster spat. It is speculated that polysaccharides secreted from or contained within the sheaths of the endolithic algae may provide a desirable substrate for fouling organisms and/or spat to settle. On the other hand, endolithic algae could inhibit spat set due to some chemical interaction or to physical deformation of the shell surface by algal borings. Two specific objectives are being examined. These are (1) to determine the distribution of boring endolithic algae in the Lower James River as a possible function of depth and salinity during the spring, summer and fall seasons, and (2) to evaluate the possible impact of the presence or absence of endolithic algae on setting of oyster spat in the Lower James River, Virginia.

Methods

I. Endolithic Algae Distribution on Oyster Shell in Relation to Depth and Salinity

To ascertain the distribution of endolithic algae in relation to salinity, oyster cultch was dredged from four stations in Lower James River during the spring and fall seasons. These stations are Deep Water Shoal, Wreck Shoal Offshore, Naseway Shoal, and Nansemond Ridge. The distribution of endolithic algae in relation to depth was investigated by dredging samples from four different depths at Wreck Shoal Inshore and Offshore during the summer season. Analysis of each sample involved: (1) choosing ten shells at random from each dredge sample; (2) removing all epilithic organisms from both shell surfaces; (3) treating each shell with EDTA to expose the algal mat; (4) preparing slides for enumeration and species composition; and (5) removing all algae from the outer surface of each shell to estimate the abundance of endolithic algae per cm^2 of shell surface via chlorophyll extraction technique.

II. Impact of Endolithic Algae Growing in Oyster Shells on Setting Intensity, Growth, and Spat Survival

A dredge sample was obtained from Wreck Shoal Offshore and eighty shells were chosen according to present criteria (whole shells 5-6 cm^2 long; minimal erosion of surface and minimal macrofouling by barnacles). Ten shells were analyzed for chlorophyll content and algal species composition; the remainder were made into fourteen shellstrings. The shells of the first set of seven shellstrings were scrubbed to remove macrofouling, leaving only live endolithic algae. The shells of the second set of seven shellstrings were boiled and scrubbed to kill all epilithic and endolithic organisms. The third series of seven shellstrings was made up of dried shell which was scrubbed to remove any dried macrofouling. All shellstrings were placed at Wreck Shoal Inshore and allowed to obtain a set of oyster spat for a period of two weeks. After the two-week exposure period, the shellstrings were removed from the river, dried, and the number of spat per cm^2 of shell surface was determined. This procedure was repeated for each of the three field experiments.

A second series of three experiments involved exposing shell of the same three treatments to laboratory-spawned larvae for a twenty-four hour period to obtain a set. The shells were then placed in flowing filtered (50 μm) seawater; mortality and growth of the spat were evaluated on a weekly basis. For each experiment a representative ten shell sample was analyzed for chlorophyll content and algal species composition.

<u>Accomplishments</u>

Data analysis for both field and laboratory studies is almost complete for chlorophyll analysis (to determine algal density) and spat settlement. Work on species composition within the algal mat is continuing.

Analysis of the data describing initial settlement in the field from both inside and outside shell surfaces indicated that shells containing live endolithic algae obtained less initial set than did shell in which algae were killed. This initial set data of Experiments 1 and 2 was analyzed using an analysis of variance (ANOVA) test and indicate that endolithic algae does not affect spatset. A borderline significant difference ($P = 0.0134$) in spatset between treatments was seen using ANOVA testing in the first experiment; however, the differences were so slight that the alternative analysis technique of multiple range tests showed no difference between treatment means.

Analysis of field collected data on percent survival on both shell surfaces indicates that spat on shell containing live endolithic algae have similar survival rates to those on shells with killed algae. These data suggest that endolithic algae do not affect survival of oyster spat.

In each of the three laboratory experiments initial set appeared to be lower on shells containing live endolithic algae than on shells without. All three experiments, being conducted under the same conditions, were combined and statistically analyzed using a randomized complete block (RCB) design ($P = 0.05$). The RCB design allowed for an increase in sample size in addition to an increase in the power of the statistical test. This test indicated that no differences in spat set existed between treatments. To substantiate that the use of the RCB design was justified, an analysis of variance (ANOVA) test was performed on the initial set data for each experiment separately. No statistical difference was found between treatments in any of the three experiments, proving that the RCB design was justified.

No clear pattern was evident in the magnitude of percent survival values between treatments in laboratory experiments. Spat on shell without algae had higher percent survival values than those on shell with algae in the first two experiments, with the opposite being the case in the third experiment. An analysis of variance test was performed on the percent survival data for each experiment separately. The analysis suggests that endolithic algae do not affect survival of oyster spat.

The analysis of variance tests performed on percent survival and initial set data for the field studies failed to find any statistical difference between treatments; i.e. endolithic algae do not appear to impact spatset or survival. It is true that this in fact may be the case, but it is also possible that a relationship between endolithic algae and oyster spat might be masked by the interaction of many uncontrolled and/or unidentified environmental factors. The investigation of a possible impact of endolithic algae on spat conducted in the field has been useful in obtaining an estimate of the type and amount of variability involved; however, due to the presence of many extraneous environmental factors, further experiments will be confined to the laboratory.

Statistical analysis of data generated during the laboratory experiments also showed little or no difference in spat set, growth and survival between treatments. The inability to show a difference in the present studies may be due to small sample sizes in addition to uncontrolled and unmeasured variability. Laboratory experiments proposed for the 1985 oyster setting season will be conducted under more controlled conditions to reduce possible variability stemming from experimental design. The proposed experiments will measure the inherent variability in set due to normal larval setting behavior by replacing the dry shell treatment of 1984 experiments with ground glass plates as substrates in 1985 experiments.

Acknowledgements

The material reported here are the collective efforts of various individuals working in the oyster section of the Department of Fisheries at the Virginia Institute of Marine Science under the direction of Dr. R. Mann. Those contributing individuals are, in alphabetical order R. Blaylock, C. Cox, D. Eggleston, Y. K. Hsu, S. Maurer, K. McCarthy, R. Morales-Alamo, E. Pafford, R. Rheinhardt, K. Walker and J. Whitcomb.

PROJECT 3

Impact of Chlorinated Sewage on Oyster Spatfall and Oyster Spat in the James River

Purpose

Larvae are particularly sensitive stages in the life history of the oyster. Unlike the adult, the larvae cannot isolate themselves from the environment even temporarily by closing their shell. Larvae are therefore vulnerable to negative effects following exposure to numerous substances. One might expect that larvae, during the period when they attach to the bottom and undergo a distinct metamorphosis, would be especially vulnerable. The value of the James River seed beds rests to a significant degree on the success or failure of this phase in the life cycle of the oyster.

Traditional wisdom has linked the decline in spat production to the appearance of the devastating disease MSX in Virginia waters in the early 1960s. Coincident with the appearance of MSX was the establishment of sewage treatment plants in the James River at which chlorination was and remains a standard practice. Chlorinated sewage additions to the James River were previously linked with population effects on finfish. Laboratory studies have shown that the oyster, during early larval stages, is among the most sensitive estuarine species to chlorine and chlorinated sewage. A subsequent study suggested that chlorinated sewage mixed with estuarine water at concentrations thought to be realistic of possible conditions at the point of discharge depresses the ability of larvae to attach to a substrate. Dechlorination was found to have some beneficial effects.

The present project was designed to verify and expand the prior research on effects of chlorination on spatfall. During the first year, experiments were conducted to determine the amount of dilution necessary for chlorinated sewage before larval setting recovered. In addition, a field spatfall survey centered around a chlorinated sewage discharge into an historically important spatfall area was mounted to evaluate whether the effects observed in the laboratory can be demonstrated in the field.

Accomplishments

Laboratory Experiments

A series of experiments were performed in which oyster pediveligers were exposed to chlorinated sewage/estuarine water mixtures (5, 2.5, 1.25, and 0.6%), chlorinated-dechlorinated sewage/estuarine water mixtures (same dilutions), and sewage/estuarine water mixture (5%) under flow-through conditions. The chlorination of sewage was accomplished in the laboratory in a system designed to provide 30 minute chlorine contact before dilution with estuarine water or dechlorination. The initial 5% dilution of chlorinated sewage or chlorinated-dechlorinated sewage was then serially diluted to produce the lower concentrations.

The results of these experiments are summarized in Figure 1. The sewage/estuarine water mixture (5% sewage) resulted in reduced attachment of pediveligers in the first and second experiments, but not the third and fourth experiments. Variable response to different collections of sewage from a single treatment plant is not unusual, reflecting temporal differences in composition.

In the first experiment, serial dilution of the chlorinated sewage did not result in significant improvement of attachment of larvae. In the next three experiments, however, the greatest dilution (0.6% sewage/estuarine

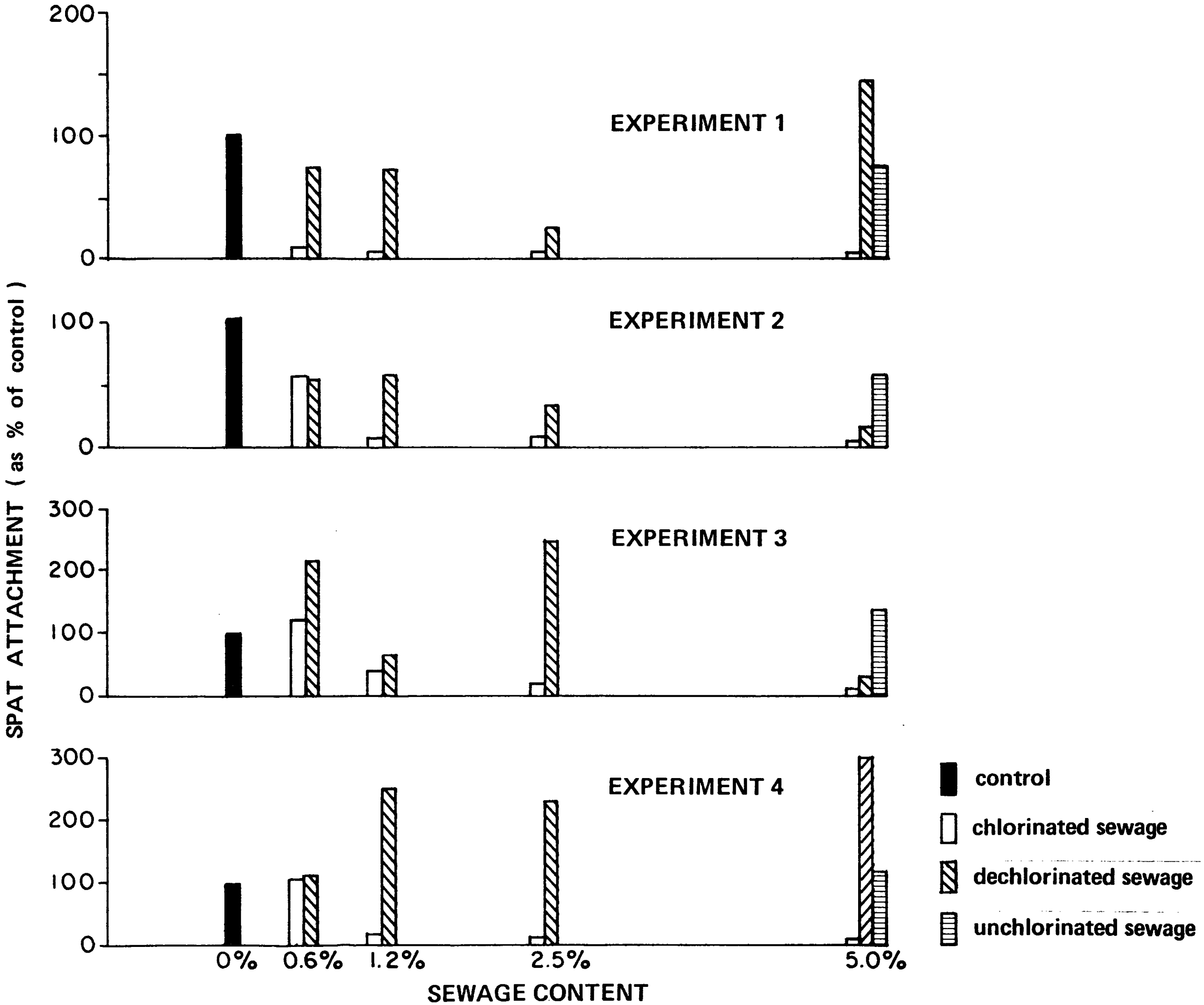


Fig. 1. Attachment of oyster pediveligers to artificial setting substrates when exposed to various concentrations of chlorinated, chlorinated-dechlorinated, and unchlorinated secondary clarifier effluent from the James River Sewage Treatment Plant (JRSTP). Attachment is expressed as a percentage of the control attachment over the time interval of the experiment.

water mixture) resulted in attachment comparable to the sewage control (5% sewage). In all experiments, dechlorination resulted in dramatic improvement in settlement of larvae. Least dramatic was the second experiment in which improvement was only to a degree equivalent to the sewage/estuarine water mixture, whereas in the other experiments, settlement well above that for the estuarine water control was observed.

One might reason that the enhanced settlement in chlorinated-dechlorinated sewage/estuarine water mixtures results from oxidation of a toxicant by chlorine, resulting in reduced toxicity of the complete mixture after dechlorination to remove the toxic chlorine. In the second experiment, the sewage/estuarine water mixture was the most toxic. A reasonable interpretation of the results for the chlorinated-dechlorinated sewage/estuarine water mixtures might be that the toxic agent in this case is not oxidizable to a non-toxic species. In the absence of analytical chemical data or identification of specific toxicant(s), this interpretation must remain a speculation.

Field Experiments

Spat collector plates were deployed in the James River at thirteen stations located in the vicinity of the James River Sewage Treatment Plant discharge beginning on 26 July 1984 (Figure 2). Plates were collected weekly from 2 August until 6 September. The number of spat and other members of the fouling community were determined for each plate after return to the laboratory and expressed as number/m^2. Spatfall was low at all stations except Stations 4 and 5 during the first two weeks of the survey. Stations 4 and 5 are the two stations located nearest to the channel and Wreck Shoal. During the third week of the survey, a major peak in oyster settlement occurred, with settlement at Station 1, nearest to the sewage

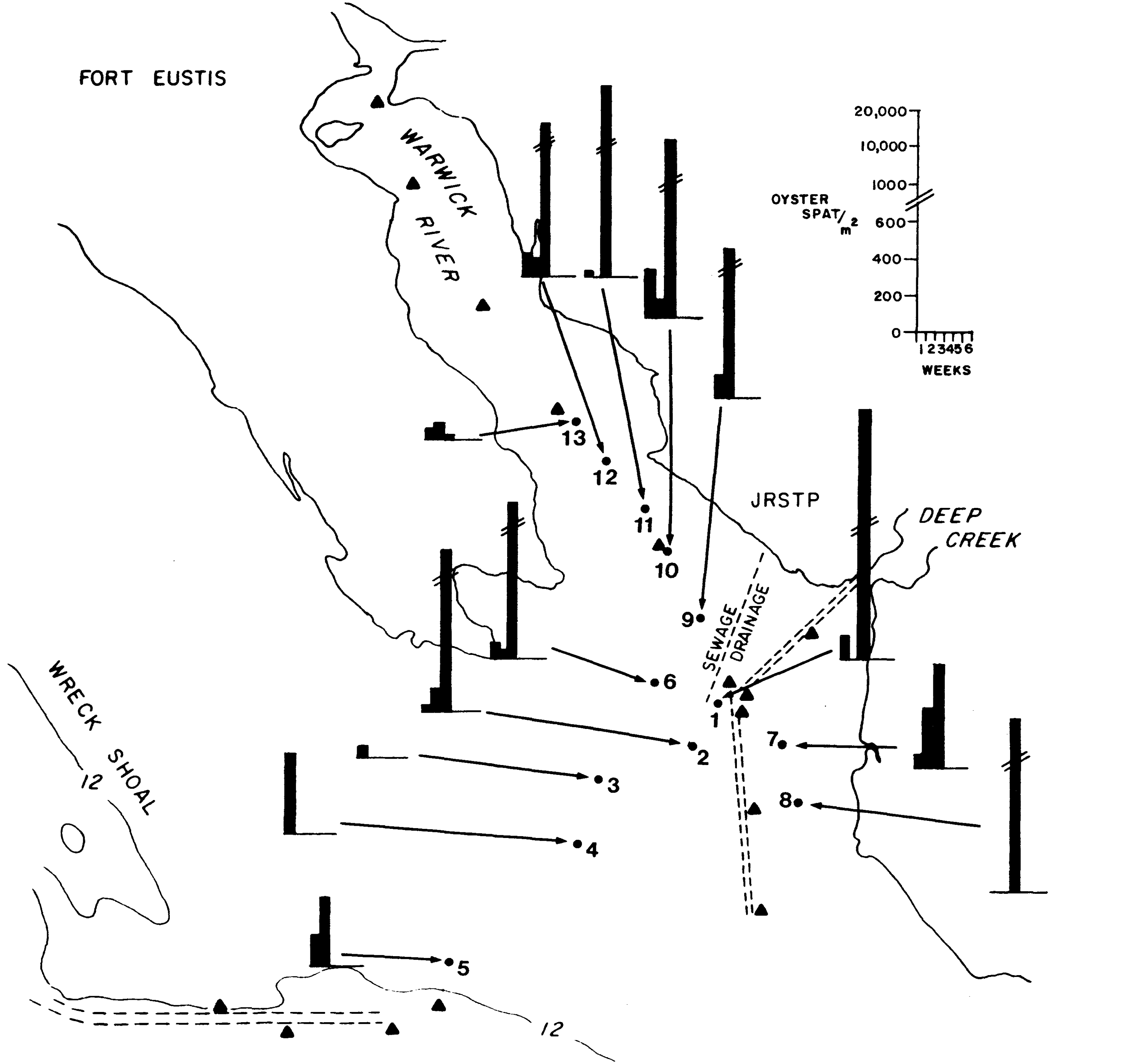


Fig. 2. Oyster spatfall at thirteen stations located in the vicinity of the outfall from the JRSTP. Spat abundance on the setting plates at each sampling interval is represented by a histogram for each station.

discharge point, being slightly higher than anywhere else in the study area. During this time, there was no settlement at Stations 3, 4, and 5; in other words, settlement was concentrated in the mouth of the Warwick River where it discharges into the James River.

During the final three weeks of the survey, no oysters were observed to settle at any station in the study area. The survey probably did not continue long enough to observe a fall peak in setting if one occurred during 1984.

Barnacles were the dominant member of the fouling community, occurring in numbers even greater than oyster spat (Figure 3). Over the survey period, barnacle settlement was high during the first three weeks, then declined for two weeks, with perhaps a secondary peak in settlement during the final week of the survey. Variation in settlement intensity over time with peaks at about monthly intervals have been observed elsewhere for barnacles.

Spatially, barnacle settlement was greatest at Station 4 near the James River channel, and at stations in the mouth of the Warwick River. Among the thirteen stations, barnacle settlement was lowest at Stations 7 and 8, located farthest downstream in the James River. Stations 3, 4, and 13 also had relatively low barnacle settlement. Station 1, nearest the outfall from the sewage plant, had barnacle attachment more like that at Stations 5 and 8 than Stations 6 and 7.

In summary, there was no apparent effect of the sewage outfall on the distribution of oyster or barnacle settlement in this reach of the James River during the summer of 1984. Factors other than chlorinated sewage would seem more appropriate to explain the observed distribution of larvae.

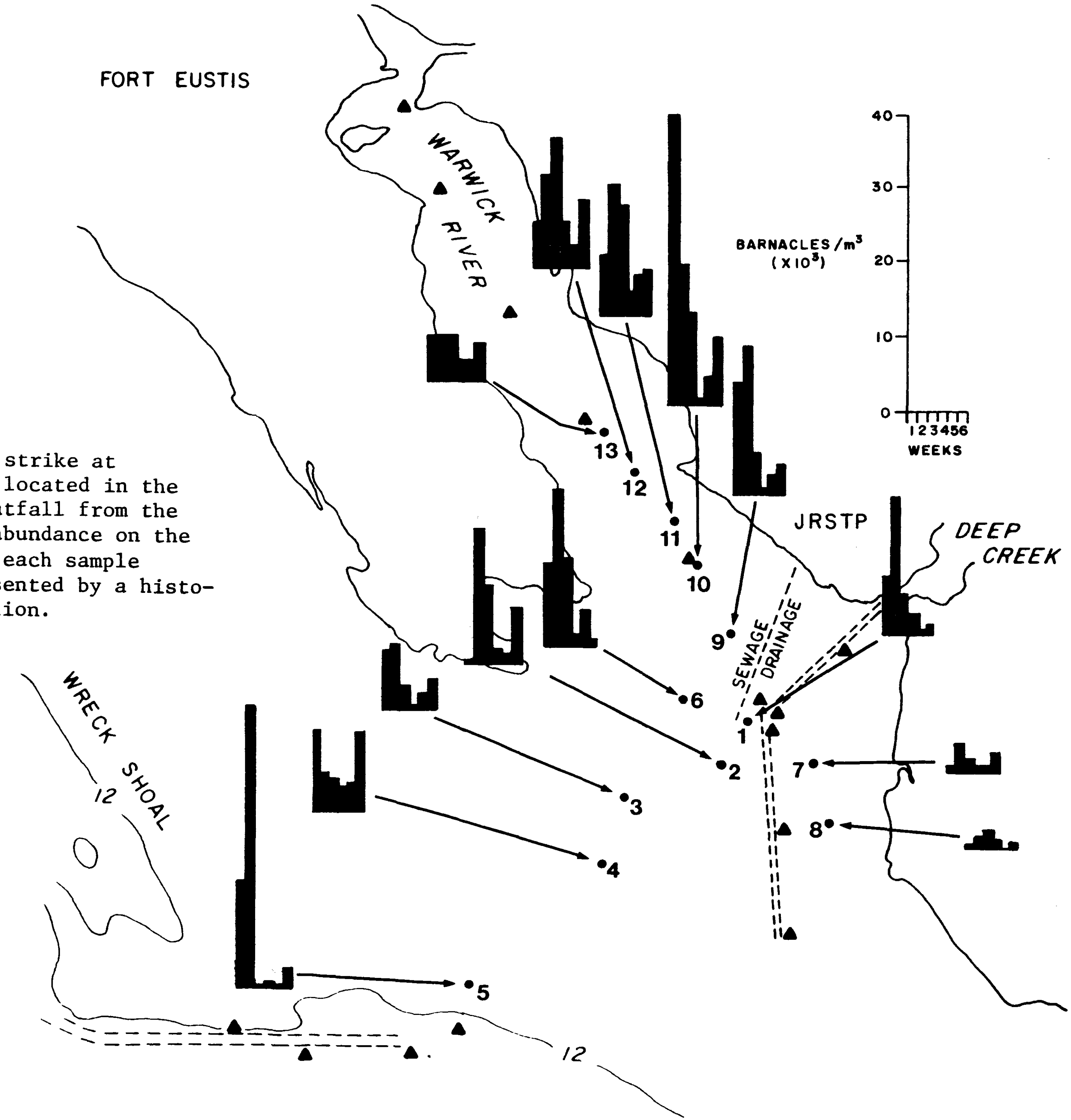


Fig. 3. Barnacle strike at thirteen stations located in the vicinity of the outfall from the JRSTP. Barnacle abundance on the setting plates at each sample interval is represented by a histogram for each station.

While it was not possible during this survey to analyze water samples at Station 1 for a chlorine residual because of high usage of the available analytical equipment in the laboratory, previous sampling of water at this approximate site in the recent past has not revealed any measurable chlorine produced oxidant residual, and there is no reason to believe that an oxidant residual would have been found in the summer of 1984.

In the laboratory experiments, a measurable oxidant residual ranging from about 0.1 down to 0.025 mg/l was produced in the three treatments with the highest percentage of sewage. At the most dilute sewage mixture, a measurable residual was not observed, but the concentration is calculated to have been $\leq$0.01 mg/l, at or below the detection limit for the amperometric titration method. If dilution of sewage at the point of discharge in the James River results in a sewage/estuarine water mixture of <0.6%, no measurable oxidant residual would be expected.

Continuing Effort

Laboratory studies to date have focused on attachment by pediveligers. No attempt was made to evaluate the ability for attached larvae to metamorphose and grow. Therefore, experiments this summer are designed to evaluate the success of spatfall in terms of both attachment and metamorphosis under the same experimental conditions used in prior experiments which focused on attachment alone.

A field survey has been mounted again this year to verify that the distribution of oyster spatfall is not affected by proximity to the sewage outfall. Three additional stations have been located in the James River proper on a transect extending due west from Station 6 toward Wreck Shoal. Additionally, the field effort has been expanded this year to include periodic placement in the field of plates with laboratory cultured spat already on them. Before placement, each plate is photographed to record the position and size of the oyster spat. After one or two weeks exposure to conditions at the thirteen stations, the plates are recovered and rephotographed. By comparing the two photographs and measuring the spat, it is possible to determine directly both survival and growth rate of the spat.

Prior to the week of 28 June-5 July 1985, there was no set of oyster larvae at any station. In a preliminary examination of plates recovered on 5 July, there appears to have been a strike of oyster spat during the preceeding week. Barnacle settlement has been extremely heavy, exceeding the highest sets observed last year. The first two sets of plates with preset oyster spat have been recovered and rephotographed. Analysis of the photographs is in progress. Observation while photographing the plates suggests marked growth of spat during the week of field placement with little or no mortality. Without comparing the before and after photographs, however, one cannot distinguish any differences in growth rate related to station placement.

PROJECT 4

Determination of the Mechanisms of Water Circulation in the Lower James River/Hampton Roads as a Means of Predicting the Transport Pathways of Oyster Larvae and/or Dissolved Pollutants

Introduction

The general goal of this project is to improve our understanding of the circulation in the lower James River and to apply that understanding to the management of the James River seed oyster beds. If there is to be revitalization of the James River seed oyster industry, then it will be necessary to manage more effectively both the resource and those activities which affect the resource. Thus we need to know how the system functions, which aspects are positive and which things have detrimental effects. We need to know as much as possible about the James River and in addition to be able to contrast the James with other estuaries for many persons believe that the James provides a uniquely satisfactory environment for the production of seed oysters.

This report gives the progress of the work to date along with projections for future efforts, and a technical discussion of the research on a subproject by subproject basis.

A problem anticipated in these circulation studies is the conflict for resources (equipment, vessels, and especially manpower) required to continue the seed oyster bed circulation study at the earlier projected pace and also necessary to evaluate the effects of the proposed New Port Island off Newport News Point. It will be virtually impossible to maintain the previously projected schedule for the circulation studies while at the same time conducting the field work and interpretation necessary to make the assessment mandated by the Legislature in House Bill 1396. It is recommended that the schedule for the seed oyster bed studies be revised to allow more time for completion of the work. An extension to at least December 1986 is requested. It should be noted that the circulation near Newport News Point potentially plays an important role in the dispersion and retention of oyster larvae in the Hampton Roads area. Thus the detailed study of the area required for the New Port Island project will provide information and knowledge that will be useful for the larger circulation questions regarding the seed oyster industry.

We further recommend:

a.) That a state-of-the-art three-dimensional numerical hydrodynamic model be acquired, configured for the James River, and interfaced on the VIMS computer.

b.) That the model be applied to the problem of larval transport and transport of dissolved substances.

PROJECT 4A

Horizontal Circulation and Characterization of Larval Movement and Retention

Purpose

The purpose of this phase of the James River studies is twofold:

1. To determine the general horizontal circulation in the estuarine portion of the river in order to predict the most probable pathways followed by oyster larvae from expected brood stock areas to seed beds, and
2. To estimate the retention of oyster larvae within the seed bed region during their setting period (10 to 20 days after spawning) when released from expected brood stock areas.

Accomplishments

First Quarter

. Processing and preliminary analysis of the 1968 hydraulic model dye tests.

. Modified the vertically-averaged math model of the James River estuary to use real tidal records for boundary conditions and to simulate dye dispersal.

Second Quarter:

. Completed the analysis of the 1968 dye release studies.

Six historically important oyster rocks have been ranked in terms of the transport of dye to the seed beds and in terms of retention of dye in that area. The analysis has been extended to include factors which influence larval production such as the size of the beds, relative fecundity, etc.

The analysis of the dye studies has been presented at two scientific conferences. A manuscript has been prepared documenting primarily the physical transport aspect of the studies and this will be included in the proceedings of the VIMS conference on Estuarine Circulation. The second presentation was to the National Shellfisheries Association, a group that has many oyster biologists as members. The presentation included a video recording of the dye study results using computer generated graphical displays. The enthusiastic response that this presentation garnered suggests that it will be possible to interact in a productive manner with the biologically oriented scientists.

. The modified math model has been used to simulate the previously mentioned dye releases in the hydraulic model and a real-world dye release.

Comparison of hydraulic model and math model dye releases suggests that general patterns of transport and dispersion are simulated well, but that neither may reproduce the details observed in the real world. In particular it appears that the hydraulic model has insufficient lateral mixing while the math model has too much. However, only one real-world dye release is available for evaluating the models. Therefore an additional field study is planned to acquire data to confirm or refute the perceived deficiencies of the models.

It should be noted that it will be possible to adjust the math model, or if that is insufficient, to reformulate the algorithms for estimating lateral dispersion rates. The measurements of tidal currents (Project 4C) also could be used to recalibrate the model. The reason why such an effort may be required and desired is that the math model allows a broad range of options and conditions to be tested easily and economically, and therefore it can provide useful guidance to managers.

Summary of Accomplishments;

The hydraulic model studies have been used to provide first order estimates of the movement of oyster larvae. Consequently six historically important oyster rocks have been ranked (1) in terms of their contribution of larvae to the seed oyster bed region and (2) in terms of the likelihood that oyster shells planted in those areas would receive a good spatfall.

The math model has been modified and refined to better reproduce the features observed in nature. However, there are some indications that the lateral dispersion of materials may not be accurately simulated. This deficiency in the model could be corrected if additional field dye releases are made.

Continuing Effort:

. Conduct a dye dispersion study in the James River. The results of this field study will be used to evaluate and compare the ability of the hydraulic model and the math model to simulate natural mixing phenomena.

. Summarize information on circulation patterns in the lower James using results of hydraulic model experiments, field studies, and math model simulations.

PROJECT 4B

Role of Mesoscale Circulation in Larval Transport

Purpose

The purpose of the mesoscale studies is:

1. To identify the location and characterize water convergence zones (fronts) that are likely to affect larval and dissolved pollutant transport processes, and
2. To determine whether the formation and dissipation of fronts play a significant role in oyster larval transport processes.

Accomplishments

First Quarter:

. Made aerial overflights to document when and where fronts or convergence zones exist in the vicinity of seed oyster beds.

. Familiarization of staff with use of dual frequency echo sounder.

. Completed preliminary echo sounder surveys near Newport News Point and at Wreck Shoals, with measurements of conductivity, temperature, and depth to characterize water masses.

Second Quarter:

. Continued the analysis of early field efforts.

It is important to recognize that the mesoscale studies incorporate an entirely new approach to the study of physical transport processes in Virginia's estuaries. Although the methods and instrumentation have been applied to a some estuaries in other countries, this effort is the first of its kind for Virginia waters and in fact similar efforts are underway in only a few other areas in the country.

Consequently the mesoscale studies have an evolutionary aspect. First, the instrumentation is new to both the professionals and the technical support staff and some time is necessary for them to become proficient in use of the instruments and to learn ways to maximize the acquisition of scientifically useful information. During the past year the field efforts have become progressively more sophisticated. First efforts were geared towards use of the echosounder to show that scattering layers existed. Those echo images showed highly variable midwater scattering features, including a variety of orderly wavelike patterns, as well as chaotic layer splitting and disintegration. Subsequently a CTD (conductivity-temperature-depth sensor) was used to document ambient conditions in general. This work showed the correlation of density interfaces with the scattering layers. The inferred vertical displacements of the density interface (pycnocline) suggest that motions associated with lowest mode internal waves were fairly common. More striking were large vertical excursions, apparently associated with lee waves or internal hydraulic jumps. These were observed in the preliminary surveys near Wreck Shoal and Newport News Point. The most recent field efforts included simultaneous recordings of echograms and density profiles so that the two types of information could be correlated even more closely. For that field study the newly acquired, profiling CTD was used.

The rapid change in computer technology means that data logging and processing capabilities are constantly improving and those changes can be incorporated into the methodology to improve data acquisition. More specifically, portable, battery-powered microcomputers are being used to log data from the profiling CTD . The goal is to be able to see how the density structure varies while in the field so that the sampling patterns can be adjusted to maximize the acquisition of useful data or to focus on areas of maximum interest. Additionally, we are attempting the development of data processing packages which will allow the data to be entered into a main frame computer rapidly so that more detailed analyses can be conducted.

. Completed design of and carried out more intensive field studies of fronts in the vicinity of Wreck Shoals.

. Monitored conditions in the vicinity of Newport News Point.

In 1974 field studies related to the design of the outfall for the Small Boat Harbor sewage treatment plant showed that a convergence zone developed just west of Newport News Point during flood tide. During the past quarter regular (typically three times per week) surveys of the area were made to determine whether that convergence zone developed on most flood tides or only during certain meteorological or tidal conditions. The preliminary results of this several week monitoring effort indicate that the front varies in location and other characteristics during flood tide but that it is a regular and persistent feature throughout the lunar cycle.

Summary of accomplishments:

In the first year we have identified important mesoscale features near Wreck Shoal and near Newport News Point. Both preliminary and more detailed field efforts have been mounted near Wreck Shoal. The effort near Newport News Point was of a somewhat different nature in that its purpose was to document that the convergence is a persistent feature during flood tides. The processing and anlysis of the recent field efforts (May at Wreck Shoals and June near Newport News Point) has not yet been completed; a report will be prepared in the near future describing those field efforts and the results obtained.

Continuing Effort:

. Continued analysis of field results and interpretation of same in light of comparable studies of stratified flow in fjords and other water bodies.

. Continued field efforts to characterize the formation and dissipation of fronts and how those processes vary in response to ambient conditions such as degree of stratification and lunar phase.

Field capabilities will continue to evolve and improve during the coming year. A newly acquired microcomputer that is appropriate for taking into the field is being programmed to allow for some processing of data on the spot. This enhanced computer capability will allow simultaneous recording of the echo patterns, position, current speeds and directions, and other important variables. In addition, the ability to process the data, even if only partially, will allow the scientists to constantly refine and adjust the sampling protocol so that data can be acquired in the most important locations or in such a manner to best elucidate the mechanisms under study.

PROJECT 4C

Role of Temporal Variations in Gravitational Circulation

Purpose

The purpose of the gravitational circulation studies is:

1. To document the fortnightly variation in density driven gravitational circulation as well as changes in salinity gradients, and to ascertain the importance of other temporal scales, and

2. To infer the role of temporal and spatial variations as they affect oyster larval transport.

Accomplishments

First Quarter;

. Processed density data from 15 daily slackwater surveys of the James River estuary in August 1983 and prepared longitudinal-depth density plots for each cruise.

. Deployed four current meters at one station above the James River Bridge for 6 weeks in October and November.

. Evaluated and acquired state-of-the-art instruments for measuring currents, conductivity, temperature, and depth.

Second Quarter:

. Analyzed the August 1983 slackwater survey data

The analysis of the 1983 data has as its goal a better understanding of how variations in tide range and tidal currents, which are associated with the phase of the moon, affect the stratification in the river. Preliminary review of the data indicates that longitudinal density gradients are strong in the vicinity of Wreck Shoal, suggesting that this topographic feature plays an important role. Analysis of the mixing sequence is expected to provide insights as to the role of features such as oyster rocks, other changes in channel depth or direction, exchange between shallow flanks and the main channel, etc.

. Tested and deployed the newly acquired InterOcean current meters.

The new vector-averaging current meters were deployed in April to test the instrument and perfect our ability to moor these instruments and acquire the data in the desired format. These meters, equipped with microprocessors, may be programmed to sample at desired frequencies. The stored data is then transferred to a larger computer for processing. Although some problems were encountered, the trial run was successful in that much was learned for later applications.

Current meters were deployed in early June just upriver of the James River Bridge and near Deep Water Shoals. A salinity chain was moored with the current meters near Deep Water Shoals. Tide gages were installed at several locations, and salinity and temperature sensors were deployed from one of the ships in the "idle fleet". When this upstream/downstream deployment is completed in early July, all of the meters will be moored along the transect near the bridge.

. Current measurements from a station just upriver of the James River Bridge between October 19 and December 2, 1984 have been processed and analyzed.

When tidal currents are averaged over several tidal cycles or longer, one typically observes a net movement. The classical pattern for these netflows was developed by Dr. Donald Pritchard who used data acquired in the James River in the early 1950's. In that formulation saltier water near the river bottom tends to flow upriver while fresher water near the surface tends to flow downriver towards the bay. Recent studies in the Potomac River estuary and elsewhere have suggested that this pattern applies only about half of the time. Our 1984 measurements, however, confirm this net circulation pattern in the James. During the period of observation, the non-tidal current near the surface (2.4m depth) was in the ebb direction except for two days in early November, while that at 9.1 m depth was in the flood direction all of the time. (See Figure 4C-1)

Tidal height records for that same period showed an interesting feature (Figure 4C-2). When the variations due to the movement of the sun and the moon are removed from the record, the remaining non-tidal part of the record shows variations that were anticipated but were not expected to be as large as those observed. Previous studies have shown that local winds interact with the Bay and cause fluctuations with periods of 3 to 8 days. For the late-1984 period, however, a 15 to 20 day cycle is apparent in the record. This long-term variation in mean water surface elevation is believed to be due to onshore/offshore winds which affect the coastal seas and estuaries that are tributary to those coastal seas. The magnitude of this variation was around 0.6 m, which is nearly as large as the typical tide range observed in the lower James (about 0.8 m). The two-day period during which the near surface net currents were in the upriver direction corresponded to the period when the non-tidal surface elevation at Sewell's Point (Figure 4C-2) reached its maximum.

. The modified 2-dimensional math model (vertical and longitudinal) has been used to investigate the response of the estuary to the lunar cycle.

It has been observed in some estuaries that the mean water surface (averaged of a tidal cycle) in the upper estuary rises going from neap tide to spring tide. The water stored in that part of the river is then released

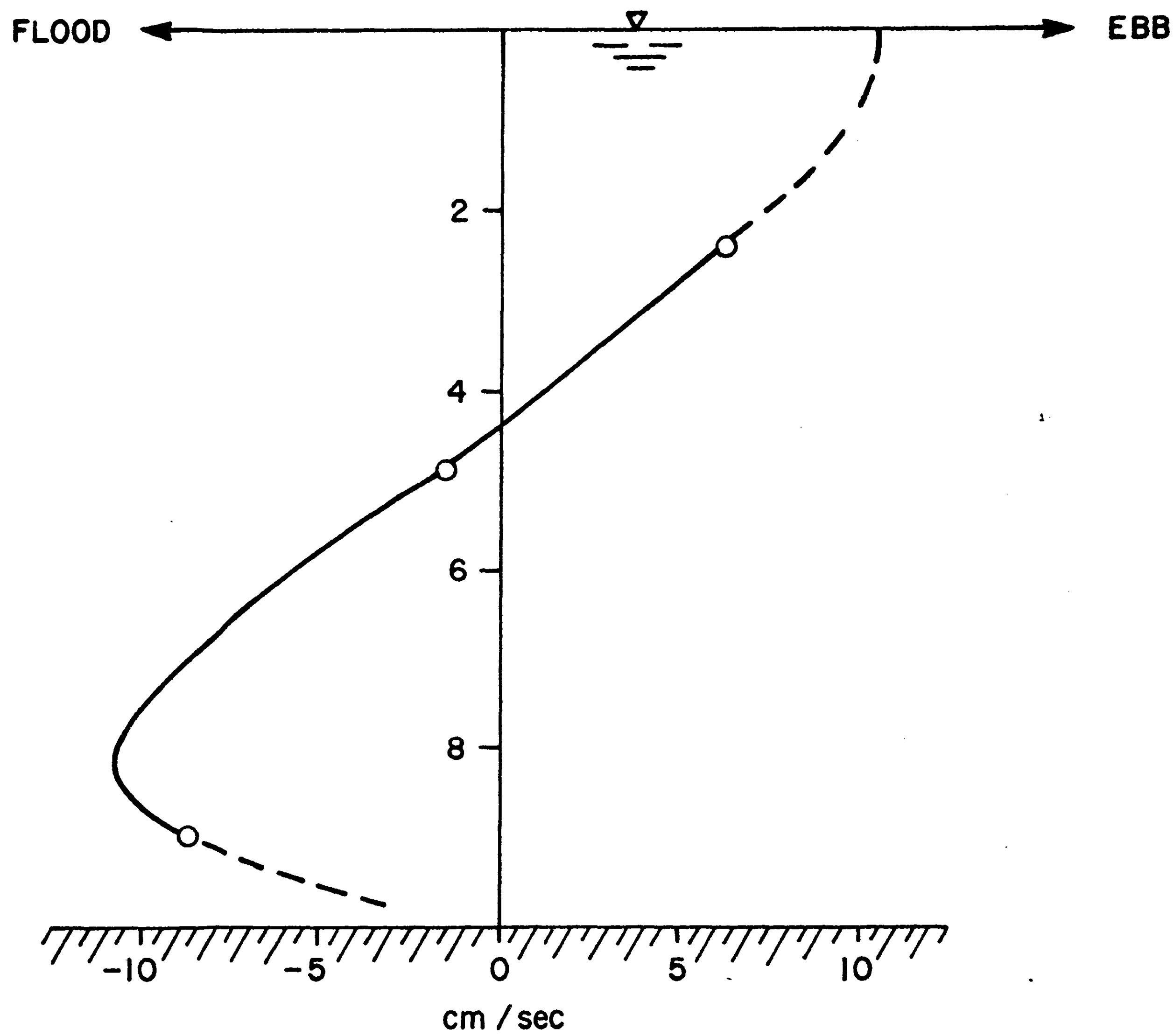


FIGURE 4C-1 **Vertical distribution of average longitudinal velocity.**

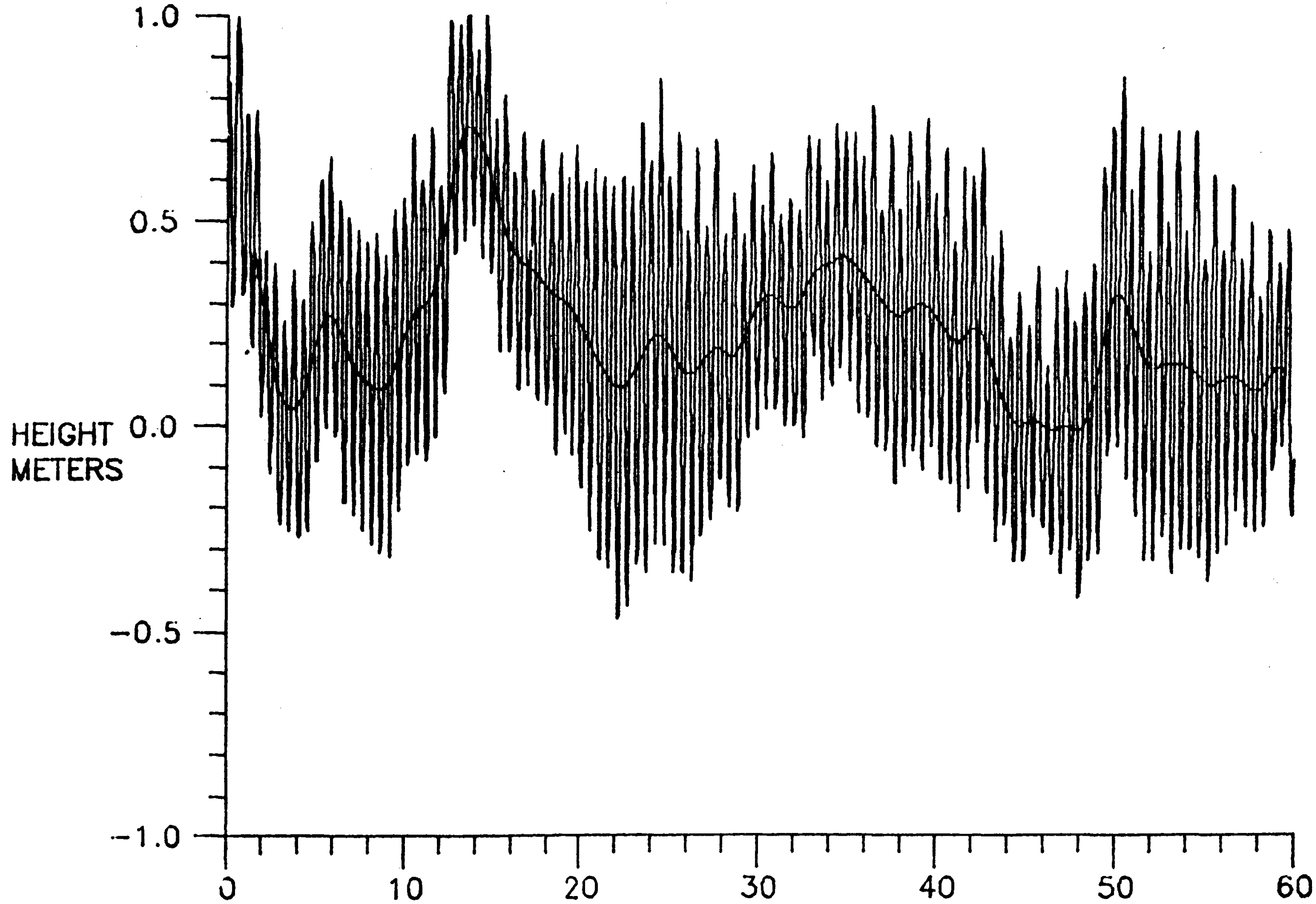


Figure 4C-2. Tidal record and residual at Sewell's Point referred to NGVD. (starting 0000 hr, Oct. 1, 1984) during the spring tide to neap tide portion of the lunar cycle. When the model of the James was run to reproduce a lunar cycle for typical summer flow and stratification the model prediction showed the aforementioned build-up of water and indicated that the amplitude of the variation increases in the upestuary direction. Additional model runs were made to investigate the cause of this variation. That work suggests that greater amounts of energy are dissipated when tidal currents are strong (spring tide) than when currents are weak (neap tide). If the river flow is constant over that period, then the energy loss due to friction must be counterbalanced by an increased water elevation difference between the upper estuary and the river mouth.

Summary of Accomplishments

The data from the field study of the fortnightly salinity variations have been processed and are available. The data are being interpreted and are expected to provide insights which will be of value to the research efforts in the other subprojects. Math model exercises indicate that the model simulates real world behaviour well and have provided insights into the causes of the storage of water in the upper estuary during spring tides.

Analysis of the late 1984 current meter deployment indicate that the net circulation patterns in the James follow the classical pattern nearly all of the time, even though mean water surface elevation was seen to vary over nearly the same range as the typical semi-diurnal tides.

A draft technical report which fully discusses methods, analysis, and results has been prepared and is available upon request.

Continuing Effort:

. The current measurements will be processed to show temporal and spatial variations in the net movement of water.

. The data will be analyzed to relate observed variations in water movement and density structure with factors such as river flow, tides, wind patterns and so on.

PROJECT 4D

Micro-Circulation and Sedimentation Processes at Wreck Shoal

Purpose

The objective is to determine the sedimentation processes on oyster rocks and to formulate criteria for judging site suitability for shell plantings.

Sedimentation processes on oyster rocks are very poorly documented or understood. In addition to being located within a zone of relatively high natural sedimentation the oysters themselves produce very large volumes of sediments as fecal materials. How are the oyster rocks maintained in this environment of deposition? This project is intended to provide answers to this question. The significance of the project rests on the fact that if additional replenishment areas are needed, we must have a better understanding of oyster rock sedimentation. With such understanding we will be able to evaluate the risk of planting shell at various sites.

Wreck Shoal, one of the most important seed rock areas, has been selected for study. Since this seed bed area contains distinctly different types of oyster beds in adjacent areas, the site offers the opportunity to compare and contrast the oyster habitat in terms of sediment type and hydraulic energetics.

Accomplishments

In order to understand the present condition of seed rocks we must also consider the recent past because antecedent conditions may exert a dominant influence. This is particularly the case in estuarine systems since the present day rocks are thought by many to be the result of evolving growth as sea level has risen. Therefore this project is divided into joint examination of the recent (125 yrs) morphological changes, and the modern processes acting.

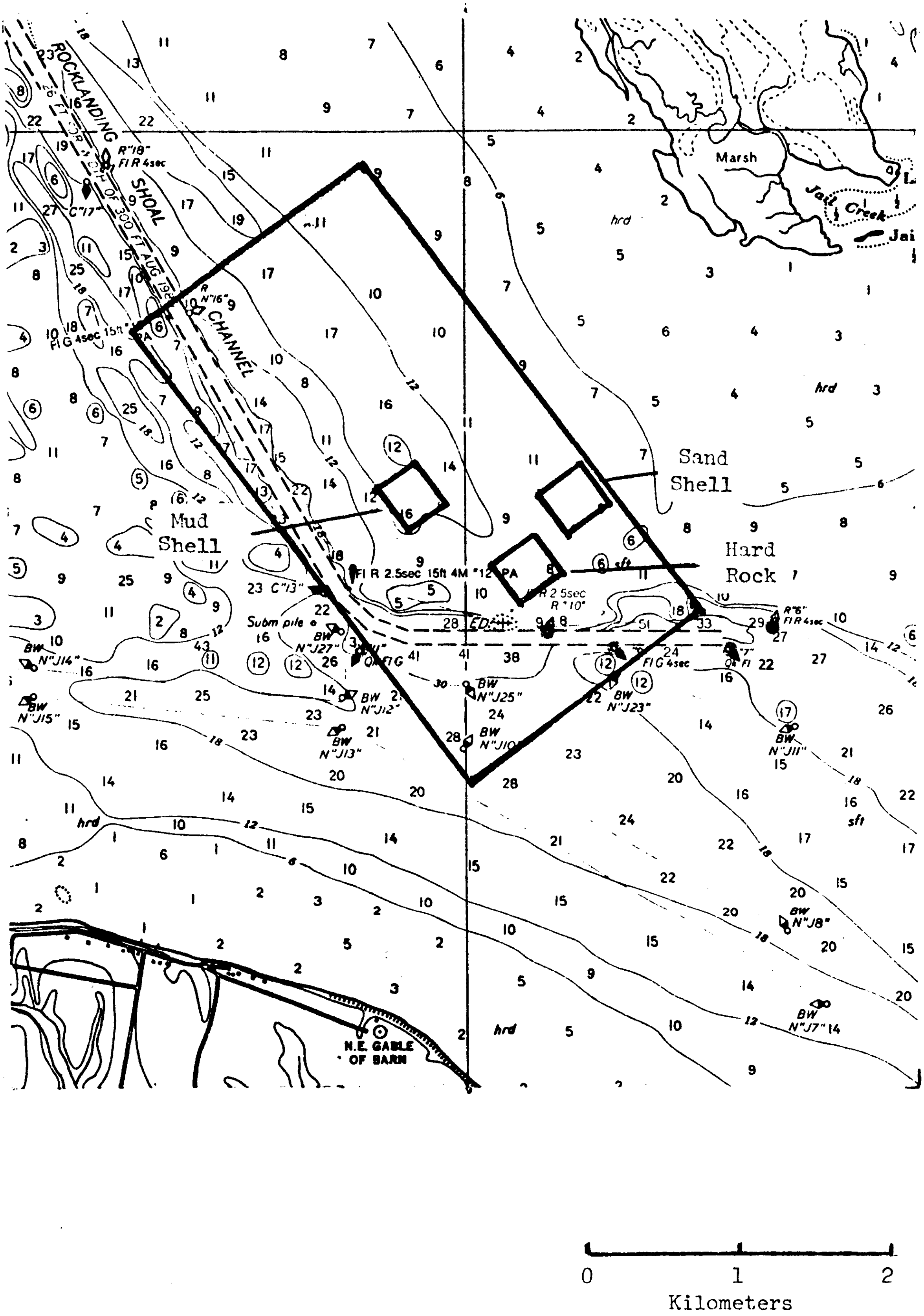
FY 1984-1985 Accomplishments

. Comparisons were made between bathymetric data of 1854, 1871, 1910, 1946, and 1984.

These comparisons, made over the area including Burwell Bay, Point of Shoal, and Wreck Shoal, delineate those areas showing significant elevation changes. While Burwell Bay channel has shoaled, Wreck Shoal/Point of Shoal has been relatively stable. However, the results clearly indicate that oyster harvesting at the principal rocks has deflated the bottom by several feet.

. The principal types of oyster producing bottoms in the Wreck Shoal area were identified and sub-plots were established for intensive investigation.

Previous reconnaissance mapping of the seed oyster beds by Haven, Whitcomb and Kendall (1981, VIMS) distinguished gradations in bottom types: Based on these studies three types of sub-environments were selected at Wreck Shoal, hard-rock, sand-shell, and mud-shell. A twenty-five acre plot was estblished in each sub-environment and 25 patent tong samples were secured from each site. This sampling scheme was developed to test for simularities between sub-environments with respect to biological (oyster) and sediment characteristics (Fig. 4D-1). Subsequent statistical analysis indicates that the sand-shell substrate is not significantly different from hard-rock but both are significantly different from the mud-shell substrate. These differences are important when viewed in the context of micro-circulation.



MAP OF STUDY AREA
WRECK SHOAL, JAMES RIVER, VIRGINIA

FIGURE 4D-1

. Side-scan sonar imagery was utilized to investigate meso-scale substrate roughness.

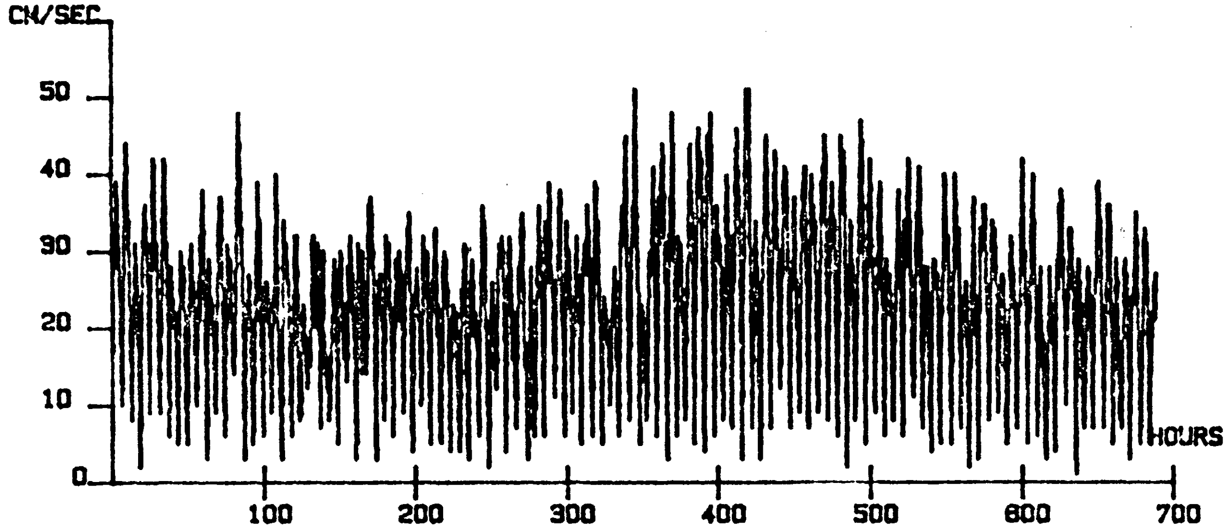
The level of fluid shear exerted on the substrate and available for sediment resuspension is dependent upon substrate roughness as well as sediment size, and flow speed. Therefore, a side-scan sonar survey was performed to determine the distribution of mesoscale roughness (sand waves and/or ripples). In addition to flow related features long furrows across the oyster beds were noted. These features, with vertical relief up to one meter, are attributed to ships over-passing the beds. A second side-scan survey was performed in May, 1985, in order to determine whether the scars persist and if new tracks were evident. Analysis of this data is in progress.

. Micro-circulation measurements were secured in summer, 1984, the results analyzed, and from these results the expected sediment erosion/ accretion tendencies have been inferred.

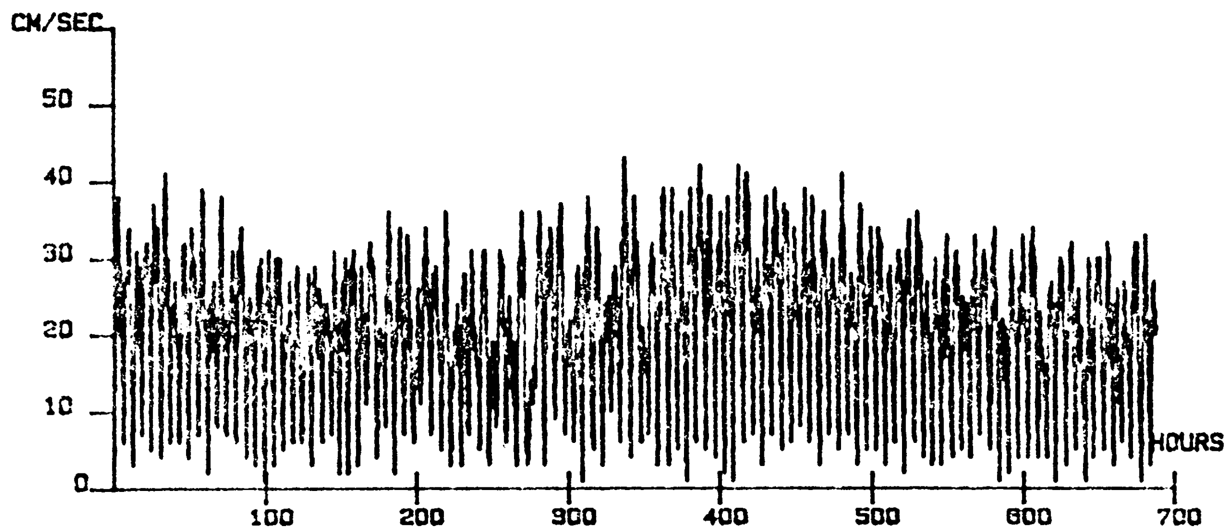
In order to ascertain the micro-circulation a one-month in-situ current meter deployment was made with one meter in each sub-environment. While these installations recorded temporal variations (Fig. 4D-2), spatial variations in bottom current between and around the sites were observed at 8 additional stations for one-half tidal cycle.

The results of a micro-circulation study of Wreck Shoal indicate considerable spatial variation in the strength of the bottom current regime. The magnitude of the bottom current is inversely correlated to mean water depth. Temporal variations in a bottom current are accounted for by a net downstream current and a strong periodic tidal component. The "hard-rock" oyster reefs experience relatively strong bottom currents while the "mud-shell" oyster reefs experience relatively weak bottom currents. The

BOTTOM CURRENT, STATION HARD ROCK, WRECK SHOAL



BOTTOM CURRENT, STATION SAND SHELL, WRECK SHOAL



BOTTOM CURRENT, STATION MUD SHELL, WRECK SHOAL

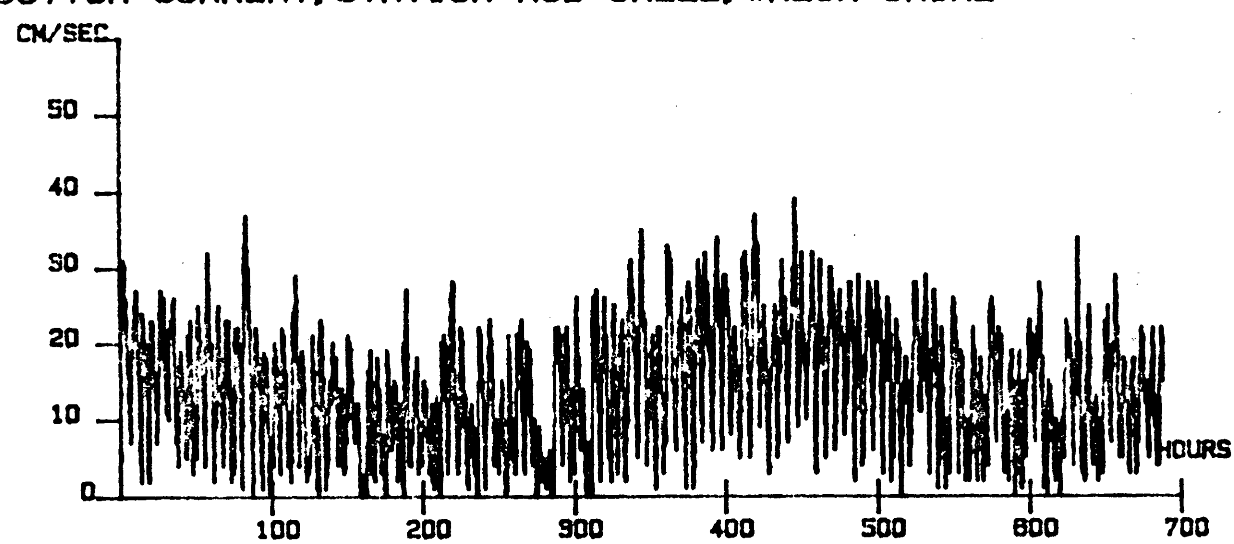


FIGURE 4D-2

bottom shear stress and therefore sediment transport power of the bottom currents for the two oyster reefs are substantially different and this is reflected in the observed sedimentation processes. The contemporary sedimentation processes and the productivity of the oyster reef are correlated to the magnitude of the bottom current and to the geomorphology of the shoal.

A technical report describing the details of the methods, analyses, results, and discussion has been prepared. This will be published in a symposium proceedings on Estuarine Circulation.

Continuing Effort

During FY 85/86 the plan is to complete field observations and to integrate these results into a general interpretation of the sedimentation processes on Wreck Shoals. This will then be generalized into criteria for ranking substrates as to suitability for shell replenishment. Field observations will include:

a.) Sub-bottom seismic studies to investigate the recent geological history of Wreck Shoals.

b.) Deployments of a tripod with near bottom current meter and turbidity sensors to corroborate our hypotheses on sediment resuspension.

PROJECT 5

Development of Models Relating Environmental Variations with Strength of Recruitment of Virginia's Populations of Spot and Summer Flounder

Purpose

Estimates of annual recruitment to fishable stocks of the principal commercial and recreational finfish species of Virginia waters are needed in the development of Fishery Management Plans (FMP's) by the Virginia Marine Resources Commission. This project will develop models for prediction of recruitment for spot and summer flounder, based on a model successfully developed recently for the Atlantic croaker.

The Chesapeake Bay is an important nursery area for all three of these species which spawn on the continental shelf in the fall-winter. Therefore, it is important to investigate the environment both on the shelf and in the rivers. It is likely that they respond to similar wind and temperature regimes.

The 30-year juvenile fish monitoring data base at VIMS, used in development of models for the three species, will also be readied for future model development for weakfish and striped bass.

The main objective of this project is to identify the trends and cyclic components of recruitment together with the concomitant environmental variables and to incorporate them into a model which will provide accurate assessments of future juvenile finfish stock size, and hence recruitment.

Accomplishments

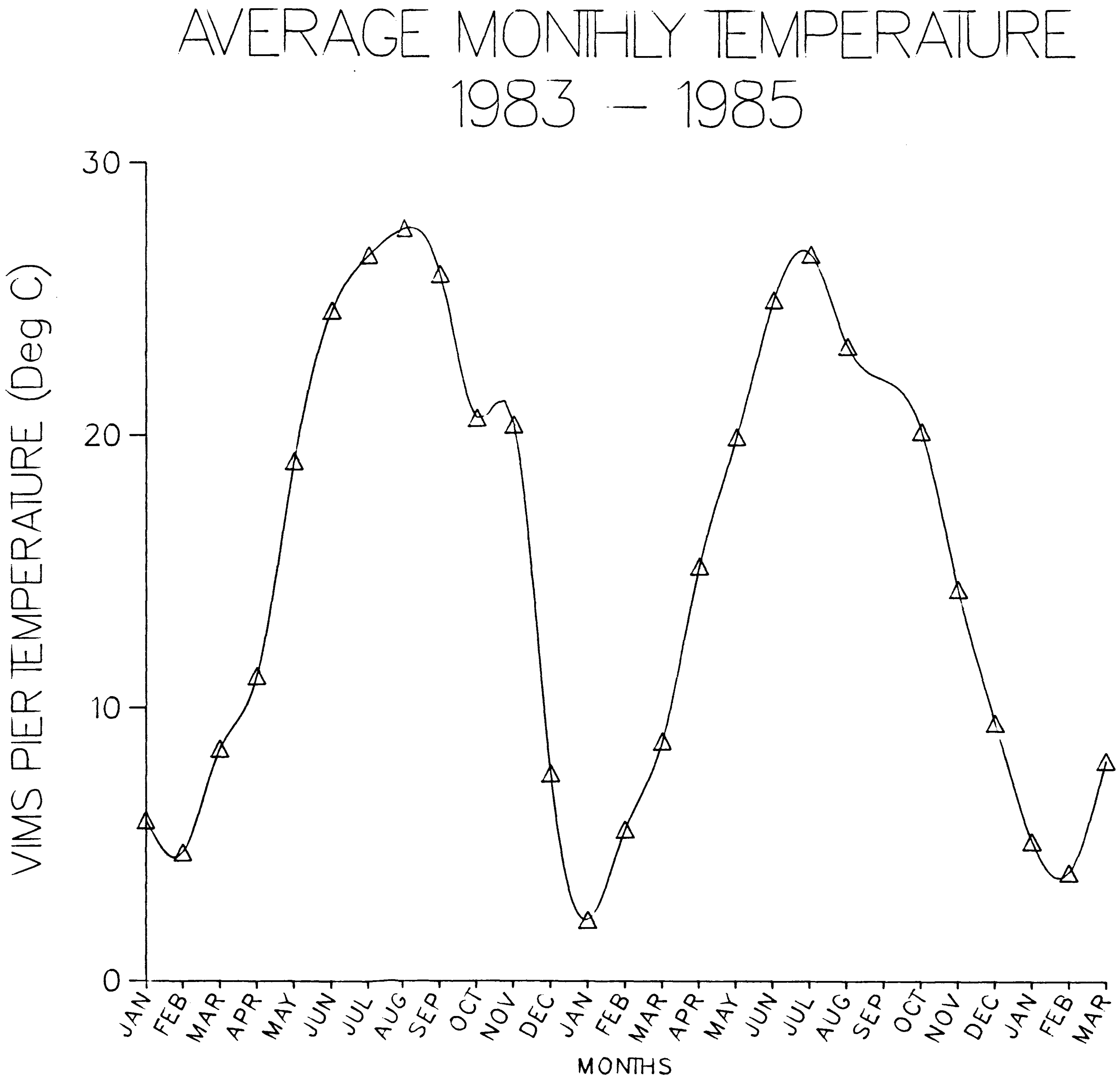
Average daily Norfolk, VA wind vectors (source: Local Climatological Data) for 1983 and 1984 (24 months) have been added to the existing base (now 1948 - 84). These data will be used to analyze larval transport on the shelf.

Daily VIMS pier temperatures are now available in a useable form on the computer through March 1985. These data came from strip chart readings and from the automated recording system which was in use for early 1984 and part of 1985. We have also incorporated data from other observations taken by Institute scientists since there were gaps in the data. These temperature data (Figure 1), which will be updated quarterly, will be correlated to juvenile survival.

Minimum and maximum daily temperatures, 1955 - 1985, are also being entered into the Prime. These data may become important when average monthly temperatures are insufficient as predictor variables.

Analysis of vertical distribution of croaker larvae at the mouth of the Chesapeake Bay shows that the larvae are not all concentrated on the bottom, but rather, spread throughout the water column in in-flowing waters. This has implications for spot and flounder transport, as they recruit into the Bay after peak croaker recruitment.

Trawl survey data for croaker in 1983 have been corrected and incorporated in the existing SIR data base. Monthly length/frequency charts have been analyzed individually to separate year classes. The results of monthly length/frequency evaluations for croaker, 1954 - 1983, have been compared to the simpler, quick method currently used which uses the same "average" length of 149 mm to separate year classes each month and each year. There is however, a greater degree of error caused by using this



AVERAGE MONTHLY TEMPERATURE
1983 – 1985
VIMS PIER TEMPERATURE (Deg C)
30
20
10
0
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR
MONTHS

Figure 1

easier method. While usually adequate for January - May, the error increases as growth increases in the summer (June - September) and is compounded when the new year class appears in the fall. Because modal size and growth rates of croaker change with year-class strength and winter temperature, year classes cannot be standardly assigned based on size. Although it is much more time comsuming to analyze each month's length/frequency plot, it appears to be the only reliable method.

Since flounder and spot have early life cycles similar to that of croaker, the indication is that it will be important to examine each month's length/frequency distribution individually in order to determine year-class size.

The modelling techniques used for the croaker were recalculated and reevaluated, including additional analyses of residuals. A common critique of the present croaker model has frequently been that there are no confidence limits associated with it. Computer statistical packages cannot accommodate multivariate models. Therefore, since these are necessary for all models which are developed, a program has been developed which allows calculation of confidence limits for the present croaker model and similar multiple regression models which are to be developed for flounder and spot.

A paper was presented on the development of the croaker model at the 5th International Symposium on Forecasting. At that meeting, the model was discussed with several people from other disciplines that use forecasting. The general concensus was that we have followed standard econometric forecasting techniques and have reasonably manipulated the data to develop indices which could be used as variables. At a Forecasting Workshop on more advanced statistical techniques, discussions showed that the present model is actually quite sound. In fact, the resolution is more than adequate to assign levels of recruitment needed by management (i.e. failure, poor, average, good, and dominant year classes) (Figure 2).

A major problem with the VIMS trawl survey data base is that the gear has changed over the 30 years of collections. This was unimportant in the past as no attempts have been made to use the data base in its entirety, until now. Parallel studies of gear comparison are scheduled in 1985 that will address these questions. These studies, funded by the National Oceanic and Atmospheric Administration, were designed with these gear problems in mind.

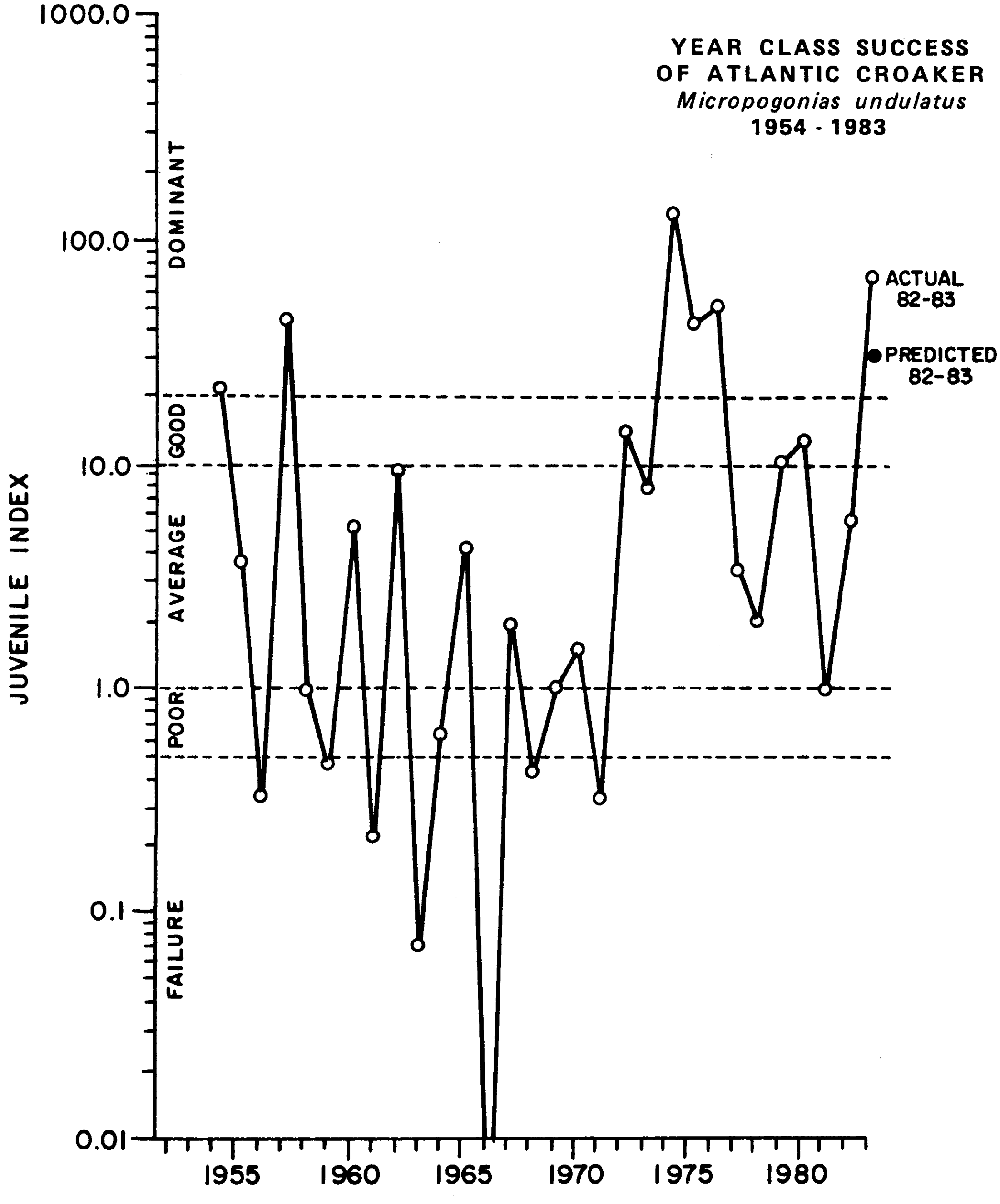
The VIMS trawl survey data have been checked for errors through 1979, and 1983-1984. Individual errors have been identified, and will be corrected by hand search of the original records. Members of the Fisheries Department are working on this and a new graduate student working on another project will also assist with the corrections.

The data base management programs (Fortran, SIR, SPSS) for preprocessing, checking and analyzing species data from the VIMS trawl surveys have been tested and modified for 1983 croaker. Because of the length of time that is necessary to correct and process each year's data, it may be necessary to use this same base and to enter flounder and spot collections and lengths. It must be noted that the current data base does not include hydrographic and station data, but only collections of croaker by river and date. It is anticipated that it will require the entire next project quarter just to correct the spot and flounder (not including striped bass and weakfish) data and incorporate it into the current SIR system.

Interim Findings

The basic model of life history stages in which recruitment potential may be affected was developed for croaker and will be applied to flounder

Figure 2

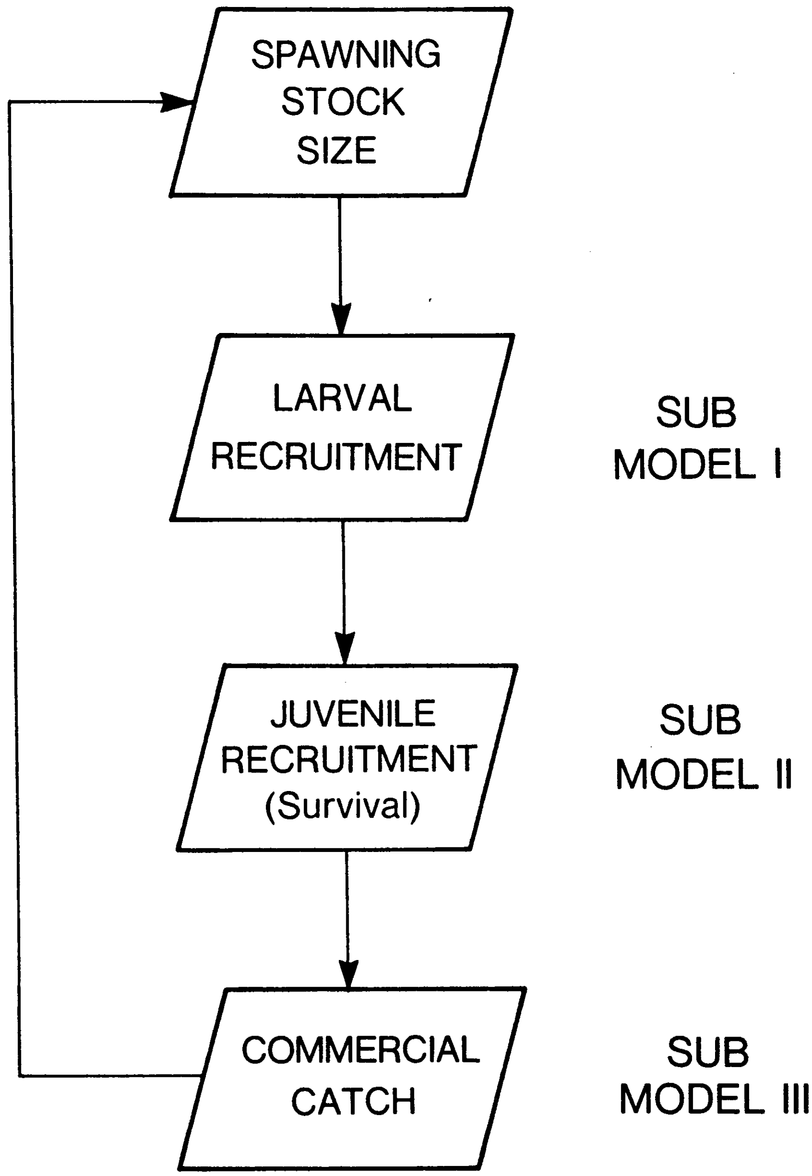

YEAR CLASS SUCCESS
OF ATLANTIC CROAKER
Micropogonias undulatus
1954 - 1983
JUVENILE INDEX
1000.0
100.0
10.0
1.0
0.1
0.01
DOMINANT
GOOD
AVERAGE
POOR
FAILURE
ACTUAL
82-83
PREDICTED
82-83
1955
1960
1965
1970
1975
1980

and spot (Figure 3). Larval recruitment in both species is likely to be affected by wind driven transport between the shelf spawning site and the estuarine nursery, while juvenile recruitment is dependent upon factors affecting survival within the estuary. Working hypotheses for flounder and spot have been developed.

Flounder are known to be affected adversely by cold temperatures, i.e. they are weakened by the cold and succumb to parasite infestations. Therefore, this is the most likely parameter to first quantify for this species. However, it is likely that an average temperature may not be appropriate for analysis of this relationship. Flounder, as well as croaker, may be affected by the magnitude and time scale of the change in temperature. Terms shorter than monthly and/or 3-monthly averages used for the croaker model will be investigated. Flounder are thought to spawn just outside the mouth of the Chesapeake Bay. The juveniles are affected by winter conditions within the Bay. Transport into the Chesapeake Bay versus Pamlico Sound is also considered important. Because adult stocks seem to advance northward yearly, it may actually be that good juvenile recruitment to warmer waters of North Carolina determines the success of the fishery in Virginia. However, since an index of juvenile recruitment in North Carolina is not readily available, wind transport data will be used as an indicator.

Spot recruit to the Bay later, after danger from cold temperatures. Although the literature says that spot spawn in the Mid-Atlantic Bight, analysis of seasonal bottom temperatures has lead me to conclude that the appropriate warm (16°C) water is not present during the winter spawning season. It is suspected that spot spawn at or south of Cape Hatteras and are transported into the Chesapeake Bay as juveniles. Another indication that spot do not spawn near the Bay entrance as croaker do, is that they are

Figure 3


SPAWNING
STOCK
SIZE
LARVAL
RECRUITMENT
SUB
MODEL I
JUVENILE
RECRUITMENT
(Survival)
SUB
MODEL II
COMMERCIAL
CATCH
SUB
MODEL III

much larger (20-25mm) than croaker (5-10mm) when entering the Chesapeake. It is hypothesized that the peculiar winter offshore/onshore transport regime south of Cape Hatteras keeps the fall/winter spawners offshore and out of the estuaries during the winters. There may be a mechanism which connects this physical regime with transport around Cape Hatteras and northward into the Chesapeake Bay, so coastal transport is the first area to investigate. The extremely efficient North Carolina long-haul seine fishery takes even the smallest fish within the Bay. Therefore, if recruitment of spot is to North Carolina, the post-recruitment mortality (i.e. due to fishing), may be significantly higher than mortality at the same time in the Chesapeake Bay. The result would be that transport into the Chesapeake Bay favors juvenile survival and resultant stock size of spot.

PROJECT 6

Striped Bass Egg Production and Vitality on the Pamunkey River Spawning Grounds with Special Reference to Acid Runoff

Purpose

Although factors affecting striped bass abundance are multiple and complex, current evidence suggests that principle variations in survival occur during early development. As a result, investigations intended to describe specific sources of mortality in the egg or larval stage may aid in understanding reasons for the perceived decline in abundance of striped bass stocks. Two factors recently raised as possible causes of the decline are reduced levels of egg viability (the percentage of developing eggs/embryos deposited) and increases in acidity of tidal freshwater; the first as a result of previous studies in North Carolina, the second as a result of preliminary findings in Maryland. Both require examination in Virginia. The objectives of the present study are: to estimate striped bass egg production on the Pamunkey River spawning grounds in spring 1985 and to assess striped bass egg vitality in relation to environmental variables (primarily pH, alkalinity and temperature).

Accomplishments

Tables 1 and 2 summarize field sampling activity during the spring spawning period. Weekly or twice weekly sampling along a 25-mile segment of the Pamunkey River was accomplished during the period 2 April - 15 May 1985. The spawning area was divided into 3-mile strata from which stations were randomly selected. Collections at each station included a stepped oblique bongo tow and measurements of surface and bottom temperature, salinity and

Table 1. Physical data and water volume filtered (m^3) from striped bass egg production survey of the Pamunkey River, spring 1985. Mean temperature (°C), salinity (o/oo) and dissolved oxygen concentrations (mg/l) are presented. Inclusive dates: 2-19 April 1985

Strata		Date 4/2	4/5	4/9	4/15	4/19
30-32	River mile	30	31	31	32	30
	Temp	12.5	14.1		15.3	17.0
	Sal	11.6			9.2	6.1
	DO_2	8.4	8.6		7.9	7.6
	Volume	117.5	247.0		97.6	63.7
33-35	River mile	34	35	33	35	33
	Temp	13.0	14.4	13.8	15.5	17.1
	Sal	8.8			7.9	3.1
	DO_2	7.7	8.3		7.8	7.7
	Volume	91.1	85.7	80.0	126.3	89.3
36-38	River mile	36	37	37	37	36
	Temp	13.3	14.4	13.7	15.3	17.1
	Sal	5.1		.4	6.7	1.1
	DO_2	8.0	9.9	9.1	7.9	8.4
	Volume	122.3	102.3	78.6	114.7	28.4
39-41	River mile	41	39	41	40	40
	Temp	13.5	14.7	13.7	15.2	17.2
	Sal	.6		.1	2.7	.3
	DO_2	9.2	9.2	9.3	8.4	8.6
	Volume	143.3	110.7	149.9	69.2	69.6
42-44	River mile	43	44	44	43	42
	Temp	13.6	14.5	13.6	15.0	17.4
	Sal	.23		.133	.7	.180
	DO_2	9.5	9.3	8.8	8.5	8.6
	Volume	78.9	136.2	99.2	69.2	102.8
45-47	River mile	45	46	47	45	46
	Temp	14.3	14.9	13.7	15.3	17.6
	Sal	.2		.1	.2	.1
	DO_2	9.4	9.8	8.9	8.9	9.1
	Volume	147.8	89.4	119.2	68.7	90.4
48-50	River mile	49	50	49	48	50
	Temp	13.7	15.9	14.1	15.0	18.1
	Salinity	.1		.1	.1	.1
	DO_2	9.7	10.2	9.1	9.16	8.9
	Volume	132.9	181.3	142.6	55.8	119.6
51-53	River mile	51	52	51	51	52
	Temp	14.5	16.0	13.8	15.9	18.9
	Sal	.3		.1	.1	.1
	DO_2	9.4	10.0	8.9	9.3	9.1
	Volume	99.6	115.0	125.0	131.6	

Strata		Date				
		4/2	4/5	4/9	4/15	4/19
54-56	River mile	55	55	55	56	56
	Temp	14.9	16.8	13.0	16.7	19.9
	Sal.	.1		.1	.1	.1
	DO_2	9.2	9.4	8.80	8.74	8.9
	Volume	84.6	78.8	89.8	80.0	

Table 2. Physical data and water volume filtered (m^3) from striped bass egg production survey of the Pamunkey River, spring 1985. Mean temperature (°C), salinity (o/oo) and dissolved oxygen concentrations (mg/l) are presented. Inclusive dates: 23 April - 14 May 1985.

Strata		Date 4/23	4/26	4/30	5/14
30-32	River mile				
	Temp				
	Sal	n.s.	n.s.	n.s.	n.s.
	DO_2				
	Volume				
33-35	River mile	33	34	34	
	Temp	20.3	20.4	22.0	
	Sal	7.8	5.2	6.3	n.s
	DO_2	6.7	6.6	6.3	
	Volume	70.5	39.0	126.8	
36-38	River mile	38	37	37	38
	Temp	20.4	20.4	21.4	23.8
	Sal	5.0	1.9	2.5	4.5
	DO_2	7.4	7.6	7.1	6.8
	Volume	112.5	50.3	50.9	94.4
39-41	River mile	41	39	39	41
	Temp	20.0	20.9	21.4	23.4
	Sal	1.3	.8	1.2	1.8
	DO_2	7.6	7.5	7.1	7.0
	Volume	72.3	93.5	186.7	72.8
42-44	River mile	43	42	44	44
	Temp	19.9	22.1	22.3	23.4
	Sal	.6	.3	.2	.7
	DO_2	7.9	8.5	9.2	7.3
	Volume	170.8	152.1	147.4	72.0
45-47	River mile	45	46	47	45
	Temp	20.1	23.0	22.3	23.5
	Sal	.3	.1	.1	.6
	DO_2	8.7	9.8	9.4	7.3
	Volume	144.7	165.9	370.7	93.3
48-50	River mile	48	50	50	49
	Temp	21.2	23.6	22.8	23.9
	Sal	.1	.1	.1	.1
	DO_2	9.5	10.5	9.2	7.2
	Volume	127.5	343.4	85.2	158.7
51-53	River mile	53	51	52	51
	Temp	22.0	23.0	23.4	24.2
	Sal	.1	.1	.1	.1
	DO_2	8.8	10.2	9.6	7.3
	Volume	181.9	160.5	56.5	113.9

Table 2 (Cont'd)

Strata		Date			
		4/23	4/26	4/30	5/14
54-56	River mile			56	56
	Temp			23.5	24.8
	Sal	n.s.	n.s.	.1	.1
	DO_2			6.8	6.5
	Volume			106.0	83.0

dissolved oxygen. Sampling yielded 74 plankton collections from which eggs and larvae of striped bass are currently being sorted and identified. To date approximately one-third of these collections have been processed. Eggs (n=156) and larvae (n=8) were present at three stations on 2 April indicating that spawning had commenced sometime prior to the initial cruise. Eggs were absent from collections on the last two sampling dates. Estimates of total egg production and calculations of female biomass required to produce observed egg deposition must await completion of sample processing.

Previous observations of salt penetration in the Pamunkey River (a pilot striped bass survey in 1980 and subsequent surveys in 1983 and 1984) reveal that, while tidally variable, furthest upstream penetration of salt rarely occurs above river-mile 35. Taking 0.5 o/oo salinity as the upper limit for fresh water, the Pamunkey is generally fresh above river-mile stratum 30-32 during spring months with average rainfall. Analysis of surface and bottom salinity data during April-May 1985 indicated a significant departure from normal salt distributions. In 1985, the Pamunkey was fresh above river-mile stratum 42-44 (Figure 1) reflecting a potential loss in total available spawning reach of 10-14 miles, a reduction which resulted from severe drought conditions throughout most of the eastern seaboard in late 1984 through spring 1985. Our assessment of the extent to which the loss of spawning area affected 1985 egg production will be forthcoming.

Examination of the potential effects of atmospheric acid deposition on the spawning grounds was conducted through the deployment of continuously recording pH meters (Beckman Model 31 equipped with Orion electrodes) on existing piers at two locations: unit 1 at the Olsson Estate near river-mile 38 and unit 2 at Lestor Manor near river-mile 52. These 24-hour surface pH

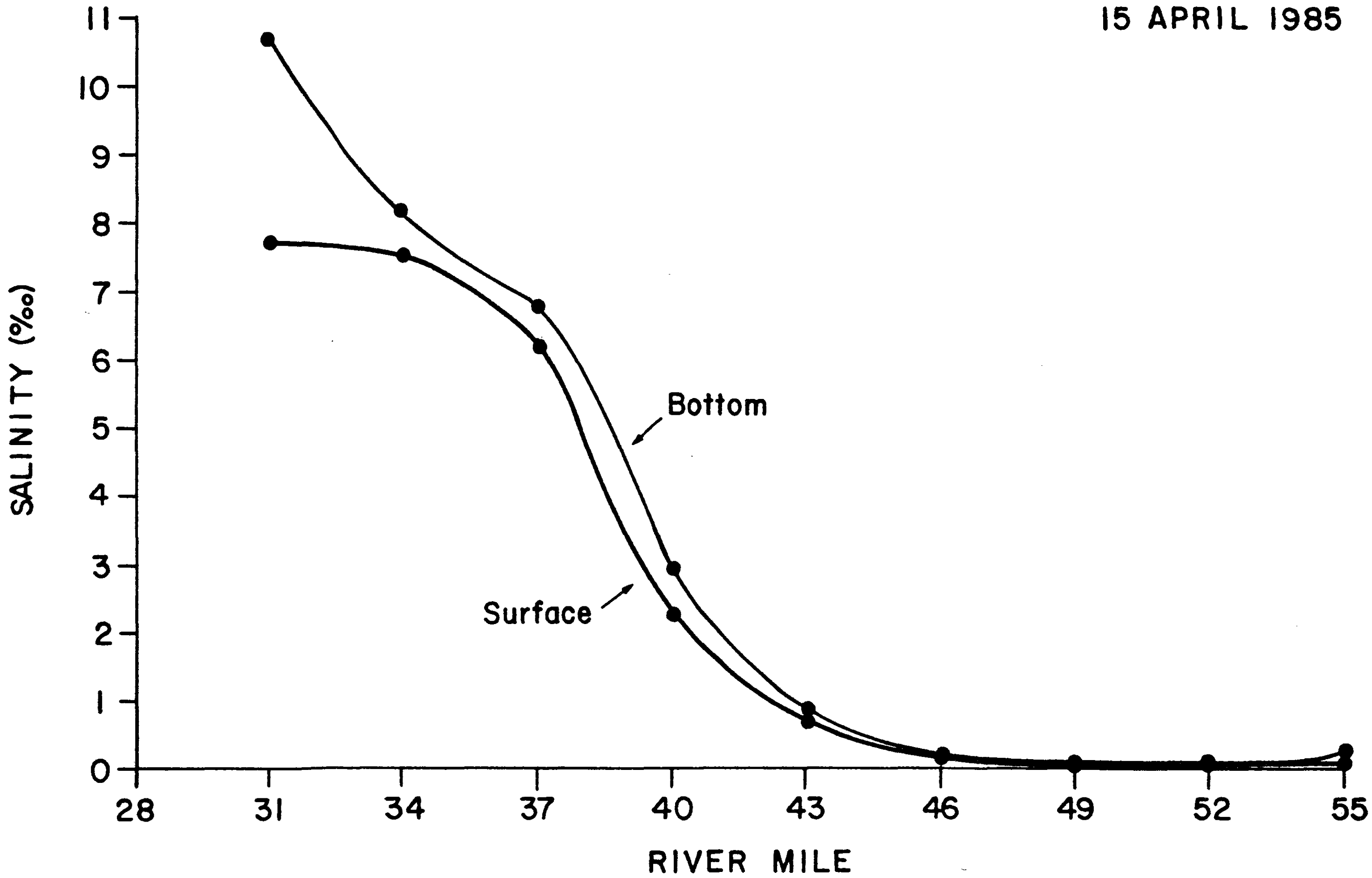


Fig. 1. Upstream penetration of salt on the Pamunkey River, April 1985.

data were supplemented by thrice weekly replicate alkalinity samples taken at each of the above locations during periods of pH meter calibration. As a result of the unusually dry conditions, unit 1 (river-mile 38) was under the influence of saline water and data resulting from that deployment were not appropriate to our objectives. Data resulting from the deployment of unit 2 (river-mile 52) are depicted in Figures 2 and 3. Throughout the 56-day period, pH values never varied significantly from normality (range 6.6 - 7.9) and alkalinity values never fell below .27 meq/l (range .27 - .70).

Collections designed to provide preliminary assessment of striped bass egg vitality resulted from the separate deployment of a Hansen egg net consisting of a bridled 1-meter plankton net fitted with 202 micron mesh Nitex. Samples (n = 10, Table 3) were obtained from vertical hauls (bottom to surface) of this gear at stations where eggs were present. In these preliminary trials, effort was focused on developing shipboard and laboratory protocol which would minimize capture damage. Replicate collections were either preserved in an histological fixative (samples 6 - 10 in Table 3 await processing) or examined unpreserved (samples 1-5, Table 2). Unpreserved samples were brought to the laboratory within 2-4 hours of collection where live vs. dead eggs were separated under a stereomicroscope (Observation 1, Table 3). Living eggs were sorted into developmental categories (Stage I - early cleavage - blastula; II - embryonic streak; III - tail-free stage; IV - late embryo). Dead eggs were categorized as Dead I (eggs opaque, protein contents denatured) and Dead II (eggs clear but containing little or no organized tissue). Both living and dead eggs were incubated for 12 hours, then re-examined, sorted and staged (Observation 2, Table 3). These data (Table 3) suggested: (1) initial sorting resulted in no classification error since differences between vitality estimates before

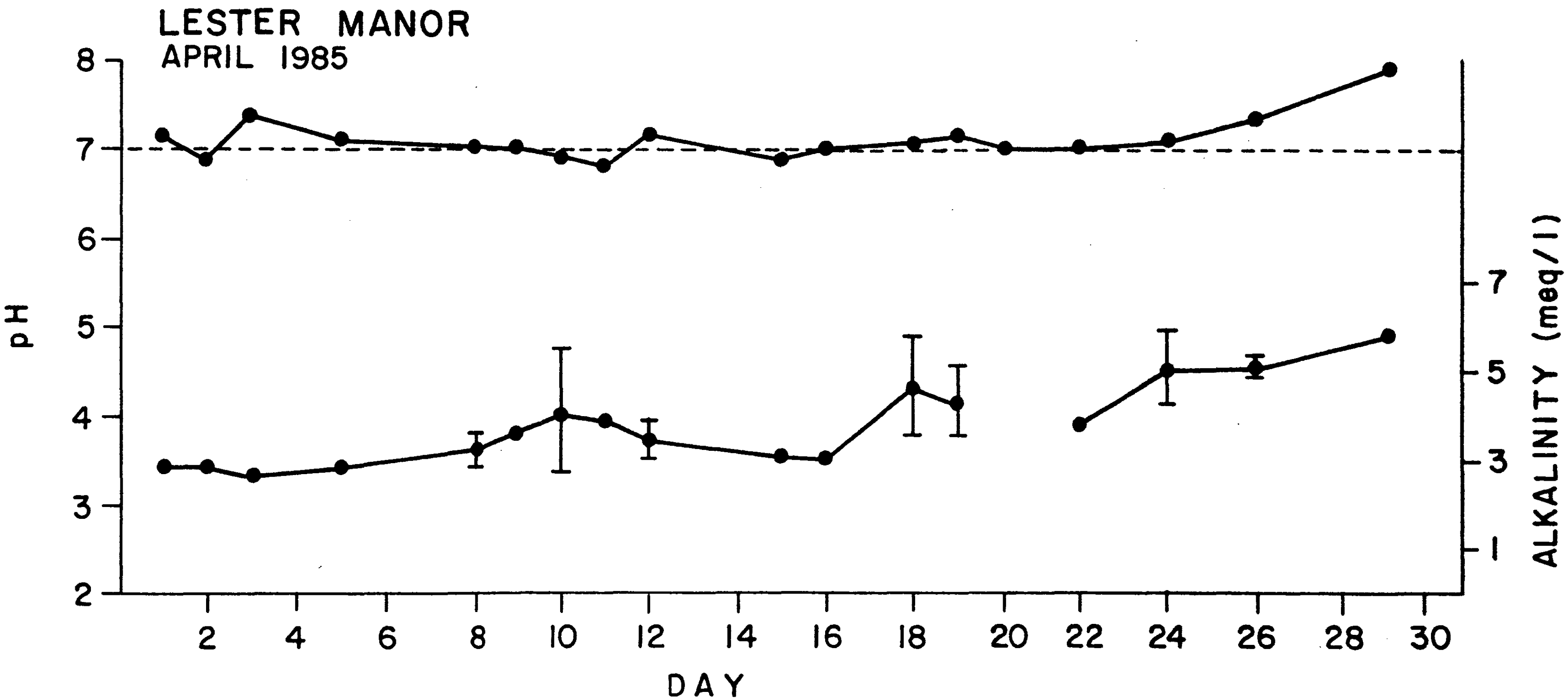


Fig. 2. Alkalinity (lower line) and pH (upper line) variability on the Pamunkey River, April 1985.

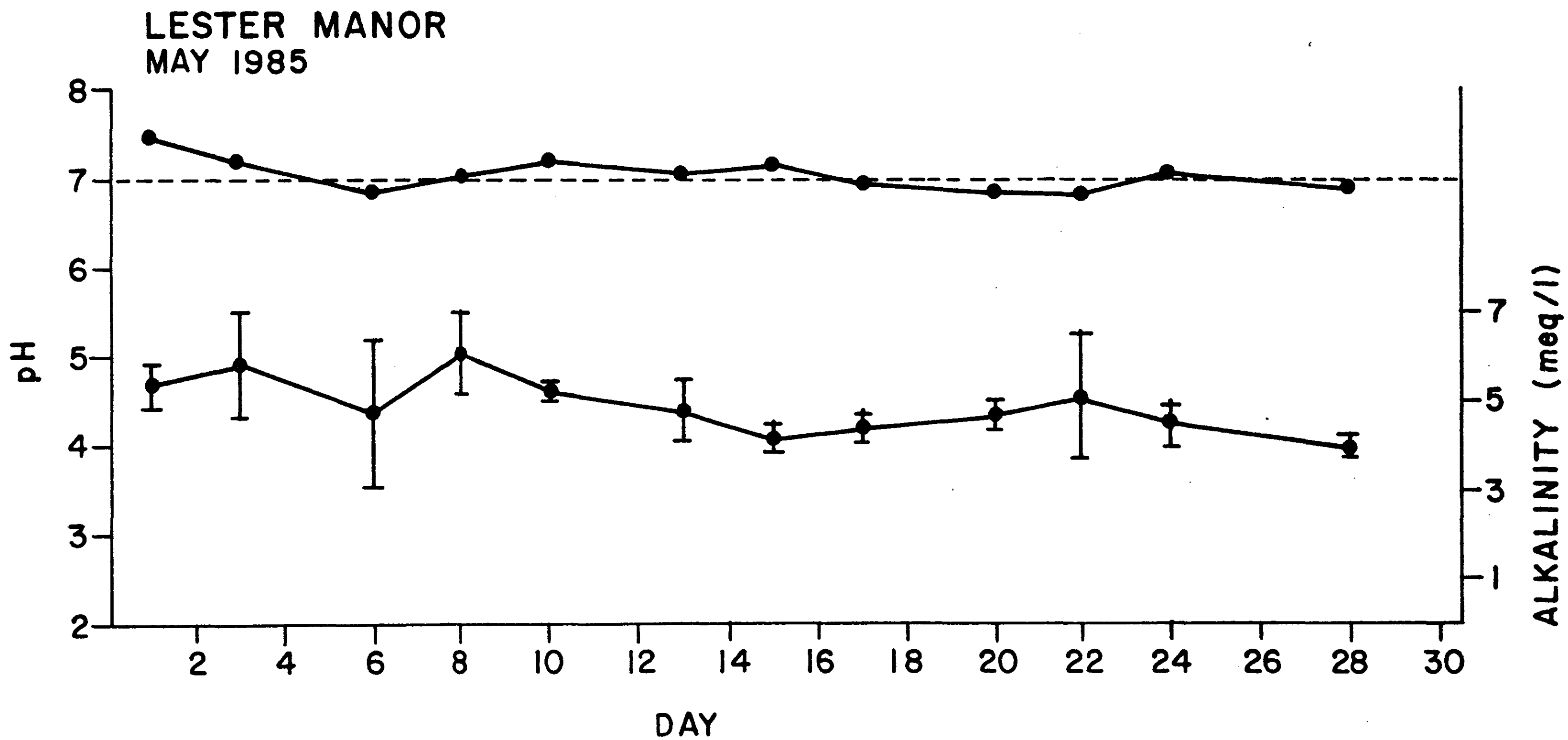


Fig. 3. Alkalinity (lower line) and pH (upper line) variability on the Pamunkey River, May 1985.

Table 3. Egg Vitality Data, Spring 1985

N	STA	DATE	OBS	TIME	I	II	III	IV	HATCH	DEAD I	DEAD II	TOTAL	% LIVE
1	P43	15 IV	1	1549	0	0	0	0	0	6	2	8	0
		16 IV	2	1000	0	0	0	0	0	6	2	8	0
2	P45	15 IV	1	1549	4	0	0	0	0	25	12	41	9.7
		16 IV	2	1000	0	0	4	0	0	25	12	41	9.7
3	P45	18 IV	1	1600	2	0	1	0	0	20	0	23	13.0
		19 IV	2	0900	0	0	0	0	3	20	0	23	13.0
4	P46	18 IV	1	1700	0	0	3	1*	0	9	1	14	28.5
		19 IV	2	0900	0	0	0	1*	3	9	1	14	28.5
5	P47	18 IV	1	1800	0	0	7	0	0	30	5	42	16.7
		19 IV	2	0900	0	0	0	2	5	30	5	42	16.7
6	P43	15 IV	1	1146		Preserved and unprocessed							
7	P45	15 IV	1	1247		Preserved and unprocessed							
8	P46	19 IV	1	1125		Preserved and unprocessed							
9	P47	19 IV	1	1200		Preserved and unprocessed							
10	P49	19 IV	1	1215		Preserved and unprocessed							

* Capture damage

and after incubation were not observed; and (2) between sample variability was great (range 0 - 28.5) but values never exceeded 28.5% viable eggs. The extent to which these values reflect post-collection handling damage must await processing of shipboard preserved replicates.

PROJECT 7

Chemical Poisons in Virginia's Tidal Waters - Fate and Effect of Polynuclear Aromatic Hydrocarbons

Purpose

The effort is designed to correct the current problem which exists with respect to detecting metabolites of polynuclear aromatic hydrocarbon compounds. Coupling or interfacing of a microbore high performance liquid chromatograph (HPLC) with a quadruple mass spectrometer (MS) which has the capability of electron impact as well as both positive and negative chemical ionization, is being accomplished. The milder separation conditions of HPLC should allow us to separate and identify many of the metabolites.

ACCOMPLISHMENTS

Mass Spectrometers

Both mass spectometers have been set up at their new location and are operating. The DuPont mass spectrometer, because it has been used continuously for several years, needed to be overhauled. New bearings have been installed in the turbomolecular pump by the manufacturer, the oil diffusion pumps have been cleaned and refilled with fresh oil, four vacuum lines have been cleaned or replaced, a new gas chromatograph has been installed and some of the electronic circuitry has been replaced.

Liquid Chromatography-Mass Spectrometry

The quadrupole mass filter was interfaced with a high performance liquid chromatograph (HPLC). To simplify these initial experiments no column was included in the system. Since the interface is constructed for the direct injection of solvent (DLI), the solvent must exit through a narrow, 5 μm aperture in a stainless steel diaphragm. To maintain a source pressure of approximately 300 microns (~ optimum for chemical ionization with acetonitrile/water as reagent gas) the turbomolecular pump is complemented by cryogenic pumping, using liquid N_2 as a coolant.

A jet of solvent droplets emerging coaxially with the probe must be observed before the interface probe can be inserted into the mass spectrometer source. Experience quickly indicated that maintenance of the jet over longer periods of time (>10 minutes) was a major problem, because of the closing of the aperture in the diaphragm by particles or fibers. The jet either stopped abruptly or it emerged at an angle. A first step to correct the problem was to filter the solvents and rinse all interface parts. We have now been able to maintain jets for approximately 45 minutes and appear to be making significant progress towards the reliable maintenance of jets with even longer duration.

The short periods of time during which the liquid chromatograph-mass spectrometer interface was functioning, however, demonstrated that very stable ion beams could be generated with mixtures of acetonitrile and water and that good control of the chemical ionization conditions could be achieved by regulating the injection pressure. So far, the capacity limit of the cryogenic pump has not been reached.

<u>Chemical Ionization Mass Spectrometry</u>

In order to be able to look for and identify metabolites of polynuclear aromatic hydrocarbons (PAHs) it is necessary to understand the mass spectrometric properties of such compounds. This information must be obtained using chemical ionization conditions because the DLI interface that is being used to couple the HPLC with the mass spectrometer requires the use of chemical ionization. Because of the difficulties encountered with the DLI interface, the initial experiments have been carried out using a direct insertion probe for the introduction of the compounds and methane as the chemical ionization reagent gas. The compounds tested included 1 and 2 naphthol, 9-hydroxy-fluorene, and a variety of hydroxylated benzo(a)pyrene (B(a)P) derivatives.

The spectra obtained can be explained on the basis of the thermal stability of the compounds being analyzed. The naphthols and monohydroxy-B(a)Ps all gave spectra with the $[M+H]^+$ ion as the base peak (Figures 1 and 2). As would be expected, the chemical ionization spectra of isomers [e.g. 1 and 2 naphthol, and 3, 7 and 9 hydroxy B(a)P] are indistinguishable and chromatographic retention time will be required for additional identification. Figure 3 shows the spectrum of 9-OH-fluorene. Again the $[M+H]^+$ is a major ion but here the base peak is the $[M-OH]^+$ ion. The fact that all these compounds give clean spectra with very little fragmentation means that for quantification of small quantities selected ion monitoring can be used to enhance sensitivity by as much as a factor of 10^3.

The B(a)P derivatives with more than one hydroxyl group are much more labile. Representative spectra are shown in Figures 4, 5 and 6 for B(a)P-9,10-dihydrodiol, 7,8,9-trihydroxy-7,8,9,10-tetrahydro-B(a)P and B(a)P-7,8,9,10-tetrahydrotetrol respectively. All these spectra are characterized by an intense ion at m/z 269. This ion is apparently the result of thermal degradation of these compounds to a monohydroxy derivative during the heating of the probe tip to volatilize the sample. The protonated molecular ions of the di and trihydroxy compounds are present at relatively low

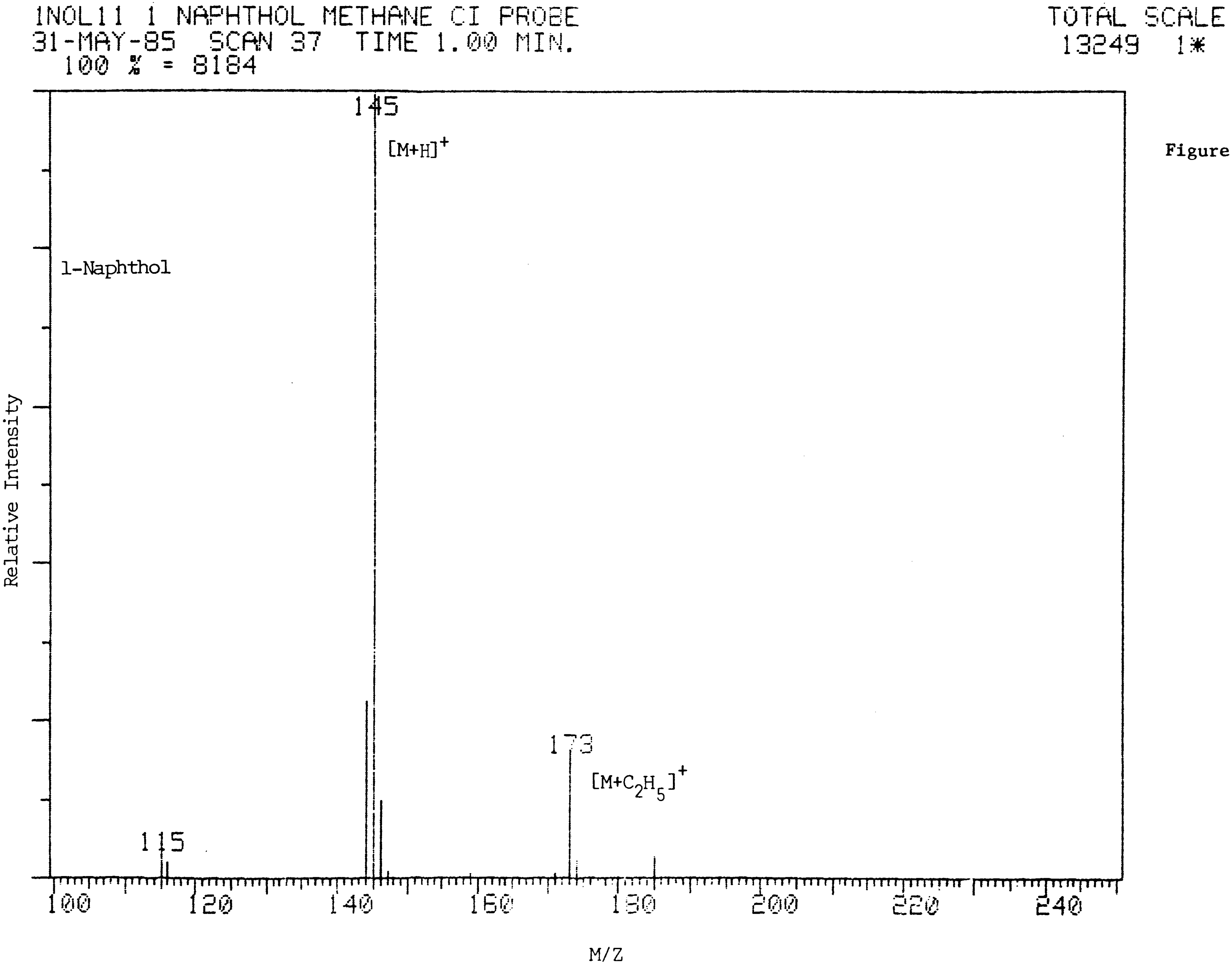
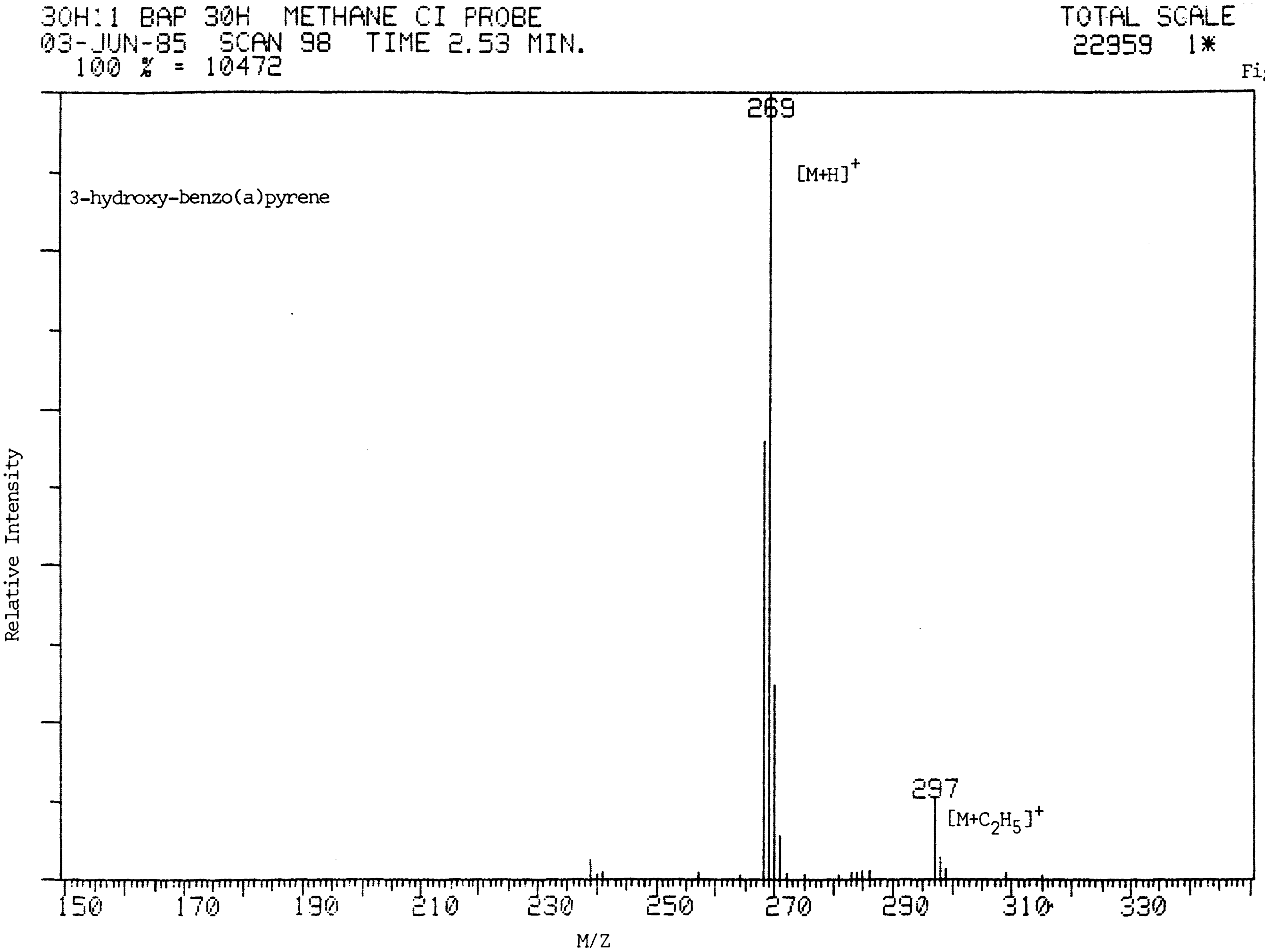
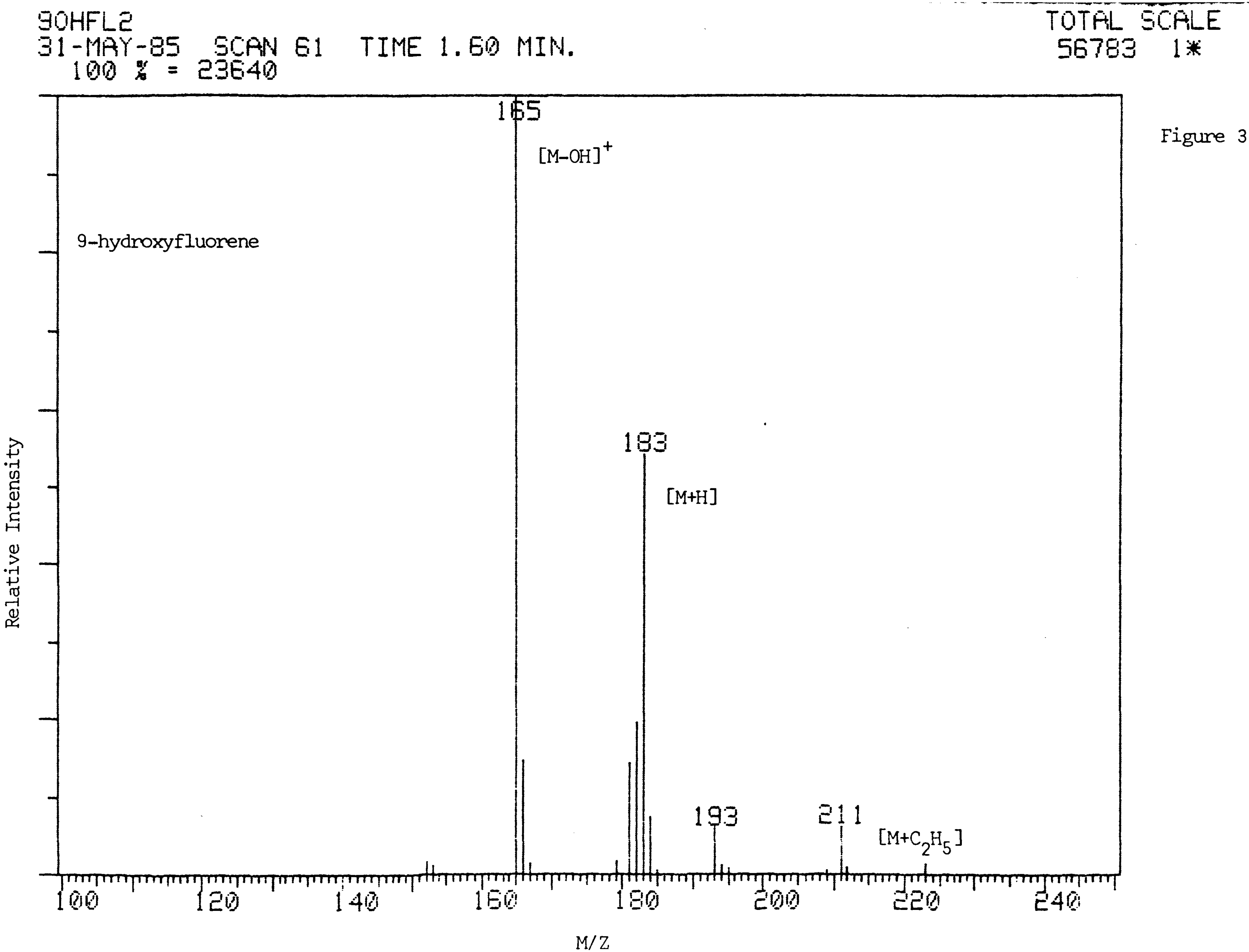

1NOL11 1 NAPHTHOL METHANE CI PROBE
31-MAY-85 SCAN 37 TIME 1.00 MIN.
100 % = 8184
TOTAL SCALE
13249 1*
145
[M+H]+
1-Naphthol
173
[M+C2H5]+
115
Relative Intensity
100
120
140
160
180
200
220
240
M/Z

Figure 1

Figure 2


30H:1 BAP 30H METHANE CI PROBE
03-JUN-85 SCAN 98 TIME 2.53 MIN.
100 % = 10472
TOTAL SCALE
22959 1*
3-hydroxy-benzo(a)pyrene
269
[M+H]+
297
[M+C2H5]+
Relative Intensity
150 170 190 210 230 250 270 290 310 330
M/Z



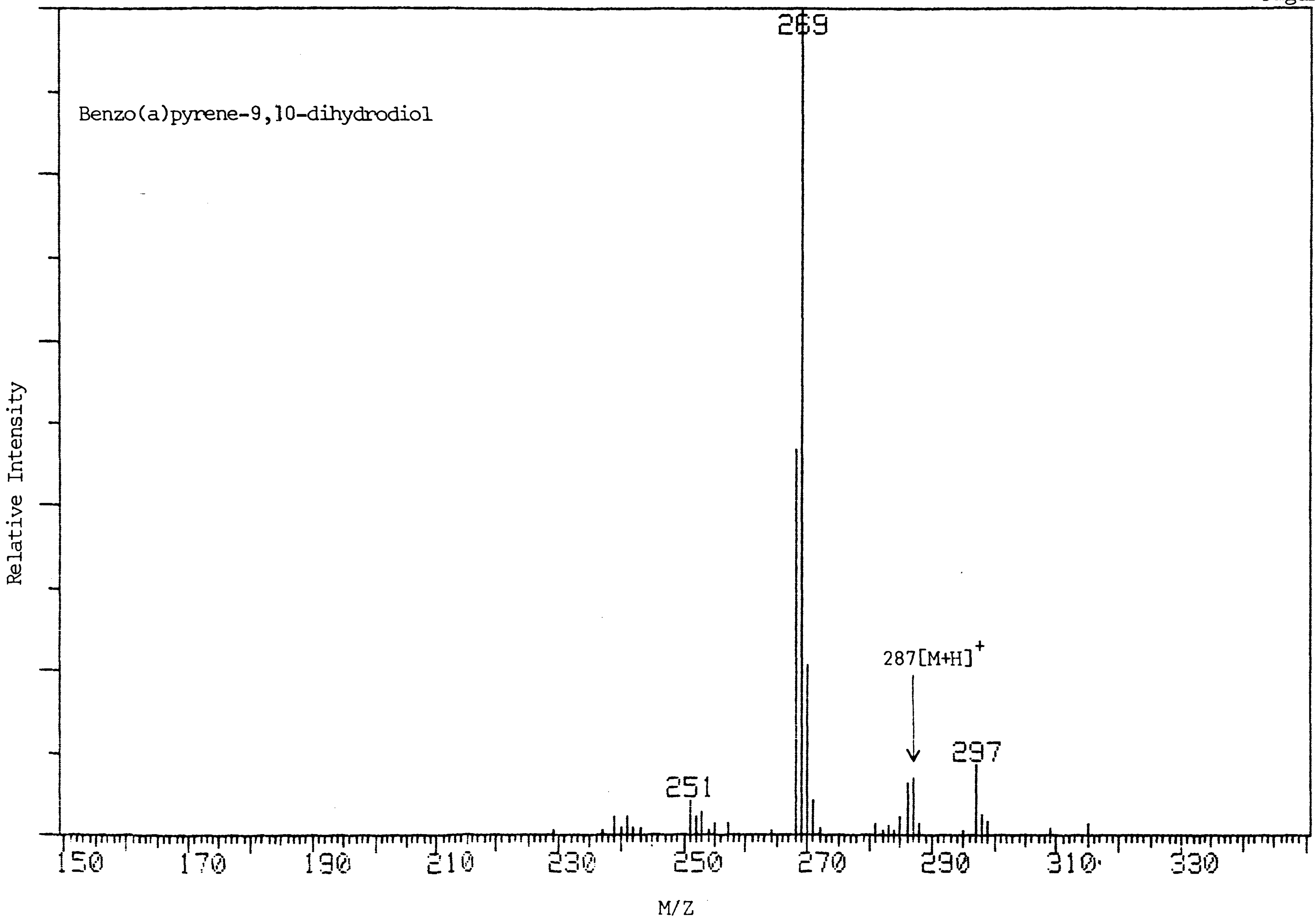
9OHFL2
31-MAY-85 SCAN 61 TIME 1.60 MIN.
100 % = 23640
TOTAL SCALE
56783 1*
165
[M-OH]+
9-hydroxyfluorene
183
[M+H]
193
211
[M+C2H5]
Relative Intensity
100
120
140
160
180
200
220
240
M/Z

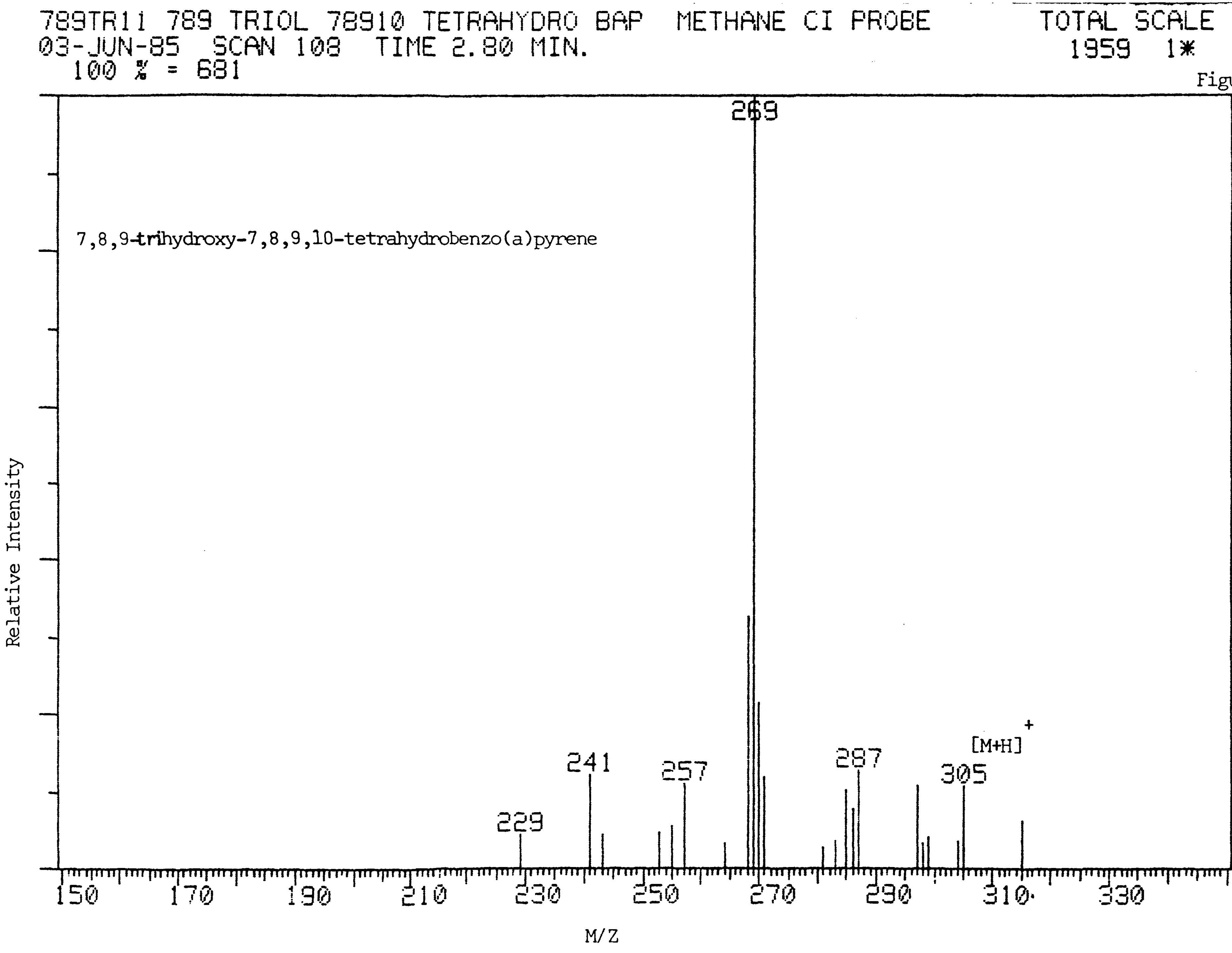
Figure 3

910DL1 BAP 910 DIOL METHANE CI PROBE
03-JUN-85 SCAN 89 TIME 2.32 MIN.
100 % = 9944

TOTAL SCALE
23469 1*

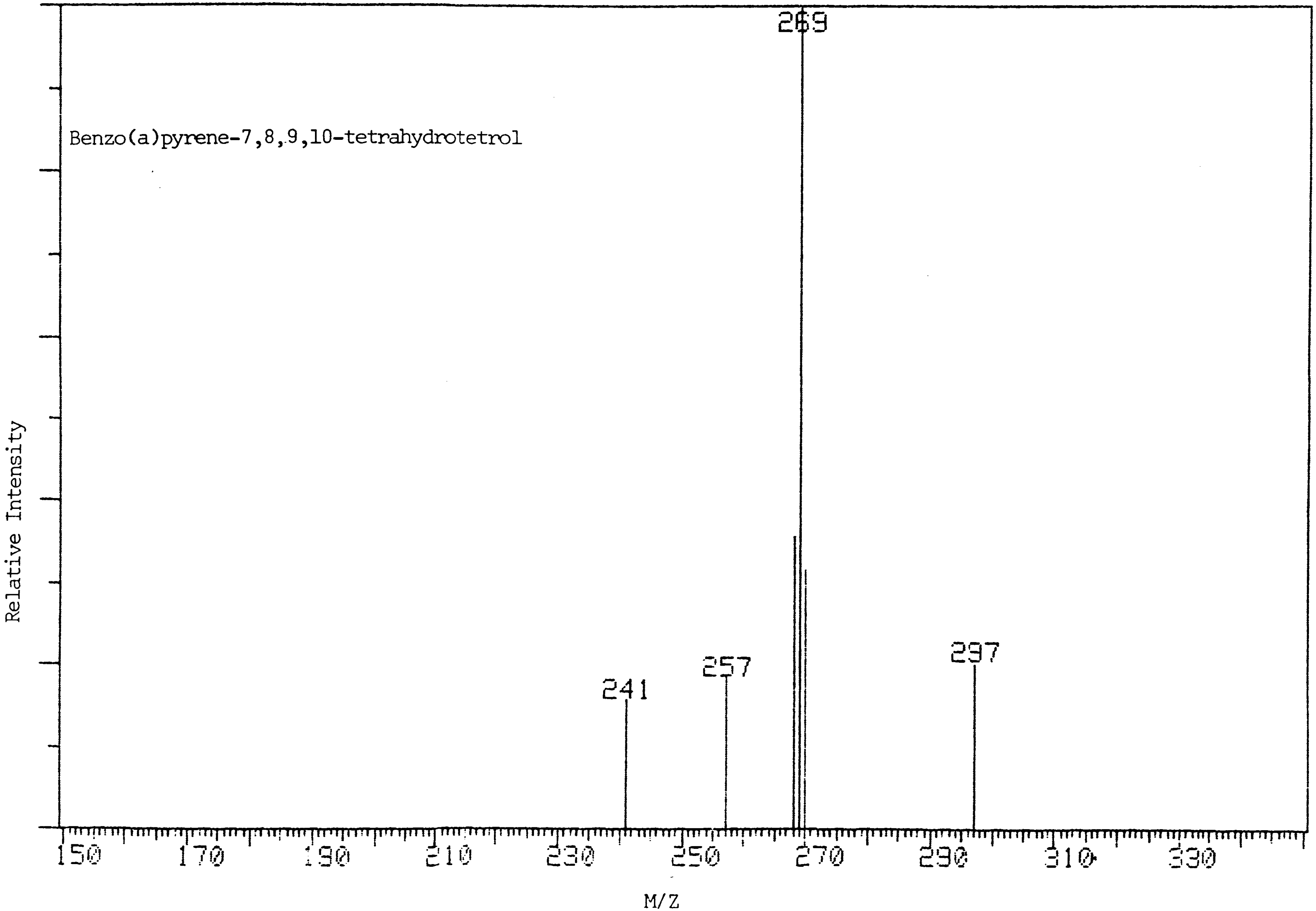
Figure 4




789TR11 789 TRIOL 78910 TETRAHYDRO BAP METHANE CI PROBE
TOTAL SCALE
03-JUN-85 SCAN 108 TIME 2.80 MIN.
1959 1*
100 % = 681
7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene
269
241
257
229
287
305
[M+H]+
150
170
190
210
230
250
270
290
310
330
Relative Intensity
M/Z
Figure 5

78910T 78910 TETRAHYDROTETROL BAP METHANE CI PROBE TOTAL SCALE
03-JUN-85 SCAN 127 TIME 3.27 MIN. 470 1*
100 % = 212

Figure 6



intensities. This ion is completely absent in the tetrahydroxy derivative a reflection of the greater lability of this compound. The thermal decomposition rather than mass spectrometric fragmentation with successive loses of water of these compounds is confirmed by two features of the spectra (a) the existence of m/z 297 which is the $[M+C_2H_5]^+$ of a monohydroxy B(a)P and (b) the presence of the m/z 269 ion in the tetrahydrotetrol spectrum because if fragmentation by successive losses of water from this molecule had occurred this would have given an m/z 267 ion.

The problem of thermal instability may be avoided when the direct liquid interface is fully operational because the compounds will not have to be volatilized from a surface prior to entering the ion source, but will remain in the gas phase as the solvent is evaporated from the droplets entering the desolvation chamber ion source. It is therefore anticipated that $[M+H]^+$ ions will be enhanced and that intact conjugated (glucuronides and sulphates) forms of the metabolites may be analyzable. The latter is an exciting prospect as the analysis of such compounds without prior hydrolysis has only rarely been achieved.

High Performance Liquid Chromatography

Reverse phase HPLC of different metabolites of PAHs, e.g. benzo(a)pyrene, naphthalene and fluorene was performed using C_{18} -bonded silica columns with detection by UV absorbance (λ = 254 nm) and fluoresence (λex=389 nm, λem=418nm). The most common mobile phase was acetonitrile/water: 75/25. Potential metabolites being studied are B(a)P-3-glucuronide, 9-OH B(a)P, B(a)P-9-sulphate, B(a)P 4,5-dihydrodiol, B(a)P-7,8-dihydrodiol, B(a)P-9,10-dihydrodiol, B(a)P-7,8,9,10-tetrahydro- 7,8,9-

triol, B(a)P-7,8,9,10-tetrahydrotetrol, 1-naphthol, 2-naphthol and 9-hydroxyfluorene. All of these compounds eluted as well defined peaks with retention times varying between 0.9 to 2.6 minutes while 7-hydroxy-B(a)P eluted at 3.4 min (mobile phase was programmed from aretonitrile/water: 75/25 to 100% acetonitrile over 10 minutes). B(a)P-3-glucuronide and B(a)P-9-sulphate as well as 1-naphthol, 2-naphthol and 9-hydroxyfluorene eluted close together under the conditions used. This study was carried out using both analytical and microbore columns.

A range/response relationship study to check the linearity of the UV detector and the fluorometer gave linear plots for -log (range) versus log (height). The responses were found to be linear and gave correlation coefficients of 0.97 and 0.99 respectively.

Determination of the number of theoretical plates/meter (40,000) for two microbore columns coupled together shows that the coupling of microbore columns is approximately additive.

Recovery experiments for different metabolites from grey trout (Cynoscion regalis) and bluefish (Pomatomus saltatrix) bile collected from the York River were undertaken. Fish bile was spiked with metabolite standards and extracted with ethyl acetate (by vortexing). The solvent was evaporated under nitrogen, the residue dissolved in acetonitrile and analyzed by HPLC. The recovery values varied from 25% (for 2-naphthol) to almost 100% for B(a)P-9-sulphate and B(a)P-7,8,9,10-tetrahydro-7,8,9-triol but changed from day to day even with the same methodology. A possible cause of this variability may have been an inability to recover the compounds from the glass or their decomposition on the glass. To examine this possibility the effect of evaporation to dryness was compared with incomplete evaporation that left approximate 100 µl of liquid residue. The latter gave better recoveries though the values still varied. The improved recoveries can be best demonstrated in the cases of 1-naphthol (39% improved to 73%), 2-naphthol (25% improved to ~100%) and 7-hydroxy benzo(a)pyrene (25% to 90%). Henceforth, the method of evaporation to leave a liquid residue was adopted.

It is planned to fractionate the environmental samples on an analytical HPLC column followed by injection of collected fractions on a microbore column interfaced with a mass spectrometer. The recovery of sample from the analytical column is thus critical. In an initial experiment collection of B(a)P-9-sulphate from reverse phase analytical HPLC column and reinjection gave 87% recovery.

Because of the difficulties in obtaining reproducible extraction efficiencies using the liquid:liquid procedure a liquid: solid extraction method has been investigated. Solid phase extraction of bile spiked with PAH metabolites was done by first loading a Fisher Prep Sep (C_{18}) extraction column with the spiked bile and then eluting the adsorbed compounds with methanol. Both polar (e.g. sulphate and phosphate esters and glucuronides) and less polar (e.g. phenols, dihydro diols, tetrahydrotriols, tetrahydrotetrols) can be extracted by this method.

Initial experiments on bile samples from grey trout from the highly contaminated Elizabeth River were made using this methodology. Both, aqueous and organic fractions were analyzed. A solvent program (100% water to 100% acetontirile in 20 minutes and back in 15 minutes) showed a number of peaks in both the aqueous residue and methanol extracts for both control and Elizabeth River fish bile. A doublet appears between 1.8 to 2 min while a multiplet of peaks as a poorly resolved envelope appears between 9 and 14 minutes. In one experiment, methanol extract showed particularly intense peaks for Elizabeth River grey trout bile (Figure 7), possibly due to PAH contamination and metabolism when compared with the control bile (Figure 8). Additional study is necessary to verify this interpretation.

Dr. Ashok Deshpande, an organic chemist with extensive experience in organic synthesis, has joined the Chemistry Department. Prior to VIMS, he was doing research at the University of Nebraska Medical Center.

The most important immediate problem to be solved is to get the LC-MS interface working reliably. Without continuity in the liquid jet, it will be difficult if not impossible to get useful data. The solving of this problem is currently a major effort in this project.

Another problem of importance concerns the stability of the metabolites. If it should turn out that quasimolecular ions are missing for some of these compounds, other approaches must be found. The composition of the chemical ionization gas may have to be changed, the compounds may first have to be derivatized or fast atom bombardment (FAB) ionization may have to be used.

Future work using HPLC will involve 1) recovery experiments from bile, 2) recovery experiments for collection of fractions from the analytical column and reinjection onto analytical/microbore columns, 3) optimizing conditions for the use of Fisher Prep Sep extraction columns, 4) analysis of Elizabeth River fish bile for different species and location, 5) collection of fractions from Elizabeth River fish bile from an analytical column and reinjection on microbore columns, comparison to obtain well resolved peaks

FIGURE 7

June 3, 1985
Elizabeth River Gray Trout Bile (MeOH Extract)

Column: Perkin Elmer Analytical HC-OD5

Mobile Phase: 100% H_2O $\xrightarrow{\text{20 min}}$ 100% CH_3CN
$\xleftarrow[\text{15 min}]{}$

Fluorometer (detector): $\lambda ex = 380$ nm
$\lambda em = 418$ nm

Range: 0.1 μA

Sample Injection: 50 μL

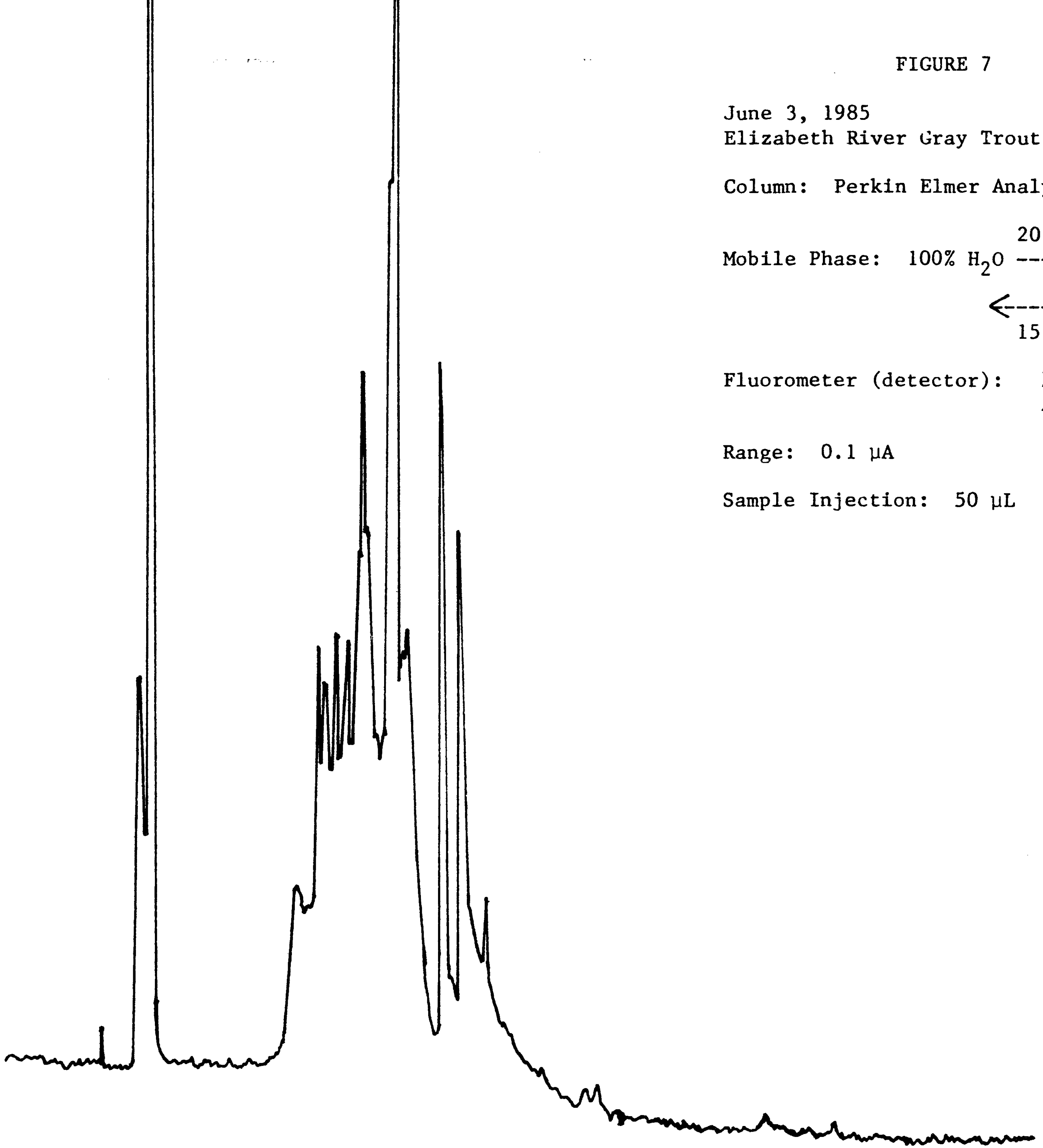


FIGURE 8

June 3, 1985
Control Gray Trout Bile (MeOH Extract)

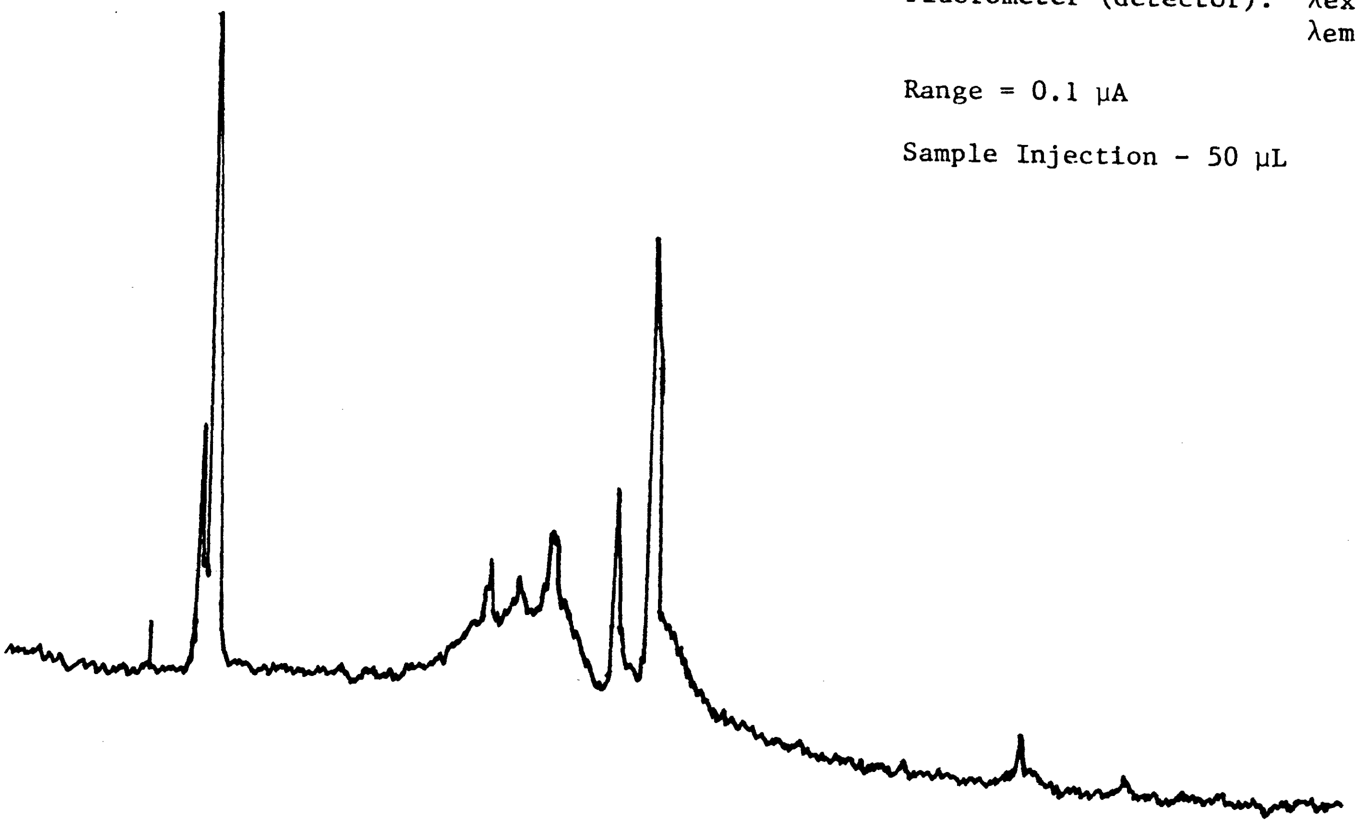
Column: Perkin Elmer Analytical HC-OD5

Mobile Phase: 100% H_2O $\xrightarrow{20\ min}$ 100% CH_3CN
$\xleftarrow[15\ min]{}$

Fluorometer (detector): λex = 380 nm
λem = 418 nm

Range = 0.1 μA

Sample Injection - 50 μL



for retention times with standards and obtaining mass spectra for structural confirmation, and 6) study HPLC of quinones.

During the HPLC phase of this project the main technical difficulty encountered was that the seals on the high pressure pistons of the HPLC pumps leaked. Perkin Elmer representatives have assured us of good service and have repaired the pumps twice so far.

Continuing Effort

It is anticipated that prior to July 1986 a number of identifications of metabolites will have been made by using a combination of high performance liquid chromatography and mass spectrometry. Because of the highly complex nature of the contaminants that are present, one of the goals of the July 1986 - June 1988 period is to continue the identification process. By this time, it is contemplated that changes in the interface between the LC and the MS will be desirable. One of the goals, for example, is to eliminate the cyrogenic pump, since the need for a constant supply of liquid nitrogen is impractical and too inflexible. Additional changes concern modifications to allow source tuning and calibration under LC-MS conditions.

The major effort will, however, concern the correlation of the disease state of fish with the relative amounts of individual PAH metabolites. This effort will be conducted on both an intra- and interspecific level. In the former case the intention will be to correlate the extent of disease (e.g. integumental lesions, cataracts) with the extent of PAH metabolism. In the latter case (interspecific) the intention will be to see if differences can be found between the matabolites produced by those species of fish that display disease symptoms and those species that are apparently resistant to the affects of the pollutants.

Attaining a greater degree of understanding of disease processes associated with the pollution will assist in the development of a strategy by which the effect of the pollutants on biota will be minimized. The estimated cost of this continuation effort is $290,000.

PROJECT 8

Early Warning System for Pollutants in Seafood

Purpose

The objectives of this research initiative were to integrate and supplement elements of on-going monitoring and research studies to produce a comprehensive monitoring programs for potentially toxic organic chemicals in Virginia seafood.

Accomplishments (funded specifically by this initiative)

Equipment

The primary GC, a Tracor 560 with a Hall Electrolytic Conductivity detector, is functioning with consistant efficiency. The GC has been converted to accept a fused silica capillary column. An SE-54 column has been fitted and a second column is on hand for backup.

The GC has been interfaced with the HP3354 Data System for data acquisition and processing. The computer is extremely critical for calculating relative retention indices, concentrations and assigning identifications.

Sampling

Samples of bluefish, grey trout, croaker and spot were collected during the summer and fall of 1984. Samples of oysters, crab, clams and fish have been or will be collected during the spring, summer and fall of 1985.

Sampling will proceed on a monthly frequency through the summer and fall of 1985.

Analysis

The analytical techniques required for an extensive study of chlorinated hydrocarbons in Virginia's seafood are complex and require the coordination of computer programming, analytical instrumentation and up to date research information. If only PCBs, chlordane and toxaphene were analyzed, 209 possible congeners for PCBs, 177 compounds for toxaphene and 50 compounds for chlordane could be present. To analyze for these compounds in addition to 35 other pesticides and industrial compounds requires precise operation of analytical equipment, state-of-the-art analytical techniques and close tolerance coordination with data acquisition equipment.

A review of research literature has provided identification of most of the PCB congeners (Albro, et al., 1979 & 1981; Ballschmiter, et al., 1980; Kozloski, 1985; Mullin, et al., 1984). Although some inconsistencies exist between researchers, most compound structures and elution orders of the PCB congeners are consistent. Thirty-two PCB congener standards have been acquired to help resolve the identities of some of the compounds in question.

The literature is not as complete for chlordane or toxaphene. General molecular formulae are known for the compounds in toxaphene. Only a few of the compounds in chlordane have been identified.

The most commonly accepted procedure for quantitation of mixtures is to match peaks with the standard mixture and quantitate. This method will be used for chlordane and toxaphene. Quantitation of PCB concentrations will be considerably more refined than for toxaphene and chlordane. Individual congener quantitation for PCBs will be used providing a more accurate concentration measurement.

Analysis of some of the samples from '84 and '85 indicate two possible trends in PCB concentrations of Virginia's seafood. The first trend is, as would be expected, that PCB concentrations appear to increase through the summer into early fall. The second trend is that certain PCB congeners are more prevalent than others. Some of these isomers are in significantly higher concentration with respect to back ground PCB level. Many of the prevalent congeners present in existing samples could be used as indicator of total PCB content as suggested in a draft paper by Kerkhoff, 1985. Further analysis will reveal which congeners are of most significant concern analytically and toxicologically.

Extracts are being archived for further analysis for chlorinated phenols and herbicides. Analysis of these compounds requires completely different sample preparation including derivatization. The analysis for these compounds will begin in September.

Analyses will be more frequent and extensive during 1985 since much of the analytical development has been completed.

Continuing Effort

Until this project there was no systematic monitoring program of Virginia's seafood to determine chlorinated organic contaminant levels. The results from the present two-year monitoring efforts will give a good picture of the status of such pollutants in our marine products. However, a monitoring system is effective only if it continues. What we will have after the present effort is a "snapshot" of existing conditions. To help guarantee that new toxicants do not reach harmful concentrations and to determine existing trends, the program must continue. The objectives of the continuing effort will be to extend the monitoring of chlorinated organic chemicals in Virginia's seafood and supply the State Water Control Board and Health Department with the data required to keep abreast of contaminated burdens in Virginia's fish, shellfish and crabs. If necessary the states' regulatory agencies can take appropriate actions to control the sources of the contaminants and hopefully avoid the unfortunate social, economic and biological effects of a contaminated resource. The cost of this continuation for the 86-88 biennium is estimated to be $85,000.

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PROJECT 9

Re-establishment of Submerged Aquatic Vegetation Initiative

Purpose

Beds of submerged aquatic vegetation (SAV) were once common features of the many shoal areas along the tributaries and mainstem of the Chesapeake Bay. The recent decline of these important systems during the last 15 years has generated much interest at both the state and federal level as to the causes of the decline, the effects of this decline on the productivity of these denuded areas and the feasibility of replanting these areas.

The overall goals of the initiative work have been to attempt to re-establish SAV at selected sites in the lower bay and by doing so to investigate the factors that affect the growth and distribution of SAV.

Accomplishments

Transplant Survivorship

The original transplant effort conducted from September 26 through November 15, 1984 resulted in 15 acres being planted at 10 sites in five different river systems (York (4.5 acres), Piankatank (4 acres), East (0.5 acres), Rappahannock (4 acres), and Coan Rivers (2 acres)). One-half of the 15 acres were fertilized with a slow release fertilizer (osmocote) and each site, except the Gloucester Point and East River sites, had both a fertilized and unfertilized area. At each site, replicate sub-plots were established to assess the survivorship of the individual units of eelgrass.

Survivorship plots were monitored during three different periods following the transplant effort: November - December, April and June. Plots were monitored for presence-absence during these three sampling periods, while areal spread of the individual surviving units was measured in April and June and shoot density per plot recorded in June (Tables 1 and 2; Fig. 1).

Survivorship rates for all sites in April was 74% but 89% when the Mumfort Island and Clay Bank sites are excluded. The survivorship decreased to 50% for all sites in June and 61% when the two York River sites are excluded. The poor survivorship rates at Mumfort Island and Clay Bank sites may be attributed to low initial rates of success following the transplant effort and ice scouring during the winter months. Several other sites had low percentages of units present by the June period. Both fertilized and unfertilized areas at the Healy Creek site exhibited poor survival. The large loss between April and June may be attributed to the uprooting of many plantings by ray or crab activity.

The Queens Creek unfertilized plot 1 had no remaining units by April. Considerable sand movement at the site suggests that most plantings were simply washed out or covered with sand. Plot 2 was located on the opposite side of the creek channel along a gradient of water depth ranging from 0.3 m at the inshore row to 1.0 m(MLW) at the offshore row. Sediments were much finer than at site 1. Survival here varied but, overall, was significantly higher than plot 1. By June units along the inshore row were generally missing or very sparse while units at the deeper rows were much more robust. Large holes were present at plot 2 in June and were attributed to cownose ray activity, although none of the units appeared to have been lost by the activity.

The Coan River site had much lower survivorship in June compared to April. Inspection of the site in June found numerous, wooden stakes

Table 1. Percent of eelgrass units surviving initial transplant effort. Estimates made on counts in replicate subplots at each site.

		Date Planted	Date Checked/% Units Surviving		
			Nov/Dec	Apr	Jun
YORK RIVER					
-Gloucester Point	unfert plot 1	Nov 9	100%	80%	76%
	unfert plot 2	Nov 9	100%	83%	80%
-Mumfort Island	unfert plot 1	Oct 3, 4	73%	53%	33%
	unfert plot 2	Oct 3, 4	73%	44%	33%
	fert plot 1	Oct 5	67%	33%	33%
	fert plot 2	Oct 5	83%	25%	5%
-Clay Bank	unfert plot 1	Sep 26,27	92%	39%	8%
	unfert plot 2	Sep 26,27	90%	36%	2%
	fert plot 1	Oct 12	37%	19%	5%
	fert plot 2	Oct 12	64%	28%	0%
PIANKATANK RIVER					
-Queens Creek	unfert plot 1	Oct 9	94%	50%	0%
	unfert plot 2	Oct 9	100%	97%	89%
-Burton Pt.	unfert plot 1	Nov 15	100%	100%	89%
	unfert plot 2	Nov 15	100%	94%	58%
	fert plot 1	Oct 11	100%	77%	67%
	fert plot 2	Oct 11	100%	92%	80%
-Healy Creek	unfert plot 1	Nov 13	100%	94%	6%
	unfert plot 2	Nov 13	96%	81%	8%
	fert plot 1	Nov 13	100%	81%	53%
	fert plot 2	Nov 13	100%	72%	40%
MOBJACK BAY					
-East River	fert plot 1	Oct 19	100%	100%	100%
	fert plot 2	Oct 19	100%	100%	100%
RAPPAHANNOCK RIVER					
-Parrott Island	unfert plot 1	Oct 16	97%	94%	55%
	unfert plot 2	Oct 16	100%	92%	25%
	fert plot 1	Oct 18	100%	94%	64%
	fert plot 2	Oct 18	100%	94%	44%
-Morattico	unfert plot 1	Oct 23	92%	94%	89%
	unfert plot 2	Oct 23	94%	94%	97%
	fert plot 1	Oct 25	97%	100%	100%
	fert plot 2	Oct 25	100%	100%	100%

Table 1 (concluded)

		Date Planted	Date Checked/% Units Surviving		
			Nov/Dec	Apr	Jun
POTOMAC RIVER					
Coan River	unfert plot 1	Nov 1	100%	78%	25%
	unfert plot 2	Nov 1	100%	56%	44%
	fert plot 1	Nov 6	100%	81%	47%
	fert plot 2	Nov 6	100%	75%	47%

Table 2. Aerial dimensions of transplanted units in April and June, 1985, and shoot density in June compared with initial estimates from transplant period in the fall, 1984. *Estimated, based on counts made from fert plot 1.

		Initial Area (cm^2)	Initial Shoot #	April Area (cm^2)	June Area (cm^2)	June Shoot #
YORK RIVER						
-Gloucester Point	unfert plot 1	150	31	172	453	80
	unfert plot 2	150	31	227	485	84
-Mumfort Island	unfert plot 1	150	31	236	902	87
	unfert plot 2	150	31	88	575	93
	fert plot 1	150	31	314	1146	218
	fert plot 2	150	31	177	736	122
-Clay Bank	unfert plot 1	150	31	107	very few present	
	unfert plot 2	150	31	130	sparse shoots	
	fert plot 1	150	31	389	" "	"
	fert plot 2	150	31	98	" "	"
PIANKATANK RIVER						
-Queens Creek	unfert plot 1	700	123	461	0	0
	unfert plot 2	700	123	870	2146	644
-Burton Pt.	unfert plot 1	700	123	553	1358	81
	unfert plot 2	700	123	461	1682	115
	fert plot 1	700	123	609	2899	858
	fert plot 2	700	123	709	3462	1240
-Healy Creek	unfert plot 1	700	123	532	very few left	
	unfert plot 2	700	123	475	" "	"
	fert plot 1	700	123	675	1830	356
	fert plot 2	700	123	566	1775	424
MOBJACK BAY						
-East River	unfert plot 1	700	123	959	2075	870
	unfert plot 2	700	123	1004	2126	733
RAPPAHANNOCK RIVER						
-Parrott Island	unfert plot 1	700	123	537	1565	397
	unfert plot 2	700	123	282	350	6
	fert plot 1	700	123	442	1209	407
	fert plot 2	700	123	341	939	331
-Morattico	unfert plot 1	700	123	1223	992	174
	unfert plot 2	700	123	1302	1213	277
	fert plot 1	700	123	1502	3285	1583
	fert plot 2	700	123	1381	3715	1583*

Table 2 (concluded)

		Initial Area (cm^2)	Initial Shoot #	April Area (cm^2)	June Area (cm^2)	June Shoot #
POTOMAC RIVER						
-Coan River	unfert plot 1	700	123	762	1201	206
	unfert plot 2	700	123	598	1661	434
	fert plot 1	700	123	530	1527	215
	fert plot 2	700	123	406	857	102

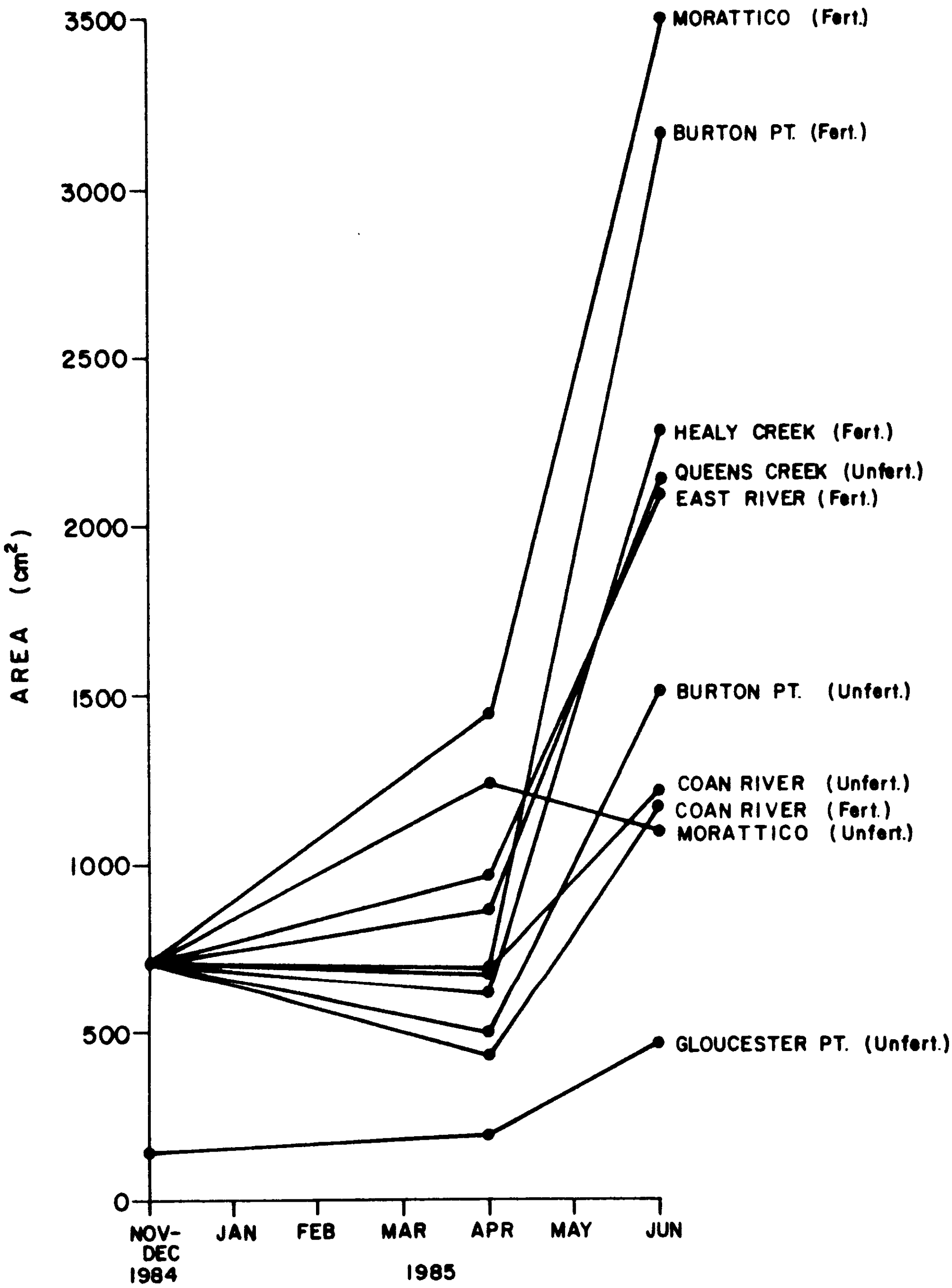


Fig. 1. Growth of eelgrass units planted at the different sites along the western shore of the bay in the fall of 1984. (Data only from those sites where significant numbers of individual units have survived through June, 1985. See Tables 1 and 2 for additional information).

throughout the transplant area and recently deposited oyster shells present on the bottom. The Coan River has a number of active oyster growers and one had apparently established some of his grounds in our transplant area. During our April field check, we observed several watermen harvesting oysters adjacent to the transplant area indicating that indeed this was an active oyster site. We believe that many of the units have been lost due to the boat and dredging activity related to the use of the area by the watermen. There has, however, been growth and spread of those units that have still remained (Table 2).

The most successful sites for transplant survivorship have been at the East River and Morattico sites. There has been 100% success at the East River area where only a fertilized plot was planted, while the success rate at the unfertilized site and fertilized site at Morattico in June was 93% and 100%, respectively. The growth of the fertilized units at the Morattico site has been considerable (Table 2, Fig.1). Aerial coverage has increased from 700 cm^2 to almost 3500 cm^2 in just seven months.

A comparison of the fertilized and unfertilized areas indicate a significant effect of the fertilizer treatment on the growth of the transplant unit. This was especially true at the Morattico and Burton Pt. sites. The units were significantly denser and much more robust than the unfertilized units. Leaf lengths were generally two to three times longer than plants from adjacent unfertilized areas. The only exception was the Coan River site where both fertilized and unfertilized areas were similar in size and density of shoots. Fertilizer has had a significant effect on the growth of the transplant unit and corroborates our experimental work conducted earlier with both laboratory reared seedlings and transplanted whole plants under field conditions.

Seedling Experiments

Seeds collected in the spring, 1984, were planted at two sites in the York River (Gloucester Point and Clay Bank) using four different methods of planting. These methods included 1) raking the bottom to create furrows and randomly releasing the seeds over the furrows, 2) incorporating seeds in a cotton gauze tube and burying the tube just beneath the sediment surface, 3) injecting the seeds into the sediment with a large bore syringe, and 4) placing the seeds directly on the sediment surface and allowing seeds to become naturally incorporated in the sediment matrix.The experimental design was constructed using a randomized block design with three replicates per treatment. Seeds were placed in the experimental plots in November, 1984. Germination of eelgrass seeds occurs from late October through April, thus our first examination of the test plots were conducted in late April.

Table 1 presents the results from Clay Bank and Gloucester Point. Data from the Gloucester Point site indicates that the control, rake and injection method yielded similar numbers of germinated seeds while the gauze yielded significantly fewer seeds (Table 3). Data from the Clay Bank site indicated that raking the bottom yielded the greatest number of seeds per plot while the other three methods had similar seedling densities and were similar to the numbers recorded at the Gloucester Point site for the most successful methods (Table 3). Based on the data from these two sites, we believe that creating furrows along the bottom and placing the seeds in the furrows would produce the greatest success if large numbers of seeds were to be used for reseeding denuded areas as an alternative to using whole plants which is the current method employed by most replanting efforts.

Our plans are to attempt a larger scale revegetation effort using seeds this fall. In order to acquire a large number of seeds, reproductive shoots

Table 3. Number of germinated seedlings (mean of three replicates) in test plots at Gloucester Point and Clay Bank incorporating different methods of planting (checked April 26).

Site and Treatment	# of Seedlings	% of Viable Seeds (est) Which Germinated Within Experimental Area
Clay Bank - Control	76	5.3
Gauze	65	4.5
Baster	41	2.7
Rake	193	13.5
Gloucester Point - Control	48	15.2
Gauze	4	1.3
Baster	51	16.1
Rake	52	16.5

with viable seeds must be collected before they are released and fall into the sediment where they are very difficult to collect. To accomplish this task, we collected a considerable number of eelgrass reproductive shoots this past spring (the last two weeks of May and the first week of June). Seeds will be held in running seawater over the summer and will be used in a designated transplant area this fall. We are presently conducting preliminary tests to enable us to automatically plant the seeds using an underwater planter.

Because of the interest in our program by the public and our use of volunteers during the fall planting, we solicited the help of several volunteer groups to assist our staff in collecting reproductive shoots. Three groups volunteered during the spring and provided us with a number of young adults. These were a boy scout group from Seaford (9), a 4-H group from Chesapeake (9), and a dive club from York County (7).

Environmental Monitoring

A bi-weekly sampling program was initiated in the fall of 1984 to monitor a number of parameters at five shallow water study sites along the York River. Data measured include: total organic and inorganic filterable matter, chlorophyll *a*, nitrate, nitrite, ammonia, phosphate, apparent downwelling diffuse light attenuation, broad band photosynthetically active radiation, wind speed and direction, tidal stage, water depth, air and water temperature, and salinity. The study sites (Guinea Marsh, Allens Island, Gloucester Point, Mumfort Island, Claybank) are located in the shallow littoral zone (depth at MLW 1.0 m) at areas that currently support or previously supported submerged aquatic vegetation. Guinea Marsh is the vegetated donor site for vegetation used in the transplantation experiments while Gloucester Point and Claybank are sites of current transplant efforts.

Allens Island and Mumfort Island are spaced along the York River between these three and are sites of previous as well as current transplantation experiments.

Summer is the most critical time for survival of vegetation in this region of the Chesapeake Bay. Therefore, analysis of the monitoring data is only preliminary at this point. However some of the data is presented here in graphical form for summarization.

Figures 2A and 2B illustrate the air and water temperatures evident over the sampling period to date. Overall there is no significant difference in temperatures between the sites. Lowest temperatures occurred at the end of January with a minimum water temperatures of -1.0 C. At that time ice was present over much of the York River including three of the five study sites.

Salinities (Fig. 3A) varied between the sites with the most upstream site, Claybank, having the lowest salinities. Overall, the mean here was 14.2 ppt. Salinities were significantly greater at the next downriver site, Mumfort Island, where the mean was 16.9. There was no significant difference among the three downriver sites where the means ranged between 17.8 and 18.6. Salinities vary both with riverflow and tidal stage as well as other factors at any one site. However, as the data here were collected at near low water, they should represent minimums for the locations. Seasonally lowest values occurred in January and February. The dry spring season observed in 1985 accounts for the relatively high values observed during that period.

Total suspended solids varied considerably among the sites and between sampling dates (Fig. 3B). Much of this was apparently due to resuspension of bottom material by wind action. Most of this material was inorganic

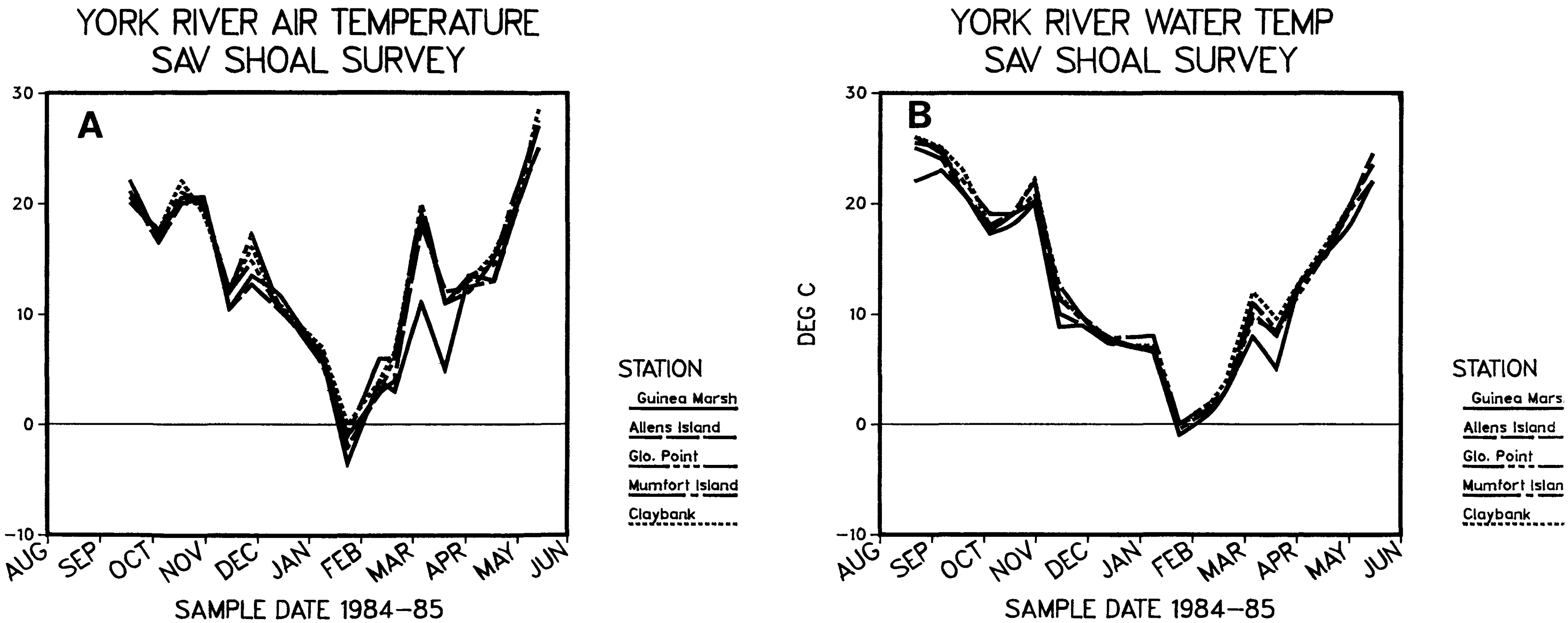


FIG. 2 Air (A) and water (B) temperature for the York River shoal survey from August 1984 through June 1985.

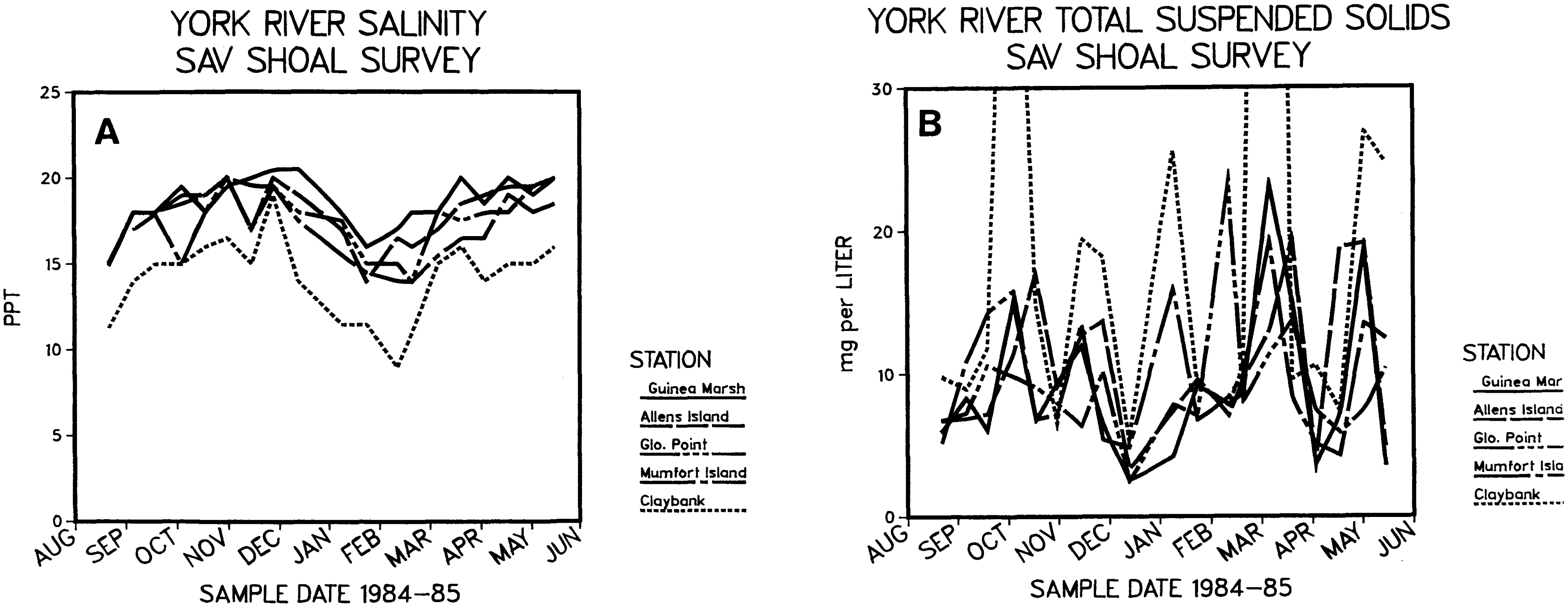


FIG. 3 Salinity (A) and total suspended solids (B) for the York River shoal survey from August 1984 through June 1985.

matter (Fig. 4A) which when subtracted out reveals a more homogeneous pattern evident in the organic fraction (Fig. 4B). Several peaks in organic matter are evident with the most pronounced occurring in the spring. Intrasite comparisons reveal no significant difference overall between the downstream three sites, with significantly higher values at each of the upstream sites.

Nutrients (Figs. 5A, 5B, 6A, and 6B) illustrate varying trends with highest levels, as expected, occurring during the winter months when biological activity is at a minimum. Nitrite showed the most pronounced shift with a precipitous drop in mid-February. Overall the upstream station showed significantly higher levels of all nutrient species than the downstream stations.

Chlorophyll levels showed considerable variation (Fig. 7A) and, as expected, levels were highest in the spring. Intrasite comparisons reveal that, although overall mean levels of chlorophyll were higher at the upstream stations, statistically the differences were not significant.

Light readings (Fig. 7B) which are presented as diffuse downwelling attenuation coefficients (K), are influenced in part by the resuspension of bottom material at the sites with peaks occurring when the wind direction and speed were suitable for stirring of the bottom. Generally, the vegetated site, Guinea Marsh, had the lowest light attenuation (highest transparency) and Claybank the highest attenuation.

Plant Monitoring

Eelgrass samples have been collected bimonthly since the fall of 1984 from the vegetated donor site (Guinea Marsh) and 2 transplant sites (Gloucester Point, Clay Bank) in the York River. At each date, eelgrass density and macrophyte and epiphyte biomass were measured from random

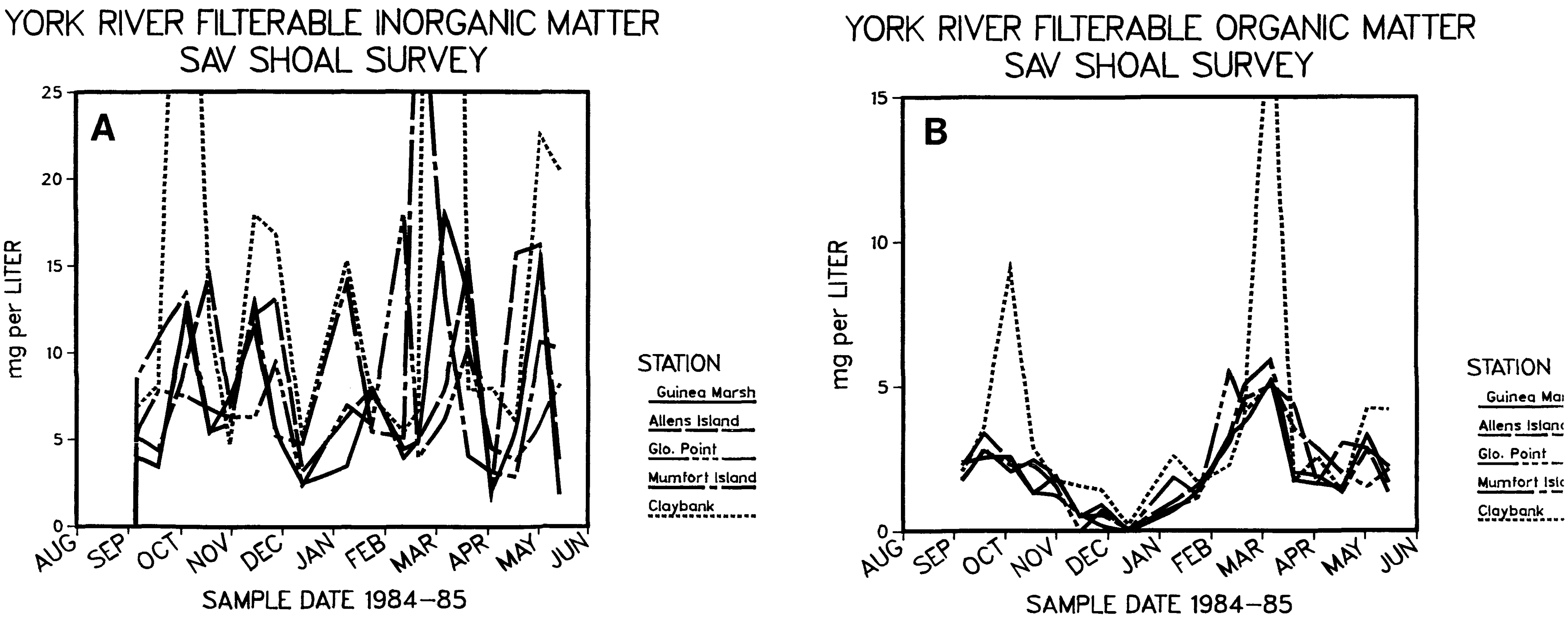


FIG. 4 Filterable inorganic (A) and organic (B) matter for the York River shoal survey from August 1984 through June 1985.

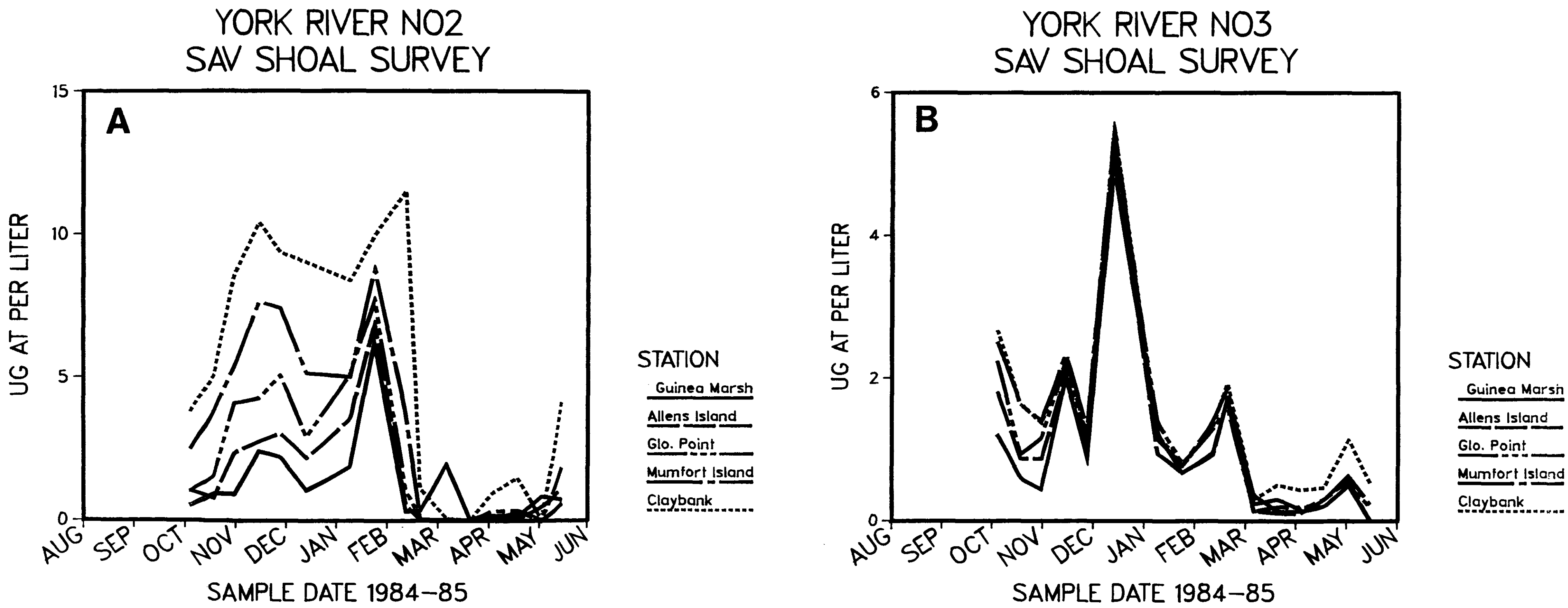


FIG. 5 Nitrite (NO_2) (A) and nitrate (NO_3) (B) for the York River shoal survey from August 1984 through June 1985.

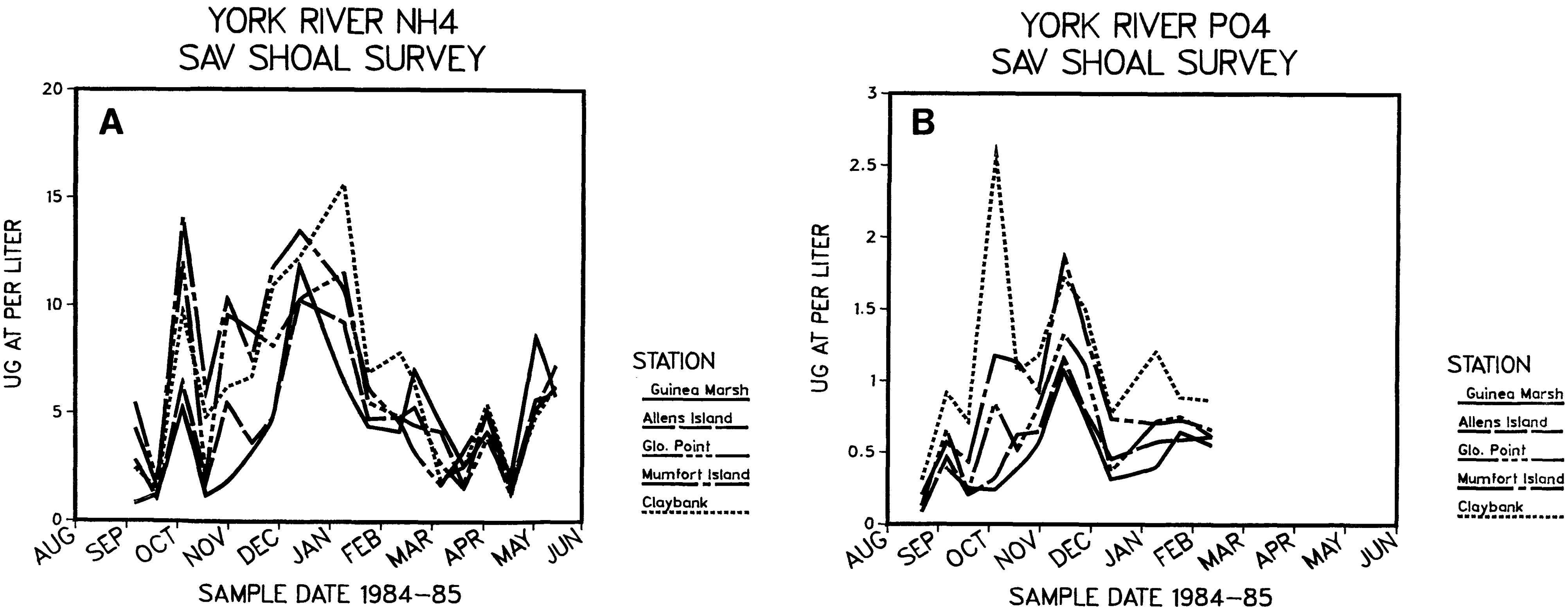


FIG. 6 Ammonium (NH_4) (A) and phosphate (PO_4) (B) for the York River shoal survey from August 1984 through June 1985.

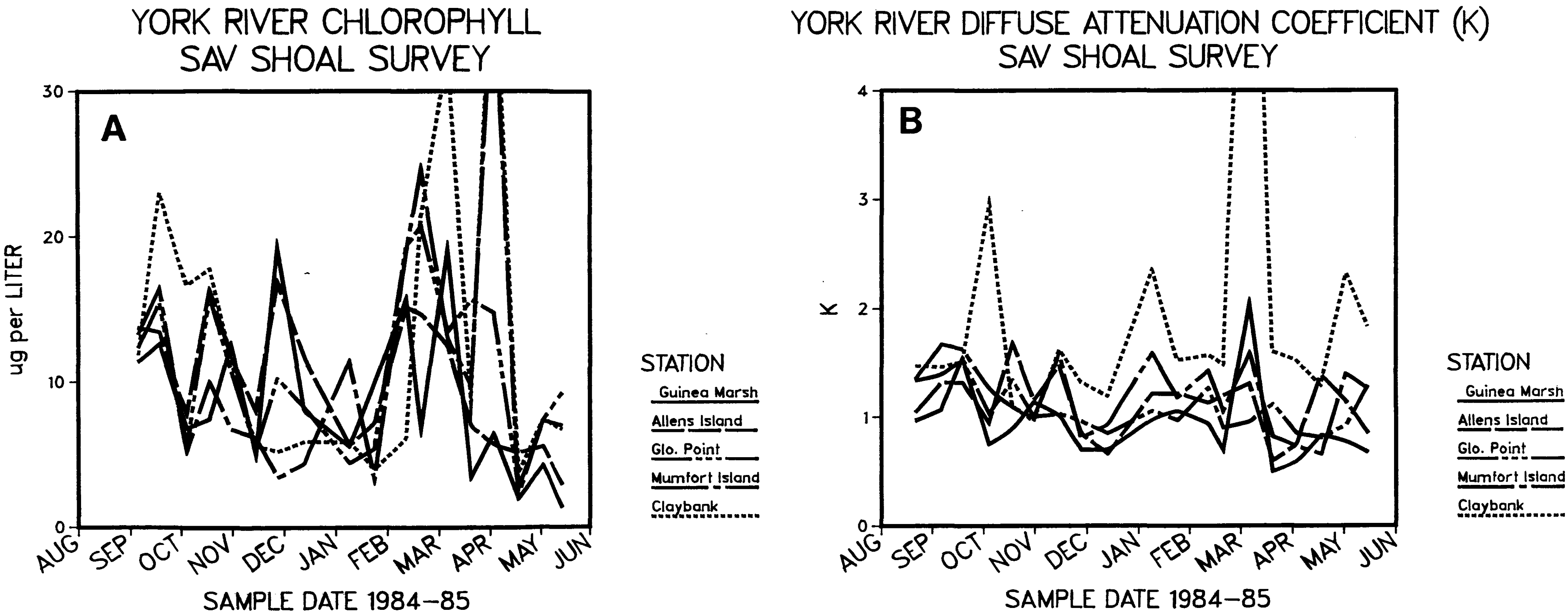


FIG. 7 Cholorophyll (A) and attenuation coefficient (B) for the York River shoal survey from August 1984 through June 1985.

locations at Guinea Marsh and surviving transplanted patches at Gloucester Point and Clay Bank. Concurrent with field investigations, laboratory experiments were done to determine the photosynthetic efficiency of plants at each site. Comparisons of these data among sites and correlation with measured physical parameters will elucidate the factors controlling eelgrass distribution and abundance in the lower Chesapeake Bay.

Preliminary analysis of field data gathered to date reveals considerable variation in eelgrass density both seasonally and among sites (Fig. 8). By March, however, the plant density at both transplant sites was lower than that at Guinea Marsh. Aboveground biomass of eelgrass plants remained similar among sites until the spring growth period (Fig. 9A); by May, shoot weight of Guinea Marsh plants greatly exceeded that of the transplants. In contrast, the biomass of belowground plant organs was significantly higher at Gloucester Point and Guinea Marsh than at Clay Bank during January (Fig. 9B), which influenced patterns of total biomass per plant (Fig. 9C).

The combination of eelgrass shoot density and biomass yields information on total plant biomass per unit area of substrate. Following the initial sampling period, aboveground biomass per unit area at Gloucester Point remained lower than that of the other 2 sites (Fig. 10A). By May, the aboveground biomass per unit area at Guinea Marsh was more than double that of the transplant sites (Fig. 10A). No significant differences in belowground or total biomass per unit area were observed until May, when values for Guinea Marsh again exceeded those for Gloucester Point and Clay Bank by more than 100% (Figs. 10B, 10C).

The size of eelgrass patches (Fig. 11A) and the number of shoots within each patch (Fig. 11B) increased during the spring growth period at both

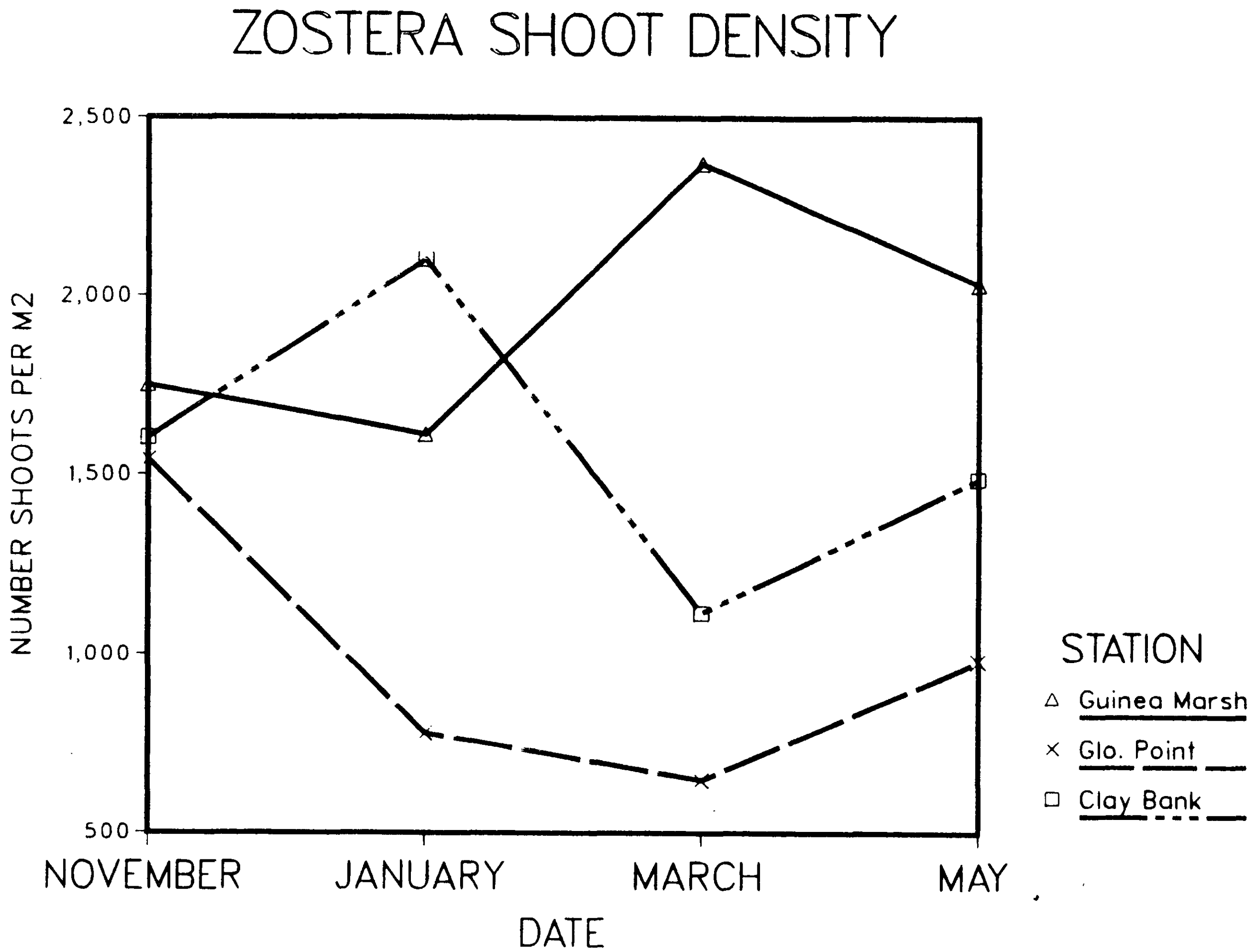
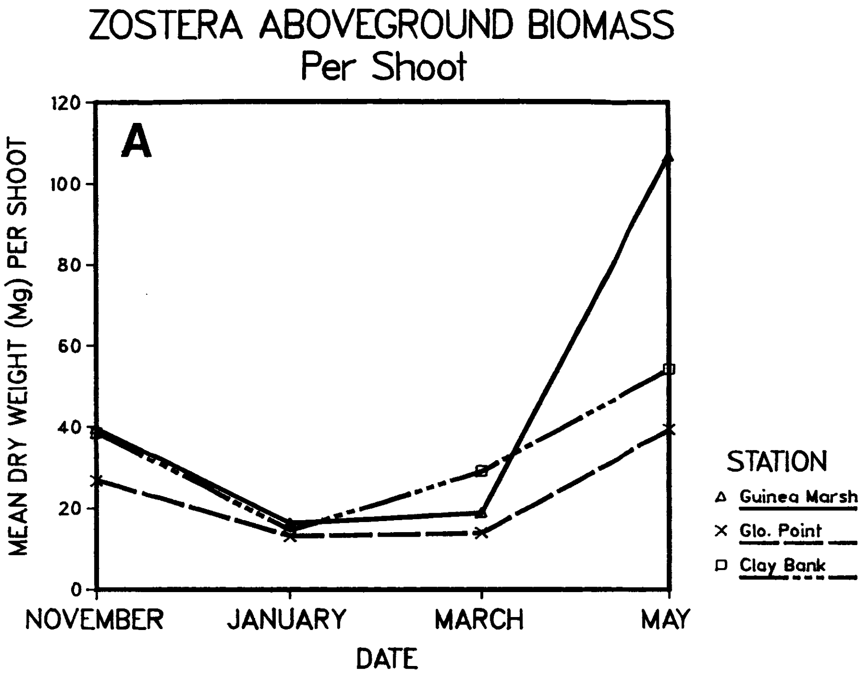
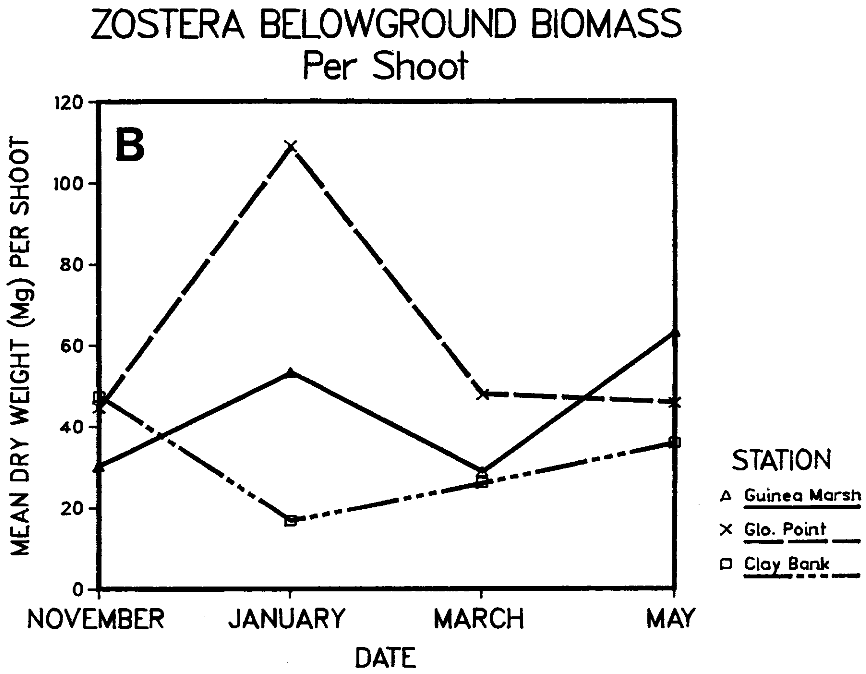


FIG. 8 Zostera shoot density at Guinea Marsh, Gloucester Point, and Clay Bank from November 1984 through May 1985.





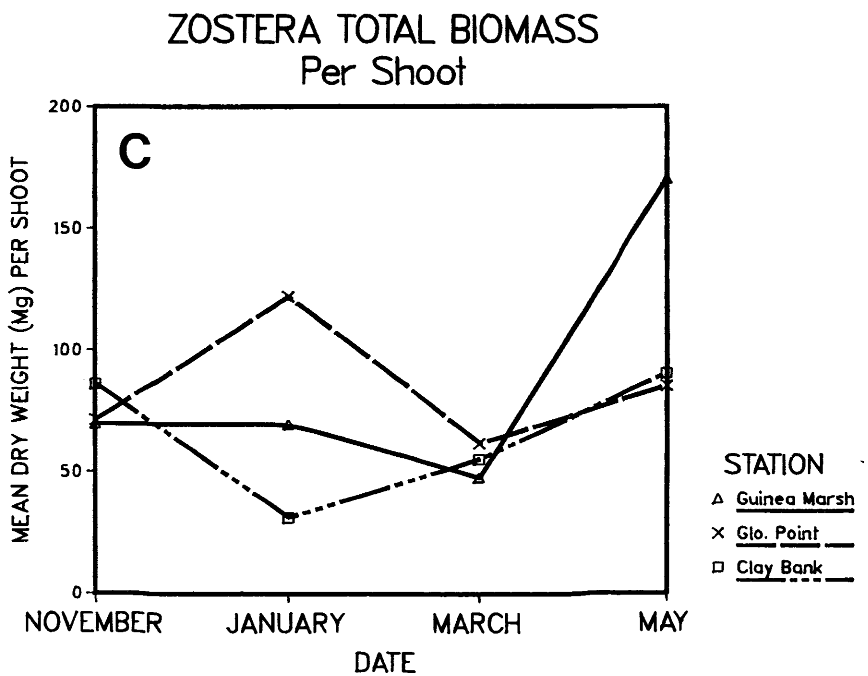
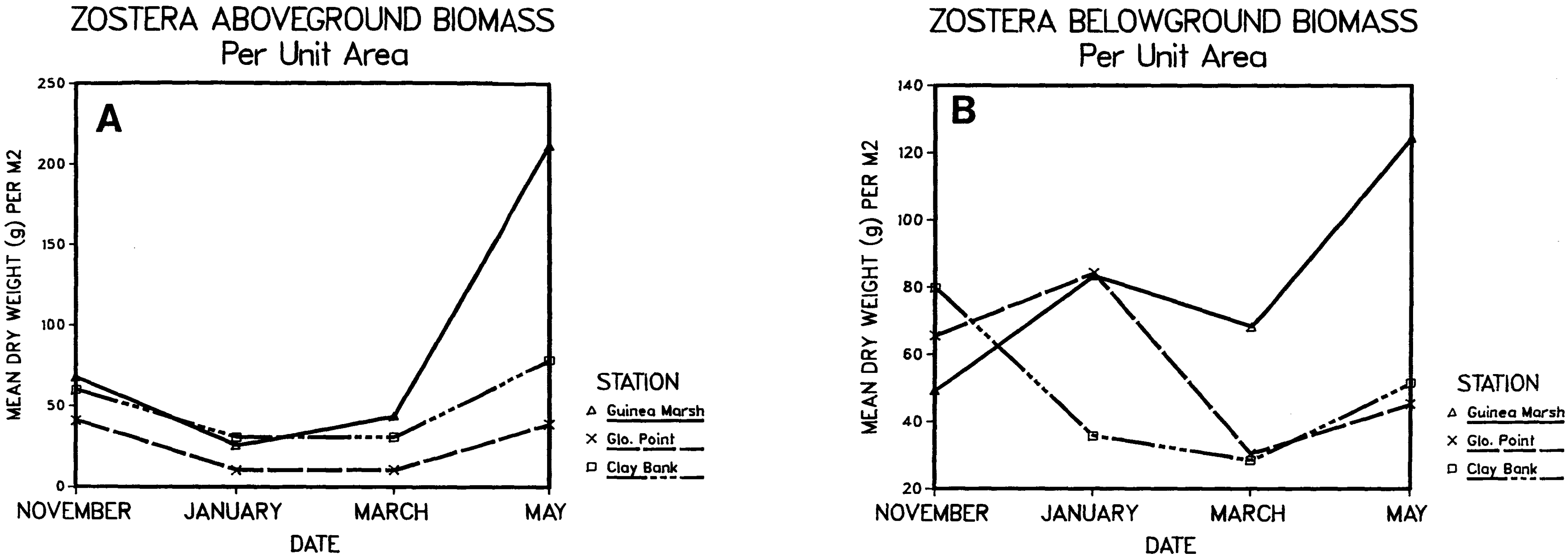


FIG. 9 Aboveground (A), belowground (B), and total (C) biomass per shoot for Zostera at the three York River sites from November 1984 through May 1985.



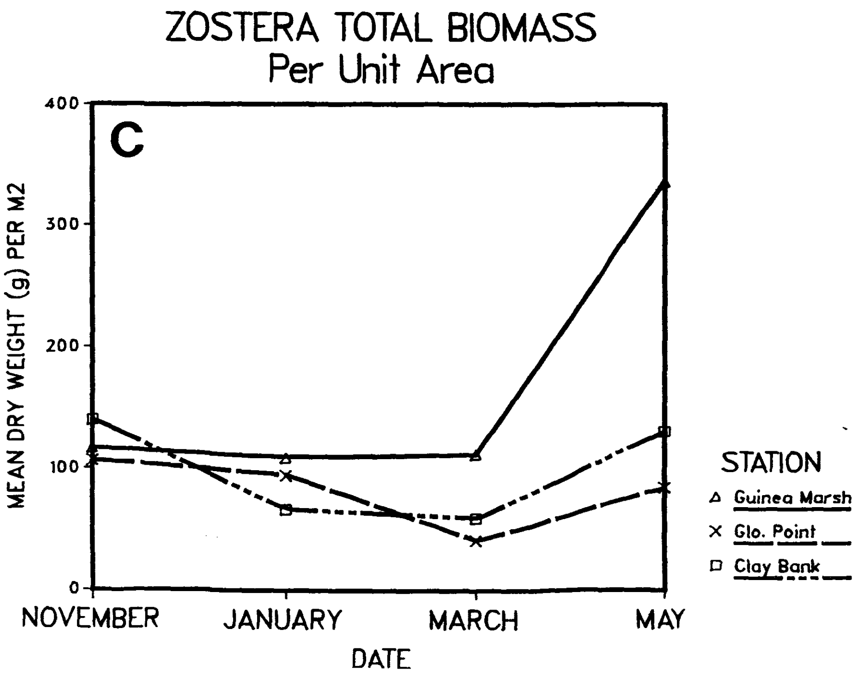


FIG. 10 Aboveground (A), belowground (B) and total (C) biomass per m^2 for *Zostera* at the three York River sites from November 1984 through May 1985.

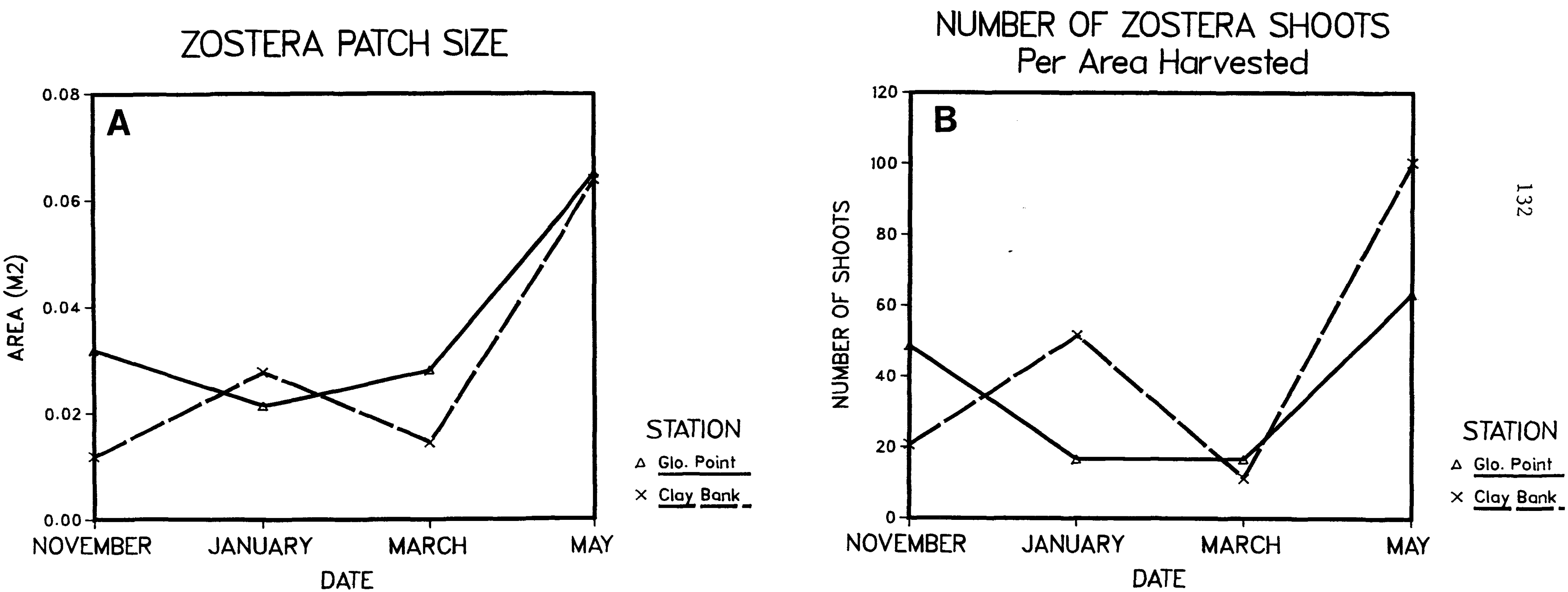


FIG. 11 Zostera patch size (A) and number of shoots within each patch (B) for the Gloucester Point and Clay Bank transplant sites from November 1984 through May 1985.

transplant sites. No significant differences in these parameters between transplant sites were observed. Conclusions regarding pattern of biomass accumulation at each site, comparisons among sites, and correlation with other environmental variables await incorporation of data from summer and early fall periods.

Concurrent with the biomass sampling beginning in May 1985 eelgrass productivity, leaf turnover and plant growth is being measured using 30cm by 50cm by 15cm boxes containing whole eelgrass plants and natural sediments. The plants were obtained from the Guinea Marsh donor site in the spring of 1985. After a period of equilibration at the Gloucester Point site the boxes were placed at the Gloucester Point and Claybank transplant sites. All the shoots located within randomly placed quadrats in the boxes were banded, numbered, measured and marked using a new technique developed for this study. At 7 to 10 day sampling intervals the boxes are retrieved, the shoots remeasured and marked and the boxes returned to the study sites. This experiment will enable us to closely follow the process of leaf turnover and develop rate measurements not possible with the biomass and standing crop studies.

Long-term Light Records

Long-term, continuous, integrated records of PAR insolation have been continuing at the Gloucester Point site. Broad band (400 to 700 nm) spectral submarine intensity and attenuation has been followed since last fall at this same site using cosine-corrected, underwater sensors and recorded using microprocessor-controlled variable scale, integrating light meters. Arrays of two units with the sensors deployed at two different depths are being used to provide continuous records of submarine irradiance intensity at the level of the plant canopy and to allow calculation of the apparent downwelling diffuse light attenuation coefficient. Software programs have been developed to read and analyse the recorded data and input the information to a retrieval system maintained on our PRIME 850 mainframe. Recently a second set of sensors has been deployed at the Claybank site, a location that our biweekly monitoring has identified as having the poorest light quality of those being studied.

Continuing Effort

During the next six months our transplanting efforts will be directed toward the following tasks:

1. Design and test seed planter for replanting efforts this fall.
2. Plant 13 acres of eelgrass at predetermined locations. The locations will be based on survivorship data from the previous year's transplanting effort.
3. Plant seeds using method developed in task 1 at one location where survivorship data from 1984 efforts indicates our greatest success.

Environmental water quality monitoring will continue through the fall of 1985 so as to complete an annual data base. Bimonthly biological monitoring will also continue with sampling in July, September and November. During the summer months when water temperatures are at their highest, natural populations of submerged aquatics generally experience a significant reduction in growth and, in some cases, complete dieback. It is essential that biological, chemical and physical monitoring continue through this period. Considerable time will also be spent in the upcoming months on data analysis and modelling efforts. Additionally, plans for monitoring next falls transplants will be finalized based, in part, on the results of this year's work.

EXHIBIT 1

VIRGINIA INSTITUTE OF MARINE SCIENCE

CHESAPEAKE BAY INITIATIVE
Statement of Revenues and Expenditures
Year Ended June 30, 1985

REVENUES:

Appropriation Transfer from the Council on the Environment	$700,000
Appropriation Transfer from the Health Department	33,000
U. S. Fish & Wildlife Services	3,280
Agency Funds, VIMS	104,219
Total Revenues	$840,499

EXPENDITURES:

Study:	Personnel	Maintenance and Operations	Total
Revitalization of the James River Seed Oyster Industry	$295,455	$244,269	$539,724
Factors Effecting Recruitment of Virginia's Critical Finfish Population	92,162	7,621	99,783
Chemical Poisons in Virginia's Tidal Waters	98,747	102,245	200,992
Total, Chesapeake Bay Initiative	$486,364	$354,135	$840,499

EXHIBIT 2

VIRGINIA INSTITUTE OF MARINE SCIENCE

SUBMERGED AQUATIC VEGETATION
Statement of Revenues and Expenditures
Year Ended June 30, 1985

REVENUES:	
Appropriation Act, Chapter 619	$ 75,000
Agency Funds	85,453
Total Revenues	$160,453
EXPENDITURES:	
Personnel	$132,972
Maintenance & Operations	27,481
Total Expenditures	$160,453