1-1986

Third Quarterly Progress Report for the Period July 1, 1985 - December 31, 1985

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

THIRD QUARTERLY PROGRESS REPORT

FOR THE PERIOD JULY 1, 1985 - DECEMBER 31, 1985

CHESAPEAKE BAY RESEARCH

AND

SUBMERGED AQUATIC VEGETATION

INITIATIVES

by the

Virginia Institute of Marine Science

of the

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EXECUTIVE SUMMARY

This is the third semi-annual report by the Virginia Institute of Marine Science on Chesapeake Bay Initiatives funded for the 1984-1986 biennium. Highlights of progress in the past six months are:

- Genetic differences among lower James River broodstocks of oysters are being examined in greater detail and compared with stocks from Maryland and other Virginia locales.
- Monitoring of spatfall at 20 stations in the lower James River was continued in the summer and fall of 1985. Setting levels have continued high in the 1980's and may have been enhanced in 1985 by a stable salinity and temperature regime.
- Sex ratio and reproductive conditions of oysters from Wreck Shoal and Brown Shoal are very similar, but those from Horsehead are quite different. These studies are continuing as part of our efforts to characterize and evaluate potential of different broodstock.
- Experiments on fouling of shell placed in the James River showed that clean shell placed on the river bottom between May 21 and July 1 are 60% covered with fouling organisms by mid-August. Maximum fouling coverage appears to coincide with highest water temperatures (mid-August).
- Experiments with endolithic algae (those growing below the surface of shells) show better survival and growth of oyster spat on shell containing the algae than on shell treated to kill the algae or on glass substrate.

- Studies of predation by mud crabs on oyster spat have revealed a plateau in predation rates, with predation rates increasing directly with spat density until a density of 470 spat per sq. meter, at which point predation rates level off.
- Although it now appears that predation on oyster spat by crabs (both mud and blue crabs) is significant, control measures do not appear feasible.
- Actual field examination of effects of chlorinated sewage discharges on oyster setting and spat survival in the lower James River does not indicate any effect of proximity to the discharge point. This project should be essentially complete within the original 2-year span.
- Circulation in the lower James River moves larvae in a general counter-clockwise pattern, while gravitational circulation causes salty water on the bottom to slowly move upstream. This is the mechanism allowing larvae to remain in, and be transported to, the principle seed beds.
- Studies of circulation patterns show that Hampton Flats might once have been an important source of oyster larvae before MSX appeared, but that Wreck Shoals may today provide the principle source.
- Existing 2-dimensional mathematical models will not adequately predict the movement of oyster larvae, but studies conducted thus far will be useful in calibration of a 3-D model of tidal flow when that tool is made available to us.
- Fronts, or convergence zones, are a regular feature of estuaries such as the lower James River. They are known to be of importance in concentration and transport of planktonic organisms such as oyster larvae, so are being identified and surveyed in the study site. In

addition to the important, semi-permanent front off Newport News Point, several fronts have been observed in the Wreck Shoal area.

. A strong lunar effect on stratification and destratification is evident in the lower James, aiding mixing, preventing bottom depletion of oxygen and, perhaps, explaining why oyster larvae survive better than in other river systems.

. Carbon-14 dating of Wreck Shoal oyster shell shows a thin layer of live oysters over a substrate of shell formed about 400 years ago. The current "cull law" is insufficient to maintain the Wreck Shoal reef.

. Passage of large ships over Wreck Shoals is producing scars due to prop wash, churning several acres of the oyster rocks (revealed by surveys with side-scan sonars).

. A review has resulted in a generalized distribution chart of summer flounder in the Middle Atlantic Bight by season and life stage. There are indications that two stocks exist, the more southern maturing at a smaller size. A conceptual model of the summer flounder's life history has been developed.

. Egg production by striped bass has been calculated from past data sets (1980-1983) from the James, York and Rappahannock rivers. Comparisons show greatest reproduction on the Rappahannock River during 1982 and 1983.

. Attempts to examine the potential effects of "acid rain" on the striped bass spawning grounds were inconclusive due to lack of rain. Drought conditions persisted in the area throughout the normally high precipitation months of April and May.

- Sampling for chemical pollutants in Virginia's seafood was completed for 1985, and included grey trout, bluefish, spot, croaker, flounder, blue crab, oysters and clams. No samples have yet been found to contain pesticides or PCB concentrations that exceed the action levels.
- Fifteen acres of eelgrass were planted in the fall of 1985 in areas that that showed best success from the 1984 plantings, and in some new experimental sites.
- Survival of 1984 eelgrass transplants varied from 0 to 100%. All plants were gone from the Clay Bank (York River) and Healy Creek (Piankatank River) sites, while greatest survival (100%) occurred in the East River.

The Oyster Hatchery

In addition to the above regular Initiatives, it can be reported here that the hatchery is functional and operating, and is being further prepared for full production in 1986. Winter months are being used to complete a facility for growing algae as food for larvae and for enlargement of the cold room so more spawning stock can be held.

PROJECT 1

Composition and Distribution of Broodstocks

Introduction

Studies on oyster biology during the latter half of 1985 have focused on five areas, three of them concerned with broodstocks and treated as subprojects within this Project 1. These three subprojects include: (a) the identification of discrete broodstocks in the James River, (b) the monitoring of spatfall during the summer and fall, and (c) the examination of sex ratios in James River broodstocks. The remaining two subprojects are included under Project 2 and deal with effects of fouling and predation on oysters in the James River.

Project 1A

Oyster Broodstocks in the James River

Description and Objectives

In future management of the James River oyster seed beds, it will be important to know (a) whether there are distinct and separate broodstocks, and (b) if so, which are the best producers of eggs and larvae.

Status

Previous work on this project has revealed the presence of genetically distinct oysters at opposite ends of an environmental gradient, represented by oysters from Nansemond Ridge and from Horsehead Rocks. More recently, efforts have centered on providing comparable genetic information from oysters lying between these extremes.

Expected Accomplishments Next Quarter

During January-March 1986 these allele frequency data will be statistically examined in fine detail to determine the degree of relatedness among these oyster rocks. To determine if variations in the genetic population structure among these oyster rocks is a unique occurrence or universal, the genetic structure of Rappahannock River oysters is being examined. As a quality control measure an additional group of oysters from the Tred Avon River in Maryland is being examined. This population of oysters has been genetically characterized previously by scientists at The University of Maryland Horn Point Environmental Research Lab. Work is proceeding as planned on all samples with project completion planned for April 1986.

Problems, Delays

None yet experienced or anticipated next quarter.

Interim Findings

Oysters from three primary oyster rocks in the James River (Horsehead, Nansemond Ridge and Wreck Shoal) have been examined using gel electropheresis techniques at eight enzyme loci. The loci examined include leucine aminopeptidase-1, leucine aminopeptidase-2, phosphoglucose isomerase, phosphoglucosemutase-1, phosphoglucosemutase-3, alanopine dehydrogenase, strombine dehydrogenase and aminopeptidase-3. An additional locus, aminopeptidase-2 has been examined in oysters from Horsehead and Wreck Shoal. Each of these loci are polymorphic, and an initial examination of allele frequencies revealed considerable differences among the oyster rocks.

Project 1B

Temporal and Spatial Variation in Oyster Spatfall in the James River

Description and Objectives

Each year VIMS effects a weekly survey of oyster spatfall at various locations from June through October. Spatfall is quantified by counting numbers of newly-settled oysters on "strings" of oyster shells. This subproject was designed to expand the number of shellstring monitoring stations in the James River to more comprehensively describe spatial and temporal variability in setting.

Status

The twenty spatfall monitoring stations reported in an earlier quarterly report for the James River addendum studies were continued in 1985. When the shellstrings were collected salinities and temperatures were taken at the surface and at the bottom. The stations and transect locations were reported in the earlier quarterly report.

Expected Accomplishments Next Quarter

The January-June period is the "off-season" for this oyster setting project. June represents the initiation of seasonal monitoring, however, so spring months are used for preparation of shellstrings.

Problems, Delays

None, except for tampering with certain shellstrings (see below).

Interim Findings

The potential for setting, as reported on shellstrings (Table 1), was similar to the high levels of setting in the 1980's, with the exception of four stations. Salinities were above normal in the late winter and early spring. Setting on shellstrings began by the fourth standard week, the third full calendar week in June, and extended for nineteen weeks. Setting peaked in the seventh standard week, at least five weeks earlier than in 1984, and declined to a very low level in the twelfth standard week. Secondary peaks occurred in the first week of September and second week of October. The bottom temperature was 24^{o} Centigrade, or higher, at all stations measured on June 25th. Setting on shellstrings did not stop until the temperature dropped below 19^{o} Centigrade on the bottom.

Setting was, perhaps, enhanced in 1985 by a stable salinity and temperature regime that did not have abrupt variations or changes. The Deepwater Shoal data is not representative of the environment because the

Table 1
Spatfall on Shellstrings*
1985
JAMES RIVER

June 17 - Nov. 8, 1985

Standard Week	Deepwater Shoal	Horsehead Bar	Mulberry Swash	Point of Shoal	Long Rock	East End	Dry Shoal	Rock Wharf Shoals	Wreck Shoals
3 (Jn. 17)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.2	1.0	0.5	1.7	1.4	0.7	2.8	--	0.7
6	--	2.4	1.7	6.1	8.0	2.7	5.0	--	3.9
7	--	17.7	27.1	14.7	16.7	26.2	50.7	138.2	10.0
8	0.5	9.1	0.9	3.9	9.9	1.2	5.4	14.3	3.9
9	--	2.5	3.2	2.7	1.2	0.5	7.3	7.7	0.6
10	0.1	0.3	2.2	0.1	1.3	0.3	0.3	0.6	0.1
11	0.0	0.3	2.2	0.5	0.6	2.0	1.3	0.9	0.4
12	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
13	0.0	0.0	0.0	0.3	0.7	0.0	2.9	0.2	0.1
14	0.0	1.1	0.0	0.6	3.5	0.2	3.6	0.1	3.6
15	0.0	0.3	0.0	0.1	0.0	0.3	1.1	0.0	1.1
16	0.0	0.0	0.0	0.0	0.8	0.2	0.1	0.2	0.1
17	--	0.2	0.1	0.0	0.5	0.1	0.1	0.2	0.0
18	0.0	0.2	0.0	0.0	0.2	0.1	0.1	0.0	0.1
19	0.3	0.9	0.1	0.5	0.7	0.2	6.1	0.0	1.6
20		0.0	0.1	0.0	0.4	0.1	0.2	0.6	0.0
21		0.0	0.0	0.0	0.3	0.1	0.1	0.5	0.0
22		0.0	0.0	0.0	0.1		0.0	0.0	
23		0.0		0.0	0.1		0.0	0.0	
TOTALS	1.1	36.0	38.1	31.2	46.5	34.9	87.1	163.5	26.3

Standard Week	Wreck Shoal Inshore	Days Point	White Shoals	Miles Watch House	Brown Shoals	Dog Shoal	Naseway Shoal	Horne Brothers	Cruiser Shoal	Ridge	Hampt Flats
3 (Jn. 17)	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.1	0.0	0.0
4	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
5	0.3	0.7	--	0.4	0.4	3.2	1.3	1.2	3.8	0.0	0.4
6	8.6	19.1	0.5	5.3	2.1	11.9	29.8	5.0	7.3	5.1	9.3
7	10.2	78.0	4.9	4.9	8.8	276.0	292.3	78.2	192.1	50.9	50.3
8	2.9	6.0	4.4	1.8	5.1	225.3	104.6	23.2	13.2	2.5	5.3
9	1.0	1.7	0.6	2.0	0.9	16.3	10.8	3.6	--	2.5	5.3
10	0.7	0.6	0.4	0.7	0.3	0.6	0.0	2.6	0.3	0.9	1.0
11	0.5	0.6	0.4	0.5	0.7	3.4	2.2	3.9	3.9	0.6	0.8
12	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.6	0.0	0.1	0.8
13	0.7	3.6	0.2	0.6	0.8	5.8	2.2	2.0	0.1	0.0	1.5
14	1.4	5.9	1.1	1.4	2.5	9.4	15.9	3.6	1.9	5.5	3.8
15	0.0	0.8	0.2	0.2	0.1	3.3	3.1	2.5	1.2	0.3	0.9
16	0.0	0.2	0.2	0.0	0.1	0.4	0.3	2.0	0.1	0.1	1.1
17	0.0	0.1	0.0	0.8	0.0	0.3	0.1	1.5	0.1	0.1	0.9
18	0.0	0.6	0.1	0.4	0.2	0.7	0.1	1.0	1.0	0.3	0.6
19	--	2.4	0.3	1.6	1.1	1.5	0.4	0.6	0.7	0.2	0.1
20	0.0	--	0.4	0.1	2.8	5.4	2.6	3.2	1.7	0.3	2.5
21	0.0	--	0.4	0.0	0.3	2.5	0.1	0.5	--	0.2	0.7
22	0.1	0.0		0.1	0.3	2.5	0.1	0.4	--	0.1	0.7
23	0.1	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
					0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTALS	26.5	120.3	14.3	20.9	26.6	568.8	465.9	135.6	227.6	69.7	86.5

*Shows spat per shell (smooth side only, mean of 10 shells)
--Data not received.

station was disturbed by unusual sports fishing activity. Frequently either the shellstring or the weight was missing on the collection dates.

Project 1C

Examination of Sex Ratios in Oysters in the James River

Description and Objectives

Oysters have the ability to change sex, the dogma stating that first maturation is predominantly male with a progressive increase in the percentage of females occurring as oysters increase in size. Clearly, a balance of males and females is required to optimize reproduction. In order to assess the potential impact of any "imbalance" in sex ratios, an examination of sex ratios in present oyster stocks and archived specimens was undertaken.

Status

This is a continuing project, particularly suitable for those months when intensive field monitoring is slack. Further examinations of monthly samples of oysters collected since 1964 and stored in the VIMS archives are now in progress.

Interim Findings

Histological mounts of oysters collected since 1960 and stored in the VIMS archives are being examined to determine their reproductive condition and sex ratios. We are using a stereology method based on point-count volumetry to assess the proportion of gonadal tissue occupied by developing or ripe gametes and which is reported as the Gonad Volume Fraction (GVF). The GVF can range from zero for a reproductively inactive oyster to one for an oyster showing maximum reproductive development.

Figure 1 shows the percent frequency of GVF values grouped in three ranges for oysters collected in June 1964 from three rocks in the James River. There is very little difference between Wreck Shoal and Brown Shoal or between the sexes at each of those two rocks; however, the Horsehead oysters show a difference between the sexes and a significant difference in reproductive condition from those of Wreck Shoal and Brown Shoal. Horsehead oysters appear to be lagging behind oysters from the other sites in their development. Figure 1 illustrates the usefulness of the point-count volumetry method in comparison of the reproductive development of oysters from different rocks and at different times of the season.

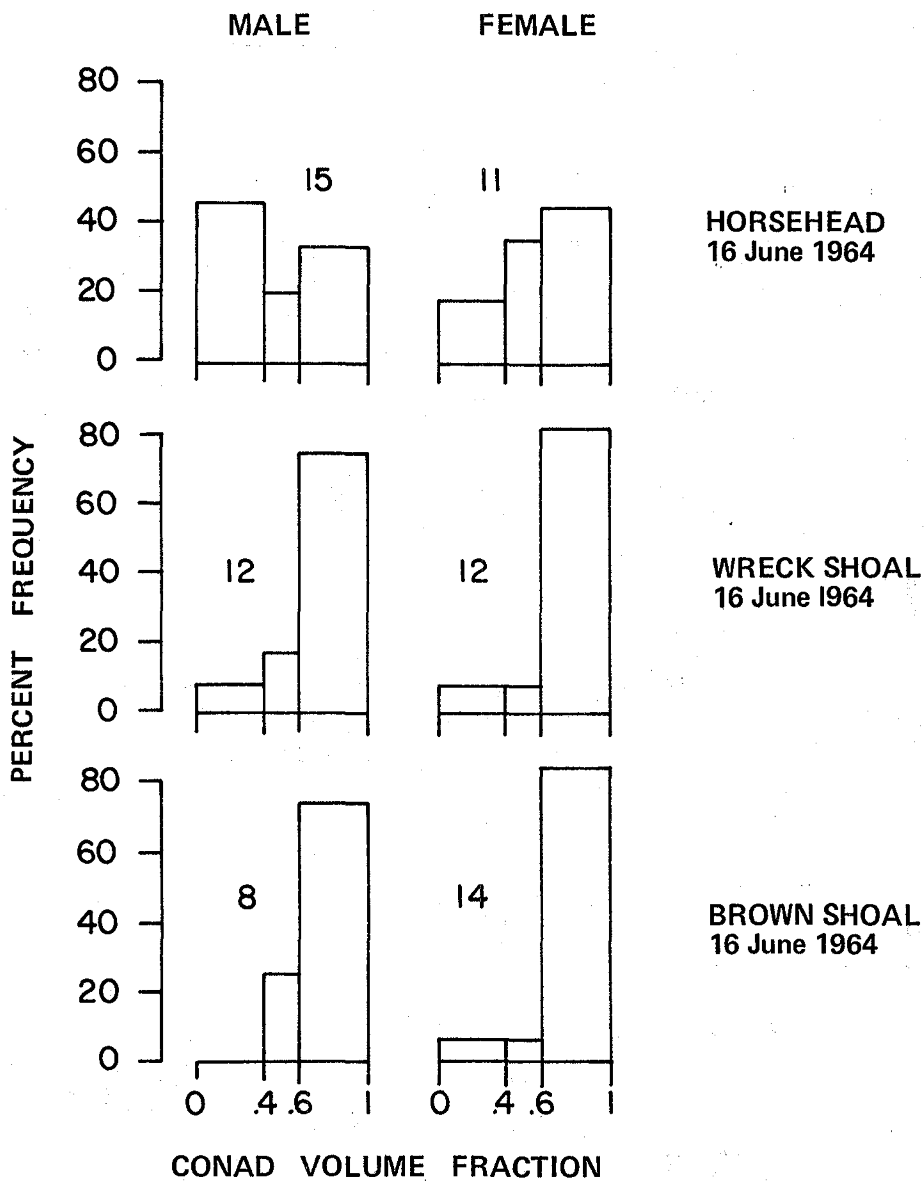


Figure 1. Percent frequency of gonad volume fraction values by sex for oysters collected in 1964. Based on examination of mounted histological sections in VIMS archives. Number of oysters examined is given by each histogram.

PROJECT 2

Effects of Predators and Fouling on Spat Settlement and Survival

Introduction

A possible increase in abundance of either fouling organisms or predators could conceivably have caused the decline in lower James River seed oysters, or if not the cause, could be preventing recovery of oyster abundance to pre-1960's levels. Several projects were designed to examine both fouling and predation rates and effects; these have now been reorganized into two subprojects.

Project 2A

Development and Influence of Fouling Communities on Oyster Settlement

Description and Objectives

Knowledge of the seasonality and relative density of fouling organisms, as well as effects on spat settlement and growth, is critical to effective management of the resource, especially in regard to cultch planting. Timing of cultch placement can be crucial to the success of oyster setting. This project was designed to provide those details necessary for a rational consideration of fouling.

Status

Continuing.

Interim Findings

During the period May-October 1985 a field experiment was effected using diver deployed and collected series of clean shell substrate on Wreck Shoal in the James River. Preliminary data from the study suggests that clean shells placed on the river bottom between May 21 and July 1 exhibit a rapid increase in fouling coverage over time. Maximum fouling coverage appears to coincide with the period of maximum water temperature (mid-August), irrespective of when (between May 21 and July 1) the shells are placed on the bottom. Approximately 60% of the shell surface (both sides) are covered by fouling by mid-August and continue at this level of coverage through early October.

In addition to determining rates and extent of fouling progression over time, the compositional change over time of the fouling communities is being studied. Measured environmental parameters are also being assessed to determine possible relationships between the parameters and community structure. The high rate of fouling combined with the maintenance of the community has clear implications on the timing of shell planting operations in the James and other sites in Virginia. Completion of community and statistical analysis related to this project in spring 1986 promise significant managerial guidelines for future shell plant operations.

The natural reefs of the James River also contain significant communities of fouling organisms. Amongst these are the endolithic algae. During the latter half of 1985 studies on endolithic algae have continued with the purpose of determining their distribution in the Lower James River as a possible function of depth and salinity during the spring, summer and fall seasons, and quantifying their influence on oyster settlement and early post settlement survival.

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 of shell surface via chlorophyll extraction technique.

Data for the spring, summer, and fall chlorophyll samples was analyzed using analysis of variance (ANOVA) tests ($\alpha = 0.05$) performed on each of the three data sets. A borderline significant difference ($P = 0.0211$) in algal abundance per cm of shell surface between stations was seen in the spring sample, whereas the difference in algal abundance between stations in the fall sample was strongly significant. For both spring and fall samples the highest algal abundance values were found at the most upriver station (Deep Water Shoal) with values continually decreasing downriver, Nansemond Ridge having the smallest abundance of algae. The analysis of variance test performed on the data from the summer sample, where algal abundance was measured at four depths at Wreck Shoal Offshore and Inshore, showed no significant difference, suggesting that depth at this station does not influence algal abundance. Work on species composition within the algal mat for these previous samples and others is continuing. To date, three species of bluegreen algae (Masigocoleus testarum, Schizothrix calcicola and Entophysalis deusta) and one species of green algae (Gomontia sp.) have been identified.

To complement the aforementioned field studies laboratory experiments on the influence of endolithic algae on oyster settlement were completed using the following method. Oyster shells were obtained from Deep Water

Shoal (DWS) and shells chosen according to present criteria (whole shell 5-6 cm long; minimal erosion of surface and minimal macrofouling by barnacles). Ten shells were analyzed for chlorophyll content; however, samples collected at DWS at the same depth and time the previous year will be used for algal composition information. The remaining shell was scrubbed to remove all epilithic organisms then cut into one inch square pieces with a geologic saw. The pieces of shell substrate were held in flowing filtered (50μ) seawater until the beginning of the experiment. Sandblasted double-strength glass was also cut into one inch square pieces, acid cleaned, washed, and soaked in filtered seawater to remove any remaining chemicals.

Metamorphically competent oyster larvae were obtained from the VIMS hatchery twenty-four hours before the setting of the larvae. All substrate pieces were scrubbed, the shell substrate divided into Treatments A and B, substrate of Treatment B boiled, and substrate of all treatments arranged in a random order in the setting tray filled with 1μ filtered seawater. Treatments A, B, and C are as follows: shell containing live endolithic algae; shell containing dead algae; and glass, respectively. Thirty-six thousand pediveligers were then introduced to the setting tray, maintained and fed for six days at which time the experiment was terminated, the spat dyed with methyl red and removed to air dry.

The parameters measured were magnitude of spat set and post settlement survival. The values for spat set consisted of the number of live and dead spat found on the outer surface of each piece of substrate. Post settlement survival was defined as those spat that had settled, were larger than 280 and were actively growing when the experiment was terminated. Values for this parameter consisted of the number of spat on the outer substrate surface meeting the criteria set forth by the definition of post settlement survival.

Spat set data of Treatments A (live algae), B (dead algae), and C (glass) was analyzed using an analysis of variance (ANOVA) test ($\alpha = 0.05$). A significant difference between treatments was found; however, the multiple range test showed that set on substrate on Treatment A was different (larger) from that of Treatment C and set on shell of Treatment B was different (larger) than set on glass of Treatment C, but the number of spat setting on the substrates of Treatments A and B were statistically equal.

An ANOVA test was also performed on the post settlement survival data for Treatments A and B; data from Treatment C was not statistically considered because of too few data points. A significant difference in survival between treatments was found, suggesting that oyster spat survive and grow to a larger degree on shell containing endolithic algae.

Statistical analysis of chlorophyll data generated during the spring, summer and fall seasons suggests that the abundance of endolithic algae is partially influenced by salinity but not by the depth at which it exists. It is possible that a larger species of algae exists in larger densities at the upriver stations than in the more saline waters of the downriver stations, thereby containing a larger amount of chlorophyll per unit volume. This would explain the larger algal abundance values seen at the upriver stations. Data generated from identification and quantification of algal filaments on the species composition slides will provide this information.

Sampling of oyster shell over the past few years has shown the widespread occurrence of endolithic algae throughout the Lower James River. Results of the 1985 laboratory experiments indicate that endolithic algae does not influence the setting of oyster larvae; however, once the oyster larvae have set on a shell containing the algae their survival appears to be enhanced.

Project 2B

Predation on Oysters After Settlement

Description and Objectives

See overall objectives for Project 2.

Status

This project included both field studies of seasonal variation in the abundance of oyster spat and predators at Wreck Shoal in the James River, and specific examination of crab predation on oysters in both the laboratory and the field. Field studies of oyster spat and predator abundance have been described in detail previously. By December 1985 we had examined all samples collected through November 1984 and 83% of all samples collected.

Interim Findings

Figure 2 shows the density of *Odostomia*, *Stylochus* and oyster spat in samples collected between August and November 1984. Densities are high, and it appears that the densities of spat and predators are negatively correlated between the middle of August and the middle of September but positively correlated from there on. The density of *Odostomia* was significantly higher than that of spat from the middle of September through

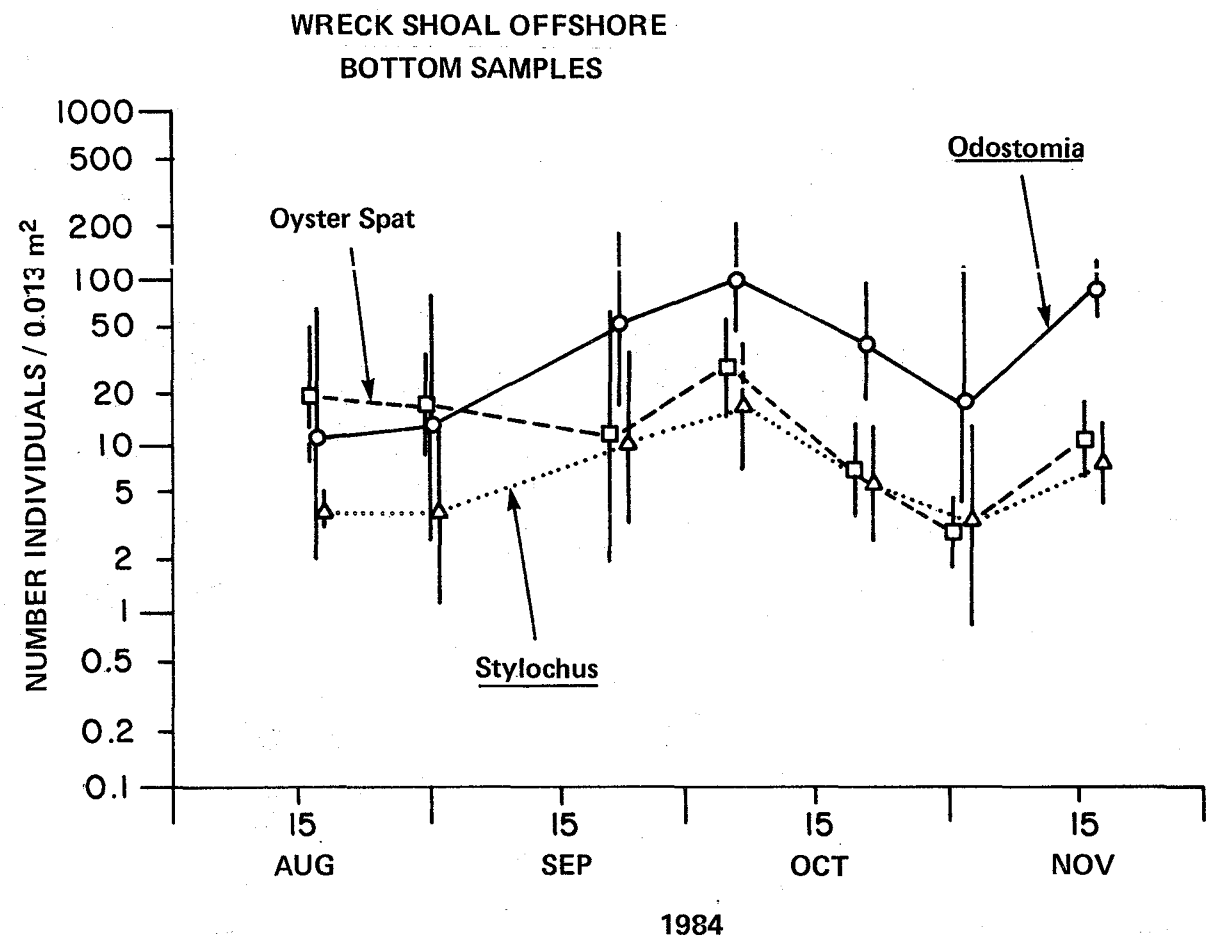


Figure 2. Number of individuals per 0.013 m^2 of oyster spat and two of their predators in bottom samples collected at Wreck Shoal between August and November 1984. Mean and 95% confidence intervals are given.

November, while the number of Stylochus was about the same as that of spat for the same period.

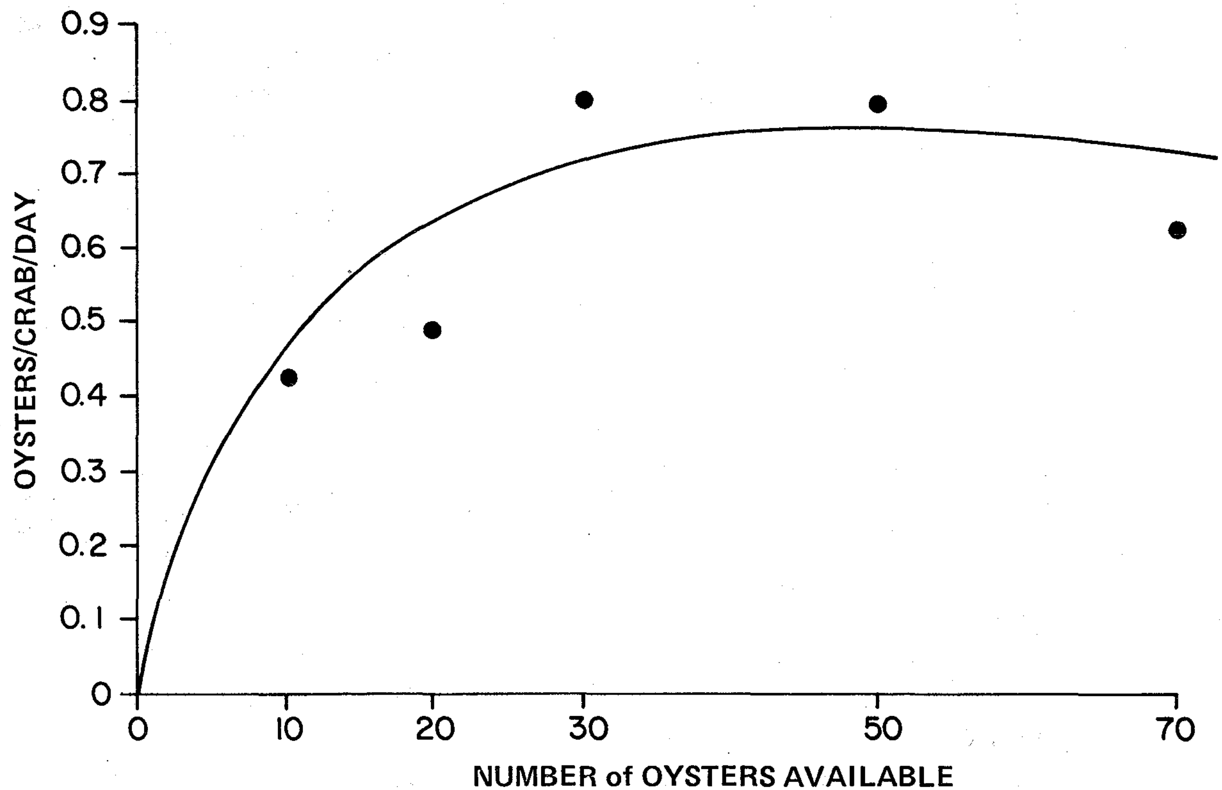
Densities of mud crabs and blue crabs are not shown because they were always either absent or extremely low in our samples.

From 10 June through 11 November, 1985, four series of laboratory experiments were conducted to assess mud crab, Panopeus herbstii, predation on Crassostrea virginica of the 20-40 mm right valve length. In addition to obtaining an overall predation rate it was considered desirable to examine predation in terms of the predator's functional response. The functional response is a model which describes the increase in predation with an increase in the number of prey available. There should be an upper limit to the predation rate beyond which there should be no increase in predation with the additional availability of prey.

The mean overall predation rate over 32 treatments with varying densities of spat was 0.65 ($\pm$ 0.485 S.D.) spat/crab/day. Predation rates ranged from 0 to 1.60 spat/crab/day. Even though these results are highly variable, they are significant (one tailed t-test, α = .10).

The functional response curve is not clear-cut at low prey densities because of the high variability in predation rates between individual crabs; however, it is the asymptote which is of importance. As shown in Figure 3, there is an obvious asymptote above a prey availability level of 30 spat per crab. This corresponds to a density of 472 spat/m^2. The importance of this is that predation will increase with an increase in prey up to this density level, whereas above this density predation rates will be maintained at a steady level. This suggests that the effects of predation are of relatively less importance once this prey density threshold is exceeded.

Figure 3. The functional response of *Panopeus herbstii* on *Crassostrea virginica*.



Although the original research plan examining crab-oyster predator-prey focussed on the mud crab Panope, the opportunity was taken to expand the study to include work with the blue crab *Callinectes sapidus*. Specific objectives were to quantify the level of oyster spat mortality due to blue crab predation under laboratory conditions, determine if there is a prey size selectivity by a given size blue crab, measure the relationship between prey density, feeding rates, and number of spat consumed, and quantify the seasonal predation rate of both Xanthid and blue crabs on oyster spat at Wreck Shoal seed bed, James River, Virginia.

Preliminary laboratory experiments were performed from July 12 through October 2, 1985, in order to assess the validity and sensitivity of experimental design. The data obtained were used to statistically determine the sample size and number of replicates needed to achieve the desired power in hypothesis testing. The experiments are of matrix design examining predation rate in relation to predator and prey sizes, and varying prey densities. The experiments utilize a flow through seawater system in which the seasonal changes in temperature are the physical variable influencing predation rates. The spat were attached to cultch and varied from 15-40 mm. Male crabs were used. The overall predation rate for crabs with an average carapace width of 146.4 mm was 5.48 ($\pm$ 6.53 SD) spat/crab/day with a range of 0-24 spat/crab/day. This rate was over 13 treatments with a mean water temperature of 25.9^{o}C. During September and the later part of August the mean temperature rose to 27.6^{o}C. This appeared to be the upper lethal temperature as numerous crabs died or became very lethargic. During October the mean predation rate for crabs with an average carapace width of 148.8 mm was 1.3 ($\pm$ 0.59 SD) spat/crab/day with a range of 1-4 spat/crab/day. This rate was over 3 treatments with a mean water temperature of 22.5^{o}C. Crabs with a mean carapace width of 116.3 mm did not demonstrate any predation during the experimental period. The data has yet to be analysed with respect to objectives 2 and 3.

The field component of this study utilizes a mark and recapture method in order to quantify the level of crab predation at the 1984 Wreck Shoal VMRC shell plant area. The mean density of spat over 3 treatments was 177 ($\pm$ 14.9 SD) spat/0.25 m^2. The mean predation rate for Xanthid and blue crabs combined was 0.38 ($\pm$ 0.21 SD) spat/0.25m^2/day. This corresponded to a mean water temperature of 27.4^{o}C. A continuation of this work is planned for summer 1986.

At this time it is clear that crab predation has significant impact on oyster populations in the James River. Control of such predation does not, at this time, appear practical.

PROJECT 3

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

Description and Objectives

Larvae are particularly sensitive stages in the life history of the oyster. They are vulnerable to negative effects following exposure to numerous substances especially during the period when they attach to the bottom. 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. The objective of this portion of the research was to verify prior laboratory results indicating great sensitivity of oyster larvae to chlorinated sewage during settlement and secondly to evaluate whether adverse effects can be found in the estuary near the outfall of a sewage plant discharging chlorinated sewage.

Status

Further laboratory testing has supported earlier research that indicated larvae are sensitive to chlorinated sewage at low concentrations. The concentrations, expressed in terms of chlorine residuals, which impact on larvae are at or below measurable concentrations. Therefore the potential for a serious negative impact at a sewage discharge location cannot be readily evaluated by mere chemical analyses.

To evaluate actual impacts in a field situation, three considerations are important: the success of initial settlement, the survival of newly set oyster spat, and the initial growth of spat. Oyster settlement has been monitored at 16 stations in the James River during the summers of 1984 and 1985. All samples from this phase of the study have been counted and tabulated for data analysis. During the summer of 1985, substrates with spat newly set in the laboratory were photographed, placed at the 16 stations in the James for a week, and rephotographed. By comparing the photographs, one can obtain an estimate of survival and growth of the spat. In addition, the entire fouling community was enumerated after the plates were photographed the second time. The images on each before and after photograph have been transferred to paper, and the initial and final shell lengths are being measured as an index of growth.

Problems and Delays

The quantification of the photographic data has been delayed during the fall by changes in personnel. The staff has been returned to full complement and a major effort is being made to complete digitization of the data by the middle of February 1986.

Findings

The laboratory experiments conducted in 1984 regarding the dosages of chlorinated sewage inhibiting settlement of pediveligers confirmed earlier results. In the summer of 1985, the experiments considered settlement and metamorphosis. A preliminary examination of the results suggests that the effective concentrations expressed a chlorine residual and are about the same as those for settlement (simple attachment).

During the initial field study of 1984, it became clear that oyster spat attach to suitable substrates located within several hundred feet of the discharge point as well as they do at more remote sites. Differences in settlement among the stations sampled bore no relationship to proximity to the discharge point. Similarly, other members of the marine fouling community were unaffected by the discharge of chlorinated sewage at the present concentrations. Results during 1985 are similar.

It is clear that newly set spat placed at the 16 stations are subject to significant mortality at all locations. A cursory examination of the photographic plates does not indicate any effect of proximity to the discharge point. Shell growth was observed at all stations, although it is too soon to determine whether there was a difference in growth among the stations.

Continuation of the Project

It is expected that the data analysis can be completed before the termination of the present project period. These data should be sufficient to reach a conclusion whether the potential for inhibition of oyster settlement suggested by laboratory studies is in fact realized in the James River with the present treatment practices and discharge rates. Less intensive monitoring routinely conducted by the Institute should provide adequate evaluation of changes in settlement over extended periods. The more intensive monitoring of the present project will be terminated.

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

Management Summary

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 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.

Background Information on the Circulation Studies of the Seed Oyster Beds

Although all of Virginia's tidal waters are considered potential shellfish growing waters, in fact that potential varies greatly from estuary to estuary and also from one part of an estuary to another. Oysters require at least brackish water to survive, and therefore they are found only where the salinity averages about five parts per thousand (ppt) or more. In the James, Wreck Shoals is the approximate upper limit of the principal oyster rocks. Within the brackish portions of the bay, the quality and quantity of available food and other factors result in some areas being good "seed" areas, others being good "grow out" areas, and some areas being generally poor for oyster culture in general. In the James the reach above the James River Bridge is an excellent seed oyster area, while the Hampton Roads area below the bridge and adjacent parts of Chesapeake Bay historically were used as grow out areas.

The moderate salinity (5 to 15 ppt) region in the James River seems to be exceptionally well suited to the production of seed oysters. Decade after decade large numbers of small (roughly one to two inches) oysters have been harvested from the oyster rocks in this region. Once the James seed oysters reach this size range, they grow slowly; this results in a relatively thick shell which makes them ideal for transplanting, since blue crabs and oyster drills are not able to attack the thick shells easily. Consequently, James River seed oysters have been transplanted to other estuaries across the state, and the James continues to be the lynchpin of the Virginia oyster industry. Should some catastrophe wipe out the seed oyster beds of the James, the effects would be felt widely and oyster production for the state would drop to levels much, much smaller than at present. Some estimates suggest that up to 90% of the oysters harvested in Virginia originate in the James. It, therefore, is obvious why attention is being given to the seed oyster beds.

Until about 1960 large parts of the bottoms on Hampton Flats, Willoughby Bank and other areas were leased by planters and shucking houses. However, when the oyster disease MSX became prevalent, mortalities were sufficiently large to make further planting uneconomical, and Hampton Roads ceased to be used as a grow out area. Coincident with those oyster mortalities, there was a decline in the setting of oyster larvae. Shell strings placed in the river since around 1960 show spatfall only about one-tenth as abundant as that measured before 1960.

One might ask why circulation, as well as oyster biology, is being studied. The answer is that the circulation in the James appears to be one of the most important factors which makes it such an outstanding producer of seed oysters. Some estuaries have very light spatfall (attachment of larval oysters to shell or other hard surfaces on the bottom) most years with exceptional spatfall infrequently. Neither situation results in high productivity because the light spatfall results in low densities of adult oysters and the heavy spatfall results in crowding and competition among the small oysters. That, in turn, results in mortality for many of the oysters, and those which do survive have peculiar shapes and grow slowly due to the crowding. In contrast, the James has moderate spatfall through much of the spawning season and experiences relatively consistent spatfall year after year. One possible explanation is that the circulation patterns in the James result in the dispersion of the oyster larvae, but most of the larvae are retained in the growing areas. Hence, the spatfall is moderate rather than light or intense. Because there are a number of large and productive oyster rocks in the James River, spatfall is consistent from year to year. In summary, the great productivity of the James River appears to be based in part on the circulation patterns which disperse oyster larvae over a broad area, much of which provides suitable habitat for the organism. The circulation and the existence of numerous oyster rocks results in a set of juvenile oysters that is consistent over the years and appears to be in densities that are near optimal. Despite a dramatic drop in the spatfall which occurred around 1960, the James River continues to provide seed oysters that are grown to market size in other estuaries in the state.

Preliminary Findings

The circulation patterns in the James disperse larvae over a large area with the general pattern being a counter-clockwise movement of water. In addition, the so-called gravitational circulation resulting from salinity differences causes the saltier bottom waters to move upstream slowly. Thus, there is a mechanism to both replenish the bottom waters (which often exhibit poor water quality in other estuaries) and transport larvae upriver to the seed beds. The preliminary results of current measurements suggests that the gravitational circulation is especially strong in the James.

By weighting the results of dye studies made in the hydraulic model, it has been possible to compare the likely contributions of Wreck Shoal and Hampton Flats prior to 1960. It appears that Hampton Flats and Wreck Shoal had roughly equivalent strengths as sources for larvae, suggesting that Hampton Flats was an important source of larvae prior to the impact of MSX. As well, it suggests that Wreck Shoal is indeed the most important remaining source for larvae.

Convergence zones or fronts have been observed at a number of locations in the James. Those occurring near Newport News Point appear on most flood tides and dramatically alter the pathway of water (and whatever else) flowing across the front. The existence of that front may be due in part to the large scale characteristics of the flow pattern as well as local changes in bottom geometry and salinity structure. Although the importance of these fronts to larval transport in general remains an unanswered question, it is clear that the front at Newport News Point is important to larval transport processes.

The results from the Wreck Shoal sedimentation studies indicate that mean currents influence the degree to which oyster shells remain clean or accumulate sediments. Further generalization of those results will lead to criteria for ranking substrate suitability for shell planting. These findings, coupled with the 1985 current measurements in the general circulation studies, will provide additional means of ranking potential shell planting areas. The final result, then, will be maps showing areas where the circulation should keep the substrate clean and bring in oyster larvae.

The Wreck Shoal sedimentation studies also suggest that oyster harvesting since 1870 has significantly modified patterns of shoaling in Burwell Bay. Moreover, the existing reef surface is fossil shell (about 400 years old) with a veneer of live shell. The management implications are discussed in the section on Project 4D.

Recommended Extension in the 1986-88 Biennium

While much will be achieved by July 1986, certain of our objectives will not be met. In large measure this is due to the need to give much expanded attention to the frontal system at Newport News Point. It will be necessary to extend our activities into the 1986-88 biennium to include further integration of the results from the component studies and additional field and analytical study of mesoscale features such as fronts. Funds for these activities are being programmed from the agency's expanded general fund base.

In addition, we recommend that a state-of-the-art, three-dimensional numerical hydrodynamics model be acquired, configured for the James River, and interfaced on the VIMS computer. This model may then be applied to the larval transport problem. Funds for the model have not been identified.

Project 4A

Horizontal Circulation and Characterization of Larval Movement and Retention

Description and Objectives

The larvae of oysters are microscopically small, and although they can swim upwards (or sink when they cease swimming), for the most part their transport and dispersion occurs due to tidal flows. Stated somewhat differently, the natural circulation patterns determine where oyster larvae will be located when they reach the stage of setting on hard surfaces. Thus, circulation plays an important role in the continued recruitment of the oyster population. For this specific project, the objectives are twofold: (1) to determine from the general horizontal circulation patterns the most probable pathways followed by oyster larvae, and (2) to estimate the retention of oyster larvae within the seed bed region during the setting period (10-20 days after spawning).

Status

Pre-existing data sets from the James River hydraulic model in Vicksburg, Mississippi, have been used to understand transport processes. The dye study results have been further adjusted to account for abundance of spawning oysters, the likely viability of the resulting larvae, and other biological factors. This phase of the study is essentially complete.

The study of other aspects of the flows in Hampton Roads has increased awareness of the complexity of the circulation in this region. Efforts to upgrade our understanding of horizontal circulation and its role in larval transport have been only partially successful, as will be discussed in the Findings section below.

Problems

Although the initial studies of circulation and larval transport will be completed within the biennium, efforts to improve that understanding through use of mathematical models will not be completed by the end of this project. The reasons for this situation are presented in the following section.

Findings

The hydraulic model study results illustrate some of the difficulties with trying to understand complex physical and biological systems. The dye results indicated that the present seed oyster beds were the best sources of larvae and the best areas for setting. However, when the abundance and fecundity of the oyster populations on the existing and previously productive areas are considered, the results suggest that the oyster population on Newport News Bar and other parts of Hampton Flats were important to the James River oyster production.

Those results suggest that biological aspects should be incorporated into the modelling scheme. The hydraulic model has a number of limitations, not the least of which are its distant location and the difficulty one has in making modifications to it. Mathematical models, once developed, provide a useful and relatively inexpensive tool for simulating a variety of situations, including differing management schemes.

Two pre-existing 2-dimensional models (one in the horizontal plane and the other in the vertical plane) have been used in this project. The model which simulates the vertical differences in currents and salinity has been helpful in improving our understanding of density driven circulation but is not suitable for most management applications. The horizontal plane model, on the other hand, appeared to have the potential to reproduce the movement of a cloud of oyster larvae. Careful examination of model predictions suggested that lateral dispersion may not be adequately simulated, but existing real-world measurements were inadequate for testing and revising the model. Thus, a major remaining task is the conduct of a dye study in lower James/Hampton Roads area to determine which model (hydraulic versus mathematical) gives the better estimate of lateral dispersion.

The insights gained in this and other subprojects have illustrated a number of the complexities of the circulation in Hampton Roads. The present opinion of the scientists involved in these studies is that only a 3-dimensional model will be able to reproduce the important physical features at a scale and in a manner that will be useful to managers. It should be noted that the aforementioned dye study, measurement of currents and other field portions of this project will provide the bulk of the data necessary for the calibration of such a 3-D model of tidal flows.

Continuation

Much work will remain at the end of this biennium. If our ability to predict the movement and dispersion of oyster larvae and pollutants is to be moved forward, then a 3-dimensional model is required. Assuming that such a model is secured, then the data from the present study must be synthesized for use in that model.

Project 4B

Role of Mesoscale Circulation in Larval Transport

Description and Objectives

Although it is necessary to understand the general circulation pattern, smaller scale features also can be very important affecting both the local environment as well as the overall circulation. Of particular concern are fronts or zones where two water masses converge. The objectives of this project are: (1) to identify where and when these convergence zones (fronts) occur, and (2) to determine whether these fronts play a significant role in oyster larval transport processes.

Status

Extensive and intensive studies of the fronts occurring near Newport News Point have been conducted. Detailed measurements also were made in the vicinity of Wreck Shoal, but other parts of the seed bed area have been studied to a limited extent only. The data from the field studies are being interpreted and also placed in the broader context of fronts as studied around the world.

Problems

The primary problem, such that it is, is that the study of fronts in a complex and large water body such as Hampton Roads requires more than two years for completion. It should be noted that this field of study has been developed only in the last decade or so; therefore, the technology and the understanding are progressing rapidly. Given the paucity of information on fronts in estuaries in general and the Chesapeake Bay region in particular, it is not surprising that much remains to be accomplished.

Although much progress has been made during the past 18 months, much remains to be done. The occurrence of fronts and much of the physics will be documented by the end of this project, but the importance of fronts to biological processes will be only partially assessed.

Findings

Perhaps the most important finding has been the documentation that fronts are a regular feature of the estuary. A group of fronts has been found to exist during flood tide near Newport News Point, and this feature has been observed repeatedly during varying meteorological and water column conditions. Additionally, water flowing through the front plunges from the surface to depths of five meters or more below the surface. Oyster larvae entrained in the flow are thus passively injected to depths where the net flow is upriver. Thus, this frontal system is likely to play a strong role in larval transport processes.

Fronts and mesoscale features of a different nature also have been observed near Wreck Shoal. There the highly variable bottom topography, the nature of the salinity stratification, and the strength of the tidal currents appear to be the controlling variables that determine the characteristics of the flow patterns. Near Newport News Point density stratification plays an important role, but it appears that larger scale circulation patterns also are important and may be the reason that these fronts occur on virtually every flood tide.

Published studies of fronts in other environments are being reviewed to better understand the dynamics of fronts occurring in Virginia and to gain insights as to other times and places where fronts might be encountered. A summary of our findings within the general context of front development and dynamics will be completed by the end of the project.

Continuation

Continued study of fronts in the lower James River and the Hampton Roads area definitely is warranted. Areas that have already been studied will be further studied to elucidate the physics of the processes. In addition, other parts of the river will be surveyed to see if similar fronts exist or if fronts develop due to other factors. And finally, as the understanding of the physics of fronts becomes more sure and clear, field studies will be initiated to document how the fronts affect distributions and transport of phytoplankton and larvae.

Project 4C

Role of Temporal Variations in Gravitational Circulation

Description and Objectives

Although the tidal flows are the most obvious feature of estuaries, important transport processes occur at other time scales. In particular, density differences due to variations in salinity give rise to the so-called gravitational circulation. When tidal flows are averaged over several tidal cycles, one can observe a net flow of the saltier bottom water upriver and a net flow of the fresher surface waters toward the ocean. The specific objectives of this project are: (1) to document any fortnightly variation in this density-driven circulation, and (2) to infer the role of temporal and spatial variations as they affect oyster larval transport.

Status

Data from 15 consecutive daily slackwater surveys made in August 1983 have been analyzed to examine the fortnightly lunar cycle. Current meters were deployed briefly at one station in the Fall of 1984, and a major field study was conducted during the Summer and Fall of 1985. The data from the latter survey are being processed and analyzed to provide information on temporal and spatial variations in both tidal currents and the long-term-average currents.

Problems

No major problems have been encountered nor are any anticipated. However, the long-term deployment of several tide gages, a string of 12 conductivity and temperature sensors, and approximately a dozen current meters, some of which were equipped with conductivity and temperature sensors, has produced volumes of data. Although the essential components of the analysis should be completed within the fiscal year, the integration of these with other components of the study will require additional time.

Findings

The studies of the horizontal circulation suggested that the lower James acts as a large mixing bowl. The results of the slackwater surveys support this general concept in several ways. First, strong longitudinal salinity gradients in the Burwell Bay area suggest that the constriction at Mulberry Point serves as a partial barrier to upstream transport. This in effect provides the upper limit to the study area. Near the mouth of the river, the water column was consistently stratified which suggests that there is a constant exchange of water with the Bay. The intermediate reaches, however, responded to the lunar cycle by mixing and then restratifying. This lunar effect may be an important contribution to the "mixing bowl effect".

The preliminary current meter deployment suggests that in the James gravitational circulation is strong most of the time. Review of dissolved oxygen and salinity data for the major tributaries corroborates this finding. Although wastewater loadings to the James are quite large, it experiences problems of low dissolved oxygen very infrequently, whereas the more pristine York and Rappahannock estuaries often exhibit low concentrations of dissolved oxygen in bottom waters near the mouth. The strong gravitational circulation is believed to be the reason why the James experiences few of these water quality problems, which in turn may explain why survival of oyster larvae is better in the James than in the other tributaries.

Continuation

It is anticipated that the objectives of this project will be met by the end of the fiscal year. However, we also anticipate that the Commonwealth will benefit from this project for many years to come. First, the state-of-the-art equipment purchased will be available for future studies such as the effect of channel dredging on the circulation patterns and the currents at specific points. In addition, the data sets which have been collected will provide much information that will be useful to both scientists and students in future studies which may or may not be oriented toward the management perspective.

Project 4D

Micro-Circulation and Sedimentation Processes at Wreck Shoal

Description and Objectives

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 better able to estimate 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. And, of course, the results are directly applicable to replenishment efforts in the Wreck Shoal area.

Status

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 years) morphological changes and the modern processes acting.

All of the field work and much of the analysis has been completed. These include: (1) comparisons of bathymetric data of 1854, 1871, 1910, 1946, and 1984, in order to identify recent morphological changes; (2) sub-bottom seismic surveys to trace the evolution of the Wreck Shoal area as it pertains to existing oyster reef areas; (3) quantitative surface sampling to discriminate the different types of oyster producing bottoms in the area; and (4) measurements of the hydraulic regime operating over the different types of oyster producing bottoms.

The results of these activities are being integrated to provide a general interpretation of the sedimentation processes. This will be completed during the January-June time period.

Interim Finding

The analyses to date lead to the following tentative conclusions which have ramifications for resource management:

1) The comparisons of bathymetry between 1870 and later years indicate rather pronounced changes have occurred in the Burwell Bay-Point

Shoal/Wreck Shoal complex. In 1870, the natural channel coursing through Burwell Bay was quite deep (about 35 feet). At this time there were extensive intertidal oyster reefs on Point of Shoals, and the fringe reefs on Wreck Shoal, while subtidal, had very shallow depths. The intertidal and shallow subtidal reefs acted to inhibit tidal flow over Point of Shoal-Wreck Shoal and focused the tidal flow through the Burwell Bay channel, thus maintaining the relatively large depth. By 1940, dramatic changes occurred at the Point of Shoal-Wreck Shoal oyster reefs. The reefs on Point of Shoal and Wreck Shoal were significantly reduced in elevation; those on Point of Shoal were no longer subtidal. Correspondingly, the Burwell Bay channel shoaled dramatically from about 35 feet to 15 feet. This appears to represent a case where strong oyster harvesting pressures removed a flow barrier which resulted in flow reduction in the main channel and consequent shoaling. Man again intervened in the mid-1940's with the excavation of the Rocklanding Shoal channel to accommodate the loss of sufficient depths in the natural channel.

2) The deflation of the oyster reef by harvesting is corroborated by carbon-14 isotope dating on shells now within a foot of the oyster reef surface. A thin layer of live oysters overlie a substrate of oyster shell formed about 400 years ago. Thus, the current "cull law" (separation of market and seed oysters from cultch and return of cultch to bottom) is not sufficient to maintain the Wreck Shoal oyster reef. The continued loss of elevation will eventually deplete the fossil reef shell stock. With further reduction in

elevation, tidal currents will diminish, siltation will increase, and productivity will decrease.

These findings suggest that shell replenishment will be required to balance the loss of cultch. This could be achieved by "sprinkling" shell over the most productive reef areas in order not to bury the existing live oysters.

3) Repetitive side-scan sonar imagery repeated a year apart has documented that passage of large commercial vessels over Wreck Shoal is impacting the oyster grounds. Scars due to prop wash, with width up to 10 meters, have churned about 5 acres of oyster bottom. While this is a relatively small area, the impact could be avoided by requiring commercial vessels to use the marked navigation channels.

PROJECT 5

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

Description and Objectives

The Chesapeake Bay is considered to be an important nursery area for flounder, spot and croaker, which spawn on the continental shelf in the fall-winter. Therefore, it is important to investigate the climatic environment both on the shelf and in the estuary. It is likely that these species respond to similar wind and temperature regimes in these areas. The emphasis is placed on the flounder research first, because VMRC has expressed an interest in the development of a juvenile flounder index which they hope to use in directing the management of this fishery.

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.

Status

The average daily Norfolk, Va wind vector data base is continuously updated as data become available. The daily VIMS pier temperature data base is updated through November 1985 and past recorder problems have been dealt with. A new data logger was installed in late November 1985. Work continues on computerizing historic daily maximum and minimum temperatures as well as interpretting data from the new recorder.

The VIMS trawl survey data have been checked for errors through 1980. Approximately 7% of these data did not pass the error checking routines. Because of time constraints, flounder data from the VIMS trawl survey are being incorporated into the current SIR (Scientific Information Retrieval) data base which was developed for croaker. This data base format does not include hydrographic and station data, but only collections of fish by river and date. All discrepancies that are identified will be examined by hand.

Expected Accomplishments Next Quarter

1) Final error check and manual correction of flounder data from VIMS trawl survey.
2) Based on the VIMS trawl survey, development of a juvenile index for I^+ summer flounder as an index of recruitment to the Chesapeake Bay.
3) Development of a fall-winter wind index by which to quantify the successful juvenile recruitment of summer flounder spawned in the Middle Atlantic Bight.
4) Development of a statistical equation which explains juvenile flounder recruitment based on winds on the shelf one fall and winter temperatures in the estuary the following year.

Problems, Delays

A major problem with the VIMS trawl survey data base is that the gear has changed over the 30 years of collections. Parallel studies of gear comparison that were scheduled for 1985 to address these questions have been postponed. The Fisheries Department decided that such studies could not be undertaken until all past trawling methods had been recorded. Members of the Fisheries Department are currently documenting each trawl made by Fisheries at VIMS for the past 30 years with these gear problems in mind. Therefore, at this time the numbers of flounder, croaker and spot collected by the VIMS trawl survey cannot yet be corrected to compensate for gear differences.

In the previous model developed for the Atlantic croaker, successful recruitment into the Chesapeake Bay was indicated by a measure of juvenile croaker inside the Bay in the fall. This index of fall recruitment was regressed on fall winds to yield an algorithm for croaker transport. However, careful examination of the trawl survey data reveals no record of juvenile flounder in the Bay in the fall. In fact, juvenile flounder are not regularily sampled by the VIMS trawl until June of the following summer, when they are nearly 100 mm. Inspection of data collected throughout the Mid-Atlantic states and North Carolina reveals a substantial gap in data of summer flounder newly recruited to the estuaries. This appears to be due to gear avoidance and to sampling in the wrong areas. Juvenile flounder seem to prefer the more saline areas of the lower estuary, therefore, the Fisheries Department is extending their monthly trawl surveys to include the lower Bay.

We will analyze the abundance of flounder (0^+) collected during the summer by the VIMS trawl survey, but are not confident that this is an appropriate measure of wind-driven transport. The Chesapeake Bay and the the eastern shore of Virginia and the sounds of North Carolina are thought to be the primary nurseries for summer flounder. Since an observed index of juvenile flounder recruitment is not now available for either state, the wind transport data will be used as an indicator without correlating it to estuarine collections of flounder in the fall and winter. We suggest that collections efforts be directed at the more saline areas of Chesapeake Bay and Pamlico Sound to verify the mathematical relationships which result from this study.

Interim Findings

Summer Flounder

A thorough examination of the literature on summer flounder (*Paralichthys dentatus*), as well as contacts with prominent researchers in the field, lead to the creation of a generalized distribution chart of summer flounder in the Middle Atlantic Bight by season and life stage (Figure 1). The uncertain distribution of juvenile summer flounder is indicated within the estuaries of Delaware Bay, Chesapeake Bay and the North Carolina sounds.

There are indications of a northern stock in which both adults and juveniles are found nearshore and in the estuaries in the summer, but only juveniles are found nearshore in the winter, with mature adults concentrated offshore north of Chesapeake Bay. Around Cape Hatteras, a different pattern emerges, with both adults and juveniles found nearshore in the summer and winter. If there are two stocks, it is possible that the southern stock produces individuals which mature at a smaller size and age.

Eggs and larvae are found in the continental shelf waters. Thus planktonic larvae are vulnerable to prevailing currents as well as wind-driven water movements. It is 'common knowledge' according to the literature that juvenile (0^+) summer flounder use estuaries as nursery grounds, although they are rarely captured. Based on this assumption, our analysis of winds will consider wind-driven transport toward estuarine inlets to yield successful recruitment. Winds blowing offshore would result in the loss of larvae. In general, in September, October and November, when summer flounder larvae have been captured north of the Chesapeake Bay, the direction of the wind is toward the south and west. This, combined with the mean southerly longshore flow, would then result in transport of flounder into the Chesapeake Bay (larval drift in Figure 1). However, successful transport may not necessarily be into the Chesapeake Bay, but rather into Pamlico Sound, through Oregon Inlet in North Carolina. With two stocks geographically separated by Cape Hatteras, Pamlico Sound is hypothesized as a common nursery, with entering and exiting of the estuary occurring through the same inlets, i.e. north and south of Hatteras. Because adult stocks seem to advance northward yearly, it may actually be that good juvenile recruitment to the Chesapeake Bay supplies adults for the fisheries farther up the coast while juvenile recruitment to warmer waters of North Carolina determines the success of the fishery in Virginia.

It is unknown whether newly recruited flounder are affected by winter conditions within the Bay, since they are not sampled at this time. Flounder are known to be affected adversely by cold temperatures during

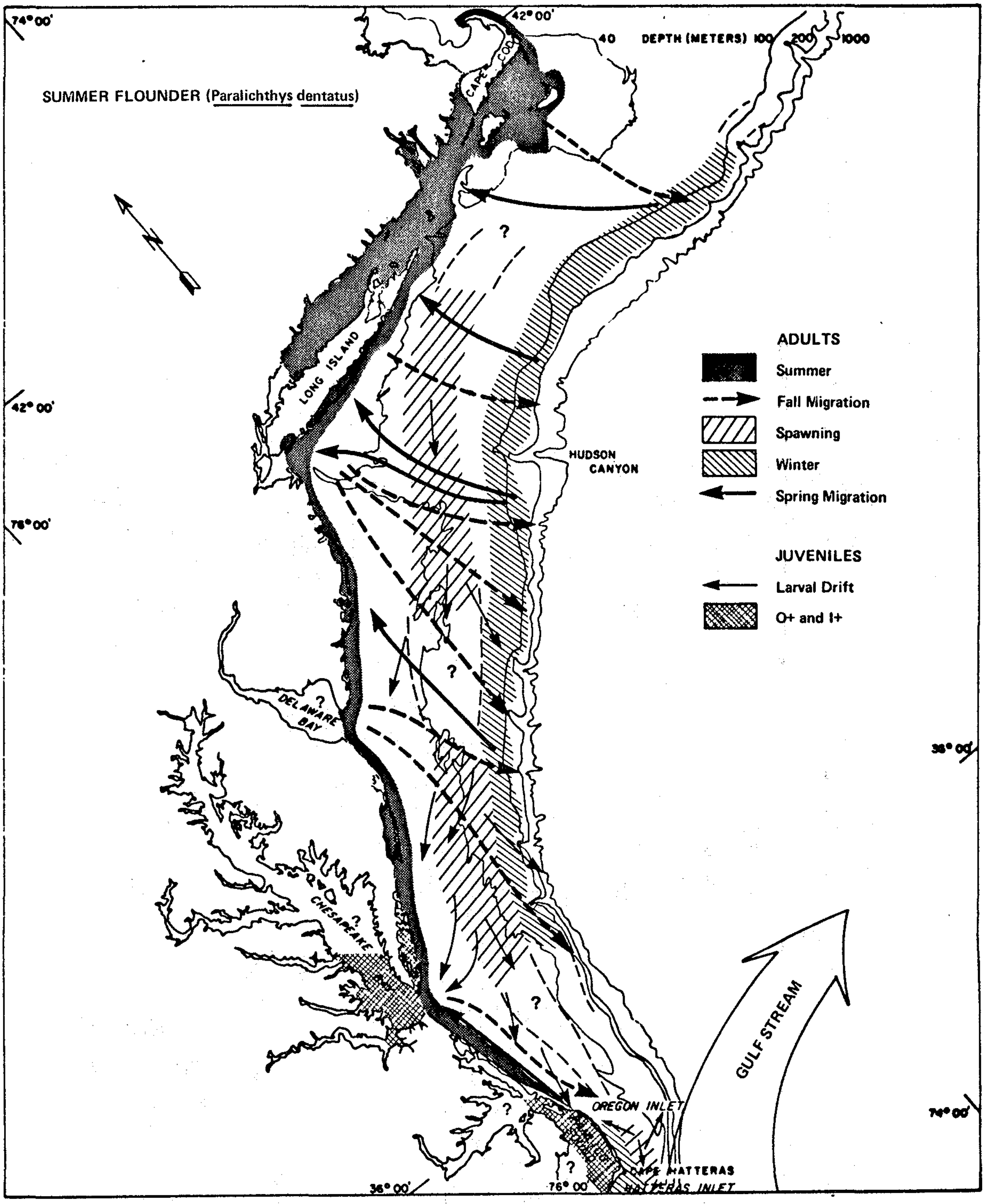


Figure 1. Seasonal migration and distribution of adult and juvenile summer flounder.

their second winter (I^+). E. Burreson (VIMS) has shown that flounder are weakened by cold temperatures and succumb to infestations of the parasite Trypanoplasma bullocki. Since no experimental data are available, we will empirically quantify the temperature effect by regressing on it the survival of summer flounder (I^+), as indicated by the abundance in the VIMS trawl survey the following summer.

The above discussion is summarized in a conceptual life history model of summer flounder (Figure 2). This model identifies the periods in the life history which are vulnerable to environmental perturbations. The key effects are catagorized with postitive and negative outcomes, and the potential reduction of flounder recruitment is clearly labelled at each life stage. This approach also identifies input data to use to run the model, and points out significant gaps in general knowledge and data collections for this species. The present study addresses the first and second years in this life history, i.e., from after spawning on the shelf through I^+ year-class strength in the estuary.

Spot

The literature says that spot spawn in the Mid-Atlantic Bight from late winter to early spring, and recruit to the Chesapeake Bay from December through May. However, analysis of seasonal bottom temperatures (1968-1981, December to May) has lead me to conclude that the appropriate warm water (16^{o}C) is not present during the winter spawning season. I hypothesize that spot spawn south of the warm water convergence zone near Cape Hatteras and are transported into the Chesapeake Bay as juveniles. The peak time of spot entrance to the Chesapeake Bay is from April through June, after danger from

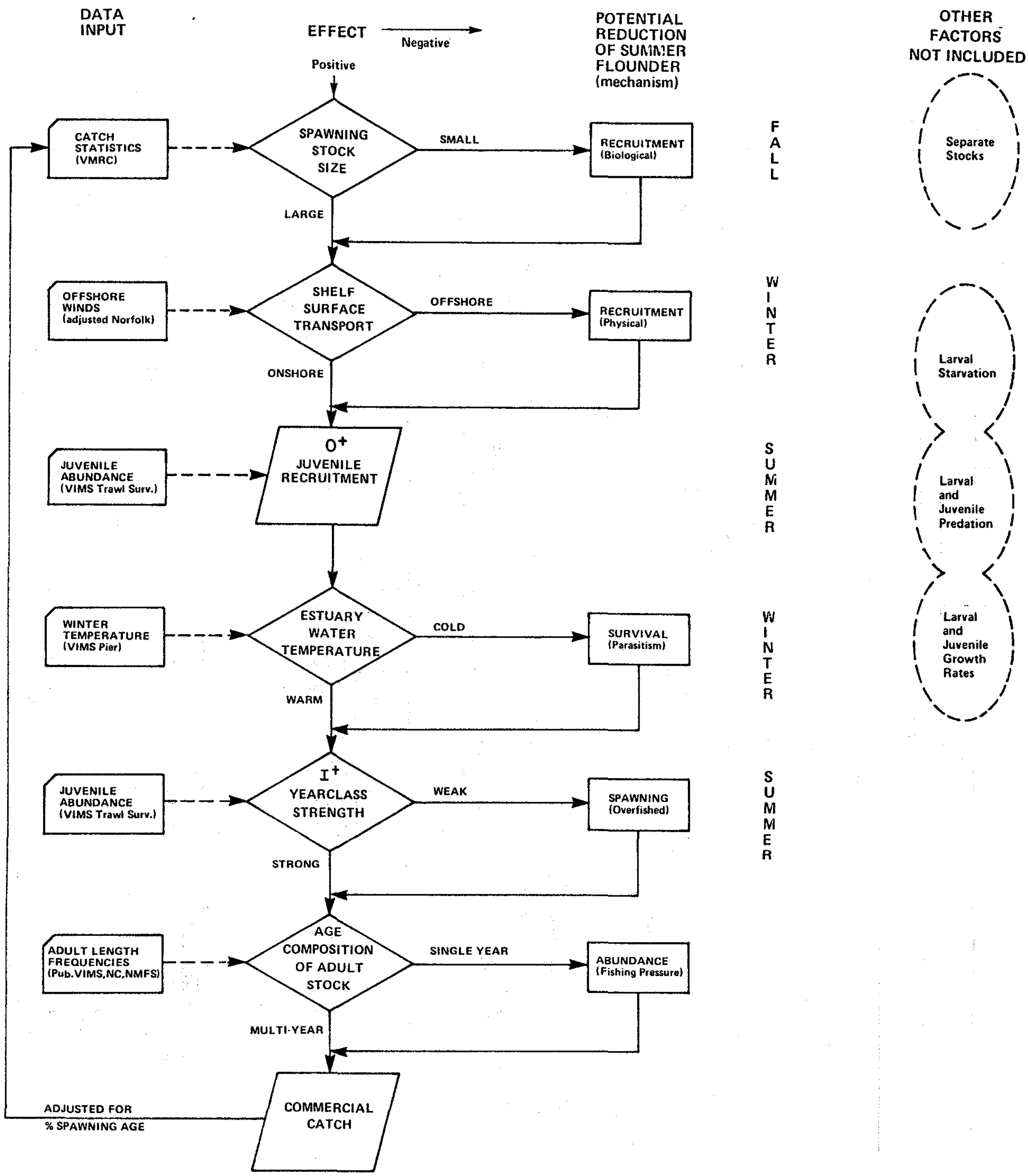


Figure 2. Flow-chart model of summer flounder emphasizing points in life history which are vulnerable and most likely to affect recruitment.

cold temperatures is past. Spot are larger and older when entering the estuary than croaker because they have spent more time on the shelf in transport from spawning areas, i.e. they travelled farther than the croaker larvae. Also, the shelf waters are colder in the winter than they were in the fall, thus slowing the growth rate of spot.

Recruitment of spot to estuarine nursery areas will be to the Chesapeake Bay, or to Pamlico Sound through Oregon Inlet north of Cape Hatteras, as discussed for flounder, or through Hatteras Inlet, or one of the other inlets south of the Cape. Because recruitment to the estuary is in the spring, cold water temperatures do not affect spot either by direct mortality as croaker or indirect parasite-induced mortality as flounder. Therefore, winter water temperatures will not be a factor in the spot model.

Young-of-the-year spot are major components of North Carolina long-haul seine and pound net fisheries. Post-recruitment mortality may be significantly higher in Pamlico Sound (i.e. due to fishing) than mortality at the same time in the Chesapeake Bay. The result would be that transport into the Chesapeake Bay favors juvenile survival and increased stock size of spot. Thus the combination of transport success and fishing pressure are likely to be important factors in the spot model.

Continuation

Burreson's work brings up interesting question. He states that he has never encountered a juvenile (0^+) flounder within the Chesapeake Bay in the summer with a positive antibody titer, which would indicate that it had be exposed to the parasite previously. This could only be because (a) flounder did not spend their first winter on the bottom of the estuary and therefore were not exposed to the parasite, (b) all flounder that were previously infected died, or (c) small flounder are intentionally avoided by the leach which is the intermediate host of the parasite. If 'a' is occurring, we need to determine where they are the first winter. If 'b' is occuring, a sizeable reduction of recruitment would result which should be investigated. The likelihood of 'c' 100% of the time is remote according to Burreson (pers. comm.). This reiterates the need for winter sampling in search of newly recruited flounder larvae/juveniles.

Continuing Efforts

1) Field sampling for flounder on the shelf and newly recruited to the estuary to verify the model.
2) Continued error checking of spot data from VIMS trawl survey.
3) Development of a juvenile recruitment index for spot.
4) Further investigation of winter circulation patterns around Cape Hatteras and wind-induced transport at that time.
5) Development of a predictive equation for spot based on hypothesis stated here.

PROJECT 6

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

Description and Objectives

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. In addition, recent management efforts directed towards Chesapeake Bay stocks by Maryland and Virginia have restricted landings, thereby reducing availability of fishery data useful for stock assessment. An alternative approach is the utilization of ichthyoplankton data to generate egg production estimates and calculations of adult biomass required to produce the observed egg deposition. In consideration of these needs, the objectives of the present study are: (1) to calculate striped bass egg production and female biomass on the Pamunkey River spawning grounds in spring 1985, (2) to assess suitability of existing survey data (1980-1983) for egg production and biomass calculation, (3) to examine the possible effects of acid rain on striped bass spawning grounds, and (4) to assess striped bass egg viability in relation to environmental variables (primarily pH, alkalinity and temperature).

Status

Laboratory processing of 1985 plankton collections (objective 1) continues. Eggs and larvae of striped bass are currently being sorted, identified and enumerated in remaining samples. Final data analysis for objectives 2-4 is now complete and summarized below. Initiation of spring 1986 sampling is planned for March.

Expected Accomplishments Next Quarter

1) Sorting and identification of remaining spring 1985 samples.
2) Development of egg production and female biomass estimates from 1985 Pamunkey River data.
3) Initiation of spring sampling in March.

Problems, Delays

Sample processing of 1985 collections has been delayed due to temporary reassignment of technical personnel to a federally funded (NSF) plankton ecology study at the Chesapeake Bay mouth. No further delay is expected and, upon completion of processing and analysis of these samples, all original objectives (1-4) will be successfully accomplished.

Interim Findings

Objective 1: 1985 Pamunkey Egg Production

Weekly or twice weekly sampling along a 25-mile segment of the Pamunkey River was accomplished during the period 2 April - 15 May 1985. Collection data and ancillary hydrographic results were previously reported in the First Annual Progress Report. Sampling yielded 74 plankton collections from which eggs and larvae of striped bass are currently being sorted and identified. 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. Completion of analysis is projected for June 1986.

Previous observations of salt penetration in the Pamunkey River 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 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. Recent analysis of previous data on the James River, however, suggests that drought conditions may not alter spatial characteristics of spawning activity.

Objective 2: Analysis of Existing Survey Data

Ichthyoplankton data sets resulting from the 1980-1983 surveys of the James, York (Pamunkey and Mattaponi) and Rappahannock rivers (Table 1) have been reexamined to assess suitability of these data for estimation of egg production and female biomass. Details of data collection are summarized in a series of final reports to the National Marine Fisheries Service (Grant and Olney 1981, 1982; Olney et al. 1983, 1985).

Egg production estimates were calculated following a modification of the techniques of Houde (1977) after Sette and Ahlstrom (1948) and Ahlstrom (1954, 1959). Fecundity values were derived following Setzler et al. (1980:13) using the equation $F = 2.18 \times 10^5 W - 1.17 \times 10^4$ where W is weight in kg. A value of 3.4 kg was used in this relationship since this value represented the mean weight of a 1978 yearclass female striped bass captured in pound nets on the Rappahannock River in spring 1983 (Loesch and Kriete 1983). This estimated fecundity value was used in calculations of female biomass in 1980 and 1982, although fishery data were not available on the York River system in 1980 and 1978 yearclass fish may not have contributed significantly to the spawn on the Rappahannock River in 1982. Comparable values of F are reported by Hardy (1978) on a range of female sizes from 2.3 - 29.5 kg. Fecundity values expressed as eggs per unit body weight may not vary significantly with age. In Hardy's (1978) data set, F ranged from 1.42×10^5 eggs/kg (22.6 kg individual) to 2.79×10^6 eggs/kg (14.5 kg individual).

Temporal variability in cruise egg production values (P_i) was observed between years on all rivers and can be related to mid-spawning temperature depressions observed on the Pamunkey and Rappahannock rivers in 1983 during

Table 1. Summary of striped bass egg and larval survey data from major Virginia tributaries, 1980-1983.

1980-1982	PAMUNKEY	MATTAPONI	JAMES	RAPPAHANNOCK
First sampling date	16 Apr 1980	18 Apr 1980	22 Apr 1981	5 Apr 1982
Last sampling date	13 Jun 1980	14 Jun 1980	18 Jun 1981	23 Jun 1982
Total survey cruises	13	13	9	19
Total stations	108	100	123	174
Total eggs	500	720	428	1976
Total larvae	162	153	431	4792
1983				
First sampling date	5 Apr 1983	-	8 Apr 1983	9 Apr 1983
Last sampling date	11 May 1983	-	8 May 1983	13 May 1983
Total survey cruises	6	-	5	6
Total stations	50	-	41	49
Total eggs	569	-	776	1174
Total larvae	41	-	155	439

the week of 22 April (Figures 1-3). On the James River (Figure 2), temporal trends were obscured due to insufficient sampling effort. Cruise production estimates on all rivers ranged from 0.004 - 1.615 (eggs X 10^8) in 1982 and 1983 (Tables 2-4).

Values of annual egg production calculated from 1980-1983 surveys of Virginia's major spawning grounds (Tables 2-4) varied from 1.499 - 7.242 (eggs X 10^8). The upper value of this range may be more justifiably reported as 5.81 X 10^8 eggs (Rappahannock 1983) since large variance estimates and confidence intervals associated with James River data (Table 3) indicate considerable error. These values are comparable to annual egg production estimates reported during some years in the Roanoke system (Hassler et al. 1981), larger than values reported by Boynton (cited by Setzler et al. 1980:46) on the Potomac but smaller than Potomac River production values calculated by Polgar (1977).

Biomass of 1978 yearclass females (average weight, 3.4 kg; calculated fecundity given in equation 4) necessary to produce the observed 1983 egg deposition was estimated to be 2707 kg ($\pm$ 1498 kg) on the Rappahannock River and 1022 kg ($\pm$ 756 kg) on the Pamunkey. These values were not calculated for the James in 1983 due to the large errors associated with James River egg production estimates. Summing the upper and lower biomass estimates for the Rappahannock and Pamunkey rivers yields a total biomass range of 1475 - 5983 kg necessary to produce the cumulative egg deposition in these two rivers in 1983. Thus, if only 1978 yearclass females (average weight, 3.4 kg) were spawning in 1983, these values indicate that as few as 434 or as many as 1760 individuals could have produced the observed spawn in these two rivers.

Table 2. Annual spawning and female biomass estimates for the Rappahannock River, 1982-3. Abbreviations used are: D_i - days represented by each cruise; d_i - duration of the egg stage; P_i - cruise egg production estimates; $S^2_{P_i}$ - variance estimate; CI - 95% confidence interval.

Year	Cruise Date	Daily Spawning Estimate (eggs X10^6)	D_i (days)	d_i (days)	P_i (eggs X10^8)	$S^2_{P_i}$ (X10^{16})	Annual Egg Production (eggs X10^8)	CI (egg X10^8)	Female Biomass (Kg)
1982	5 Apr	1.323	5.5	3.0	0.073	0.038			
	8 Apr	3.915	5.5	3.7	0.215	0.082			
	16 Apr	4.595	6.5	2.9	0.299	1.304			
	21 Apr	38.054	3.5	2.4	1.332	0.317			
	23 Apr	15.682	4.5	2.6	0.706	0.663			
	30 Apr	3.693	5.5	2.3	0.203	0.300			
	4 May	4.055	5.5	1.9	0.223	1.799			
	11 May	0.095	7.5	1.7	0.007	0.229			
Annual Total			44		3.058	4.733	5.45	± 1.508	2540
1983	9 Apr	0.910	11.0	3.1	0.100	2.533			
	15 Apr	2.469	6.5	2.9	0.161	0.055			
	22 Apr	0.140	7.0	3.3	0.009	0.037			
	29 Apr	24.846	6.5	2.6	1.615	10.167			
	5 May	3.237	7.0	1.8	0.227	1.974			
	13 May	0.541	6.0	1.7	0.032	1.366			
Annual Total			44		2.144	16.132	5.81	± 3.214	2707

Table 3. Annual spawning and female biomass estimates for the James River, 1981 and 1983. Abbreviations used as in Table 20 .

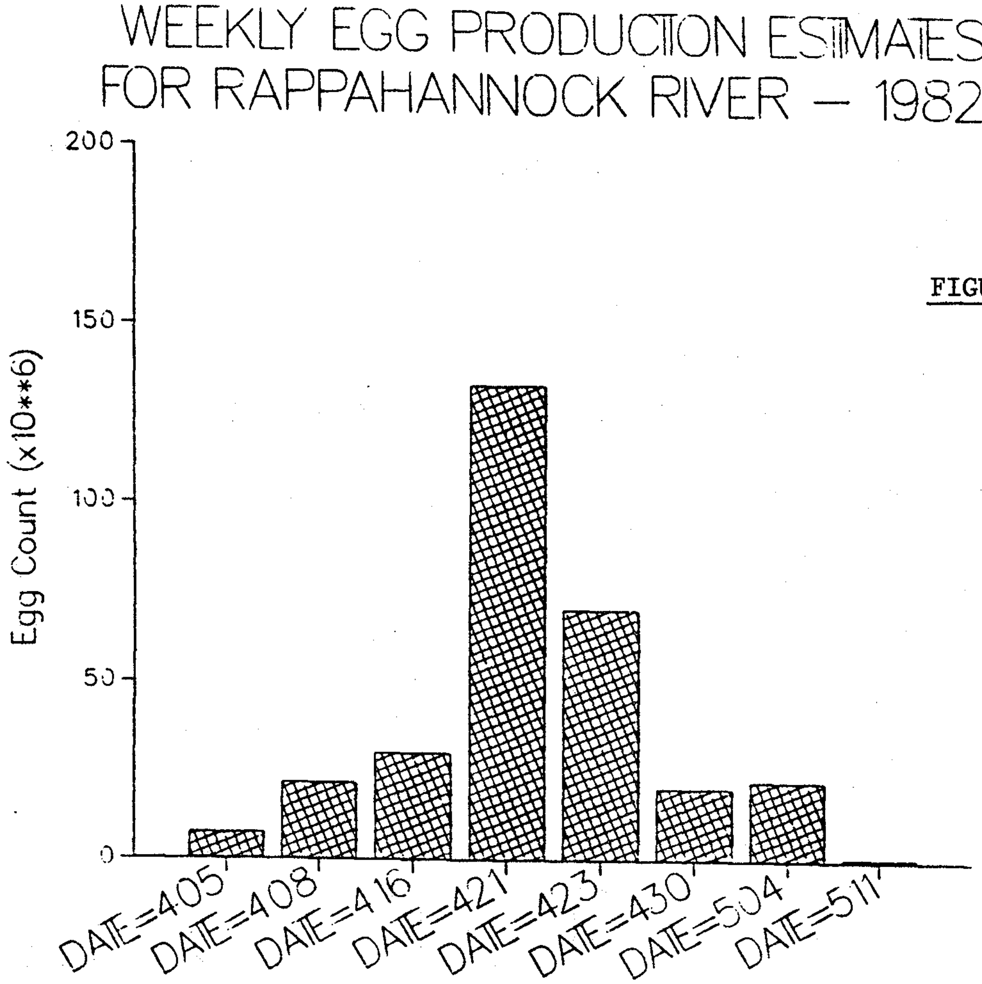
Year	Cruise Date	Daily Spawning Estimate (eggs X10^6)	D_i (days)	d_i (days)	P_i (eggs X10^7)	$S^2_{P_i}$ (X10^{17})	Annual Egg Production (eggs X10^8)	CI (eggs X10^8)	Female Biomass (Kg)
1981	22 Apr	0.967	24	2.3	2.321	4.678			
	27-28 Apr	2.108	7	1.9	1.475	0.269			
	5-6 May	3.919	8	1.8	3.135	2.168			
	13-15 May	0.377	5	1.7	0.189	0.001			
Annual Total			44		7.120	7.115	4.380	± 8.268	Not calculated
1983	8 Apr	0.003	10	3.1	0.263	0			
	14 Apr	0.973	9	2.9	0.876	0.016			
	26 Apr	2.503	9	3.0	2.253	1.269			
	2 May	31.699	6	1.9	19.020	2.934			
	8 May	0.800	10	1.7	0.800	0.895			
Annual Total			44		23.212	5.114	7.242	± 6.269	Not calculated

Table 4. Annual spawning and female biomass estimates for the Pamunkey River, 1980 and 1983. Abbreviations used as in Table 20 .

Year	Cruise Date	Daily Spawning Estimate (eggs X10^6)	D_i (days)	d_i (days)	P_i (eggs X10^6)	$S^2_{P_i}$ (X10^{15})	Annual Egg Production (eggs X10^8)	CI (eggs X10^8)	Female Biomass (Kg)
1980	16 Apr	0.615	18	2.6	11.065	2.601			
	22 Apr	6.725	4	2.1	26.901	8.522			
	24 Apr	1.223	4	1.9	4.893	1.333			
	30 Apr	1.575	4	2.1	6.301	3.270			
	2 May	1.268	4	2.0	5.072	2.640			
	8 May	0.390	7	1.9	2.733	0.982			
	16 May	0.006	8	1.5	0.502	4.263			
Annual Total			45		57.467	23.611	1.723	± .581	803
1983	5 Apr	0.090	5	3.2	0.452	0			
	13 Apr	0.601	9	2.8	5.412	7.959			
	23 Apr	0.176	7	3.1	1.233	0.486			
	27 Apr	10.593	5.5	2.8	58.264	12.203			
	4 May	2.641	7	2.1	18.487	15.399			
	11 May	0.616	7.5	1.7	4.618	5.159			
Annual Total			44		88.466	41.206	2.193	±1.624	1022

Variance estimates of cruise egg abundance and total egg production based on Houde's (1977) model are forced by sampling frequency and river area. As a result, James River variance estimates are large since sampling effort was reduced relative to other rivers and total James River volume (508.75 X $10^6 m^3$) exceeds the volume of all other rivers combined (370.58 X $10^6 m^3$). Thus, our original objective (Olney et al. 1985) of examining the relative contribution of three rivers based on annual egg production fails since reliable data from the James in 1983 are not available. Examination of cruise production estimates on each river, however, provides some insight into relative spawning activity on these rivers since cruise production values of have lower variances than annual production values. During periods of peak spawning activity in 1982 and 1983, Rappahannock River production was one order of magnitude above James River estimates and two orders of magnitude above those from the Pamunkey River. These data suggest that the greatest spawning activity occurred on the Rappahannock in spring 1982 and 1983.

As discussed by Houde (1977) variance estimates so obtained are subject to considerable uncertainty and probably do not account for all sources of variability in these data sets. Among those unaccounted sources are day-to-day variability in spawning and within stratum variability. Increases in sampling frequency can improve these estimates and within stratum replication (presently absent in our sampling strategy) could improve production estimate reliability. Some balance between these increases in sampling effort and cost effectiveness of ichthyoplankton survey data must be reached, however. We believe that despite the uncertainty surrounding production values presented herein, these data are useful first-order


WEEKLY EGG PRODUCTION ESTIMATES
FOR RAPPAHANNOCK RIVER — 1982
Egg Count (x10**6)
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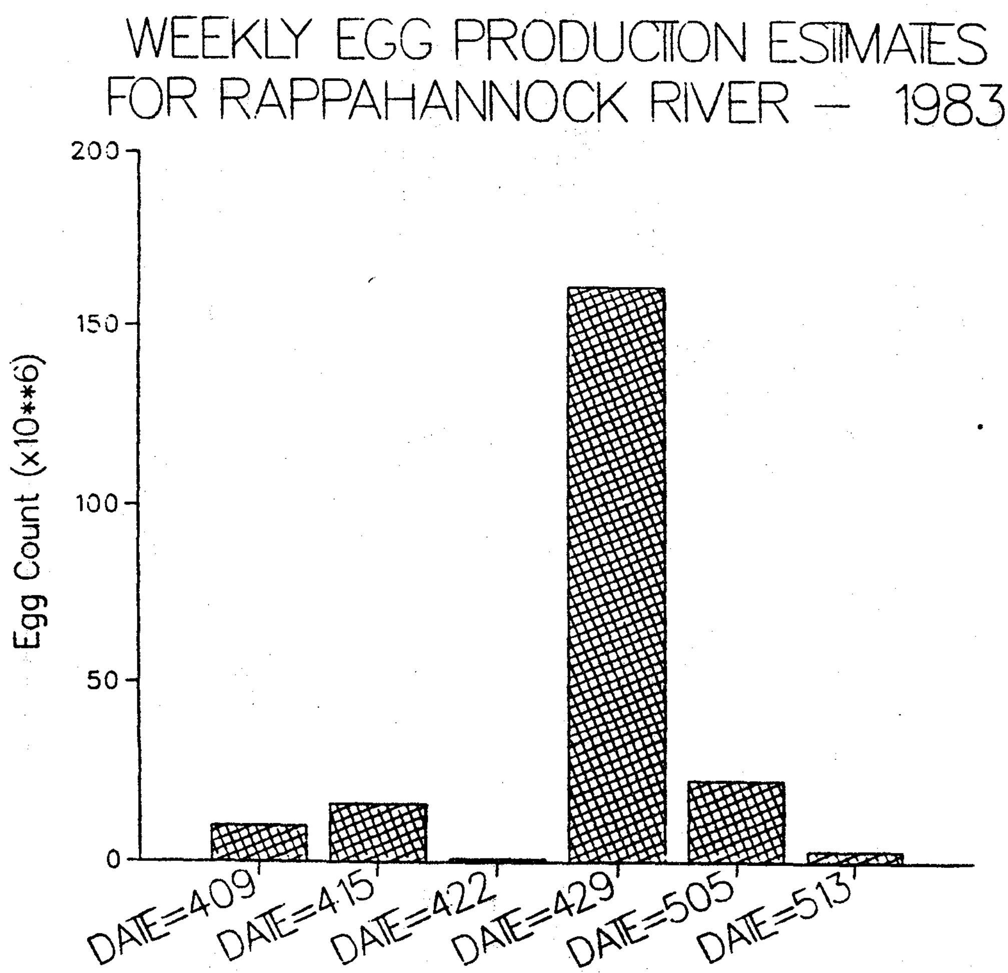
150
100
50
0
DATE=405
DATE=408
DATE=416
DATE=421
DATE=423
DATE=430
DATE=504
DATE=511

FIGURE 1


WEEKLY EGG PRODUCTION ESTIMATES
FOR RAPPAHANNOCK RIVER — 1983
Egg Count (x10**6)
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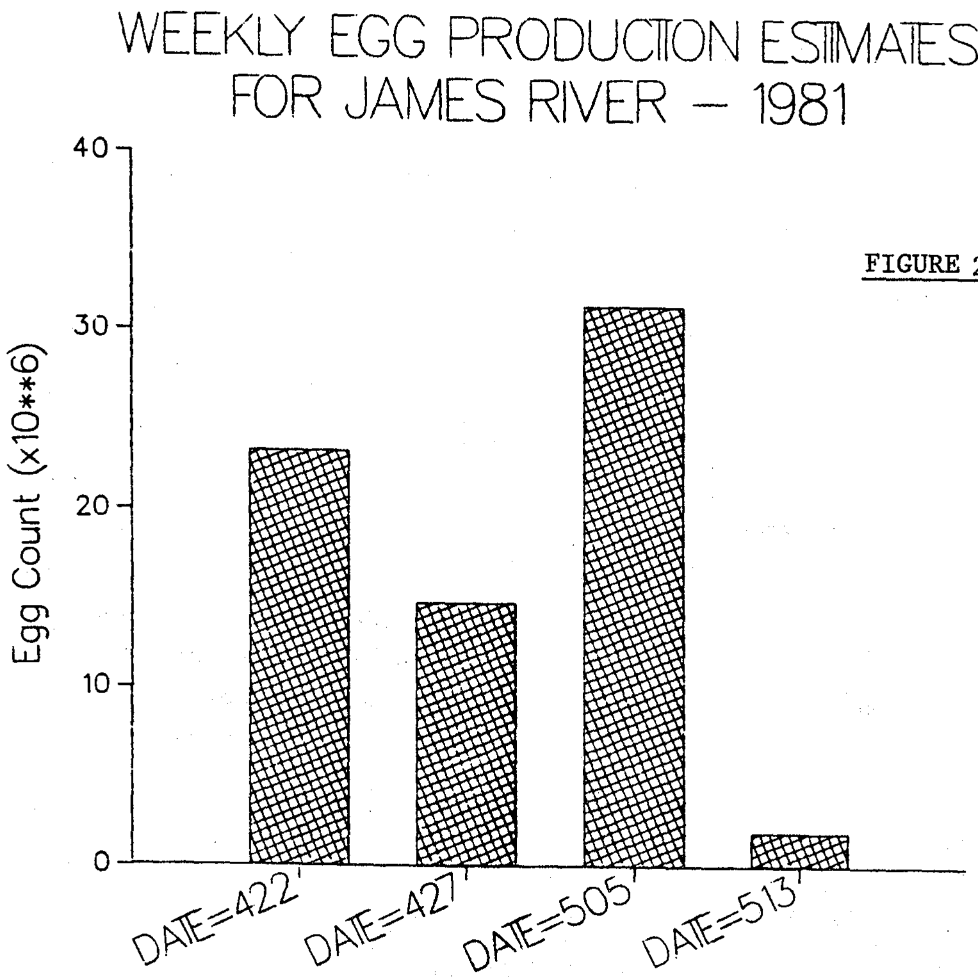
150
100
50
0
DATE=409
DATE=415
DATE=422
DATE=429
DATE=505
DATE=513

FIGURE 2


WEEKLY EGG PRODUCTION ESTIMATES
FOR JAMES RIVER — 1981
Egg Count (x10**6)
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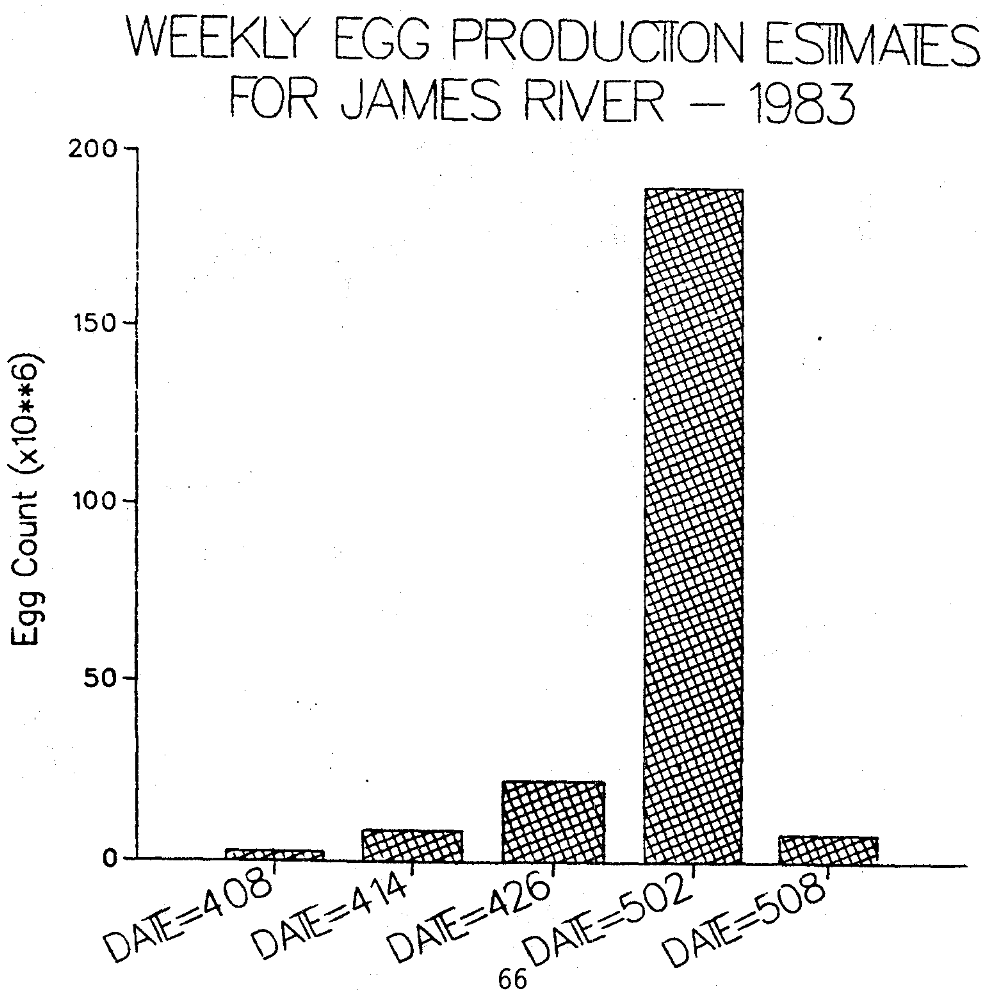
10
20
30
40
DATE=422
DATE=427
DATE=505
DATE=513


WEEKLY EGG PRODUCTION ESTIMATES
FOR JAMES RIVER — 1983
Egg Count (x10**6)
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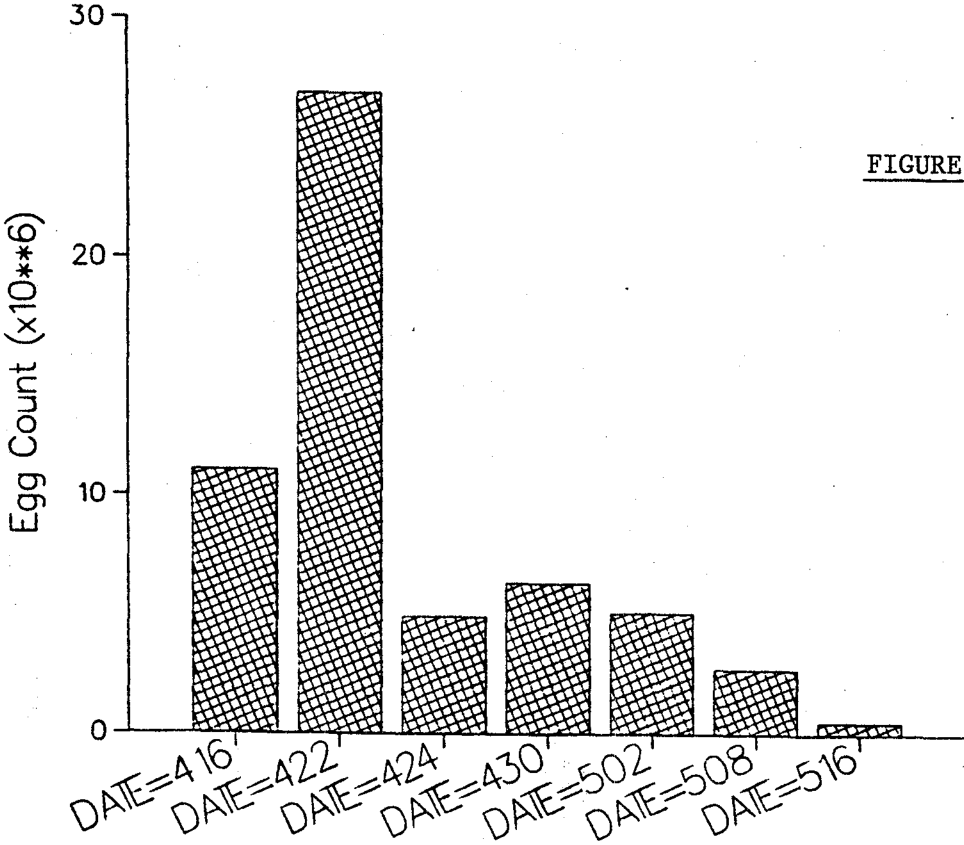
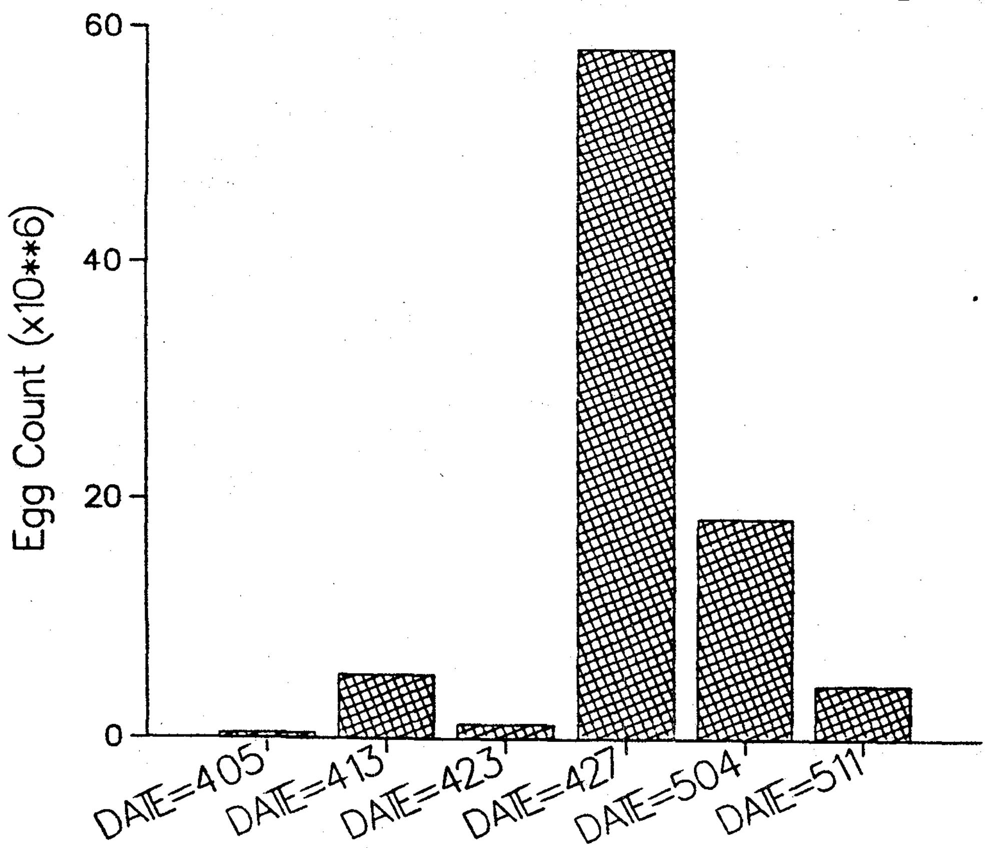
50
100
150
200
DATE=408
DATE=414
DATE=426
DATE=502
DATE=508

WEEKLY EGG PRODUCTION ESTIMATES
FOR PAMUNKEY RIVER — 1980

FIGURE 3



WEEKLY EGG PRODUCTION ESTIMATES
FOR PAMUNKEY RIVER — 1983



estimates of Virginia's contribution to the total Chesapeake Bay striped bass egg production.

Objective 3: Acid Rain

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 on the Pamunkey River. Analysis of these data is complete, and results were summarized in the First Annual Progress Report. Acidity never varied significantly from neutral (pH range was 6.6-7.9), and alkalinity did not fall below 0.27 meg/liter. Results are inconclusive, however, due to the lack of rainfall and drought conditions of April and May 1985.

Objective 4: Egg Viability

Collections designed to provide preliminary assessment of striped bass egg viability resulted from the deployment of a Hansen egg net consisting of a bridled 1-meter plankton net fitted with 202 micron mesh Nitex. Samples (n = 10, Table 5) were obtained from vertical hauls (bottom to surface) of this gear at stations where eggs were thought to be 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 5) or examined unpreserved (samples 1-5, Table 5). Unpreserved samples were brought to the laboratory within 2-4 hours of collection where

Table 5. Egg Vitality Data, Spring 1985

N	STA	DATE	OBS	TIME	I	II	III	IV	HATCH	DEAD I	DEAD II	DEAD III	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	9	0	1	0	-	0	0	7	17	58.8
7	P45	15 IV	1	1247	15	0	0	0	-	2	0	24	41	36.6
8	P46	19 IV	1	1125	0	0	0	0	-	0	0	3	3	0
9	P47	19 IV	1	1200	0	1	1	0	-	0	0	1	3	66.7
10	P49	19 IV	1	1215	0	0	0	0	-	0	0	2	2	0

* Capture damage

live vs. dead eggs were separated under a stereomicroscope (Observation 1, Table 5). 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), Dead II (eggs clear but containing little or no organized tissue), and Dead III (eggs clear but containing disorganized tissue). Both living and dead eggs were incubated for 12 hours, then re-examined, sorted and staged (Observation 2, Table 14). Eggs sorted from preserved collections (samples 6-10, Table 5) were examined under dissecting microscope and classified into appropriate developmental or dead categories. Analysis of these samples was considerably less time consuming than previous analysis of unpreserved replicates. Resultant data (Table 5) revealed that viability estimates based on examination of unpreserved samples (method I, samples 1-5) were lower than estimates based on analysis of replicate samples preserved in histological fixative (method II, sample 6 - 10). Between sample variance was high in data sets resulting from both treatments (unpreserved treatment mean 13.6, standard error 4.6; preserved treatment mean 32.4, standard error 14.1), but three preserved treatments containing both live and dead eggs (samples 6, 7 and 9) produced consistently higher viability estimates. These results suggest that post capture handling does bias vitality estimates based on examination of live material. The extent to which shipboard procedures affect estimates are as yet unknown.

Continuing Studies

Recent analysis of existing survey data (Table 1) reveals the extent to which certain sampling variables, especially sampling frequency, within stratum replication and stratum size, affect egg production and biomass estimates. We are continuing examination of these data in an attempt to visualize a sampling protocol that would result in the smallest variance associated with egg production estimates, yet be cost effective. An approach will be to subject the model to sensitivity analysis in order to establish critical sampling levels. A revised survey methodology might then be proposed and evaluated in light of effort and cost estimates.

In keeping with the egg production model evaluation described above, we are assessing the possibility of acquiring a recently developed submersible photographic system. The Benthos Model 373 Plankton camera provides in-situ silhouette photographic images of plankton concentrated at depth and allows accurate density estimates of plankton without actual collection of specimens. As a result, sampling effort can be significantly increased but processing time minimized. This gear has not been used in tidal freshwater systems, and its feasibility as a quantitative sampler of striped bass eggs must be established.

PROJECT 7

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

Description and Objective

The objective of this project is to provide information on the fate and effects of polynuclear aromatic compounds, including polynuclear aromatic hydrocarbon (PAH) metabolites. The methodology intended to be used in these studies relies on the interfacing of mass spectrometry (MS) with microbore high performance liquid chromatography (HPLC). The latter provides the separation of mostly labile, very polar and involatile compounds while the former gives information on molecular weights and thus the identities of eluting compounds.

Status

1. High Performance Liquid Chromatography

A number of difficulties were encountered with the microbore HPLC columns obtained from Alltech (a chemical supplier): 1) clogged frits were difficult to clean, 2) concatenation of the columns to increase separative power was suggested by the manufacturer, however, the company did not assume the responsibility of the possible consequences (e.g. deterioration of column performance) by this action, 3) high back pressures encountered as a result of concatenation caused leakage of the column packing material into the detectors endangering the life of the flow-cell, and 4) the column bed stability was in question as the 1/16" stainless steel tubing used for the columns was susceptible to easy bending and twisting.

Different manufacturers were contacted and, finally, new rigid columns were obtained from Scientific Glass Engineering, Austin, Texas. At present, the initial testing of the columns is in progress. Once the column performance has been determined to be satisfactory, more columns will be ordered and concatenation will be attempted again. In our last purchase order, C-18 bonded columns were ordered along with amino bonded and cyano bonded columns. Unfortunately, the amino bonded column was shipped in a box meant for the C-18 column. The mobile phase used for C-18 columns was not compatible with the amino bonded column, and eventually high back pressures were observed. The company has promised that the column will be replaced.

Dr. John Dolan, author of the "Trouble-Shooting" column in the LC Magazine, has suggested that we consider using a technique of recycling chromatography as an alternative to concatenation. In this technique, compounds eluting from one column are recycled into another with the help of a column switching valve. These same compounds, as they elute from the second column, can then be recycled into the first column until a desired resolution is obtained. Column performance is, however, sensitive to void volume, and the effect becomes pronounced in the microbore HPLC. The column switching valves require the use of a long piece of tubing between the column and the valve port. To achieve optimum resolution, bent columns should be used, and they should be bent before they are packed. The scientists at Scientific Glass Engineering are assisting us in this problem and are testing columns that have been manufactured in the correct configuration.

2. High Performance Liquid Chromatography - Mass Spectrometry

One of the most serious problems previously encountered in the HPLC-MS system was our inability to achieve and maintain reproducible and stable operating conditions. In addition, the combination of HPLC with MS is basically very inflexible and is capable of operating only in a very narrow range. It was quickly learned that only a systematic, step-by-step approach could provide an understanding of the problems at hand and allow us to make progress. This has allowed us to overcome several problems:

a. Inability to achieve and maintain the solvent jet into the ionization chamber (caused by clogging of 5 micron aperture in the interface). The 5 micron aperture was replaced by an aperture of 10 micron diameter. This successfully reduced the frequency of clogging but resulted in a larger solvent flow rate into the MS source and a corresponding increase in pressure which exceeded the maximum operating conditions of the MS. To bring the source pressure down to acceptable values, pumping in addition to the two turbomolecular and the cryogenic pump was necessary. It was provided by a simple mechanical pump connecting directly to the ionization volume. As a fringe benefit of this change, it seems that the cryogenic pump, and with it the need for liquid N_2, can be eliminated. This provides easier instrument operation and a considerable reduction in operating costs.

An additional measure to maintain a reliable solvent jet was the purchase and use of a laminar flow hood, providing a clean working environment for the cleaning and assembly of interface components. These changes allow us to establish a solvent jet without difficulty and result in extremely stable working conditions over days.

b. Tuning of the MS under HPLC-MS operating conditions. The design of the MS does not allow the use of the standard calibration and tuning probe once the HPLC interface is installed. On the other hand, retuning under the conditions of operation for LC-MS is an absolute necessity. Methodology to introduce suitable compounds for tuning into the source during solvent injection has been developed.

Due to these changes, it has been possible to start concentrating on the real problems of HPLC-MS analysis. A reverse phase HPLC column was mounted in the system and operated with methanol/H_2O as CI gas: predictable spectra for phenanthrene and pyrene were recorded (Figures 1 and 2). The major ion in both cases is the quasi-molecular ion that results from a protonation of the molecule. In Figure 3, the same two compounds were recorded by the selected ion monitoring (SIM) technique that normally allows an increase in sensitivity by a factor of approximately 10^3. SIM also is an established method of quantification.

Attempts to separate and detect hydroxylated PAHs, the type of compound that can be expected to be produced by the metabolism of PAH, initially failed to produce results. Direct injection of these substances into the solvent, however, revealed the reason: these compounds did not form a quasimolecular (M+1) ion, but fragmented according to the probable equations indicated in Figures 4 to 8. Although it will now be easy to get the mass

Figure 1 Phenanthrene

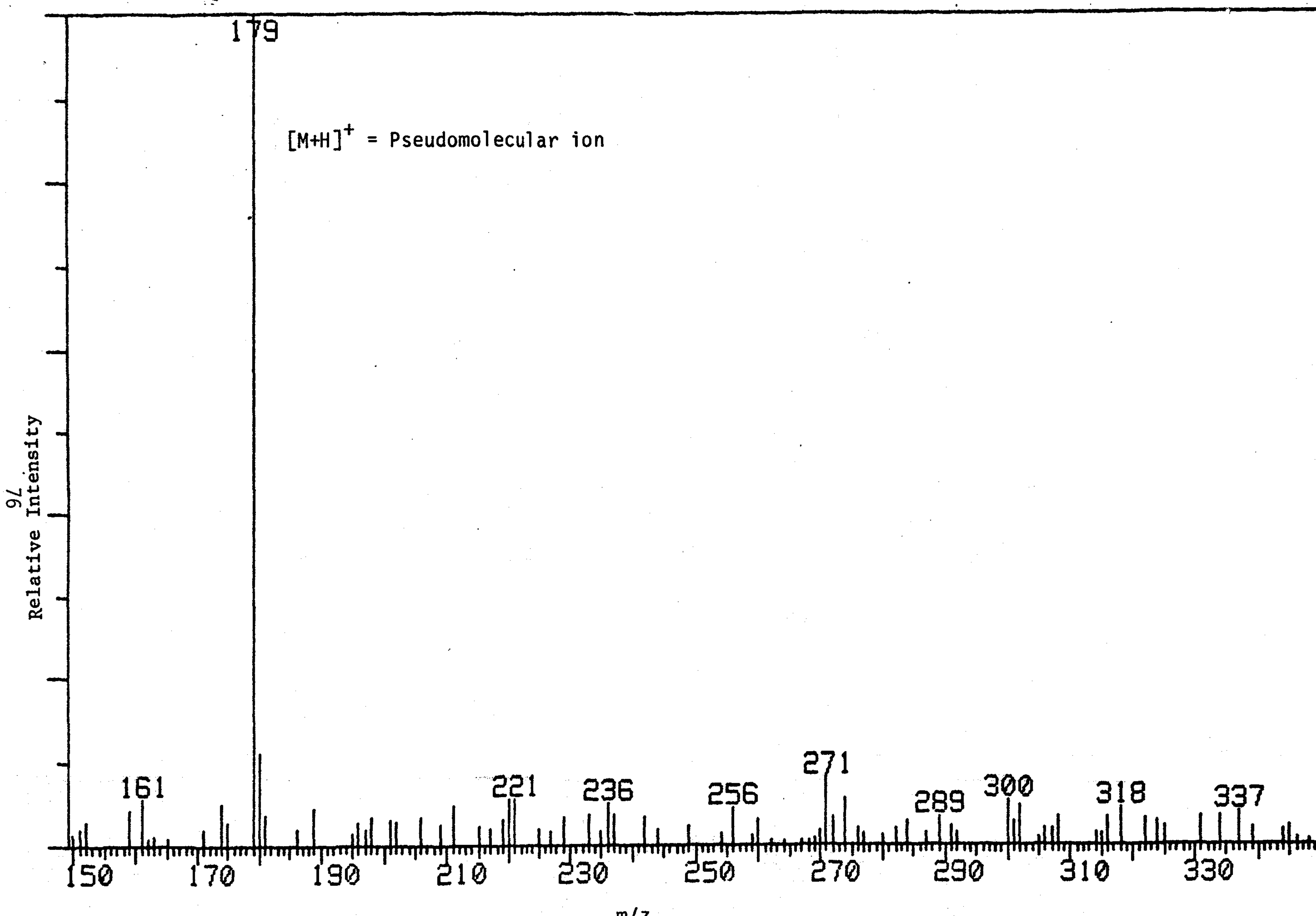


Figure 2 Pyrene

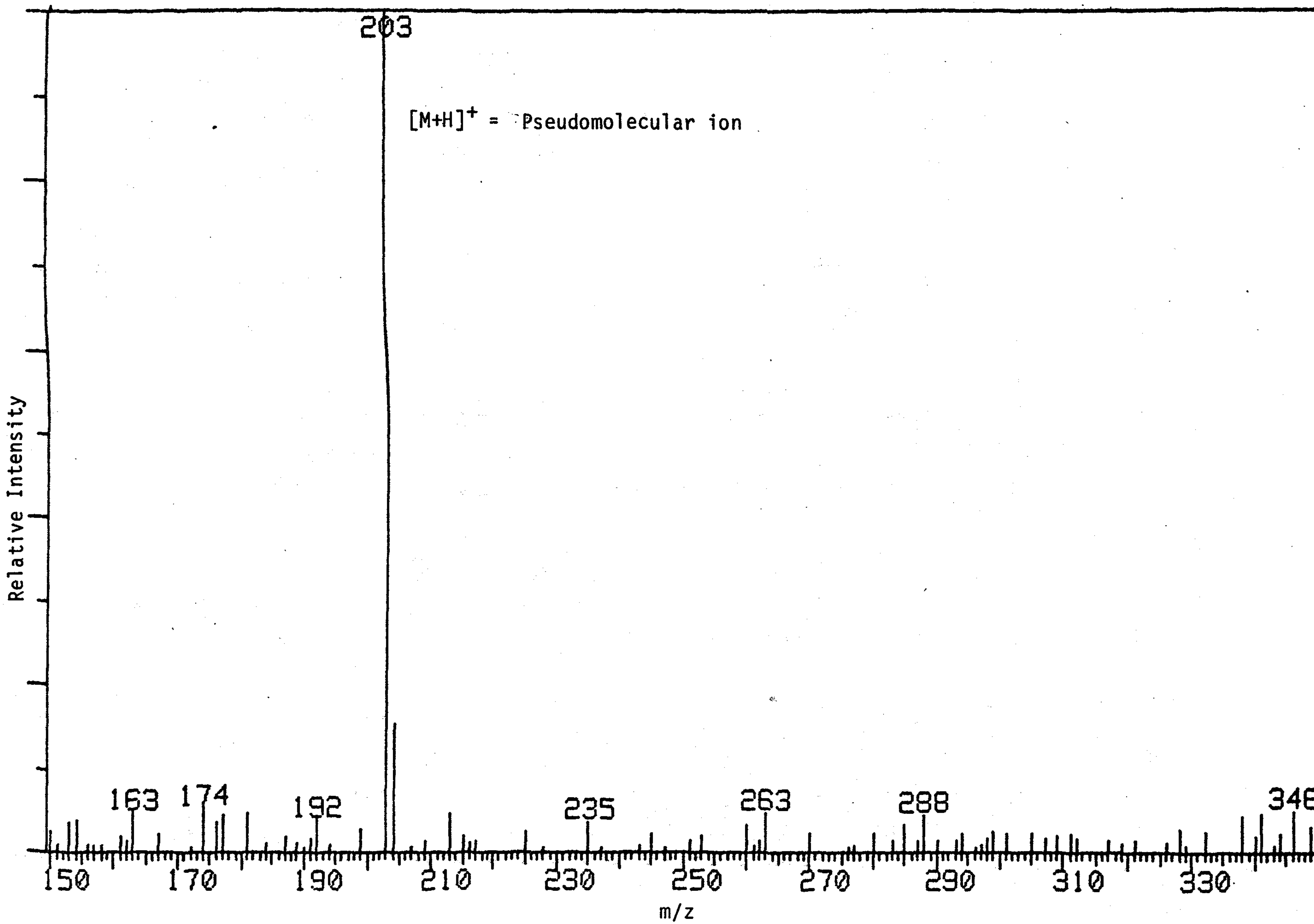
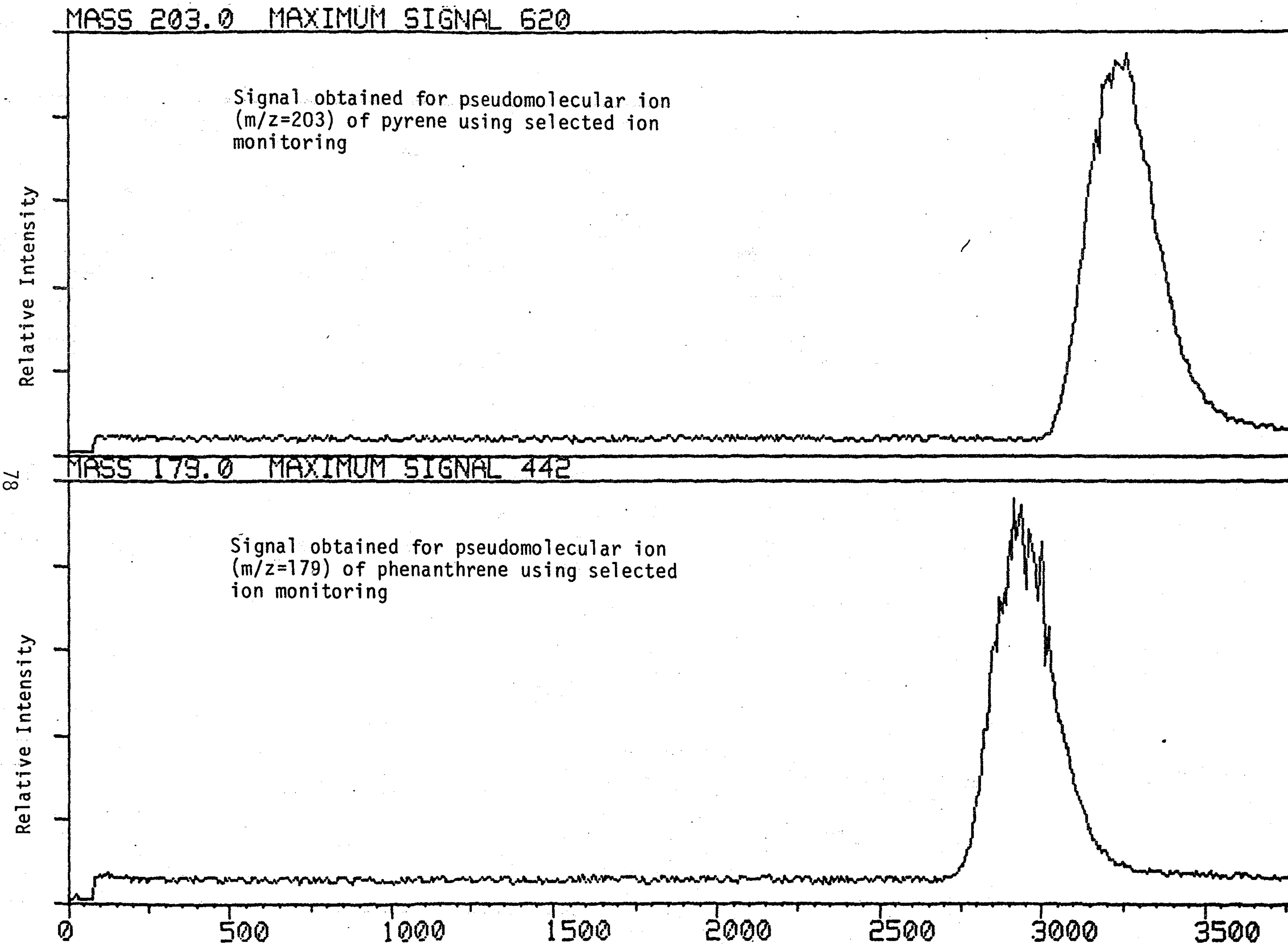


Figure 3



MASS 203.0 MAXIMUM SIGNAL 620
Signal obtained for pseudomolecular ion (m/z=203) of pyrene using selected ion monitoring
Relative Intensity
MASS 179.0 MAXIMUM SIGNAL 442
Signal obtained for pseudomolecular ion (m/z=179) of phenanthrene using selected ion monitoring
Relative Intensity
0
500
1000
1500
2000
2500
3000
3500

Figure 4

Benzo(a)pyrene,6-hydroxy-

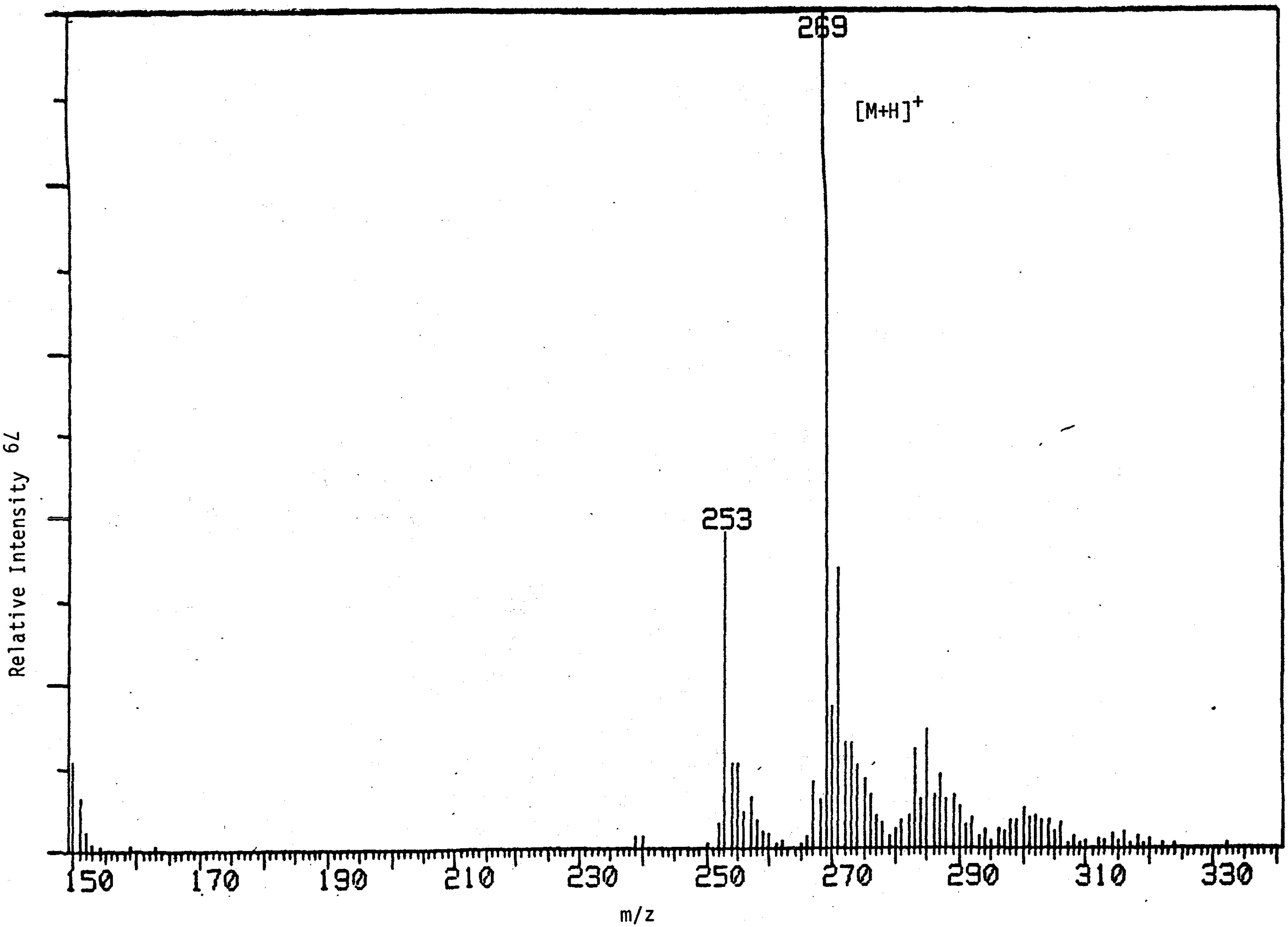


Figure 5 Benzo(a)pyrene-9,10-dihydrodiol

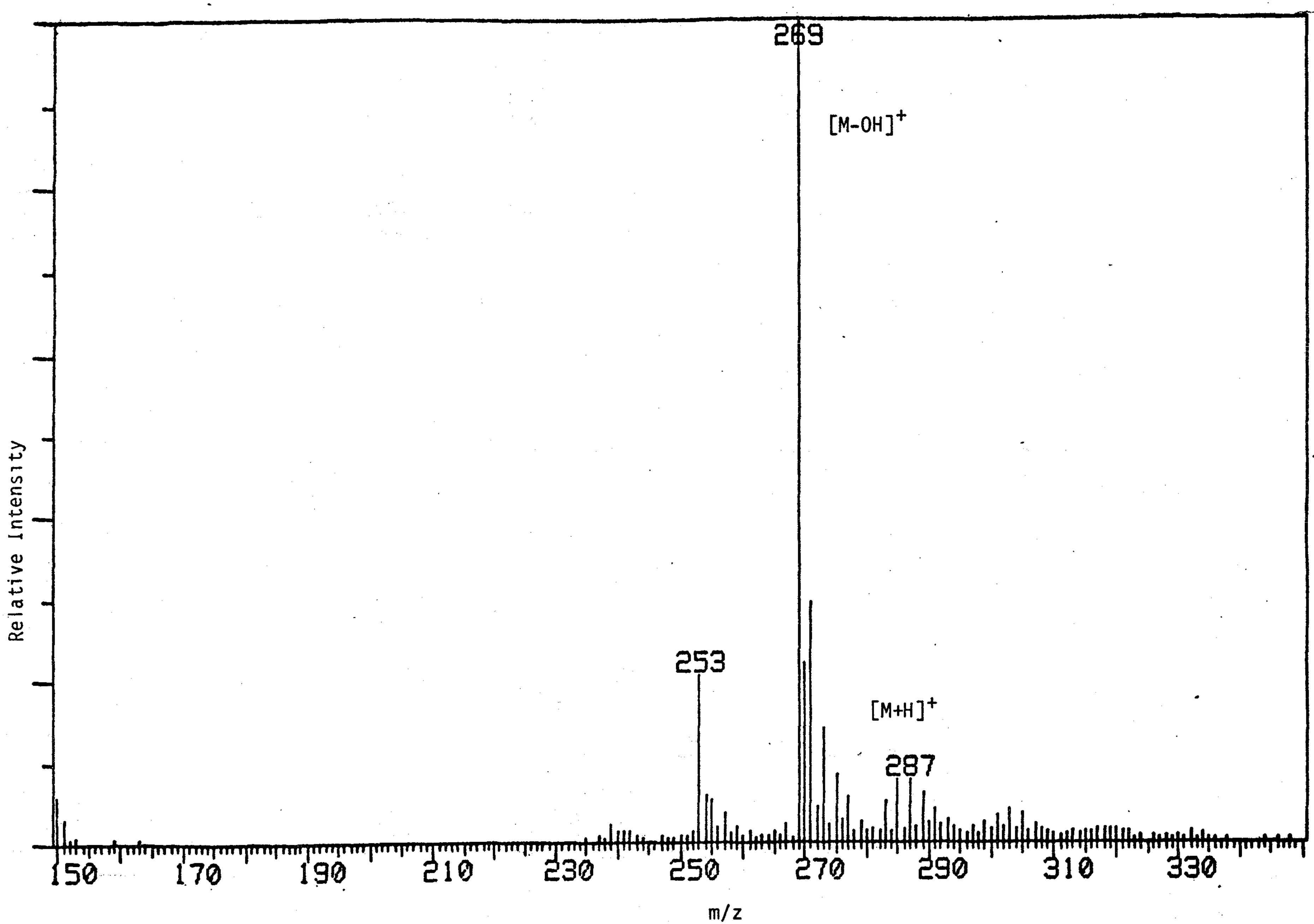


Figure 6 Benzo(a)pyrene-7,8.9,10-tetrahydro-7,8,9-tiol.

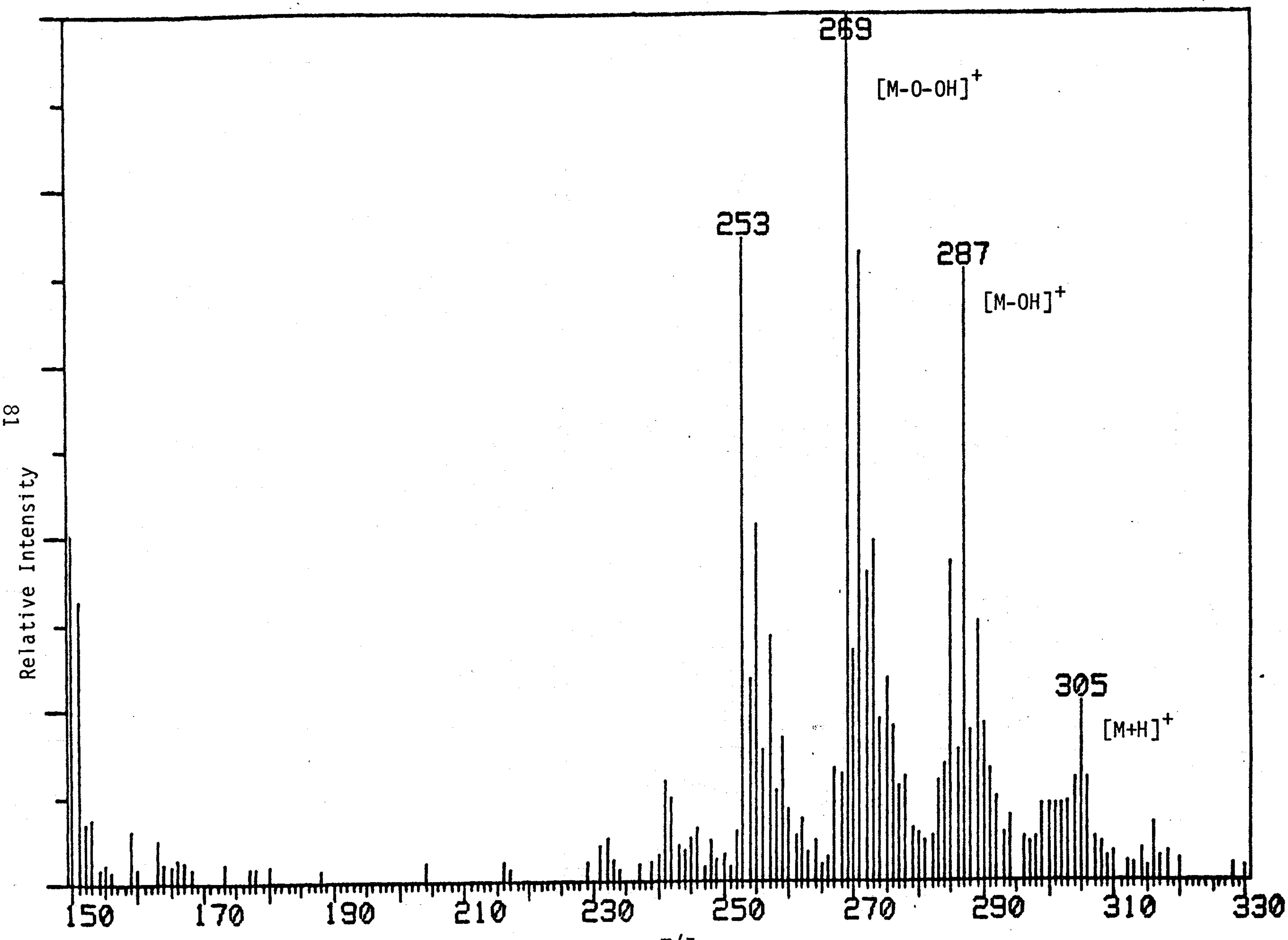


Figure 7 Benzo(a)pyrene-6-glucuronide

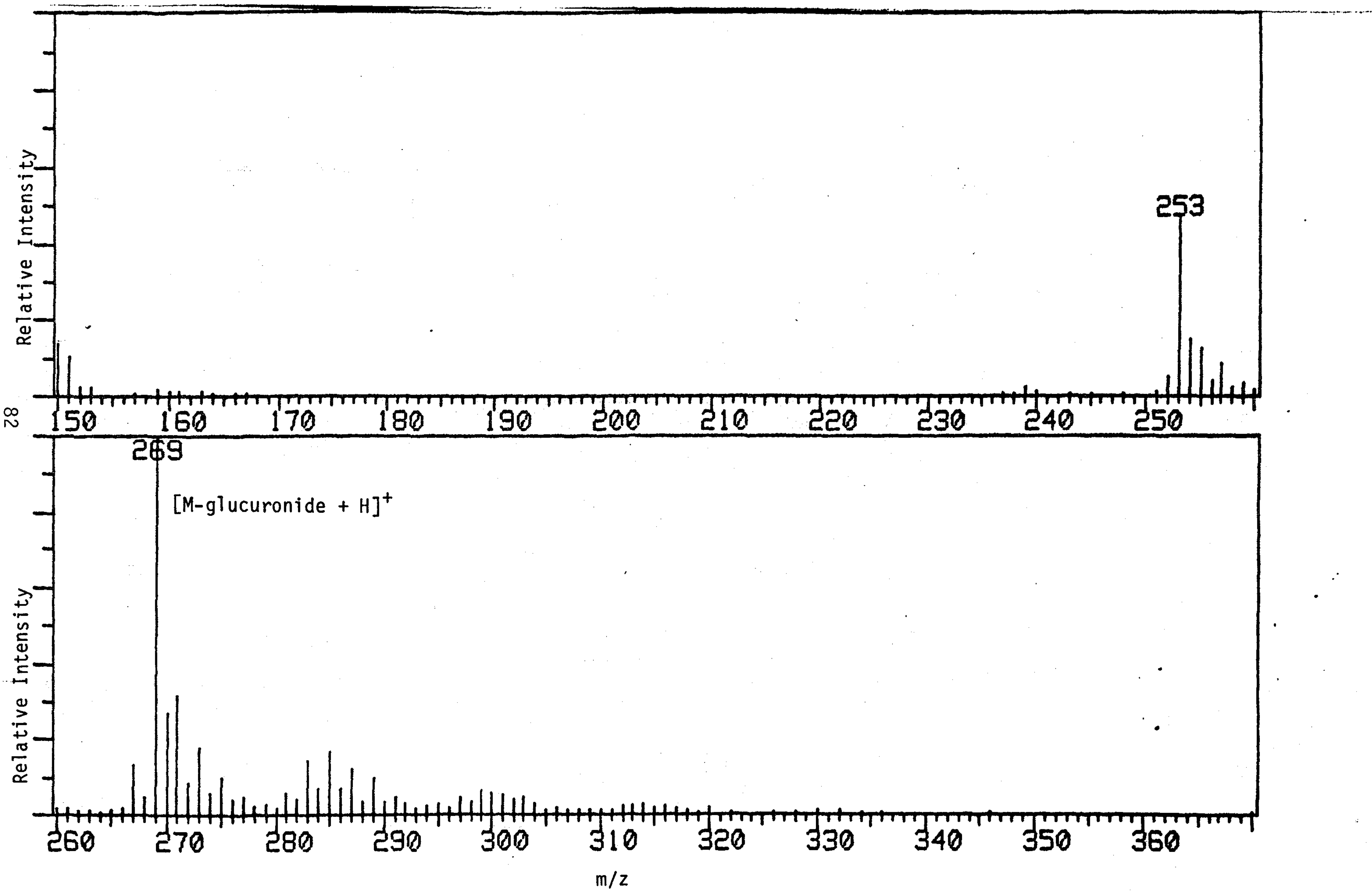
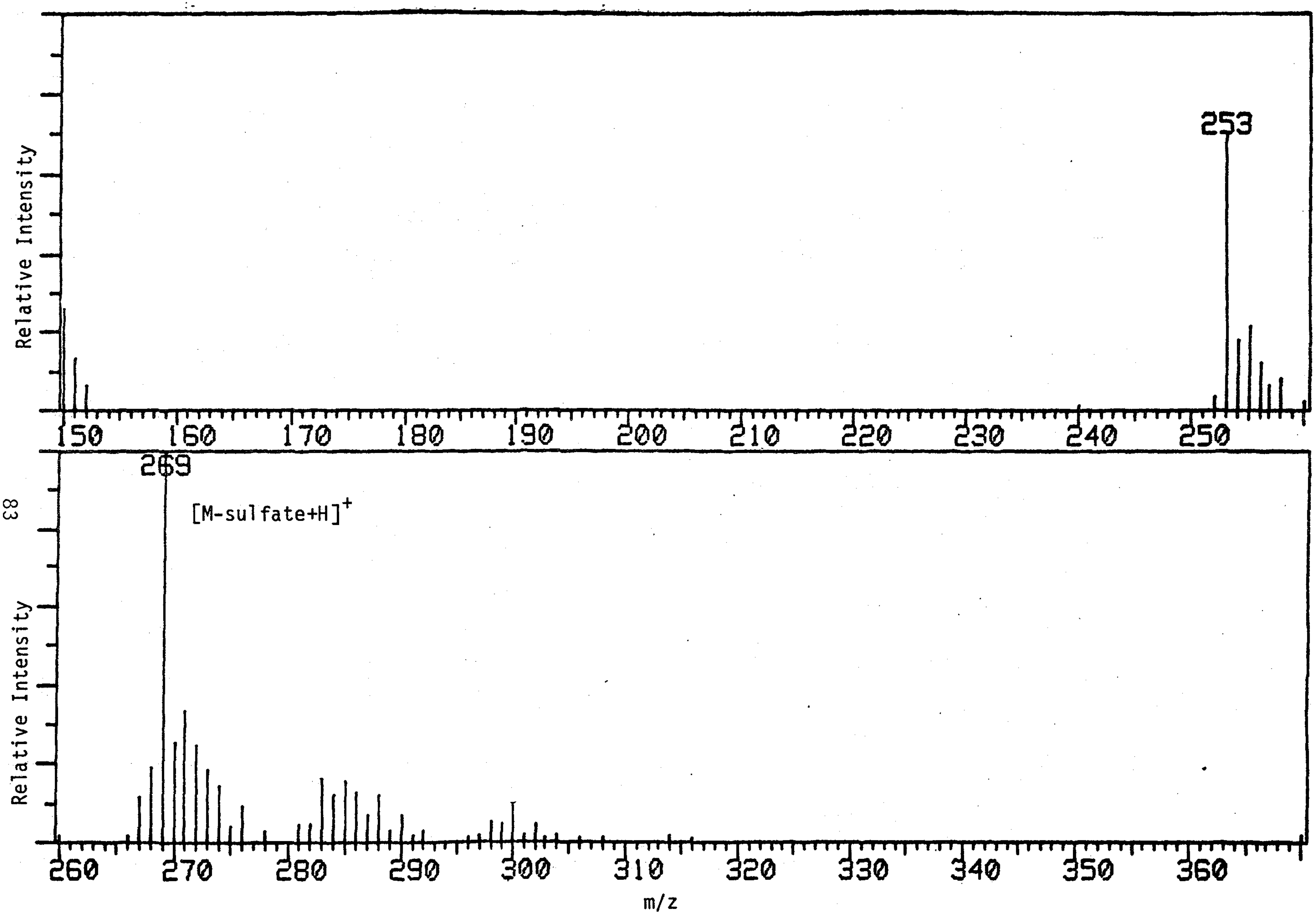


Figure 8

Benzo(a)pyrene-9-sulfate



spectra of individual PAH metabolites by HPLC-MS, it appears from the few spectra derived under the specific conditions used at the present that the identification of PAH metabolites may not be as unambiguous as one would wish. The data in Figures 4-8 suggest the presence of stable substructural fragment ions which are very similar. This, however, may not be true when other solvents and source temperatures are used or when negative ions are observed.

Continuation of the Project

After some initial technical difficulties that were due to the fact that HPLC-MS is not yet fully developed, we have made considerable progress and at the present have reached a stage where we can work on problems relevant to the interests of the State of Virginia. To our knowledge, this analytical system is one of few within the U.S. to work with almost routine reliability, and we are the only marine science institution to operate such a system.

One of the next steps will be to investigate other possible solvent systems that satisfy both the separating capability and the ionization process. With respect to the latter, the goal is to find a CI gas that imparts less energy to the molecule in the primary ionization process. In addition to positive chemical ionization, negative chemical ionization will be investigated.

For HPLC aspects, it is planned to study both the concatenation and the recycle chromatography in efforts to optimize the separation of PAH metabolites.

For the period beyond 30 June 1986, we anticipate having enough basic information on PAH metabolites to investigate bile extracts from Elizabeth River fish. An additional effort concerns the correlation of the disease state of fish with the relative amounts of individual PAH metabolites. Finally, other toxic compounds of concern, like arenes and nitro PAH, must be studied.

Funding for this work is being programmed within the Agency's expanded base funds.

PROJECT 8

Early Warning System for Pollutants in Seafood

Description and Objectives

The objective of this research is to provide background information about chlorinated hydrocarbon concentrations in Virginia's seafood. This information will be integrated with and supplement elements of on-going monitoring and research studies to produce a comprehensive monitoring program. Such a program will help ensure that Virginia's seafood remains a strong, viable and wholesome resource.

Status

Virginia's seafood is being analyzed for chlorinated hydrocarbons which are comprised of pesticides, industrial chemicals and PCBs. The use of congener analysis for PCBs, coupled with the analytical instrumentation chosen for chlorinated hydrocarbons, is providing a more complete and detailed picture of PCB contamination in seafood. The data acquisition system and programs are providing excellent quantitation of PCBs and other compounds in the complex sample mixtures.

Sampling has been completed for 1985. Samples from Virginia distributors were collected at six-week intervals from May through December. Species collected were grey trout, bluefish, spot, croaker, flounder, blue crab, oysters, and clams, depending on the supplier, season and availability. The sources of the seafood were the James River, York River, Rappahannock River, upper and lower Chesapeake Bay. Approximately 240 samples were collected through the year.

Problems, Delays

The primary gas chromatography (GC) with a Hall Electrolytic Conductivity Detector (HECD) has suffered serious electrical and mechanical problems. A second GC with an HECD is being set up to prevent loss of analytical capabilities due to instrument failure. The instrument failure has caused a backlog of samples. However, with the additional instrumentation this backlog should be eliminated in a relatively short period of time. It is estimated that analysis of the samples will be completed within 16 weeks.

Findings

The delay due to instrumentation failure makes it inappropriate to make firm judgements about chlorinated hydrocarbon trends in Virginia's seafood at this time. Trend analysis will commence shortly now that the existing instrumentation is operable and additional instrumentation is anticipated.

To date, no samples have been found to contain pesticides or PCB concentrations which exceed the action levels. Characteristically, samples contain PCBs, chlordanes, DDE and BHCs in the part per billion range.

A number of samples have been found to contain unknown compounds. An effort is continuing to identify these unknowns to improve the characterization of chlorinated hydrocarbon content of Virginia's seafood.

Continuation of the Project

It is important that this survey of chlorinated hydrocarbons in Virginia's seafood be continued. Changes in pesticide and PCB concentrations in seafood may occur due to a number of reasons. Among these are climate, point and non-point discharges. Chlorinated hydrocarbon baseline levels and temporal trends of these levels become apparent only through additional sampling and analysis of Virginia's seafood. Without these data, Virginia's regulatory agencies will be hampered in their efforts to manage the Chesapeake Bay. Funding for this work is being programmed within the agency's expanded base funds.

PROJECT 9

Re-establishment of Submerged Aquatic Vegetation

Description and Objectives

Submerged aquatic vegetation (SAV) is an important natural resource of the Chesapeake Bay and the Commonwealth of Virginia. Extensive stands of SAV once covered the shallow water areas of the Potomac, York, Rappahannock and Piankatank rivers. The SAV Initiative was developed because of the dramatic and unprecedented decline of SAV in the Bay and the resultant loss of many important functions of SAV to the estuarine system.

The objectives of the SAV Initiative are to undertake a replanting effort to develop methodologies necessary to efficiently reestablish these important grasses in areas that once supported dense beds, and to use these transplants as tools to better understand how these grasses may respond to potentially limiting factors such as excess nutrients and turbidity.

Interim Findings

1985 Transplant Results

Fifteen acres of eelgrass were planted in the Fall of 1985 in areas that had the best survivorship rates from the 1984 plantings, as well as in new areas that would be used as experimental sites for our intensive biological monitoring (Figure 1). This was two acres more than had been proposed in the original research plan.

Six acres were planted at the Belle Isle site in the upper Rappahannock River near Morattico, six acres in the Piankatank River, two acres in the East River and one acre in the York River. All sites except the York River were planted with eelgrass placed on five meter centers and fertilized with a slow release fertilizer, Osmocote. Small, unfertilized test plots (10 x 10m) with plants placed on one meter centers were planted at five sites in the York River (Guinea Marsh, Gloucester Point, Mumfort Island, Carters Creek and Clay Bank). Identical fertilized plots were placed at all sites in the York River except Guinea Marsh.

Three experimental test plots (10 x 10m on one meter centers) were established in the lower Rappahannock River. One plot was placed at Parrott Island in the same location as the 1984 plantings. A second plot was placed behind the island for more protection from strong north winds. A third plot was placed several kilometers upriver from the Parrott Island site. This area had eelgrass in the 1960's and is being examined as a potential transplant site for future work. The physical characteristics here are very similar to those of the Belle Isle site.

The method of planting used in 1985 was changed from the method used in 1984 as we continued to develop new and improved procedures that resulted in more economical transplantation. Individual shoots, rinsed free of sediment, are tied to a small metal anchor with a twist tie. These prefabricated units are then transported to the transplant site. Initially, a small metal trowel is inserted into the sediment and moved back and forth creating a small excavation. The entire transplant unit is then placed in this hole. The advantages of this method are the ease of transporting the

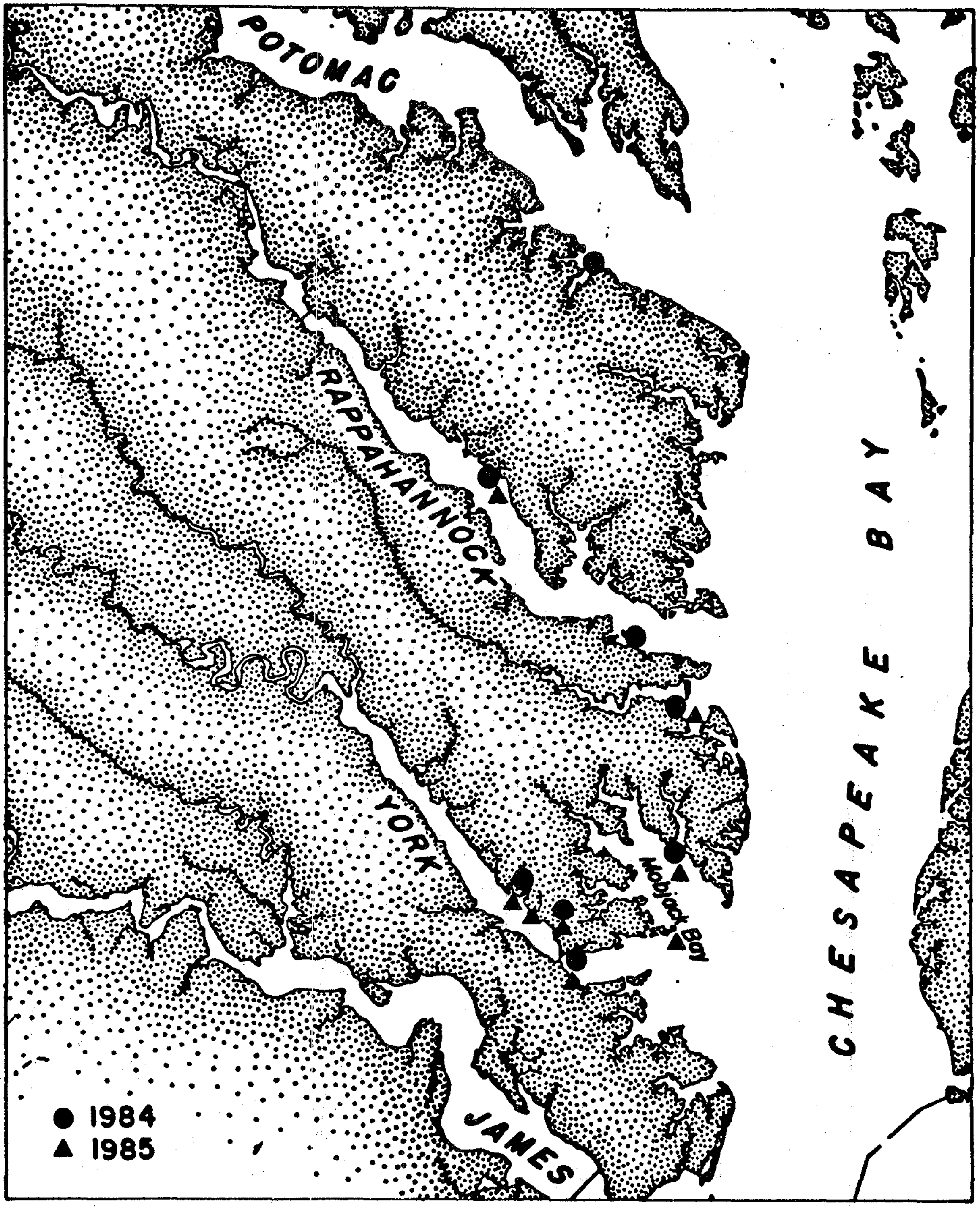


Fig. 1. Major transplant sites in lower Chesapeake Bay, 1984, 1985.

planting units, since no sediment is involved, and the shorter time needed in the field for both collecting and planting the eelgrass. Greater time is needed in the laboratory this year for the fabrication of the units; last year whole mats of grass and sediment were planted. However, there is more flexibility permitted in the logistics of transplanting to remote sites.

Volunteer assistance was again provided by members of the Save the Ole Piankatank group. Approximately 10 people participated in the fabrication of the units that were to be planted in the Piankatank River.

1984 Transplant Survivorship

Table 1 presents survivorship data for all sites planted in the Fall of 1984. All transplants were field checked in August 1984, except the Coan River site in the Potomac River. The intensive transplant effort in the Fall of 1985 precluded field checking all sites for the remainder of 1984. However, several of these sites which were again replanted in 1985 (e.g. Mumfort Island, Piankatank River) were visually checked during the time of the transplanting.

All transplants at Clay Bank had disappeared by August. In addition, the seedlings in the experimental plot established in 1984 did not survive. Although there were some plants still present in August at Mumfort Island, these were no longer present by the fall. There were, however, still some surviving units outside the survivorship plot. Transplant units at Gloucester Point were still present in August although survivorship had declined to 64%. These were still evident in the late fall.

In the Rappahannock River, only a few units remained at the Parrott Island site in August and were gone by late fall. At Morattico, all units

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	Aug
YORK RIVER						
-Gloucester Point	unfert plot 1	Nov 9	100%	80%	76%	64%
	unfert plot 2	Nov 9	100	83	80	64
-Mumfort Island	unfert plot 1	Oct 3, 4	73	53	33	14
	unfert plot 2	Oct 3, 4	73	44	33	25
	fert plot 1	Oct 5	67	33	33	17
	fert plot 2	Oct 5	83	25	5	0
-Clay Bank	unfert plot 1	Sep 26,27	92	39	8	0
	unfert plot 2	Sep 26,27	90	36	2	0
	fert plot 1	Oct 12	37	19	5	0
	fert plot 2	Oct 12	64	28	0	0
PIANKATANK RIVER						
-Queens Creek	unfert plot 1	Oct 9	94	50	0	0
	unfert plot 2	Oct 9	100	97	89	28
-Burton Pt.	unfert plot 1	Nov 15	100	100	89	39
	unfert plot 2	Nov 15	100	94	58	14
	fert plot 1	Oct 11	100	77	67	61
	fert plot 2	Oct 11	100	92	80	78
-Healy Creek	unfert plot 1	Nov 13	100	94	6	0
	unfert plot 2	Nov 13	96	81	8	0
	fert plot 1	Nov 13	100	81	53	0
	fert plot 2	Nov 13	100	72	40	0
MOBJACK BAY						
-East River	fert plot 1	Oct 19	100	100	100	100
	fert plot 2	Oct 19	100	100	100	100
RAPPAHANNOCK RIVER						
-Parrott Island	unfert plot 1	Oct 16	97	94	55	0
	unfert plot 2	Oct 16	100	92	25	0
	fert plot 1	Oct 18	100	94	64	8
	fert plot 2	Oct 18	100	94	44	6
-Morattico	unfert plot 1	Oct 23	92	94	89	0
	unfert plot 2	Oct 23	94	94	97	0
	fert plot 1	Oct 25	97	100	100	97
	fert plot 2	Oct 25	100	100	100	97

Table 1 (concluded)

		Date Planted	Date Checked/% Units Surviving			
			Nov/Dec	Apr	Jun	Aug
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	*

* not checked

were gone in the unfertilized plot, but 97% of the units in the fertilized survivorship plots were still present.

In the Piankatank River all units were gone at the Healy Creek site; when a visual check of the entire transplant area was made in November, it revealed only one unit left. However, this unit was healthy and measured approximately one meter in diameter. At the Queens Creek site, 28% of the units remained in unfertilized plot 2 in August but were gone by November. Only one unit could be found in the entire area. At Burton's Point, 25% of the unfertilized units remained in August, but many were gone by November; therefore, the final survivorship rate would have been less by November. At the Burton's Point fertilized plot, 70% remained in August and many of these were still present in November.

Transplants at the East River site were the most successful with 100% of the units still present in August. These units were field checked in November and were still quite healthy.

Transplants at the Coan River site were not checked in August because of logistical problems, nor were they checked in the fall. This site will be checked in the spring to determine whether the remaining units present in June have survived.

Monitoring Studies

Biweekly water quality monitoring at five sites along the York River has continued over the past six months. Parameters measured include light, suspended matter, dissolved nutrients, dissolved oxygen, temperature and salinity. In the Fall of 1985 an additional upstream site was added and a downstream site eliminated. This was done to provide more precise data for analysis of factors potentially limiting to SAV in this region.

Eelgrass production studies were initiated in May 1985. Sods of eelgrass including whole plants, roots, rhizomes and sediments to a depth of 15cm were removed from an established bed at Guinea Marsh. The material was put in plastic boxes and placed at comparable depths at the Gloucester Point and Claybank transplant sites in the York River. Individual plants within the boxes were labeled, marked and measured, then remeasured at weekly intervals to provide precise measurements of plant production. Standing crop and biomass measurements of eelgrass and its epiphytic communities commenced in the Fall of 1984 and were continued at bimonthly intervals until the Fall of 1985.

Results of the eelgrass production studies indicate a bimodal pattern of plant production at both sites, with highest levels in the spring and fall (Figure 2). During these periods of maximum productivity, production was higher in the downriver Gloucester Point site than the upriver Claybank site. Similar low levels of production were evident at both sites during the summer. Water column nutrients were higher at the Claybank site throughout the year. Water clarity varied throughout the year but was low at both sites during the summer and greater at the Gloucester Point site during the spring and fall. Suspended inorganic matter in the water correlates highest of all parameters measured with light attenuation, suggesting phytoplankton may play only a minor role in the turbidity at these sites.

Biomass data obtained from the Fall 1984 transplants indicates a greater aboveground to belowground ratio during the winter following transplanting at Claybank as compared to Guinea Marsh. This suggests the

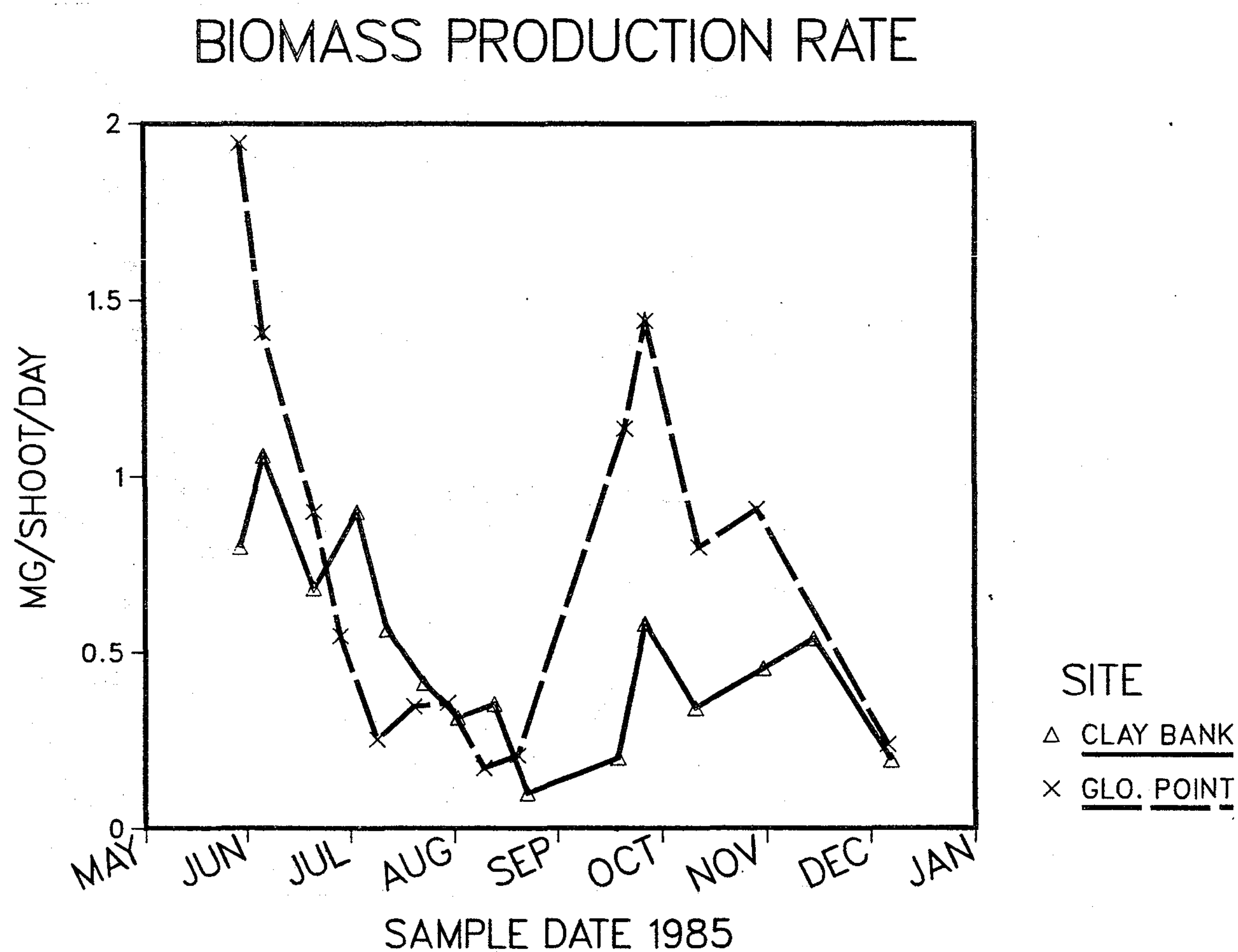


Fig. 2. Biomass production rate (mg/shoot/day) for eelgrass at Clay Bank and Gloucester Point.

plants at the upriver site may be responding to a poorer light environment by placing an increasing proportion of production into photosynthetic tissue. It may be that failure of plants to persist throughout the summer at various sites is due to lower light availability during spring and fall growth periods resulting in not only lower overall production but proportionally less belowground reserves.

Problems

A 4 November storm had a devastating effect on the York River region and caused serious damage to our permanently deployed light sensors at Gloucester Point and Clay Bank. All sensors are now being repaired and will be re-deployed once they are returned. This will result in a loss of data from our continuous light record from November to approximately March or April when we anticipate having everything operational.

1986 Research Objectives

In 1986, all survivorship plots at the transplant sites (both 1985 transplant sites and 1984 sites that still have plants) will be checked in April once the plants have started to show significant growth. In addition, water column monitoring and plant productivity studies will be continued, and investigations into belowground plant structure will begin. Also, data on the sedimentary environment, particularly the nutrients, will be gathered. We are planning to collect reproductive shoots again this spring for eelgrass seeds to be used in transplant experiments during the fall.

Also, we will be initiating efforts to get the greenhouse operational for the laboratory experiments that are planned for the next biennium.

Research Objectives - 1986-1988

Goals for the 1986-1988 biennium include the continuation of the transplanting effort at sites that are showing successful re-establishment of SAV. During each year of the biennium, we propose to plant 13 acres of eelgrass. We are planning to test the effectiveness of using seeds on a larger scale in the Fall of 1986 if adequate numbers of seeds can be collected in the Spring of 1986.

The monitoring effort of the York River transplant sites will continue as well as the continuous light recording at Gloucester Point and Clay Bank. Sediment-nutrient work, production measurements of aboveground and belowground growth, initiated in early 1986, will continue into the next biennium. Laboratory experiments to determine the effects of light, nutrients and epiphytes will be initiated pending the startup of the greenhouses.

The work proposed for the 1986-88 biennium is expected to be funded from the Chesapeake Bay Initiative package.

EXHIBIT A

Virginia Institute of Marine Science

Chesapeake Bay Research - Summary

	1984-85 Expenditures	1985-86 Budget [1]	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$424,159	$ 657,300	$116,280	$249,318	$ -0- [2]	$407,982
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	308,841	342,700	83,441	175,079	24,025	143,596
TOTALS	$733,000 [3]	$1,000,000	$199,721	$424,397	$24,025	$551,578

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] FY-1986 budgets are subject to revision, with funds being reprogrammed to reflect actual expenditure distribution.

[2] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

[3] Does not include VIMS match of $107,499.

EXHIBIT A

Study 1: Revitalization of the James River Seed Oyster Industry[1]

	1984-85 Expenditures	1985-86 Budget[2]	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$257,667	$368,645	$48,534	$123,802	$ -0-[3]	$244,843
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	213,027	180,990	51,360	116,189	17,806	46,995
TOTALS	$470,694	$549,635	$99,894	$239,991	$17,806	$291,838

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] Includes Projects 1, 2, 3, 4A, 4B, 4C, 4D as identified in the Institute's Initiative Revised Plan, 9/84.

[2] FY-1986 budgets are subject to revision, and funds will be reprogrammed to reflect actual expenditure distribution.

[3] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

Study 2: Factors Effecting Recruitment of Virginia's Critical Finfish Population[1]

	1984-85 Expenditures	1985-86 Budget[2]	Expenditures 10/1/85- 12/31/85	Expenditures 7/1/85- 12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$80,375	$ 83,322	$22,87	$33,619	$ -0- [3]	$49,703
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	6,646	49,670	14,557	14,589	1,422	33,659
TOTALS	$87,021	$132,992	$37,384	$48,208	$1,422	$83,362

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] Includes Projects 5 and 6 as identified in the Institute's Initiative Revised Plan, 9/84.

[2] FY-1986 budgets are subject to revision, and funds will be reprogrammed to reflect actual expenditure distribution.

[3] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

Study 3: Chemical Poisons in Virginia's Tidal Waters[1]

	1984-85 Expenditures	1985-86 Budget[2]	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$ 86,117	$205,333	$44,919	$ 91,897	$ -0-[3]	$113,436
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	89,168	112,040	17,524	44,301	4,797	62,942
TOTALS	$175,285	$317,373	$62,443	$136,198	$4,797	$176,378

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] Includes Projects 7 and 8 as identified in the Institute's Initiative Revised Plan, 9/84.

[2] FY-1986 budgets are subject to revision, and funds will be reprogrammed to reflect actual expenditure distribution.

[3] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

Virginia Institute of Marine Science

Submerged Aquatic Vegetation

	1984-85 Expenditures	1985-86 Budget [1]	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$132,972	$153,884	$38,995	$71,602	$ -0- [2]	$82,282
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	27,481	22,150	5,642	12,522	2,791	6,837
TOTALS	$160,453	$176,034	$44,637	$84,124	$2,791	$89,119

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] FY-1986 budgets are subject to revision, and funds will be reprogrammed to reflect actual expenditure distribution.

[2] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

EXHIBIT C

Virginia Institute of Marine Science

Oyster Hatchery - Operating

	1984-85 Expenditures	1985-86 Budget [1]	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/85
Personal Services	$ -0-	$104,220	$24,776	$35,089	$ -0- [2]	$69,131
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	-0-	45,780	11,129	16,497	13,359	15,924
TOTALS	$ -0-	$150,000	$35,905	$51,586	$13,359	$85,055

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.

[1] FY-1986 budgets are subject to revision, and funds will be reprogrammed to reflect actual expenditure distribution.

[2] Personal service costs are not encumbered; budgets are used to identify personal service obligations.

EXHIBIT D

Virginia Institute of Marine Science

Oyster Hatchery, Non-Operating

	1984-85 Expenditures	1985-86 Budget	Expenditures 10/1/85-12/31/85	Expenditures 7/1/85-12/31/85*	Current Obligations**	Balance 12/31/8
Personal Services	$ -0-	$ -0-	$ -0-	$ -0-	$ -0-	$ -0-
Nonpersonal Services (Travel, supplies, rent, equipment, grants, etc.)	18,428	170,572	25,248	103,145	10,908	56,519
TOTALS	$18,428	$170,572	$25,248	$103,145	$10,908	$56,519

* Includes quarters one and two.

** Not included in second quarter expenditure amounts.