The Applicability of Single Species Bioassay for Estimating the Effects of a Refinery Effluent on an Estuarine Environment

HEALTH & ENVIRONMENTAL SCIENCES
API PUBLICATION NO. 4401
JULY 1985

American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

THE APPLICABILITY OF SINGLE SPECIES BIOASSAY FOR ESTIMATING THE EFFECTS OF A REFINERY EFFLUENT ON AN ESTUARINE ENVIRONMENT

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ESE No. 82-301-0304-2130

July 1985

ABSTRACT

The ability of single-species aquatic toxicity bioassays to predict the impacts of industrial effluents in receiving water ecosystems was evaluated. A refinery discharging into an estuary was chosen for the assessment. Effluent plume dispersion characteristics, analytical chemistry data, acute and chronic bioassay results, and biological ecosystem analyses were correlated to evaluate whether the bioassay results accurately reflected receiving water ecosystem impact findings. Bioassays indicated no toxic effects of the effluent (at concentrations of waste effluent occurring in the ecosystem), and no ecosystem impacts were discerned. In this limited sense, therefore, ecosystem condition was reflected by toxicity data.

The raw study data contained within the Appendix of this report related to Physical Characterization (Appendix A), Chemical Characterization (Appendix B), Field Biology Studies (Appendix C), and Bioassay (Appendix D) are available for consultation and review in the American Petroleum Institute library, 1220 L Street NW, Washington, D.C. 20005.

KEY WORDS

Bioassay	Single-Species Bioassay
Effluent Bioassay	Industrial Bioassay
Virginia	NPDES--Refinery Effluents
Chesapeake Bay	York River
Mysidopsis bahia	Refinery Effluents

ACKNOWLEDGMENTS

ESE acknowledges with appreciation the assistance and information provided by the Amoco Oil Company, Yorktown Refinery personnel. ESE is also grateful to the Hampton Roads Sanitation District for access to their water quality and biological data. The American Petroleum Institute (API) W-24 Task Force provided technical input, assistance, and review. This study was conducted under a contract from API.

EXECUTIVE SUMMARY

The objective of this study was to evaluate the ability of single-species aquatic toxicity bioassays conducted on industrial effluents to predict the impacts of the effluents in receiving water ecosystems. This study was initiated in response to EPA protocols recommended for NPDES permits and by concern that draft EPA biomonitoring policy was placing excessive reliance on the information obtainable by such bioassays. This case study was conducted at the Standard Oil Company of Indiana (Amoco) refinery in Yorktown, Virginia, which discharges its process effluents and noncontact cooling waters into the York River estuary. The studies were conducted throughout 1982 and 1983.

The study had four major tasks: (1) characterization of the effluent discharge plume's dispersion behavior; (2) chemical characterization of the effluents, receiving waters, and York River sediments; (3) laboratory toxicity bioassays on the effluents; and (4) evaluation of ecosystem impacts as indicated by benthos and fouling plate studies. The results of these four study components were evaluated to determine whether the bioassay data accurately predicted ecosystem impact findings.

Plume characterization studies indicated that the receiving water body and its transport of the waste plume are exceedingly complex. In the refinery vicinity, the York estuary is stratified for most of the year. This stratification causes the summer chronic dissolved oxygen (DO) depletion in the deeper water. This study also showed that the DO can change on a bi-weekly basis when the spring tides are of sufficient magnitude and the freshwater flows are sufficiently reduced to allow vertical mixing, thus replenishing bottom DO. This biweekly cycle does not always occur, but during the summer low-DO period it can occur regularly.

The biweekly tidal cycle also affects the physical character of the refinery effluent plume within the York River. Regardless of whether

the plume is headed inland during the flood tide, headed seaward during the ebb, or spreading during the slack water period, its vertical distribution is controlled by the spring and neap tides. During a spring tide, vertical mixing results in up to 0.02 percent of the refinery process water reaching the bottom even in deeper areas. During the stratified neap tide conditions, the plume does not reach the bottom at the deeper stations. Because the stratified conditions occur approximately 75 percent of the time, this means that, at a minimum, 75 percent of the time the refinery plume is caught above the pycnocline and therefore has little or no quantifiable effect on water deeper than about 10 meters.

The plume studies also showed that the process water concentration in the York River estuary did not exceed 0.35 percent at any time during the survey and that, typically, the value was nearer 0.02 percent within the study area.

The chemistry studies found constituents to be typical for a refinery discharge.

Toxicity bioassays indicated demonstrable toxicity of the process effluent in acute (96-hour and shorter) tests and sublethal effects during a chronic (26-day) test. However, no effects occurred at test concentrations corresponding to effluent concentrations occurring in the York River.

Benthos stations were selected on the basis of metal and hydrocarbon contents of the sediments. This allowed comparisons of stations with high versus low concentrations of these parameters. Three replicates of samples collected twice at 30 stations were thoroughly analyzed to determine benthic community characteristics. No impacts attributable to the discharge of refinery effluents were discerned within the benthos study area.

Similarly, fouling organism communities collected on artificial fouling plate arrays distributed in the area of effluent discharge were extensively examined. Again, no impacts from the refinery effluent were evident.

The bioassays did accurately reflect the finding of "no impact" on the ecosystem based on environmental exposures resulting from effluent dilution. Nevertheless, this refinery would have failed bioassay-related regulatory criteria used in some states, based on the least favorable acute bioassay of the several tests conducted during this study.

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1.0 INTRODUCTION

This study was funded by the American Petroleum Institute (API) and was conducted by Environmental Science and Engineering, Inc. (ESE) during 1982 and 1983. It was initiated because of questions arising from draft regulations and policy discussions promulgated by the U.S. Environmental Protection Agency (EPA). The subject of the study and the approaches taken to attempt clarification of the issues are discussed below. Study results and conclusions are presented in subsequent chapters.

1.1 THE ISSUES

Federal and state agencies interact for the protection of water quality by controlling the discharge of toxic substances in toxic amounts as mandated in the Clean Water Act. Sections 308 and 402 of the Clean Water Act authorize EPA and the states to require National Pollutant Discharge Elimination System (NPDES) permit applicants to provide chemical and biological data necessary to assure compliance with water quality standards.

In January 1981, EPA issued a draft document (EPA, 1981) proposing the use of effluent toxicity testing as a criterion for Second Round NPDES Permit application evaluation. In this draft document, EPA stated:

> "Toxicity may be used to identify dischargers with significantly toxic discharges, determine receiving water impacts, and establish permit limits..." (page 8)

Although EPA subsequently discussed the types of additional data required to evaluate receiving water impacts, this document nevertheless initiated API concerns that EPA was becoming oriented toward a great reliance on effluent toxicity data for making such evaluations. API concerns were specifically raised by the statement in the draft EPA document that:

> "Water-quality-based limits are designed to prevent chronically toxic conditions from occurring in the receiving water. The

impact of a discharge is a function of the toxicity of the effluent and the dilution capacity of the receiving water at low flow." (page 29)

API was concerned that this and similar statements indicated a de-emphasis of the myriad of additional factors affecting ecosystem impacts, such as receiving water quality, sediment types in the receiving water body, biotic communities present, population dynamics of the specific ecosystem, and similar ecosystem characteristics.

A further point of concern was initiated by the draft statement that:

"EPA has developed a standard testing procedure to screen wastewater discharges for acute toxicity. This procedure is detailed in 'Effluent Toxicity Screening Test Using Daphnia and Mysid Shrimp.'" (page 20)

This implied that EPA was inclined toward using single-species bioassays to extrapolate to the impacts of effluents on receiving water ecosystems. API questioned whether a sufficient scientific understanding of ecosystem dynamics exists to realistically permit such a simplistic approach.

This concern was subsequently emphasized by a previous draft of the present EPA Statement of Policy entitled "Policy for Development of Water Quality-Based Permit Limitations for Toxic Pollutants" (EPA, 1984). This policy states:

"There is now a general consensus that an evaluation of effluent toxicity, when adequately related to instream conditions, can provide a valid indication of receiving system impacts."

That such a consensus exists within the scientific community is questionable; there is considerable doubt that enough is known to "adequately" relate instream conditions to single-species bioassays in order to predict effluent impacts on complex ecosystem dynamics, except in

extreme circumstances (i.e., conditions of extreme, catastrophic toxicity).

API's concern was echoed by the Environmental Effects, Transport and Fate Committee of EPA's Science Advisory Board (EPA Board, 1983a), which concluded:

> "...laboratory toxicity tests fail to account for interactions between species, ecosystem level effects, interactions with other chemicals, and modifications by local water quality characteristics. In addition, the non-specific stresses in a laboratory setting...are assumed to be equivalent to the non-specific stresses encountered in the environment. The species tested in the laboratory are assumed to reflect significant or important species in the environment."
>
> "The Committee concluded that the sum of such assumptions made it essentially impossible to discern a logical framework which would guarantee achieving the protection of the environmental integrity of aquatic systems."

To address these issues, API sponsored this research project to attempt determination of whether information derived from single-species toxicity testing can be correlated with ecosystem data collected in the field. API is concerned that the ability of laboratory tests to predict potential ecosystem impacts has not been adequately verified by field studies. This project is one of many such studies that must be conducted to properly evaluate these issues.

1.2 STUDY RATIONALE

ESE conducted a series of studies to attempt determination of relationships between single-species bioassay data and measurable effects of a petroleum refinery effluent on an estuarine environment. The studies were conducted at the Standard Oil Company of Indiana (Amoco) Yorktown

Refinery, Yorktown, Virginia. This site was selected by API as representative of the very complex estuarine conditions existing at many coastal refineries. In API's view, studies conducted at less complex sites are occasionally dismissed as simplistic; this potential fate was avoided by selection of the Yorktown site.

Through a framework of onsite bioassays, biological field studies (benthic and fouling communities), plume studies, extensive chemical characterization (effluent, receiving water, and sediments), and background information review, a comprehensive data base was assembled. From this information, correlations between laboratory predictions and ecosystem impacts were evaluated.

To achieve the study objectives, a phased approach was followed. This report presents and discusses the results obtained from all laboratory and field studies. Task efforts included: (1) physical characterization of the discharge plume; (2) acute and chronic bioassays using several laboratory-reared and indigenous species; (3) chemical characterization of effluents, ambient river water, and river sediment samples; (4) sediment particle size distribution; and (5) benthic and fouling community studies.

The following sections discuss the study site, the materials and methods of each task, and the task-specific results and discussion. The final section of the report integrates all the phases of this study, presents correlations between laboratory and field studies, and relates these findings to the proposed EPA (1983b) guidelines.

2.0 SITE DESCRIPTION

2.1 ENVIRONMENTAL SETTING--THE YORK RIVER BASIN

The purpose of the following discussion of historical data is to describe the York River and the factors that affect the physical nature of the river and to gain an understanding of how these factors might affect the physical character of the effluent plume issuing from the Amoco refinery.

The York River basin (Figure 2-1) is located in Virginia and has an area of 6,790 square kilometers (km^2), comprised predominantly of forests, farmland, and marshland. Only 2 percent of the area is classified as urban (Weston, 1976). The major industries are lumbering, pulp and paper, petroleum refining, fabricated metals, textiles, fishing, and shellfishing. Figure 2-1 and Table 2-1 present the major point source industrial and municipal discharges for the York River basin.

Characteristically, the area has hot summers [July mean daily maximum air temperature of 31 degrees Celsius (°C)], relatively dry autumns, and mild wet winters (January mean daily minimum air temperature of -1°C). Precipitation, which annually averages 114 centimeters (cm), is generally lowest from September to January and highest in July and August, due to thunderstorms. An example of extreme precipitation was tropical storm Agnes which dropped 71 cm within 2 days in 1972. The prevailing winds are from the south to southwest at 4.5 meters per second (m/s) [10 miles per hour (mph)].

The York River proper begins approximately 50 kilometers (km) up-estuary of its mouth at the confluence of the Pamunkey and Mattaponi Rivers, and actually occupies only a small portion of the total York River basin. Typically, the York River is straight, wide [approximately 3,200 meters (m)], and deep (in excess of 7.5 m) throughout its length except for the area of Gloucester Point, where the river bends and its width is reduced to 780 m.

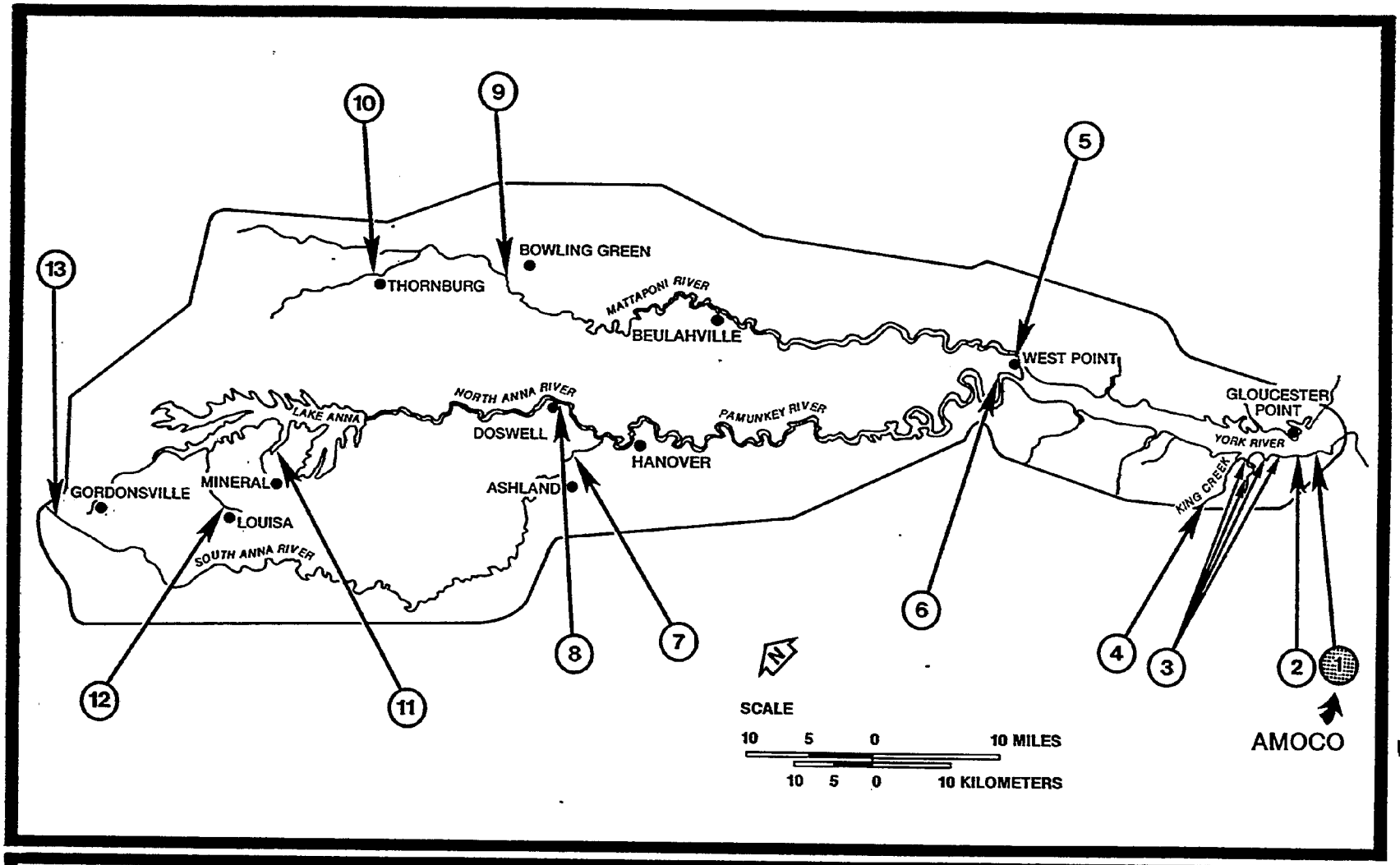

Figure 2-1
YORK RIVER BASIN AND MAJOR POINT DISCHARGES

SOURCE : WESTON, 1976.

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Table 2-1. Major Point Sources in the York River Basin (Measured Discharge Levels, Except Where Indicated Otherwise)

Major Point Sources	Map Reference Number*	Flow† (m^3/day)	BOD_5** (kg/day)	Oil and Grease (kg/day)
Amoco (Petroleum Refinery)	1	4,958††	322	283
VEPCO (Power Plant)	2	3,595,372	***	90
Combined Navy Weapons (Sewerage)	3	1,893†††	1,841†††	***
York and James City Co. Sewer District No. 1	4	1,211	14	***
West Point POTW****	5	1,476	50	***
Chesapeake Corporation (Pulp and Paper)	6	47,691	10,699	***
Ashland POTW	7	2,385	76	***
Doswell POTW	8	4,966	134	***
Bowling Green POTW	9	125	4	***
Thornburg POTW	10	95	3	***
Mineral POTW	11	76	2	***
Louisa POTW	12	106	4	***
Gordonsville POTW	13	1,351	58	***

*Refer to Figure 2-1.
†Annual average daily flow for 1981.
**Annual average daily BOD_5 load for 1981.
††Process water only.
***Not measured.
†††Based on projection by Weston, Inc. (1976) for 1977.
****Public Owned Treatment Work.

Source: Woodsin, Personal Communication, Virginia State Water Control Board, 1982.

The freshwater flow in the York River is generally greater from January through April, and less in July through September. Hyer et al. (1975) reported that the summer thunderstorms and associated precipitation have less effect on the river flow than do the spring rains. The York River is tidally influenced throughout its entire length and, therefore, does not have a flow gaging station. Flows cited for the York River are calculated from the sum of the flows of the Pamunkey and Mattaponi Rivers and multiplied by the drainage area ratio of 1.54 (Haas, 1977). Table 2-2 presents summary flow data. With an average flow of only 70 cubic meters per second (m^3/s), the York River contributes only about 3.2 percent of the total fresh water to the Chesapeake Bay drainage basin. In fact, Hyer et al. (1975) states that the salinity at the mouth of the York River is controlled more by freshwater flow from the other major rivers in Chesapeake Bay than by freshwater flow from the York River.

The surface salinity at the mouth of the York River generally ranges from 15 to 24 parts per thousand (ppt). Hyer et al. (1975) reported that salinity at the source of the York River (West Point) can reach as high as 17.2 ppt in the late fall, but decreases to less than 1 ppt in the spring.

Salinity, more than temperature, is responsible for density gradients that lead to stratification in the York estuary. According to Hyer et al. (1975), vertical salinity differences between top and bottom can vary from <1 to 8 ppt. These density gradients determine the depth and strength of the pycnocline (the region in the water column where density changes sharply with depth) which generally forms a distinction, or even a virtual barrier, between two distinct water masses. The pycnocline generally occurs between 4.5 and 12 m.

In addition to salinity, the depth and strength of the pycnocline are affected by several other factors, including bottom depth, river flow, wind-induced mixing, and tides. Hydroscience, Inc. (1975) reported that during the summer, at shallow sites (depth <9 m), the density gradient

Table 2-2. Summary Flow Data for the York River and its Tributaries

River	Maximum (m3/s)	Mean (m3/s)	Minimum (m3/s)
Pamunkey River at Hanover	1140	28.52	0.34
Mattaponi River at Beulahville	479	16.99	0.17
York River	--	70.09*	4.25†

*Calculated using drainage area ratio of 1.54.
†Calculated 7-day, 10-year low flow from Hydroscience, Inc. (1975).

Source: ESE [from U.S. Geological Survey (USGS), 1979].

was 0.056 $_t$ units per meter (units/m), whereas the deep site gradient was 0.144 $_t$ units/m.

According to both Hydroscience, Inc. (1975) and Haas (1977), freshwater flow in the York River affects the density gradient and, therefore, the degree of stratification. They agree that the higher freshwater flows in winter produce the greatest density gradients and, therefore, the greatest degree of stratification. Their findings are to be expected when one considers the dynamic classification of estuaries as defined by Pritchard (1967). The three major categories, in order of increasing tidal dominance over river flow, are: (1) highly stratified or salt-wedge estuary, (2) moderately stratified or partially mixed, and (3) vertically homogeneous or completely mixed. During the winter when freshwater flows are greater, the York estuary is similar to the highly stratified estuary where river flow is dominant over tidal action. As the river flow decreases, the York River more closely resembles a moderately stratified estuary.

In addition, Hydroscience, Inc. (1975) and Haas (1977) reported that winds affect the degree of stratification to a lesser extent. Haas (1977), however, reported that one of the dominant factors also affecting stratification in the York estuary is the tides. The semi-diurnal tides of the York estuary have a rather small range of 0.7 m and usually generate tidal currents on the flood and ebb tides of approximately 30 centimeters per second (cm/s). It is not, however, the daily fluctuation of the tides that affects the stratification in the York estuary. Rather, it is the biweekly cycle of lunar spring and neap tides, their associated currents, and time differences between tidal stages within the Chesapeake Bay in the vicinity of the York River that affect stratification.

Webb and D'Elia (1980) and Hayward et al. (1982) also reported that there was a strong positive correlation between destratification (and, therefore, complete vertical mixing) and spring tides. Destratification

was usually most intense approximately 4 days after the spring tide. Following destratification, the estuary once again began to stratify and became most stable during the neap tides. Not all spring tides produced vertical homogeneity. The spring high tide height had to be of sufficient magnitude (0.8 m, according to Hayward et al., 1982) to induce destratification. The periods of homogeneity were more numerous and intense in summer because of the annual cycle in magnitude of high tides. Also, because of decreased freshwater flow in the summer, destratification was more likely.

However, all authors (Hydroscience, 1975; Haas, 1977; Webb and D'Elia, 1980; and Hayward et al., 1982) maintain that variations in river flow and meteorological conditions are of secondary importance. For instance, during the increased flows associated with the tropical storm Agnes in 1972, the cycles of stratification and mixing proceeded according to the tides and were not measurably affected by increased flows.

To reiterate, the primary regulating factor for the York estuary's stratification-destratification cycle is the biweekly variation in tidal currents rather than the annual variation in river flows. This cycle is of particular importance when examining water quality parameters. This section of the report will only briefly discuss water quality as it will be examined in more detail in other sections of the report.

Perhaps the water quality parameter of most interest, both biologically and physically, is dissolved oxygen (DO). DO in the lower York River frequently drops below the Virginia State water quality standard of 4.0 milligrams per liter (mg/L), particularly during the summer and at depths greater than 12 m. Webb and D'Elia (1980) stated that:

> "...the spring tidal destratification phenomenon has substantial effects on the distribution of oxygen and nutrients in the lower York River. Vertical mixing accompanying destratification replenishes oxygen in deep water, allowing aerobic processes to proceed again until oxygen is depleted. This mixing will also accelerate the input of benthic regenerated nutrients into the

euphotic zone. This pulsing of nutrients into the euphotic zone may support phytoplankton growth during stable conditions of the stratified period immediately after the destratification event."

It is emphasized that such DO "violations" in the lower York River are the result of natural rather than man-made phenomena.

To understand the dynamic physical structure of the York River, 14 data sets spanning 2 years (Appendix, Table A-1) provided by the Hampton Road Sanitation District (HRSD, 1982) were examined. These data were used because HRSD has done extensive and frequent sampling in the study area adjacent to the refinery.

Table 2-3 presents summary data for five of the HRSD stations for all 14 dates. The five stations examined by ESE are shown in Figure 2-2.

Historical data from two particular days were examined in greater detail. The first day, July 13, 1981, occurred during a neap tide; the second day, August 24, 1981, was during a spring tide. Selected parameters for three stations on both days were plotted versus depth (Figures 2-3 and 2-4). Figure 2-3 shows temperature, salinity, and density (as $_{t}$) versus depth; Figure 2-4 shows DO and DO as percent of saturation versus depth.

These two figures demonstrate several features of the York estuary discussed previously. The most obvious feature is the stratification that existed on July 13 during neap tide, where the lunar first quarter occurred on July 8 and the full moon occurred on July 16. On August 24 (2 days after a full moon, i.e., spring tide), the estuary was virtually homogeneous. The DO depletion, particularly in the deep water as presented in Figure 2-4, was very pronounced on July 13 (neap tide), whereas on August 24 there was only a slight decrease in DO with depth.

A final feature revealed in these figures is the tendency toward greater stratification in the deeper water. The density gradient between 6 and

Table 2-3. Summary HRSD Water Quality Data for the York River from October 1979 through September 1981

	YA-2			YA-1			YB-1			YO-4			YU		
	Max	Mean*	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min
Temperature (°C)	28.67 12 Aug 80†	17.14 —	4.34 19 Feb 81†	28.74 13 Jul 81	19.85 —	3.04 19 Feb 81	28.90 13 Jul 81	19.48 —	2.56 19 Feb 81	29.32 13 Jul 81	19.92 —	2.86 19 Feb 81	29.68 04 Aug 81	19.52 —	2.76 19 Feb 81
Salinity (ppt)	24.42 19 Feb 81	19.90 —	14.97 22 May 80	24.22 19 Feb 81	20.86 —	15.34 31 Oct 79	25.50 22 Jul 80	21.28 —	15.70 22 May 80	23.73 19 Feb 81	20.78 —	15.74 22 May 80	25.85 10 Sep 81	21.22 —	14.28 22 May 80
$t_B - t_S$**	14.11 24 Aug 81	6.86 —	-0.22 07 Apr 81	1.18 13 Jul 81	0.41 —	-0.08 04 Aug 81	5.72 22 Jul 80	1.65 —	-0.19 24 Aug 81	1.62 22 Jul 80	0.65 —	0.09 18 Jun 80	5.23 22 Jul 80	1.74 —	-0.05 24 Aug 81
Dissolved Oxygen (mg/L)	11.7 19 Feb 81	8.4 —	5.5 12 Aug 80	11.9 19 Feb 81	7.1 —	2.6 22 Jul 80	12.25 19 Feb 81	6.9 —	1.4 13 Jul 81	12.4 19 Feb 81	7.5 —	1.4 13 Jul 81	13.7 04 Aug 81	7.1 —	1.3 13 Jul 81
Oxygen Saturation (%)	109.6 24 Aug 81	94.6 —	66.4 22 May 80	116.2 13 Jul 81	85.3 —	48.5 10 Sep 81	136.2 13 Jul 81	82.9 —	18.8 13 Jul 81	131.5 13 Jul 81	88.7 —	49.2 13 Jul 81	203.0 04 Aug 81	85.1 —	17.5 13 Jul 81
Total Kjeldahl Nitrogen (mg/L)	0.88 22 Jul 80	0.46 —	0.06 19 Feb 81	1.95 22 May 80	0.47 —	0.05 05 Nov 80	0.99 20 Mar 80	0.47††	0.15 10 Sep 81 19 Feb 81	1.03 18 Jun 80	0.48††	0.07 19 Feb 81	1.64 18 Jun 80	0.53††	0.01 19 Feb 81
Nitrate-Nitrite (mg/L)	4.21 10 Sep 81	0.52 —	0.02 18 Jun 80	4.40 10 Sep 81	0.64 —	0.01 18 Jun 80	4.21 10 Sep 81	0.66††	0.02 20 Mar 80	3.68 10 Sep 81	0.30 —	0.01 18 Jun 80	4.26 10 Sep 81	0.56 —	0.02 22 Jul 80
Ammonia (mg/L)	0.72 20 Mar 80	0.23 —	0.00 19 Feb 81	0.84 22 May 80	0.17††	<0.001 18 Jun 80	0.48 20 Mar 80	0.19††	<0.001 18 Jun 80	0.96 20 Mar 80	0.21††	0.00 19 Feb 81	0.08 20 Mar 80	0.18††	0.00 19 Feb 81
Orthophosphate (mg/L)	0.15 13 Jul 81	0.06 —	<0.001 22 May 80	0.39 24 Aug 81	0.09 —	0.01 18 Jun 80	0.22 13 Jul 81	0.08 —	0.02 18 Jun 80	1.16 24 Aug 81	0.13 —	0.02 22 May 80	2.34 24 Aug 81	0.17††	<0.001 22 May 80
BOD (mg/L)	3.3 19 Feb 81 07 Apr 81	1.4 —	0.5 20 Mar 80	3.2 13 Jul 81	1.2††	0.3 18 Jun 80	5.6 13 Jul 81	1.3 —	0.3 12 Aug 80	5.3 13 Jul 81	1.6††	0.2 18 Jun 80	4.4 20 Mar 80	1.3 —	0.1 12 Aug 80
Total Suspended Solids (mg/L)	29 22 Jul 80	16 —	1 19 Feb 81	110 20 Mar 80	21 —	1 19 Feb 81	59 20 Mar 80	19 —	0 19 Feb 81	42 24 Aug 81	18 —	5 12 Aug 80	254 20 Mar 80	40 —	5 31 Oct 79

*Mean calculated from surface and bottom values for all dates.
†Date of occurrence of maximum and minimum values.
**Bottom t minus surface t.
††Values below detection limit present in data set.

Source: ESE (from Hampton Roads Sanitation District, 1982).

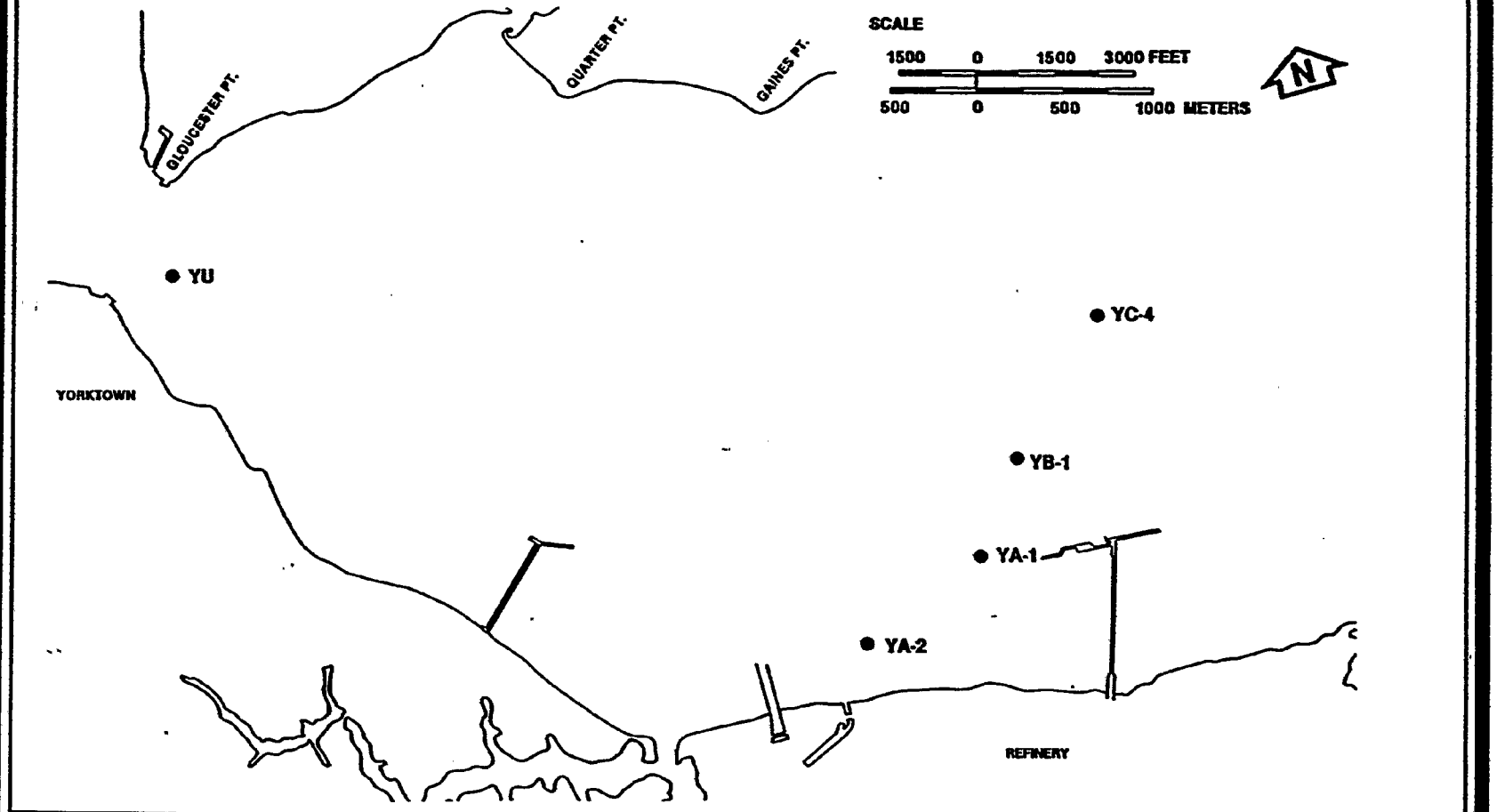

Figure 2-2
HAMPTON ROADS SANITATION DISTRICT (HRSD) WATER QUALITY STATION LOCATIONS ON THE YORK RIVER, VIRGINIA

SOURCE: HRSD, 1982.

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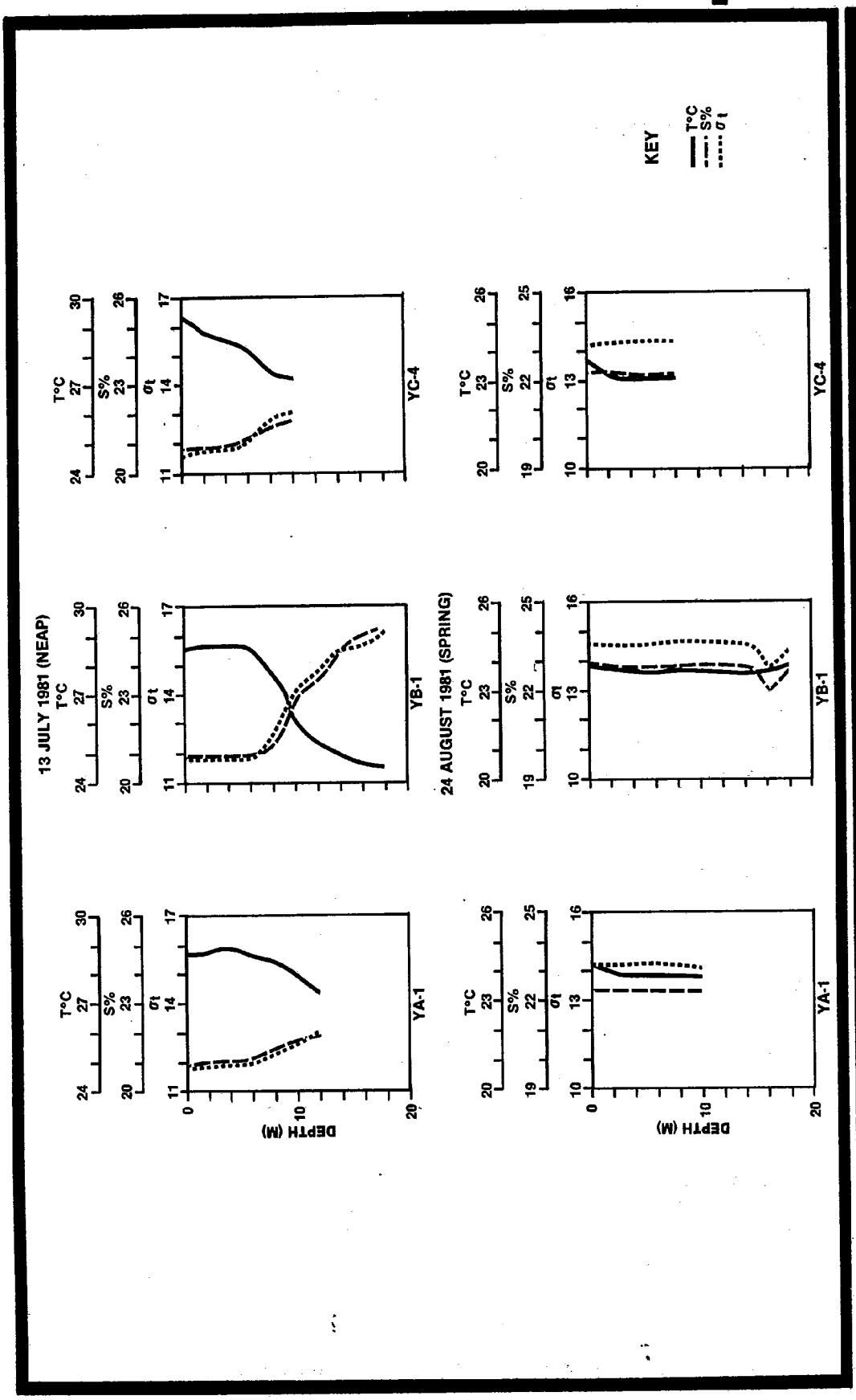

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Figure 2-3
VERTICAL PROFILES OF TEMPERATURE, SALINITY AND SIGMA-T FOR THREE STATIONS ACROSS THE YORK RIVER DURING NEAP AND SPRING TIDES (FROM HAMPTON ROADS SANITATION DISTRICT)

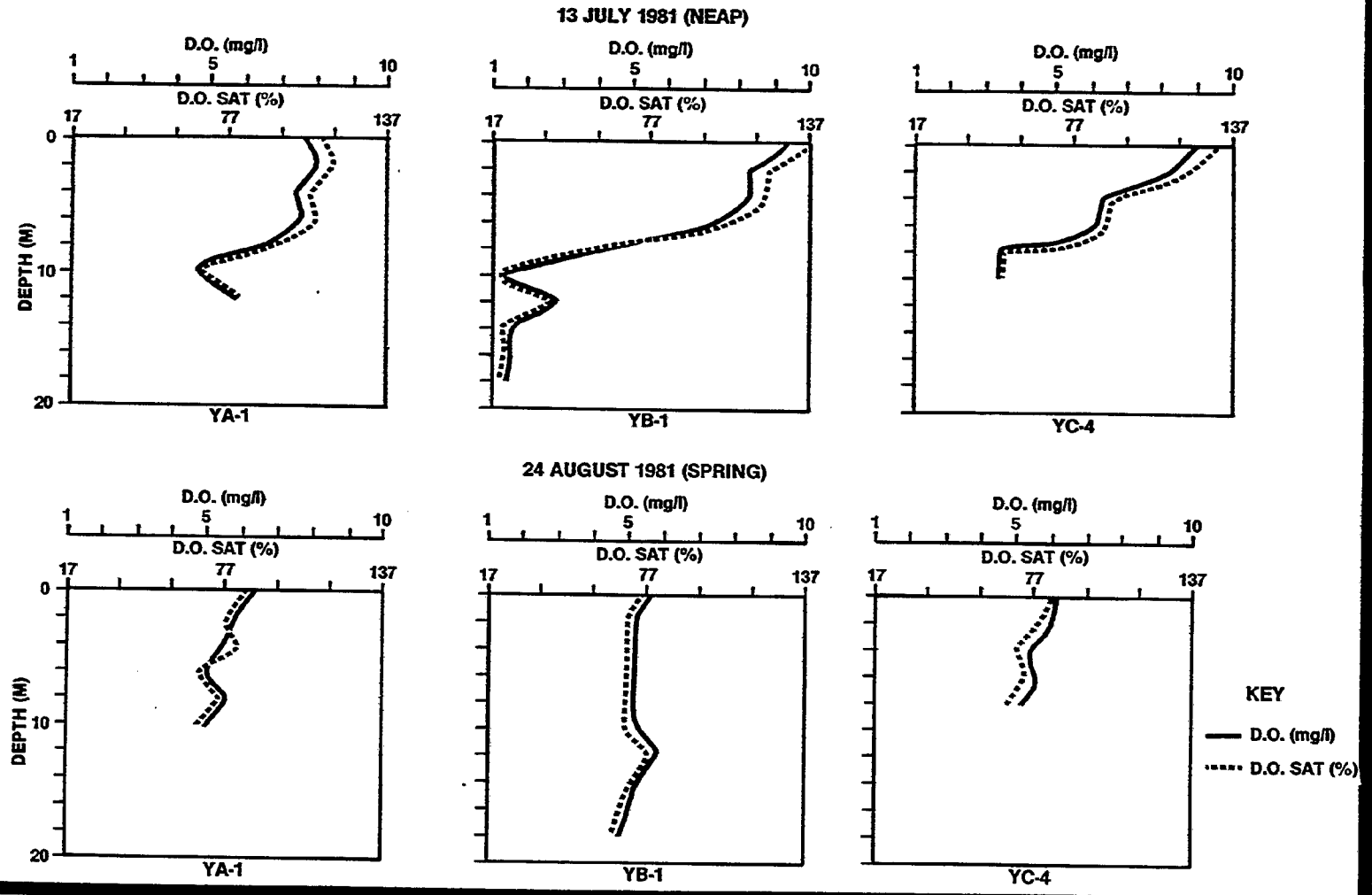

Figure 2-4
VERTICAL PROFILES OF DISSOLVED OXYGEN CONCENTRATION AND SATURATION FOR THREE STATIONS ACROSS THE YORK RIVER DURING NEAP AND SPRING TIDES (FROM HAMPTON ROADS SANITATION DISTRICT)

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10 m is 0.5 $_t$ units/m at Station YB-1 (depth = 18 m) and approximately 0.2 $_t$ units/m at Stations YA-1 and YC-4 (depth $\leq$12 m).

2.2 STUDY SITE--THE AMOCO YORKTOWN REFINERY

The Amoco refinery was chosen by API for this research study. The refinery and waste treatment facility operate continuously. The major products of the refinery are gasoline, butane, heptane, LPG, coke, sulfur, and No. 2 fuel oil. The major raw material is crude oil. The Federal Standard Industrial Code (SIC) for Amoco is 2911.

Figure 2-5 is a wastewater treatment diagram for the refinery (Wilson et al., 1981). The process wastewater (Outfall 101) joins the non-contact cooling water (Outfall 201) to form the final effluent (Outfall 001), which is discharged into the York River through a 60-inch (in) line approximately 600-m offshore and at a depth of approximately 6 m. The only noticeable change in the physical appearance of the receiving water at the point of discharge was the appearance of air bubbles in the river water.

According to Wilson et al. (1981), water enters the refinery treatment plant from fresh water, salt water, storm runoff, and tanker (ship) ballasts. The fresh water is used in the crude oil refining process and in boiler steam generation. This water then goes through the oily water sewer to the API separator. Area runoff combines with the plant wastewater and the ship ballast water and are sent to the API separator for initial treatment. These wastewaters pass through a chemical equalization basin followed by an aeration basin. The effluent is pre-filtered and again aerated. Backwash from the prefilters is discharged to a backwash pond before entering a clarifier. Effluent from the second aeration tank is then discharged into the activated sludge plant and then into a clarifier to reduce settleable material before the final filters. Sludge from the clarifier goes to the digestor and then to a land farm. The effluent produced after final filtration comprises Outfall 101, the NPDES sampling point for the refinery.

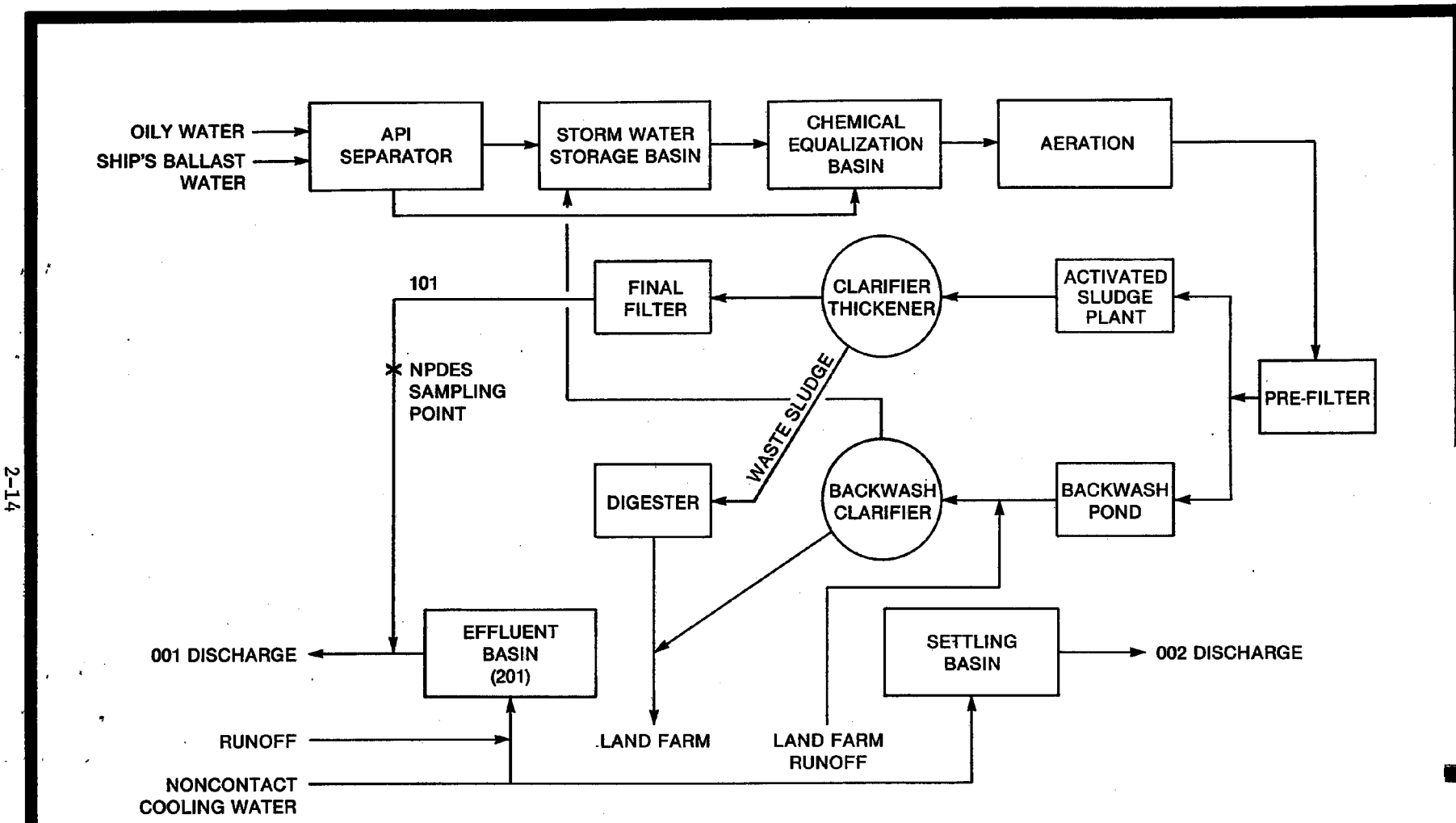

Figure 2-5
AMOCO YORKTOWN REFINERY WASTEWATER TREATMENT DIAGRAM

SOURCE: Wilson et al., 1981.

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Salt water is used for area washdowns and non-contact cooling water. The non-contact cooling water (Outfall 201) is stored in the effluent holding basin prior to combining with final treated process water (Outfall 101) for discharge (Outfall 001). The process water [1.008 million gallons per day (MGD)] is diluted by the non-contact cooling water (56.067 MGD) prior to discharge (Outfall 001). Therefore, based on these projected average values (NPDES Permit Number VA0003018 for Amoco), the percent treated process water at time of discharge (Outfall 001) should average 1.8-percent process water.

3.0 MATERIALS AND METHODS

3.1 PHYSICAL CHARACTERIZATION

3.1.1 Objectives

The objective of this task was to describe the physical characteristics of the Amoco effluent plume discharged into the York River estuary. The effects of the environment on the physical behavior of the refinery plume and the plume effects on the environment were also investigated.

The results from this task aided in the determination of possible environmental impacts from the Amoco plume by defining the effluent concentration isopleths in the York estuary, thereby allowing a direct demonstration of the areas of anticipated possible acute and chronic impact, if any. In addition, the plume characterization physically portrayed the position of the effluent plume (and concentrations) under various environmental conditions so that not only the effluent concentrations were known, but the periods of exposure of the different communities could be estimated. The physical characterization study also defined the positions of the plume with respect to the benthic and water column communities; these results were used in the program design of the field biology sampling schemes.

ESE conducted a historical data survey and two field surveys to physically characterize the York River estuary and evaluate the dispersion of the refinery discharge plume. The historical data survey was conducted throughout the study period. The field surveys were conducted in March and June 1982 during spring and neap tides, respectively. Each field survey consisted of hydrographic and water quality sampling, and dye plume surveys. Techniques are described in the following sections.

3.1.2 Historical Data Survey

The first step in the historical data survey was to conduct a computer search of eight data bases using specific geographic and topical key words. This search yielded more than 100 citations. The most relevant

articles or their abstracts were obtained and examined in detail. The computer search also included an EPA STORET retrieval. This procedure retrieved existing water quality and discharge data for the general study area.

In addition to the computer searches, ESE conducted a search throughout the University of Florida and the Virginia Institute of Marine Sciences (VIMS) libraries.

In an effort to obtain unpublished data, several institutions and agencies were contacted or visited, including VIMS, the Virginia State Water Control Board, and HRSD. HRSD regularly conducts water quality sampling in the vicinity of the refinery and made its data readily available to ESE.

3.1.3 Hydrographic and Water Quality Survey

The hydrographic and water quality surveys consisted of *in situ* measurements of temperature, conductivity (and, hence, salinity), DO, pH, currents, and water quality sampling for laboratory analyses.

In situ physico-chemical measurements were made with a Model 4041 Hydrolab®. Current speed and direction were measured with an ENDECO® Model 110 deck readout current meter. All data were recorded on field data sheets.

Surface grab samples were taken for laboratory water quality analyses. The preservation and analytical techniques used are described in the chemical characterization sections of this report.

A physical and chemical reconnaissance survey of the York River in the vicinity of the Amoco Yorktown Refinery was conducted on March 9, 1982.

Representative ambient water was collected on March 5, 1982, to be used in fluorometer calibrations and pre-dye injection tests. These tests

included comparisons of ambient water and effluent water background fluorescence levels with distilled water, as well as recovery ratio tests.

The background tests were conducted to determine if any variability existed in background fluorescence or if the background fluorescence was unusually high. In either case, the amount of dye injected would be increased. The background level was determined by placing ambient water and effluent water in a cuvette and reading its fluorescence on a fluorometer. This reading was then compared to readings made with distilled water.

The recovery ratio tests involved adding a known amount of dye to ambient water or effluent discharge water, and distilled water. Fluorescence and temperature were then measured for each sample periodically throughout a 96-hour period. Any changes in the fluorescence of the discharge or ambient water versus the distilled water indicated that the dye was affected by constituents in the discharge or ambient water. If this had occurred, the amount of dye injected would have been increased to compensate for this effect.

The remainder of the ambient water was used to calibrate both the laboratory and survey fluorometers. In each case, the calibration involved the serial addition of a known amount of dye to the ambient water producing solutions of known concentrations. The fluorescence and temperature of each solution were then measured. Solution temperature is an important parameter as fluorescence of Rhodamine WT dye varies with temperature. The fluorescence was corrected to 25.0°C, and these values were plotted against dye concentrations to yield calibration curves. These curves were then used to convert fluorescence and temperature measurements made in the field to dye concentrations.

ESE used fluorescent Rhodamine WT dye continuously injected for a minimum of 2 days into the refinery effluent to tag the effluent plume.

The injection rate of the concentrated dye was 15 milliliters per minute (ml/min). The injection rate was checked several times per day to ensure constant dye delivery. Fluorescent dye was chosen for these studies because the dye could be tracked in the field, whereas chemical tracers existing in the discharge would be ambiguous and require chemical analysis. To define the dye plume, ESE used its automated high-speed data acquisiton system (DAS). The DAS was controlled by an Esterline-Angus® Model PD 2064 Data Logger, which was interfaced with a thermistor (accurate to $\pm$0.1°C), a Turner Designs® Model 10 Fluorometer, and a DECCA® Model 202-MS20 Trisponder® Navigation System (DTNS). The DTNS is a short-range microwave navigation system which provides accurate triangulation position fixing based on the distances from two shore-based reference stations. This system is accurate to $\pm$1 m and has a range of up to 80 km. The fluorometer was calibrated before each study using known dye standards.

Each dye survey was conducted in two parts. The first part was a complete horizontal survey of the surface concentrations of dye. Surface water (approximately 0.5 m deep) was pumped continuously through a fluorometer located on the survey vessel. The fluorescence, water temperature, and position from the DECCA were automatically recorded by the data logger. The survey vessel, instrumented in this manner, traversed the plume along several transects with the data logger automatically recording fluorescence, temperature, sampling location, and time at approximately 15-second intervals. In this manner the distribution of surface dye concentration was mapped.

Immediately following the horizontal survey, a survey of the vertical distribution of dye and temperature was conducted. Dye, temperature, and position were sampled at several depths at each station using the same instrumentation. The sampling at depth was done by lowering the intake hose to the proper depth prior to activating the data logger.

The magnetic data cassette from the data logger (and its backup paper tape) was returned to ESE for analysis. The data from the horizontal and vertical surveys were transferred to ESE's computer. The computer converted the two ranges for each station into X/Y coordinates and plotted these stations at a predetermined scale. The data were then tabulated and plotted for inclusion in this report.

3.2 CHEMICAL CHARACTERIZATION

3.2.1 Objective

Chemical data were gathered from the Amoco effluent, from the receiving and mixing waters, and from sediment samples taken from areas near the outfall. It was the objective of the chemical characterization studies to aid in the assessment of the wastewater toxicity and in defining the area of impact of the Amoco wastewater plume. Chemical characterization data of the effluent, the receiving water, and sediments were incorporated with the ecological data, to provide a more thorough evaluation of the wastewater's impact to the estuarine system. The chemical characterization data obtained from the sediment analyses were used extensively in the design of the benthic study.

The study objective was achieved through a phased approach. Phase I was a reconnaissance trip designed to develop a broad data base on the effluent. Phase II involved further characterization of the effluent concurrent with bioassay tests and also included the analysis of 60 sediment samples to define the area impacted by the plume, if any. The third phase of the study involved the analysis of effluent and 30 additional sediment samples to provide additional information on the zone of presumed impact.

3.2.2 Reconnaissance--Phase I

The reconnaissance trip had two major objectives. The first was to establish a broad chemical data base on the effluent and the receiving water with a special effort to characterize the unknown organic compounds by gas chromatography/mass spectrometry (GC/MS). Chemical characterizations of (1) the discharge plume under two sets of tidal conditions, (2) an ambient (dilution) water sample, and (3) a sediment sample collected near the outfall were conducted. The second objective of the reconnaissance trip was to attempt to limit the number of parameters necessary to characterize future samples with respect to their biological significance and potential toxicity.

Three types of samples were collected for chemical characterization during the reconnaissance trip: one wastewater effluent sample, three receiving water samples (one ambient and two plume samples), and one sediment sample.

The effluent composite sample (Outfall 101, process water) consisted of automatically composited subsamples and manual grab samples taken according to methods published in the Federal Register, Volume 45 (1980), Number 98 for NPDES outfall sampling. The effluent sample was collected over a 24-hour period. An ISCO automatic sampler set to sample 400 milliliters (ml) every 15 minutes was used to collect a 24-hour composite for all chemical fractions except cyanide, volatile organics, and hydrocarbons. These three fractions were manually collected composites (one-fourth of each sample was collected every 6 hours).

The receiving water samples (one ambient and two plume samples) were grab samples; the various fractions were preserved similarly to the effluent sample. The sediment sample was taken with a Ponar® grab, and the sample was iced for preservation.

The sampling locations were:

- Effluent: Sample taken from the NPDES sampling point (sump) for Outfall 101 (process water).
- Plume 1: Sample taken next to outfall, Station 1EBB of physical characterization study (Figure 3-1)
- Plume 2: Sample taken from Station 1FL00D of the physical characterization study (Figure 3-1).
- Ambient: Sample taken from the York River, upstream and across the river from the Amoco refinery. The exact sampling point was below the Highway 17 bridge, at Gloucester Point. This was the dilution water source for bioassay.
- Sediment: Sample taken from near the outfall pipe (near plume 1 sample, Figure 3-1).

The analytical methods utilized in the analyses of the samples are listed in Tables 3-1 through 3-3. The methods used in the analyses of the water samples were EPA-approved methods, while the methods for the analyses of the sediment sample utilized modifications of the

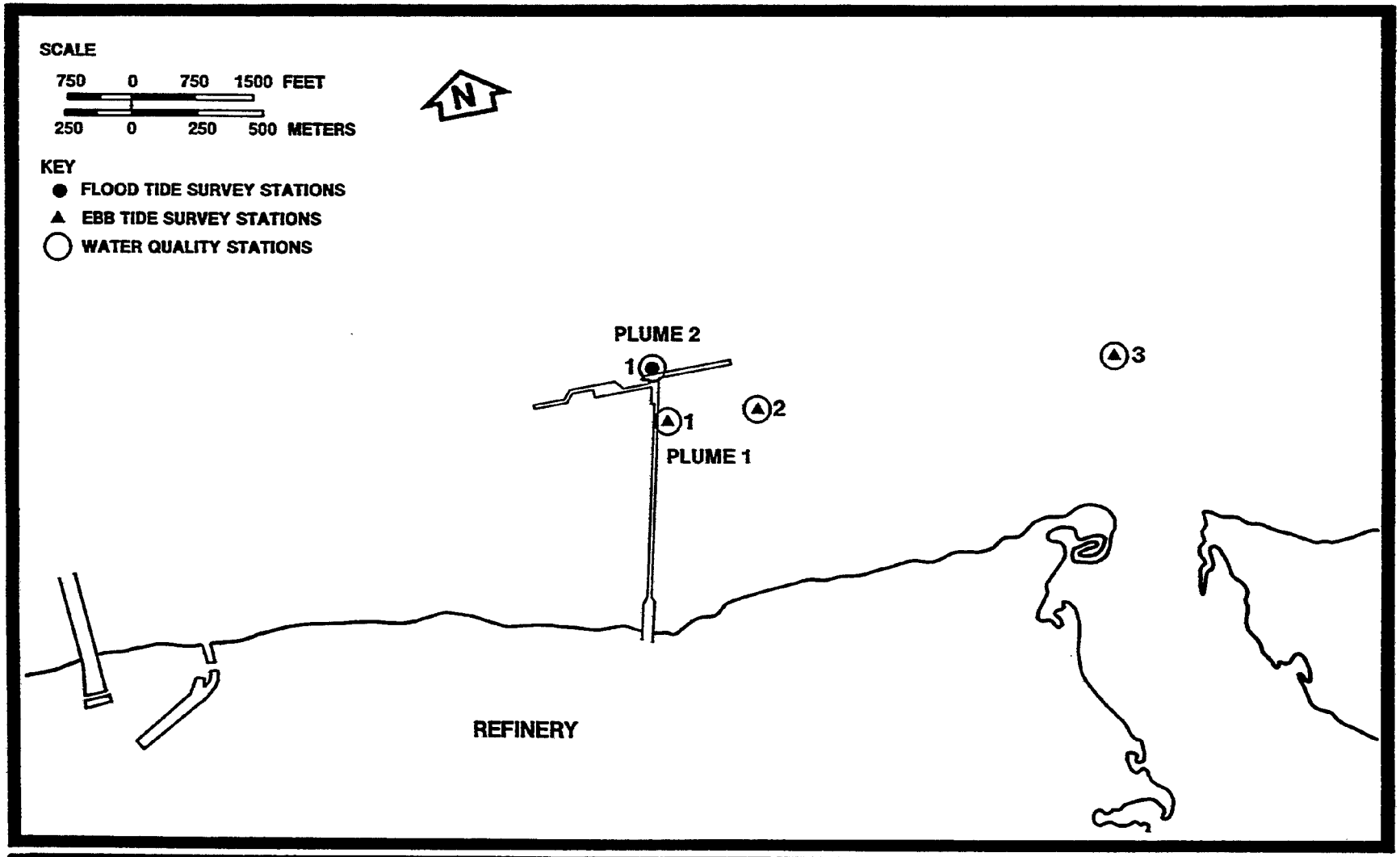

Figure 3-1
VERTICAL DYE AND WATER QUALITY STATION LOCATIONS FOR FLOOD AND EBB TIDE SURVEYS ON MARCH 9, 1982, IN THE YORK RIVER, VIRGINIA

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Table 3-1. Phase I Analytes and Methods for the Chemical Characterization of Wastewater

Analytes	Methods Utilized
A. Traditional Analyses	
Biochemical Oxygen Demand (BOD)	BOD Method* (No. 507)
Chemical Oxygen Demand (COD)	COD Method† (No. 410)
Total Organic Carbon (TOC)	TOC Method † (No. 415.1)
Total Suspended Solids (TSS)	TSS Method* (No. 209)
Ammonia	Ammonia Method* (No. 417)
Bromide	Bromide Method† (No. 320.1)
Sulfate	Sulfate Method† (No. 375.1)
Cyanides	Cyanide Method† (No. 335.3)
Temperature (Field)	Thermometric Method† (No. 170.1)
Dissolved Oxygen (Field)	Membrane Electrode Method† (No. 360.1)
pH (Field)	Electrometric Method† (No. 150.1)
B. Metals Analyses	
Aluminum	ICAP Method**
Barium	ICAP Method**
Boron	ICAP Method**
Cobalt	ICAP Method**
Magnesium	ICAP Method**
Manganese	ICAP Method**
Tin	ICAP Method**
Antimony	ICAP Method**
Chromium	ICAP Method**
Nickel	ICAP Method**
Thallium	ICAP Method**
C. Petroleum Hydrocarbon Analysis	Extraction and Capillary GC/MS
D. Volatile Compound Analyses	
Xylenes	EPA Method 624**
Benzene	EPA Method 624**
Toluene	EPA Method 624**
Ethyl Benzene	EPA Method 624**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches
E. Base/Neutral Compound Analyses	
Acenaphthene	EPA Method 625**
Acenaphthylene	EPA Method 625**
Anthracene	EPA Method 625**
Benzo(a)anthracene	EPA Method 625**

Table 3-1. Phase I Analytes and Methods for the Chemical Characterization of Wastewater (Continued, Page 2 of 2)

Analytes	Methods Utilized
Benzo(a)pyrene	EPA Method 625**
3,4-Benzo-fluoranthene	EPA Method 625**
Benzo(ghi)perylene	EPA Method 625**
Benzo(k)fluoranthene	EPA Method 625**
Bis(2-chloroethoxy)methane	EPA Method 625**
Chrysene	EPA Method 625**
Dibenzo(ah)anthracene	EPA Method 625**
Fluoranthene	EPA Method 625**
Fluorene	EPA Method 625**
Naphthalene	EPA Method 625**
Phenanthrene	EPA Method 625**
Pyrene	EPA Method 625**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches
F. Acidic Compound Analyses	
Cresols	EPA Method 625**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches

Sources: ESE.
*APHA et al., 1980.
†EPA, Environmental Monitoring and Support Laboratory, 1983b.
**EPA, 1979a.

Table 3-2. Phase I Analytes and Methods for the Chemical Characterization of Receiving Water Samples

Analytes	Methods Utilized
Level 1. Routine Field Parameters	
Temperature	Thermometric Method† (No. 170.1)
Dissolved Oxygen	Membrane Electrode Method† (No. 360.1)
pH	Electrometric Method† (No. 150.1)
Conductivity	Specific Conductance Method† (No. 120.1)
Level 2. Intensive Analysis	
A. Traditional Analyses	
BOD	BOD Method* (No. 507)
COD	COD Method† (No. 410)
TOC	TOC Method† (No. 415.1)
TSS	TSS Method* (No. 209)
Ammonia	Ammonia Method* (No. 417)
Bromide	Bromide Method† (No. 320.1)
Sulfate	Sulfate Method† (No. 375.1)
Cyanides	Cyanide Method† (No. 335.3)
B. Metals Analyses	
Aluminum	ICAP Method**
Barium	ICAP Method**
Boron	ICAP Method**
Cobalt	ICAP Method**
Magnesium	ICAP Method**
Manganese	ICAP Method**
Tin	ICAP Method**
Antimony	ICAP Method**
Chromium	ICAP Method**
Nickel	ICAP Method**
Thallium	ICAP Method**
C. Petroleum Hydrocarbon Analysis	Extraction and Capillary GC/MS
D. Volatile Compound Analyses	
Xylenes	EPA Method 624**
Benzene	EPA Method 624**
Toluene	EPA Method 624**
Ethyl Benzene	EPA Method 624**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches

Table 3-2. Phase I Analytes and Methods for the Chemical Characterization of Receiving Water Samples (Continued, Page 2 of 2)

Analytes	Methods Utilized
E. Base/Neutral Compound Analyses	
Acenaphthene	EPA Method 625**
Acenaphthylene	EPA Method 625**
Anthracene	EPA Method 625**
Benzo(a)anthracene	EPA Method 625**
Benzo(a)pyrene	EPA Method 625**
3,4-Benzo-fluoranthene	EPA Method 625**
Benzo(ghi)perylene	EPA Method 625**
Benzo(k)fluoranthene	EPA Method 625**
Bis(2-chloroethoxy)methane	EPA Method 625**
Chrysene	EPA Method 625**
Dibenzo(ah)anthracene	EPA Method 625**
Fluoranthene	EPA Method 625**
Naphthalene	EPA Method 625**
Phenanthrene	EPA Method 625**
Pyrene	EPA Method 625**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches
F. Acidic Compound Analyses	
Cresols	EPA Method 625**
Identify Other Major Unknown Peaks	Manual and Computerized Library Searches

Sources: ESE.
*APHA et al., 1980.
†EPA, Environmental Monitoring and Support Laboratory, 1983b.
**EPA, 1979a.

Table 3-3. Phase I Analytes and Methods for the Chemical Characterization of a Sediment Sample Taken Near the End of Outfall 001 Pipe

Analytes	Methods Utilized
Volatiles	
Benzene	Modified EPA Method 624*
Xylenes (total)	Modified EPA Method 624*
Toluene	Modified EPA Method 624*
Ethylbenzene	Modified EPA Method 624*
Extractables	
Acenaphthene	Modified EPA Method 625*
Acenaphthylene	Modified EPA Method 625*
Anthracene	Modified EPA Method 625*
Benzo(A)anthracene	Modified EPA Method 625*
Benzo(A)pyrene	Modified EPA Method 625*
Benzo(B)fluoranthene	Modified EPA Method 625*
Benzo(K)fluoranthene	Modified EPA Method 625*
Benzo(g,h,i)perylene	Modified EPA Method 625*
Bis(2-chloroethoxy)methane	Modified EPA Method 625*
Chrysene	Modified EPA Method 625*
Dibenzo(a,h)anthracene	Modified EPA Method 625*
Fluoranthene	Modified EPA Method 625*
Fluorene	Modified EPA Method 625*
Naphthalene	Modified EPA Method 625*
Phenanthrene	Modified EPA Method 625*
Pyrene	Modified EPA Method 625*
Cresols (total)	Modified EPA Method 625*
Metals	
Antimony	ICAP Method*
Chromium	ICAP Method*
Thallium	ICAP Method*
Nickel	ICAP Method*
Aluminum	ICAP Method*
Barium	ICAP Method*
Boron	ICAP Method*
Cobalt	ICAP Method*
Magnesium	ICAP Method*
Manganese	ICAP Method*
Tin	ICAP Method*
Traditionals	
Oil and Grease	Method 739 Oil and Grease in Sediments
Moisture Content	EPA Method for Moisture Content**

Sources: ESE.
*EPA, 1979a.
†EPA, 1979b.
**EPA, 1969.

EPA-approved methods. These modifications involved changes in the sample workup. The volatiles were analyzed by taking an aliquot of the sediment and adding 5 ml of organic-free water in the purging chamber. The sample was then analyzed as described in EPA Method 624. The extractable fractions were prepared by taking an aliquot of the sediment, adding 100 ml of organic-free water, adjusting the pH to 12, and serially extracting three times utilizing methylene chloride and a Polytron Tissumizer®. This base/neutral fraction was then concentrated and analyzed according to EPA Method 625. Another 100 ml of organic free water was added to this same sediment aliquot, adjusted to pH 2, and serially extracted as described for the base/neutral fraction. This acid extract was then concentrated and analyzed according to EPA Method 625. The metals analysis utilized a nitric acid digestion of the sediment followed by analysis by ICAP. The oil and grease determination was by a freon Soxhlet extraction procedure followed by gravimetric measurement. The moisture content of the sediment was done by standard EPA methods utilizing a weight difference after drying the sample.

3.3.3 Phase II.

At the conclusion of the reconnaissance phase, the data were reviewed by ESE and API personnel. A list of parameters was developed to help define the area of impact of the plume. Based on the plume characterization results, a sampling grid was established and 60 sediment samples were taken (September 1983) to provide data from the anticipated zone of impact. The parameters selected included 7 metals, total organic extractables (TOE), and petroleum hydrocarbons. The specific parameters are listed in Table 3-4. Each of these parameters was selected on the basis of its occurrence in the reconnaissance survey sample and its presumed utility in helping to trace pollutant accumulation areas in sediments of the receiving water body. For example, manganese occurred at a much higher concentration in the reconnaissance survey effluent sample then in the ambient water sample. It was, therefore, analyzed in subsequent samples to attempt determination of its fate after discharge and its areas of accumulation, if they exist. Because the primary

Table 3-4. Analytes for the Chemical Characterization of the Sediment Samples Taken During Phase II of the Study

Analytes
Moisture Content
Total Extractable Organics
Total Petrogenic Hydrocarbons
Aliphatic Petrogenic Hydrocarbons
Aromatic Petrogenic Hydrocarbons
Unresolved Petrogenic Hydrocarbons
Total Petrogenic Hydrocarbons, Including Unresolved
Cadmium
Chromium
Lead
Manganese
Selenium
Vanadium
Zinc

Source: ESE.

purpose in this effort was to differentiate between zones that have been impacted by the refinery discharge and zones that have not been impacted, similar reasoning was used for selection of the remaining analytical parameters. They all either were found to occur in higher concentrations in the effluent than in ambient receiving waters or were presumed to present that potential by virtue of their occurrence in refinery raw materials or process schemes. In addition, one sample from Outfall 001, three from Outfall 101, one from Outfall 201, and one from the plume and ambient waters were collected from June through September 1982. These samples were analyzed for parameters listed in Table 3-5.

The methods utilized in the analyses of the outfalls, plume, and ambient water samples were the same as those outlined in Table 3-2. The methods utilized to analyze the metals in the 60 sediments are the same as those outlined in Table 3-3. The methodology used to determine the TOE petroleum hydrocarbons in the sediments involved extraction with freon and final measurement using infrared (IR) spectroscopy. The method is based upon the EPA oil and grease method (EPA Method 413.2) and the American Society for Testing and Materials (ASTM) method for oil and grease and petroleum hydrocarbons in water (ASTM-03921-80).

The ASTM method defines oil and grease as that matter which is extractable by freon and measured by IR absorption (i.e., TOEs). Petroleum hydrocarbons are also defined by the ASTM as that portion of the oil and grease which is not adsorbed by silica gel.

The sediment extraction procedure for TOE and petroleum hydrocarbons involved acidifying the sediment, drying it with magnesium sulfate monohydrate, and extraction with freon in a Soxhlet apparatus for 4 hours at 20 cycles/hour. The extract was filtered through anhydrous sodium sulfate and analzyed by IR at 2,930 cm^{-1}, which is the characteristic absorption band for the carbon-hydrogen bond. This measurement determines the presence of TOE. Silica gel was then added to the extract to remove any adsorbable polar compounds. The IR

Table 3-5. Analytes for the Chemical Characterization of Effluents and Receiving Water Samples Collected During Phases II and III of the Study

Analytes	
Dissolved Oxygen	Manganese
pH	Nickel
Temperature	Selenium
Carbon, TOC	Vanadium
Salinity	Oil and Grease
Ammonia	Zinc
TSS	Chromium

Source: ESE.

measurement was repeated to yield the aliphatic petroleum hydrocarbons. The IR was then changed to absorption band 3,050 cm^{-1}, calibrated, and utilized to measure the aromatic petroleum hydrocarbons in the samples.

The IR method as described above achieved detection limits of 20 micrograms per gram (ug/g) for the aliphatic petroleum hydrocarbons and 200 ug/g for the aromatic petroleum hydrocarbons. Since only 1 of the 60 samples analyzed had detectable levels of petroleum hydrocarbons, an alternate procedure capable of achieving lower detection limits was subsequently utilized.

The subsequent procedure used consisted of concentrating the silica gel treated extract and then analyzing the concentrated extract by capillary gas chromatography utilizing a flame ionization detector (GC/FID). This method is capable of achieving detection limits of less than 0.1 ug/g for aliphatic and aromatic petroleum hydrocarbons.

3.2.4 Phase III

Phase III sampling was conducted in May 1983. One effluent sample was taken from Outfall 101. The 60 sediment stations were again sampled, but only 30 samples were analyzed. The 30 samples selected for analysis were the same 30 stations used in the benthic study. A detailed discussion of station selection is presented in the Results and Discussion section of this report.

The Outfall 101 sample was analyzed according to the methods outlined in Table 3-2. The 30 sediments were analyzed for TOE by the IR method described in Phase II (Section 3.3). The extracts from the TOE analyses were treated with silica gel, concentrated to 1 milliliter (ml), and analyzed by GC/FID, as discussed in Phase II. The IR method was utilized for TOE only and not for the petroleum hydrocarbon data. The metals were acid digested and analyzed by ICAP as outlined in Table 3-3.

3.3 FIELD BIOLOGY STUDIES

3.3.1 Benthos

Two field efforts for the collection of York River benthos samples were conducted (September 1982 and May 1983). Stations were located using a DECCA Trisponder® Navigation System (station coordinates are included in Appendix B). During each field effort, 60 stations were sampled by collecting five replicate petite Ponar® (225 cm^2 each) grab samples at each station. Samples were sieved in the field using a 0.5-millimeter (mm) mesh screen and preserved in 10-percent buffered formalin containing Rose Bengal stain. Of the 60 stations sampled, 30 stations were selected for analysis of benthos, based on the silt, hydrocarbon, and metals content of the sediments. Selection criteria are discussed in the results and discussion sections of the report.

From each of the 30 stations selected for analysis, three of the five replicate grab samples collected were completely sorted and the taxa identified to the species level, whenever possible. The standard error as a percent of the mean was calculated for the three replicate samples at each station as an index of precision (Elliott, 1977), with the standard error calculated by the equation presented by Downing (1979). The standard error of the mean indicates the amount of error in the sample mean when it is used to estimate the population mean. The ratio of standard error to sample mean is an index of precision and can be used to calculate the number of samples necessary to obtain estimates of the population mean within a particular range of precision (Elliott, 1977). Statistically reliable estimates of benthos populations may be obtained with a standard error equal to 20 percent of the mean (Elliott, 1977) to 30 percent of the mean (Dauer et al., 1979).

The number of taxa and mean density of individuals were plotted against station, with stations arranged from the lowest to highest sediment hydrocarbon concentration. As a comparison of the qualitative distributions of taxa, a presence-absence matrix of taxon occurrences

was constructed with stations arranged in ascending sediment hydrocarbon concentration.

The Shannon-Weaver diversity index (Shannon and Weaver, 1963) was calculated for each benthos collection at a station. These data were compared with the diversity index calculated in previous studies conducted in the York River.

Normal cluster analyses were performed on faunal density data collected in September 1982 and May 1983 using the CLUSTAN computer package (Wishart, 1982). The Bray-Curtis dissimilarity coefficient was used to measure the resemblance between stations. This coefficient is calculated as:

$$D_{jk} = \frac{\Sigma_1 \left| X_{ij} - X_{ik} \right|}{\Sigma_1 (X_{ij} + X_{ik})}$$

where: X_{ij} is the density of species i in the jth collection, and

X_{ik} is the density of species i in the kth collection.

All density data were log transformed [X = log (X + 1)] and clustered using group average sorting.

Statistical analyses were performed using the Statistical Analysis System (SAS) (1982). The hypothesis tested was that there was no significant difference in benthos densities due to sediment hydrocarbon concentrations. To test the hypothesis, linear regressions were computed using mean benthos density per station (individuals/m^2) as the dependent variable and total sediment hydrocarbon content (ug/g) as the independent variable. All density data were log transformed [x = log (x + 1)] to stabilize the variance or remove dependence of variance on the means.

Linear regressions were also computed using log transformed mean densities of individual taxa as the dependent variable and total

sediment hydrocarbon content as the independent variable. Only taxa occurring in at least 20 of the 30 stations were used in these regressions.

The hypothesis that there was no significant difference in benthos densities due to sediment metals content was also tested by regression. Stepwise regressions were computed using log transformed benthos densities as the dependent variable and sediment metals content (mg/kg dry weight) as the independent variables. Metals used as independent variables were cadmium, chromium, lead, manganese, selenium, and vanadium.

3.3.2 Artificial Substrates (Colonization Samplers)

Artificial substrates were deployed in the York River at nine locations on May 18, 1983 (see Figure 3-2). Five locations (designated A through E) were at the Amoco refinery pier, two locations (F, G) were approximately 500 and 1,000 meters upstream of the pier in approximately 4.5 m of water, and two locations (H, I) were 500 and 1,000 m downstream of the pier in approximately 4.5 m of water. Two deployment methods were used for artificial substrates. Racks at Locations A through E were bolted directly to pilings in the refinery pier. These racks (see Figure 3-3) consisted of frames made of two-by-fours with PVC pipe extending down into the water. The artificial substrates, 15 x 7.5 cm Plexiglas plates, were attached in a vertical position at the bottom of the PVC pipe. Each rack consisted of six PVC pipes, each holding two Plexiglas plates. Racks at Locations F through I were suspended in the water column. These racks consisted of PVC frames hung from floats and anchored at each end. Six plates were attached to each side of these racks in a vertical position. All artificial substrates were submerged to approximately 1.5 m at low tide.

Two replicate plates chosen by random number were retrieved from each location at approximately monthly intervals (Time Series Panels). Plates were retrieved by unbolting the PVC pipe to which they were

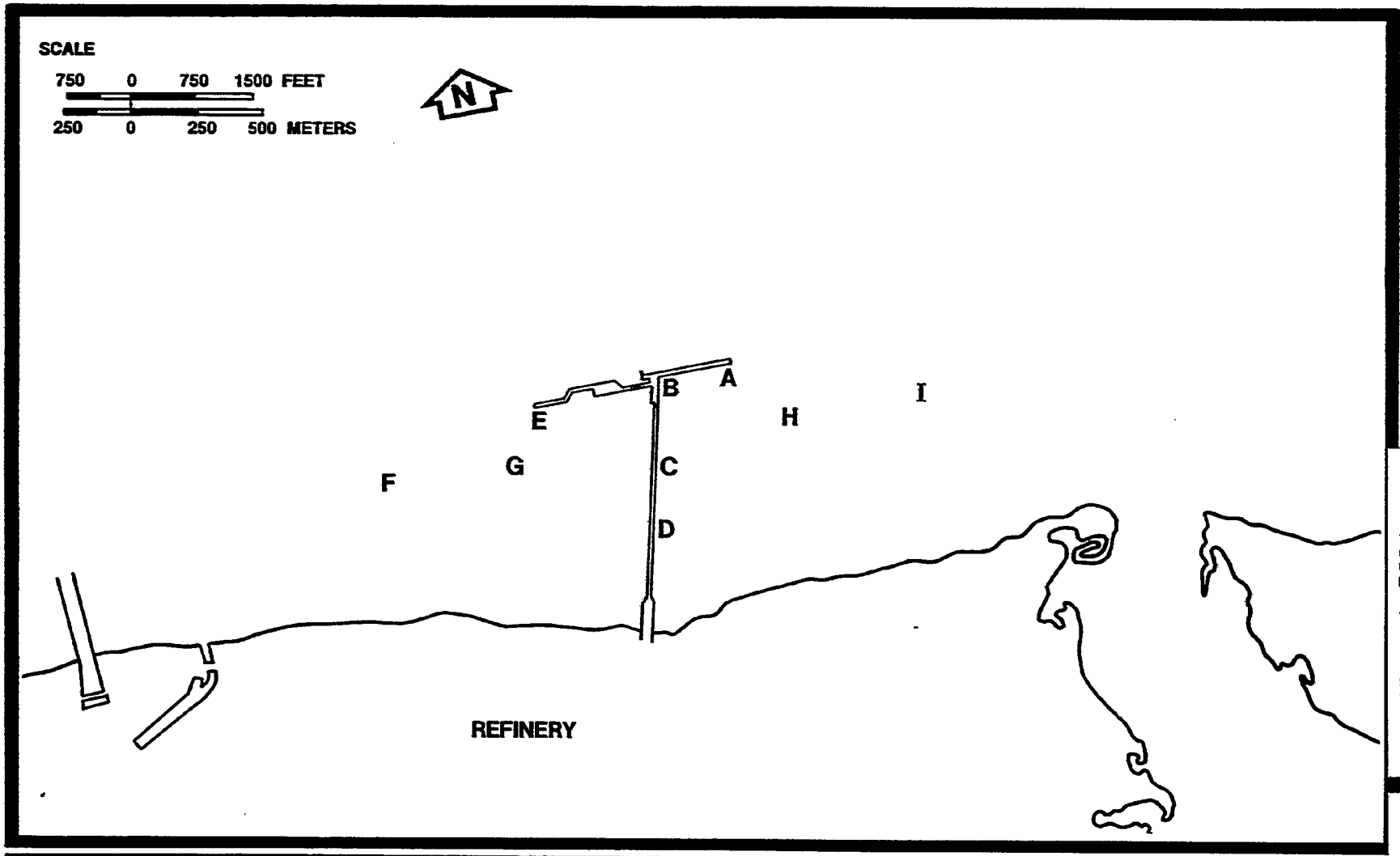

Figure 3-2
LOCATION OF FOULING PANELS

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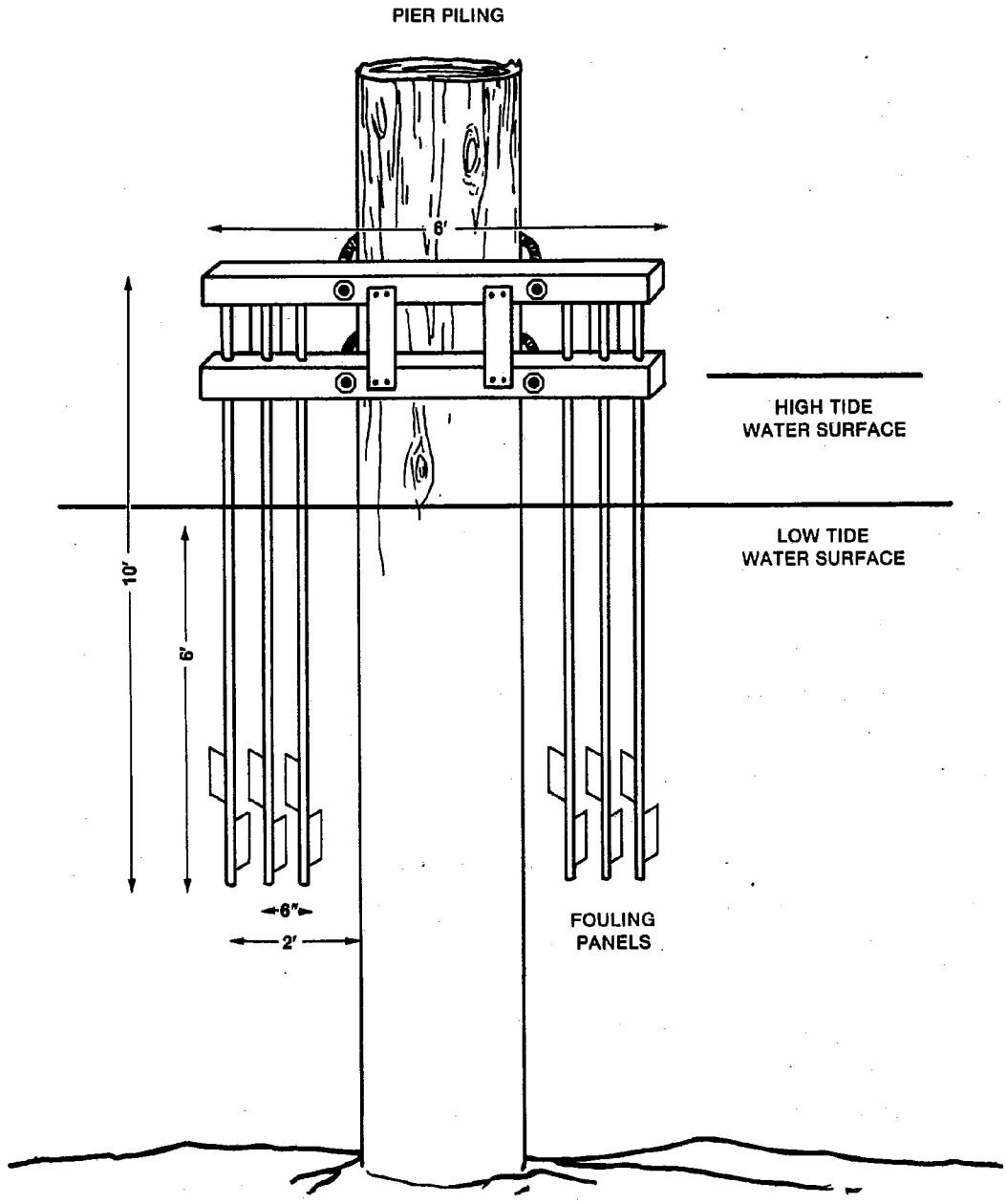

Figure 3-3
PIER-ATTACHED FOULING PANELS

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attached (see Figure 3-3), lifting the pipe to the surface, and unfastening the plates. Dean (1981) cited data which indicated abundances of most species are unaffected by lifting artificial substrates out of the water as opposed to bagging them underwater. In addition, two replicate plates were deployed at each location when plates were retrieved, and were picked up 1 month later (Monthly Recruitment Panels). The retrieved plates were placed in separate sample bags, labeled, and then placed in gallon jars which contained a 10-percent buffered seawater and formalin mixture. Plate deployment and retrieval dates are listed in Table 3-6.

Water temperature and salinity were measured on each collection date. Measurements were made at the depth of the artificial substrates.

Artificial substrates were examined both for primary fouling species and secondary fouling species. Primary foulers are those species which attach directly to the substrate and include organisms such as barnacles, tunicates, hydroids, and ectoprocts. Primary fouling species may be either solitary or colonial organisms. Secondary fouling species are those which live among the primary fouling species. They may be either motile or sedentary and include taxa such as polychaetes, amphipods, isopods, copepods, gastropods, and flatworms.

Primary Fouling Species--Percent cover of primary fouling species was estimated by recording species that occurred at each of 50 randomly selected points on the surface of the artificial substrate. Points placed on clear Plexiglas plates were superimposed over the artificial substrates, and animals occurring under the points were identified and counted. Only the area between the attachment straps was censused because the physical structure created by the straps induces an additional variability to the plates. The area censused on each plate measured 13 x 7.5 cm [97.5 square centimeters (cm^2)].

Table 3-6. Schedule of Artificial Substrate Deployments and Retrievals

Trip No.	Deployment Date	Retrieval Date
Time Series Panels		
1	May 15, 1983	June 18, 1983 (A,H,I) July 2, 1983 (B,C,D,E,F,G)
2	May 15, 1983	August 3, 1983
3	May 15, 1983	September 5, 1983
4	May 15, 1983	October 7, 1983
5	May 15, 1983	November 9, 1983
6	May 15, 1983	December 16, 1983
Monthly Recruitment Panels		
1	June 18, 1983 July 2, 1983	August 3, 1983 (A,H,I) August 3, 1983 (B,C,D,E,F,G)
2	August 3, 1983	September 5, 1983
3	September 5, 1983	October 7, 1983
4	October 7, 1983	November 9, 1983
5	November 9, 1983	December 16, 1983

Source: ESE.

Sutherland and Karlson (1977) found 75 random points adequate to estimate cover in plates which measured 225 cm^2, while Mook (1980) used 80 random points on panels measuring 225 cm^2. To estimate the number of points necessary to obtain adequate estimates of percent cover for panels in this study, replicate counts were conducted on a panel using densities of 40, 50, and 75 random points (Table 3-7). Inspection of estimates of percent cover (Table 3-7) revealed only slight variation in estimates between the three densities. Mann-Whitney U tests between sample pairs showed no significant differences in the counts between dot densities. The 50-dot density was chosen for both processing time considerations and ease of calculation of percent cover. Once the 50-dot density was chosen, all percent-cover estimates for each plate were made using three replicate counts with three different dot patterns, and percent cover was reported as the mean of the replicate estimates. New dot patterns were used each collection period.

Secondary Fouling Species--Secondary fouling species are those species living among the primary foulers but not attached directly to the substrate. To estimate numbers of secondary foulers, plates were scraped cleaned. The material and organisms removed from each plate were concentrated on a 100-micron (u) mesh screen. Since there were so many individual organisms in the material scraped off the plates, the entire sample could not be processed in a reasonable amount of time, and it was necessary to subsample the secondary fouler taxa. Subsampling was accomplished by placing the plate scrapings in a Matoda plankton splitter and splitting the material to a point where the subsample could be sorted in a 30-minute period.

To provide reliable estimates of the complete sample, a subsample must be drawn from a randomly distributed population. To test whether the organisms were randomly distributed in the plankton splitter and subsampling was valid, a chi-square test was used to check for agreement with a Poisson series (Elliott, 1977). Five replicate 1/64 splits

Table 3-7. Percent Cover of Primary Fouling Species for Replicate Counts at Three Random Dot Densities

Number of Dots	Taxa	Percent Cover Rep. 1	Rep. 2	Rep. 3	$\bar{X}$
40	Free Space	0.0	5.0	N.D.	2.5
	Balanus spp. (live)	57.5	57.5	N.D.	57.5
	Molgula manhattensis	2.5	5.0	N.D.	3.75
	Balanus/Membranipora	5.0	0.0	N.D.	2.5
	Balanus spp. (dead)	17.5	20.0	N.D.	12.5
	Membranipora spp.	0.0	0.0	N.D.	0.0
	Balanus/Stylochus	17.5	12.5	N.D.	15.0
50	Free Space	2.0	4.0	4.0	3.3
	Balanus spp. (live)	58.0	46.0	58.0	54.0
	Molgula manhattensis	8.0	4.0	4.0	5.3
	Balanus/Membranipora	2.0	8.0	4.0	4.6
	Balanus spp. (dead)	14.0	26.0	20.0	20.0
	Membranipora spp.	0.0	2.0	0.0	0.7
	Balanus/Stylochus	16.0	10.0	10.0	12.0
75	Free Space	8.0	5.3	1.3	4.9
	Balanus spp. (live)	50.7	54.7	53.3	52.9
	Molgula manhattensis	4.0	5.3	8.0	5.8
	Balanus/Membranipora	1.3	8.0	6.7	5.3
	Balanus spp. (dead)	20.0	12.0	16.0	16.0
	Membranipora spp.	1.3	2.7	2.7	9.8
	Balanus/Stylochus	14.7	12.0	12.0	12.9

N.D. = No data; only 2 replicates counted.

Source: ESE.

(split necessary for 30-minute sorting time) from one panel were sorted, the organisms identified and enumerated, and the chi-square test applied. Since the five replicate subsamples agreed with a Poisson series at the 95-percent probability level, it was concluded that the organisms were randomly distributed in the plankton splitter before subsampling, and therefore, the subsampling procedure was valid; one subsample was therefore considered adequate to estimate numbers of individuals per plate (Elliott, 1977). One subsample was then analyzed for all subsequent plate samples.

All plates could not be analyzed using the same split because organisms in some samples were much more plentiful than in others. To allow comparison of organism numbers between plates, the split was multiplied by an appropriate factor to bring the numbers of organisms in a subsample to numbers of organisms per plate. For example, if a 1/64 split was analyzed, all taxa counts were multiplied by 64 to give the number of individuals per plate.

Data Analysis--To compare panels, numbers of primary fouler and secondary fouler taxa were plotted versus collection date. As a further comparison of panels, mean percent cover of taxa greater than or equal to 10 percent of the cover on any date was plotted versus collection date. Compound taxa identifications created to gain information on panel structure were combined by the predominant taxa for the percent cover plots. As an example, Sabella microphthalma and Sabella/Membranipora would be plotted as S. microphthalma. Sabella/Membranipora indicates that Sabella covered the plate and had Membranipora encrusting the tubes.

Presence/absence matrices were constructed to show the qualitative distributions of secondary fouling taxa across panel locations.

The hypothesis that there was no significant difference in the community which colonized artificial substrates in relation to location around the

refinery discharge was tested by one-way analysis of variance and Duncan's multiple range test. These tests were run separately for primary fouling taxa and secondary fouling taxa, since the units of measurement were not the same. The tests were also run separately for each collection period to eliminate differences at a location due to collection date.

Statistical analyses of primary fouling taxa were run using log transformed dot counts of primary foulers as the dependent variable. Tests on secondary fouling taxa used the estimated mean numbers of individuals per plate as the dependent variable. These data were log transformed.

Primary and secondary fouling taxa data were log transformed to meet the assumptions of equality of variance and independence of variance from the means. An examination of plots of the standard deviation versus the mean for primary and secondary fouler taxa indicated that variances were equal and independent of the means.

3.4 BIOASSAYS

Bioassays were conducted to evaluate the possible toxicity of Amoco refinery effluents by using available EPA guidelines and the most updated methods available. Information from all other project tasks was utilized in the bioassay predictions of environmental impact.

Aquatic toxicity tests were conducted on Amoco Oil Refinery process water (Outfall 101) from March 7 through March 12, 1982 (reconnaissance study), from June 5 through July 15, 1982, and in May 1983. Additional toxicity tests were conducted on non-contact cooling water (Outfall 201) and on the combined final discharge (Outfall 001). The NPDES sampling point for treated process water is Outfall 101. There is no NPDES monitoring at Outfall 001. Under normal operating conditions Outfall 101 represents from 1 to 5 percent of the final effluent (Outfall 001).

The Amoco process effluent consists of adulterated freshwater that is discharged into an estuarine (saltwater) environment. Therefore, the problem exists of whether to evaluate the effluent toxicity using freshwater or saltwater species. Current EPA thinking is to use aquatic animals that could be affected by the discharge, hence saltwater animals were used. EPA-standard estuarine test species at the time of this study included the mysid shrimp (Mysidopsis bahia) and the sheepshead minnow (Cyprinodon variegatus). Both were tested to evaluate the possible effluent toxicity. To test saltwater animals in a freshwater effluent, seasalts were added to the static high effluent concentrations to avoid mortality of test species due to ionic inbalance.

Ninety-six hour flow-through tests were conducted as part of the reconnaissance phase of this study to determine possible toxic effects of the process water (Outfall 101) on mysid shrimp (Mysidopsis bahia) and sheepshead minnow (Cyprinodon variegatus).

Based on the reconnaissance results, definitive static and flow-through acute test, and a chronic life cycle test, were conducted using mysid shrimp. Mysid shrimp were chosen for the chronic test due to their higher sensitivity to the effluent than the sheepshead minnows. The chronic mysid life cycle test monitored growth and reproductive success of the test animals exposed to Amoco's process water. Additional static toxicity tests were also conducted on the sheepshead minnows. Reference toxicant tests using sodium dodecyl sulfate (SDS) were conducted to assess the sensitivity of the test species. Furthermore, acute tests were conducted on two indigenous species collected from the York River, a grass shrimp (Palaeomonetes spp.) and a fish (Leiostomus xanthurus).

ESE's mobile bioassay laboratory was located in the Amoco waste treatment area next to the Outfall 101 sump. Electricity and tapwater hook-ups were provided by Amoco personnel.

No NPDES bioassay toxicity limitations were in effect for Amoco at the time of testing. Previous bioassay tests were conducted by Monsanto Research Corporation (Monsanto) and are discussed in the following section.

3.4.1 Background

Previous toxicity testing was conducted on Amoco wastewater by Monsanto's Dayton Laboratory (Wilson et al., 1981), under contract to EPA, Chemical Processes Branch, Industrial Environmental Research Laboratory. The objective of the Monsanto project was to conduct an in-depth study of the Chesapeake Bay basin to assess factors adversely affecting water quality of the bay. Their objective was partially achieved through the performance of aquatic bioassays that supplied data on the acute toxicity and mutagenicity of 10 wastewater outfalls, including the Amoco Yorktown Refinery. Aquatic toxicity tests were conducted using freshwater species [fathead minnows (Pimephales promelas) and daphnids (Daphnia magna)] and marine species [sheepshead minnows (Cyprinodon variegatus) and mysid shrimp (Mysidopsis bahia)].

The following test results were obtained by Wilson et al. (1981) for bioassays conducted on Amoco's process wastewater (Outfall 101):

1. Fathead Minnow Test (total length 30 mm): 96-hour LC50 greater than 100-percent wastewater.
2. Daphnia Test: 48-hour LC50 of 45-percent wastewater; 95-percent confidence interval of 37- to 56-percent wastewater.
3. Mysid Shrimp Test (6 to 8 days old): 40-percent mortality in 100-percent wastewater after 96 hours of exposure.
4. Sheepshead Minnow Test (total length 6 to 11 mm): 30-percent mortality in 100-percent wastewater after 96 hours of exposure.

For the saltwater species, the salinity of the effluents was adjusted to 20 ppt by adding Rila® marine salt mix. All tests were conducted under static conditions.

3.4.2 Toxicity Test Methods

All acute toxicity tests for the present ESE study were conducted according to Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms (Peltier, 1978). The mysid life cycle chronic test was conducted according to Draft 2 ASTM Mysid Methodology, January 1982.

According to Peltier (1978), EPA specifications for physical and chemical parameters for industrial bioassays are as follows: (1) the test temperature should not vary more than ±2°C of the acclimation temperature (22°C for these tests), (2) the dissolved oxygen concentration in the test solution should not be permitted to fall below 40-percent saturation, and (3) no limitations are described by EPA methods for test alkalinity or pH.

3.4.3 Test Animals

Mysid shrimp (M. bahia) and sheepshead minnows (C. variegatus) were the laboratory-cultured species used in the onsite tests. These organisms

were cultured in ESE facilities from stocks originating from EPA laboratories, with one exception. Mysid shrimp used in the life cycle study were from a commercial supplier (Sea Plantations, Inc.). The mysid shrimp life cycle test was originally started using mysids cultured onsite from ESE stocks. After 24 hours of testing, it was discovered that the Nitex® mesh originally used in the test chambers was not small enough to contain the smallest mysids. Consequently, the youngest mysids escaped through the mesh and the test was invalidated. To restart the test immediately, 500 additional mysids less than 30 hours old were needed. Enough animals could not be obtained from the onsite cultures; therefore, mysids were ordered from ESE's commercial supplier, Sea Plantations, Inc. New chambers with smaller Nitex mesh were also made and soaked in flowing water for a couple of days before using.

Grass shrimp (Palaeomonetes spp.) and spot (Leiostomus xanthurus) were collected from an upstream relatively unpolluted area of the York River unaffected by the discharge. Bioassay tests were conducted with these indigenous species in order to assess the sensitivity of receiving water fauna. These tests were conducted in May 1983 (Phase III).

Young fish are usually more sensitive to toxicants than adults; therefore, sheepshead minnows used in the tests were less than 2 weeks old. Based on average growth rates in ESE cultures, 2-week-old minnows weigh less than 1.0 milligram (mg). Mysids used in acute tests were 7 to 14 days old. Based on ESE cultures, the weight of mysids of this age averages 0.94 mg. Mysids used in the life cycle study were less than 30 hours old. The field-collected grass shrimp were non-ovigerous adults, and the fish were juveniles.

Field testing conditions of laboratory-cultured species were similar to ESE culture conditions (i.e., temperature, photoperiod, and salinity). Marine test organisms were maintained onsite in ESE's laboratory at 20-ppt salinity. They were acclimated to test site dilution water for a

minimum of 24 hours by periodically replacing partial volumes of the transportation water with dilution water of the appropriate salinity. For acute tests, animals were acclimated to two salinities (14 and 18 ppt) so that no animals would be subjected to a differential of greater than 3 ppt when transferred to test solutions. During the reconnaissance study, the 56.0-percent effluent flow-through replicates were exposed to a 5 ppt salinity change. The brevity of this initial field study did not allow time to acclimate mysids to the most diluted saltwater concentration of 9 ppt. This was considered appropriate due to the screening nature of the tests during the reconnaissance period.

3.4.4 Physical Test Facilities

Flow-through tests were conducted using a solenoid-controlled, intermittent-flow proportional diluting system calibrated to provide a minimum of six volume turnovers of test solution per day (Figure 3-4). The number of diluter cycles and total elapsed time of diluter operation during the study were recorded by the diluter control system. The average turnover rate for the test was calculated from these values. Test chamber volume was 6.5 liters (L). Water depth in the test chambers cycled from 10 to 15 cm, with volume fluctuating concomitantly from 3.5 to 6.0 L. Minnows and mysids were contained within nylon screened (Nitex®) cylinders in test chambers. Aeration was not used. Test temperature was controlled by regulating laboratory ambient air temperature and by adjusting process and dilution water temperature with a constant temperature bath. Coils of stainless steel tubing in the bath received process water from the NPDES sampling point and dilution water from a 300-gallon fiberglass holding tank, respectively. Process and dilution water were supplied at protocol-prescribed temperatures to the diluting system.

Static mysid and minnow tests were conducted in 1,700-ml culture dishes; the volume of test solution was 1,000 ml. Aeration was not used. The temperature was controlled by regulating ambient air temperature in the laboratory. A 14-hour light/10-hour dark cycle was used for all acclimation and testing.

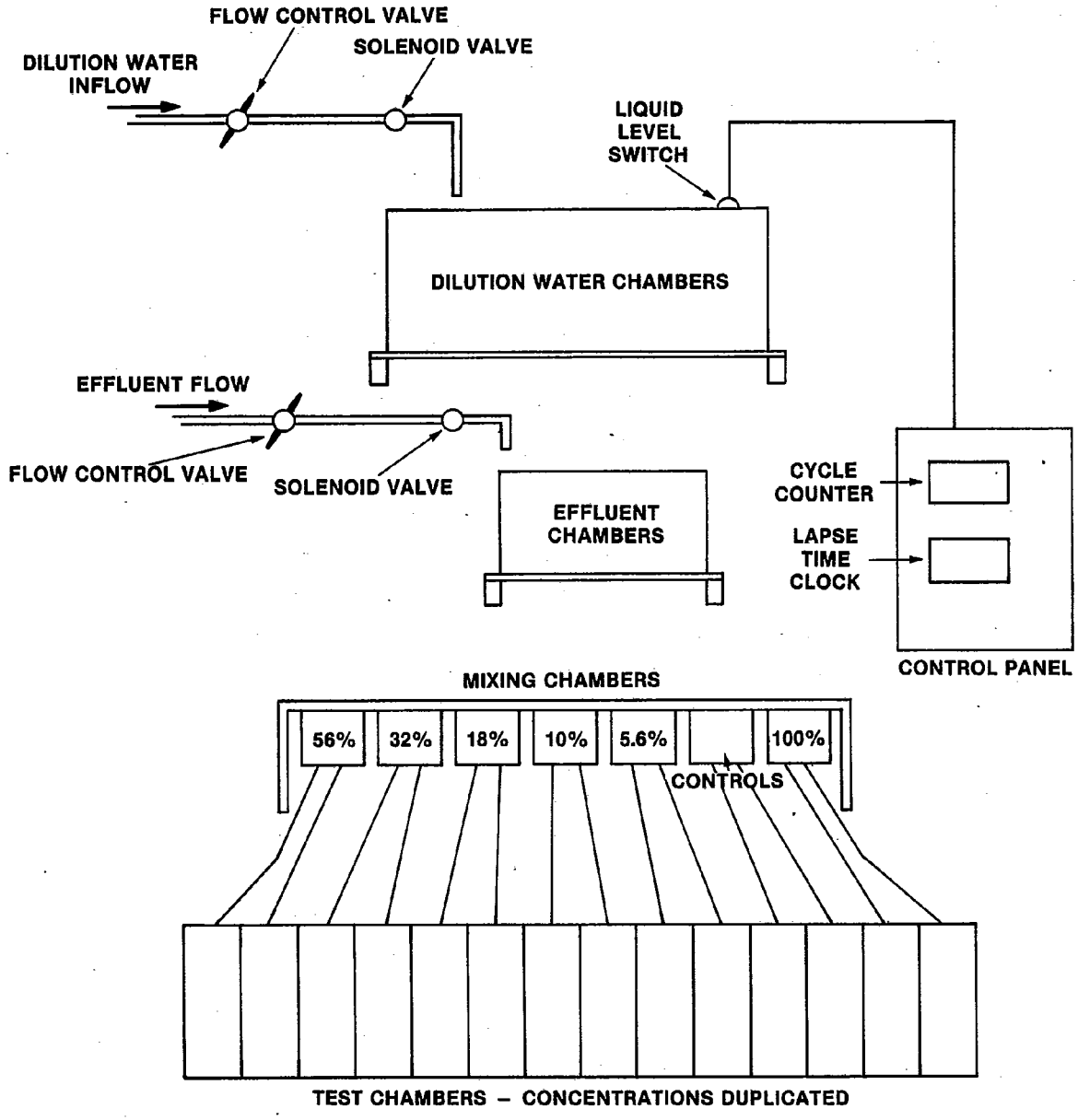

Figure 3-4
SCHEMATIC DIAGRAM OF SOLENOID-VALVE DILUTER SYSTEM

SOURCE: ESE (modified from Peltier, 1978).

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3.4.5 Loading Rates

The loading rate for each static test was determined by dividing the weight of the test animals in each test chamber by the volume of test solution in each chamber. Loading rates in flow-through tests were determined similarly, with one exception. When more than one species was tested, in separate mesh containers, in a common aquarium, the weight of animals of both species was summed. The minimum water volume at the low point of the fluctuating aquarium level cycle was used as the test solution volume. This summed weight divided by the minimum water volume per aquarium gave the maximum loading rate occurring any time during the test.

According to Peltier (1978), loading must not exceed 5 grams per liter (g/L) at temperatures of 20°C or less, or 2.5 g/L at temperatures above 20°C, for flow-through tests. Static test loading must not exceed 0.8 g/L at temperatures of 20°C or less, and 0.4 g/L at temperatures above 20°C. Loading for all tests was below allowed maximums.

3.4.6 Toxicity Tests

Static and flow-through acute and chronic tests were conducted on Amoco wastewaters. Wastewaters for testing were sampled from Outfall 101 (process water), Outfall 201 (non-contact cooling water), and Outfall 001 (final combined wastewater). For flow-through tests, a continuous sample was pumped with a Prosser® centrifugal pump through approximately 40 feet (ft) of Teflon® tubing to the mobile laboratory. At that point, flow was split for the diluter and for monitoring of chemical parameters. Static tests were conducted using 24-hour composites (collected with an ISCO® automatic sampler) or grab samples.

Diluent salt water was collected from Gloucester Point, an area west and across the river from the Amoco plant. Two hundred gallons of dilution water were collected for each day of testing. The chemical

characteristics of the dilution water are presented in Section 4.4 of this report.

All chemical monitoring probes were calibrated daily. All new tubing was used in the diluter. Test water supply systems were flushed for 24 hours prior to testing to allow for initial leaching of possible contaminants from pumps, hoses, tubings, diluter, and test chambers. This also allowed time for the test chamber solutions to chemically and thermally equilibrate. The diluting system was calibrated prior to test initiation and again at test conclusion; delivery rates of wastewater and dilution water to the dilution system were monitored and recorded at least twice daily. Flows were adjusted accordingly to ensure maintenance of constant flow rates during the test period.

Reference toxicant tests using the reference toxicant SDS (Fisher® 90-percent pure) were conducted as part of the quality assurance program for this study.

The following subsections discuss methods specific to the acute and life cycle tests.

Acute--

Chemical Monitoring

Wastewater was monitored continuously for dissolved oxygen prior to thermal or other adjustments. Salt water, used for dilution water, was monitored at time of collection for DO, temperature, pH, and salinity.

Flow-through test solutions were analyzed initially and again at 24, 48, 72, and 96 hours for DO and pH. Initial alkalinity and salinity were also recorded. One test chamber was also monitored continuously for temperature during testing. Static test solutions were analyzed initially and again at 24 and 48 hours for DO, temperature, and pH.

Tests Conducted

During the reconnaissance trip (March 1982), the following tests were conducted: one 96-hour acute flow-through test using mysid shrimp, one 96-hour acute flow-through test using sheepshead minnows, one 24-hour screening static test using daphnids, and two 24-hour reference toxicant (SDS) tests (mysids and minnows).

For the final phase of the study (June and July 1982), one 96-hour flow-through acute mysid test and one 48-hour flow-through sheepshead minnow test were conducted. Numerous static toxicity tests were conducted, including: twelve 48-hour acute toxicity tests on mysids, five 48-hour acute toxicity tests on sheepshead minnows, and three reference toxicant tests on mysids and sheepshead minnows.

Life Cycle Test--A life cycle test was conducted on process water (Outfall 101) using mysid shrimp (*M. bahia*) approximately 30 hours old. Life cycle test concentrations were chosen based on the results of all the flow-through acute tests conducted. The highest concentration was no greater than the concentration producing adverse effects in the acute tests. Based on this rationale, the life cycle concentrations were: 3.20-, 1,80-, 1.00-, 0.58-, 0.32-, 0.18-, and 0.00-percent wastewater.

Forty-eight mysids were tested per concentration, 24 per duplicate test chamber. To avoid crowding and to facilitate growth and survival observations, mysids from each concentration were distributed among six nylon-mesh retention cups (three cups per replicate chamber, eight mysids per cup). The cups in test chambers were labeled A, B, and D. Test organisms were fed once daily using brine shrimp (*Artemia* sp.) nauplii, less than 24 hours old. The duration of the test was 26 days, which complied with the method requirement (ASTM, 1982) that the test be conducted for 7 days past the average time of brood release in the controls.

Daily counts were made by placing each retention container on a light table. Survivors were recorded by sex, number, females with or without brood pouch, and all other pertinent information observed (such as behavior, brood appearance, etc.). Dead mysids were removed daily and, when possible, categorized by sex.

Juveniles dropped during testing were counted and removed daily. They were held in separate containers for observation of latent effects. Furthermore, reference toxicant tests were conducted on some of these juveniles to evaluate whether their sensitivity to SDS changed due to their exposure to the wastewater during their embryologic development. These reference toxicant tests were conducted on juveniles dropped in the controls, 1.0-, and 3.2-percent process water concentration chambers.

DO concentration and pH were measured periodically in each test chamber. Test temperature was continuously monitored. Test chambers were cleaned every other day by carefully siphoning all bottom debris. This debris was carefully checked for adult bodies and juveniles. Diluter flow rates were checked daily and adjusted periodically. All diluter delivery pipes and tubing, as well as solenoid valves, were cleaned and/or repaired as needed. The wastewater pump (placed in the sump) was pulled out for cleaning three times during testing.

3.4.7 Data Analysis

Acute Tests--Each test result is expressed as an LC50 value (that concentration at which 50 percent of the organisms die). Such values are reported, where possible, for each organism after 24, 48, 72, and 96 hours of exposure to the wastewater. Additionally, upper and lower 95-percent confidence limits (UCL and LCL) are reported for each data set.

A single set of LC50 values with confidence limits is reported for each 24-hour exposure period of each test. All results were calculated using

a computer-implemented Moving Average method (Dryer, 1980), the Probit Method (Finney, 1971), or the Binomial Test. The Moving Average method incorporates angular transformations of the mortality data and log-transformation of the wastewater concentration to improve the linearity of the mortality curve. When the data could not be calculated using the Moving Average method, then the Binomial Test was used.

The final values of results presented for each data set were selected according to the following criteria, ranked in order of priority:

1. A complete set of results obtainable (an LC50, an upper 95-percent confidence limit, and a lower 95-percent confidence limit), and
2. Calculations based on the maximum number of data points possible (a minimum of data discarded as aberrant).

Life Cycle Test--Computerized regression analyses and analyses of variance (ANOVA) were conducted on all life cycle data to quantify the "effect" and "no-effect" levels.

4.0 DISCIPLINE-SPECIFIC RESULTS AND DISCUSSION

4.1 PHYSICAL CHARACTERIZATION

4.1.1 Hydrographic and Water Quality Survey

In situ hydrographic and water quality data were collected throughout the water column at selected stations during the March 9 (spring tide) and June 14 and 15, 1982 (neap tide) field surveys. Appendix A contains data not presented in this section.

Current speed and direction were measured versus depth during the March survey; however, because of boat motion, the variability in currents due to time differences between stations, and interference caused by the refinery dock, the data were complex and not readily comparable with other stations. Generally, however, the currents measured, agreed with the tidal currents reported by Hyer *et al.* (1975). These currents were set up-estuary on a flood tide, down-estuary on an ebb tide, and with an average velocity of approximately 30 cm/s in either direction.

In situ conductivity, temperature, DO, and pH measurements were made during the March and June 1982 surveys. During the March survey, four stations were occupied (Figure 4-1) with the emphasis on high resolution with depth. The data are presented in Table 4-1.

The water temperature during March ranged from 6.6 to 5.4°C and showed a slight tendency to decrease with depth. The salinity ranged from 15 to 19 ppt. Generally, salinity increased with depth, but the shallower stations (EBB-2 and EBB-3) were nearly uniform with depth. Only FLD-1, with a bottom depth of 12 m, showed any pronounced salinity gradient. The DO saturations at all depths were almost entirely in excess of 100 percent (ranging from 98 to 114 percent). This was the result of winter conditions and the spring tides which had induced vertical mixing. The pH ranged from 7.3 to 7.8 and showed a slight tendency to increase with depth.

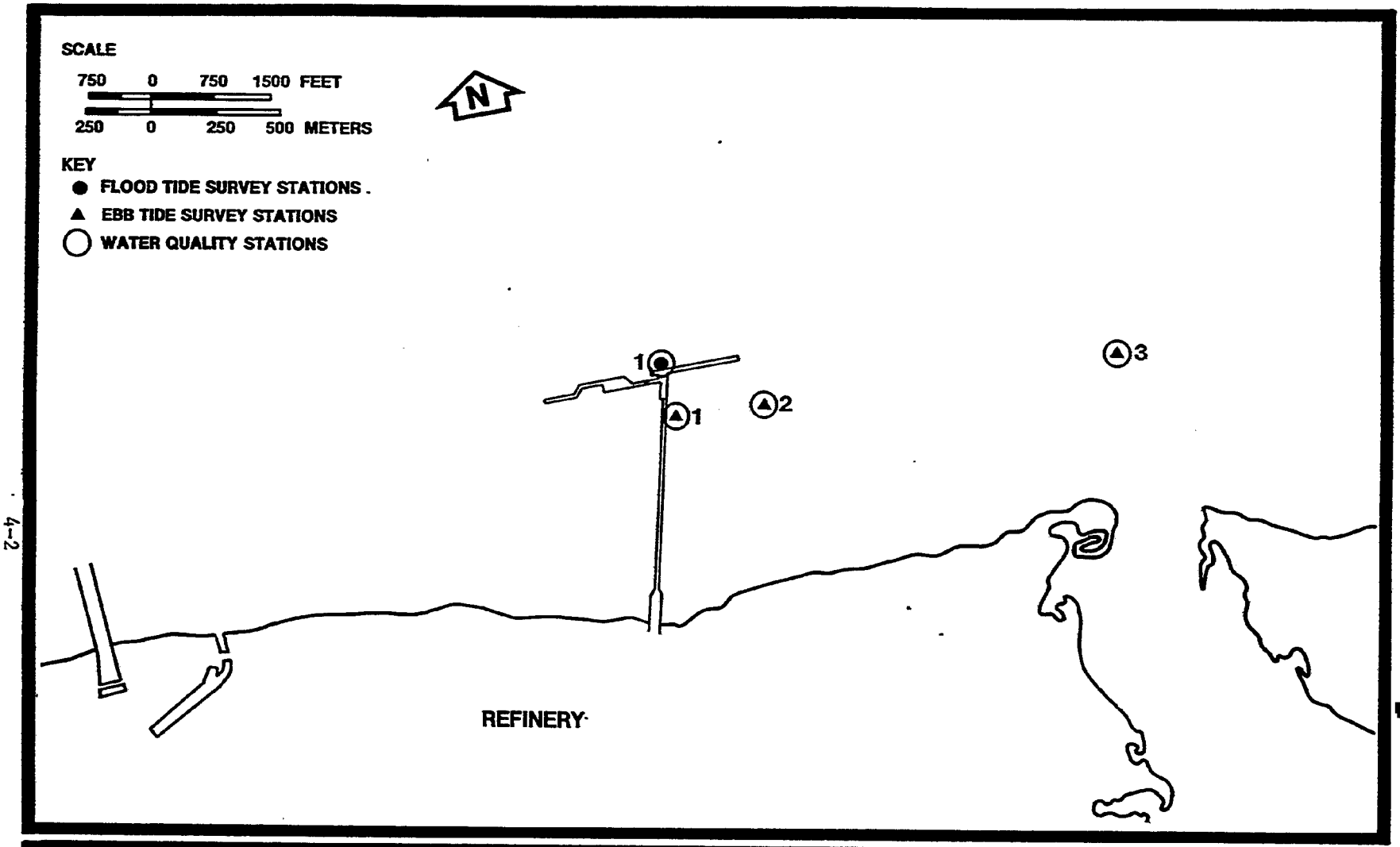

Figure 4–1
VERTICAL DYE AND WATER QUALITY STATION LOCATIONS FOR FLOOD AND EBB TIDE SURVEYS ON MARCH 9, 1982, IN THE YORK RIVER, VIRGINIA

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Table 4-1. Physico-Chemical Parameters Versus Depth on March 9, 1982, During Spring Tides in the York River, Virginia

Station No.	Time (EDT)	Tide*	Depth (m)	Temp. (°C)	Cond. (umhos/cm)	Sali. (ppt)	DO (mg/L)	DO Sat. (%)	pH (SU)
1**	1225	EBB	1	6.4	14,000†	8.11†	11.8	100	7.3
			2	6.4	18,500†	10.97†	11.3	98	7.5
			3	6.5	20,000†	11.94†	11.4	99	7.6
			4	5.9	25,500	15.56	11.4	100	7.7
			5	6.0	27,400	16.84	11.4	101	7.7
			6	5.8	29,300	18.13	11.3	101	7.7
			7	5.5	29,800	18.47	11.2	100	7.7
			8	5.5	31,000	19.29	11.1	99	7.7
			9	5.5	31,100	19.35	11.0	98	7.7
			9.5	5.4	31,500	19.63	11.0	98	7.7
2	1400	EBB	1	6.3	29,000	17.92	12.7	114	7.4
			2	6.1	29,900	18.53	12.2	110	7.6
			3	6.0	30,400	18.88	12.0	108	7.6
			4	6.1	30,500	18.94	11.7	106	7.6
			5	6.0	30,600	19.01	11.7	106	7.7
			6	6.0	30,700	19.08	11.6	105	7.7
			7	6.0	30,700	19.08	11.6	105	7.7
			8	6.0	30,700	19.08	11.4	103	7.8
			9	5.9	30,800	19.15	11.3	102	7.7
3	1425	EBB	1	6.1	29,600	18.33	12.4	112	7.4
			2	6.1	30,200	18.74	11.9	108	7.5
			3	6.1	30,300	18.81	11.7	106	7.6
			4	6.0	30,600	19.01	11.5	104	7.6
			5	6.0	30,600	19.01	11.5	104	7.6
			6	6.0	30,600	19.01	11.4	103	7.6
			6.5	5.9	30,700	19.08	11.3	102	7.6
1**	1705	FLD	1	6.6	24,600	14.86	12.4	110	7.5
			2	6.5	26,600	16.30	12.0	108	7.6
			3	6.5	28,200	17.38	11.7	106	7.6
			4	6.4	29,400	18.19	11.6	105	7.7
			5	6.4	29,600	18.33	11.4	103	7.8
			6	6.4	29,700	18.40	11.3	103	7.8
			7	6.3	29,800	18.47	11.4	103	7.8
			8	6.5	29,800	18.47	11.4	104	7.8
			9	6.4	30,000	18.60	11.3	103	7.8
			10	6.3	30,300	18.81	11.2	102	7.8
			11	6.3	31,000	19.29	11.2	102	7.8
			12	6.3	30,700	19.08	11.8	108	7.7

*Stage of tide: EBB—maximum ebb; FLD—maximum flood; HWS—high water slack; LWS—low water slack.
†Air bubbles in water affecting conductivity probe (and, hence, salinity).
**Water samples collected for laboratory analysis.

Source: ESE.

The emphasis of the vertical survey during the June (neap tide) study was on greater areal resolution at the expense of depth resolution. Therefore, 12 stations (Figures 4-2 and 4-3) were sampled for *in situ* water quality parameters with the sampling depth intervals being greater. Table 4-2 presents the data collected from these stations.

The temperatures were above those measured in March and ranged from 21.3 to 24.6°C. Again the temperature decreased with depth. The salinity during June was slightly higher than March salinities (ranging from 17 to 24 ppt). The salinity differences between surface and bottom (especially at the deep stations) were approximately 6 ppt (similar to that found in March).

The most notable difference between March and June occurred with DO. DO concentration decreased because of the elevated water temperatures; however, DO saturation values over 100 percent were infrequent as compared to March. Again, DO decreased with depth, but whereas in March the maximum change at any one station was from 114- to 102-percent saturation; in June the maximum change was from 135 to 9 percent. The lowest DO concentration (0.4 mg/L or a saturation of 5 percent) measured was at Station HWS-3, where the total water depth was 18 m. The pH was somewhat higher in June than in March (ranging from 7.9 to 8.7).

Table 4-3 compares the extreme values of temperature, salinity, DO, and DO saturation measured by ESE during the two surveys. The same parameter extremes measured by HRSD over 2 years, 1979-1981 (14 data sets), are also presented. This comparison was made in an effort to establish how "normal" conditions were during ESE's survey. These data and other historical data indicate that conditions during the ESE survey were typical for the York estuary. Therefore, the behavior of the refinery plume as measured by ESE with fluorescent dye tracers could also be expected to be reasonably representative of normal plume behavior.

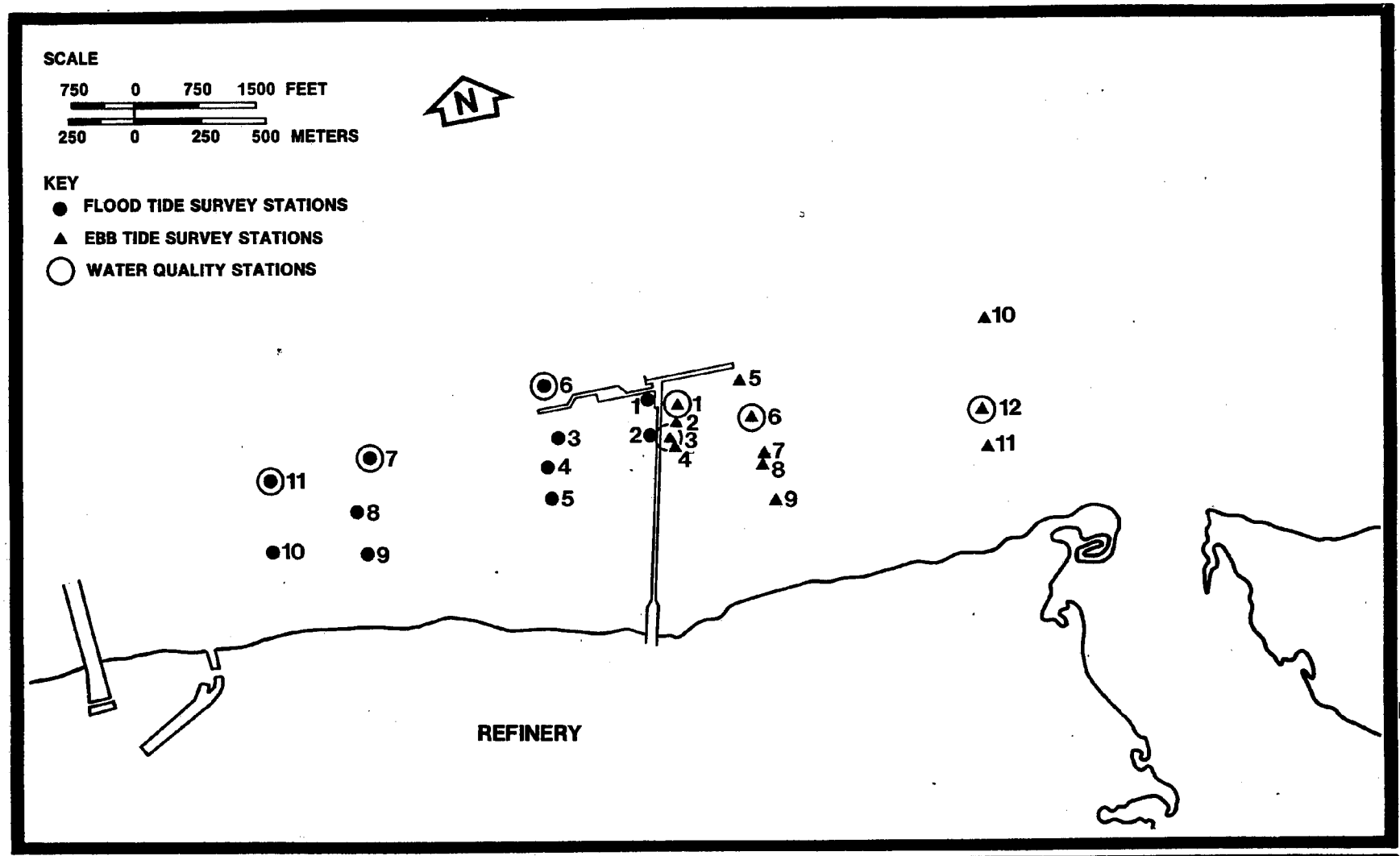

Figure 4-2
VERTICAL DYE AND WATER QUALITY STATION LOCATIONS FOR FLOOD AND EBB TIDE SURVEYS ON JUNE 14, 1982, IN THE YORK RIVER, VIRGINIA

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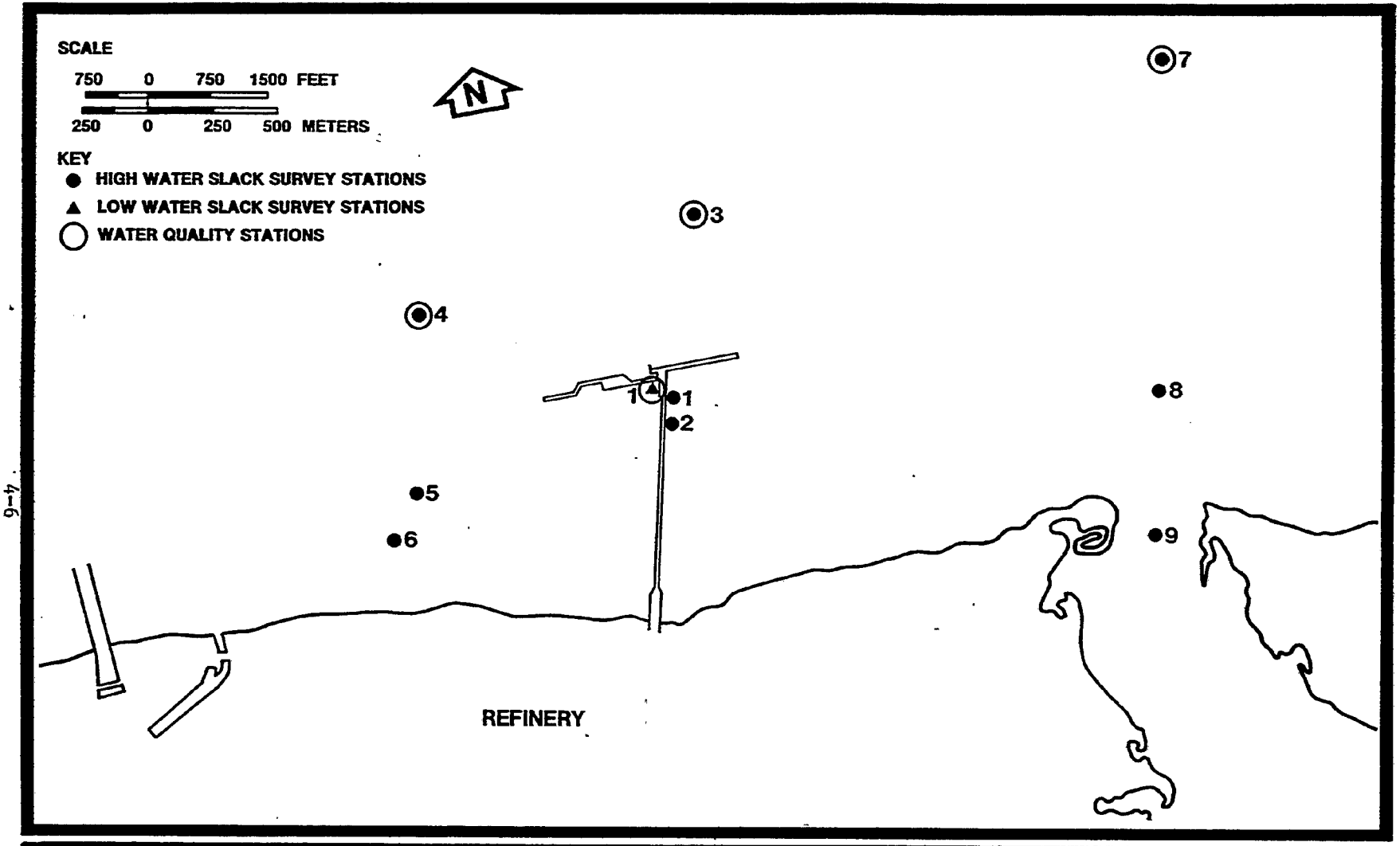

Figure 4-3
VERTICAL DYE AND WATER QUALITY STATION LOCATIONS FOR HIGH AND LOW WATER SLACK TIDE SURVEYS ON JUNE 14 AND 15, 1982, IN THE YORK RIVER, VIRGINIA

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Table 4-2. Physico-Chemical Parameters Versus Depth on June 14 and 15, 1982, During Neap Tides in the York River, Virginia

Station No.	Time (EDT)	Tide*	Depth (m)	Temp. (°C)	Cond. (umhos/cm)	Sali. (ppt)	DO (mg/L)	DO Sat. (%)	pH (SU)
1	1005	EBB	0.0	22.9	29,900	18.53	7.0	89	8.0
			2.0	22.1	30,200	18.74	6.8	86	8.1
			4.0	22.3	30,200	18.74	6.4	81	8.1
			11.0	22.3	30,200	18.74	6.2	78	8.0
3	1027	EBB	0.0	23.0	20,500†	12.26	7.8	96	8.4
			2.0	22.6	30,200	18.74	6.8	86	8.3
			4.0	22.6	30,100	18.67	6.5	82	8.3
			6.0	22.3	30,100	18.67	6.6	83	8.2
6	1051	EBB	0.0	22.6	30,100	18.67	7.7	98	8.3
			2.0	22.3	30,100	18.67	7.0	88	8.3
			9.0	22.0	30,200	18.74	6.4	80	8.2
12	1133	EBB	0.0	22.6	29,900	18.53	7.5	95	8.4
			2.0	22.4	30,300	18.81	7.2	91	8.4
6	1512	FLD	0.0	23.8	29,700	18.40	9.0	117	8.2
			2.0	23.3	30,000	18.60	7.6	98	8.3
			4.0	22.6	30,000	18.60	7.2	91	8.3
			12.0	22.0	33,800	21.21	2.6	33	8.0
7	1532	FLD	0.0	24.0	30,000	18.60	8.4	109	8.4
			2.0	23.6	30,100	18.67	8.0	104	8.4
			4.0	23.0	30,100	18.67	7.4	95	8.4
			6.0	22.8	30,200	18.74	7.3	93	8.3
			8.0	22.7	30,100	18.67	6.8	86	8.3
11	1556	FLD	0.0	23.5	29,900	18.53	8.8	114	8.5
			2.0	23.2	30,100	18.67	7.8	100	8.4
			4.0	22.9	30,200	18.74	7.6	97	8.4
			7.0	22.7	30,300	18.81	6.3	80	8.2
3	1751	HWS	0.0	23.6	30,300	18.81	9.7	126	8.7
			2.0	23.4	30,500	18.94	8.0	103	8.6
			4.0	22.4	30,500	18.94	6.9	87	8.5
			6.0	22.6	30,600	19.01	6.6	84	8.4
			8.0	22.4	30,700	19.08	6.4	81	8.4
			10.0	22.4	31,000	19.29	5.5	70	8.3
			12.0	21.8	33,300	20.87	3.6	46	8.3
			14.0	21.6	35,500	22.39	2.0	26	8.1
			18.0	21.4	37,700	23.93	0.4	5	8.0

Table 4-2. Physico-Chemical Parameters Versus Depth on June 14 and 15, 1982, During Neap Tides in the York River, Virginia (Continued, Page 2 of 2)

Station No.	Time (EDT)	Tide*	Depth (m)	Temp. (°C)	Cond. (umhos/cm)	Sali. (ppt)	DO (mg/L)	DO Sat. (%)	pH (SU)
4	1810	HWS	0.0	23.8	28,500	17.58	10.0	129	8.6
			2.0	23.6	29,700	18.40	8.8	114	8.5
			4.0	22.6	30,300	18.81	7.2	92	8.4
			6.0	22.5	30,500	18.94	6.6	84	8.3
			10.5	22.0	32,900	20.59	3.5	44	8.1
7	1905	HWS	0.0	23.6	30,400	18.88	10.4	135	8.7
			2.0	23.5	30,700	19.08	7.9	102	8.5
			4.0	22.6	30,800	19.15	6.6	84	8.4
			14.5	21.3	37,500	23.79	0.7	9	7.9
1**	1425	LWS	0.0	24.6	28,700	17.72	8.6	113	8.4
			2.0	24.0	29,400	18.19	7.6	99	8.4
			4.0	23.5	29,300	18.13	7.3	94	8.4
			6.0	23.2	29,500	18.26	6.8	87	8.3
			8.0	22.5	31,600	19.70	4.0	51	8.1
			10.0	22.4	32,000	19.97	4.0	51	8.1
			12.0	21.9	34,300	21.56	2.0	26	7.9
			12.5	21.8	34,300	21.56	1.8	23	7.9

*Stage of tide: EBB—maximum ebb; FLD—maximum flood; HWS—high water slack; LWS—low water slack.
†Air bubbles in water affecting conductivity probe (and, hence, salinity).
**Water sample collected for laboratory analysis.

Source: ESE.

Table 4-3. Comparison of Selected Parameters Measured by HRSD (1979-1981) and ESE (1982)

	HRSD		ESE	
	Maximum	Minimum	Maximum	Minimum
Temperature (°C)	29.7	2.6	24.6	5.4
Salinity (ppt)	25.8	14.3	23.9	15.0
DO (mg/L)	13.7	1.3	12.7	0.4
DO Saturation (%)	203	18	135	5

Sources: HRSD, 1981.
ESE.

4.1.2 Dye Surveys

The background variability tests of ambient and combined discharge water indicated a slight natural fluorescence when compared with distilled water. The fluorescence, however, was sufficiently low to negate the need for increasing the amount of dye injected. Recovery ratio tests also indicated there was no need to increase the dye injection rate. Tests conducted with discharge, ambient, and distilled water showed no measurable effect on the dye. The dye was not affected appreciably, therefore, by constituents in either the discharge or ambient water.

Refinery plume dye surveys were conducted on March 9 (spring tide) and June 14-15, 1982 (neap tide). Injection was begun on March 6, 1982, approximately 3 days prior to the initial survey, to allow the dye plume to approach stabilization in the river. Fourteen checks of the dye injection rate indicated a mean dye injection rate of 14.9 ml/min, with a maximum variation of $\pm$3 percent. On March 8, 1982, at approximately 1700 hours, the dye injection was interrupted by Amoco personnel after they found that the dye was interfering with their ammonia analyses. Following conversations with plant personnel, the injector was moved to a tap approximately 600-m landward of Outfall 001. The injection resumed at 1830 hours. The 1.5-hour gap was deemed insignificant for the 3-day injection period.

Water samples from the discharge were taken to measure the initial dye concentration (C_o) values for the combined discharge. The samples collected were read on the laboratory fluorometer, values were temperature corrected, and dye concentrations were calculated. The dye concentrations varied from 139 to 249 parts per billion (ppb), with an average concentration of approximately 200 ppb. These C_o values must be either measured or calculated from flow rates to determine plume dilution ratios. These measurements also enabled ESE to calculate flow

values for the combined discharge at the time of sampling. The discharge flows varied from 23 to 41 MGD, which is well within the estimated range provided by Amoco.

The surveys consisted of two parts: (1) a surface horizontal survey, and (2) a vertical survey of selected stations. The purpose of the horizontal survey was to delineate the areal extent of the plume; the vertical survey was conducted to provide an indication of vertical structure. Both surveys had to be conducted as quickly as possible to measure the plume characteristics at the desired tidal stage. This timing was very important in the tidal York estuary, where the nature of the plume changed rapidly with the tides. Figure 4-4 presents plots of predicted tidal currents (NOAA, 1982) for the March and June survey days. Brackets containing the letters A through H indicate the particular type of sampling done with respect to tidal stage. From this figure, it is apparent that there is a very small temporal window in which to conduct surveys for a selected tidal stage.

When examining the results of a horizontal or vertical plume, the temporal variation should be considered as much as spatial variations. Because of temporal variation, there is not an exact correlation between the results of a horizontal survey and a vertical survey conducted some time later. The vertical survey is not designed to describe the areal plume; it provides, within the allotted time, an indication of the vertical structure that existed during the vertical survey.

The refinery plume delineated by the dye was represented by dye concentration isopleths. The 1-ppb isopleth was equivalent to approximately 0.02 percent of the refinery process wastewater (based on an initial dye concentration in the process water of 4,390 ppb); the 0.5-ppb isopleth, therefore, represented 0.01 percent of the process wastewater. It should be noted that the process water (Outfall 101) is diluted approximately 40 to 50 times after combining with the non-contact cooling water (Outfall 201) to form the final effluent (Outfall 001) which empties into the York River.

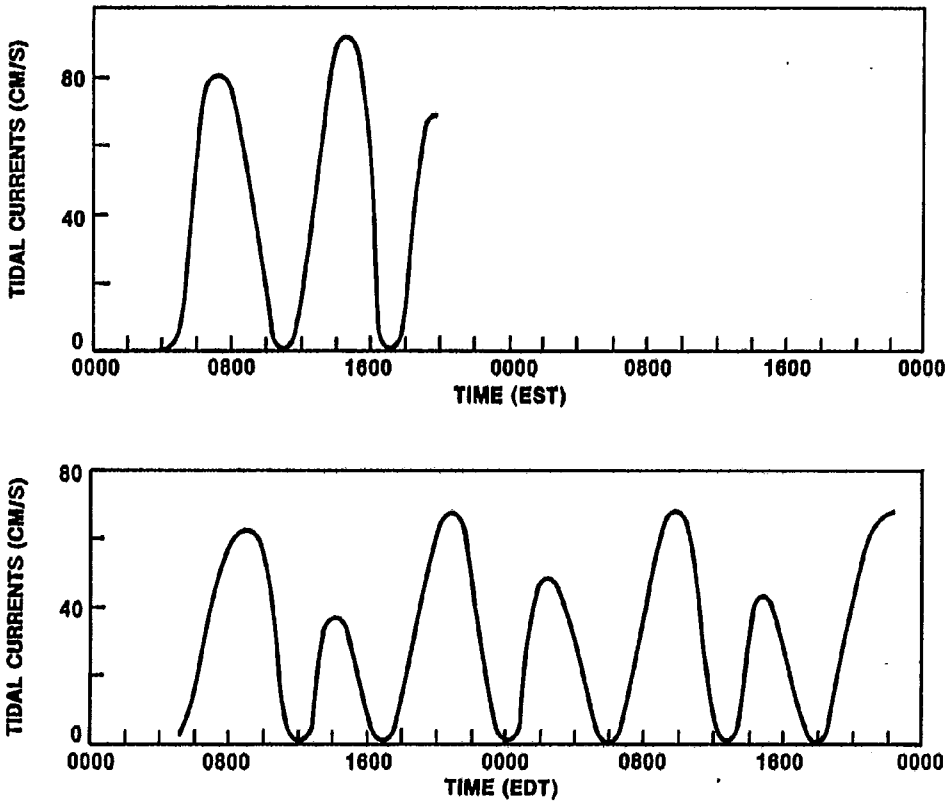

Figure 4-4
YORKTOWN PREDICTED TIDES AND TIDAL CURRENTS DURING ESE SURVEY PERIODS — SPRING TIDES (MARCH) AND NEAP TIDES (JUNE)

SOURCES: NOAA 1982.

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The goal in March was to conduct surveys during the ebb and flood tides; however, due to technical problems, the flood tide was missed.

The March horizontal survey ebb tide plume shown in Figure 4-5 was directed down-estuary and was rather narrow; the 1-ppb isopleth never extended more than 900 m offshore, roughly parallel to the shore.

Figure 4-6 and Appendix Table A-2 present the results of the vertical survey. Three stations (shown in Figure 4-1) were occupied down the center line of the plume. The dye concentration at the bottom was approximately 1 ppb (0.02-percent process water). Higher concentrations were found higher in the water column in the near vicinity of the discharge (the highest concentration was 13.7 ppb or 0.31-percent process water); however, at distances greater than 1,000 m the concentration had already decreased to 2 ppb (0.04-percent process water) in surface waters.

The objective during the June survey was to map the effluent plume during neap, ebb, flood, high water slack, and low water slack tides. The surveys conducted on June 14 and 15, 1982, again consisted of a series of horizontal and vertical surveys (vertical survey data are presented in Appendix Table A-3).

The results of the ebb tide survey are presented in Figure 4-7. As in March, the plume trended down-estuary. This time, however, the plume was noticeably wider, as delineated by the 1-ppb (0.02-percent process water) contour, and extended as much as 1,000 m offshore.

The vertical cross sections of the ebb plume, presented in Figures 4-2 and 4-8, reveal a definite vertical stratification. The plume did reach the bottom, but only at Station 12 did the concentration reach 1 ppb (0.02-percent process water). Dye concentration at the bottom was as low as 0.2 ppb (0.005-percent process water). The highest dye

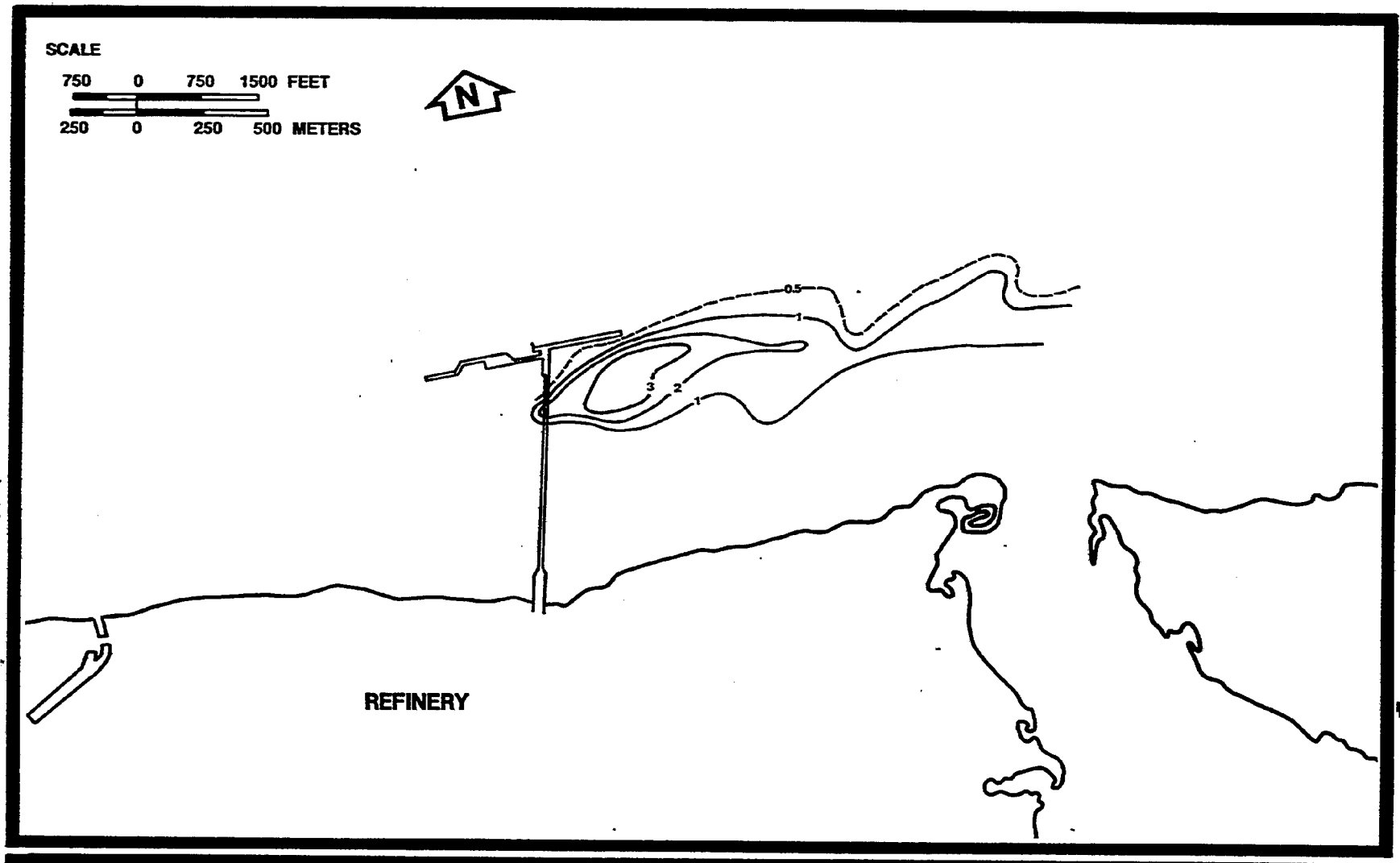

Figure 4–5
EBB TIDE REFINERY EFFLUENT PLUME FOR MARCH 9, 1982. DYE CONCENTRATION ISOPLETHS ARE IN PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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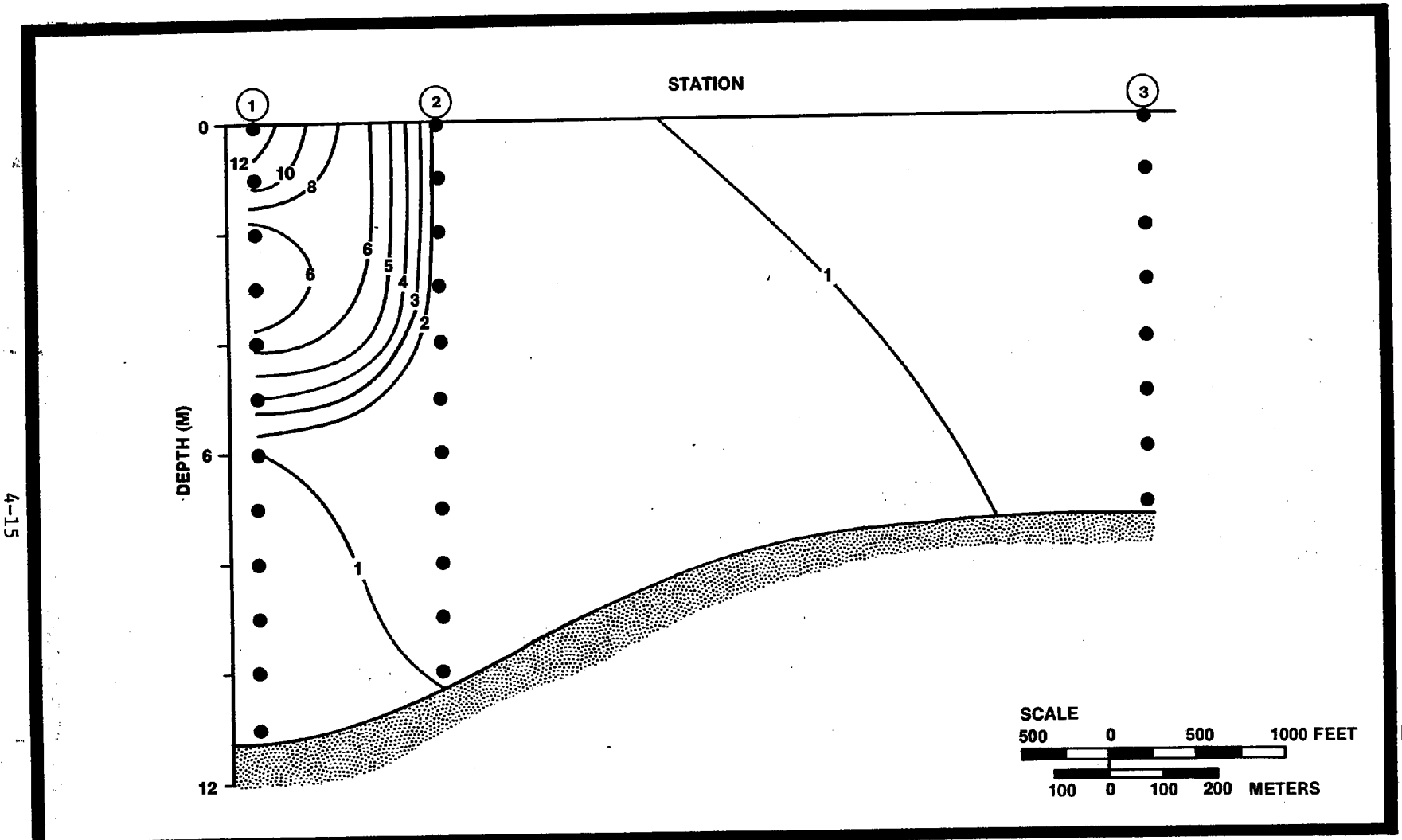

Figure 4-6
LONGITUDINAL CROSS SECTON OF REFINERY EFFLUENT PLUME DURING EBB TIDE (MARCH 9, 1982). CONTOURS ARE DYE CONCENTRATION IN PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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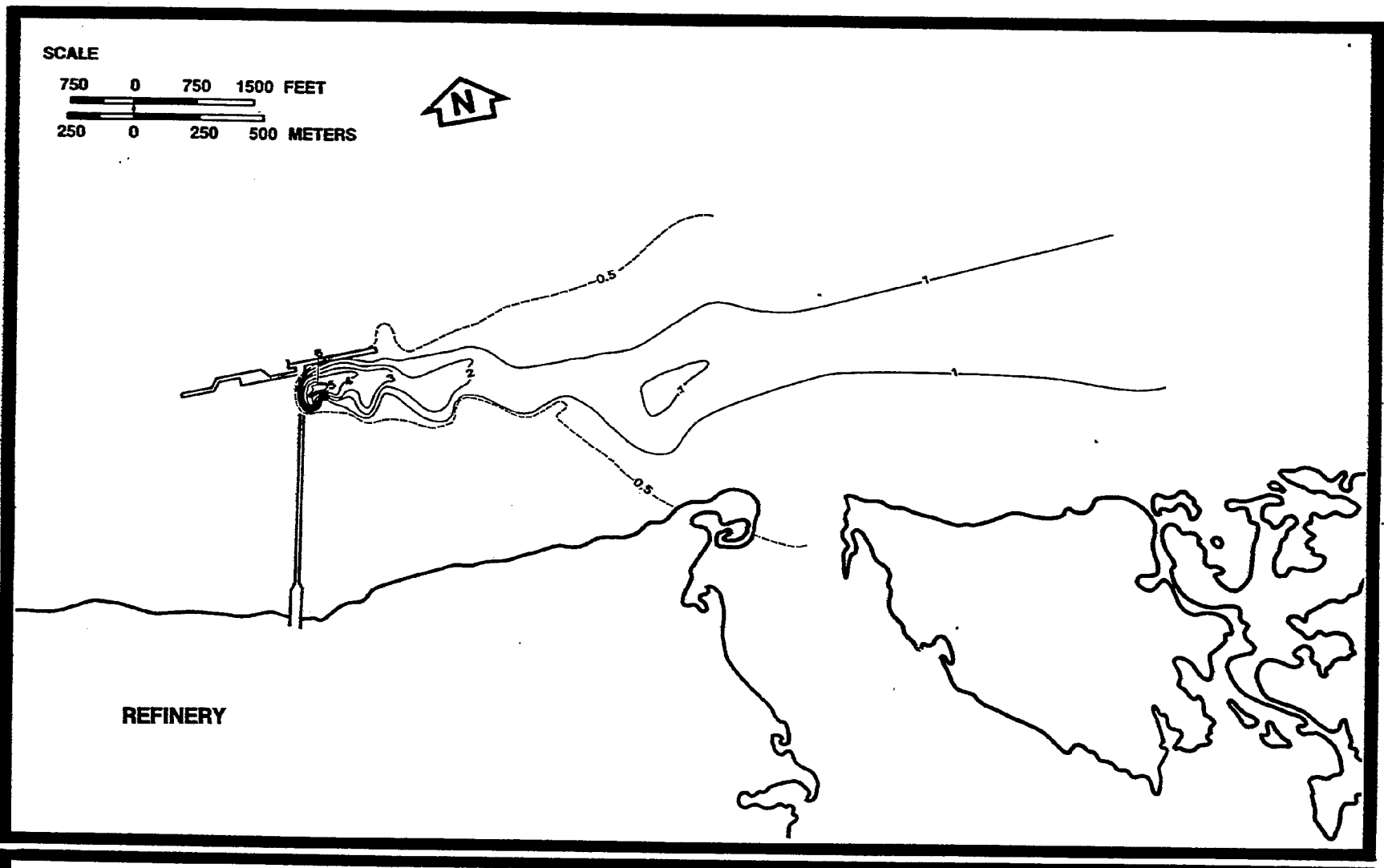

Figure 4-7.
EBB TIDE REFINERY EFFLUENT PLUME FOR JUNE 14, 1982. DYE CONCENTRATION ISOPLETHS ARE PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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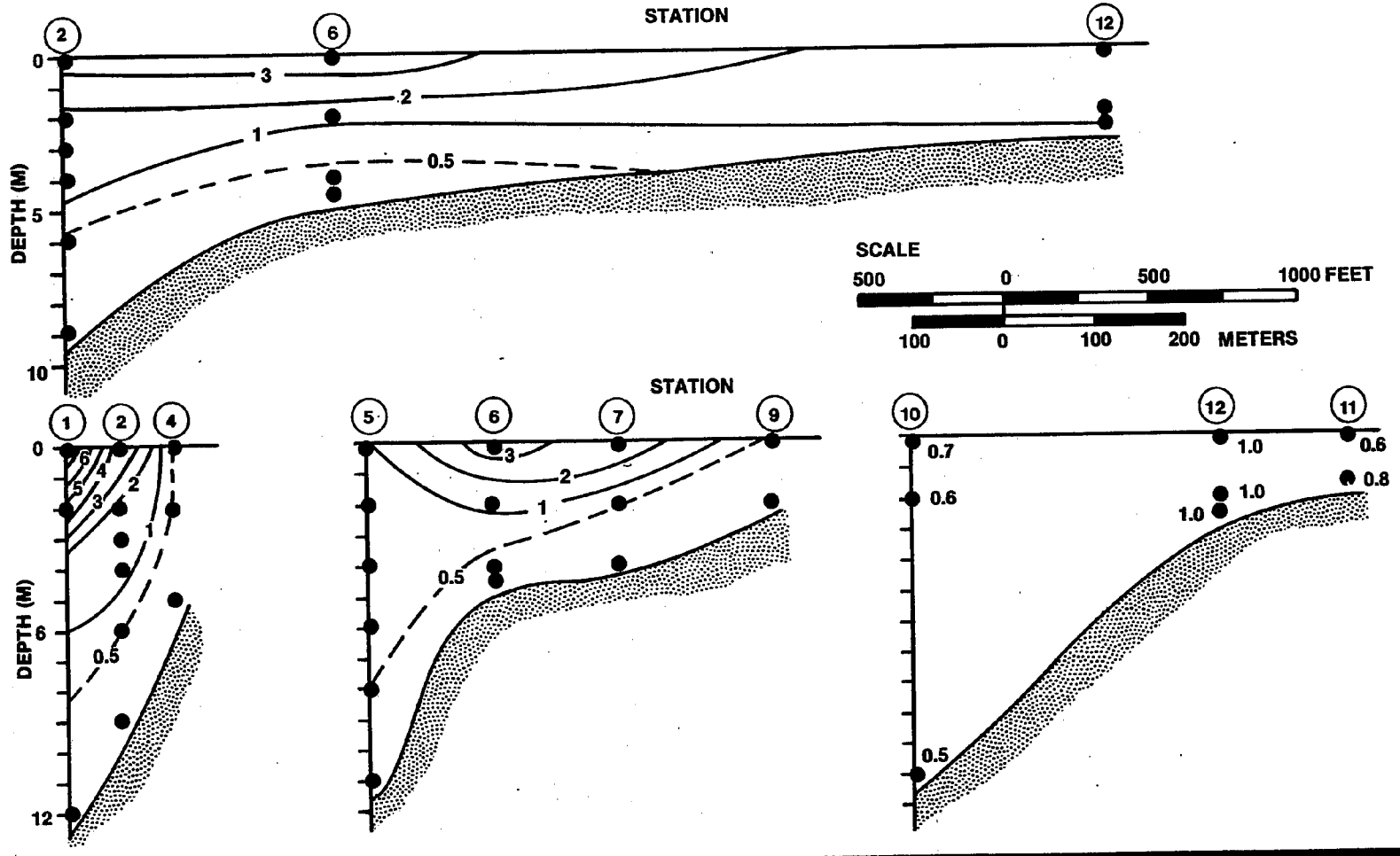

Figure 4-8
LONGITUDINAL AND LATERAL (LOOKING DOWN ESTUARY) CROSS SECTIONS OF REFINERY EFFLUENT PLUME DURING EBB TIDE. CONTOURS ARE DYE CONCENTRATION IN PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER (JUNE 14, 1982)

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concentration, 7.7 ppb (0.18-percent process water), was at the surface near the discharge.

The flood tide survey was conducted on the same day (June 14, 1982) as the ebb tide survey. The plume, presented in Figure 4-9, was headed up-estuary. It differed from the ebb plume in that there was some dye down-estuary of the discharge (no higher than 1 ppb) and the plume was more complex. These differences were probably due to the small differences in tidal stage during the two surveys. An examination of the tide curves in Figure 4-4 reveals that the ebb plume survey was conducted at maximum ebb (based on tidal current curve), whereas the flood plume survey was conducted just prior to maximum flood. Had the flood survey occurred later, the plume would probably have been fully developed (similar to the ebb plume).

This "mirror image" characterization for the ebb and flood tides is based on the fact that net flow for the York River results in average velocities far smaller than the tidal velocities. Haas (1977) reported average tidal velocities (both ebb and flood) of approximately 30 cm/s, while velocities resulting from York River flow were calculated to be 0.3 cm/s or one one-hundredth of the tidal velocity. This net flow-induced velocity was calculated by dividing the average York River flow of 70 m^3/s by the average cross-sectional area of 24,000 square meters (m^2).

The extent of the plume during this particular tide stage, as delineated by the 0.5-ppb contour (0.01-percent process water), was approximately 1,700 m up-estuary and 1,100-m offshore. Figure 4-9 indicates many small pockets of dye and ambient water which added to the complexity of the plume.

The vertical structure of the plume (Figures 4-2 and 4-10) again reveals the vertical stratification. Some dye was evident at every station except Station FLD-6 and, again, the 1-ppb contour (0.02-percent process

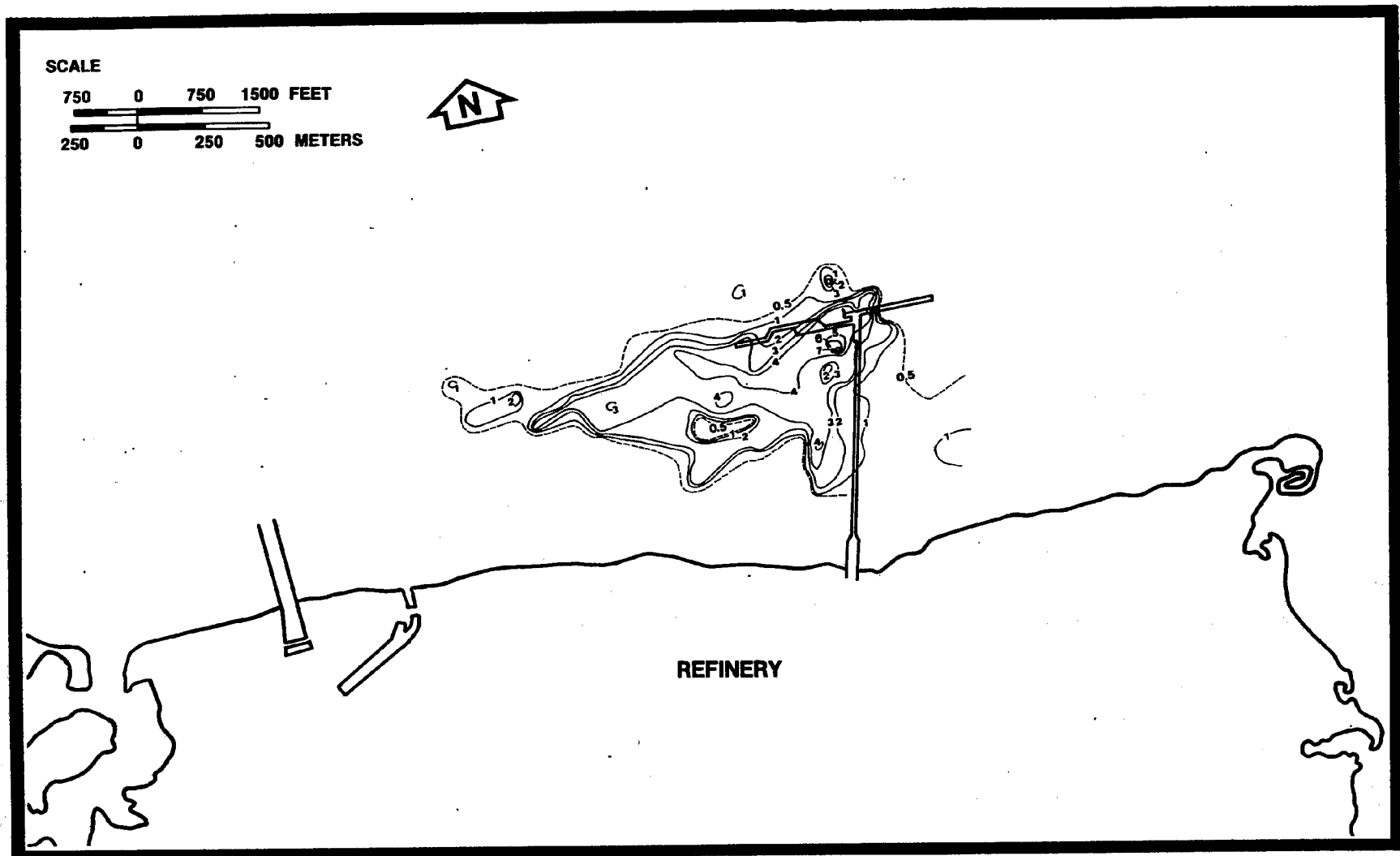

Figure 4-9
FLOOD TIDE REFINERY EFFLUENT PLUME FOR JUNE 14, 1982. DYE CONCENTRATION ISOPLETHS ARE PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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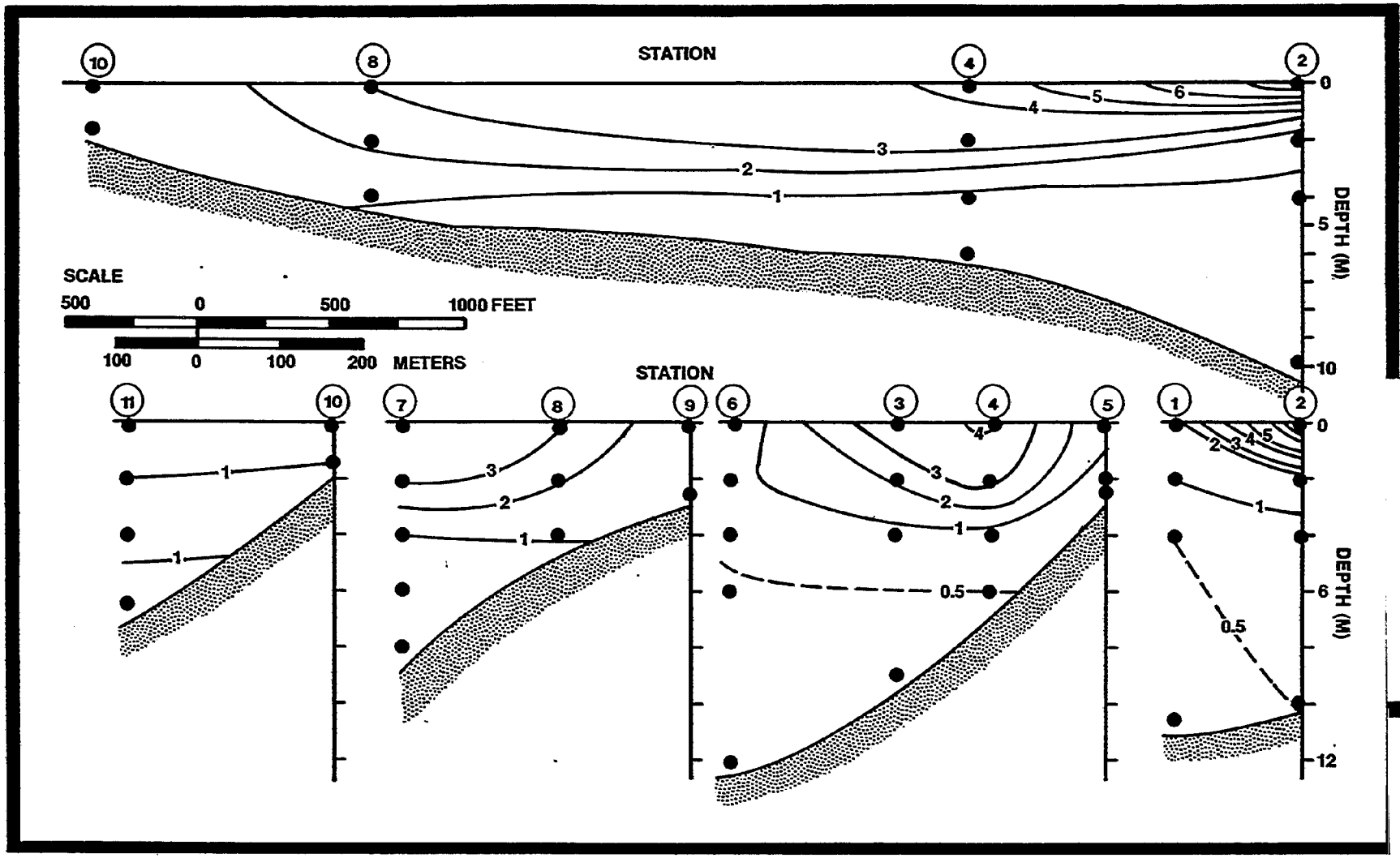

Figure 4-10
LONGITUDINAL AND LATERAL (LOOKING DOWN ESTUARY) CROSS SECTIONS OF REFINERY EFFLUENT PLUME DURING FLOOD TIDE. CONTOURS ARE DYE CONCENTRATION IN PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER (JUNE 14, 1982)

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water) did intersect the bottom at the shallower stations. The highest concentration, 7.4 ppb (0.17-percent process water), again occurred at the surface and near the discharge.

The procedure for conducting the high water slack tide plume was slightly modified due to the extent of the plume and to the consequent amount of time required to conduct the survey. The vertical survey for this plume was interspersed with the horizontal survey rather than waiting until the completion of the horizontal survey.

The results of the horizontal survey are presented in Figure 4-11. The areal extent was best delineated by the 1-ppb or 0.02-percent process water contour. The 0.5-ppb (0.01-percent process water) contour could not be completely mapped within the time constraints of the tides and was located only between the discharge and shore, indicating that during this stage of the tide, the plume only slightly impacted the near-shore area adjacent to the refinery dock.

Within the area mapped, the 1-ppb contour extended 2,000-m offshore and revealed further offshore extension. The up-estuary extent of the 1-ppb contour was approximately 1,400 m; the down-estuary extent was not mapped. The plume was somewhat complex with pockets of dye occurring throughout the plume.

Nine vertical stations (Figure 4-3) were occupied during the June 14, 1982 survey; the results are presented in Figure 4-12. The vertical stratification was again evident, and the higher dye concentrations occurred near the discharge. This was not the case during the horizontal survey conducted near the discharge; this was probably more the result of the transient nature of the plume rather than actual conditions at any instant in time.

The vertical dye concentrations varied from 0 to 9.2 ppb (0.21-percent process water). The apparent dye concentration was actually as low as

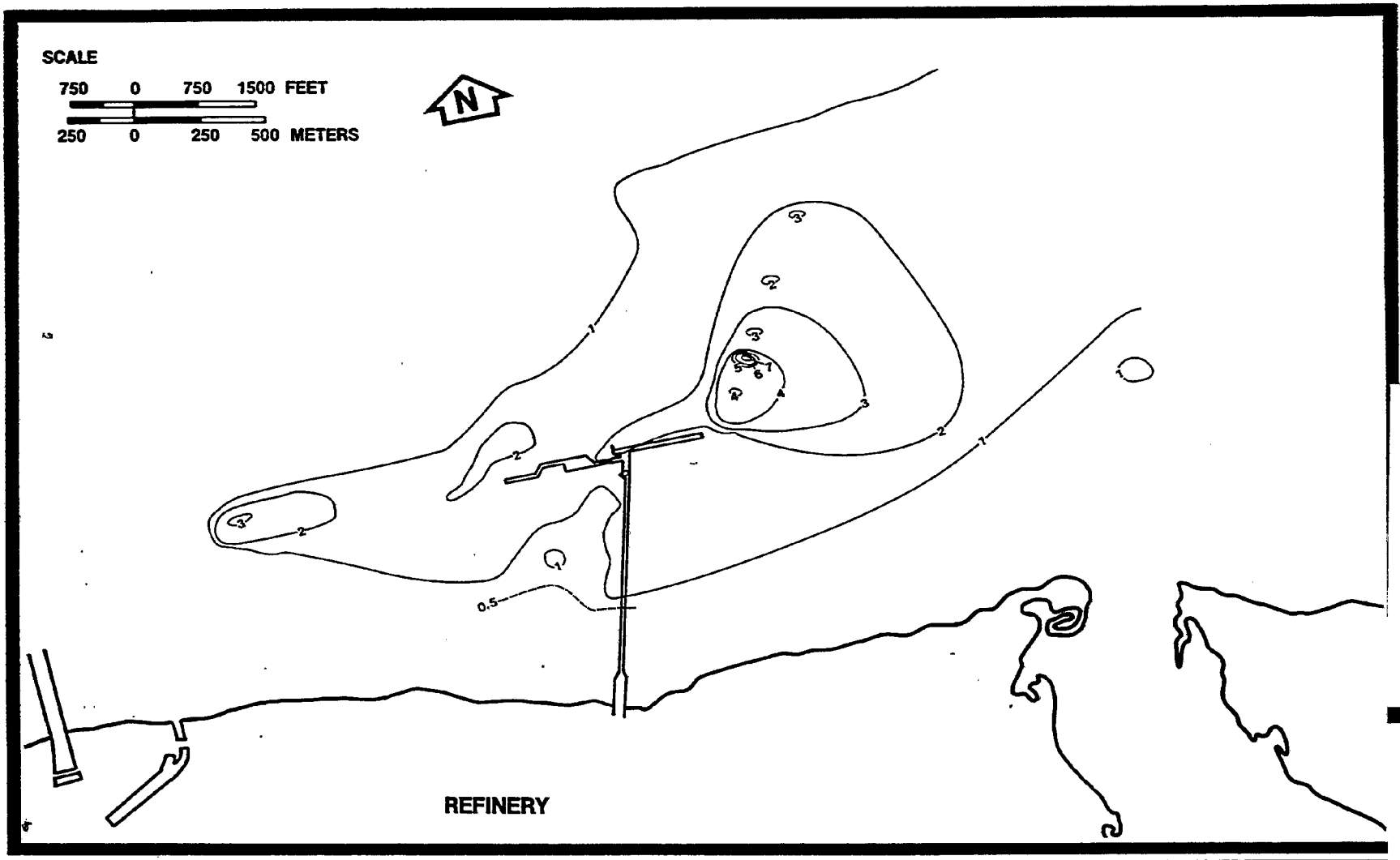

Figure 4-11
HIGH WATER SLACK TIDE REFINERY EFFLUENT PLUME FOR JUNE 14, 1982. DYE CONCENTRATION ISOPLETHS ARE PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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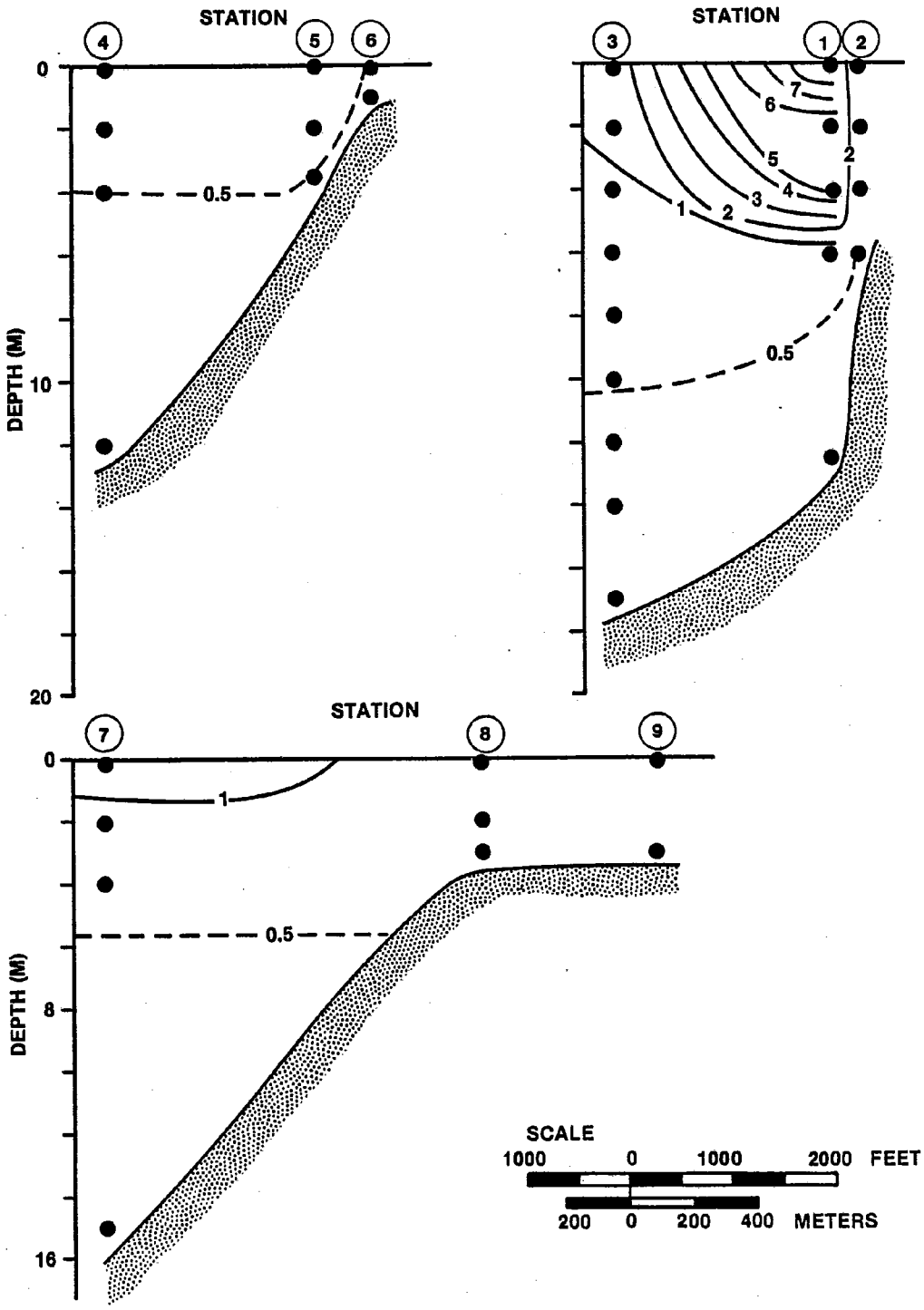

Figure 4-12
LATERAL (LOOKING DOWN ESTUARY) CROSS SECTIONS OF REFINERY EFFLUENT PLUME DURING HIGH WATER SLACK SURVEY. CONTOURS ARE DYE CONCENTRATION IN PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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-0.1 ppb. This apparent negative concentration was the result of differences in natural background fluorescence between the deeper ambient water and the ambient water used for the fluorometer calibrations.

The low water slack tide survey, conducted on June 15, 1982, was further complicated by tugboat activity occurring as a result of docking a tanker at the refinery dock. The horizontal survey was initiated slightly before the ebb tide had ceased. Figure 4-13 shows the remnant ebb tide plume. As the survey continued, the dye concentration suddenly dropped to near ambient conditions. This occurred just as the two tugboats began pushing the tanker into the dock. A reconnaissance (with the recorder shut off) in the area surrounding the discharge revealed ambient conditions, except for areas directly astern of one of the tugboats where only a small portion of the dye was evident. It became apparent that the tugboats were drawing the plume through their screws where it was being mixed rather vigorously. The propwash was so turbulent as to preclude any detailed sampling where the dye was measurable at all. After the tugboats withdrew, the dye was finally located between the bow of the down-estuary tanker (drawing 11 m) and the stern of the up-estuary tanker (drawing 8 m). The dye revealed a plume (Figure 4-13) of limited areal extent and with the highest concentrations measured to date (15.3 ppb or 0.35-percent process water). By the time this survey was completed, there was time to occupy only a single vertical station at the discharge before the tides reached maximum flood. Therefore, no vertical cross sections were possible nor would they have been very representative of a lower water slack plume due to the tugboat activity.

The results of the five plume surveys conducted by ESE not only provided information on the physical character of the refinery effluent plume, but also on how the physical character changed with changes in the York estuary. The most obvious plume characteristic observed was the location and areal extent of the plume resulting from the stage of the daily

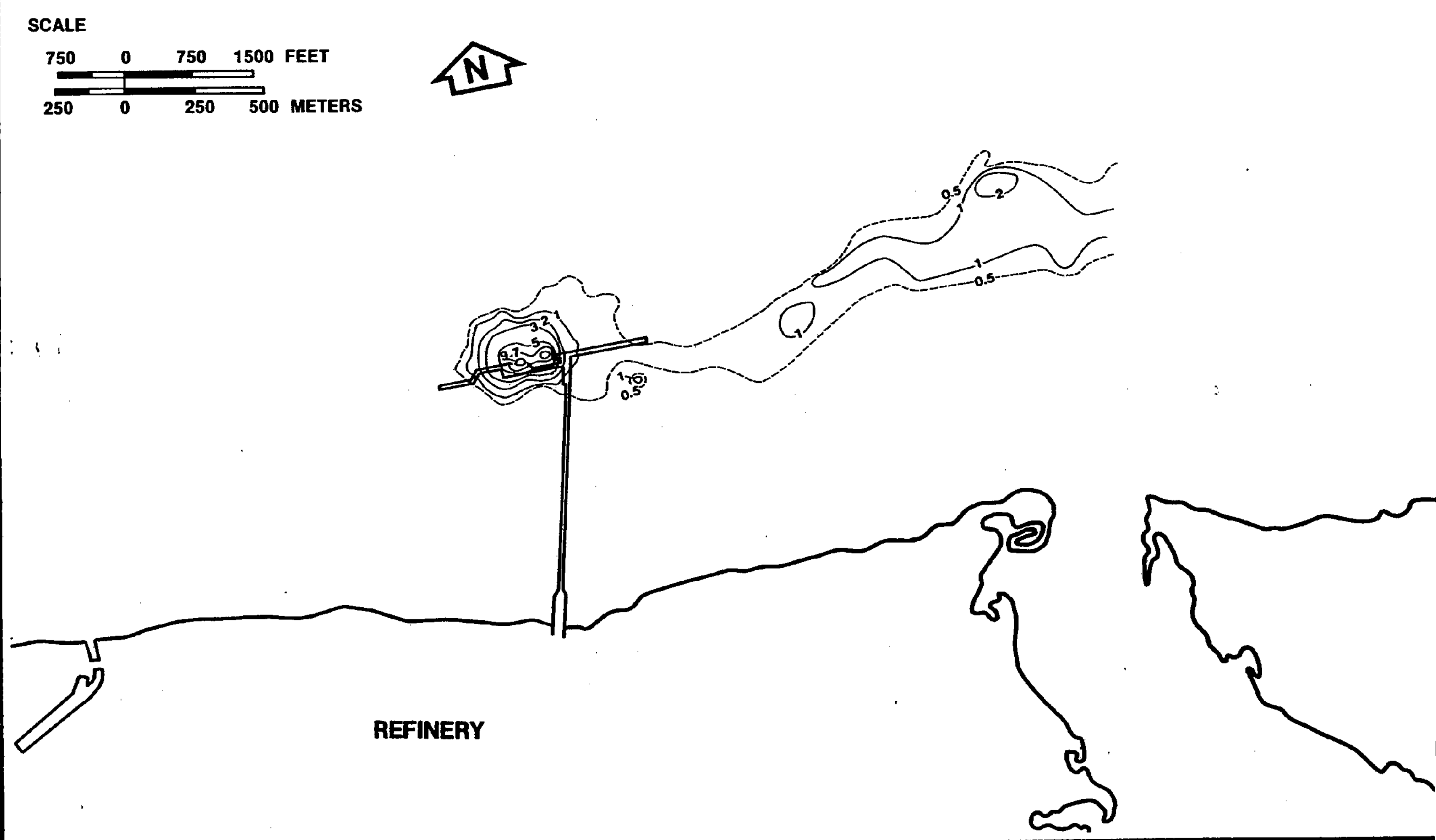

Figure 4-13
LOW WATER SLACK TIDE REFINERY EFFLUENT PLUME FOR JUNE 15, 1982. DYE CONCENTRATION ISOPLETHS ARE PPB WITH 1 PPB REPRESENTING 0.02-PERCENT PROCESS WATER

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tides. The plume had a tendency to elongate and head up-estuary or down-estuary, depending on whether the tides were maximum flood or maximum ebb, respectively. As the tides approached slack, the plume tended to spread, as demonstrated by the high water slack tide plume. The low water slack tide plume is considered atypical due to the tug-boat activity.

Less obvious characteristics included the stages of plume development as shown by the flood and ebb tide plumes. The flood tide plume mapped just prior to the maximum, as shown in the tidal curve (Figure 4-4), is more complex and of limited areal extent, whereas the ebb tide plume mapped during the maximum ebb revealed a fully developed plume.

The plume surveys also indicated the effluent concentrations that could be expected both spatially and temporally in the York estuary. The dye concentrations ranged from ambient to 15.3 ppb. This indicated that at no time did there exist more than 0.35-percent process water in the environment, and that this occurred only in the immediate vicinity of the discharge. More typically, for an area extending approximately 1,000-m offshore and paralleling the shore either up-estuary or down-estuary, the average process water contribution was about 0.02 percent.

Finally, among the most important, but less obvious features revealed were the changes in the physical character of the effluent plume with spring and neap tides. The March survey, during a spring tide with higher tidal currents (Figure 4-4), revealed a plume that was almost vertically homogeneous and extended to the bottom, with process water contributing approximately 0.02 percent. During the neap tide survey in June (Figure 4-4), stratification was evident and the 1-ppb contour (0.02-percent process water) intersected the bottom only at very shallow locations. At stations where the bottom was deeper than 10 m, the dye concentration was at or very near 0 ppb (ambient conditions).

4.1.3 Conclusions

The York River basin consists primarily of forests and farmland. Large industries that discharge into the York River are limited to a pulp and paper mill, a fossil-fuel power plant, and a petroleum refinery (Amoco). The York River is a large tidal estuary with relatively small (70 m^3/s) freshwater flow. As a result, physical processes that occur in the York estuary are primarily the result of the tidal flows.

This physical characterization study has shown that the York estuary, in the refinery vicinity, is stratified for most of the year. This stratification causes the typical summer DO depletion in the deeper water. This study has also shown that the DO can change on a bi-weekly basis when the spring tides are of sufficient magnitude and the freshwater flows are sufficiently reduced to allow vertical mixing, thus replenishing bottom DO. This bi-weekly cycle does not always occur, but during the summer, low-DO period, it can occur regularly.

The bi-weekly cycle also affects the physical character of the refinery effluent plume. Regardless of whether the plume is headed up-estuary during the flood tide, down-estuary during the ebb, or spreading during the slack water period, its vertical distribution is controlled by the spring and neap tides. During a summer spring tide, vertical mixing results in as much as 0.02 percent of the refinery process water reaching the bottom even in deeper areas. During the stratified neap tide conditions, the plume does not reach the bottom at the deeper stations. This means that, at a minimum, 75 percent of the year (i.e., those times during neap tide or when river flows are high enough to discourage destratification even during spring tides) the refinery plume has little or no effect on water deeper than about 10 m. Therefore, the selection of benthic stations that would provide the most information on refinery effluent effects in the York estuary must consider the horizontal spatial arrangement as well as depth.

The plume studies also showed that the process water concentration did not exceed 0.35 percent at any time during the survey and that, typically, the value was nearer 0.02 percent within the study area.

However, as shown in Section 4.4.5 (Table 4-26), there are rare instances when cooling water is not available to dilute the process water. This occurred in both October and November 1983 during plant maintenance and reparations. This results in the discharge of 100-percent process water rather than the 0.02-percent process water normally discharged from Outfall 001. It is conceivable, based on this information and results of the dye surveys, that the concentration of process water in the estuary (in the vicinity of the discharge) could be 50 times higher than the 0.35 percent measured, or 17.5 percent. However, while this discharge would be 100-percent process water, the volume discharged would be approximately 50 times less, and, therefore, dilution of this water would occur rapidly, resulting in process water concentrations similar to or slightly higher than concentrations normally measured in the estuary distant from the immediate vicinity of the discharge.

4.2 CHEMICAL CHARACTERIZATION

Complete chemical characterization of the York River Estuary and all discharges into it is far beyond the scope of this project. Rather, a chemical sampling program was conducted for two primary reasons:

1. To aid in interpretation of toxicity bioassay results; and
2. To identify and use tracer compounds to attempt delineation of estuarine zones that have been impacted by the Amoco refinery effluents, thereby enabling comparisons with "non-impacted" zones.

Chemical data, therefore, were reviewed to identify potentially toxic compounds in the effluent. Furthermore, in the attempt to clarify impacted versus non-impacted areas, four basic criteria were used with the objective of chemically tracing the effluent within the receiving estuary:

1. Concentrations significantly higher in the effluent than in the ambient water,
2. Concentrations higher in the effluent than in the plume,
3. Concentrations higher in the effluent and in the plume than in the ambient water, and
4. Concentrations considered high enough to impact existing biological communities.

The water quality characteristics selected for analysis, therefore, consisted primarily of materials potentially occurring in refinery effluents. Parameter lists were refined as additional data became available.

The specific uses of these data are discussed in Sections 4.3 and 4.4. In this section, the data are presented primarily for reference and as introduction to the sections indicated above.

4.2.1 Reconnaissance--Phase I

Aqueous Samples--The effluent (Outfall 101) sample was designated as OUTF 1, and was subjected to the analyses listed in Table 3-1. The two plume samples (Figure 3-1) were designated PLUM 1 and PLUM 2, and the ambient sample was designated AMBT 1. They were subjected to analyses as shown in Table 3-2. The data for the effluent (Outfall 101) sample, two plume samples, and the ambient sample are shown in Table B-1 (Appendix B).

The 5-day BOD was 54 mg/L in the wastewater, which was significantly higher than the two plume samples (1.2 mg/L and 1.9 mg/L for plumes 1 and 2, respectively) and the ambient sample (<1 mg/L BOD). The COD in the wastewater was 142 mg/L; this concentration significantly exceeds the two plume samples (36 mg/L COD for plume 1 and 45 mg/L COD for plume 2) and the ambient sample (COD of 54 mg/L). The TOC in the plume and ambient samples ranged from 3.7 to 4.2 mg/L, while the TOC in the wastewater was 17.3 mg/L. The concentration of these three parameters in the effluent, although higher than the concentrations in the receiving water, are not uncommon for petroleum refinery effluents, and their levels as noted are significantly reduced in the mixing waters.

The 5-day BOD for the effluent is higher than expected for a refinery treated effluent. Since ammonia level was high and TOC does not account for the BOD, it is feasible that nitrification was occurring.

Ammonia was at a concentration of 25.7 mg/L in the effluent, while the plume and ambient samples ranged from 0.08 to 0.14 mg/L. The concentration of ammonia, although elevated compared to the receiving body of water, is not excessively high for a petroleum refinery effluent. Manganese was significantly higher in the effluent [269 micrograms per liter (ug/L)] than in the plume and ambient samples, which ranged from 19 to 34 ug/L.

The GC/MS analyses for the effluent, ambient, and plume samples indicated that volatile compounds and acid and base/neutral compounds were all less than 10 ug/L.

The petroleum hydrocarbon scan of the effluent showed the presence of hydrocarbons, though specific compounds were all less than 10 ug/L. The effluent oil and grease concentration was 6 mg/L. The petroleum hydrocarbon scan of the plume and ambient samples did not show any appreciable response to hydrocarbons and oil and grease; results were below the detection limit.

Sediments--A sediment sample taken near the discharge pipe was subjected to analyses for specific metals, petroleum hydrocarbons, volatile compounds, acidic compounds, and base/neutral compounds. Objectives of the analyses of this sample were to obtain data on the possible accumulation of effluent compounds in the sediment and also to analyze the sediment extract by GC/MS to look for possible effluent degradation products that might be accumulating in the sediment.

The analytical data for the sediment sample are given in Table 4-4. Low levels of benzene and toluene were detected, but overall the volatile analysis did not detect any volatiles greater than 1 ppm.

The acid and base/neutral scan did not detect any specific compounds greater than 1 ppm. There were some polynuclear aromatic compounds detected [benzo (g,h,i) perylene, fluoranthene, and pyrene], but these were all less than 1 ppm. The scans did not detect any compounds greater than 10 ppm. The largest peak was octasulfur.

The petroleum hydrocarbon scan had a series of aliphatic and aromatic hydrocarbons, but specific hydrocarbons were all less than 1 ppm. The oil and grease for the sediment was 1,450 milligrams per kilogram (mg/kg) dry weight. This particular oil and grease determination was done by a non-specific gravimetric analysis. This procedure not only provides quantitative data for petroleum hydrocarbons (oils) and greases, but also includes fatty acids, resin acids, humic substances, and other large molecular weight compounds that may not be amenable to quantitation by GC/MS.

Table 4-4. Chemical Characterization of a Sediment Sample Taken Near the End of Outfall 001 Pipe

	Outfall Sediment 159700 mg/kg (dry weight basis)
Volatiles	
Benzene	0.07
Xylenes (total)	<0.01
Toluene	0.27
Ethylbenzene	<0.02
Extractables	
Acenaphthene	<0.27
Acenaphthylene	<0.15
Anthracene	<0.19
Benzo(A)anthracene	<0.37
Benzo(A)pyrene	<0.13
Benzo(B)fluoranthene	<1.00
Benzo(K)fluoranthene	<1.00
Benzo(g,h,i)perylene	0.20
Bis(2-chloroethoxy)methane	<1.00
Chrysene	<0.19
Dibenzo(a,h)anthracene	<0.24
Fluoranthene	0.32
Fluorene	<0.25
Naphthalene	<0.12
Phenanthrene	<0.21
Pyrene	0.16
Cresols (total)	<0.10
Metals	
Antimony	<9
Chromium	24
Thallium	22
Nickel	19
Aluminum	12,800
Barium	27
Boron	24
Cobalt	<2
Magnesium	2,690
Manganese	105
Tin	<9
Traditionals	
Oil and Grease	1,450*
Moisture Content	70% wet weight

*Also includes non-petrogenic hydrocarbons.

Source: ESE.

The sediment sample collected near the outfall showed detectable levels of several metals at approximately 20 mg/kg. Manganese was detected at 105 mg/kg, while magnesium and aluminum showed levels at 2,690 mg/kg and 12,800 mg/kg, respectively. As shown in Table 4-4, magnesium and aluminum levels were much higher in receiving water samples than in the effluent indicating that the refinery is probably not the source of these materials.

4.2.2 Phase II

Table B-2 (Appendix B) lists the results of the metals and petroleum hydrocarbons analyzed in the 60 sediments. The total petrogenic hydrocarbons, aliphatic petrogenic hydrocarbons, and aromatic petrogenic hydrocarbons (STORET Numbers 99207, 99208, and 99209, respectively) were obtained by IR as described in the previous section. The hydrocarbon data presented across from STORET Numbers 99162, 99163, 99164, 99028, and 99029 were obtained from the concentrated extract analyzed by GC/FID.

Presented in Table B-3 (Appendix B) are the results of one plume sample, one ambient sample, one Outfall 001 sample, one Outfall 201 sample, and three Outfall 101 samples. These monitoring samples were taken in June 1982, except for one Outfall 101 sample taken in September 1982. The June 1982 samples were collected during the mysid life cycle test.

The sediment results are discussed in further detail in the Field Biology Studies (Section 4.3). The data presented in Table B-3 are discussed in Section 4.4, Bioassays.

4.2.3 Phase III

Chemical data of Outfall 101, taken in May 1983 and analyzed in June 1983, are presented in Table B-4 (Appendix B). The results from the 30 sediment analyses are shown in Table B-5. The use of these data is discussed in Section 4.3 of this document.

4.3 FIELD BIOLOGY STUDIES

As a result of the complexity of the York River system and the wide dispersion of the effluent plume, no control area similar to the study area was available. To overcome the lack of a control area, a station grid system was established, and benthos sampling stations were selected from this grid based on sediment grain size, sediment hydrocarbon content, and sediment metals content. In addition to infaunal benthos, colonization of artificial substrates in the upper water column was investigated by placing artificial substrates around the Amoco refinery discharge. Infaunal benthos and fouling communities were analyzed with the objective of determining whether or not negative impacts of the refinery effluent could be determined by traditional field studies.

4.3.1 Benthos

Sampling Station Array--The original intent of the benthic survey was to characterize the areal extent and degree of impact of the refinery effluent by comparing impacted zones with non-impact (control) zones. This approach requires the definition of a control zone that is not subjected to the effluent discharge, but is otherwise identical (or nearly so) to the impacted zone.

The York River estuary is a very complex system. The basic difficulty in program design was that the effluent plume contact with the bottom is aperiodic. The plume moves up and down through the water column, toward and away from shore, and up and down estuary. Furthermore, these patterns of shift change throughout the year and are not readily predictable.

These complexities precluded definition of a traditional control area because:

1. Spreading of the effluent during periods of stratification indicated that no location in the river with conditions

similar to the area of discharge is free from its influence. During the stratified periods, the discharge plume extends so far down current that by the time it becomes sufficiently diluted to allow establishment of a control area, the bottom substrate and salinity regime are different from the experimental, or "impact," area.

2. Establishment of impact and control stations along transects running orthogonally from the shoreline was inappropriate, because substrate and depth differences along these transects are very substantial. These differences rendered potential stations along such transects statistically incomparable.
3. The multidirectional effluent dispersion patterns induced by tidal influences, and the complex dispersion patterns caused during tidal transitions, rendered calculation of time-exposure relationships impossible with the limited data available. Collection of sufficient plume data to satisfy this aim would have been impractical for this program.

To overcome the lack of a suitable, or traditional, control area, sediment chemistry data were used to attempt definition of potentially impacted areas. Considering the shifts in effluent dispersion patterns, it was concluded that, over time, some benthic locations are exposed to more or less of the effluent in the aggregate than are others. That is, among the hypothetical Locations A, B, and C, Location A will have been exposed to a greater amount of effluent over the last several years than Location B, which will have been exposed, in turn, to more than Location C. This may be supposed true even if the locations are exposed to the same absolute concentrations of effluent, because the aggregate duration of exposure will have been different among the locations.

In addition, it was recognized that the value of benthic infauna as environmental indicators lies in their capacity for integrating long-term exposure to pollutants. Thus, if the benthic community at

Location A were exposed to more of the pollutant over a longer (in aggregate) period of time than that at Location B, the two communities would exhibit different characteristics, which would presumably be amenable to analysis. Rather than search for a presumed concentration gradient, which might have been possible in a more stable and less complex system, sediment chemistry data were utilized to attempt definition of potential impact gradients.

A close-order (200 x 200 m) grid was used to sample 60 benthic stations in the ebb tide plume area (Figure 4-14); samples were collected for physico-chemical analysis, and five replicates were collected from each station for benthos analysis. Sediments from each station were analyzed for particle size distribution (PSD), hydrocarbon content, and selected metals. Analytical results are detailed in Section 4.2. It was anticipated that distribution patterns in hydrocarbon and metals concentrations would become apparent because of the time-exposure phenomenon discussed above, and because of the tendency of these pollutants to adsorb onto sediment particles. Once such patterns were discerned, stations would be selected for benthos analysis based on identified exposure gradients, as evidenced by hydrocarbon and metals concentrations in the substrate.

Potential problems with this approach were recognized. If sediments had been exposed to effluent over a long period of time, it is conceivable that all sediments had become saturated with hydrocarbons, preventing the emergence of patterns. This was considered unlikely, because plume studies indicated that bottom sediments were subjected only to low effluent concentrations. Microbiological degradation, solubilization, and similar phenomena were expected to preclude this problem. A second potential problem existed in that the amount of hydrocarbons and metals adsorbed varies with sediment type. Thus, stations must be compared only within similar groups, as determined by PSD analysis.

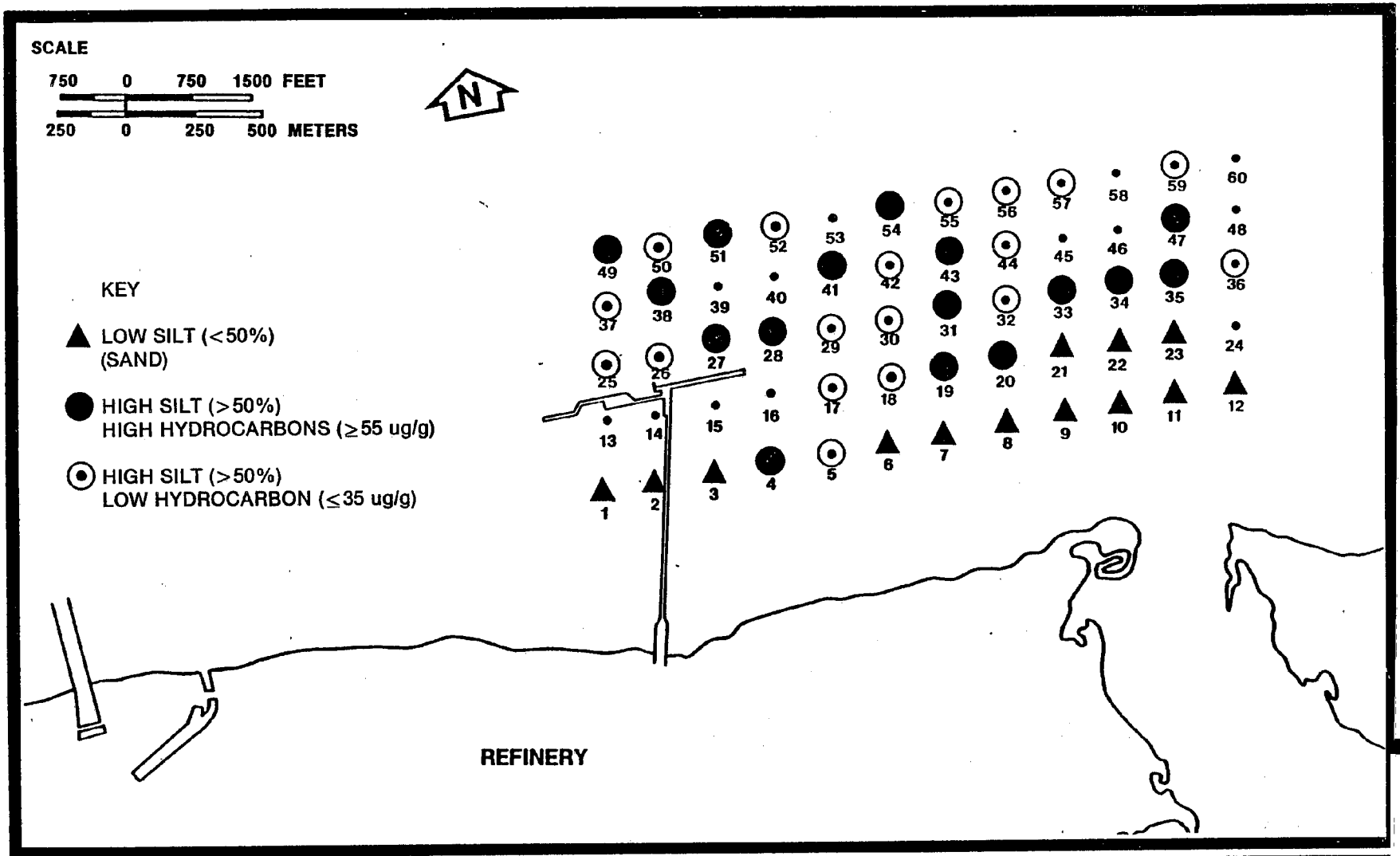

Figure 4-14
SPATIAL DISTRIBUTION OF SAND AND SILT SUBSTRATE AND RELATIVE HYDROCARBON LEVELS

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Of the 60 stations sampled in September 1982, 30 were selected for benthos analysis. Steps and criteria for selection were as follows:

1. Analysis and Grouping of Sample Locations Based on PSD and Moisture Content--Sediments were classified into those having high silt content (>50-percent silt) and high sand content (<50-percent silt). Based on these criteria, 13 locations were classified as "high sand content" and 47 as "high silt content" (Figure 4-14). The hydrocarbon concentrations of all but one of the high sand stations were $\leq$3.0-ug/g dry weight. Because of this factor and because of the small numbers of high sand stations, it was concluded that insufficient data were available to statistically analyze the biotic impacts among the high sand stations. Only high silt stations, therefore, were included in the selection.

2. Analysis and Determination of Sample Locations with High Metals Content--The procedure reviewed by Huggett (1981) was applied to determine which stations in the array contained the metals cadmium, chromium, lead, manganese, selenium, vanadium, and zinc in excess of the amount expected as a result of the solids content. Substantial excess metal content relative to the background can be used as an indicator of sediment contamination.

   Two types of distribution relative to total solids content were observed using a computerized Statistical Programs for the Social Sciences (SPSS) correlation program. The relationship of cadmium and lead showed very little slope of the line of best fit relative to solids content. Chromium, manganese, selenium, vanadium, and zinc showed a sharp slope to the regression line. All metals were highly correlated to solids. Seven sample locations contained excess metals, as per the reasoning of Huggett (1981). In three of these locations lead

and vanadium were high. One additional station contained excess lead only, and one contained vanadium only. One station contained excess cadmium, and one contained excess manganese.

No general point source impact relative to the discharge was shown by the distribution of the locations of excess metals in the sampling grid. Selection of stations for biological analysis was made to include the locations which showed excess lead and vanadium in order to attempt quantification of metals impact alone, as well as the interaction of hydrocarbon concentration with excess lead and/or vanadium on the benthic community structure.

3. Classification of Sample Locations by Hydrocarbon Content--All high-silt stations were grouped on a histogram (Figure 4-15) by total petrogenic hydrocarbon content. Concentrations ranged from 8.20- to 439-ug/g dry weight. If Station 4 (439 ug/L) is excluded, the highest concentration was 88.8 ug/g. No spatial pattern of hydrocarbon concentration versus station location relative to the refinery outfall was discerned.

4. Selection of Station Locations for Characterization--Selection of the 30 high-silt stations for benthos characterization based on hydrocarbon content was weighted toward the lower and the higher hydrocarbon concentrations to maximize the likelihood of discerning biological differences. A representative selection of stations with intermediate hydrocarbon concentrations was included to permit analysis of impact gradients if they were suspected after examination of biological data. Stations previously determined to have elevated metals content were also included in this selection.

Stations selections were intended to allow testing of the hypothesis that no significant differences exist in benthos

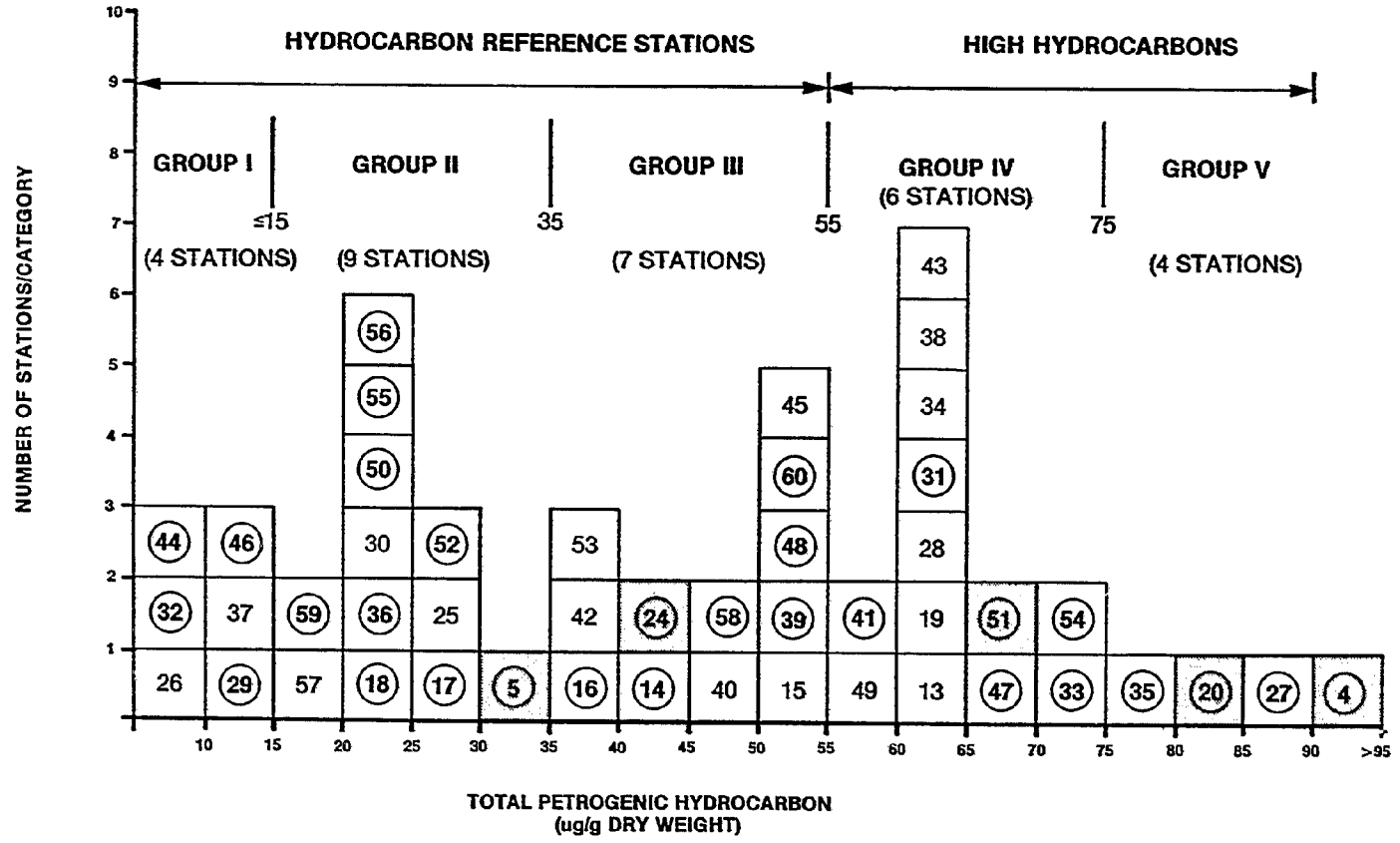

Figure 4-15
RANGE OF TOTAL PETROGENIC HYDROCARBON CONTENT

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densities due to sediment hydrocarbon concentrations. Stations were selected with the widest range of hydrocarbon concentrations to allow the use of linear regression in hypothesis testing. A significant regression indicates a cause and effect response of the dependent variable by the independent variable. In this case a significant and negative regression would indicate that elevated hydrocarbon concentrations (independent variable) caused a decrease in benthos densities (dependent variable).

The following 30 stations were selected for biological characterization to represent the stations most likely to show either a hydrocarbon concentration or metal-related resolvable effect on the benthic community (Figure 4-16).

Stations

4	27	39	50
5	29	41	51
14	31	44	52
16	32	46	54
17	33	47	55
18	35	48	56
20	36		58
24			59
			60

Sampling Efficiency--The standard error as a percent of the mean density for each station was calculated as an index of precision (see Section 3.3.1). The calculated standard errors and standard errors as a percent of the mean density are presented in Tables 4-5 and 4-6. As a percent of the mean, the standard errors for the 30 benthos stations ranged from 11 to 24 percent (September 1982) and 10 to 17 percent (May 1983). Since these errors were within 20-30 percent of the mean, 3 replicates was considered an adequate sample size to give reliable estimates of benthos densities (see Section 3.3.1).

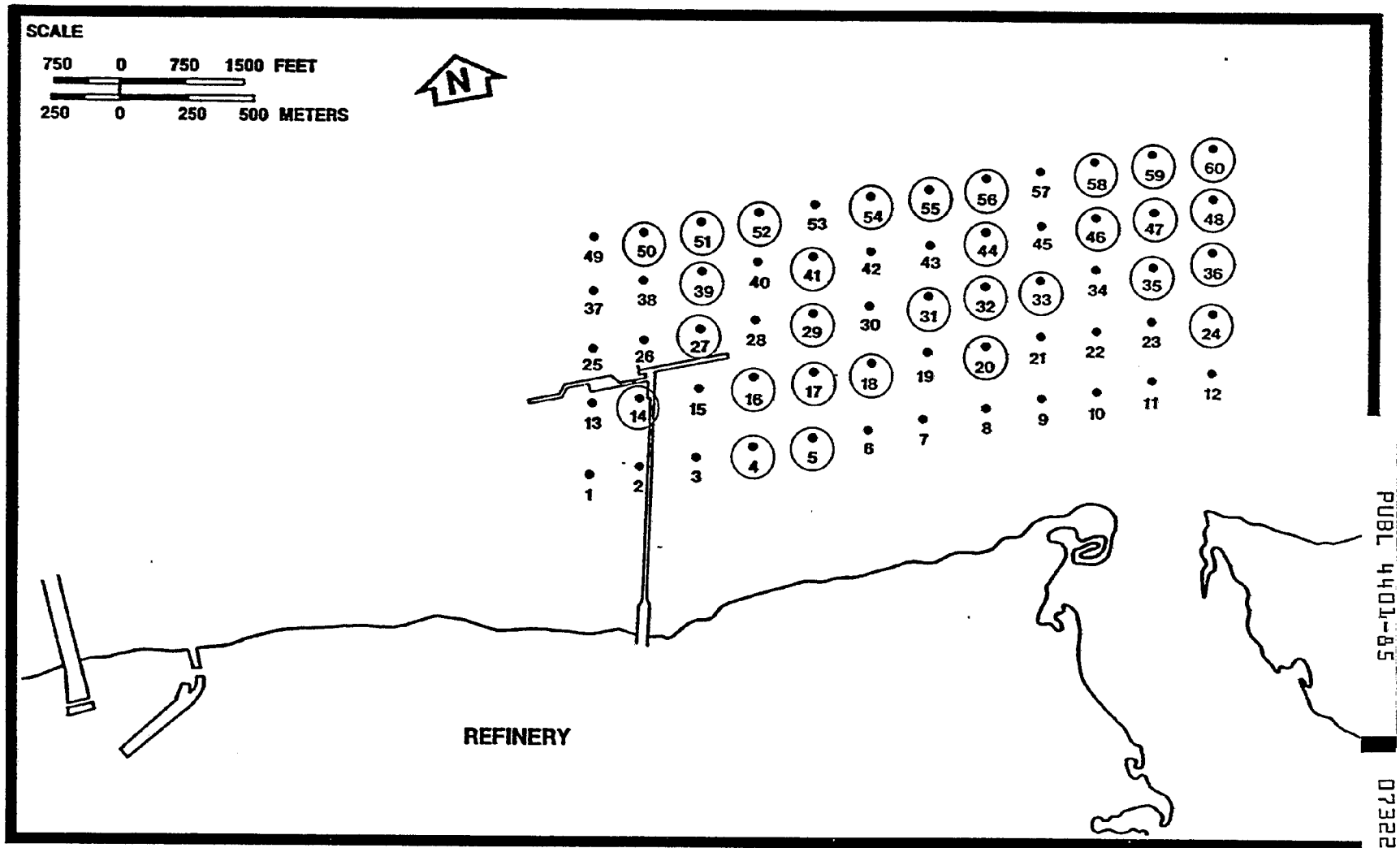

Figure 4-16
BENTHIC ANALYSIS PATTERN-STATIONS SELECTED

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Table 4-5. Number of Taxa, Total Individuals (3 Replicates), Mean Density, Standard Error, and Standard Error as Percent of the Mean for 30 Stations, York River, Virginia, September 1982

Hydro-Carbon Group	Station	No. of Taxa	Total Individuals	Mean Density $\bar{X}$ No./m^2	SE	SE/$\bar{X}$
Group I	29	19	408	6,044	±786	0.13
<15 ug/g	32	19	368	5,452	±763	0.14
	44	17	338	5,007	±701	0.14
	46	16	404	5,985	±838	0.14
Group II	5*	12	110	1,630	±326	0.20
15-<35 ug/g	17	17	352	5,214	±730	0.14
	18	17	457	6,770	±880	0.13
	36	11	214	3,170	±507	0.16
	50	16	166	2,459	±443	0.18
	52	13	189	2,800	±476	0.17
	55	20	372	6,511	±772	0.14
	56	16	338	5,007	±701	0.14
	59	23	534	7,910	±949	0.12
Group III	14	22	117	1,733	±329	0.19
35-<55 ug/g	16	19	254	3,763	±602	0.16
	24*	13	66	978	±235	0.24
	39	22	351	5,199	±728	0.14
	48	20	628	8,429	±1,011	0.12
	58	14	362	5,363	±751	0.14
	60	22	360	5,348	±748	0.14
Group IV	31	17	333	4,933	±691	0.14
55-<75 ug/g	33	13	233	3,452	±483	0.14
	41	16	252	3,733	±597	0.16
	47	18	709	10,503	±1,155	0.11
	51*	13	320	3,496	±559	0.16
	54	17	459	6,799	±884	0.13
Group V	4*	22	258	3,822	±612	0.16
≥75 ug/g	20*	18	288	4,266	±639	0.15
	27	18	215	3,170	±507	0.16
	35	15	174	2,578	±438	0.17

*Stations with excess metals

Source: ESE.

Table 4-6. Number of Taxa, Total Individuals (3 Replicates), Mean Density, Standard Error, and Standard Error as Percent of the Mean for 30 Stations, York River, Virginia, May 1983

Hydrocarbon Group	Station	No. of Taxa	Total Individuals	Mean Density $\bar{X}$ No./m^2	SE	SE/X
<15 ug/g	20	30	1,088	16,117	±1,612	0.10
	48	23	456	6,755	±880	0.13
	36	26	791	11,717	±1,291	0.11
	27	20	178	2,637	±457	0.17
	39	29	496	7,347	±933	0.13
	4	62	1,062	15,732	±1,585	0.10
	46	25	522	7,733	±967	0.13
	35	28	827	12,251	±1,332	0.11
15-<35 ug/g	41	30	512	7,584	±954	0.13
	32	29	684	10,132	±1,167	0.12
	47	22	317	4,696	±683	0.15
	33	37	853	12,532	±1,361	0.11
	14	33	1,550	10,325	±1,182	0.11
	31	22	595	8,814	±1,059	0.12
	52	27	513	7,599	±955	0.13
	29	22	425	6,296	±838	0.13
	44	28	231	3,422	±548	0.16
	24	34	765	11,332	±1,261	0.11
35-<55 ug/g	58	27	191	2,844	±482	0.17
	16	32	732	10,873	±1,225	0.11
	5	29	695	10,266	±1,177	0.11
	18	26	1,186	17,569	±1,711	0.10
	17	28	657	9,732	±1,134	0.12
	50	31	413	6,133	±823	0.13
	51	28	326	4,829	±697	0.14
	59	23	260	3,851	±595	0.15
	55	24	706	10,458	±1,193	0.11
	56	23	236	3,496	±556	0.16
55-75 ug/g	60	24	380	5,629	±775	0.14
	54	26	501	7,421	±939	0.13

Source: ESE.

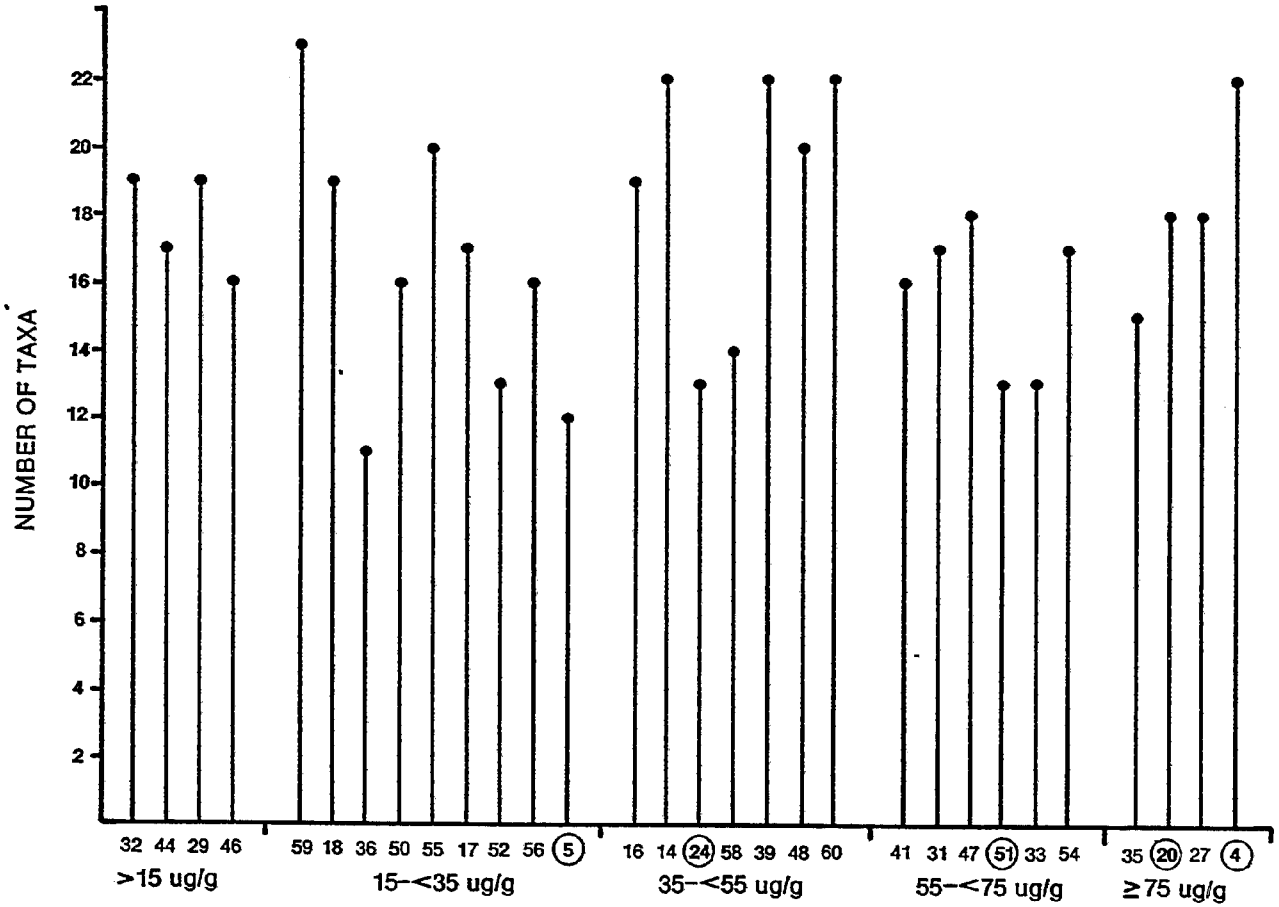
Community Composition--In the September 1982 collections, 67 individual taxonomic observations were made over the 30 stations. Of taxa encountered, 54 percent were polychaetes, 22 percent were crustaceans, and 9 percent were molluscs. The remaining 16 percent of the taxa consisted of hydrozoans, nemertines, sipunculids, phoronids, ectoprocts, ophiuroids, holothuroids, and tunicates.

In the May 1983 collections, 99 individual taxonomic observations were made over the 30 stations. Of these observations, 45 percent of the taxa were polychaetes, 25 percent were crustaceans, 17 percent were molluscs, and 3 percent were oligochaetes. The remaining 10 percent consisted of phoronids, ectoprocts, pycnogonids, echinoderms, tunicates, nemertines, turbellarians, cnidarians, and, on one occasion, chironomids.

Number of Taxa

The number of taxa per station in September 1982 ranged from 11 to 23. No discernible pattern concerning the number of taxa per station is evident when the stations are grouped by total hydrocarbons (Figure 4-17). The 5 stations (4, 5, 20, 24, 51) having excess metals included the station with the next lowest number of taxa (12 at Station 5) and ranged to a high of 22 taxa at Station 4; thus, no correlation of number of taxa versus high metal content was discerned.

The number of taxa per station in May 1983, ranged from 20 to 62. With respect to sediment hydrocarbon content, there is no readily apparent pattern of the number of taxa (Figure 4-18). The high number of taxa (62) encountered at Station 4 results from the substrate at the station. The bottom was composed of a high percentage of sand (87 percent) and also contained oyster shell. The sand/oyster shell creates habitat allowing more taxa to occupy the bottom. Twenty-four of the 62 taxa were encountered only at Station 4. The sabellid



NOTE: CIRCLED STATION NUMBERS INDICATE STATIONS WITH EXCESS METALS.

Figure 4-17
NUMBER OF TAXA PER STATION;
YORK RIVER, VIRGINIA, SEPTEMBER 1982

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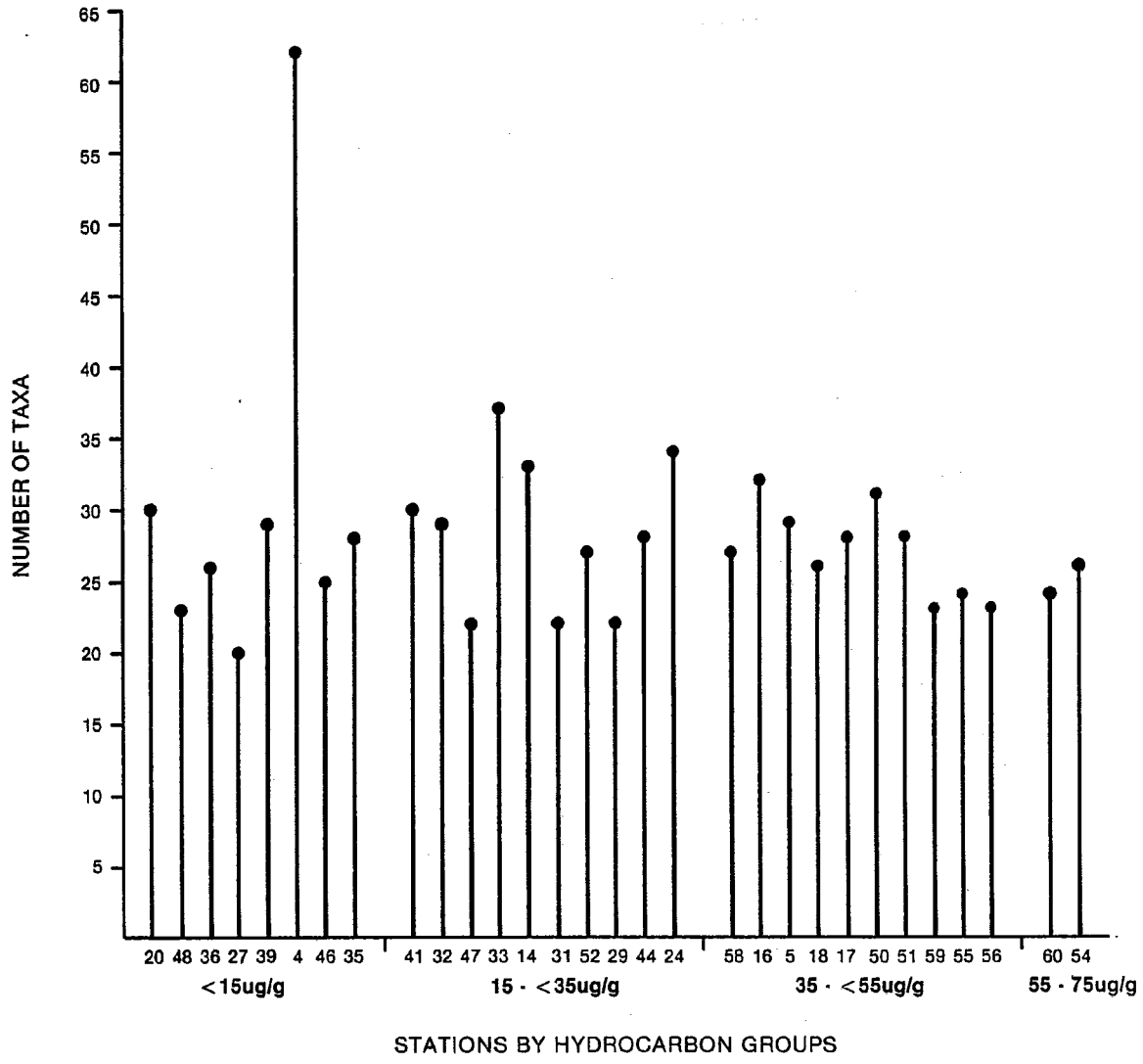

Figure 4-18
NUMBER OF TAXA PER STATION;
YORK RIVER, VIRGINIA, MAY 1983

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polychaete, Sabella micropthalma, numerically dominated Station 4 as a direct result of the oyster shell present. This is a large, tube-building polychaete which requires a firm substrate to which it attaches its tube.

Density of Individuals

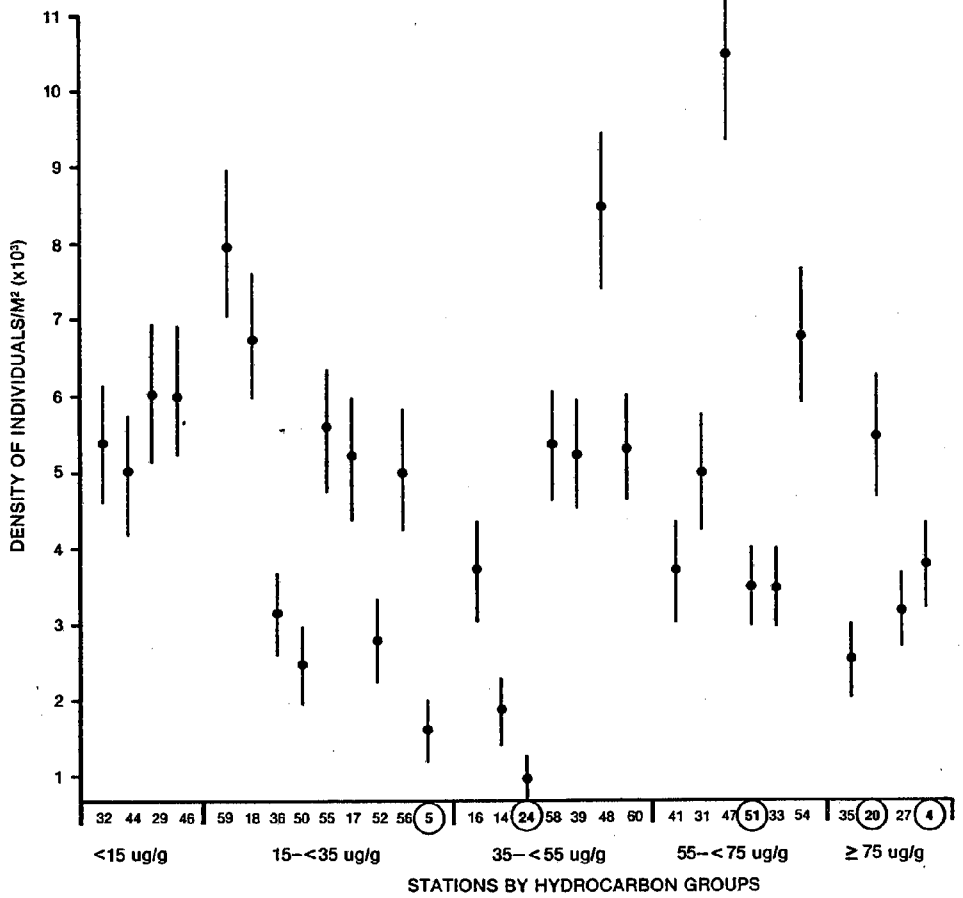
The mean density of individuals per square meter (no./m^2) in September ranged from a low of 978/m^2 at Station 24 to a high of 10,503/m^2 at Station 47. No pattern of density in relation to hydrocarbon levels within the sediments is readily apparent (Figure 4-19). The two lowest mean densities were calculated for Stations 5 and 24, both of which had an excess of metals present in the sediments.

The mean density of individuals per square meter in May ranged from a low of 2,637/m^2 at Station 27 to a high of 17,669/m^2 at Station 18. No pattern of density in relation to hydrocarbon levels within the sediments is readily apparent (Figure 4-20).

Densities encountered in May were generally higher than those encountered in September. Twenty-two of 30 stations had an increased density in May. The higher densities in May 1983 appear to be predominantly due to increased populations of the capitellid polychaete, Mediomastus ambiseta and oligochaete species.

Species Occurrence--The qualitative distribution of taxa among stations reveals essentially two groups of taxa: (1) taxa which are common and occur at a high percentage of the stations, and (2) relatively rare taxa which occur at few stations. This pattern exists for both benthos collections.

In September 1982, Ampelisca abdita, Paraprionospio pinnata, and Neomysis americana were found at all 30 stations. A total of 10 taxa occurred at 24 or more of the 30 stations. Six taxa occurred at 13 to 19 of the 30 stations. Fifty-one taxa occurred at less than 10 of the



NOTE: CIRCLED STATION NUMBERS INDICATE STATIONS WITH EXCESS METALS.

Figure 4-19
MEAN DENSITY OF INDIVIDUALS PER SQUARE METER (± STANDARD ERROR); YORK RIVER, VIRGINIA, SEPTEMBER 1982

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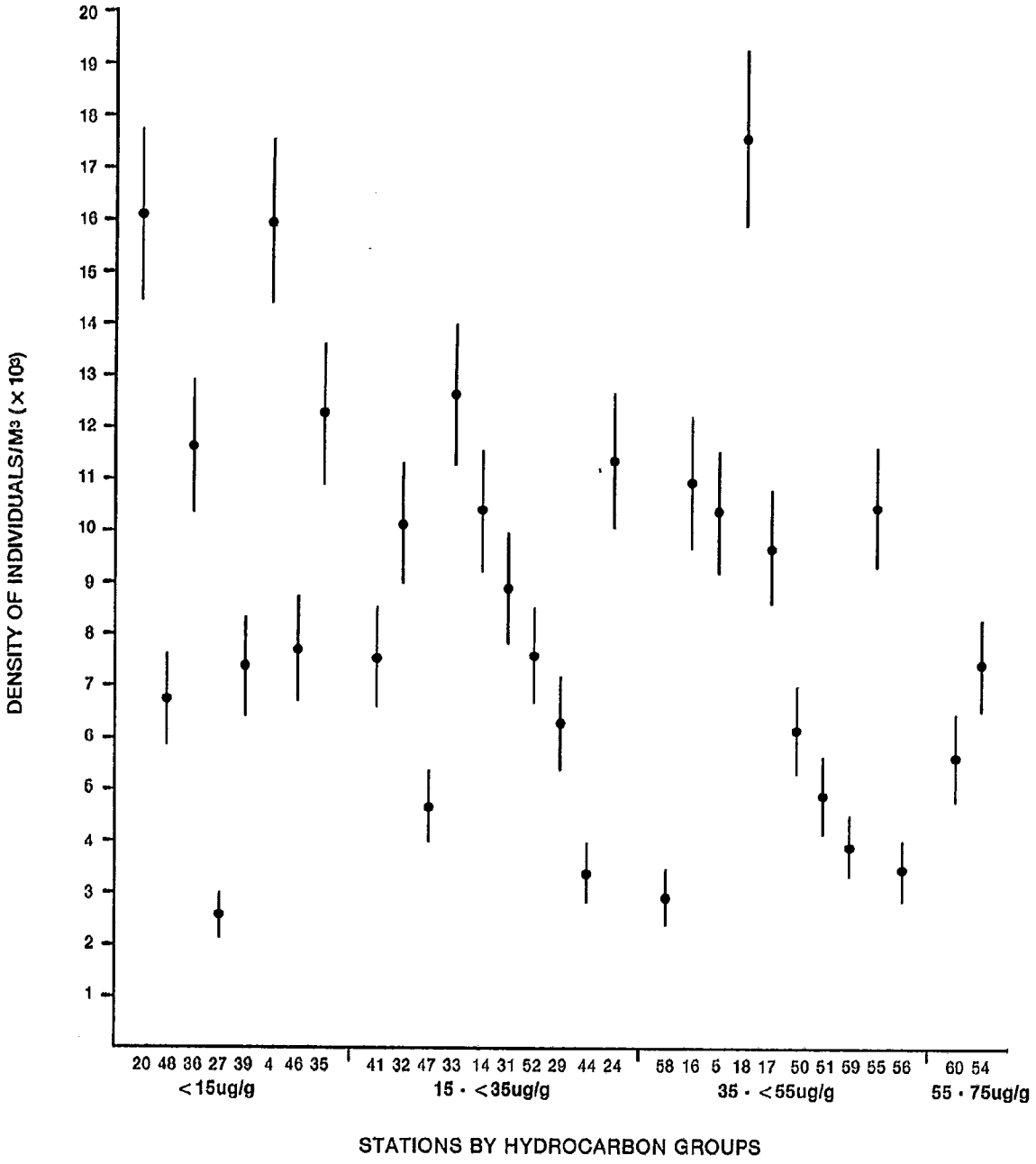

Figure 4-20
MEAN DENSITY OF INDIVIDUALS PER SQUARE METER (± STANDARD ERROR); NUMBER OF TAXA PER STATION; YORK RIVER, VIRGINIA, MAY 1983

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30 stations, with 20 of these being recorded at only 1 station each (Table B-6, Appendix B). In relation to hydrocarbon levels within the sediments, no pattern appears evident in the distributions of the less common taxa.

The dominant species collected in the September 1982 benthos samples was the spionid polychaete, P. pinnata. P. pinnata was the most abundant species at 23 of the 30 stations and was ranked second numerically at the remaining 7 stations. It accounted for 25.8 to 67.8 percent of total individuals across the 30 stations. The gastropod Acteocina canaliculata was also a commonly collected species and was numerically dominant at 6 stations; it also ranked second at 11 stations and ranked third at 6 stations. Ranked abundance tables for each station are contained in Appendix B.

In May 1983, 7 taxa (A. abdita, A. canaliculata, Eteone heteropoda, Leucon americanus, M. ambiseta, P. pinnata, and Scoloplos spp.) were collected from all 30 stations (Table B-7, Appendix B). An additional 14 taxa occurred at 20 to 29 of the 30 stations. Seventy-eight taxa occurred at less than 20 stations, 37 of which occurred at only 1 station.

The most commonly collected organism was M. ambiseta (Appendix B). It was numerically dominant at 26 of the 30 stations. Oligochaeta spp. numerically dominated 1 station and ranked second at 10 stations. P. pinnata, which numerically dominated the majority of stations in the September collections, was numerically dominant at 3 stations, ranked second at 12 stations, and ranked third at 4 stations.

Cluster Analysis

The dendrograms resulting from the cluster analyses are presented in Figure 4-21. All 30 stations from September form a fairly tight cluster with the highest dissimilarity at the 0.447 level, or conversely, all stations show a high degree of similarity (0.553). No distinct clusters appear in relation to hydrocarbon concentrations or levels of metals

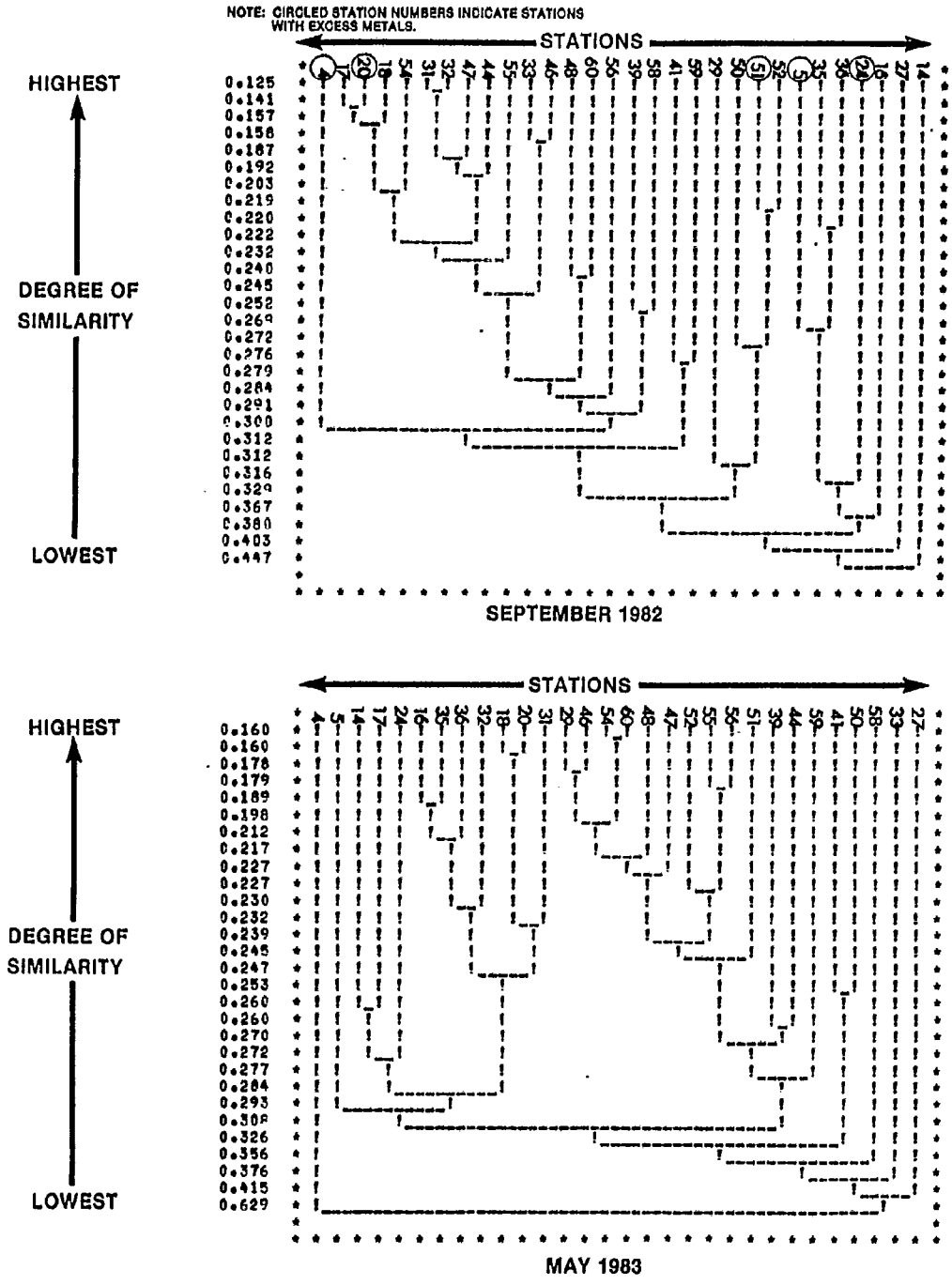

Figure 4-21
DENDROGRAMS PRODUCED FROM NORMAL CLASSIFICATION OF 30 BENTHOS STATIONS, YORK RIVER, VIRGINIA

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measured in the sediments. Stations linking together appear from the entire range of hydrocarbon concentrations measured, and stations with excess metals do not necessarily link together.

Dissimilarity of stations appears to be based primarily on distributions of infrequently occurring taxa rather than on gross differences in community composition. For example, Station 14 displays the greatest dissimilarity to the collection. The late clustering of this station results from the rare taxa which occurred at the station. From the total of 22 taxa recorded, six were found only at Station 14. These were found in very low numbers, generally equalling only one individual. These taxa were an unidentified sabellid polychaete, a barnacle (Balanus improvisus), an amphipod (Elasmopus levis), a xanthid crab (Rhithropanopeus harissi), an ectoproct species, and a tunicate (Molgula sp.).

All 30 stations from the May 1983 collections cluster at the 0.629 dissimilarity level, with Station 4 the last to link in the dendrogram. As previously mentioned, 24 taxa were unique to Station 4 as a result of the substrate, including the dominant organism, S. microphthalma. These unique taxa, the high number of taxa, and the dominant taxa at Station 4 result in the dissimilarity shown for this station.

The remaining 29 stations cluster at the 0.415 dissimilarity level (0.585 similarity level). No clustering of stations in relation to sediment hydrocarbon content is evident.

Two sub-clusters may be identified in the station linkages: sub-cluster I (endpoints Station 5 and Station 31) and sub-cluster II (endpoints Station 29 and Station 59). Sub-cluster I forms at the 0.293 dissimilarity level, and sub-cluster II forms at the 0.277 dissimilarity level. The two sub-clusters link at the 0.308 dissimilarity level. These two sub-clusters appear to relate to depth rather than to

hydrocarbons or metals, as the stations forming sub-cluster I are generally shallower than the stations forming sub-cluster II.

In general, cluster analyses performed on the September 1982 and May 1983 benthos collections show a low dissimilarity (high similarity) between benthic communities sampled at the individual stations. The most dissimilar stations result from the presence of some unique taxa at these stations, which are in turn attributable to substrate type.

Statistical Analysis--Regression analysis performed using benthic data and sediment chemistry data collected in September 1982 showed no significant negative correlations between the benthic community and/or hydrocarbon content and depth ($\alpha = 0.05$).

Stepwise regression analysis of station densities versus sediment metals content showed no significant negative correlation of density on metals. The first metal entered in the model was lead ($n = 30$, $\alpha = 0.05$, $P > 0.0517$). At the 0.05 significance level, this result is apparently almost significant. However, the model explains only 13 percent ($r^2 = 0.13$) of the variability in benthos populations. This result is primarily guided by low densities at three stations (24, 5, and 51) which had higher than average lead content. More stations with high lead content would be necessary to show whether lead was truly regulating the density of the benthic community.

Regression analysis on May 1983 data showed no significant correlation of benthos densities versus sediment hydrocarbon content ($n = 30$, $\alpha = 0.05$). Variability in benthos densities was due to factors other than sediment hydrocarbons, since sediment hydrocarbons accounted for only 6 percent ($r^2 = 0.06$) of benthos density variability. One factor which may be regulating benthos density is depth, since regression of density versus depth was significant ($P = 0.006$, $\alpha = 0.05$).

No significant negative correlations between individual species' densities versus sediment hydrocarbon content were observed in either May benthos collections.

Stepwise regression on May benthos densities versus sediment metal concentrations resulted in a significant correlation with a three variable model containing chromium, lead, and selenium ($n = 30$, $F = 5.38$, $P < 0.05$, $r^2 = 0.383$). The following table shows the slopes, F values, and probabilities for the three individual metals:

	B Value	F	Prob < F
Chromium	-0.01705558	7.8	0.0097
Lead	0.04347962	4.37	0.0466
Selenium	1.46002036	8.48	0.0073

Note that the slopes for lead and selenium are positive, indicating increased density with increasing metal concentrations. This correlation, therefore, does not indicate a negative environmental impact.

Comparisons With Historical Data--Previous studies conducted in the York River by the Virginia Institute of Marine Science (VIMS) and HRSD revealed a benthic community dominated by species which are referred to as euryhaline opportunists. These species are predominantly polychaetes and include, but are not limited to, P. pinnata, Nereis succinea, E. heteropoda, Glycinde solitaria, Scoloplos fragilis, and Tubificoides gabriellae. Appendix C tables show that the benthic community found in the present study is dominated by these same euryhaline opportunists as well as by several other ubiquitous species. These species dominate in the frequency of occurrence over the 30 stations and in their numerical abundance at the 30 stations.

Dominant Taxa

Comparison of the ranked abundances for the 30 stations (Appendix C) and the ranked abundance for HRSD stations (Table 4-7 and Figure 4-22)

Table 4-7. Rank Abundance of Dominant Species from York River, Virginia, September 1982

YA-1	% Total	YA-2	% Total	YA-3	% Total	YA-4	% Total
Paraprionspio pinnata	70.7	Paraprionospio pinnata	39.0	Paraprionospio pinnata	68.9	Paraprionospio pinnata	72.2
Acteocina canaliculata	12.7	Phoronis sp.	15.9	Acteocina canaliculata	8.2	Acteocina canaliculata	11.3
Eteone heteropoda	4.5	Acteocina canaliculata	11.8	Sigambra tentaculata	4.4	Tubificoides gabriellae	5.7
Ogyrides limicola	4.5	Mediomastus ambiseta	11.3	Tubificoides gabriellae	4.4	Sigambra tentaculata	2.5
Sigambra tentaculata	2.2	Glycinde solitaria	7.7	Eteone heteropoda	2.0	Eteone heteropoda	1.1
Neomysis americana	1.9	Cirratulidae sp.	3.1	Pyramidella spp.	1.8	Mediomastus ambiseta	1.1
	96.5	Pyramidella spp.	2.6	Neomysis americana	1.5		93.9
			91.4	Ogyrides limicola	1.3		
				Glycinde solitaria	1.3		
					93.8		

YB-1	% Total	YB-3	% Total	YC-1	% Total
Paraprionospio pinnata	76.2	Paraprionospio pinnata	83.5	Paraprionospio pinnata	78.9
Acteocina canaliculata	10.6	Sigambra tentaculata	3.5	Acteocina canaliculata	6.9
Eteone heteropoda	2.6	Acteocina canaliculata	3.0	Eteone heteropoda	2.9
Glycinde solitaria	1.6	Neomysis americana	2.6	Nereis succinea	2.9
Nereis succinea	1.6	Eteone heteropoda	2.0	Scoloplus robustus	2.2
Tubificoides gabriellae	1.6	Scoloplos robustus	1.5	Ogyrides limicola	2.2
Neomysis americana	1.6		96.1		96.0
	95.8				

YC-2	% Total	YC-3	% Total	YU	% Total
Paraprionospio pinnata	86.5	Paraprionospio pinnata	84.2	Paraprionospio pinnata	78.0
Pyramidella spp.	2.2	Sigambra tentaculata	4.6	Neomysis americana	4.0
Acteocina canaliculata	2.0	Eteone heteropoda	3.0	Pectinaria gouldii	1.9
Sigambra tentaculata	1.5	Acteocina canaliculata	2.5	Anadara transversa	1.6
Tubificoides gabriellae	1.3	Glycinde solitaria	2.5	Ancistrosyllis jonesi	1.6
Neomysis americana	1.3	Neomysis americana	1.1	Sigambra tentaculata	1.4
	94.5		97.9	Glycinde solitaria	1.3
				Ampelisca sp.	1.3
				Nereis succinea	1.1
					92.2

Source: HRSD, Unpublished data.

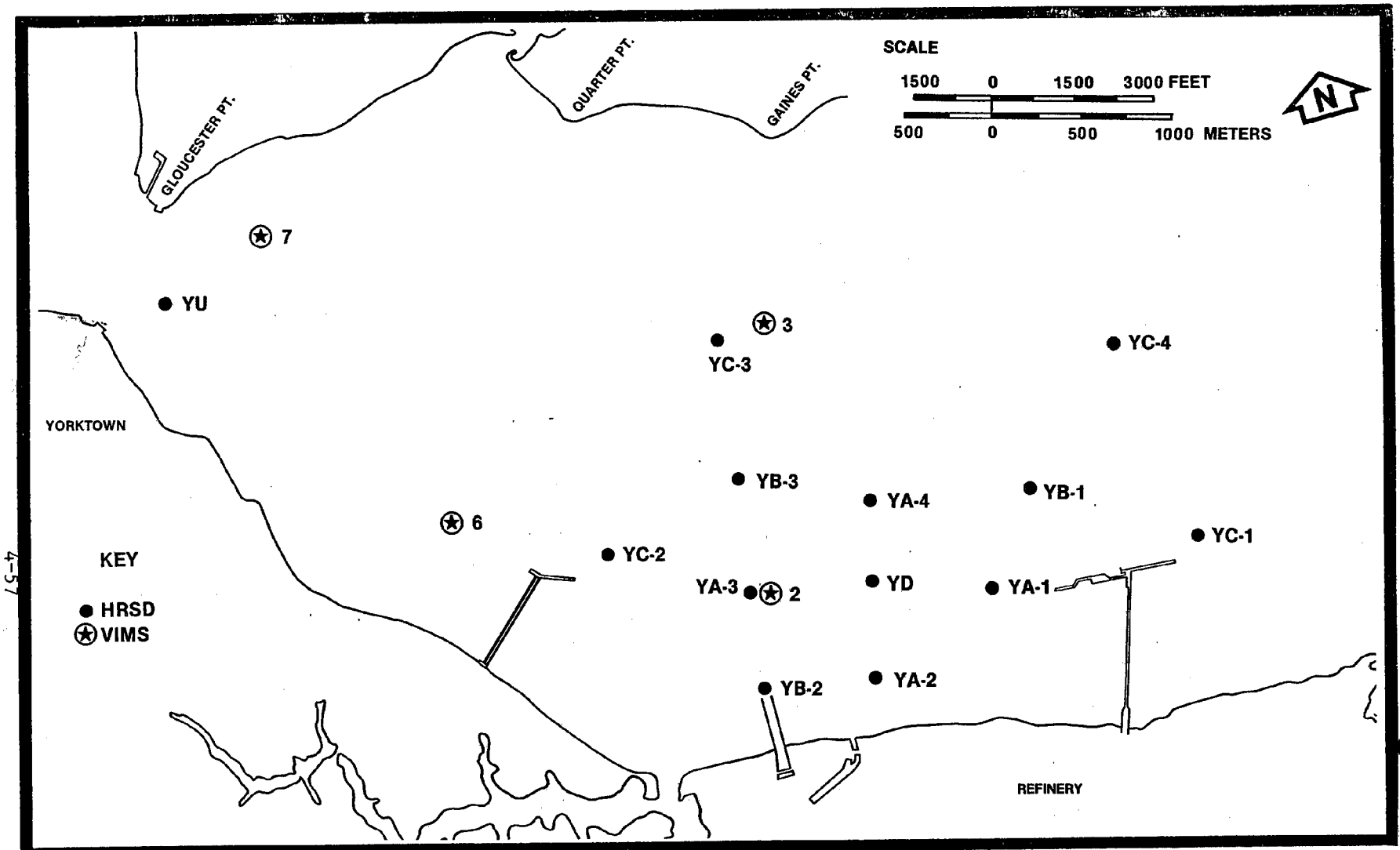

Figure 4-22
HAMPTON ROADS SANITATION DISTRICT (HRSD) AND VIRGINIA INSTITUTE FOR MARINE SCIENCE (VIMS) STATION LOCATIONS ON THE YORK RIVER, VIRGINIA

SOURCES: HRSD, 1981; JORDAN et al., 1975.

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show a nearly identical pattern of species abundances. P. pinnata numerically dominates at all HRSD stations and dominates at 23 of the 30 stations in the present study. In HRSD stations, P. pinnata generally comprises a greater percentage of total individuals than in the present study. The opisthobranch gastropod, A. canaliculata, ranked second at 5 of the 10 HRSD stations and ranked first or second at 17 of the 30 stations in the present study. The other dominant species at HRSD stations and the present study stations generally consist of the previously mentioned euryhaline opportunists and other ubiquitous species.

Comparison of HRSD May 1980 data (Table 4-8) and the May 1983 data of the present study (Appendix C) show several similarities. Both data sets are (1) dominated by the previously mentioned ubiquitous euryhaline opportunistic species, (2) show a decrease in the number of stations numerically dominated by P. pinnata as opposed to the respective September 1982 collections, (3) show a decrease in the percent composition of total individuals by P. pinnata as opposed to September 1982 collections, and (4) show an increased importance of oligochaetes (T. gabriellae in HRSD data) and capitellid polychaetes (Capitella capitata-HRSD, M. ambiseta-ESE) in May 1983 collections.

A further comparison with the study's dominant species as listed by Jordan et al. (1975) (Table 4-9) shows that all three data sets are very similar in the species which numerically dominate the York River benthic community. Comparison of dominant organisms of the York River with dominant organisms of previous studies in the Chesapeake Bay region shows great similarity among studies. The areas studied included a heavily industrialized estuary (Boesch, 1971, 1973; Hawthorne and Dauer, 1983), a residentially developed area (Tourtellotte and Dauer, 1983), a relatively pristine area (Ewing and Dauer, 1982), and a 9-meter mud habitat in the Calvert Cliffs region of upper Chesapeake Bay (Holland et al., 1977).

Table 4-8. Rank Abundance of Dominant Species from York River, Virginia, (HRSD Data) May 1980

YA-1	% Total
Paraprionspio pinnata	22.0
Acteocina canaliculata	15.0
Capitella capitata	15.0
Streblospio benedicti	9.6
Macoma balthica	5.0
Glycinde solitaria	4.3
Mulinia lateralis	4.3
Tubificoides gabriellae	4.0
Sigambra tentaculata	3.6
Eteone heteropoda	3.3
	86.1

YA-2	% Total
Steblospio benedicti	26.0
Mya arenaria	24.0
Polydora ligni	21.0
Capitella capitata	5.4
Eteone heteropoda	4.5
Tubificoides gabriellae	2.9
Neomysis americana	2.0
Glycinde solitaria	1.8
Paraprionospio pinnata	1.6
Gammarus mucronatus	1.3
	69.5

YA-3	% Total
Capitella capitata	23.0
Streblospio benedicti	13.5
Paraprionospio pinnata	12.3
Tubificoides gabriellae	12.2
Acteocina canaliculata	7.6
Nereis succinea	4.6
Polydora ligni	4.1
Eteone heteropoda	3.8
Mulinia lateralis	3.3
Macoma balthica	2.5
	86.9

YA-4	% Total
Capitella capitata	30.0
Acteocina canaliculata	19.5
Tubificoides gabriellae	16.2
Mulinia lateralis	11.6
Paraprionospio pinnata	6.4
Streblospio benedicti	5.6
Eteone heteropoda	2.1
Glycinde solitaria	1.2
Leucon americanus	1.2
	93.8

YB-1	% Total
Acteocina canaliculata	20.2
Amygdalum papyrium	16.5
Paraprionospio pinnata	12.8
Neomysis americana	6.2
Eteone heteropoda	4.9
Ogyrides limicola	4.5
Pyramidella sp.	4.1
Glycinde solitaria	3.7
Macoma balthica	2.9
	75.8

YB-3	% Total
Capitella capitata	37.5
Tubificoides gabriellae	15.6
Acteocina canaliculata	13.2
Mulinia lateralis	9.4
Streblospio benedicti	7.4
Paraprionospio pinnata	5.9
Glycinde solitaria	1.3
Eteone heteropoda	1.2
Macoma balthica	1.2
Amygdalum papyrium	1.2
	93.9

YC-1	% Total
Paraprionospio pinnata	29.8
Acteocina canaliculata	29.4
Nereis succinea	7.7
Capitella capitata	6.4
Polydora ligni	4.7
Glycinde solitaria	3.3
Streblospio benedicti	2.7
Eteone heteropoda	2.7
Mulinia lateralis	2.3
Leucon americanus	1.7
Pectinaria gauldii	1.7
	92.4

YC-2	% Total
Paraprionospio pinnata	45.7
Capitella capitata	9.7
Acteocina canaliculata	8.4
Eteone heteropoda	5.0
Sigambra tentaculata	4.7
Glycinde solitaria	3.9
Streblospio benedicti	3.1
Corophium tuberculatum	2.6
Leucon americanus	1.8
Asabellides oculata	1.6
Mulinia lateralis	1.6
	88.1

VC-3	% Total
Paraprionospio pinnata	24.1
Capitella capitata	24.1
Mulinia lateralis	11.9
Streblospio benedicti	11.1
Tubificoides gabriellae	6.4
Acteocina canaliculata	5.0
Glycinde solitaria	4.2
Eteone heteropoda	3.6
Sigambra tentaculata	1.9
Polydora ligni	1.4
Ancistrosyllis jonesi	1.4
	95.1

YU	% Total
Paraprionospio pinnata	25.0
Mulinia lateralis	15.6
Tubificoides gabriellae	12.2
Capitella capitata	9.9
Leucon americanus	7.7
Acteocina canaliculata	3.6
Monoculodes edwardsi	3.6
Streblospio benedicti	3.3
Glycinde solitaria	3.3
Molgula manhattensis	2.3
	86.5

Source: HRSD, Unpublished data.

Table 4-9. Dominant Species in Muddy Sediments from York River, Virginia, in Order of Decreasing Importance as Found by Jordan et al. (1975)

Paraprionospio pinnata (P)
Pseudeurythoe sp. (P)
Acteocina canaliculata (G)
Glycinde solitaria (P)
Sigambra tentaculata (P)
Heteromastus filiformis (P)
Leucon americanus (Cu)
Ogyrides limicola (D)
Phoronis muelleri (Ph)
Nephtys incisa (P)

Key: Cu = cumacean
G = gastropod
P = polychaete
Ph = phoronid
D = decapod crustacean

Source: Jordan et al., 1975.

Diversity

Shannon-Weaver diversities (Shannon and Weaver, 1963) in the present study (Table 4-10) showed little variation over the 30 stations. In September 1982, the index ranged from 1.77 to 3.06. The low value (1.77 at Station 36) results from this station having the fewest taxa and an uneven distribution heavily skewed towards P. pinnata.

In May 1983, the index ranged from 2.43 to 4.35. The high value (4.35) is again due to the high number of taxa recorded at Station 4. Diversity values are generally slightly higher for the May 1983 benthos collections, due primarily to the higher number of taxa recorded in May 1983 rather than the evenness component of the benthos distributions.

Shannon-Weaver diversities calculated by HRSD for September 1981 (see Table 4-11) also showed little variation over their mud stations, but were generally lower than those calculated in the present study. This difference results from the greater percentage of total individuals P. pinnata in the HRSD samples as opposed to the present study. The evenness of samples was higher in the September 1982 collections in the present study than in the HRSD study (see Table 4-11). The high diversity values which appear in the HRSD data (Stations YD, YB-2, YC-4) result from the fact that these stations were located in sandy substrates as opposed to high silt/clay substrates and showed a greater species richness. Boesch (1972) and others have found higher species richness and thus diversity in sandy sediments compared to high silt/clay sediments.

Diversity values for HRSD May 1980 (Table 4-12) calculations are similar to those for the present May 1983 collection and show the same trend of higher diversity due to a greater species richness component.

Table 4-10. Shannon-Weaver Diversity Index and Evenness for York River Benthos, September 1982 and May 1983

Station No.	Diversity		Evenness	
	September 1982	May 1983	September 1982	May 1983
4	2.75	4.35	0.62	0.73
5	2.59	3.08	0.72	0.63
14	3.06	3.38	0.69	0.67
16	2.46	2.98	0.58	0.60
17	2.61	3.10	0.64	0.64
18	2.81	2.73	0.69	0.58
20	2.69	2.53	0.64	0.52
24	2.67	3.06	0.72	0.60
27	2.23	3.20	0.53	0.74
29	2.44	2.97	0.58	0.67
31	2.85	2.44	0.70	0.55
32	2.64	2.81	0.62	0.58
33	2.67	3.04	0.72	0.58
35	2.31	2.43	0.59	0.51
36	1.77	2.63	0.51	0.56
39	2.54	3.08	0.57	0.64
41	2.15	2.77	0.54	0.57
44	2.25	3.48	0.55	0.72
46	2.42	2.82	0.60	0.61
47	2.39	2.93	0.57	0.66
48	2.10	3.12	0.49	0.69
50	2.72	2.98	0.68	0.60
51	2.32	3.26	0.63	0.68
52	2.17	3.10	0.59	0.65
54	2.61	3.00	0.64	0.64
55	2.72	2.69	0.63	0.59
56	2.13	3.18	0.53	0.70
58	2.35	3.14	0.62	0.66
59	2.51	3.27	0.56	0.72
60	2.20	2.83	0.49	0.62

Source: ESE.

Table 4-11. Community Parameters for HRSD Stations, September 1981

Site	No. of Species	No. of Individuals	Individuals/ m^2 (±SE)	Shannon-Weaver Diversity	Even-Ness
YD*	32	227	1,455 (±302)	3.55	0.71
YA-1	12	267	1,712 (±338)	1.61	0.45
YA-2	18	195	1,250 (±272)	2.84	0.68
YA-3	22	453	2,904 (±489)	2.00	0.45
YA-4	21	353	2,263 (±411)	1.68	0.38
YB-1	14	189	1,212 (±266)	1.47	0.39
YB-2*	38	379	2,429 (±432)	3.79	0.72
YB-3	18	535	3,429 (±549)	1.18	0.28
YC-1	11	275	1,763 (±345)	1.34	0.39
YC-2	19	453	2,904 (±489)	1.06	0.25
YC-3	11	436	2,795 (±476)	1.07	0.31
YC-4*	37	374	2,397 (±428)	2.69	0.52
YU	27	696	4,461 (±659)	1.67	0.35

*Indicates stations with predominantly sand-size sediments.

Source: HRSD (unpublished data).

Table 4-12. Community Parameters for HRSD Stations, May 1980

Site	No. of Species	No. of Individuals	Individuals/ m^2 (±SE)	Shannon-Weaver Diversity	Even-Ness
YD*	27	261	1,673 (±333)	3.86	0.81
YA-1	23	303	1,942 (±369)	3.56	0.79
YA-2	29	445	2,853 (±483)	3.12	0.64
YA-3	33	608	3,897 (±600)	3.70	0.73
YA-4	25	518	3,321 (±537)	3.01	0.65
YB-1	32	243	1,558 (±317)	3.97	0.79
YB-2*	40	824	5,282 (±741)	3.96	0.74
YB-3	26	598	3,833 (±593)	2.97	0.63
YC-1	24	299	1,917 (±366)	3.11	0.68
YC-2	33	383	2,455 (±435)	3.21	0.64
YC-3	22	361	2,314 (±417)	3.20	0.72
YC-4*	41	696	4,462 (±659)	3.58	0.67
YU	29	392	2,513 (±442)	3.62	0.74

*Indicates stations with predominantly sand-size sediments.

Source: HRSD (unpublished data).

Number of Taxa

In terms of number of taxa encountered per station, the HRSD data and data of the present study are very similar. September 1982 counts ranged from 11 to 27 taxa at mud stations in the HRSD study and from 11 to 23 taxa in the current ESE study. May 1983 counts ranged from 22 to 33 taxa at mud stations in the HRSD study and from 20 to 37 taxa (excluding 62 taxa at Station 4) in the present study. The number of taxa collected by Jordan et al. (1975) from mud stations ranged from 6 to 41 taxa but was generally between 11 and 25 taxa.

Density

The mean number of individuals per square meter found by HRSD (Tables 4-11 and 4-12) was generally slightly lower than densities found in the present study, but of the same order of magnitude, with a range from 1,212/m^2 to 4,461/m^2 in September 1982 and a range of 1,558/m^2 to 5,282/m^2 in May 1983.

4.3.2 Artificial Substrates

Mean water temperature and salinity at the times of panel collections are shown in Figure 4-23. Water temperature remained fairly constant through the first three collection periods and then decreased from October to December. Salinity remained within the upper mesohaline to lower polyhaline range throughout the study period.

Panels were originally placed at nine locations around the Amoco refinery in May. During the September collection period, panel arrays at Locations F through I were lost, and the study was continued with Locations H through E only. At the time of the December collection, time series panels at Location C were no longer in place.

Monthly Recruitment Panels--

Number of Taxa

Numbers of primary fouler taxa (Figure 4-24) ranged from a low of zero taxa (Trip 5) to a high of eight taxa (C2, Trip 1). Generally, the

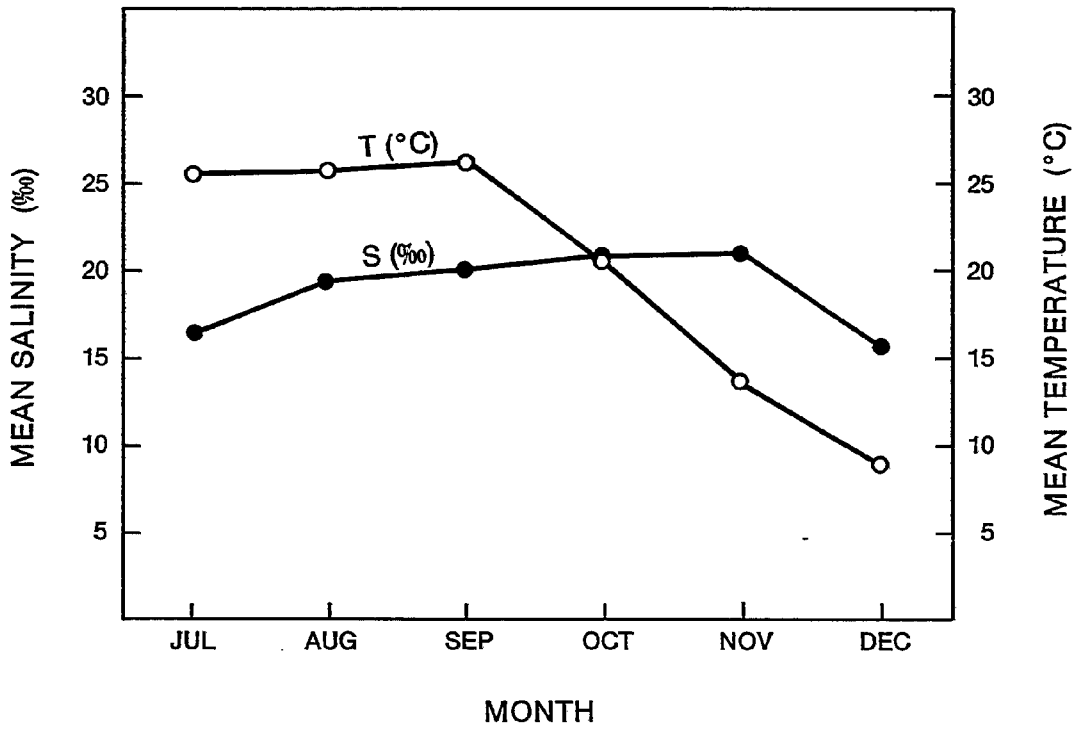

Figure 4-23
MEAN SALINITY AND TEMPERATURE AT TIMES OF ARTIFICIAL SUBSTRATE RETRIEVAL, 1983

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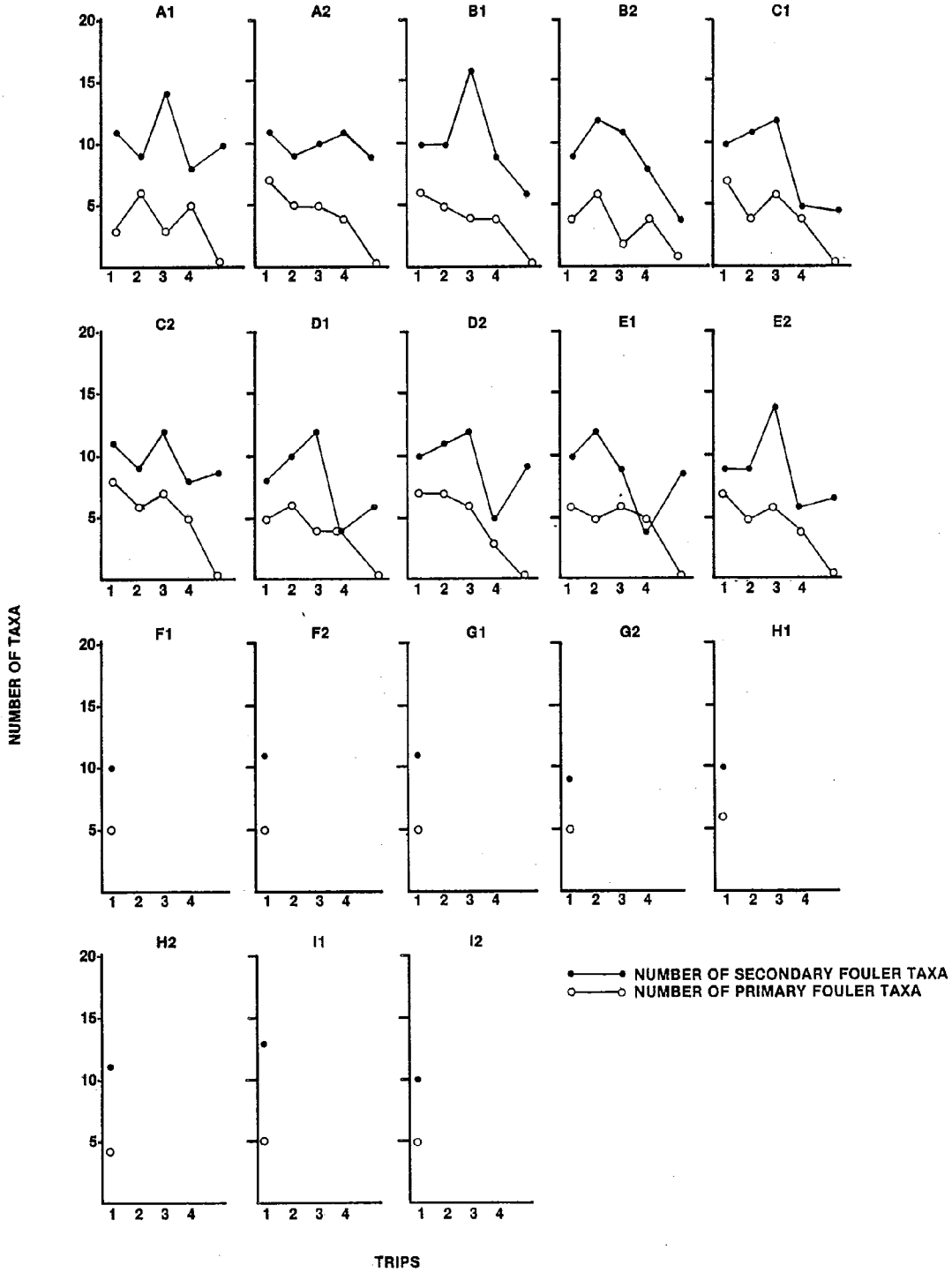

Figure 4-24
NUMBERS OF PRIMARY AND SECONDARY FOULING TAXA RECORDED ON MONTHLY RECRUITMENT PANELS, 1983

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number of primary fouler taxa was between four and seven. There is no apparent pattern relevant to the number of primary fouler taxa between panels during the same collection date or across the first four collection dates. For example, Panels A1 and A2 contained 3 taxa (A1) and 7 taxa (A2) on the first collection date. Across dates the number of taxa on Panel A1 increased and decreased whereas the number of taxa on Panel A2 decreased from Trip 1 to Trip 4. On the first collection date, numbers of primary taxa on panels around the Amoco refinery pier (Panels A through E) were comparable to panels (F through I) located upstream and downstream of the pier. There was little or no recruitment of primary fouling taxa during the November to December recruitment period.

The numbers of secondary fouler taxa (Figure 4-24) ranged from a low of four taxa to a high of 16 taxa. There is no apparent pattern to the number of secondary fouler taxa between panels within the same collection period. All panels except A2 showed a decrease in the number of taxa from the third to fourth collection period. This decrease coincides with decreasing water temperature and decreasing recruitment of primary fouler taxa. Field (1982) found that the number of secondary fouler species was dependent on the morphological heterogeneity of the primary fouler assemblage.

Percent Cover

Generally, either Membranipora spp. (encrusting ectoproct) and/or Molgula manhattensis (solitary tunicate) dominated recruitment panels in August (Collection 1; Figure 4-25). After the first collection, Membranipora spp. cover declined to low levels and panels became dominated by M. manhattensis in both the second and third recruitment periods. The only exception to this pattern was at Location E, where Balanus spp. (barnacles) dominated panels in the second collection period. On Panel F1, Hydrozoa sp. A comprised a significant percent cover in addition to barnacles. On Panel E2, M. manhattensis comprised 30 percent of the cover.

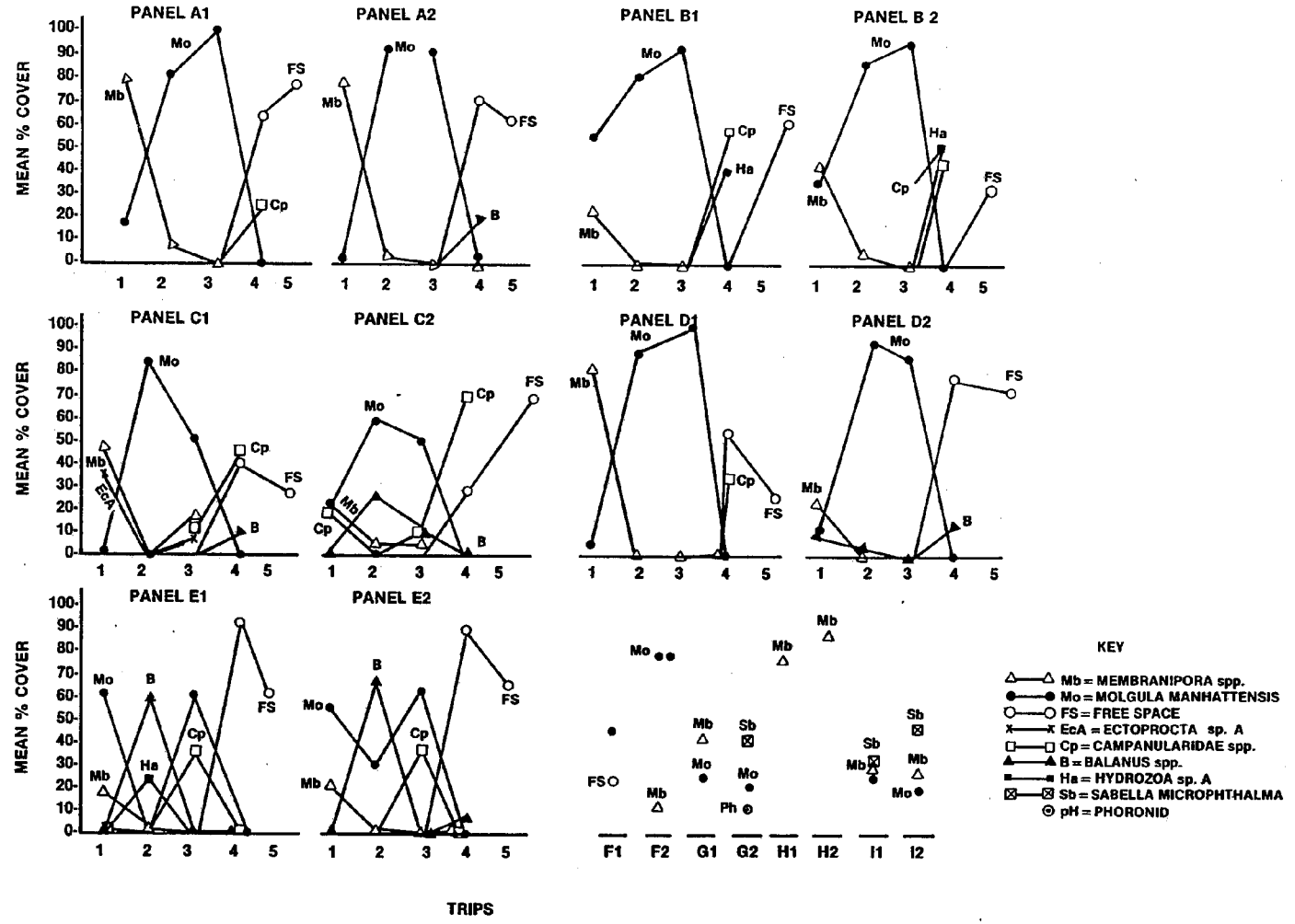

Figure 4-25
MEAN PERCENT COVER WITH DOMINANT PRIMARY FOULER TAXA ON MONTHLY RECRUITMENT PANELS, 1983

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Primary fouler species recruitment dropped off on the November 1983 (Trip 4) panels, with free space (bare panel) predominating. The only panels on which free space was not prevalent were those at Location B, which were almost entirely covered by hydrozoans (Campanularidae spp. and Hydrozoa sp. A). The Campanularidae spp. were also important on Panels A1, C1, C2, and D1.

During the November 1983 to December 1983 recruitment period, there was little or no settlement of primary fouling species. Panels were predominantly free space and/or covered with a thin layer of algae (Appendix B). Algae which occurred on panels included a filamentous blue-green (Spirulina sp.), a filamentous green, and diatoms. The lack of invertebrate settlement coincides with reduced water temperatures and decreased reproduction during the winter. Balanus spp. Campanularidae, and Ectoprocta were found on the panels, but only in very low numbers or percent coverage.

Qualitative Species Distributions--Seventeen taxa of secondary foulers were recorded on monthly recruitment panels during the first collection period (Appendix C). Six taxa (N. succinea, Polydora ligni, S. microphthalma, harpacticoid copepods, Corophium spp., and Stenothoe sp.) were recorded on all panels. Stylochus ellipticus was recorded on all panels except A2. Melita nitida was recorded from all locations except Location E, and Pleusymtes glaber occurred at all locations except B. The remaining taxa had sporadic occurrences with no apparent pattern.

Eighteen taxa were recorded on the second set of recruitment panels (Appendix C). Six taxa (S. ellipticus, P. ligni, S. microphthalma, harpacticoid copepods, Corophium spp., and Stenothoe sp.) were recorded on all panels. N. succinea and M. nitida were recorded at all locations but not on all panels. The occurrences of the remaining taxa were sporadic.

Twenty-two taxa were recorded on Trip 3 recruitment panels (Appendix C), and four (N. succinea, P. ligni, harpacticoid copepods, and Stenothoe sp.) were recorded on all panels. S. ellipticus, S. microphthalma, Nudibranchia sp. A, Corophium spp., Pycnogonida sp., and M. nitida were recorded at all locations.

Fifteen taxa were recorded on Trip 4 recruitment panels (Appendix C). Harpacticoid copepods, Corophium spp., P. glaber, Stenothoe sp., and Caprellidae spp. were recorded at all locations.

Sixteen taxa were recorded on Trip 5 recruitment panels (Appendix C). The number of taxa recorded from any one panel ranged from 4 to 10. Harpacticoid copepods, Balanus spp., Corophium spp., Stenothoe sp., and Caprellidae spp. were recorded from all locations. Polydora ligni was recorded from all locations except Location B next to the refinery discharge. The remaining taxa were recorded only sporadically. Harpacticoid copepods and amphipods were the most commonly occurring animals. Qualitatively, the animals found on the December panels were similar to the previous collections, but numbers of organisms were reduced due to the onset of winter and the lack of a well-developed primary fouler assemblage.

The qualitative distributions of secondary fouling taxa showed essentially two groups of taxa. One group was ubiquitous over the artificial substrates, and the second group occurred sporadically. The ubiquitous taxa, particularly harpacticoid copepods and the amphipods, were generally the numerically dominant taxa on artificial substrate panels (see rank abundance tables in Appendix C).

Statistical Analysis--Plots of standard deviation versus the mean showed that in most cases the log transformation of both the primary and secondary fouler taxa was adequate to meet the assumptions of ANOVA. The only case in which the log transformation did not distribute the standard deviation versus the mean totally adequately was for Trip 4

secondary foulers. In this case the ANOVA can be considered only approximate. In no case did ANOVA and Duncan's multiple range test detect a significant difference ($a = 0.05$) in the fouling community between locations of panels around the Amoco refinery discharge.

Time Series Panels--

Number of Taxa

Numbers of primary fouler taxa ranged from one to eight over all panels and trips (Figure 4-26). The lowest number of taxa was generally recorded during the first collection period when barnacles dominated the panels. After the first collection period, the diversity of primary fouler taxa increased on all panels and generally ranged between five and seven taxa across all panels and trips. There was no apparent relation between number of primary fouler taxa and location.

The numbers of secondary fouler taxa ranged from 6 to 17 over all panels and trips (Figure 4-26). There was no apparent pattern between the number of secondary fouler taxa and location.

Percent Cover

Mean percent cover estimates of dominant primary fouler taxa are illustrated in Figure 4-27. In general, percent cover of primary foulers follows a similar pattern over all locations. During the first collection period, Balanus spp. dominated the artificial substrates, then declined in percent cover as they became overgrown by M. manhattensis, S. microphthalma, and Membranipora spp.

Otsuka and Dauer (1982) noted fall slough-offs of M. manhattensis from artificial substrates. These slough-offs of M. manhattensis as well as slough-offs of Sabella microphthalma also occurred in the present study. These slough-offs can be seen in Figure 4-27 as the rapid reductions in M. manhattensis and/or S. microphthalma percent cover which occur between Trips 3-4 or Trips 4-5.

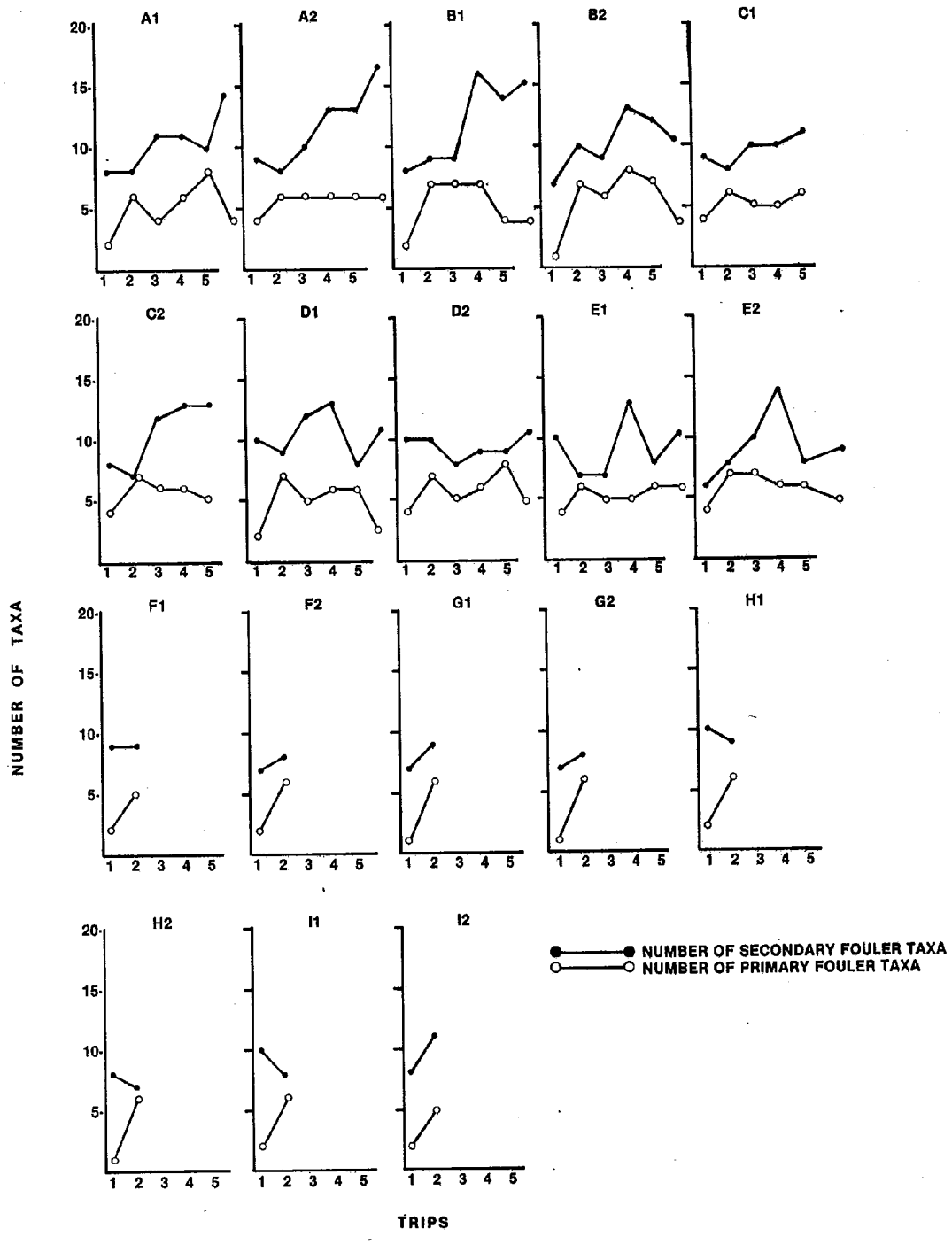

Figure 4-26
NUMBERS OF PRIMARY AND SECONDARY FOULER TAXA RECORDED ON TIME SERIES PANELS, 1983

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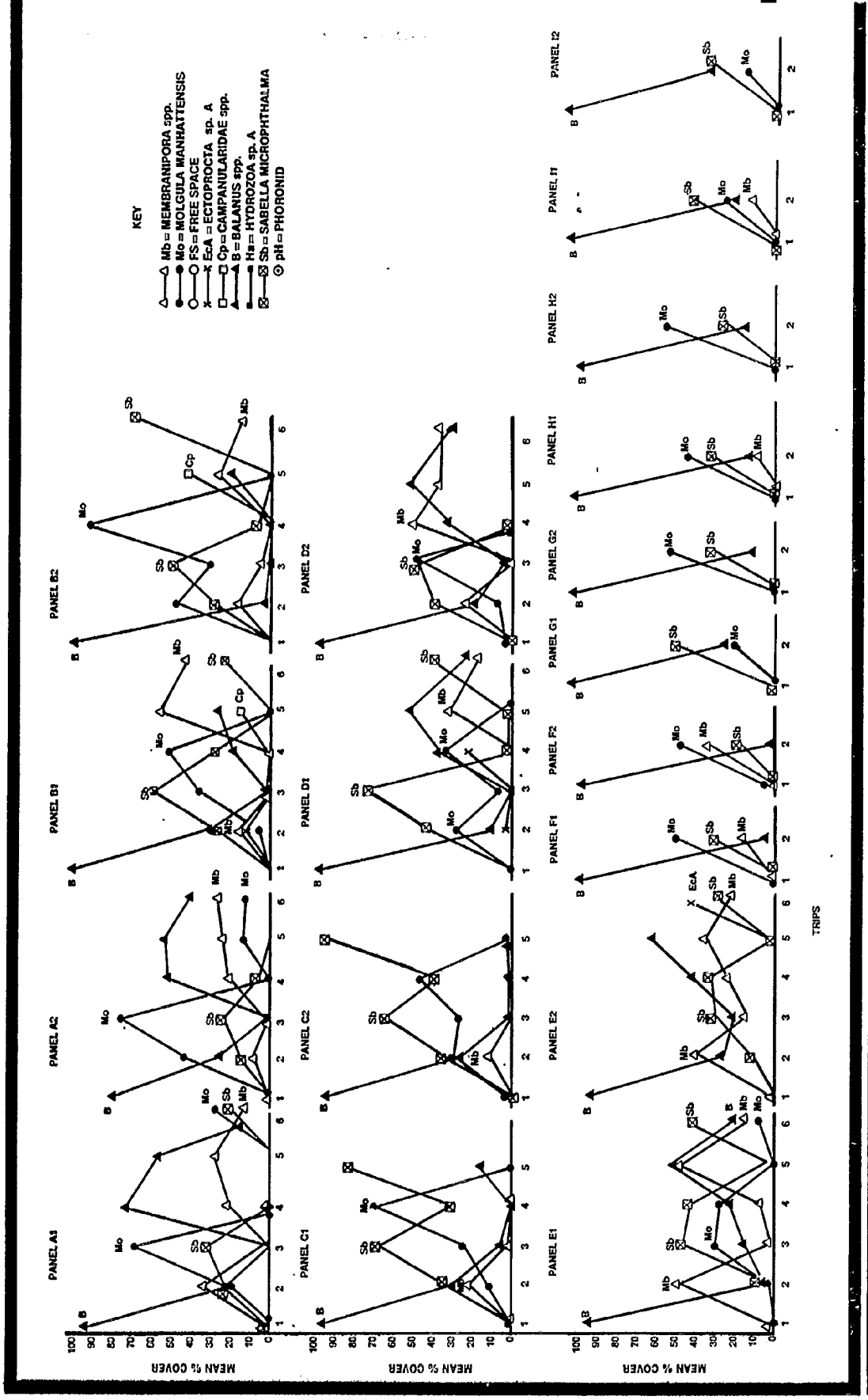

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Figure 4-27
MEAN PERCENT COVER OF DOMINANT PRIMARY
FOULER TAXA ON TIME SERIES PANELS, 1983

Qualitative Species Distributions--Seventeen taxa of secondary fouler taxa were recorded on panels during the first collection period (Appendix C). Four taxa (S. ellipticus, N. succinea, P. ligni, and Corophium spp.) were found on every panel, and harpacticoid copepods and P. glaber were recorded at all locations. The remaining taxa were less frequently collected and showed no apparent pattern in their distribution over the panels.

Fourteen taxa of secondary foulers were recorded during the second collection period. N. succinea, P. ligni, S. microphthalma, harpacticoid copepods, Corophium spp., and Stenothoe sp. were recorded from every panel. S. ellipticus was recorded from every location except E, and M. nitida was recorded from every location except C. No discernable pattern existed in relation to the occurrences of the rare taxa.

Seventeen taxa of secondary foulers were recorded during the third collection period, with P. ligni, S. microphthalma, harpacticoid copepods, Corophium spp., and Stenothoe sp. occurring on all panels. S. ellipticus, N. succinea, and M. nitida were recorded at all locations.

Seventeen taxa of secondary foulers were recorded during the fourth collection period. Seven taxa occurred on all panels and an additional four taxa occurred at all locations. Ampharetidae sp. was recorded at all locations except D, and Pycnogonida sp. was recorded at all locations except E.

Nineteen secondary fouler taxa were recorded during Collection 5. Four taxa were recorded on all panels, and five additional taxa were recorded at all locations.

Twenty two taxa were recorded from artificial substrates in the December collection. Eight taxa were recorded at all locations, and pycnogonids

were recorded at all locations except D. Harpacticoids, amphipods, and polychaetes were the most commonly and widely occurring organisms. The cnidarious and molluscs occurred sporadically.

The secondary fouler assemblage recorded on time series panels was almost identical to the secondary fouler assemblage recorded on monthly recruitment panels. There was a ubiquitous group of taxa which occurred at all locations and during all collection periods, and a rarer group of taxa which occurred sporadically within collection periods.

The ubiquitous taxa were N. succinea, P. ligni, harpacticoid copepods, Corophium spp., and Stenothoe sp., and the latter three were generally the numerically most abundant organisms. S. ellipticus occurred at the majority of locations in the early collection periods, but became rare in the November and December collections. Nudibranchia sp.A. and Doridella obscura were rarely found at all locations within a trip, but did appear at all locations through the course of the study. Caprellid amphipods and pycnogonids were rare in the early collections and became widespread in the latter collection periods.

Statistical Analysis--Plots of standard deviation versus the mean showed that in all cases except Trip 4 secondary foulers, the log transformation was adequate for the data to meet the assumptions of ANOVA. In this one case, the ANOVA can only be considered approximate. In no case did ANOVA and Duncan's multiple range test detect a significant difference (= 0.05) in the fouling community between locations of panels around the Amoco refinery discharge.

4.3.3 Conclusions

Analyses of the number of taxa, the density of individuals, and the qualitative distributions of infaunal benthic taxa across stations revealed no readily apparent patterns with respect to sediment hydrocarbon concentrations or sediment metals concentrations. Linear regressions of benthos densities versus sediment hydrocarbon concentrations produced no significant regressions and was unable to

detect negative impacts of sediment hydrocarbon concentrations on the benthic community. Stepwise regression of benthos densities versus sediment metals content did not produce a significant regression for September 1982 data. In this regression lead was almost significant due to high lead concentrations at three stations. To detect whether lead concentrations had an effect on benthos densities, more stations with a wider range of lead concentrations were necessary. Stepwise regression of benthos densities versus sediment metals on May 1983 data produced a significant regression with a three variable model including chromium, lead, and selenium. Chromium had a negative slope whereas lead and selenium had positive slopes. Comparison of benthic data from the present study and historical data showed that taxa which dominated the benthic community have remained relatively stable over the last decade.

Analyses of the fouling community which colonized artificial substrates located around the effluent discharge revealed no major differences in the community with respect to station location. Artificial substrates were analyzed for both primary and secondary fouling community composition. The one-way analysis of variance which was performed on fouling community components by trip did not show a significant difference in the community with respect to location around the discharge.

Both infaunal benthos and fouling community studies were designed to investigate the impact of the refinery effluent in the natural environment. Neither set of studies was able to detect negative impacts in the field.

4.4 BIOASSAYS

Sheepshead minnows, mysid shrimp, and daphnids appeared to be vigorous and in good physical condition during acclimation and at the beginning of all tests. All laboratory-culture test animals were fed daily during acclimation and testing periods due to their young age (EPA, 1978). Field-collected animals were not fed during testing.

Tables D-1 and D-2 (Appendix D) summarize the onsite characterization of the dilution water samples used for the aquatic toxicity tests. DO remained at 6.7 mg/L or higher at all times; this level of DO is acceptable. The river water temperatures ranged from 8 to 14°C during March 1982 and from 23 to 25°C during June and July 1982. The dilution water pH range was from 7.5 to 8.0 and showed no obvious trends of variability. Salinity range for dilution water samples was from 16 to 20 ppt. Table D-3 presents the in-depth chemical characterization of the dilution water conducted in March and June 1982. The first sample was screened for an extensive list of water parameters, and the subsequent sample was then analyzed for a smaller list of relevant parameters (Table D-3). The chemical analysis showed the dilution water to be acceptable for bioassay testing use. This conclusion was confirmed by the successful culture and reproduction of mysid shrimp in this dilution water as well as by the excellent survival of all animals during acclimation and in the controls.

The life cycle test lasted 26 days because there was no wastewater flow following a treatment plant upset on the 27th testing day. This did not affect the results because the minimum testing period had been met.

Bioassays were conducted primarily on treated process water (Outfall 101) because this is the main NPDES sampling and monitoring point. As discussed previously, the process water mixes with the non-contact cooling water (Outfall 201) to form the final plant effluent (Outfall 001). There is no NPDES monitoring of Outfall 001 nor an appropriate sampling point at this site. Based on these factors, most

bioassays were conducted on Outfall 101 with some testing of Outfalls 201 and 001 for completeness (Figure 4-28). Full strength process water was not tested using estuarine species because they are unable to survive in fresh water.

4.4.1 Reconnaissance Tests

Ninety-six-hour acute flow-through bioassays were conducted using mysid shrimp (Mysidopsis bahia) and sheepshead minnows (Cyprinodon variegatus) at the Amoco plant (Outfall 101) during the reconnaissance period (March 1982) of this study. Survival data for these tests are shown in Table 4-13. The only control mortality occurring during testing was the 10-percent mysid control mortality. Five percent of the mortality is attributed to one mysid shrimp that jumped out of the water, adhered to the side of the testing container, and died.

The estimated 48-, 72-, and 96-hour LC50 estimates for mysid shrimp exposed under flow-through conditions were 20.0-, 13.6-, and 11.7-percent treated process water, respectively. For sheepshead minnows, the estimated 72- and 96-hour LC50s were 53.4-percent wastewater. At test conclusion all minnows surviving exposure to 32.0- and 56.0-percent wastewater concentrations displayed erratic swimming movements. Table 4-14 summarizes LC50 values and their respective 95-percent confidence limits. Results were calculated using a computerized Moving Averages Method. This computerized program uses log transformation of the concentration data, as well as angular transformations to improve linearity and to stabilize variance (Bennett, 1952).

Physical and chemical conditions during tests are included in Table D-4. Salinity varied with wastewater concentration from 17 to 1 ppt (full strength wastewater), decreasing with increasing wastewater concentration. Temperature was monitored continuously and ranged from 20° to 25°C. DO concentrations were measured daily and remained at 64-percent saturation or higher. The pH ranged from 7.1 to 8.0. The

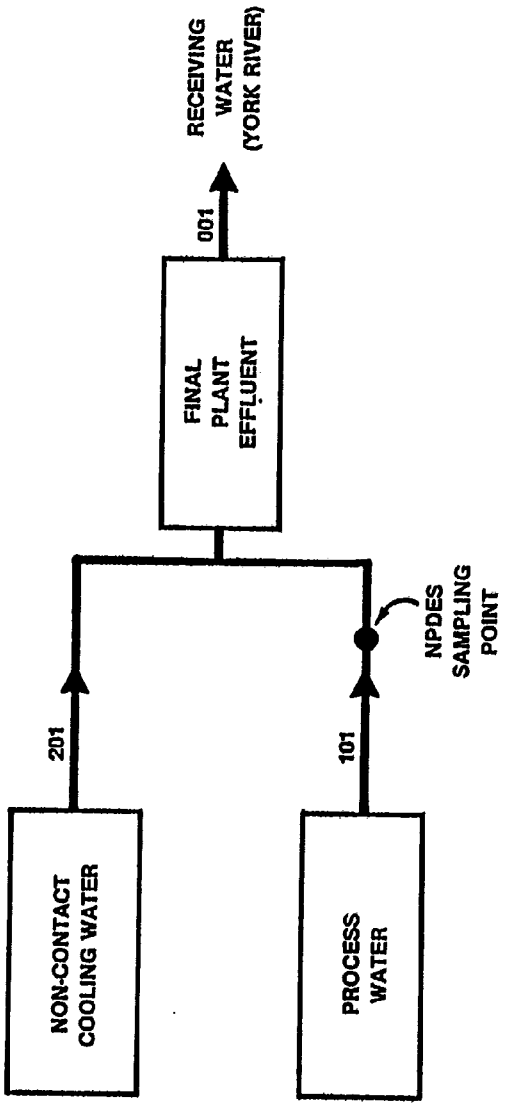

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Figure 4-28
SCHEMATIC DIAGRAM OF OUTFALLS AT THE AMOCO OIL COMPANY, YORKTOWN REFINERY, VIRGINIA

Table 4-13. Reconnaissance Study: Survival of Test Organisms Exposed to Treated Process Water (Outfall 101) at Amoco Refinery, Yorktown, Virginia (March 7 through 12, 1982)

Wastewater Concentration (%)	Survival Data† Flow-Through Tests									
	Sheepshead Minnows March 8-12, 1982					Mysid Shrimp March 7-11, 1982				
	0 hr	33* hr	57* hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0.0	10	10	10	10	10	10	8	8	8	8
0.0	10	10	10	10	10	10	10	10	10	10
5.6	10	10	10	10	10	10	9	8	8	8
5.6	10	10	10	10	10	10	10	7	7	7
10.0	10	10	10	10	9	10	9	9	9	8
10.0	10	10	10	10	10	10	9	7	6	3
18.0	10	10	10	10	10	10	10	9	5	4
18.0	10	10	10	10	10	10	10	8	6	5
32.0	10	10	10	9	9	10	8	2	1	0
32.0	10	10	10	10	10	10	9	0	0	0
56.0	10	10	4	3	3	10	6	1	0	0
56.0	10	10	7	6	6	10	8	1	0	0

*Sheepshead minnow test was started a day later. Intermediate daily readings coincide with scheduled mysid readings.
†LC50s are summarized in Table 4-19.

Source: ESE.

Table 4-14. LC50 Values and 95-Percent Confidence Limits* for Bioassays Conducted on Amoco's Treated Process Water (Outfall 101) During the Reconnaissance Phase (March 1982)

Date of Test	Test Species	Exposure Type	Exposure Period (Hr)	Concentration (% Wastewater) LCL†	LC50	UCL**
March 7, 1982	Mysidopsis bahia (mysid shrimp)	Flow-through	48	14.0	20.0	29.8
			72	9.9	13.6	17.5
			96	9.1	11.7	14.5
March 8, 1982	Cyprinodon variegatus (sheepshead minnow)	Flow-through	72	45.6	53.4	75.6
			96	45.6	53.4	75.6

*Results calculated from complete data sets using a computerized Moving Averages Method.
†LCL = 95-percent Lower Confidence Limit.
**UCL = 95-percent Upper Confidence Limit.

Source: ESE.

total alkalinity at test initiation ranged from 61.0 to 95.5 mg/L as $CaCO_3$, increasing with wastewater concentration.

The loading rate for the mysid shrimp and sheepshead minnow flow-through test was no greater than 0.003 g/L. The aquarium turnover rate for this study was 10.5 turnovers per day; EPA methods (1978) require a minimum of five turnovers per day. Considering the low loading rates used and the high turnover rates, stress (as a result of overcrowding or metabolite build up) would not be expected in this series of tests.

Bioassays were conducted without interruptions or anomalies that would be expected to affect the test results. Quality assurance tests conducted in support of this test series resulted in 24-hour LC50 values of 14.1 mg/L SDS for mysid shrimp and <0.5 mg/L SDS for sheepshead minnows. These tests were conducted onsite starting on March 10, 1982. According to ESE records, the mysid test results fell within the usual toxicity range for SDS after 24 hours. Sheepshead minnows were more sensitive than the average, possibly because the age group tested (3 to 5 days old) was younger than usual (10 to 14 days old). Younger animals were tested because of slower developmental rates of eggs exposed to lower-than-usual culture temperature during transportation to the test site.

The mysid shrimp and sheepshead minnow LC50 estimates of 11.7- and 53.4-percent wastewater, respectively, are measures of the treated process water (Outfall 101) toxicity prior to mixing with the non-contact cooling water (Outfall 201).

Based on these results, a definitive acute and chronic bioassay program was designed to evaluate the chronic effects, if any, of the Amoco discharge on the York River.

4.4.2 Definitive Bioassay Program

During June and July 1982, the following definitive static acute toxicity studies were conducted on Amoco effluents to further document and quantify fully their possible toxicity. A 24-hour range-finding test using mysid shrimp was initiated on June 6, 1982, on a grab sample from Outfall 101 (treated process water). Table D-5 summarizes the range-finding data. Sixty-percent survival was recorded, after 24 hours, in the highest effluent concentration tested (56.0-percent). No control mortality was observed.

Based on the range-finding results, a 96-hour and a 48-hour flow-through acute test was conducted on the mysid shrimp and sheepshead minnows, respectively. Table 4-15 summarizes these results.

The mysid test, started on June 8, 1982, showed the process water to be acutely toxic. Table 4-16 summarizes the LC50 values obtained. The 48-hour and 96-hour LC50 estimates were 5.8- and 4.6-percent process water. These values were estimated using a computerized probit method (Finney, 1971). Twenty-percent mortality in the controls was observed at 96 hours of testing. This control mortality is higher than desirable and may be attributable to the stress of transport of animals to the field. No control mortality was observed during the first 48 hours of testing; therefore, the 48-hour data may be a better screen of the effluent toxicity.

Ninety-five-percent survival or better was observed in all wastewater concentrations used in the sheepshead minnow test, including controls. Hence, no quantifiable toxicity was detected by the sheepshead minnows. The chemical and physical parameters for these tests are included on Table D-6. All parameters remained within acceptable limits; aeration of dilution water in the holding tank was initiated after 72 hours of testing.

Table 4-15. Acute Test: Survival Data of Mysid Shrimp (Mysidopsis bahia) and Sheepshead Minnows (Cyprinodon variegatus) Exposed Under Acute Flow-Through Conditions to Wastewater from Outfall 101, Amoco Refinery, Yorktown, Virginia (June 8 through 12, 1982)

Wastewater Concentration (%)	Mysidopsis bahia (June 8-12, 1982)					Cyprinodon variegatus (June 10-12, 1982)		
	0-Hr	24-Hr	48-Hr	72-Hr	96-Hr	0-Hr	24-Hr	48-Hr
0.0 A	10	10	10	8	7	10	10	10
0.0 B	10	10	10	9	9	10	9	9
5.6 A	10	10	5	3	3	10	10	10
5.6 B	10	9	5	4	4	10	10	10
10.0 A	10	4	3	2	2	10	10	10
10.0 B	10	2	1	1	1	10	10	10
18.0 A	10	1	0	0	0	10	10	10
18.0 B	10	0	0	0	0	10	10	10
32.0 A	10	0	0	0	0	10	10	10
32.0 B	10	0	0	0	0	10	10	10
56.0 A	10	0	0	0	0	10	10	10
56.0 B	10	0	0	0	0	10	9	9

Source: ESE.

Table 4-16. LC50 Values and 95-Percent Confidence Limits* for 96- and 48-Hour Flow-Through Acute Toxicity Tests Conducted on Process Water (Outfall 101) Using Mysid Shrimp and Sheepshead Minnows (June 8 through 12, 1982) at Amoco Refinery, Yorktown, Virginia

Date of Test	Test Species	Exposure Type	Exposure Period (Hr)	Concentration (% Wastewater) LCL†	LC50	UCL**
June 8-12, 1982	Mysidopsis bahia (mysid shrimp)	Flow-through	48	3.7	5.8	7.2
			96	1.6	4.6	6.2
June 10-12, 1982	Cyprinodon variegatus (sheepshead minnow)	Flow-through	96	NOT ENOUGH MORTALITIES		

*Results calculated from complete data sets using a computerized Probit Method.
†LCL = Lower 95-Percent Confidence Limit.
**UCL = Upper 95-Percent Confidence Limit.

Source: ESE.

To determine the possible effects of varying salinity on the test results, parallel 48-hour static acute tests using mysid shrimp were initiated on June 9, 1982. The first set of static tests was conducted using a 24-hour composite started on June 8, 1982. The compositing frequency was 250 ml each 15 minutes. The salinity of the dilution water (natural seawater) was 20 ppt. As the test concentrations (100.0-, 56.0-, 32.0-, and 18.0-percent process water) were prepared, the salinity of the test solutions increased (1, 11, 14, and 16 ppt, respectively) with decreasing wastewater content. Mysidopsis bahia is an estuarine species found at salinities of 9 ppt or higher (Nimmo et al., 1977); thus, artificial seasalts were used to test full strength effluent. Table 4-17 summarizes these test results.

Three controls were used: Control 1, using natural seawater (dilution water) at 20 ppt; Control 2, using artificial seawater at 20 ppt; and Control 3, using natural seawater at 12 ppt. Test animals, as usual, were acclimated to salinities approximating those of the test solutions. Results show identical survival (90 percent) of the three control types. This shows that the artificial seawater was acceptable for the survival of the test animals during exposure period and that animals were acclimated to test salinities and "salinity-shock" was not anticipated. The results of the wastewater static tests with and without salinity adjustments are shown in Table 4-17 and are similar to each other. No effect on test results is expected from the varying test solution salinities as long as the animals are well acclimated and not exposed to a drastic salinity change.

To further define the possible toxicity of the Amoco refinery, screening tests were also conducted from June 9 through 11, 1982, on grabs from Outfalls 201 (non-contact cooling water) and 001 (final discharge). These results are summarized on Table 4-18. Mysids tested in full strength non-contact cooling water showed 85-percent survival after 48 hours. On the other hand, 80-percent mortality was observed in full strength final discharge (Outfall 001).

Table 4-17. Static Toxicity Data Recorded by Test Container During Mysid Shrimp (Mysidopsis bahia) Acute Test Conducted on Outfall 101 at Amoco Refinery, Yorktown, Virginia (June 9 through 11, 1982)

Wastewater Concentration (%)	Number of Live Organisms			Dissolved Oxygen (mg/L)			Temperature (°C)			pH			Acclimatization Salinity (ppt)	Test Salinity (ppt)
	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	0-Hr
C1A	10	9	8	7.4	5.8	6.0	21	21	20	8.0	7.8	7.8	18	20
C1B	10	10	10	7.4	5.4	5.7	22	21	20	8.0	7.8	7.7	18	20
C2A	10	10	9	7.6	5.8	5.5	22	21	20	9.3	8.6	7.9	18	20
C2B	10	10	9	7.6	5.8	6.0	22	21	20	9.4	8.6	8.0	18	20
C3A	10	10	9	7.6	5.9	6.8	22	21	20	8.0	8.0	7.8	14	12
C3B	10	9	9	7.6	5.6	6.0	22	21	20	8.0	7.7	7.8	14	12
Salinity-Adjusted Using Artificial Seasalts, Dilution Water is that Used for C1														
18.0A	10	10	4	7.2	5.8	5.2	22	22	22	8.2	8.0	7.9	18	20
18.0B	10	9	4	7.2	5.8	5.1	22	22	22	8.2	8.1	7.9	18	20
56.0A	10	5	0	6.7	5.5	5.6	22	23	22	8.2	8.1	8.2	18	20
56.0B	10	5	0	6.6	5.3	5.0	22	23	22	8.2	8.1	8.1	18	20
100.0A	10	1	0	6.0	4.8	4.8	22	22	23	8.2	8.1	8.2	18	20
100.0B	10	1	0	6.1	5.1	4.9	22	22	23	8.2	8.2	8.2	18	20
Salinity Allowed to Vary with Wastewater Concentration, Dilution Water is that Used for C1														
18.0A	10	10	1	7.1	5.6	5.3	22	23	23	8.0	8.0	7.8	18	16
18.0B	10	8	4	7.1	5.5	5.7	21	23	24	8.0	8.0	7.9	18	16
32.0A	10	9	0	6.8	5.5	5.6	21	23	24	7.9	8.0	8.0	18	14
32.0B	10	8	1	6.8	5.6	5.7	22	23	24	7.9	8.0	8.0	18	14
56.0A	10	5	2	6.2	4.6	5.6	22	23	24	7.9	8.1	8.1	14	11
56.0B	10	5	1	6.4	4.6	5.6	22	23	24	7.9	8.1	8.1	14	11

Note:
C1 = Control using natural seawater at 20 ppt; C2 = control using artificial seawater at 20 ppt; C3 = control using natural seawater at 12 ppt.

Source: ESE.

Table 4-18. Static Toxicity Data Recorded by Test Container During Mysid Shrimp (Mysidopsis bahia) Acute Test Conducted on Outfalls 201 and 001 at Amoco Refinery, Yorktown, Virginia (June 9 through 11, 1982)

Wastewater Concentration (%)	Number of Live Organisms			Dissolved Oxygen (mg/L)			Temperature (°C)			pH			Acclimatization Salinity (ppt)	Test Salinity (ppt)
	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	0-Hr
0.0	10	9	8	7.4	5.8	6.0	21	21	20	8.0	7.8	7.8	18	20
0.0	10	10	10	7.4	5.4	5.7	22	21	20	8.0	7.8	7.7	18	20
Outfall 201—Non-Contact Cooling Water														
100.0A	10	10	9	6.5	5.6	6.9	24	23	23	8.0	7.9	7.9	18	17
100.0B	10	10	8	6.5	5.7	7.0	24	23	23	8.0	7.9	7.9	18	17
Outfall 001—Final Amoco Effluent														
100.0A	10	9	2	6.2	5.7	7.0	24	23	23	8.0	8.1	7.9	18	17
100.0B	10	10	2	6.4	5.9	7.0	24	23	23	8.0	8.0	7.9	18	17

Source: ESE.

An additional sheepshead minnow (Cyprinodon variegatus) static acute test was conducted on all outfalls on June 11 through 13, 1982. No mortalities were observed in any of the test concentrations and controls tested (Table 4-19). The Amoco effluents were not acutely toxic to the sheepshead minnow.

Based on the toxicity of the process water (Outfall 101) to the mysid shrimp under acute flow-through conditions, a life cycle test was initiated on June 19, 1982. The life cycle process water concentrations used were: 3.20-, 1.80-, 1.00.-, 0.58-, 0.32-, 0.18-, and 0.00-percent wastewater. These concentrations were chosen based on ESE's testing in March and June 1982 showing 96-hour LC50 estimates of 11.7- and 4.6-process water.

Table D-7 (Appendix D) shows the mysid survival during the 26-day life cycle exposure. Survival varied from 85 to 56 percent and showed no obvious trends. The control survival was 75 percent; this value is acceptable for a mysid life cycle test.

At the start of the life cycle test, the mysids were less than 30 hours old and were placed in a random manner in the test chambers. The tank location in the diluter system was also randomized. The diluter was calibrated before test initiation.

Three Nitex cups were placed in each test chamber, with 8 mysids each. The mysids were counted and fed daily. Records were kept on the number of females per cup so that the "female reproductive days" could be calculated.

The test was terminated on Day 26 because the Amoco Outfall 101 effluent was shut-off due to a treatment plant malfunction. This 26-day period of testing was sufficient since some of the test organisms were carrying their second brood and the test had to be finalized before the second brood was released.

Table 4-19. Static Toxicity Data Recorded by Test Container During Sheepshead Minnow (Cyprinodon variegatus) Acute Test Conducted on Outfalls 001, 201, and 101 at Amoco Refinery, Yorktown, Virginia (June 11 through 13, 1982)

Wastewater Concentration (%)	Number of Live Organisms			Dissolved Oxygen (mg/L)			Temperature (°C)			pH			Acclimatization Salinity (ppt)	Test Salinity (ppt)
	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	24-Hr	48-Hr	0-Hr	0-Hr
0.0 A	10	10	10	6.5	6.3	6.6	21	24	24	7.9	7.7	7.5	17	19
0.0 B	10	10	10	6.5	6.0	6.6	21	24	24	7.9	7.7	7.8	17	19
Outfall 001														
100.0A-001	10	10	10	6.4	6.3	6.7	21	24	24	7.9	7.8	7.8	17	17
100.0B-001	10	10	10	6.2	6.5	6.7	21	24	24	7.8	7.7	7.8	17	17
Outfall 201														
100.0A-201	10	10	10	6.2	5.9	6.5	21	24	24	7.9	7.7	7.8	17	17
100.0B-201	10	10	10	6.2	6.1	6.8	21	24	24	7.9	7.7	7.8	17	17
Outfall 101														
56.0A-101	10	10	10	5.7	6.4	6.7	21	24	24	7.8	7.8	8.0	14	11
56.0B-101	10	10	10	5.7	6.4	6.4	21	24	24	7.8	7.8	7.9	14	11

Source: ESE.

Table 4-20 summarizes the number of young dropped per test concentration throughout the test. The column under Day 26 represents the final number of young (cumulative number) dropped per concentration replicate. Regression analyses were conducted on the cumulative number of young dropped per concentration versus effluent concentration. No statistically significant correlations between these two parameters were found. Furthermore, multiple range tests on these data showed no difference among final number of young exposed to the control and effluent concentrations tested.

A more accurate approach to the effluent effect on reproduction is the evaluation of the total number of young released per female versus effluent concentration. The total young released per female is calculated by dividing the cumulative number of young released by the "female reproductive days." "Female reproductive days equal the number of females in each exposure cup multiplied by the number of days they are alive from the first brood release to the end of the test" (ASTM, 1983). This approach accounts for varying number of females, female mortality, and non-reproductive females during test.

Table 4-21 summarizes the cumulative number of young, the female reproductive days, and the number of young released per female reproductive day. Regression analysis were performed on number of young released per female reproductive day versus effluent concentration. The correlation was significant showing fewer young released per female reproductive day as effluent concentration increased. Multiple range tests showed 3.2-percent concentration to be significantly different from controls. Thus, for this measure of reproductive success, 3.2 percent process water is the lowest observed effect level.

Another parameter monitored during testing was the number of days to the first release of young as related to effluent concentration. The last two columns in Table 4-20 summarize by concentration replicate the number of days until first release of young. Regression analysis showed

Table 4-20. Chronic Reproduction Data of Mysid Shrimp (Mysidopsis bahia) Exposed Under Flow-Through Conditions to Wastewater from Outfall 101, Amoco Refinery, Yorktown, Virginia (June 19 through July 15, 1982)

Wastewater Concentration (%)	Test Container	Total Number of Young Released by Day of Exposure														Number of Days to First Release of Young	Average Number of Days to First Brood Release by Treatment
		13	14	15	16	17	18	19	20	21	22	23	24	25	26		
0.00	14A	0	0	0	6	6	6	6	13	18	19	19	19	19	19	15	
0.00	14B	0	0	0	13	13	13	13	13	30	30	30	30	30	30	15	
0.00	14D	0	2	7	13	21	22	25	35	37	37	46	46	46	55	13	
0.00	13A	0	0	0	0	4	5	5	5	6	6	11	11	11	20	16	
0.00	13B	0	0	3	11	11	11	14	14	14	19	26	30	30	38	14	
0.00	13D	0	0	14	15	22	26	26	26	39	39	52	56	56	66	14	14.5
0.18	10A	0	0	0	0	0	4	4	10	10	10	27	30	30	30	17	
0.18	10B	0	0	0	0	0	0	0	0	3	3	10	10	10	17	20	
0.18	10D	0	0	8	12	13	16	16	16	33	33	38	38	38	48	14	15.5
0.32	8A	0	0	4	4	4	12	12	12	17	17	32	32	32	32	14	
0.32	8B	0	0	0	5	5	8	8	8	10	10	25	25	25	31	15	
0.32	8D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*	
0.32	7A	0	0	0	8	8	8	8	8	8	8	15	17	17	17	15	
0.32	7B	0	0	0	0	0	8	8	14	24	24	24	24	24	29	17	
0.32	7D	0	0	0	3	5	5	5	5	5	10	15	15	15	15	15	15.2
0.58	6A	0	0	0	0	1	1	1	1	1	1	4	4	4	4	16	
0.58	6B	0	0	3	10	10	10	12	14	14	14	14	17	17	25	14	
0.58	6D	0	0	0	9	9	9	9	9	9	16	28	32	32	32	15	
0.58	5A	0	0	0	2	2	2	2	2	6	6	6	10	10	16	15	
0.58	5B	0	0	0	2	7	7	7	9	13	17	30	41	41	41	15	
0.58	5D	0	0	0	13	14	14	14	14	14	14	29	29	33	33	15	15.0

Table 4-20. Chronic Reproduction Data of Mysid Shrimp (Mysidopsis bahia) Exposed Under Flow-Through Conditions to Wastewater from Outfall 101, Amoco Refinery, Yorktown, Virginia (June 19 through July 15, 1982) (Continued, Page 2 of 2)

Wastewater Concentration (%)	Test Container	Total Number of Young Released by Day of Exposure														Number of Days to First Release of Young	Average Number of Days to First Brood Release by Treatment
		13	14	15	16	17	18	19	20	21	22	23	24	25	26		
1.00	4A	0	0	0	0	5	16	16	16	25	30	41	41	48	48	16	
1.00	4B	0	0	0	0	3	16	16	18	18	18	31	38	38	48	16	
1.00	4D	0	0	7	7	9	21	21	21	27	32	44	44	44	44	14	
1.00	3A	0	0	0	0	0	2	2	2	2	4	8	11	14	14	17	
1.00	3B	0	1	13	13	13	16	24	29	29	32	44	48	49	49	13	
1.00	3D	0	0	0	0	0	11	11	12	12	12	21	21	22	22	17	15.5
1.80	2A	0	0	0	0	0	0	0	0	0	1	4	4	4	10	21	
1.80	2B	0	0	6	6	6	16	16	16	16	16	16	29	29	29	14	
1.80	2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*	
1.80	1A	0	0	0	0	0	0	6	6	6	6	12	18	19	19	18	
1.80	1B	0	0	0	0	0	1	3	3	3	3	3	10	11	11	17	
1.80	1D	0	0	0	0	4	13	13	13	13	13	23	32	32	32	16	17.2
3.20	12A	0	0	0	0	0	0	4	4	7	7	7	10	15	21	18	
3.20	12B	0	0	1	1	1	3	3	3	3	3	8	19	19	19	14	
3.20	12D	0	0	0	0	0	0	0	0	3	9	9	9	9	9	20	
3.20	11A	0	0	0	0	0	6	6	6	6	6	6	11	11	14	17	
3.20	11B	0	0	0	1	1	5	5	5	5	5	5	19	19	25	15	
3.20	11D	0	0	6	12	12	18	18	18	37	46	46	46	46	46	14	16.3

* All males in this cup.

Source: ESE.

Table 4-21. Number of Young Per Female Reproductive Day; Chronic Reproduction Data of Mysid Shrimp (Mysidopsis bahia) Exposed Under Flow-Through Conditions to Amoco Outfall 101 Wastewater (June 19 through July 15, 1982)

Wastewater Concentration (%)	Test Container	Cumulative Number of Young	Female Reproductive Days	Number of Young Released Per Female Reproductive Day
0.00	14	104	126	0.82
0.00	13	124	195	0.64
0.18	10	95	180	0.53
0.18	9	62	83	0.75
0.32	8	63	135	0.47
0.32	7	61	110	0.55
0.58	6	61	117	0.52
0.58	5	90	180	0.50
1.00	4	140	178	0.79
1.00	3	85	139	0.61
1.80	2	39	60	0.65
1.80	1	62	116	0.53
3.20	12	49	180	0.27
3.20	11	85	160	0.53

Source: ESE.

a correlation between these two parameters. Multiple range tests showed the controls to be statistically different from the 1.8-percent concentration. In this case, 1.8-percent process water is the lowest observed effect concentration (LOEC).

To reiterate, the number of young released per female reproductive day decreased with increasing process water concentrations, and 3.2-percent process water was found to cause a statistically significant observable effect when compared to the controls. The time to the release of the first brood was also affected by the process water. A concentration of 1.8-percent effluent was shown to delay the brood release of young by approximately 2.7 days.

Another parameter measured was final dry weight of test animals and its relationship to effluent concentration. Male and female data were analyzed separately. The regressions conducted on the male dry weight at end of test versus effluent concentration showed no statistically significant correlation. The female final dry weight versus effluent showed a statistically significant correlation. Multiple range tests showed that 3.2-percent process water was statistically different from controls. The females in 3.2-percent process water weighed less than those in the controls. The average weight of the controls was 0.45 mg and those in 3.2-percent wastewater weighed 0.38 mg. Hence, mysid growth (as measured by dry weight) was reduced by 15 percent in 3.2-percent wastewater. Table D-8 summarizes the average dry weights for males and females at test conclusion.

Appendix Table D-9 summarizes chemical parameters recorded by test aquarium during mysid shrimp life cycle test. DO remained at 5.0 mg/L or higher at all times (59.5-percent saturation). The temperature range was 25±2°C. The test chamber turnover rates varied from 6.1 to 7.15 turnovers per day (EPA and ASTM require a minimum of 5 turnovers per day).

Due to the known variability of industrial effluents, periodic static acute mysid shrimp tests were conducted concurrently with the life cycle test. The purpose of these tests was to document the toxicity variability of the Amoco treated process water over the period of chronic testing. Three periods of testing were conducted. The first began on June 25, 1982 (Day 7 of life cycle test). Results are summarized in Table D-10. Outfalls 201 (non-contact cooling water) and 001 (final effluent) showed no acute toxicity. The test on the process water (Outfall 101) was acutely toxic with an LC50 estimate of 42.6-percent wastewater (Table 4-22). The second period of testing started on July 3, 1982 (Day 15 of life cycle test). These results are summarized in Table D-11. Outfall 001 was again not acutely toxic, but Outfall 101 was again acutely toxic with an LC50 estimate of 42.3-percent wastewater. The third period of testing (Table D-12), starting on July 9, 1983 (Day 21 of life cycle test), showed no acute toxicity of Outfall 001 and an LC50 of 51.3-percent wastewater for the process water (Table 4-22).

To better show the variability of the process water acute toxicity during the chronic test, the LC50 estimates of all 48-hour static acute mysid shrimp and their confidence limits were plotted against time of testing (Figure 4-29). This graph clearly shows that the process water was highly acutely toxic from June 9 through 11, 1983. This toxicity to mysid shrimp was also documented by the concurrent flow-through tests (96-hour LC50 of 4.6-percent process water). As the weeks of testing continued, the toxicity decreased steadily, with a final LC50 of 51.3-percent process water.

Table 4-23 presents the data provided by the Amoco Refinery for the period of bioassay testing. As shown, during the preliminary acute tests the ammonia levels were higher than usual. This is a possible cause of the higher acute toxicity found during this period. The subsequent static acute tests concurrent with the life cycle documented lesser acute toxicity at a time when the ammonia levels had dropped

Table 4-22. LC50 Values and 95-Percent Confidence Limits* for Bioassays Conducted Concurrently with the Life Cycle Study on Outfall 101 of Amoco Refinery, Yorktown, Virginia

Date of Test	Test Species	Exposure Type	Exposure Period (hr)	Concentration (% Wastewater)		
				LCL	LC50	UCL
June 25, 1982	Mysidopsis bahia	Static	48	27.2	42.6	153.1
July 3, 1982	Mysidopsis bahia	Static	48	35.4	42.3	54.7
July 9, 1982	Mysidopsis bahia	Static	48	44.5	51.3	67.1

*Results calculated from complete data sets using the Moving Averages Method.

Source: ESE.

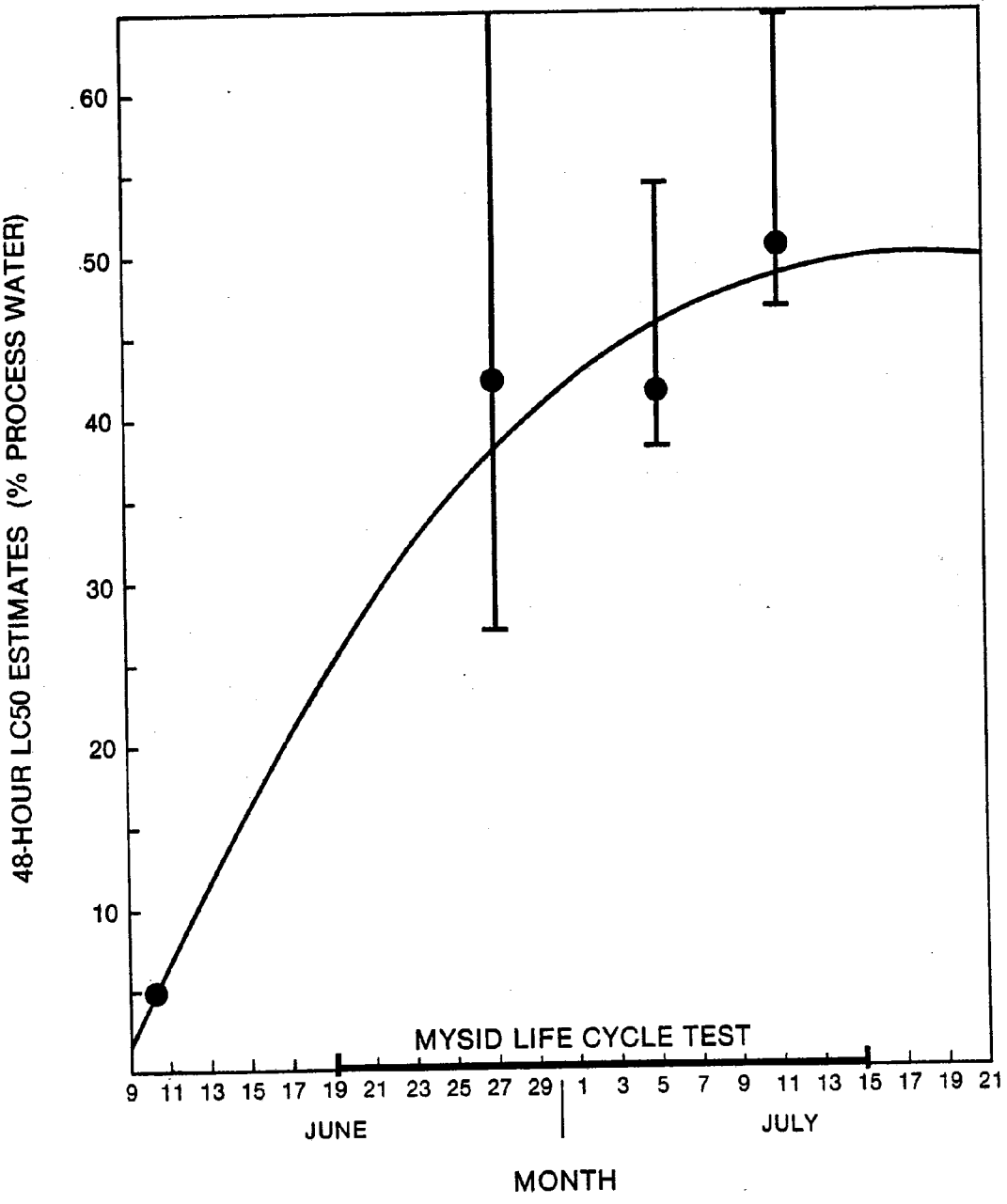

Figure 4-29
ACUTE TOXICITY (LC50 ESTIMATES) OVER TIME

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Table 4-23. Data Provided by Amoco Refinery for Period of Testing

Date (1982)	Concurrent Test	Outfall 201 Flow GPM	Outfall 101 Flow GPM	API Unit	NH_3 Influent	Effluent (101) (mg/L)
6/2		57,000	1,200	80	68	0.1
3			1,200	110	65	0.7
4			1,500	90	70	0.9
5			1,500	60	76	0.4
6			1,670	50	45	0.3
7			1,700	120	70	19
8*		56,000	1,700	60	70	20
9*			1,500	180	90	50
10*	Acute		1,300	200	130	80
11*			1,300	100	70	25
12*			1,300	160	90	45
13			1,250	150	80	28
14			1,300	90	85	53
15		58,000	Down	120	80	90
16			800	160	110	55
17			800	50	70	80
18			800	42	58	7
19†			1,200	45	68	5.5
20†			1,200	145	65	2.0
21†			1,200	39	37	0.1
22†		56,000	1,200	62	42	0.1
23†			940	115	85	1.4
24†			800	40	30	1.8
25†			800	80	65	2.1
26†	Acute		800	70	65	1.8
27†			800	85	70	2.1
28†			1,000	125	80	0.9
29†		56,000	1,000	70	72	1.9
30†			1,000	200	385	0.8

Table 4-23. Data Provided by Amoco Refinery for Period of Testing (Continued, Page 2 of 2)

Date (1982)	Concurrent Test	Outfall 201 Flow GPM	Outfall 101 Flow GPM	API Unit	NH_3 Influent	Effluent (101) (mg/L)
7/1†			1,000	30	80	0.8
2†			1,000	210	90	10.0
3†			850	40	99	9.6
4†	Acute		800	40	95	23.2
5†			800	28	50	7.0
6†		56,000	800	30	55	0.25
7†			800	110	44	0.4
8†			800	100	35	0.8
9†			800	85	45	1.0
10†	Acute		800	145	90	1.5
11†			800	80	50	0.8
12†			800	60	55	0.4
13†		57,000	800	70	60	0.5
14†			800	90	60	0.2

*Preliminary acute test.
†Life cycle study.

Source: Amoco Yorktown Refinery, 1982.

considerably. In summary, changing ammonia levels in the process water may have affected the acute toxicity of the effluent but, based on these studies, it can not be concluded that this is the sole source of the toxicity found.

4.4.3 Quality Assurance Tests

To document the sensitivity of the mysid shrimp (Mysidopsis bahia) used in the acute toxicity tests at Amoco, reference toxicant tests were conducted using sodium dodecyl sulfate (SDS). Tests conducted during the reconnaissance period and the initiation of the life cycle test show that the sensitivity of the mysids tests was within the acceptable range (LC50 range of 1 to 10 mg/L) for 48-hour exposures (Table 4-24) (U.S. EPA, 1985; Auwarter and Mousa, 1982). These tests were conducted on ESE onsite cultured animals and animals from Sea Plantations, Inc. The LC50 estimates were identical.

Additional reference toxicant tests were conducted on juveniles released by females during the life cycle test. The results are summarized in Table 4-24. Mysids released in the controls had lower LC50 estimates (6.0 mg/L) than those in the wastewater chambers (9.5 and 8.9 mg/L). This is not enough information to make definitive conclusions, but these limited data show that the mysids exposed to wastewater throughout their embryological development were less sensitive to SDS than those in the controls, although they were all within the accepted sensitivity range for mysids.

4.4.4 Indigenous Species Testing

In May 1983, two estuarine species were collected from the York River in an area unaffected by the Amoco discharge. The animals collected were a fish, spot (Leistomus xanthurus), and a grass shrimp (Paleomonetes sp). These animals were shipped to ESE Gainesville laboratories for holding and testing.

Table 4-24. Reference Toxicant Data*. Mysid Shrimp (Mysidopsis bahia) Tested for Sensitivity to Sodium Dodecyl Sulfate (SDS) Under Static Conditions, Amoco Refinery, Yorktown, Virginia

Date of Test	Source of Test Species	Exposure Period	Concentration (mg/L SDS) LCL	LC50	UCL
March 11, 1982	ESE, Gainesville cultures	24	11.1	14.1	19.8
June 19, 1982	ESE, onsite cultures	24	5.0	10.0	15.0
		48	3.8	6.3	9.1
June 19, 1982	Sea Plantations, Salem, Massachusetts	24	5.0	10.0	15.0
		48	3.8	6.3	9.2
July 10, 1982	Juveniles dropped in control chambers of chronic test	24	4.9	7.4	10.5
		48	4.5	6.0	7.7
July 10, 1982	Juveniles dropped in 1.0-percent wastewater chambers of chronic test	24	7.8	10.2	14.3
		48	6.3	9.5	15.8
July 10, 1982	Juveniles dropped in 3.2-percent wastewater chambers of chronic test	24	6.0	8.9	13.6
		48	6.0	8.9	13.6

*Results calculated from complete data sets using the Moving Averages Method, unless otherwise indicated.

Source: ESE.

On May 23, 1983, a grab sample from Outfall 101 (process water) was taken and sent to ESE for next day delivery. The effluent characteristics at time of arrival were: pH 7.8, temperature 6°C, and DO 10.4 mg/L. Forty-eight-hour static acute bioassays were started on May 24, 1983, using the grass shrimp, the spot, and mysid shrimp (Mysidopsis bahia). The tests were startd within 26 hours of sample collection. Due to animal shipment problems, not all the spot collected survived, but enough fish were available for a screening test.

Table 4-25 summarizes these tests and presents the LC50 estimates. The grass shrimp LC50 was 52.2-percent process water. All spot survived the 48-hour exposure to the effluent. The mysid shrimp LC50 was 20.7-percent effluent. The LC50 values were estimated using a computerized Binomial Method. Due to the mortality pattern, the Probit and Moving Averages Methods could not be used. The results from these tests are in agreement with previous results obtained during this study. Overall, the invertebrate species (laboratory-cultured or field collected) were sensitive to the effluent toxicity, whereas the fish were not affected by it.

4.4.5 Conclusions

According to Amoco monthly Discharge Monitoring Reports for 1983 (Table 4-26), the treated process water under normal operating conditions represents from 1.3 to 3.5 percent of the final effluent (Outfall 001). The range for 1983 is in accordance with calculations from the NPDES average flows for these outfalls [Outfall 101 at 1.008 MGD; Outfall 201 at 56.067 MGD]. Based on the acute bioassay tests conducted and non-contact cooling water dilution, the final Amoco effluent would not be considered acutely toxic at the time of discharge. Furthermore, results from ESE's Physical Characterization study (Section 4.1) indicate that complete mixing of Amoco effluent in the York River further dilute the effluent within a few meters of the discharge area. The highest effluent concentraton ever found during this study in the York River was 0.35-percent process water, but the

Table 4-25. Static Acute Toxicity Tests Conducted on Process Water (Outfall 101) Using Two Indigenous Species and Mysid Shrimp (May 25 through 27, 1983)

Wastewater Concentration (%)	TEST ANIMALS								
	Mysidopsis bahia (Mysid Shrimp)			Palaeomonetes sp. (Grass Shrimp)			Leistomus xanthurus (Spot)		
	0hr	24hr	48hr	0hr	24hr	48hr	0hr	24hr	48hr
0.0 A	10	10	9	10	9	8	5	5	5
0.0 B	10	9	8	10	10	10	--	--	--
18.0 A	10	8	5	10	10	9	--	--	--
18.0 B	10	8	6	10	10	10	--	--	--
32.0 A	10	7	4	10	10	10	5	5	5
32.0 B	10	5	3	10	10	10	--	--	--
56.0 A	10	7	5	10	5	1	5	5	5
56.0 B	10	5	4	10	9	7	--	--	--
LC50									
(% wastewater)*		20.7			52.2				†

*Results calculated using a computerized Binomial Test. Not enough partial mortalities occurred in the data to use Probit and/or Moving Averages Method.
†Not enough mortality.

Source: ESE.

Table 4-26. Actual (1983) Flow from Outfalls 101 (Process Water) and 201 (Non-Contact Cooling Water) and Calculated Percent Process Water in the Final Outfall (001)

Month (1983)	Outfall	Flow Rates (MGD) Average	Maximum	Calculated Percent Process Water at Final Discharge (001) Average	Maximum
January	101	1.20	1.53		
	201*	47.0	48.6		
	001	48.2	50.13	2.5	3.0
April	101	1.74	1.91		
	201	47.4	48.7		
	001	49.14	50.6	3.5	3.8
May	101	1.11	1.65		
	201†	46.9	49.0		
	001	48.01	50.65	2.3	3.3
June	101	1.11	1.52		
	201	77.2	77.2		
	001	78.31	78.72	1.4	2.0
July	101	0.99	1.22		
	201	75.8	76.1		
	001	76.79	77.32	1.3	1.6
August	101	1.13	1.69		
	201**	77.37	79.62		
	001	78.5	81.31	1.4	2.1

Table 4-26. Actual (1983) Flow from Outfalls 101 (Process Water) and 201 (Non-Contact Cooling Water) and Calculated Percent Process Water in the Final Outfall (001) (Continued, Page 2 of 2)

Month (1983)	Outfall	Flow Rates (MGD) Average	Flow Rates (MGD) Maximum	Calculated Percent Process Water at Final Discharge (001) Average	Calculated Percent Process Water at Final Discharge (001) Maximum
October	101	1.20	2.06		
	201††	77.2	77.2		
	001	78.4	79.26	1.5	2.6
November	101	0.93	1.5		
	201***	75.5***	75.5***		
	001	76.43	77.0	1.2 (100)†††	1.9 (100)†††

*Diverted Outfall 201 to Outfall 002 (stormwater runoff) due to leaking cooler.
†Outfall 201 was diverted to Outfall 002 from May 18 to 26, 1983, due to exchanger leak.
**On August 30 and 31, 1983, all Outfall 201 was diverted to Outfall 002 due to exchanger leak.
††Cooling water (Outfall 201) shutdown on October 17, 1983.
***Cooling water system (Outfall 201) was down from November 1 through 26, 1983. Data only represent the November 29, 1983 sample.
†††Values estimated are valid only for the period during which Outfall 201 was being discharged through final Outfall 001. At all other times, 26 out of 30 days, 100-percent process water (full strength) was being discharged into the receiving water (York River).

Source: Amoco Yorktown Refinery, Discharge Monitoring Reports, 1983.

average process water concentration near the discharge was approximately 0.02-percent process water.

The chronic life cycle test showed that at a concentration of 3.2-percent process water, the number of young released per female reproductive day was reduced and that at 1.8-percent process water, the brood release of young was delayed by 2.7 days. Hence the lowest observed effect concentration (LOEC) was 1.8 percent process water. Under normal operating conditions, this concentration would not be found in the York River due to the immediate high dilutions occurring at the point of discharge. It is important to note that, as shown in Table 4-26, full strength process water is discharged into the York River when the cooling water is diverted and that during these periods acutely toxic effluent concentrations can be expected in the immediate vicinity of the outfall (001).

5.0 CONCLUSIONS AND DISCUSSION

5.1 CONCLUSIONS

In discussing the conclusions of this study, it must be recalled that the study's primary aim was to evaluate the ability of single-species effluent toxicity bioassays to predict ecosystem impacts caused by discharge of the effluent. This requires the comparative evaluation of ecosystem impact assessments versus toxicity bioassay results.

5.1.1 Ecosystem Impact Assessment

The fundamental conclusion of the ecosystem studies is that no ecosystem impacts definitively caused by the discharge of Amoco Oil Company refinery effluents were discernible. No cause-effect relationships between the effluent discharge and ecosystem attributes were determined.

It is recognized that potential exists for factors other than the refinery discharge to be influencing the ecosystem at the present time, such as other discharges into the estuary, ship traffic, and commercial fishing operations. Particularly regarding the benthos populations, it is noted that commercial molluscan dredging operations seasonally disrupt bottom communities extensively.

The complex nature of the York River's circulation and the periodic stratification-destratification process associated with spring and neap tides is another potential source of benthos variability which could not be evaluated within resource constraints of the study. If the benthic community is periodically exposed to low oxygen conditions in the deeper water of the river, it is possible that the benthic community is continually responding to this natural perturbation through a short-term decolonization-recolonization cycle. Such a community behavior would be impossible to define without a benthic sampling program at least as frequent as the periodic disturbance. This perturbation may be causing natural community responses of such magnitude as to totally mask relatively minor refinery impacts.

Tropical storm Agnes (1972) apparently caused a major disturbance in the lower York River, resulting in the decline of many previously dominant species and the replacement of these species with Paraprianospio primata as the dominant organism in the mud bottom of the lower York River. Previous studies and the present study show that this polychaete has been a major component of the York River benthos for the last decade (Jordan et al., 1975). Ecosystem recovery may still be occurring and has the potential for masking relatively minor refinery impacts.

Nephtys incisa, reported by Boesch and Rosenberg (1981) to be a pre-Agnes dominant, was found at 24 of 30 stations in the May collections of the present study. Boesch and Rosenberg (1981) state that N. incisa has scarcely been found since 1976. In the present study, N. incisa, although widely occuring, was found at very low densities, usually only $15/m^2$ where Boesch et al. (1976) report densities higher than $300/m^2$. Whether the York River benthic community is still slowly recovering from the perturbation resulting from Agnes or has reached a new stable point is a difficult question to answer, and certainly cannot be answered by the present short-term study. The question does have implications. If the York River benthic community is presently undergoing long-term successional change toward pre-Agnes conditions, then any change observed may be due to long-term natural variation rather than pollutional effects. One of the most difficult tasks in ecology is the differentiation of natural variability from pollution effects. If the benthic community has reached a new stable point, it is a stable point dominated by euryhaline opportunists. Communities dominated by euryhaline opportunists appear to be very stable communities in the sense that they have a high resistance and resilience to perturbations. Additionally, these euryhaline opportunists often show wide fluctuations in population levels due to seasonal variation and response to environmental factors. A short-term study cannot separate the natural variation from subtle pollution effects, especially when the community is dominated by highly resistant and resilient species such as is the York River benthic community.

Within the limitations of the benthic study, it was not possible to conclusively demonstrate the presence of a zone of negative impact, or conversely a zone of non-impact, on the benthic community due to the Amoco refinery discharge. Even if such negative impacts exist, however, it is concluded that they are certainly very minor as compared to impacts of natural phenomena or of other human activities potentially affecting the local ecosystem.

Concerning this problem, Boesch et al. (1976) stated:

> "The great population variability exhibited in the communities we studied points out the extreme limitations of baseline and impact studies of short duration. Without a detailed knowledge of community dynamics, natural variations may be mistaken for the effects of a pollutant, or worse, vice versa. Knowledge of life histories is essential in the interpretation of results of impact surveys. More attention should be placed on effects on the equilibrium species in a community rather than on opportunistic 'pollution indicators' which can also sporadically exploit pristine habitats."

Two additional problems arise from the concept of using only the equilibrium species in a community, however. The first is defining which species in a community are the equilibrium species, and gaining knowledge of their life histories. Second is that the equilibrium species are unknown in the present study and may be the less common species in terms of occurrence and number of individuals encountered. Under the sampling methodology of the present study, the sample replication is adequate for total densities at a station, but is insufficient to sample with adequate precision for population density of less common taxa. To reduce the error of population estimates of these less common taxa for statistically reliable results could require as many as 20 to 25 replicate samples per station. This would take an extremely intensive, time-consuming and costly effort.

5.1.2 Toxicity Bioassay Results

A summary of all process water acute toxicity test results is presented in Table 5-1. If "acute toxicity" is defined as any effluent-induced

Table 5-1. LC50 Values and 95-Percent Confidence Limits for Bioassays Conducted on Outfall 101 of Amoco Refinery, Yorktown, Virginia (March 7 through June 11, 1982)

Date of Test	Test Species	Exposure Type	Exposure Period (hr)	Concentration (% Wastewater) LCL	LC50	UCL
March 7, 1982	Mysidopsis bahia	Flow-through	48	14.0	20.0	29.8
			96	9.1	11.7	14.5
June 8, 1982	Mysidopsis bahia	Flow-through	48	3.7	5.8	7.2
			96	1.6	4.6	6.2
June 9, 1982	Mysidopsis bahia	Static	48	0	1.8	14.0
June 9, 1982	Mysidopsis bahia	Static (salinity adjusted)	48		5.6	
June 25, 1982	Mysidopsis bahia	Static	48	27.2	42.6	153.1
July 3, 1982	Mysidopsis bahia	Static	48	35.4	42.3	54.7
July 9, 1982	Mysidopsis bahia	Static	48	44.5	51.3	67.1
March 7, 1982	Cyprinodon variegatus	Flow-through	96	45.6	53.4	75.6
June 11, 1982	Cyprinodon variegatus	Static	48	NO MORTALITIES OBSERVED		

Source: ESE.

mortality, then virtually all of the acute bioassays indicated a degree of effluent toxicity to the invertebrate species tested.

If the question to be clarified, however, is whether acute toxicity was demonstrated at process wastewater concentrations expected to occur in the York River estuarine ecosystem, the conclusion must be modified. As indicated by the plume characterization study, the highest process wastewater concentration found in the York River was 0.35 percent, and this occurred only within a few meters of the outfall (001), both vertically and horizontally.

Over most of the area in which the plume was definable, the process wastewater concentration was 0.02 percent or lower. The only acute bioassay results even remotely indicating toxicity at concentrations approaching these levels were those conducted from June 8 to 12, 1982. Data strongly indicate that the increased toxicity at this time was due to a release of unusually high ammonia concentrations from the refinery wastewater treatment plant. Under normal operating conditions, acute toxicity testing provided no results that would indicate toxicity at process wastewater concentrations occurring in the receiving water body.

Chronic mysid testing indicated sublethal effects of 1.8- and 3.2-percent process wastewater over a 26-day testing period. Once again, however, these concentrations are much higher than the levels found in the York River. Therefore, the bioassay results predicted no documentable impacts on the York River ecosystem caused by discharges from the Amoco refinery under the conditions tested. This prediction was confirmed by field surveys, which failed to document any measurable ecosystem impacts.

5.2 IMPLICATIONS RELATIVE TO PROPOSED REGULATORY GUIDELINES

EPA and the state regulatory agencies work together for the protection of water quality by controlling the discharge of toxic substances in toxic amounts. Sections 308 and 402 of the Clean Water Act authorize EPA and the states to require NPDES permit applicants to provide chemical and biological data necessary to assure compliance with water quality standards.

The EPA (1983a) draft "Technical Support Document for Water Quality-Based Toxics Control" recommends that an integrated approach, including both biological and chemical techniques, be used to assess water quality impacts from industrial and municipal effluent sources. In the EPA view, the biological and chemical approaches both have advantages and disadvantages. The type of testing that is most appropriate in each situation depends, therefore, on the effluent type and receiving water characteristics. This integrated case-by-case approach requires techniques that identify specific toxic pollutants (chemical-specific) as well as techniques that detect whole effluent toxicity (bioassays).

The chemical-specific approach measures each toxic pollutant and evaluates its specific toxic properties. Unfortunately, not all potentially toxic substances can be identified by chemical methods. Furthermore, the complex chemical interactions occurring in the final, discharged effluent affect the fate and impact of such pollutants in the receiving waters. On the other hand, bioassays provide a measure of toxicity without identifying causative agents in complex effluents.

EPA (1983a) states bioassays are useful in water quality impact assessment because: (1) the effects of complex discharges of unknown as well as known chemical constituents can be assessed, (2) bioavailability of pollutants after discharge can be measured, (3) pollutants for which there are no adequate chemical analytical methods can be addressed, and (4) potential synergistic effects can be identified. In cases where

Best Available Technology Economically Achievable (BAT) is in place, EPA believes bioassays can be used to assure that state water quality standards are not violated by the discharger, by applying them as a monitoring criterion. This approach can be used to monitor and evaluate, thereby controlling, effluent discharges beyond BAT, secondary treatment, or other technology-based requirements of the Clean Water Act.

In general, this approach seems reasonable. In reality, however, its reasonableness depends extensively on the degree of reliance on bioassay applied, the validity of specific applied interpretations made of bioassay data, and the quality of judgements made by individuals administering regulatory policy.

In the United States, 20 states use specific numerical criteria related to bioassay LC50 data. Based on the lowest 96-hour LC50 determined in this study (4.6-percent process wastewater), the Amoco refinery would have failed the criteria in at least nine (45 percent) of these states. This is true despite the fact that extensive field studies were unable to discern any environmental impacts caused by the refinery effluent. Thus, at the very least, present rules have the potential of resulting in uneven imposition of criteria on American industry.

This conclusion is based on the least favorable bioassay results. While it is true that most bioassays conducted during this study indicated lower toxicity levels, it is equally true that wastewater treatment system malfunctions occasionally occur. Bioassays conducted during any of these system malfunctions would likely yield equally unfavorable results, even though the Amoco refinery was not in violation of its chemical-specific NPDES permit conditions.

5.3 SUGGESTED APPROACHES FOR FUTURE STUDIES

Based on the experience of this study, several recommendations may be made regarding strategies for future similar study efforts. The most important suggestions concern site selection.

Scientific method suggests that in any investigation the number of variables should be reduced to a minimum, to zero if possible, except for the phenomenon under investigation. Thus the implications associated with the phenomenon of interest can be more clearly perceived. The site selected for the present study, which is representative of a typical complex estuarine system, may not have been the most appropriate for the study. The question of whether ecosystem impacts can be predicted by use of single-species bioassays should be studied first in simple systems with complexity increasing as the simpler systems are understood. ESE, therefore, believes that, to the extent practical, future studies of this type should be conducted at a site where:

1. The effluent plume reaches the bottom;
2. Immediate dilution of the plume is not so great; and
3. The effluent plume is, to a much greater degree, unidirectional.

It is important in such studies to be able to clearly document the presence or absence of impacts of the discharge on the ecosystem, because, in a sense, these documented impacts serve as a benchmark against which bioassay results will be compared. Any doubts existing about the ecosystem impacts magnify doubts about the validity of the ultimate study conclusions. Therefore, it is exceedingly important to be able to define (1) a zone of effluent impact, (2) a zone of non-impact that is otherwise comparable to the impacted zone, and (3) a transitional impact zone, so that quantifiable impacts can be related to effluent concentration characteristics, which can in turn be related to bioassay results.

For similar reasons, a site should be selected that is, to the extent possible, free of other point and non-point source discharges, which usually complicate analysis.

Depending upon the specific aims of the study, it may be appropriate to select a site where ecosystem impacts are either known to occur, or can be anticipated with reasonable certainty. It is already known that bioassays can often predict ecosystem impacts in extreme cases (i.e., when they demonstrate a total lack of toxicity, and when they demonstrate such extreme toxicity that a disastrous impact can clearly be expected). It is not known, however, to what degree intermediate bioassay results may be used to predict intermediate ecosystem impacts. To assess this relationship, a site is required where intermediate bioassay results are obtainable, and where intermediate ecosystem impacts can be reasonably anticipated. The two sets of data may then be compared using whatever indices or statistical approaches are demanded by the study plan.

Finally, regarding site selection, it might be desirable to conduct the study in a tropical or sub-tropical location, such as Puerto Rico, to test EPA's selection of Mysidopsis bahia as the standard marine test organisms. M. bahia does not occur naturally in these areas, and such a location would provide a measure of this species' applicability to the entire universe of such regulatory applications.

Regarding selection of biotopes for study, ESE would recommend using macrobenthos and fouling plate organisms to assess ecosystem impact, as was done in this study.

In selection of test organisms for screening bioassays, ESE suggests that consideration be given to the salinity of the effluent, as well as of the receiving water. It is questionable whether daphnids should be used as the sole species for screening tests of saline effluents discharged into fresh waters, or conversely, mysids for non-saline

effluents discharged into seawater. The tolerance of these test organisms to effluent salinities perhaps should be tested in parallel to tests on the whole effluent.

Regarding chronic bioassays, it is suggested that the utility of shortened chronic and partial life cycle tests be included in future studies. These tests may be performed as subsets of full chronic or life cycle tests, if they are so designed in advance.

Chronic testing and partial life cycle studies provide valuable information on toxicity of substances at the sublethal level. Chronic testing requires a greater level of effort and expertise than short-term acute tests. Hence, chronic studies are more expensive than acute tests. The value of chronic data is directly related to the level of effort and expertise of the scientists conducting the tests. Hence, a poorly conducted test can result in ambiguous data and misleading conclusions.

A chronic life cycle test requires not only expertise in bioassay techniques but knowledge of the biology, specifically life history, of the species being tested. The person conducting the life cycle test must know how to culture the species as well as be able to identify common stress signs displayed by the test organism. In other words, the technician must first know what "normal" is before attempting to observe and describe "abnormal" or "stressed" behavior and/or responses.

The animal response and behavioral parameters usually monitored in a chronic test can be highly variable and subject to judgmental observations; thus, the reduction of additional variability is an important goal in sublethal testing. Continuity in data recording is critical in a test where the response can be very subtle and its natural variability very high. ESE would recommend, therefore, that one person be assigned to "read" a chronic test throughout its duration. This recommended approach can also create problems due to the length of

the test. In most cases, the test will last about 28 days and this would mean one person working 4 weeks straight doing the same monotonous chores. Hence, the attitude and dedication of the person conducting the test are critical "human" factors that can add to the variability of the data. If shortened chronic tests are found to yield results adequate to such studies, variability caused by such human factors may be reduced.

Finally, ESE suggests the incorporation of an evaluation of the "Instream Toxicity Analysis" (EPA, 1983d, Appendix B) in future studies. According to this procedure, samples of the receiving water are collected after effluent mixing in the receiving water system and used to conduct laboratory bioassays. If such water is collected from the dispersion plume after the plume is characterized by field studies, and the effluent concentration in the sample is verified at the time of collection, the sample provides a prediluted concentration of effluent in receiving water. If several such samples are collected from various points in the plume, each having a different effluent concentration, a range of effluent dilutions may be assembled. Bioassays run on such a field-diluted effluent series could be compared to a similar series derived from standard bioassay procedures. In this manner, it may be possible to assess the influence of undocumentable or unquantifiable phenomena occurring in the receiving water, but which can affect toxicity of the effluent to the ecosystem.

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