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Statistical Summary
EMAP-Estuaries
Virginian Province - 1990 to 1993

by

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APPENDIX A

SAMPLING DESIGN, ECOLOGICAL INDICATORS, AND METHODS

A.1 Region and Estuarine Classification

EMAP-E monitoring is conducted on regional and national scales. Standardized methods are employed, and the entire Virginian Province is sampled synoptically within a defined "index" time period to ensure comparability of data within and among sampling years. EMAP-E identified boundaries for 12 estuarine regions (Holland, 1990) based on biogeographic provinces defined previously by NOAA and the U.S. Fish and Wildlife Service using major climatic zones and prevailing major ocean currents (Terrell, 1979) (Figure A-1). The 1990-1993 Virginian Province Demonstration Project included the estuarine resources located along the irregular coastline of the mid-Atlantic coast between Cape Cod, MA and Cape Henry, VA, including: Buzzards Bay, Narragansett Bay, Long Island Sound, New York/New Jersey Harbors, Delaware Bay, and Chesapeake Bay. Five major rivers within the Province were monitored: the Hudson, the Delaware, the Rappahannock, the Potomac, and the James.

A review of the literature identified potential classification variables that reduced within-class variability. These variables included physical attributes (salinity, sediment type, depth), and extent of pollutant loadings. The use of salinity, sediment type, and pollutant loadings as classification variables *(i.e., a priori* strata) would result in the definition of classes for which areal extents could vary dramatically from year-to-year or even over the index sampling period of EMAP-E. This stratification process requires establishment of a sampling frame prior to sampling; thus misclassification of sample sites within a class should be minimized. Stratification by sediment type, depth, or salinity was considered to be difficult because detailed maps of sediment and water column characteristics were not available or are often unreliable for much of the Virginian Province. These attributes were not used.

A simple classification scheme based on the physical dimensions of an estuary was used to develop three classes -- large estuaries, large tidal rivers, and small estuaries/small tidal rivers. Large estuaries in the Virginian Province were defined as those estuaries greater than 260 km^2 in surface area and with aspect ratios (*i.e.*, length/average width) of less than 18. Large tidal rivers were defined as that portion of the river that is tidally influenced (*i.e.*, detectable tide > 2.5 cm), greater than 260 km^2, and with an aspect ratio of greater than 18. Small estuaries and small tidal rivers were designated as those systems whose surface areas fell between 2.6 km^2 and 260 km^2. These designations excluded estuarine water bodies less than 2.6 km^2 in surface area. These resources were included in the sampling frame by incorporating them into the adjacent water body, but they were not sampled separately.

Application of the classification scheme based upon geometric dimensions (criteria unlikely to change in reasonable time frames) to the Virginian Province estuarine resources resulted in the identification of 12 large estuaries; 5 large tidal rivers; and 144 small estuaries / small tidal rivers.

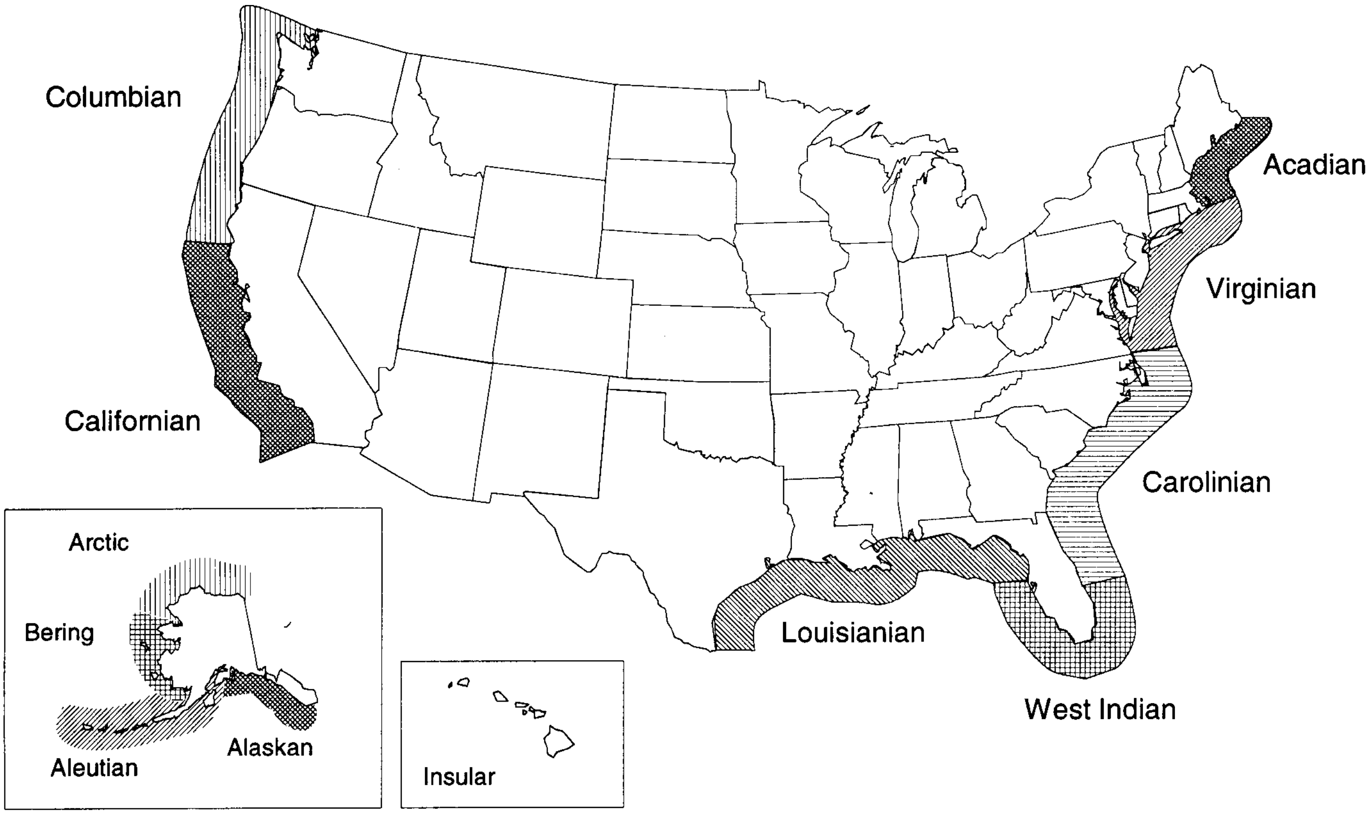

Figure A-1. EMAP-Estuaries biogeographical provinces.

A.2 Sampling Design

Sample collection in the Virginian Province focused on ecological indicators (see Section A.3) during the index sampling period (late July through September), the period when many estuarine responses to anthropogenic and natural stresses are anticipated to be most severe. The sampling design employed combines the strengths of systematic and random sampling with an understanding of estuarine ecosystems in order to provide a probability-based estimate of estuarine status in the Virginian Province. In addition, some special-study sites were sampled to collect information for specific hypothesis testing and other specific study objectives. This resulted in sampling seven types of sampling sites (stations) for the Virginian Province survey.

- Base Sampling Sites (BSS) are the probability-based sites which form the core of the EMAP-E monitoring design for all provinces, including the Virginian Province. Data collected from these sites are the basis of this preliminary status assessment. There were 446 BSS to be sampled during the four-year project.

- Index Sites (IND) were a continuation of a special study initiated in 1990. They are associated with the base sampling sites in small estuarine systems and large tidal rivers and are located in depositional environments where there is a high probability of sediment contamination or low dissolved oxygen conditions. A total of 86 IND sites were monitored.

- Long-term Trend Sites (LTT) were a select number of 1990 BSS that were revisited in 1991 through 1993. They were sampled each year to investigate the within-station annual variability. Twelve LTT sites were monitored (only 11 in 1991).

- Long-term Trends Spatial Transect (LTS) sites were located along a transect originating at selected large-estuary LTT stations. Twelve (12) LTS sites were associated with four transects in 1991 and were all located in the Chesapeake Bay. Three LTS sites were placed along each transect at 0.25, 0.5, and 1.0 statute miles from the associated LTT station to evaluate the spatial variability within a sampling cell. LTS sites were monitored only in 1991.

- Indicator Testing and Evaluation (ITE) sites were sampled to determine the reliability, sensitivity, and replicability of indicator responses for discriminating between sites with "known" environmental conditions. These sites were selected on the basis of historical information concerning dissolved oxygen concentration and sediment contamination. These sites were used to develop indices and test the discriminatory power of specific indicators. The number of ITE sites varied with year with some being revisited over multiple years. A total of 22 sampling events at ITE stations were conducted over the four-year period.

- Replicate sampling sites (REP) were randomly located in small estuarine systems. Replicate stations were designed to provide information on within-system spatial variability. Twenty-nine REP sites were sampled as part of the Demonstration Project.

- Supplemental sites (SUPP) were sites located in the Delaware Bay (large estuary) in 1990. These sites were selected using the same design used to locate large estuary sites, but on a smaller scale. The purpose of supplemental sites was to investigate the effect of scale on the design. A total of 24 supplemental sites were sampled in 1990.

A.3 Indicators

EMAP monitoring focuses on indicators of biological response to stress, and uses measures of exposure to stress or contamination as a means for interpreting that response. Traditionally, estuarine monitoring has focused on measures of exposure (*e.g.*, concentrations of contaminants in sediments) and attempted to infer ecological impacts based on laboratory bioassays. The advantage of the ecologically-based approach emphasized in EMAP is that it can be applied to situations where multiple stressors exist, and where natural processes cannot be modeled easily. This is certainly the case in estuarine systems, which are subject to an array of anthropogenic inputs and exhibit a great biotic diversity and complex physical, chemical, and biological interactions.

The implementation plan for the Virginian Province (Schimmel, 1990) listed three general indicator categories for the Demonstration Project: core, developmental and research. Table A-1 lists the EMAP-Virginian Province indicators.

Table A-1. Ecological indicators used in the Virginian Province Survey.

Category	Indicator
Core	Benthic Species Composition & Biomass Habitat Indicators (Salinity, pH, Temperature,Water Depth, % Silt-Clay)
Developmental	Sediment Contaminants Sediment Toxicity Dissolved Oxygen Concentration Gross Pathology of Fish Marine Debris Fish Community Composition & Lengths Water Clarity
Research	Histopathology of Fish Fish Biomarkers (1990 and 1991) Contaminants is Fish Tissue (1991 only)

A.4 Indicator Sampling Methods

The EMAP indicator strategy involves four types of ecological indicators (Hunsaker and Carpenter, 1990; Knapp *et al.*, 1990): Biotic condition, abiotic condition, habitat, and stressor. Biotic condition indicators are ecological characteristics that integrate the responses of living resources to specific or multiple pollutants and other stresses, and are used by EMAP to assess overall estuarine condition. Abiotic condition indicators quantify pollutant exposure and habitat degradation and are used mainly to identify associations between stresses on the environment and degradation in biotic condition indicators. Habitat indicators provide basic information about the natural environmental gradients. Stressor indicators are used to quantify pollution inputs or stresses and identify the probable sources of pollution exposure. Tables A-2 and A-3 list individual indicators.

Table A-2. Ecological indicators categorized as biotic condition, abiotic condition, and habitat indicators.

Indicator Type	Indicator
Biotic Condition	Benthic Community Composition Benthic Abundance Benthic Biomass Fish Community Composition Fish Lengths Pathology in Fish
Abiotic Condition	Sediment Contaminants Sediment Toxicity Dissolved Oxygen Concentrations Marine Debris
Habitat	Water Clarity Salinity Temperature Percent Silt-Clay pH Water Depth

Descriptions of the methods used for individual indicators have been taken from the Near Coastal Program Plan (Holland, 1990), the Virginian Province Implementation Plan (Schimmel, 1990), the 1993 Virginian Province Field Operations and Safety Manual (Reifsteck *et al.*, 1993), and the EMAP-E Laboratory Methods Manual (USEPA, 1995).

Table A-3. Subcomponents of ecological indicators.

Primary Indicator	Subcomponents
Benthos	Total abundance Species composition Species diversity Abundance by species Percentage by taxonomic group Biomass Biomass by taxonomic group
Fish	Total abundance Species composition Species diversity Abundance by species Percentage by taxonomic group Mean length by species
Gross Pathology	Type of disorder
Dissolved Oxygen	Instantaneous at sampling Continuous for 24-hr (15-min intervals)
Sediment Toxicity	*Ampelisca abdita* 10-day test
Sediment Contaminants	23 polycyclic aromatic hydrocarbons 15 metals 15 pesticides 18 PCB congeners Butyltins
Sediment Characters	Percent silt-clay Acid Volatile Sulfides (AVS: 1991-1993) Total organic carbon (TOC)

A.4.1 Biotic Condition Indicators

A.4.1.1 Benthos

Benthic invertebrate assemblages are composed of diverse taxa with a variety of reproductive modes, feeding guilds, life history characteristics, and physiological tolerances to environmental conditions (Warwick, 1980; Bilyard, 1987). As a result, benthic populations respond to changes in conditions, both natural and anthropogenic, in a variety of ways (Pearson and Rosenberg, 1978; Rhoads *et al.*, 1978; Boesch and Rosenberg, 1981). Responses of some benthic organisms indicate changes in water quality while others indicate changes in sediment quality. Most benthic organisms have limited mobility. They are not as able to avoid exposure to pollution stress as many other estuarine organisms (*e.g.*, fish). Benthic communities have proven to be a reasonable and effective indicator of the extent and magnitude of pollution impacts in estuarine environments (Bilyard, 1987; Holland *et al.*, 1988 and 1989).

Benthic samples for evaluation of species composition, abundance, and biomass were collected at all sampling sites. Samples were collected with a Young-modified van Veen grab that samples a surface area of 440 cm^2. Three (3) grabs were collected at each base, index, or long-term site. A small core (60 cc) was taken from each grab for sediment characterization. The remaining sample was sieved through a 0.5 mm screen using a backwash technique that minimized damage to soft-bodied animals. Samples were preserved in 10% formalin-rose bengal solution and stored for at least 30 days prior to processing to assure proper fixation.

In the laboratory, macrobenthos were transferred from formalin to an ethanol solution and sorted, identified to lowest practical taxonomic level, and counted. Biomass was measured for key taxa and all other taxa were grouped according to taxonomic type (*e.g.*, polychaetes, amphipods, decapods). Shell-free dry weight was determined using an analytical balance with an accuracy of 0.1 mg after drying at 60°C. Large bivalves were shucked prior to determining biomass. Smaller shells were removed by acidification using a 10% HCl solution.

A.4.1.2 Fish

There are several advantages to using fish as a potential indicator of estuarine condition. Because of their dominant position at the upper end of the estuarine food web, fish responses integrate many short-term and small-scale environmental perturbations. Fish are known to respond to most environmental problems of concern in estuaries, including eutrophication, habitat modification, and pathogenic or toxic contamination.

Fish were collected by trawling with a 15 m, high-rise otter trawl with a 2.5-cm mesh cod end. The net was towed for 10 minutes against the tide (if significant tidal current existed) between 0.5 and 1.5 m/s (1-3 knots). All fish caught in the trawl were identified to species and counted; up to 30 fish of a species from each collection were measured to the nearest millimeter.

Individuals collected in standard trawls were inspected for gross external pathological disorders at all stations where fish were collected. This inspection included checking body surface and fins for lumps, growths, ulcers and fin erosion. In 1991 only target species (Table A-4) were examined. Specimens with observed gross pathologies were preserved in Dietrich's solution for subsequent laboratory verification and histological examination. At indicator testing sites, all specimens exhibiting gross pathologies, and up to 25 pathology-free specimens of each target species (and 10 of non-target species), were preserved for quality control checks of field observations. These fish also received histopathological examinations related to liver lesions, spleen macrophage aggregates, and gill or kidney disfunction (research indicator).

Table A-4. 1991 target species examined for external pathology and saved for chemical residue analysis.

Common Name	Scientific Name
Atlantic Croaker	*Micropogonias undulatus*
Bluefish	*Pomatomus saltatrix*
Channel Catfish	*Ictalurus punctatus*
Scup	*Stenotomus chrysops*
Spot	*Leiostomus xanthurus*
Summer Flounder	*Paralichthys dentatus*
Weakfish	*Cynoscion regalis*
White Catfish	*Ameiurus catus*
White Perch	*Morone americana*
Winter Flounder	*Pleuronectes americanus*

A.4.2 Abiotic Condition Indicators

A.4.2.1 Sediment Collection Procedures

Sediments were collected using the same Young-modified van Veen grab used for benthic invertebrate sampling. The top 2 cm of 6 to 10 grabs were placed in a mixing bowl and homogenized. After approximately 2,000 cc of sediment were collected and completely homogenized, the sediment was distributed among containers for sediment characterization, sediment chemistry, and sediment toxicity testing.

A.4.2.2 Sediment Characterization

The physical characteristics of estuarine sediments (*e.g.*, grain size) and certain chemical aspects of sediments (*e.g.*, acid volatile sulfide [AVS] content, total organic carbon [TOC] content) influence the distribution of benthic fauna and the accumulation of contaminants in sediments (Rhoads, 1974; Plumb, 1981; DiToro *et al.*, 1991). Sediment silt-clay content was determined to help interpret biotic condition indicator data and sediment contaminant concentrations. AVS and TOC were collected not only as interpretive aids but also as potential covariates for toxic contaminant concentrations.

Subsamples from each benthic grab and contaminant/toxicity homogenate were retained for grain size determination. A sample for determination of AVS content was removed from the homogenate (1991) or directly from each grab being composited (1992-1993). Samples were shipped on ice to their respective processing laboratory. Samples for the determination of silt/clay content were sieved using a 63µm mesh sieve. Both an aliquot of the filtrate and the fraction retained on the sieve were dried in an oven at 60°C and weighed to calculate the proportion of silt/clay in the sample.

The AVS collection method employed in the 1991 Survey permitted the potential release of sulfides when the materials where processed on-board the sampling vessel and in subsequent shipping. The sample was collected from a homogenized composite (*i.e.*, allowing maximal exposure to oxygen). As a result, the accuracy of the 1991 AVS measurements could be in doubt although the precision may remain reliable as all samples were treated similarly. Modifications to the collection methods were incorporated into the 1992 sampling program to prevent a recurrence of these problems. Beginning in 1992 a two-cm deep core was removed from each grab included in the homogenate. These "plugs" were placed in the AVS container without mixing, thereby reducing the oxidation of the sample. The container was filled to the top to further reduce the probability of oxidation.

A.4.2.3 Sediment Contaminants

Metals, organic chemicals, and fine-grained sediments entering estuaries from freshwater inflows, point sources of pollution, and various non-point sources including atmospheric deposition, generally are retained within estuaries and accumulate in the sediments (Turekian, 1977; Forstner and Wittmann, 1981; Schubel and Carter, 1984; Nixon *et al.*, 1986; Hinga, 1988). Samples were collected from a homogenate created during sampling by combining the top 2 cm of sediment from 6-10 sediment grabs. The sediment was placed in clean glass jars with teflon liners or polypropylene containers (for organics and metals analyses, respectively), shipped on ice, and stored frozen in the laboratory prior to analysis for contaminants. Sediments were analyzed for the NOAA Status and Trends suite of contaminants (Table A-5).

A.4.2.4 Sediment Toxicity

Sediment toxicity testing is the most direct measure available for determining the toxicity of contaminants in sediments to indigenous biota. It improves upon direct measurement of sediment contaminants because many contaminants are tightly bound to sediment particles or are chemically complexed and, therefore, are not biologically available (U.S. EPA, 1989). Sediment toxicity testing, however, cannot be used to replace direct measurement of the concentrations of contaminants in sediment because such measurements are an important part of interpreting the results of toxicity tests.

Toxicity tests were performed on the composite sediment samples from each station. Tests were conducted using the standard 10-day acute test method (Swartz *et al.*, 1985; ASTM 1991) and the tube-dwelling amphipod *Ampelisca abdita*.

A.4.2.5 Dissolved Oxygen

Dissolved oxygen (DO) is a fundamental requirement for maintenance of populations of benthos, fish, shellfish, and other estuarine biota. DO concentrations are affected by environmental stresses, such as point and non-point discharges of nutrients or oxygen-demanding materials (*e.g.*, particulates, dissolved organic matter). In addition, stresses that occur in conjunction with low DO concentrations may be even more detrimental to biota (*e.g.*, exposure to hydrogen sulfide, decreased resistance to disease and contaminants). DO levels are highly variable over time, fluctuating widely due to tidal action, wind stress, and biological activity (Kemp and Boynton, 1980; Welsh and Eller, 1991).

Dissolved oxygen was sampled in three ways during the Virginian Province survey: 1) instantaneous water column profiles using a SeaBird model SBE 25 CTD, (2) point-in-time bottom oxygen conditions with a YSI (model 58) oxygen meter and the SeaBird CTD, and 3) continuous 24-72 hr measurements of bottom concentrations using a Hydrolab DataSonde 3 data logging array (1991 only). The first two measurements were taken at all sites, and the continuous measurements were taken at base stations (BSS) only, and only in 1991.

The Hydrolab DataSonde 3 data logger deployed at each 1991 Base site for 24-72 hours collected continuous DO data at 15-min intervals. The DataSonde 3 also collected salinity, temperature, water depth, and pH data. The instruments were calibrated prior to every deployment, and were checked on-board ship immediately prior to deployment and following retrieval by comparison to the YSI oxygen meter. These instruments were deployed

Table A-5. EMAP Virginian Province: Sediment Chemistry Analytes

Analyte Code	Definition
TOC	Total Organic Carbon Concentration in µg/g Dry Weight
AG	Silver Concentration in µg/g Dry Weight
AL	Aluminum Concentration in µg/g Dry Weight
AS	Arsenic Concentration in µg/g Dry Weight
CD	Cadmium Concentration in µg/g Dry Weight
CR	Chromium Concentration in µg/g Dry Weight
CU	Copper Concentration in µg/g Dry Weight
FE	Iron Concentration in µg/g Dry Weight
HG	Mercury Concentration in µg/g Dry Weight
MN	Manganese Concentration in µg/g Dry Weight
NI	Nickel Concentration in µg/g Dry Weight
PB	Lead Concentration in µg/g Dry Weight
SB	Antimony Concentration in µg/g Dry Weight
SE	Selenium Concentration in µg/g Dry Weight
SN	Tin Concentration in µg/g Dry Weight
ZN	Zinc Concentration in µg/g Dry Weight
PCB8	2,4'-dichlorobiphenyl in ng/gram
PCB18	2,2',5-trichlorobiphenyl in ng/gram
PCB28	2,4,4'-trichlorobiphenyl in ng/gram
PCB44	2,2',3,5'-tetrachlorobiphenyl in ng/gram
PCB52	2,2',5,5'-tetrachlorobiphenyl in ng/gram
PCB66	2,3',4,4'-tetrachlorobiphenyl in ng/gram
PCB101	3,3',4,4',5-pentachlorobiphenyl in ng/gram
PCB105	2,2',4,4',5-pentachlorobiphenyl in ng/gram
PCB118	2,3,3',4,4'-pentachlorobiphenyl in ng/gram
PCB128	2,2',3,3',4,4'-hexachlorobiphenyl in ng/gram
PCB138	2,2',3,4,4',5'-hexachlorobiphenyl in ng/gram
PCB153	2,2',4,4',5,5'-hexachlorobiphenyl in ng/gram
PCB170	2,2',3,3',4,4',5-heptachlorobiphenyl in ng/gram
PCB180	2,2',3,4,4',5,5'-heptachlorobiphenyl in ng/gram
PCB187	2,2',3,4',5,5',6-heptachlorobiphenyl in ng/gram
PCB195	2,2',3,3',4,4',5,6-octachlorobiphenyl in ng/gram
PCB206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl in ng/gram
PCB209	Decachlorobiphenyl in ng/gram
MBT	Mono-butyl Tin in ng/gram
DBT	Di-butyl Tin in ng/gram
TBT	Tri-butyl Tin in ng/gram
OPDDE	2,4'-DDE DDT and metabolites in ng/gram
PPDDE	4,4'-DDE DDT and metabolites in ng/gram
OPDDD	2,4'-DDD DDT and metabolites in ng/gram
PPDDD	4,4'-DDD DDT and metabolites in ng/gram
OPDDT	2,4'-DDT DDT and metabolites in ng/gram
PPDDT	2,4'-DDT DDT and metabolites in ng/gram

(Continued)

Table A-5 (continued).

Analyte Code	Definition
ALDRIN	Aldrin in ng/gram
ALPHACHL	Alpha-Chlordane in ng/gram
TNONCHL	Trans-Nonachlor in ng/gram
DIELDRIN	Dieldrin in ng/gram
HEPTACHL	Heptachlor in ng/gram
HEPTAEPO	Heptachlor epoxide in ng/gram
HEXACHL	Hexachlorobenzene in ng/gram
LINDANE	Lindane (gamma-BHC) in ng/gram
MIREX	Mirex in ng/gram
NAPH	Naphthalene in ng/gram
MENAP2	2-methylnaphthalene in ng/gram
MENAP1	1-methylnaphthalene in ng/gram
BIPHENYL	Biphenyl in ng/gram
DIMETH	2,6-dimethylnaphthalene in ng/gram
ACENTHY	Acenaphthlylene in ng/gram
ACENTHE	Acenaphthene in ng/gram
TRIMETH	2,3,5-trimethylnaphthalene in ng/gram
FLUORENE	Fluorene in ng/gram
PHENANTH	Phenanthrene in ng/gram
ANTHRA	Anthracene in ng/gram
MEPHEN1	1-methylphenanthrene in ng/gram
FLUORANT	Fluoranthene in ng/gram
PYRENE	Pyrene in ng/gram
BENANTH	Benz(a)anthracene in ng/gram
CHRYSENE	Chrysene in ng/gram
BENZOBFL	Benzo(b)fluoranthene in ng/gram
BENZOKFL	Benzo(k)fluoranthene in ng/gram
BENAPY	Benzo(a)pyrene in ng/gram
BENEPY	Benzo(e)pyrene in ng/gram
PERYLENE	Perylene in ng/gram
INDENO	Ideno(1,2,3-c,d)pyrene in ng/gram
DIBENZ	Dibenz(a,h)anthracene in ng/gram
BENZOP	Benzo(g,h,i)perylene in ng/gram
SAND_PC	Sand Content (%)
SICL_PC	Silt-Clay Content (%)

approximately 1 m from the bottom. The stored data were downloaded to a computer and the unit was serviced and recalibrated for subsequent deployment at another site. Dataloggers were also deployed in 1990 for the entire summer (*ca.* 60 days) at selected sites (Long-Term Dissolved Oxygen [LTDO] sites) to evaluate variability. Units deployed in 1990 were set to log at 30-minute intervals. The results of this special study are discussed in the 1990 Demonstration Project Report (Weisberg *et al.,* 1993).

Water column profiles for dissolved oxygen were collected at each station using a SeaBird SBE-25 SeaLogger CTD. This unit was equipped with probes to measure salinity, temperature, depth, pH, DO, light transmission, fluorescence, and PAR. The unit was equilibrated at the sea surface, and then lowered through the water column at *ca.* ¼ m/s until it reached a depth of one meter above the bottom where it was allowed to equilibrate. It was then returned to the surface and all CTD data were downloaded to an on-board computer for review and storage. If the cast appeared unusual or failed QC it was repeated. Beginning in 1991 a bottom water sample was collected using a *Go-Flo* water sampling bottle, and the dissolved oxygen concentration of the sample determined with a YSI Model 58 DO meter. This measurement served as a check on the CTD probe as well as a back-up in case the CTD failed.

A.4.2.6 Marine Debris

The kinds and amounts of floating and submerged (*i.e.*, collected in trawls) marine debris were noted at all stations. Debris was categorized as paper, plastics, metal, glass, wood, and other wastes. Only debris of anthropogenic origin was included. Wastes that were comprised of composited materials (*e.g.*, metal, wood, and plastic) were categorized based on their dominant component.

A.4.3 Habitat Indicators

Habitat indicators provide basic information about the natural environmental setting. Habitat indicator data discussed in this report include water depth, salinity, temperature, pH, water clarity, and sediment silt/clay content.

All water quality measurements were made using the Seabird model SBE 25 CTD (described earlier). This unit was equipped with probes to measure salinity, temperature, depth, pH, DO, light transmission, fluorescence, and PAR.

Measurements of water clarity are incorporated into the CTD casts that were performed at each station. Included in the CTD instrumentation package are a SeaTech transmissometer and a Biospherical PAR (Photosynthetically Active Radiation) sensor. As the CTD is lowered through the water column, transmissivity and PAR data are continually logged.

Surficial water samples were collected at all stations for determination of Total Suspended Solids (TSS). Samples were refrigerated, returned to the laboratory, filtered through a glass-fiber filter, dried and weighed.

Sediment silt/clay content was measured on samples taken from the surficial sediment (top two cm) homogenate from which chemistry and toxicity samples were also removed.

A.5 Data Collection and Sample Tracking

Each field crew was supplied with two portable computers and appropriate software to facilitate electronic recording of the data, data transfer, and sample tracking. All samples, shipments, and equipment were labelled with bar-coded labels to facilitate sample tracking and reduce transcription errors. Field computers were equipped with bar code readers to record sample identification numbers. Receiving laboratories were also equipped with bar code readers to facilitate the receiving process and to rapidly convey information concerning lost or damaged shipments.

Copies of all data entered into the field computer were stored on the hard disk and copied to diskettes. Information on the hard disk was transferred daily via modem to the Information Management Center at AED-Narragansett (RI). Backup diskettes and hard-copy data sheets were shipped weekly to the Center.

All transferred data were examined within 24-48 hours of collection by EMAP-E personnel. Errors were brought to the attention of the field crews for correction and resampling, if required. All electronic data were checked against paper data forms for verification. Further information on the details of the Near Coastal data management systems are presented in Rosen *et al.* (1990).

A.6 Analytical Methods For This Statistical Summary

Three types of analyses were conducted for this report: 1) direct descriptions of measured indicators, 2) development of modified or adjusted indicators (*e.g.*, metal contaminants in sediments), and 3) development of indices based on directly measured indicators. These analyses are documented in a Virginian Province 1990 Demonstration Report (Weisberg *et al.*, 1993) and Appendix C of this document.

A.6.1 Cumulative Distribution Functions (CDFs)

All ecological indicators collected during the Virginian Province survey were characterized using Cumulative Distribution Functions (CDFs). These functions describe the full distribution of indicators in relation to their areal extent within the Province. All observations are weighted based upon surface area associated with each sampling site. The area associated with each sampling unit in large estuaries was equal to the hexagonal spaces created by the EMAP grid (70 km^2). For tidal river and small estuary classes, the area associated with each station was determined using the ARC/INFO data model which produces areal and perimeter estimates. For the tidal river class ARC/INFO was used to delineate the extent of 25 km-long segments beginning at the mouth of the river on a 1:100,000 digital line graph. The area of a large tidal river station is equal to the area of the segment containing the station. For small estuarine systems, the station area is equal to the area of the system in which it was randomly located. The total areas associated with the three classes is: large estuaries - 16,097 km^2; large tidal rivers - 2,602 km^2; and small estuaries - 4,875 km^2.

To generate estimates across classes (strata), weights for stations within each class were adjusted so that the total of the weights for that class was equal to the total area represented by all stations (including unsampleable stations) within that class. The equations used in the generation of CDFs are described in Appendix B.

A.6.2 Adjustment To Known Covariates

In several cases, variability in observed indicators might reflect relationships to known habitat or control variables. Examples of these relationships are: variation in estuarine biota resulting from sampling throughout the salinity gradient; variation in sediment toxicity tests with different mortalities associated with the controls; and variation in sediment metals observed at a site resulting from variations in the amount of natural crustal materials at the site. In all these cases, the observed data must be adjusted in order to construct CDFs or to compare observations from different locations.

A.6.2.1 Adjustment for Natural Habitat Gradients

Estuarine biota are largely controlled by their environmental settings, both natural and anthropogenic. Natural gradients, particularly in salinity and silt-clay content, are common in estuaries. Many estuarine organisms may represent overlapping discrete distributions along these gradients. Thus, normalization of ecological measures over habitat gradients is a common tool used to interpret information when such normalization is necessary. Such relationships were examined; however, no data were normalized in the production of this report.

A.6.2.2 Adjustment for Experimental Controls

Estimates of the area in the Virginian Province containing toxic sediments were based on the results of toxicity tests using the amphipod, *Ampelisca abdita*. For this summary, a relative measure of toxicity was created to facilitate comparisons between sites over a series of bioassays. This adjustment is necessary because control mortalities vary among test series. Sediments were determined to be toxic if: survival of the test organism in test sediments was less than or equal to 80% of the survival observed in clean, control sediments; survival in test and control sediments were significantly different ($p < 0.05$); and survival in control sediments was ≥ 85%. This results in an adjustment to the observed survival rates in test sediments that accounts for variability due to differences in the controls for individual bioassays. These criteria are consistent with those established in U.S. EPA/ACE (1991).

A.6.2.3 Adjustment for Natural Crustal Properties

The extent to which anthropogenic activities have affected concentrations of metals in sediments is complicated by the natural variation of concentrations due to differing particle size distributions in sediments. Because of surface adsorptive and complexation processes, fine-grained sediments will naturally have higher trace metal concentrations than coarse sediments. In some studies, *e.g.*, the National Status and Trends program, reported concentrations are adjusted for this variation by normalizing the concentrations by the fine-grained fraction determined separately. As an alterative to actual size-fractionation measurements, a number of authors (Windom *et al.*, 1989; and Schropp *et al.*, 1990) have determined relationships between sediment concentrations of trace metals and other elements indicative of fine-grained crustally-derived material, *e.g.*, aluminum, iron and manganese. The most commonly used of these indicator elements is aluminum, due to its large natural abundance, freedom from common anthropogenic contaminant sources and significant correlation with both the fine-grained fraction and trace metal concentrations in clean, un-impacted sediments. The correlation between aluminum and trace metals in fine-grained sedimentary material has a geochemical basis related to the composition of crustal material from which the fine particles are derived and the natural adsorption and complexation processes occurring during "weathering" of the crustal material. Once background sediment metal-aluminum relationships have been determined, concentrations of metals expected from background material can be subtracted from total metal concentrations, allowing residual, presumably anthropogenic, contributions to be assessed.

Background metal-aluminum relationships are derived by linear regression of sediment concentrations of each element against aluminum concentrations in the same sediment. Some investigators have used log-transformed metals concentrations in the regression analyses. Such transformations do not improve correlation of the metals-aluminum concentrations of this data set. Furthermore, linear regressions provide direct correlation with the physical mixing and geochemical factors noted above which affect the overall concentration of metals in sediments. This correlation is lost when the concentrations are transformed. Consequently, no data transformations were performed prior to regression analysis.

Use of linear regression to determine metal-aluminum relationships in background sedimentary material can only be successful if the sediments do not include contributions from sources other than natural background sediments. The data sets used in this study were statistically screened to eliminate samples which might contain additional source materials. This was accomplished by performing linear regressions of concentrations of aluminum against each metal. The residuals (the differences between the measured concentrations and those predicted from the regression) were then tested for normal distribution. If the residuals were found not to be normally distributed, samples which had studentized residual values greater than 2 were eliminated from the data set. Regression of the reduced data set was repeated and the residuals tested again for normal distribution. This process was repeated for each metal until residuals from the regressions were all normally distributed, at which point the remaining samples were assumed to represent natural, background sediments.

The regression relationships derived for the background sediments were then applied to the original data set. Samples with trace metal concentrations exceeding the upper 95% confidence limit for that metal's regression against aluminum were designated as enriched. It should be noted that no assessment was made as to the magnitude of enriched; metal concentrations might be only slightly above the 95% confidence limit or might exceed the limit by factors of 10-100. The categorization "enriched" was applied to any sediment with a metal concentration higher than that expected from the background sediment aluminum metal relationship at the 95% confidence level.

A.6.3 The Benthic Index

A benthic index, which uses individual measures of the benthic community, was utilized to report on the condition of the benthic biological resources of the Virginian Province. The index, as used in this report, was developed from a subset of the data collected over all four years of sampling and was constructed to represent a combination of individual benthic measures that best discriminates between good and poor benthic conditions. This current index is EMAP's continued attempt to reduce many individual indicators into a single value that has a high level of discriminatory power between good and poor ecological conditions. The reader should note that the index as used in this report is a revision to prior ones used in earlier EMAP-VP reports. It has always been the intent of the program to continually revise the benthic index as more data became available (Weisberg *et al.*, 1993), and the current index represents the effort using four years of available EMAP data (Paul *et al.*, in preparation).

The process for developing an index of benthic community condition has been documented for the 1990 (Weisberg *et al.*, 1993) and, separately, for the 1990-91 (Schimmel *et al.*, 1994) data sets. This process entails several discrete steps: identification of a set of benthic parameters to define conditions that include components of faunal and functional diversity and structure; determination of the statistical relationships between these benthic parameters and habitat variables; normalization of those benthic parameters that are strongly associated with habitat condition; identification of a test data set that clearly distinguishes relatively pristine sites from those exhibiting toxic contamination, hypoxia, or both; and application of discriminant analysis to the test data set to determine those benthic parameters whose variation is most closely associated with differences in reference and impacted condition. This same process was used with the 1990-93 data set.

The benthic index developed using the 1990-91 data set suffered from poor representation of impacted and reference conditions in low salinity (< 5 ppt) in the test data set. This benthic index was highly correlated with salinity and appeared to misclassify good sites in the oligohaline and impacted sites in the meso- and polyhaline. This led to the refinement of the candidate benthic parameters, utilization of more stringent criteria for assignment of sites to the reference and impacted categories in the test data set, and revision of the benthic index based upon the four-year data set (1990-93). Statistical analyses indicated that most measures of diversity and abundance of low salinity tubificids were highly correlated with salinity and required normalization (Weisberg *et al.*, 1993).

The test data set was constructed to contain an equal number of impacted and reference sites, and equal number of sites exhibiting each condition in each of the three salinity zones (< 5, 5-18, > 18 ppt), and a relative balance of muddy and sandy sites in each condition/salinity category. For a site to be included in the reference condition test data set, all of the following were met: bottom dissolved oxygen > 7 mg/l; no more than three sediment contaminant concentrations exceeding Long *et al.* (1995) ER-L values, and none exceeding ER-M concentration; and survival in a sediment toxicity test 90% or better and not significantly different from control survival. Ten sites in each of the oligo-, meso-, and polyhaline zones were selected. Thirty impacted sites were selected based on criteria that included: low bottom dissolved oxygen (< 2 mg/l); low survival in the toxicity test and multiple concentration exceedances of ER-M values. The test data set contained 60 cases; 30 were categorized as impacted and 30 were reference.

The discriminant analyses identified a series of highly correlated benthic indices that correctly classified reference and impacted sites in the test data set. The benthic parameter common to the candidate indices that accounted for the greatest degree of variability was a measure of species richness, Gleason's D (Washington, 1984). The benthic index that was chosen (1) maximized classification efficiency using the test data set (goal of ca. 90% correct classification), (2) provided a good degree of cross-validation with the test data set (goal of ca. 80% cross-validation), (3) produced a good classification efficiency with a validation data set (goal of ca. 80% correct classification), and (4) had the individual parameters contribute to the overall score consistently with our understanding of benthic communities. The stations that met the reference and impacted site criteria, but were not used in the test data set for the discriminant analysis, were used as a validation data set (52 cases).

The three benthic parameters of the index were: salinity-normalized expected Gleason's D for infaunal and epifaunal species; salinity-normalized expected number of tubificids and abundance of spionids. The richness measure is associated with reference conditions (positive contribution) and the latter two measure are associated with impacted conditions (negative contribution).

The discriminant score calculation normalizes the individual parameters based on the mean and standard deviation for the parameter in the test data set. The critical value for discriminating between reference and impacted sites was determined to be zero using the following equation:

Benthic Index Score =

1.389 (pct expect Gleason - 51.5) / 28.4
- 0.651 (normalized tubificid abundance - 28.2) / 119.5
- 0.375 (spionid abundance - 20.0) / 45.4

Where:

Percent Expected Gleason diversity index value =

Gleason / (4.283 - 0.498*bottom salinity
+ 0.0542 * bottom salinity^2
- 0.00103* bottom salinity^3) * 100

Normalized Tubificid Abundance =

Tubificids - 500 * $e^{-15*\text{bottom salinity}}$

APPENDIX B

ESTIMATION FORMULAE FOR EMAP SAMPLING IN THE LOUISIANIAN AND VIRGINIAN PROVINCES

Acknowledgements: The equations described in this section for large estuary, large tidal river, and whole province estimates were formulated by Douglas Heimbuch and Harold Wilson of Coastal Environmental Services Inc., Linthicum, MD and Stephen Weisberg of Versar Inc., Columbia, MD

B.1 INTRODUCTION

This appendix describes the equations used for making the four-year estimates of the areal extent of conditions of interest (and estimates of variances for these estimates) reported in this document. Equations were formulated using data from the first four years of EMAP-Estuaries (EMAP-E) sampling conducted in the Virginian and Louisianian Provinces. The recommended methods were chosen to be consistent with the sampling designs employed in each of the estuarine system classes (large estuaries, large tidal rivers, and small estuaries). This appendix describes the generic equations for each class, followed by specific instructions for application to Virginian Province data. The equations and associated SAS programs were provided to the EMAP-Virginian Province team by EMAP-Estuaries.

The reader should note that the large estuary and large tidal river equations differ from those used for generating single-year estimates in the 1991 and 1992 Statistical Summaries. These equations represent a refinement of the earlier equations. It should be noted that these equations are still under review and may be further refined in the future to address additional measurements of variability. Any alterations to the equations should result in only minor changes. A comparison of the confidence intervals generated using the "old" equations with those reported in this section shows only small changes result.

The 95% confidence intervals reported in this document are calculated as 1.96 times the standard error of the estimate, with the standard error (of the estimate) being the square root of the variance of the estimate.

B.2 LARGE SYSTEM RECOMMENDED METHODS

The recommended method for large system estimation is based on a sampling design in which sampling stations are selected within hexagons of a randomly overlaid grid. If a station within a hexagon is on land, then no sample is obtained from that hexagon. The estimated subnominal (*i.e.*, impacted) area for one year of the survey is based on Horvitz-Thompson estimation methods and is given by:

$$\hat{Y}_t = A\frac{\sum_{i=1}^{n} z_{ti}x_{ti}}{\sum_{i=1}^{n} x_{ti}} = A\frac{N}{D}$$

where,

$\hat{Y}_t$ = the estimated subnominal area in year t

A = the known total area of large systems in the province

$N = \sum_{i=1}^{n} z_{ti}x_{ti}$

$D = \sum_{i=1}^{n} x_{ti}$

n = the total number of hexagons subject to sampling in the province

z_{ti} = the response from hexagon i in year t (=1 if subnominal, 0 otherwise)

x_{ti} = 1 if a sample is obtained from hexagon i, 0 otherwise.

The indicator variable X_{ti} can also be defined in a manner to estimate the subnominal area of a particular subpopulation of the province (*e.g.*, Delaware Bay as a subpopulation within the Virginian Province). In this case, X_{ti} would be defined as 1 if the sample was obtained in the subpopulation of interest, and zero otherwise. The total area used in this calculation (A) would be the known area of the subpopulation of interest.

The variance of the estimated subnominal area is estimated using a formula based on the Yates-Grundy formula for the variance of the Horvitz-Thompson estimator, and the Taylor series expansion formula for the variance of a quotient:

$$\hat{var}(\hat{Y}_t) = \hat{var}\left(A\frac{N}{D}\right) = \left(A\frac{N}{D}\right)^2\left[\frac{\hat{var}(N)}{N^2} + \frac{\hat{var}(D)}{D^2} - \frac{2\hat{cov}(N,D)}{ND}\right]$$

where,

$$\hat{var}(N) = \sum_{i=1}^{n}\sum_{j>i}^{n} W_{ij}(z_{ti}x_{ti} - z_{tj}x_{tj})^2$$

$$\hat{var}(D) = \sum_{i=1}^{n}\sum_{j>i}^{n} W_{ij}(x_{ti} - x_{tj})^2$$

$$\hat{cov}(N,D) = \sum_{i=1}^{n}\sum_{j\neq i}^{n} W_{ij}x_{ti}(x_{ti}z_{ti} - x_{tj}z_{tj}) \quad ,$$

[which is equivalent to, $\hat{cov}(N,D) = \sum_{i=1}^{n}\sum_{j>i}^{n} W_{ij}(x_{ti} - x_{tj})(x_{ti}z_{ti} - x_{tj}z_{tj})$]

$$W_{ij} = \frac{V_{ij}}{1 - V_{ij}}$$, and

V_{ij} = the proportion of random placements of the grid which result in points *i* and *j* lying in the same hexagon.

Two stations can be jointly selected for sampling in the same year only if they are located in separate hexagons. The probability of joint inclusion is therefore related to the complement of the probability that the random placement of the grid causes the two points to be included in the same hexagon. For stations that are sufficiently distant, the probability of being included in the same hexagon is equal to zero.

The recommended method for estimating the subnominal area is based on the ratio of two random quantities: the number of actual samples associated with a subnominal response, and the total number of actual samples. The total number of actual samples is a random quantity due to the edge effect of hexagons that include both land and water area. An alternative method could be used for estimation that is based only on the product of the number of hexagons with a subnominal response and the area of a hexagon. However, this method could produce estimates of subnominal area that are greater than the total known area due to a greater than expected number of sample stations falling in water. The ratio method is recommended to insure that estimates of subnominal area are not adversely affected by actual sample size in this manner.

To estimate the four-year average subnominal area in large systems, the average of the annual estimates is calculated:

$$\hat{Y} = \frac{\sum_{t=1}^{4} \hat{Y}_t}{4}$$

where,

$\hat{Y}$ = the estimated average response over four years.

The estimated variance of the four-year average is given by:

$$var(\hat{Y}) = \frac{1}{16} \sum_{t=1}^{4} var(\hat{Y}_t)$$

The recommended method for estimating the four-year average subnominal area employs years as independent strata. An alternative method could have been used which combines data from all years into one procedure without stratifying by year. In this method, years that received more effort due to random sample sizes would receive more weight in the calculation of the four year average. By treating years as strata, each year receives equal weight in the estimation of the four-year average. The recommended method also insures that the year to year variation in the response variable will not affect the variance of the four year average estimate. This is consistent with the view of interannual variability as a fixed effect (rather than a random effect) when characterizing a specified set of years.

Application to the Virginian Province

The recommended method for estimating the subnominal area for one year can be applied to large system data from the Virginian Province. However, sampling stations in the Virginian Province were not randomly selected within hexagons, but were obtained at the center point of each hexagon. For this reason, the recommended method for the estimation of the variance of the subnominal area estimate in one year cannot be directly applied. An approximate estimate of this variance can be calculated by redefining V_{ij} as :

$V_{ij}=\frac{1}{\beta n}$ for i and j in adjacent hexagons, 0 otherwise,

where β is the proportion of all pairs of samples that are from adjacent hexagons. This approximation is based on the following relationships:

$$\pi_{ij}=(1-V_{ij})\frac{1}{a^2}$$

and,

$$\sum_{i}\sum_{j\neq i}\pi_{ij}=n(n-1)$$

where,

$n =$ number of hexagons in the grid

$a =$ area of each hexagon (in appropriate units)

$\pi_{ij} =$ joint inclusion probability for station locations i and j.

Therefore,

$$na(na-1)\beta(1-\bar{V})\frac{1}{a^2}+na(na-1)(1-\beta)\frac{1}{a^2}=n(n-1)$$

where,

$\bar{V} =$ average of non-zero V_{ij} values

$\beta =$ proportion of i,j pairs that are no farther apart than the distance between centers of adjacent hexagons,

and,

$\bar{V}=\frac{1}{\beta n}$ for large "a" (relative to the size of a sampling station).

The recommended method for estimating the variance of the four-year average can be applied to the approximate variances of the one year estimates. This approach does not account for any gain in precision that may be caused by the four-year interpenetrating design which was implemented in the Virginian Province. The potential gain in precision is due to negative covariance among the annual estimates. The magnitude of the negative covariance depends on the degree of spatial autocorrelation of distances less than the size of a hexagonal cell. Analyses of the data suggest that spatial autocorrelation in the response variables is insignificant at distances as small as 2.5 km. Therefore, little increase in precision is anticipated and the recommended method is likely to provide an adequate approximation.

B.3 TIDAL RIVER RECOMMENDED METHODS

The recommended methods for tidal rivers estimation is based on a stratified random sampling design. Each river is stratified into areas of river length equal to 25 kilometers. In each year, at least one sample is obtained in each stratum with some strata providing two samples (Lousinian Province only: see notes on Application to Virginian Province). Each 25-km segment was divided into four subsegments, with one being sampled each year. The statistical area applied to each station was equal to the area of the subsegment the station resided in plus the area of the next three upstream subsegments. The recommended method for estimating subnominal area for tidal rivers is:

$$\hat{Y}_t = A\frac{\sum_{i=1}^{n} W_i \bar{z}_{ti}}{\sum_{i=1}^{n} W_i}$$

where,

$\hat{Y}_t$ = estimated subnominal area in year t

A = the total known area of tidal river systems in the province

n = the number of sampled tidal river strata in the province

W_i = the area of stratum i

$\bar{z}_{ti} = \frac{\sum_{j=1}^{m_{ti}} z_{tij}}{m_{ti}}$ = the average response in year t and stratum i

z_{tij} = the response in year t, stratum i, sample j (1 if subnominal, 0 otherwise)

m_{ti} = the number of observations in year t and stratum i.

The recommended method can be applied to estimate the subnominal area in a particular tidal river within the province. In this application, only data from the strata of interest would be utilized, and the total area (A) would be that of only the selected tidal river.

The variance of the estimated subnominal area is calculated as:

$$v\hat{a}r(\hat{Y}_t) = \frac{A^2\bar{S}_t^2}{\left(\sum_{i=1}^{n} W_i\right)^2}\left[\sum_{i=1}^{n} W_i^2\left(\frac{1}{m_{ti}}\right)\right]$$

where,

$v\hat{a}r(\hat{Y}_t) =$ the estimated variance of the subnominal area estimate in year t,

$\bar{S}_t^2 = \dfrac{\sum_{i=1}^{n^*}\sum_{j=1}^{m_{ti}}(z_{tij}-\bar{z}_{ti})^2}{\sum_{i=1}^{n^*}(m_{ti}-1)}$ (the pooled estimate of within stratum variance)

$n^* =$ the number of strata with replicate samples ($m_{ti} \geq 2$).

The variance of the estimated subnominal area is based on the estimate of within-strata variance pooled across strata which contain at least two observations. The estimation of variance requires that at least one stratum contains two or more observations (see note on application to Virginian Province).

The estimate of the four-year average subnominal area is calculated as the average of the annual estimates:

$$\hat{Y} = \frac{1}{4}\sum_{t=1}^{4}\hat{Y}_t$$

where,

$\hat{Y} =$ the estimated four-year average subnominal area.

The estimated variance of the four-year average estimate is:

$$v\hat{a}r(\hat{Y}) = \frac{1}{16}\sum_{t=1}^{4} v\hat{a}r(\hat{Y}_t)$$

Application to the Virginian Province

The recommended method for estimating tidal river subnominal area can be applied to Virginian Province data. However, the area subject to sampling in each tidal river changed over the four-year period because the statistical area applied to each station was equal to the area of the subsegment the station resided in plus the area of the next three upstream subsegments. Stations in the first subsegment were sampled in 1990, third subsegment in 1991, second subsegment in 1992, and fourth subsegment in 1993. Therefore, in year 1 the reach from 0 km to 125 km was subject to sampling, in year 2 the reach from 12 km to 125 km was subject to sampling, in year 3 the reach from 6.25 km to 125 km, and in year 4 the reach from 18.75 km to 125 km was subject to sampling.

The tidal river sampling in the Virginian Province consisted of one sample per stratum. Since replicate observations were not obtained in any stratum, the approximate estimate of within-stratum variance applied to Louisianian Province data can also be applied to data from the Virginian Province. In this application, approximations of within-stratum variances can be calculated separately for each tidal river.

The recommended methods for the estimate of the four-year average subnominal area and corresponding variance can be directly applied to tidal river data from the Virginian Province. This approach does not account for any gain in precision that may be caused by the four-year interpenetrating design which was implemented in the Virginian Province. Similarly to the approach for large systems, the potential gain in precision is a function of the degree of spatial autocorrelation in the response variables. Since studies have suggested that the degree of spatial autocorrelation is small, little increase in precision from the interpenetrating design is anticipated, and the recommended approach is likely to produce an adequate approximation.

B.4 SMALL SYSTEM RECOMMENDED METHODS

For small estuarine systems, estimates of CDFs and associated variances were computed based on a random selection of small systems within the Province, with replicate samples taken from a subset of the selected systems (Cochran, 1977). Unlike large estuaries and large tidal rivers, only a portion of the area of this class is sampled each year; therefore, a single four-year estimate is produced from the entire dataset as opposed to pooling individual yearly estimates. This method is directly applicable to Virginian Province data without modification The resulting CDF estimate is:

$$\hat{P}_{Sx} = \frac{\sum_{i=1}^{n} A_i \bar{y}_i}{\sum_{i=1}^{n} A_i}$$

where,

$\hat{P}_{Sx}$ = CDF estimate for value x

$\bar{y}_i = \frac{1}{m_i} \sum_{i=1}^{m_i} y_{ij}$

m_i = number of samples at small system i

A_i = area of small system i

$y_{ij} = \begin{cases} 1 \text{ if response is less than } x \\ 0 \text{ otherwise} \end{cases}$

n = number of small systems sampled

Since replicate samples were only obtained at a subset of the sampled small estuarine systems, the formula for the estimated variance taken from Cochran (1977 eq. 11.30) was modified to produce the following estimate of the approximate mean squared error (MSE) of the CDF estimate:

$$MSE(\hat{P}_{sx}) = \frac{\frac{N^2}{n}(1-f_1)\frac{\sum_{i=1}^{n} A_i^2(\bar{y}_i-\hat{P}_{sx})^2}{n-1} + \frac{N}{n*}\sum_{i=1}^{n*}\frac{A_i^2 S_{2i}^2}{m_i}}{A^2}$$

where,

$f_1 = n/N$

$n*$ = number small systems with replicate samples

$$S_{2i}^2 = \frac{\sum_{i=1}^{m_i}(y_{ij}-\bar{y}_i)^2}{m_i-1}$$

A = the total area of small systems in the Province (4,875 km^2)

N = number small systems in Province (144)

B.5 ENTIRE PROVINCE RECOMMENDED METHODS

The recommended estimate for the subnominal area for the entire province (*i.e.*, across all system classes) is the sum of the subnominal area estimates of the large systems, tidal rivers, and small systems:

$$\hat{U}_t = \sum_{i=l,t,s} \hat{Y}_{ti}$$

where,

$\hat{U}_t$ = the estimated subnominal area for the entire province in year t

$\hat{Y}_{ti}$ = the estimated subnominal area in year t for system class i, (i=large, tidal, small).

The estimated variance for the subnominal area in the entire province is the sum of the component variances:

$$v\hat{a}r(\hat{U}_t) = \sum_{i=l,t,s} v\hat{a}r(\hat{Y}_{ti})$$

where,

$v\hat{a}r(\hat{U}_t)$ = the estimated variance of the subnominal area in the entire province,

$v\hat{a}r(\hat{Y}_{ti})$ = the estimated variance of the subnominal area in year t, system class i.

The recommended methods for estimation in the entire province are based on the assumption of the system classes as being independent strata. The methods can be directly applied to one-year and four-year average estimates, and to data from both the Louisianian and Virginian Provinces.

APPENDIX C

LINEAR REGRESSIONS OF INDIVIDUAL METALS AGAINST ALUMINUM USED IN THE DETERMINATION OF METALS ENRICHMENT OF SEDIMENTS OF THE VIRGINIAN PROVINCE

As discussed in Section 3.2.3.5, concentrations of individual metals were normalized against the crustal element aluminum in an attempt to provide a basis for estimating the areal extent of enrichment of these metals in Virginian Province sediments. The method utilized is described in Appendix A (Section A.6.2.3). For each metal, a regression and an upper 95% confidence interval was determined and plotted (Figures C-1 to C-8). Stations with concentrations falling above the upper 95% confidence interval were classified as enriched for that metal. This process was inefficient for several metals, but performed well for As, Cr, Fe, Hg, Mn, Ni, Sb, and Zn. Regressions and regression parameters (slope, intercept, and correlation coefficient: Table C-1) for only these metals are included in this report.

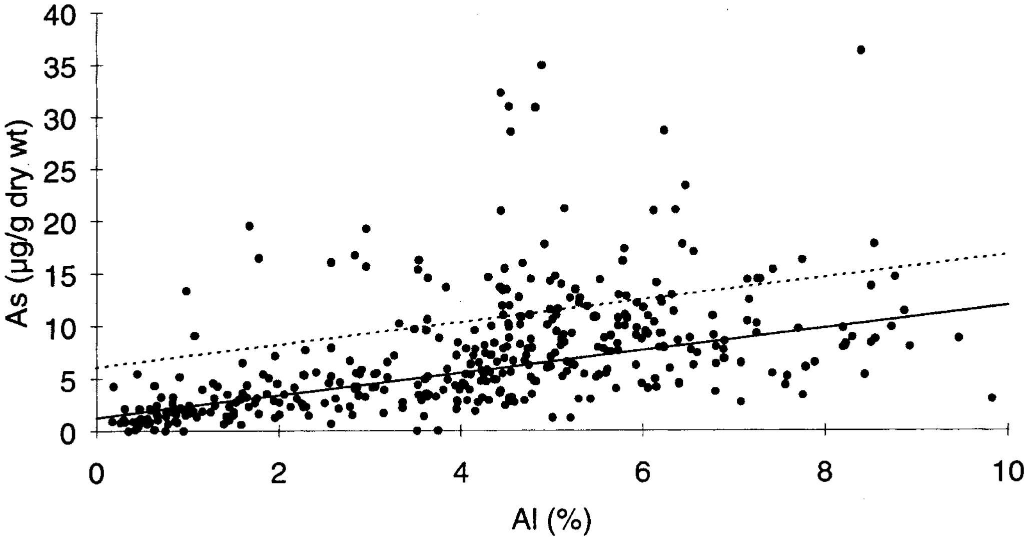

Figure C-1. Linear regression of Arsenic against aluminum. (Dashed line is the upper 95% confidence interval).

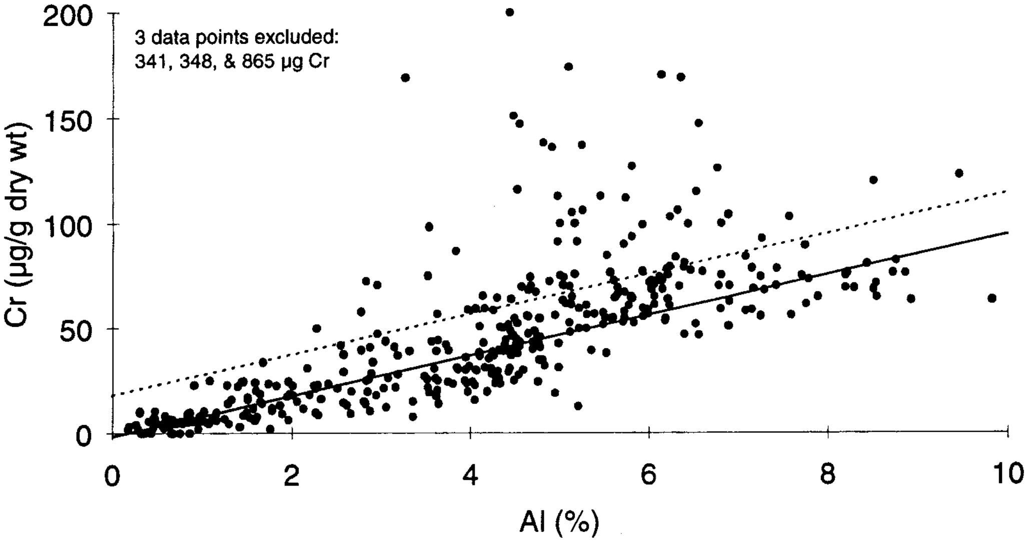

Figure C-2. Linear regression of Chromium against aluminum. (Dashed line is the upper 95% confidence interval). NOTE: Three data points were excluded for clarity.

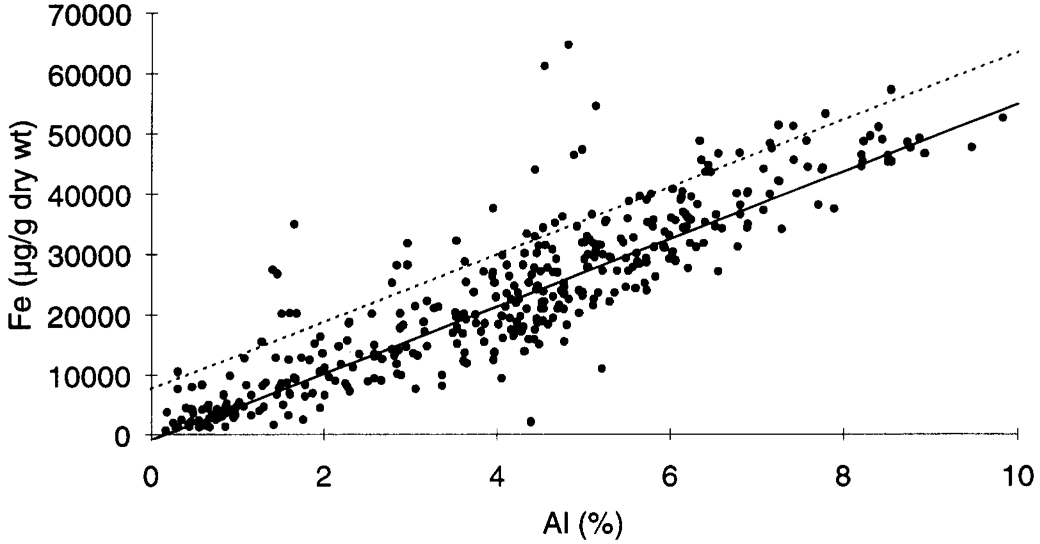

Figure C-3. Linear regression of Iron against aluminum. (Dashed line is the upper 95% confidence interval).

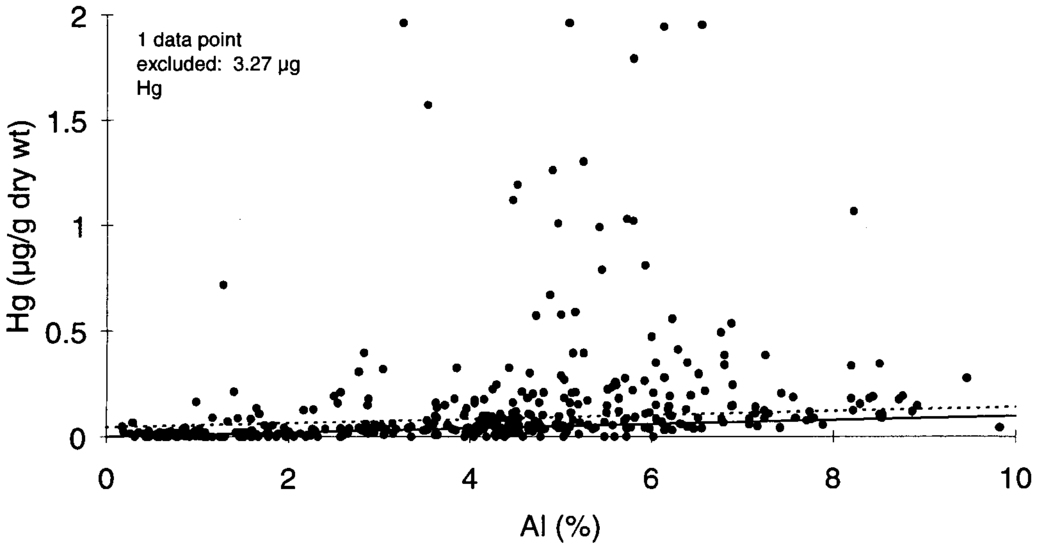

Figure C-4. Linear regression of Mercury against aluminum. (Dashed line is the upper 95% confidence interval). NOTE: One data point was excluded for clarity.

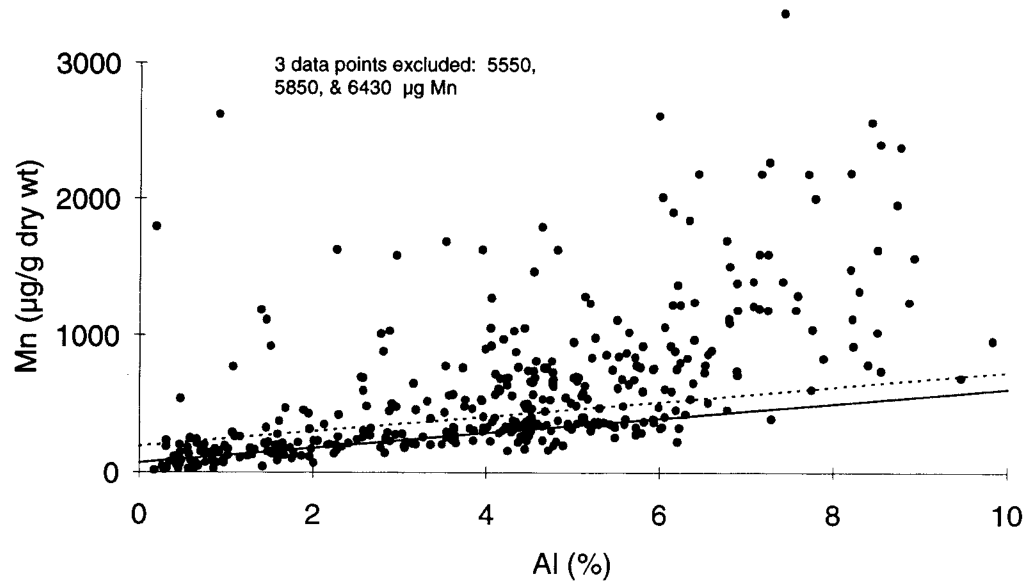

Figure C-5. Linear regression of Manganese against aluminum. (Dashed line is the upper 95% confidence interval). NOTE: Three data points were excluded for clarity.

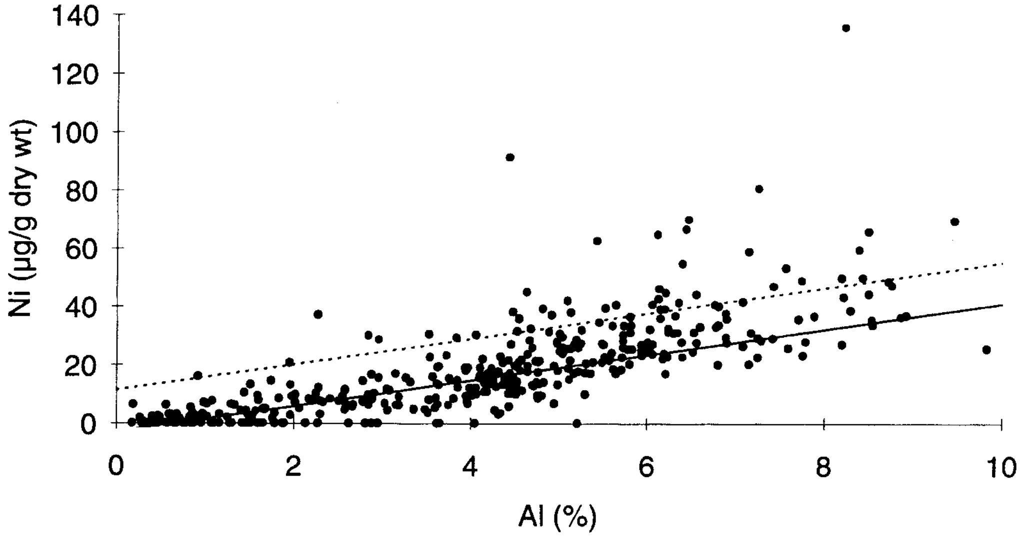

Figure C-6. Linear regression of Nickel against aluminum. (Dashed line is the upper 95% confidence interval).

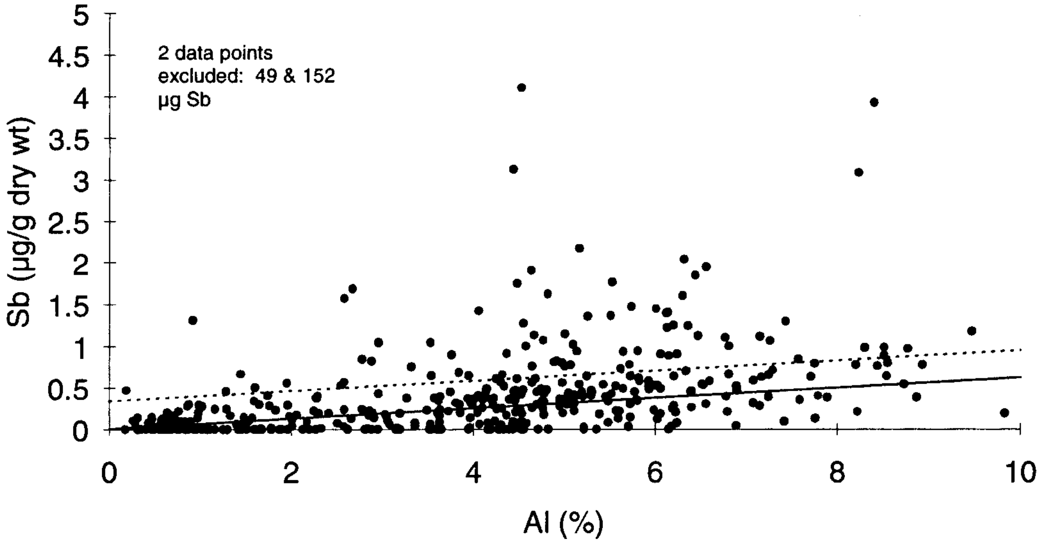

Figure C-7. Linear regression of Antimony against aluminum. (Dashed line is the upper 95% confidence interval). NOTE: Two data points were excluded for clarity.

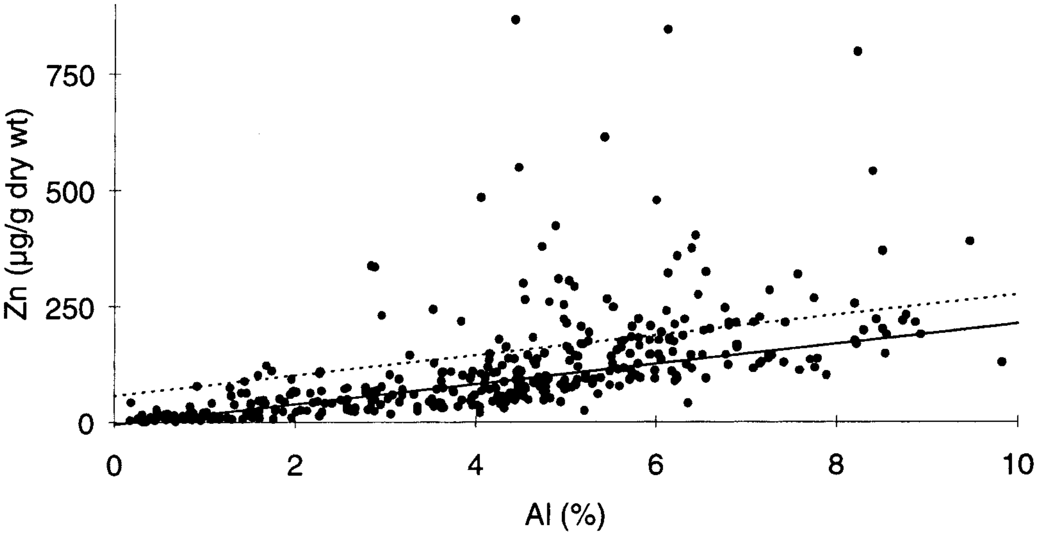

Figure C-8. Linear regression of Zinc against aluminum. (Dashed line is the upper 95% confidence interval).

Table C-1. Metal-aluminum regression parameters obtained from Virginian Province sediment data (m = slope, b = intercept, r^2 = correlation coefficient).

Element	Regression parameters		
	m	b	r^2
As	1.06	1.28	0.49
Cr	9.64	-1.55	0.82
Fe	5,581	-953	0.89
Hg	0.010	0.002	0.48
Mn	54.22	69.05	0.74
Ni	4.66	-3.40	0.76
Sb	0.006	0.006	0.39
Zn	21.83	-5.43	0.69