

1983

A statistical model to predict the incidence of pathogenic protozoa (amoebida: acanthamoebidae) in oceanic sediments using surrogate variables

Carl Robert Berman Jr
College of William and Mary - Virginia Institute of Marine Science

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**A STATISTICAL MODEL TO PREDICT THE INCIDENCE OF PATHOGENIC
PROTOZOA (AMOEBA:ACANTHAMOEBAE) IN OCEANIC SEDIMENTS
USING SURROGATE VARIABLES**

The College of William and Mary in Virginia

PH.D. 1983

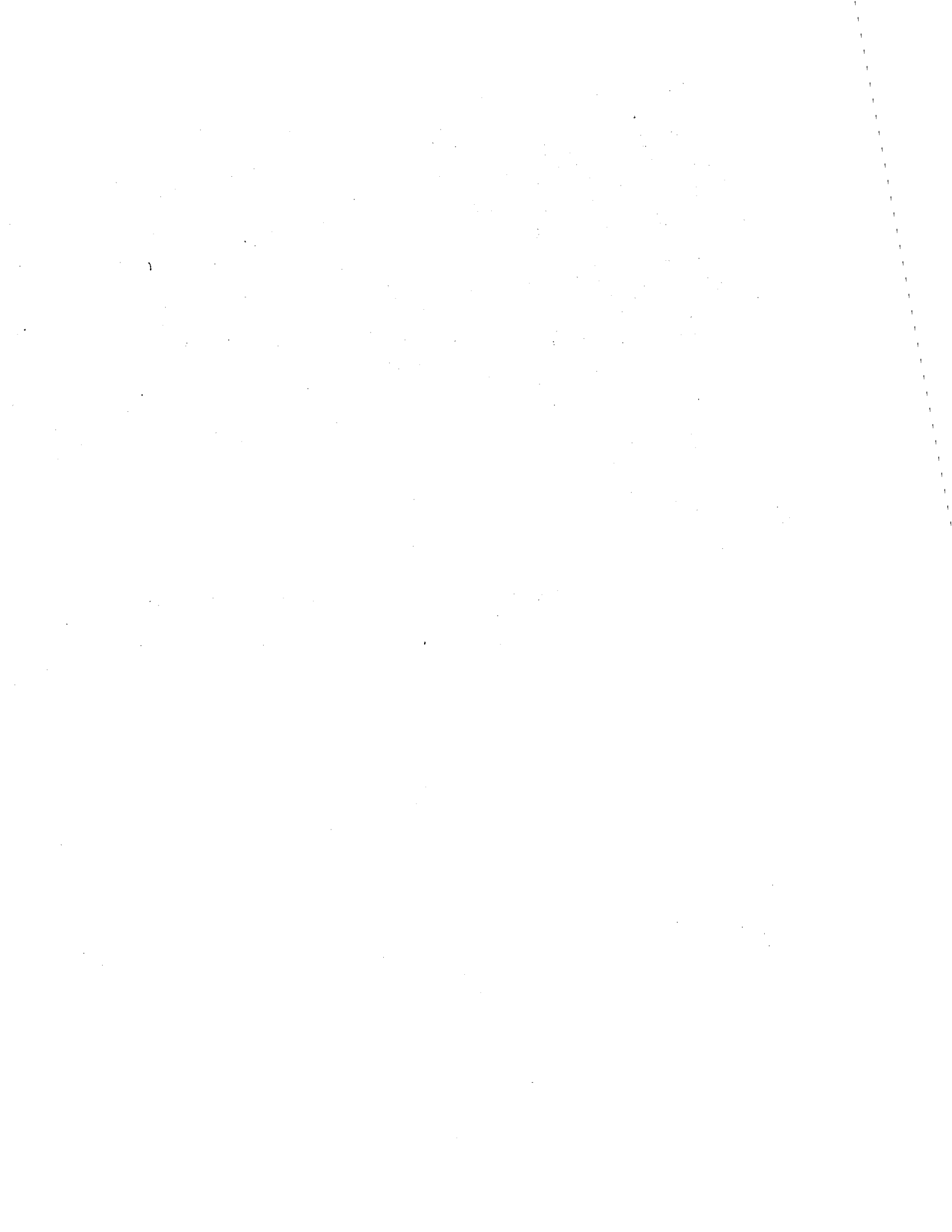
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A STATISTICAL MODEL TO PREDICT THE INCIDENCE OF
PATHOGENIC PROTOZOA (AMOEBIDA:ACANTHAMOEEBIDAE)
IN OCEANIC SEDIMENTS USING SURROGATE VARIABLES

A Dissertation

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

by

Carl Robert Berman, Jr.

Commander, NOAA

National Marine Fisheries Service

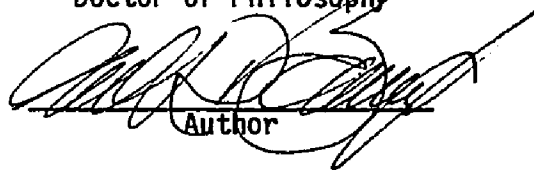
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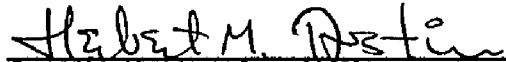
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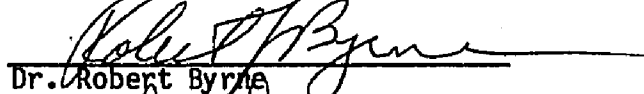
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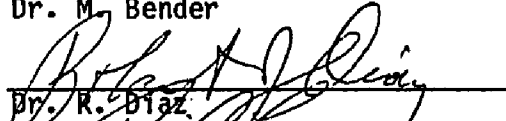

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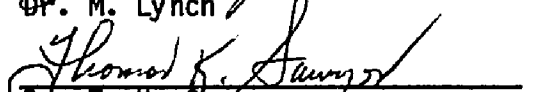

Dr. H. M. Austin, Chairman


Dr. Robert Byrne


Dr. M. Bender


Dr. R. R. Diaz


Dr. M. Lynch


Dr. T. K. Sawyer
National Marine Fisheries Service
Oxford, Maryland

DEDICATION

This dissertation is dedicated to my daughter, Suzanne Ferris Berman, who was born on the 13th of January 1981, in the hope that this work will, in some small way, make her world a better place in which to live.

TABLE OF CONTENTS

| | Page |
|-------------------------------|------|
| ACKNOWLEDGMENTS..... | v |
| LIST OF TABLES..... | vii |
| LIST OF FIGURES..... | ix |
| ABSTRACT..... | x |
| INTRODUCTION..... | 2 |
| REVIEW OF THE LITERATURE..... | 9 |
| METHODOLOGY..... | 15 |
| RESULTS..... | 22 |
| DISCUSSION..... | 43 |
| CONCLUSIONS..... | 56 |
| APPENDIX A..... | 57 |
| APPENDIX B..... | 59 |
| APPENDIX C..... | 64 |
| APPENDIX D..... | 71 |
| BIBLIOGRAPHY..... | 76 |
| VITA..... | 88 |

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LIST OF TABLES

| Table | Page |
|--|------|
| 1. Tactical (short-term, simple) and strategic (long-term, complex) variables..... | 17 |
| 2. Variable sets for the three geographic areas under study based on time/space sorting restraints of archived data..... | 19 |
| 3. Summary table for the Narragansett Bay area..... | 24 |
| 4. Stepwise regression procedure for all variables and the variable "PCUL" for Narragansett Bay..... | 26 |
| 5. General linear models procedure for "tactical" variables for Narragansett Bay..... | 28 |
| 6. The addition of total organic carbon to the variables in Table 5: distance, coliform MPN, and temperature..... | 28 |
| 7. Summary table of correlations for the New York Bight area..... | 30 |
| 8. Stepwise regression procedure for all variables and the variable "PCUL" for the New York Bight..... | 31 |
| 9. Stepwise regression procedure for "tactical" variables for the variable "PCUL" for the New York Bight..... | 32 |
| 10. Stepwise regression improvement procedure for "strategic" variables for the variable "PCUL" for the New York Bight..... | 33 |
| 11. General linear models procedure for "tactical" variables and the "PCUL" for the New York Bight..... | 34 |
| 12. General linear models procedure for "strategic" variables and the variable "PCUL" for the New York Bight..... | 34 |
| 13. Summary table of correlations for the Philadelphia-Camden dumpsite..... | 35 |
| 14. Stepwise regression procedure for all variables and the variable "PCUL" for the Philadelphia-Camden dumpsite..... | 37 |
| 15. General linear models procedure for two valid variables determined through the stepwise regression procedure and the variable "PCUL" for the Philadelphia-Camden dumpsite..... | 37 |
| 16. Correlations between PCUL and the variables DIST and LFCC for quadrants drawn through the center of the New York dumpsite..... | 39 |

LIST OF TABLES (continued)

| Table | Page |
|---|------|
| 17. Statistics for the variable PCUL correlated with the environmental variables DIST and LFCC for all stations in the Philadelphia site..... | 40 |
| 18. Values for the Durbin-Watson statistic, "d", for determining the autocorrelation residuals for a given procedure..... | 41 |

LIST OF FIGURES

| Figure | Page |
|--|------|
| 1. Narragansett Bay showing stations sampled for <u>Acanthamoeba</u> | 6 |
| 2. New York Bight apex showing stations for sampled for <u>Acanthamoeba</u> | 7 |
| 3. Site locations map for the Philadelphia-Camden dumpsite showing the dumpsite as a rectangle between the 40 and 70 m isobaths..... | 8 |
| 4. Detail of the Philadelphia-Camden wastewater sludge disposal site in the northeast Atlantic Ocean..... | 8 |
| 5. A comparison of the correlation coefficients for all variables with the variable "PCUL" for all three geographic areas under study..... | 25 |
| 6. A comparison of predicted values for the variable "PCUL" and the actual <u>in situ</u> values obtained from field studies for all geographic areas under study..... | 27 |
| 7. The spread of contaminated materials from a depositional center..... | 38 |
| 8. A "prediction table" generated by the model for the Narragansett Bay area..... | 42 |
| 9. A comparison of the models generated for the three geographic areas under study with the actual <u>in situ</u> data..... | 48 |
| 10. A prediction table for Narragansett Bay using a "background" <u>Acanthamoeba</u> population set at one positive culture/station..... | 52 |
| 11. A prediction table for Narragansett Bay using a "background" <u>Acanthamoeba</u> population set at two positive cultures/station..... | 55 |

ABSTRACT

Surrogate contaminant variables (heavy metals, organics, physical oceanographic data) can be used to predict the incidence of positive cultures of Acanthamoeba sp. in oceanic sediments. Amoebae data are drawn from five years of study involving stations in Narragansett Bay, Rhode Island, the New York Bight, and the Philadelphia-Camden dumpsite, and associated pollution parameters are drawn from literature sources, computerized marine pollution data bases, and other archives.

The Statistics Analysis System (SAS) MAXR² improvement technique (stepwise regression) and general linear model procedures are used to generate correlations for surrogate variables and produce final predictive models and tables. Model procedures for the three study areas are most valid for Narragansett Bay and the New York Bight but less valid for the Philadelphia-Camden dumpsite due to the small quantity of relevant data. The Durbin-Watson statistic is used to test for autocorrelation of model residuals and, using this test, the Philadelphia-Camden model is again found to be the least valid, although applicable within limits.

The division of contaminant variables into "tactical" (short-term, simple analysis) and "strategic" (long-term, more complex analysis) categories enhances the predictive effort through the introduction of a cost-effective procedure evaluation. Generally, the simple variables predict the incidence of positive Acanthamoeba cultures as well as the more complex data sets.

There are sufficient data and applicable computer programs to produce useful results for an investigation involving potentially pathogenic protozoans and public health management decisions may be made using the tables and formulae generated using these procedures.

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INTRODUCTION

In 1980 a microbiological study of Narragansett Bay, Rhode Island was conducted aboard the NOAA Ship GEORGE B. KELEZ (R-441). The study included stations from the vicinity of the Fields Point Sewage Treatment Plant seaward to 25 miles south of the Rhode Island shore. Statistical analysis of the data collected during this cruise indicated that there was, in this location and for these stations, a high positive correlation between bacterial MPN (most probable number of bacteria per 100 grams of sediment) and positive Acanthamoeba stations cultured from the same benthic materials (Sawyer, 1980). A negative correlation between these two microbiological measurements and distance from the Fields Point outfall also emerged as significant. Later cruises confirmed that the same correlations could be demonstrated for the New York Bight apex and portions of the inactive Philadelphia-Camden dumpsite (Lear and O'Malley, 1982; Sawyer, 1980).

During subsequent investigations, however, positive Acanthamoeba stations were recovered from sites in which no fecal coliform or streptococcal bacteria were found (Sawyer, 1980) and, the question arose as to whether or not the incidence of the Acanthamoeba could be predicted using variables other than the bacterial MPN. Since the amoebae were known to feed on bacteria which were alive (recoverable) and dead (nonrecoverable), the correlation appeared to break down in areas in which the bacteria were no longer viable. In contrast to enteric bacteria which may lose their viability after several weeks or months, most species of Acanthamoeba form resistant cysts which may survive for as long as 33 months after refrigeration of sediment samples (Sawyer, personal communication).

Statistical methods to be presented below show that significant levels of pollutants in marine sediments may be as useful as bacterial MPN's for predicting the presence of Acanthamoeba. Acanthamoeba are also an important public health consideration. The infection that these protozoans can cause may result in serious illness for healthy individuals but is more devastating, even fatal, in those persons already possessing an impaired immune system (Martinez, 1980).

The amount of sewage-related materials dumped in the oceans will increase substantially by the year 2000. During this same period, populations will increase and pressure on shoreline recreational facilities will rise accordingly (Cabelli et al., 1975; Goudette et al., 1981). For this reason, and because the study and culturing of the Acanthamoeba themselves is a highly technical and labor intensive process, pertinent statistical correlations between Acanthamoeba and other environmental contaminants which may be used to predict the incidence of the pathogenic protozoa in sediments using surrogate variables, becomes an important area for investigation.

The potential value of meaningful statistical correlations include:

1. Delineating and characterizing the dumpsite (hydrographic and geological properties);
2. Determining the degree of infestation, over time, represented by various microorganisms indigenous to waste materials;
3. Estimating potential public health considerations represented by concentrations of Acanthamoeba in or near areas of recreational fishing, swimming, or other activities associated with the marine environment;

4. Tracking the subsequent dispersal of the material in order to determine further threats to marine benthos and human populations;
5. Estimating the rate of recovery of inactive dumpsites once dumping activity ceases (Pearce, 1981).

Numbers 1-3 above were the primary considerations of the study.

Numbers 4 and 5 represented additional information which could result from the statistical applications used to analyze the data sets.

The study used five years of accumulated Acanthamoeba field data and expanded the parameters to include the following:

1. Bacterial (fecal coliform) MPN ;
2. Acanthamoeba cultures, positive and negative/station;
3. Sediment color, coded from five, black mud, to one, clean sand;
4. Sediment grain size (% silt and clay);
5. Polychlorinated biphenyls (PCBs);
6. Coprostanol (a fecal steroid);
7. Metals in sediments (copper, lead, zinc, etc.);
8. Ratio of total carbohydrates (TCH) to total organic carbon (TOC) or TCH:TOC;
9. Salinity at sample depth;
10. Temperature at sample depth;
11. Distance from point source (e.g., dumpsite depositional center or outfall pipe aperture);
12. Current patterns observed in the areas under study (direction and speed; current meter and remote sensing information).

Data sets which included but were not limited to the parameters listed above were known to exist for three different marine environments in which Acanthamoeba studies had been undertaken:

1. Narragansett Bay (estuary, inshore, surrounded on three sides by landforms; STORET, EPA, 1982; Wrens, 1953) Figure 1;
2. The New York Bight apex (shallow shelf, generally responding to meteorological conditions; Beardsley et al., 1976; NODC, EDIS, NOAA, 1982; STORET, EPA, 1982; Whitley, personal communication) Figure 2;
3. The Philadelphia-Camden dumpsite (offshore, outer continental shelf; Boesch, 1977; O'Malley, personal communication) Figures 3 and 4.

These data were used to test the hypothesis that the development of a statistically valid method for predicting the incidence of pathogenic microorganisms in and around sewage disposal sites was possible, and that the correlation between the variables outlined above and the probability of obtaining positive/negative cultures of Acanthamoeba sp. may be determined using this method. Furthermore, the use of stepwise regression techniques may permit the computation of statistically valid probabilities for the presence or absence of one group of factors which have not been sampled based upon correlations between these surrogate variables and other groups of environmental indicators for which values are known.

Figure 1. Narragansett Bay showing stations sampled for Acanthamoeba.
The Fields Point facility mentioned in the text is west of
stations 3, 4, and 5 (Sawyer et al., 1980a, in press).

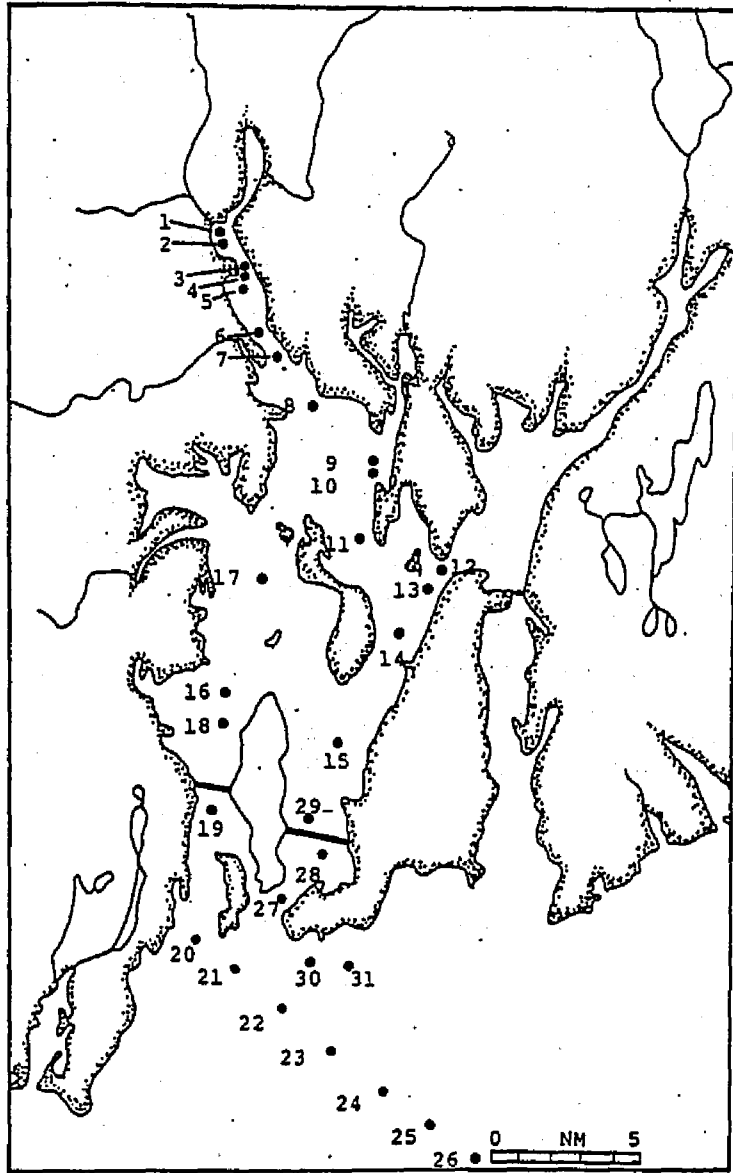


Figure 1

Figure 2. New York Bight apex showing stations sampled for Acanthamoeba. The dashed boundary represents the eastern and southeastern limits of the area closed to shellfishing (Sawyer, 1980b).

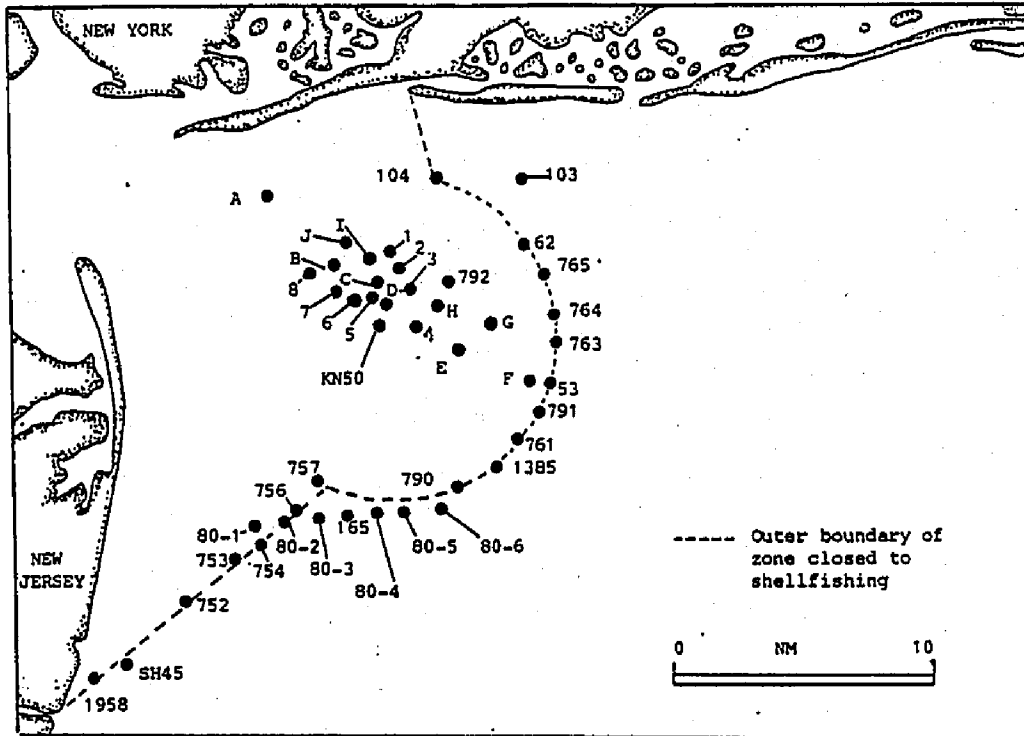


Figure 2

Figure 3. Site location map for the Philadelphia-Camden dumpsite showing the dumpsite as a rectangle between the 40 and 70 m isobaths. Large area enclosed by dashed lines has been sampled extensively (400 stations). Also shown are the transects shoreward from the 40 m isobath to the Delaware Bay and Ocean City, Maryland outfall, and the transect northward to the New York Bight apex (from O'Malley et al., 1982).

Figure 4. Detail of the Philadelphia-Camden wastewater sludge disposal site in the northeast Atlantic Ocean. Upper box outlines an inactive acid dumpsite. Lower box delineates inactive sludge dumpsite. Positions are shown for stations in this area sampled for Acanthamoeba (from Sawyer et al., 1982).

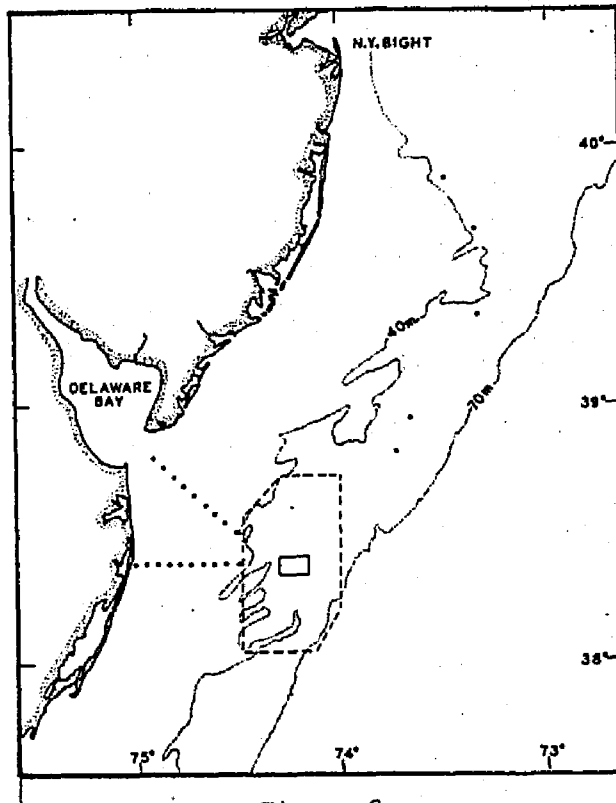


Figure 3

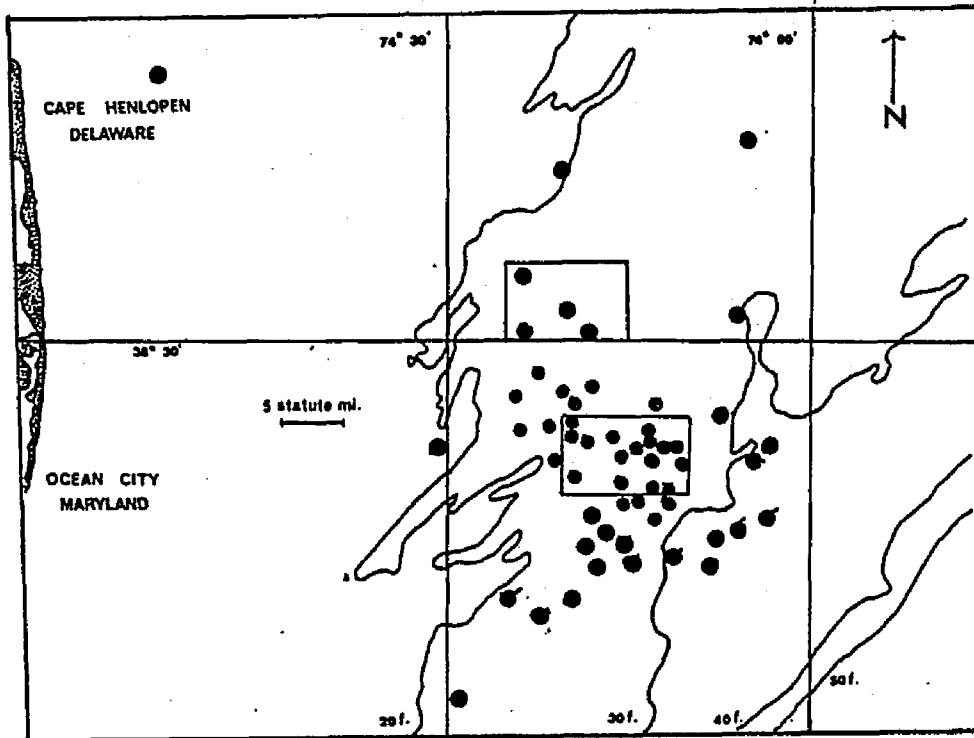


Figure 4

REVIEW OF THE LITERATURE

The purpose of the following literature review is to demonstrate that the selection of surrogate variables noted in the INTRODUCTION represents a valid approach to the problem of predicting the incidence of pathogenic protozoa (Acanthamoeba) in sediments using statistical methods. Such justification is a necessary first step in the formulation of any correlative model (Basta and Bower, 1979).

Amoebae and Bacteria

The association of species of Acanthamoeba and bacteria with sewage sludge has been noted by several investigators. Studies have included areas off the DelMarVa Coast (Lear et al., 1981; Lewis and Sawyer, 1979; Sawyer, 1979), Narragansett Bay (Sawyer et al., 1982), and the New York Bight apex (Sawyer, 1979). Acanthamoeba were abundant wherever sewage sludge was deposited. Species of Acanthamoeba included several that are known to cause death in man and animals. Page (1974) refers to these protozoans as "amphizoic", i.e., they are typically free living but may be opportunistically parasitic. The first known pathogenic species, A. culbertsoni, to be isolated from marine sediments was discovered in 1959 (Culbertson et al., 1959). Singh and Das (1972) found the same organisms in municipal sewage sludge from the city of Lucknow, India. The first recovery of this species from oceanic sediments was carried out at the National Marine Fisheries Service's Oxford Laboratory by Sawyer (1979).

Correlation analysis of Acanthamoeba culture and bacterial MPN data from field studies support the existence of a relationship between the distribution of Acanthamoeba and associated fecal indicator bacteria (coliforms and streptocci) and between these organisms and the degree of

sewage sludge contamination (Mayer, 1982). These same investigations were also valuable in pointing out those areas in which the correlation between these biotic indicators and anthropogenic wastes appeared to break down (Lear et al., 1981; O'Malley et al., 1982; Sawyer, 1980).

These reports stress the importance of laboratory procedures required to determine the coliform counts (MPNs). Generally, appropriate facilities and a trained technician are required in order to obtain valid data. Of particular importance are the inoculation procedures, preparation of proper growth media, temperature control, and accurate species identification. The culturing of the Acanthamoeba requires painstaking accuracy and inordinate patience (Sawyer, 1980).

Even if a public health laboratory has the facilities and personnel to culture and identify these organisms and then carry out temperature specific tests to isolate pathogenic species which can grow at 37°C, human body temperature (Sawyer et al., 1982), the time element may be too long if an immediate management decision is necessary.

Distance from Point Sources

These same published reports contain evidence that there is a valid negative correlation between the incidence of the Acanthamoeba and the distance at which samples are taken from the dumpsite depositional center or outfall aperture. The further away from a point source the samples are taken, the less is the probability of obtaining positive cultures of the amoebae and high bacterial MPNs (O'Malley et al., 1982; Sawyer et al., 1982).

Metals in Sediments

The contamination of marine sediments by metals has been the subject of much attention in recent years (Donozzolo et al., 1981;

Selli et al., 1977). A large body of relatively recent data also exist for the New York Bight area (Calabrese et al., 1982; Gross, 1975; Young, 1982). Mueller and Anderson (1978) compiled an extensive monograph on the results of the Marine Ecosystems Analysis (MESA) studies carried out in the Bight including sources for environmental copper, zinc, cadmium, lead, and chromium. These studies indicate that concentrations of metals in sediments are highest near the depositional centers of sewage dumpsites and that the amount of metal present decreases as distance from this center increases (Hershelman et al., 1981). In fact, concentrations of some metals may be 100 times higher than ambient environmental levels in and around sewage disposal locations (Boehm, personal communication).

Fecal Steroids (Coprostanol)

The use of fecal steroids (coprostanol) as indicators of sewage contamination has been shown to have a practical application (O'Conner et al., 1982). When the coprostanol represents a high percentage of the total environmental steroids measured (10-15%), a quantitative relationship can be computed between the percentage of steroids and the amount of sewage related organic matter (Hatcher and McGillivray, 1979). In fact, once deposited, coprostanol persists in anaerobic low-lying areas in which these materials concentrate and can be used as an indicator of previous waste disposal activities (Hatcher and McGillivray, 1979). In these studies, coprostanol from actual sewage treatment facilities was analyzed in order to demonstrate that environmental steroids were derived from both ocean-dumped and sewage outfall materials. The results showed that coprostanol could be used to delineate vertical and horizontal sediment profiles for sewage

contamination and that percentages of coprostanol decreased directly with distance from the pollution point source (Hatcher et al., 1977).

The Ratio of Total Organic Carbon (TOC) to Total Carbohydrate (TCH)

Hatcher and Keister (1976) reported that the ratio of TOC to TCH could also serve as an indicator of sewage sludge contamination. When the ratio TCH:TOC was greater than or equal to 30, the value may be attributed to materials derived from sewage. The extremely high values in the Christiaensen Basin, New York Bight, reported by Hatcher and Keister (1976), ratio values of 40 to 60, and the evident decrease in values toward the shelf edge, indicate a correlation which represents a valuable surrogate variable for the proposed correlative model.

Polychlorinated Biphenyls (PCBs)

PCBs also represent an appropriate surrogate variable for use as a sewage pollution indicator. West and Hatcher (1980) showed that:

1. "Despite the limited recent use of PCB's, sewage sludges continue to exhibit a high concentration of PCB's.
2. Upon dumping of sludge into the ocean, PCB's are rapidly removed from the water column via scavenging particulate matter. This settling of particulate matter occurs over a very short period of time and is probably an important mechanism for rapid transport of PCB's to surficial sediments.
3. The highest PCB concentrations in surficial sediments were found in a small area of the Christiaensen Basin near the sewage sludge dumpsite. These high values are probably associated with the recently deposited sludge which remains as a soupy layer above older, more consolidated muds.
4. PCB distribution in a box core suggest that high PCB concentrations have existed since the introduction of these chemicals in 1955, and their subsequent widespread use and that levels are also correlated to dramatically increased volumes of sludge deposited in the Bight Apex since 1952."

Sediment Parameters

Numerous studies have been made on the sediment regime in areas under consideration. Freeland and Swift (1978) treat the New York Bight in considerable detail. The majority of papers mentioned almost always include a description of sediment color and/or grain size for each sample taken regardless of the purpose for which the station was occupied (Douglas, 1981; Eisma, 1981). These descriptions range from "black mayonnaise" (Harris, 1974), to "clean white sand" (Hatcher and McGillivray, 1979). Sediment color, nominal data, can be coded into ordinal form (e.g. "5" for black sediments to "1" for clean sand) and used in statistical computations (Zar, 1974). There are also historical data of sufficient quality to permit comparison between archival data (literature, computer data base) and dominant sediment types/colors in the New York Bight (Gross, 1975) the outer continental shelf (Boesch, 1975), Narragansett Bay (Wrens, 1953), and for all three areas combined (National Geological Survey Marine Geology Data Inventory, 1982; ORCA, 1981; STORET, EPA, 1982).

Circulation Patterns

The circulation patterns which determine the distribution of sediments and other suspended solids are also well documented (Kavanaugh, 1980; Fisher et al., 1979). The New York Bight circulation has been described by Gross (1975), Hansen (1977), and, more recently, using satellite imagery, by Munday (1982). Data for the outer continental shelf are available in Goldsmith (1974, 1977) and Butman (1977). Narragansett Bay tidal currents are the subject of a paper by Swanson and Spauling (1979). Recent emphasis in current regime investigations has shifted somewhat from field to modelling studies. At present, many models exist for pollution dispersal and these may be

applied to all of the geographic areas involved in the present study (Hinson, 1979; Ingham, 1982; Whitley and O'Conner, personal communications).

Salinity and Temperature

Salinity and temperature represent the most complete marine data files available (NODC/EDIS/NOAA, 1982). These measurements, which are routinely taken in almost all oceanographic investigations, describe the basic environmental limits within which all marine biological activities take place and, for this reason alone, represent important parameters in any correlative model. There are indications that many of the Acanthamoeba cultured from marine sediments are in cyst form and become active when exposed to appropriate temperature and nutrient regimes in the laboratory (Sawyer, 1980). There may be a connection between the ability of the amoebae to form cysts and ambient temperature and salinity. There may be some temperatures at which the amoebae forming cysts die and another at which the viable organisms may emerge under the proper conditions. Statistical correlations may provide evidence for such a relationship if one exists.

The preceding review has cited numerous examples of surrogate variables which may follow the same general distributional trend as the Acanthamoeba in and around ocean disposal sites for municipal sewage sludge. Salinity and temperature, which tend to be positively correlated with distance from a point source in estuaries, and current patterns, which are a mechanism through which pollutant distribution may be explained, are the only two variable sets which do not follow this general pattern. The rationale for including these two measurements, however, has been noted.

METHODOLOGY

Rationale for Separation of Surrogate Variables into "Strategic" (Long-Term) and "Tactical" (Short-Term)

The primary objective of the present investigation was the identification and quantification of valid surrogate variables which could be used to predict the incidence of Acanthamoeba in sediments. A secondary, but no less important aspect of the project was the development of a procedure which could be used by public health and environmental managers to rapidly assess a given situation. These situations would be those in which the presence of Acanthamoeba would represent a threat to human health (e.g., contamination of a beach area; Martinez, 1980).

If an immediate decision in such cases was required, the parameters used must be selected so that they represent the best combination of accurate prediction (determined by statistical methods), and uncomplicated analysis. The separation of surrogate variables into "strategic" and "tactical" categories appears in Table 1.

The most complex of the "tactical" or short-term analyses is bacterial MPN which can be ascertained in a minimum of 48 hours. Although 48 hours is required for a fecal coliform analysis, public health laboratories, including those at the municipal level, maintain facilities for this purpose. The degree of contamination by other substances (metals, steroids, etc.) is generally sent to organizations with more expensive and sophisticated equipment. The reason, then, for including the MPN analysis in the short-term column is that this procedure is the most complex procedure which public health laboratories can perform on a routine basis (Adams, personal communication; American Public Health Association, 1970).

Once this division of variables had been made, the modeling procedure was repeated using each variable set (complex and rapid response) to decide if an analysis of a given environmental problem involving Acanthamoeba could be realistically accomplished using only the "tactical" set of variables. If these variables, in themselves, were not sufficient, then the variables from the "complex" set would have to be included in order to make the prediction procedure valid. The program was adjusted to reveal which variables, if any, were the most applicable in the short-term mode.

Assessment of Data Availability

The literature review indicated that several surrogate variables existed which might be incorporated into a correlative statistical model. The next step involved an evaluation of existing literature, automated archives, and data base listings to determine the availability and suitability of actual, in situ values for these parameters (see Appendix A for description of archives used during this phase).

Bibliographic files were accessed (DIALOG, SDI, etc.) using appropriate keyword entries to insure that the most recent citations were being used as source references. Other data bases (NODC, NGSDC, etc.) were queried using geographic coordinates which described the boundaries of the three areas under study (Narragansett Bay, New York Bight, and Philadelphia-Camden dumpsite).

Once the general information from each source had been received in computer printout form, data were sorted so that the archival information matched, as closely as possible, the time of sampling and location of the 134 Acanthamoeba study stations. To accomplish this sorting task, a radius of 0.05 nautical miles (nm) was drawn from the

Table 1. Tactical (short-term, simple) and strategic (long-term, complex) variables.^{††}

| Tactical* | Strategic** |
|----------------|---|
| Coliform MPNs | Heavy metal analysis |
| Temperature | PCB Analysis |
| Salinity | Organic analyses (coprostanol, organic carbon) |
| Distance | Geological analyses (grain size, percent silt and clay) |
| True bearing | |
| Sediment color | |

^{††}Since Acanthamoeba cultures are the dependent variable. This procedure is not included in the table.

*The term "tactical" refers to analyses which are relatively easy to perform or to values which can be determined by inspection in situ. The most "complex" of these is bacterial MPN. These variables, with exception of MPNs and sediment color are generally collected during any oceanographic investigation.

**The term "strategic" refers to analyses which require more time and sophisticated equipment than those listed as tactical.

center of each station location establishing a 0.10 nm circle around each of the 134 stations (the optimum repeatability of Loran "C", the most widely used navigation aid for positioning the vessels involved). The date and time of each station sampled (31 for Narragansett Bay, 44 for the New York Bight, and 59 for the Philadelphia-Camden dumpsite) were entered and sorted so that the information taken from the archival data had been sampled as closely as possible to the actual date and time of Acanthamoeba station occupation.

The data were then resorted using these time and location limits. The final product was a listing of values for surrogate variables (12 for Narragansett Bay, 20 for the New York Bight, and 12 for the Philadelphia-Camden dumpsite) matched in time and space to the Acanthamoeba stations (see Table 2).

Evaluation of Modeling Strategies

There were numerous excellent resources available which described various modeling strategies (Basta and Bower, 1979; Gold, 1977; Green, 1979; Nihoul, 1975). Because the primary objective of the present effort was the prediction of the incidence of pathogenic protozoa in marine sediments using surrogate variables, the statistical or correlative approach using stepwise regression was considered to be the most productive. This method emphasized the statistical relationship (correlation) between variables as opposed to the mass-energy modeling scheme which attempts to explain, identify, and create equations which describe the physical mechanisms which drive a particular system (Amick and Walberg, 1975; Hinson, 1979; Leedertse and Gritton, 1972, 1977; Leedertse and Liu, 1974; Tracor, 1971).

Table 2. Variable sets for the three geographic areas under study based on time/space sorting restraints of archived data.

| Variable | ADP Code | Narragansett Bay | New York Bight apex | Philadelphia-Camden Dumpsite |
|---|---------------------------|------------------|---------------------|------------------------------|
| Distance from point source or site center | DIST | X | X | X |
| True bearing, in degrees, from point source or site center | BNT | | X | |
| Percent positive <u>Acanthamoeba</u> cultures at station | PCUL (dependent variable) | X | X | X |
| Total organic carbon (using modified Schollenberger chromic acid oxidation technique) | TOC | X | X | X |
| Lead (PPM) | PB | X | X | X |
| Mercury (PPB) | HGB | X | X | X |
| Copper (PPM) | CU | X | X | X |
| Chromium (PPM) | CR | X | X | X |
| Cadmium (PPM) | CDM | X | X | |
| Zinc (PPM) | ZNM | X | X | X |
| Silver (PPM) | AGM | | X | |
| Nickel (PPM) | NIPM | | X | X |
| Coliform count (log) | LFCC | X | X | X |
| Temperature (°C) | TC | X | X | X |
| Salinity (PPT) | SPT | X | X | X |

Table 2. (continued)

| Variable | ADP code | Narragansett Bay | New York Bight apex | Philadelphia-Camden dumpsite |
|---|----------|------------------|---------------------|------------------------------|
| Sediment color (coded) | SCLR | X | X | X |
| Percent silt | PSILT | | X | X |
| Percent clay | PCLAY | | X | |
| PCBs (PPB) | PCB | | X | |
| Percent coprostanol of total steroids | PCOP | | X | |
| The ratio of total carbohydrate to total organic carbon (TCH:TOC) | TCHTOC | | X | |

Note: Some variables listed under a particular site may not have met the criteria for entry into the modeling process. See text for explanation.

At this point, the three initial steps for developing a correlative model had been completed (Basta and Bower, 1979);

1. Relevant causal surrogate variables had been selected based on a study of the system;
2. The structural form of the relationship between the surrogate variables and the dependent variable (e.g., correlation) had been hypothesized;
3. Data sets for each of the surrogate variables had been obtained from archives and the literature for use in the model.

Selection of a Suitable Computer Program

The small array of 134 stations and the availability of values for only 20 surrogate variables made maximum utilization of all information vital to the success of the project. Since the proposed correlative model was based on the validity of correlations between independent variables and positive Acanthamoeba cultures/station, a program which evaluated all variables, regardless of their individual contribution to the system, and produced a result based on an optimum selection for each factor was essential (Chatterjee and Price, 1975).

After a review of several program options (Martin, 1975; Ralston, 1983), the Maximum R^2 Improvement (MAXR; stepwise regression) contained within the Statistical Analysis System (SAS) package was selected as the best option (Goodnight, 1979).

Preparation of Data for Analysis

Raw data were extracted from the computer files which had been sorted using time/location restraints and transformed for use with the IBM 4341-2 system (8 megabytes, MVS 3.8H) at the Virginia Community College Host Computer Center.

Reported Distribution of Acanthamoeba on the East Coast

A number of other stations were considered in this study which represented a series of samples from Ocean City, Maryland to Georges Bank. These stations, which were initially clear of both Acanthamoeba and bacteria, were located from two to 100 miles offshore (Sawyer, et al., 1982). All of these stations were located in areas in which there were no known anthropogenic inputs (e.g., dumpsites or outfalls). However, repeated sampling of stations located near the mouths of major east and gulf coast rivers and estuaries, revealed pockets of Acanthamoeba not previously reported in these areas. Analysis of samples from these stations indicated that increased waste loading at these sites had caused a buildup of organic materials and subsequent decay, which encouraged the growth of the protozoans. Other offshore areas not subject to organic loading continued to remain clear of any infestation (Sawyer, personal communication).

RESULTS

The stepwise regression technique was applied to three sets of data, one from each geographic area. When this procedure yielded a sufficient mix of "tactical" and "strategic" parameters, the stepwise regression was rerun using the two different variable sets. In this way, an evaluation could be made between the complex and short-term analytical results to see if the addition of one or more of the surrogate variables requiring more complicated laboratory analysis significantly increased the R^2 values obtained.

Correlation matrices were generated for all variables from all regions. These results made possible a tabulation of all variables correlated with the positive Acanthamoeba stations for each study area. In addition, preliminary relationships between all variables in the set could be determined, by inspection, from these tables.

The general linear model package was used, in the last stage of analysis, to produce the "best" model or models from a given area based upon those variables which were selected as most highly correlated with the positive Acanthamoeba stations and which were found to significantly increase the R^2 values. For each model so produced, a prediction table was generated which could be used by an environmental manager in decision-making. This table was designed to discriminate between a preselected level of "ambient infestation" by Acanthamoeba and possible population levels elevated above this preselected baseline. The table also made possible a determination of error rate (percentage of correct or incorrect predictions) determined by the baseline selected.

Once these analyses were completed, summaries were produced for each area and comparisons made between the different environmental and hydrographic regimes. The significance of these findings is discussed below.

Data Summaries

These data summaries are arranged by geographic area from north to south. For complete listings of all raw data, see microfiche appendices.

Narragansett Bay

Table 3 contains the summary for all variables used in the Narragansett Bay study correlated with the variable PCUL (percent positive Acanthamoeba cultures/station). Figure 5a is a graphic presentation of these correlations. Appendix B (four pages) contains the complete correlation matrix for all Narragansett Bay variables.

In Table 4, the stepwise regression improvement technique is summarized. Variables are presented in the order in which the procedure added them to the model. Tables 5 and 6 contain data for the two "best" models for the Narragansett Bay stations sampled. Figure 6a presents a graph of the actual in situ values for positive Acanthamoeba cultures/station plotted against the predicted positive cultures for these same stations based on the surrogate variable model.

New York Bight

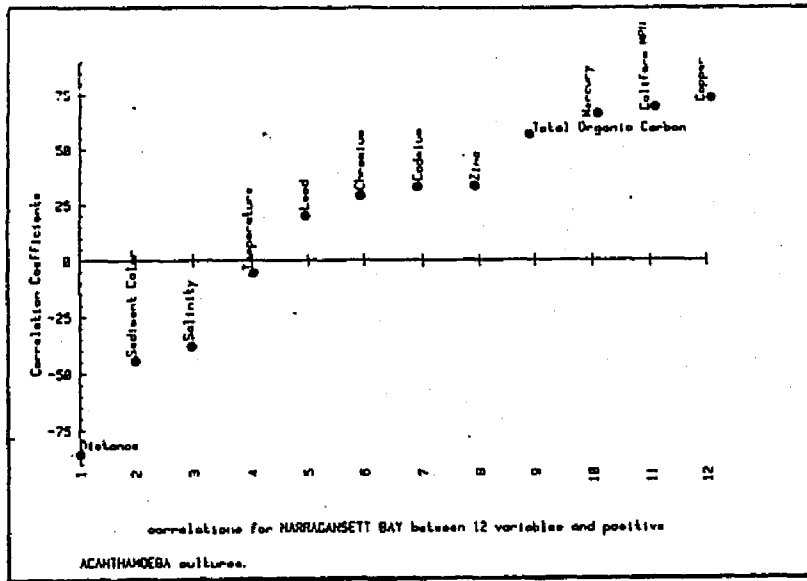
Table 7 is a summary of correlations between 20 surrogate variables and the variable PCUL. Figure 5b is a graphic representation of these same data designed to show the range of correlation coefficients. Appendix C (six pages) contains the complete correlation matrix for all 21 variables used in the New York Bight phase of the investigation.

Table 3. Summary table for the Narragansett Bay area. All variables correlated with variable "PCUL" (percent positive Acanthamoeba cultures/station).

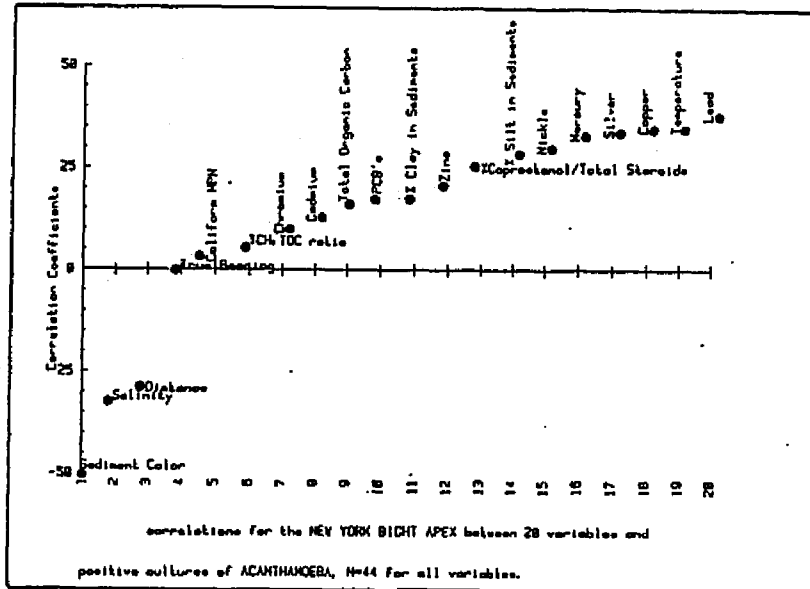
| Environmental variable | "N" for variable | Correlation with PCUL | Significance of correlation |
|--|------------------|-----------------------|-----------------------------|
| Distance in nautical miles from point source | 31 | -.83 | .0001 |
| Sediment color (coded) | 31 | -.42 | .0016 |
| Salinity | 29 | -.35 | .0614 |
| Temperature (°C) | 29 | -.04 | .8462 |
| Lead | 16 | .23 | .3936 |
| Chromium | 12 | .32 | .3053 |
| Cadmium | 14 | .36 | .2056 |
| Zinc | 12 | .36 | .2565 |
| Total organic carbon | 31 | .58 | .0006 |
| Mercury | 15 | .59 | .0199 |
| Coliform MPN | 31 | .68 | .0001 |
| Copper | 9 | .72 | .0292 |

Note: For complete listing of all raw data see microfiche appendices.

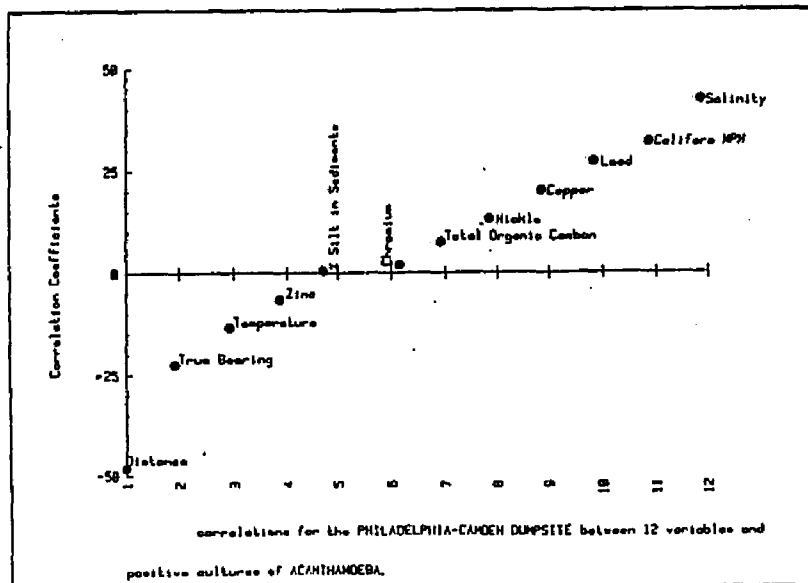
Figure 5. A comparison of correlation coefficients for all variables with the variable "PCUL" (positive Acanthamoeba cultures/station) for all three geographic areas under study.



5a.
Correlation Coeff.
Narragansett Bay



5b.
Correlation Coeff.
New York Bight



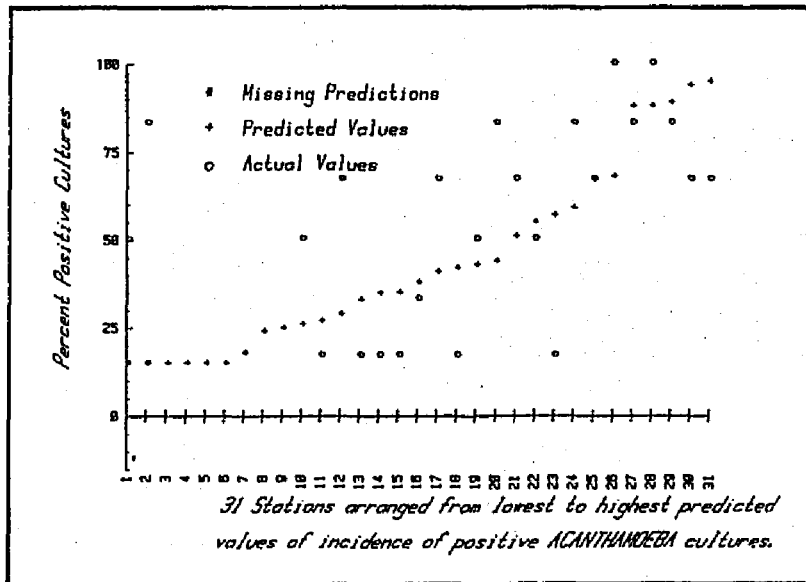
5c.
Correlation Coeff.
Philadelphia Dump
Site

Table 4. Stepwise regression procedure for all variables and the variable "PCUL" (percent positive Acanthamoeba cultures/station) for Narragansett Bay.

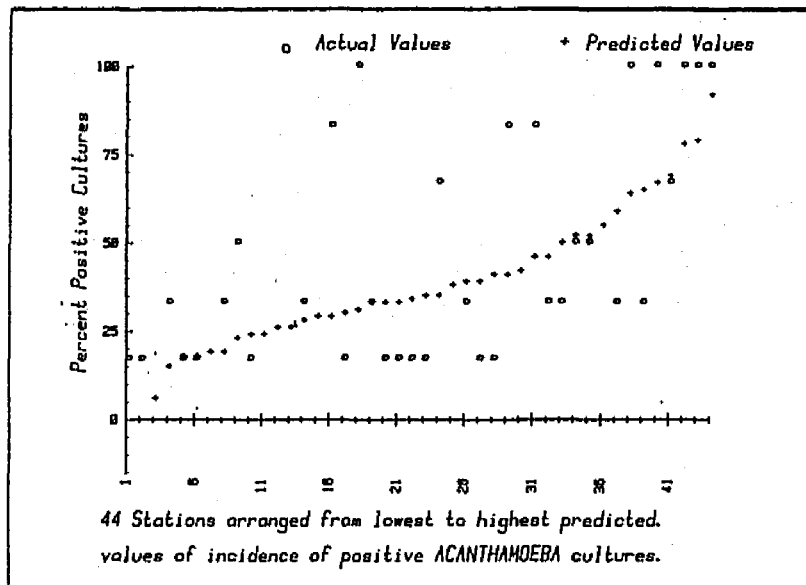
| Variables added to model in order added | Multiple R ² | Multiple R value | F value | Probability of larger F |
|---|-------------------------|------------------|---------|-------------------------|
| Distance | .73 | .85 | 71.44 | .0001 |
| Salinity | .74 | .86 | 37.07 | .0001 |
| Total organic carbon | .76 | .87 | 26.60 | .0001 |
| Temperature (°C) | .77 | .88 | 20.22 | .0001 |
| * - - - - - | | | | |
| Sediment color (coded) | .77 | .88 | 15.71 | .0001 |
| Coliform MPN | .77 | .88 | 12.53 | .0001 |

*This line represents an arbitrary cutoff point above which R² did not appear to increase.

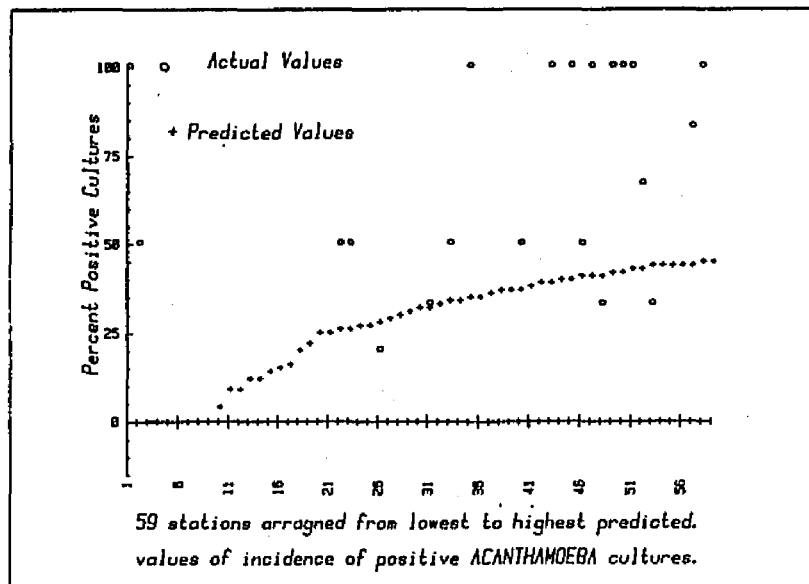
Figure 6. A comparison of predicted values for the variable "PCUL" (positive Acanthamoeba cultures/station) and the actual in situ values obtained from field studies for all geographic areas under study. Stations arranged from lowest to highest predicted culture values.



6a.
 Model vs. In-situ
 Data
 Narragansett Bay
 (Stations for all
 plots arranged
 from lowest to
 highest predicted
 value for Acantha-
moeba cultures.)



6b.
 Model vs. In-situ
 Data
 New York Bight



6c.
 Model vs. In-situ
 Data
 Philadelphia Dump
 Site

Table 5. General linear models procedure for "tactical" (short-term, or rapid analysis) variables for Narragansett Bay.

| Variables used in model | Multiple R ² for model | Multiple R value for model | F value for model | Probability of larger F for model |
|-------------------------|-----------------------------------|----------------------------|-------------------|-----------------------------------|
| Distance | | | | |
| Coliform MPN | .53 | .73 | 14.81 | .0001 |
| Temperature | | | | |

Table 6. The addition of total organic carbon (TA) to the variables in Table 5: distance, coliform MPN, and temperature, provided the only significant increase in the multiple R² value (from .53 to .56, which resulted in an increase in R from .73 to .75).

| Variables used in model | Multiple R ² for model | Multiple R value for model | F value for model | Probability of larger F for model |
|-------------------------|-----------------------------------|----------------------------|-------------------|-----------------------------------|
| Distance | | | | |
| Coliform MPN | | | | |
| Temperature | .56 | .75 | 10.67 | .0001 |
| *Total organic carbon | | | | |

*Note: The addition of total organic carbon to the variables distance, coliform MPN, and temperature, provided the only apparent increase in the R² value.

Table 8 provides an overview of the stepwise regression procedure. Variables are listed in the order chosen by the program together with pertinent statistical parameters. Tables 9 and 10 compare the stepwise regression procedures for "tactical" and "strategic" surrogate variables. In Tables 11 and 12, the models for "tactical" and "strategic" surrogate variables are compared and appropriate statistical descriptors are provided. Figure 6b is a plot of actual in situ values of the variable PCUL compared with predicted values for the same stations based on the models summarized in Tables 11 and 12.

Philadelphia-Camden Dumpsite

Table 13 is a correlation summary for all variables with the variable PCUL for the Philadelphia-Camden dumpsite. Figure 5c is a presentation of these correlation data which graphically represents the range of correlations. Appendix D (four pages) is the complete correlation matrix for all variables used in the Philadelphia-Camden dumpsite study. Table 14 provides a listing of the results of the stepwise regression technique for the Philadelphia-Camden dumpsite variables. Table 15 is a summary of the general linear models procedure for these same variables. Figure 6c represents predicted values of PCUL from the "best model" for each station compared to actual values for positive Acanthamoeba cultures. Figure 7 contains "contours" delineating areas of microbial/protozoan contamination in the Philadelphia-Camden area indicating a dispersal of materials from the depositional center of the site.

General Result Summaries

Table 16 contains the results of correlations between the variable PCUL and the variables DIST (distance) and LFCC (coliform MPN) for

Table 7. Summary table of correlations for the New York Bight area. All variables correlated with variable "PCUL" (percent positive Acanthamoeba cultures/station). N for all variables equals 44.

| Environmental variable | Correlation with "PCUL" | Significance of correlation |
|---|-------------------------|-----------------------------|
| Sediment color (coded) | -.49 | .0008 |
| Salinity | -.32 | .0343 |
| Distance in nautical miles from point source | -.30 | .0449 |
| True bearing from point source in degrees | .00 | .9959 |
| Coliform MPN | .02 | .9131 |
| Ratio of total carbohydrate to total organic carbon | .06 | .7009 |
| Chromium | .07 | .6447 |
| Cadmium | .12 | .4236 |
| Total organic carbon | .17 | .2626 |
| PCBs | .19 | .2046 |
| % clay in sediment | .19 | .2171 |
| Zinc | .22 | .1454 |
| % coprostanol in total steroids | .24 | .1205 |
| % silt in sediment | .26 | .0853 |
| Nickel | .29 | .0562 |
| Mercury | .32 | .0334 |
| Silver | .33 | .0298 |
| Copper | .34 | .0259 |
| Temperature (°C) | .34 | .0225 |
| Lead | .37 | .0123 |

Note: For complete listing of all raw data see microfiche appendices.

Table 8. Stepwise regression procedure for all variables and the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the New York Bight.

| Variables added to model in order added | Multiple R ² | Multiple R value | F value | Probability of larger F |
|--|-------------------------|---------------------|---------|----------------------------|
| Sediment color (coded) | .24 | .49 | 13.19 | .0008 |
| Temperature (°C) | .32 | .57 | 9.44 | .0004 |
| Ratio of total carbohydrate to total organic carbon | .40 | .63 | 8.88 | .0001 |
| Silver | .45 | .67 | 7.88 | .0001 |
| Chromium | .52 | .72 | 8.12 | .0001 |
| Cadmium | .58 | .76 | 8.09 | .0001 |
| PCBs | .59 | .77 | 7.51 | .0001 |
| * - - - - - | | | | |

*This line represents an arbitrary cutoff point above which R² did not appear to increase.

Table 9. Stepwise regression procedure for "tactical" (short-term, or rapid response) variables for the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the New York Bight.

| Variables added to model in order added | Multiple R ² | Multiple R value | F value | Probability of larger F |
|---|-------------------------|------------------|---------|-------------------------|
| Sediment color (coded) | .24 | .49 | 13.19 | .0008 |
| Temperature (°C) | .32 | .57 | 9.44 | .0004 |
| Salinity | .35 | .59 | 7.23 | .0005 |
| Coliform MPN | .37 | .61 | 5.67 | .0011 |
| * - - - - - | | | | |
| True bearing | .37 | .61 | 4.41 | .0029 |
| Distance | .37 | .61 | 3.58 | .0068 |

*This line represents an arbitrary cutoff point above which R² did not appear to increase.

Table 10. Stepwise regression procedure for "strategic" (long-term, or complex analysis) variables for the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the New York Bight.

| Variables added to model in order added | Multiple R ² | Multiple R value | F value | Probability of larger F |
|---|-------------------------|------------------|---------|-------------------------|
| Lead | .14 | .37 | 6.84 | .0123 |
| Cadmium | .19 | .44 | 4.69 | .0146 |
| Chromium | .24 | .49 | 4.12 | .0123 |
| Silver | .27 | .52 | 3.60 | .0136 |
| Total organic carbon | .29 | .54 | 3.18 | .0171 |
| PCBs | .33 | .57 | 3.06 | .0155 |
| Ratio of total carbohydrate to total organic carbon | .36 | .60 | 2.84 | .0183 |
| Mercury | .37 | .61 | 2.58 | .0248 |
| % clay | .38 | .62 | 2.29 | .0396 |
| * | ----- | ----- | ----- | ----- |
| % silt | .38 | .62 | 2.03 | .0622 |
| Nickel | .39 | .62 | 1.82 | .0913 |
| % coprostanol of total steroids | .39 | .62 | 1.62 | .1367 |
| Zinc | .39 | .62 | 1.45 | .1953 |
| Copper | .39 | .62 | 1.30 | .2659 |

*This line represents an arbitrary cutoff point above which R² did not appear to increase.

Table 11. General linear models procedure for "tactical" (short-term, or rapid response) variables and the "PCUL" (percent positive Acanthamoeba cultures/station) for the New York Bight.

| Variables used | Multiple R ² for model | Multiple R for model | F value for model | Probability of larger F for model |
|---------------------------|--------------------------------------|-------------------------|----------------------|---|
| Sediment color (coded) | .36 | .60 | 5.65 | .0011 |
| Temperature (°C) | | | | |
| Salinity | | | | |
| Coliform MPN | | | | |

Table 12. General linear models procedure for "strategic" (long-term, or complex analysis) variables and the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the New York Bight.

| Variables used in model | Multiple R ² for model | Multiple R for model | F value for model | Probability of larger F for model |
|--|--------------------------------------|-------------------------|----------------------|---|
| Lead | .37 | .61 | 2.58 | .0248 |
| Cadmium | | | | |
| Silver | | | | |
| Total organic carbon | | | | |
| PCBs | | | | |
| Ratio of total carbohydrate to total organic carbon | | | | |
| Mercury | | | | |
| % clay | | | | |

Table 13. Summary table of correlations for the Philadelphia-Camden dumpsite. All variables correlated with variable "PCUL" (percent positive Acanthamoeba cultures/station).

| Environmental variable | "N" for variable | Correlation with PCUL | Significance of correlation |
|--|------------------|-----------------------|-----------------------------|
| Distance in nautical miles from point source | 59 | -.47 | .0002 |
| True bearing from point source in degrees | 59 | -.22 | .0962 |
| Temperature (°C) | 8 | -.13 | .7576 |
| Zinc | 22 | -.06 | .7901 |
| % silt in sediments | 17 | .01 | .9825 |
| Chromium | 20 | .02 | .9205 |
| Total organic carbon | 20 | .08 | .7429 |
| Nickel | 8 | .12 | .7687 |
| Copper | 22 | .19 | .3882 |
| Lead | 22 | .26 | .2350 |
| Coliform MPN | 58 | .31 | .0165 |
| Salinity | 8 | .41 | .3175 |

Note: For complete listing of all raw data see microfiche appendices.

quadrants taken through the center of the New York dumpsite. Below is a plot of two transects described in the table which were used to separate the area into four equal portions.

Table 17 contains correlations between the same variables used to develop the information found in Table 16 (DIST, LFCC, and PCUL) for a northeast-southwest transect drawn through the center of the Philadelphia-Camden dumpsite, roughly parallel to the 40 m isobath.

Table 18 is the result of residual analysis and test for autocorrelation for the three "best" models, one from each geographic area, using the Durbin-Watson statistic (Chatterjee and Price, 1975).

Figure 8 is a "predication" table. This example was generated by the "best" model for the Narragansett Bay area. The use of this table in an environmental situation is discussed, in detail, below. Figures 5 and 6 compare overall results from the three geographic regions. Figure 5 compares the range and clustering of variables used in the stepwise regression technique and the general linear models procedures. Figure 6 compares the predicted values for PCUL, generated by the particular model involved, with the actual values obtained for each station in each location involved in the study.

Table 14. Stepwise regression procedure for all variables and the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the Philadelphia-Camden dumpsite.

| Variables added to model in order added | Multiple R ² | Multiple R value | F value | Probability of larger F |
|---|-------------------------|------------------|---------|-------------------------|
| Distance | .20 | .45 | 14.39 | .0004 |
| Coliform MPN | .21 | .46 | 7.21 | .0017 |

Note: No other variables were found to significantly increase R² values.

Table 15. General linear models procedure for two valid variables determined through the stepwise regression procedure and the variable "PCUL" (percent positive Acanthamoeba cultures/station) for the Philadelphia-Camden dumpsite.

| Variables used in model | Multiple R ² for model | Multiple R for model | F value for model | Probability of larger F for model |
|--------------------------|-----------------------------------|----------------------|-------------------|-----------------------------------|
| Distance Coliform MPN | .21 | .46 | 7.21 | .0017 |

Figure 7. The spread of contaminated materials from a depositional center. Analysis of stations taken near the apex of the now inactive Philadelphia-Camden dumpsite indicate that sediments containing bacteria and Acanthamoeba have been dispersed by current regimes prevalent in the area. The general dispersion pattern is to the east (down slope) and in a northeast-southeast direction. This general trend is in keeping with findings in the New York Bight apex and in Narragansett Bay (see Table 19).

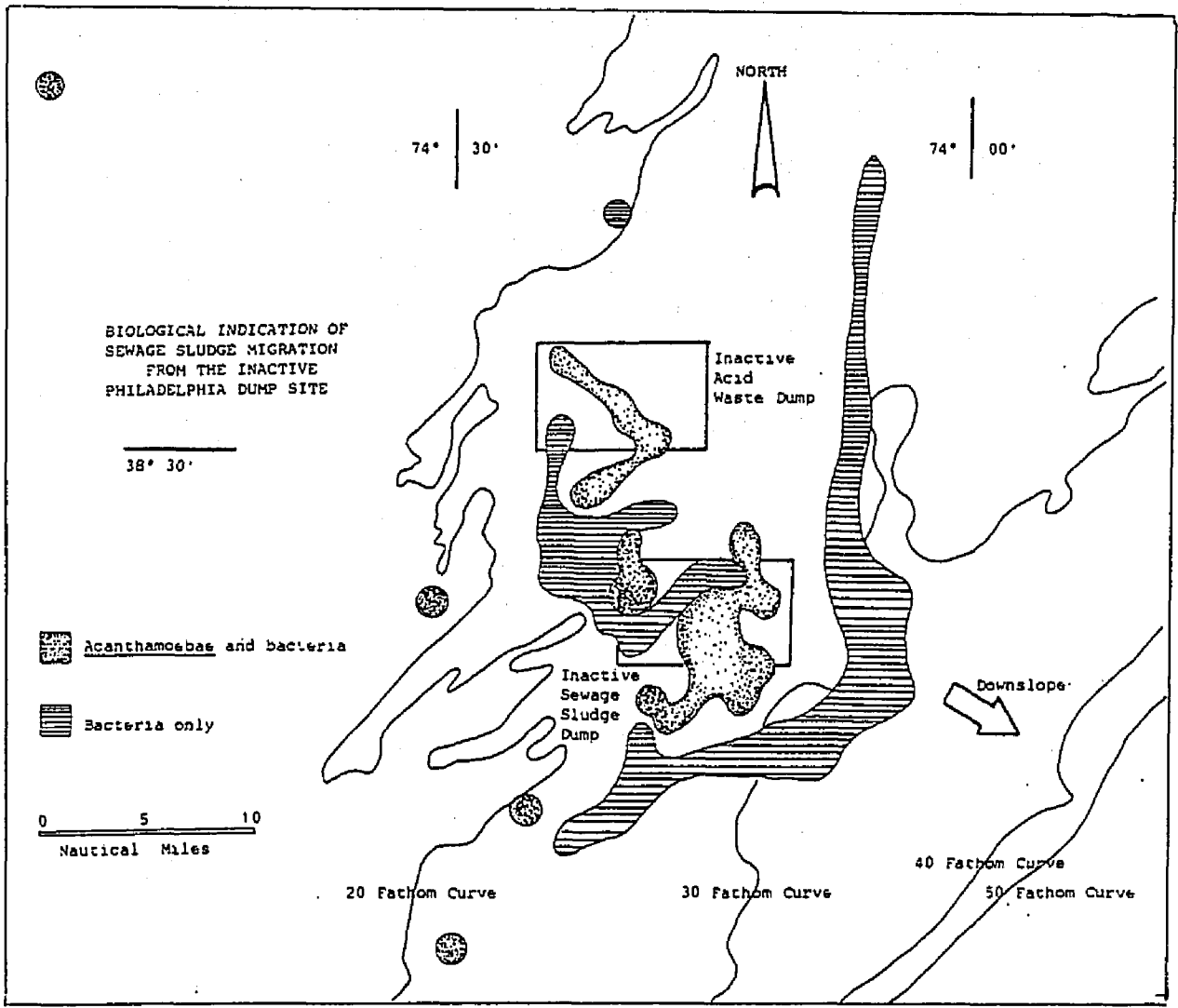
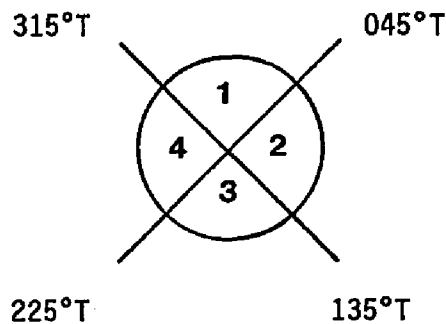


Figure 7

Table 16. Correlations between PCUL and the variables DIST and LFCC for quadrants drawn through the center of the New York dumpsite (see below).

| NEW YORK DUMPSITE QUADRANTS CORRELATION SUMMARY | | | | |
|--|----|-----------------------|------|--------------------------|
| Quadrant | N | PCUL with variable | R | Level of significance |
| 1 | 8 | DIST | .08 | .8538 |
| 2 | 12 | DIST | .28 | .3792 |
| 3 | 14 | DIST | -.54 | .0452 |
| 4 | 10 | DIST | -.69 | .0257 |
| 1 | 8 | LFCC | -.14 | .7382 |
| 2 | 12 | LFCC | .12 | .7142 |
| 3 | 14 | LFCC | -.03 | .9160 |
| 4 | 10 | LFCC | .73 | .0176 |



Quadrants through the center of the New York dumpsite (or 12-mile site). Circle represents 6 nautical miles. See figure for quadrant contours.

Table 17. Statistics for the variable PCUL correlated with the environmental variables DIST and LFCC for all stations in the Philadelphia site located east and seaward of a transect drawn through the center of the site bearing 045-225°T.

| <u>*Dependent variable PCUL correlation with:</u> | | | |
|---|----------|------------|--------------|
| | <u>R</u> | <u>"N"</u> | Significance |
| <u>DIST</u> | -0.71823 | 22 | 0.0001 |
| <u>LFCC</u> | -0.10832 | 22 | 0.6314 |

*See text for explanation.

Table 18. Values for the Durbin-Watson statistic, "d", for determining the autocorrelation residuals for a given procedure (Chatterjee and Price, 1975).

| Value for "d" for model | "N" for model | Autocorrelation | Significance level |
|-------------------------------------|------------------|-----------------|-----------------------|
| Narragansett Bay model = 1.3011 | 31 | No | .01 |
| New York Bight model = 1.4453 | 45** | No | .01 |
| Philadelphia site model = 1.4162 | 60** | Inconclusive | * |

*The value for the Philadelphia site falls between d_L and d_U in the table (A.4b) in the reference cited. In this case, the test does not present conclusive evidence that autocorrelation is not present. The other two values are both greater than the d_U for their given "N" values (1.27 and 1.38 respectively) indicating that autocorrelation is not present at the confidence level noted. As discussed below, the Philadelphia model was constructed with data some of which could not meet minimum significance level conditions imposed on the procedure.

**Tables values closest to actual "N".

Figure 8. A "prediction table" generated by the model for the Narragansett Bay area. The box below the table is used to determine error probability in an environmental-management situation. See DISCUSSION for details.

Figure 8

*PREDICTION MODEL GENERATED BY THE
 "BEST" MODEL FOR THE NARRAGANSETT
 BAY SITE

| Obs. # | Predicted% | Actual% |
|--------|------------|---------|
| 1 | . | . |
| 2 | . | . |
| 3 | 13.5995 | 0 |
| 4 | 14.0727 | 0 |
| 5 | 14.0727 | 0 |
| 6 | 14.0727 | 0 |
| 7 | 16.9115 | 0 |
| 8 | 22.9153 | 0 |
| 9 | 24.3347 | 50 |
| 10 | 25.3665 | 17 |
| 11 | 26.3128 | 67 |
| 12 | 27.7322 | 17 |
| 13 | 31.7740 | 17 |
| 14 | 33.6666 | 17 |
| 15 | 33.7360 | 33 |
| 16 | 36.8768 | 67 |
| 17 | 40.2129 | 17 |
| 18 | 40.6942 | 50 |
| 19 | 41.9827 | 83 |
| 20 | 42.8435 | 83 |
| 21 | 50.0794 | 67 |
| 22 | 53.8514 | 50 |
| 23 | 55.7440 | 17 |
| 24 | 57.6365 | 83 |
| 25 | 65.5490 | 67 |
| 26 | 67.4415 | 100 |
| 27 | 87.2227 | 83 |
| 28 | 87.2227 | 100 |
| 29 | 88.2916 | 83 |
| 30 | 93.4642 | 67 |
| 31 | 94.3197 | 67 |

| | | Actual Values | |
|------------------|------------|---|---|
| | | - cultures | + cultures |
| Predicted values | - cultures | Correct prediction | Incorrect prediction predicting - when - |
| | + cultures | Incorrect prediction predicting - when - | Correct Prediction |

*Values are arranged from the lowest to the highest predicted value for positive Acanthamoeba cultures per station.

DISCUSSION

This study was concerned with predicting the incidence of positive Acanthamoeba cultures in oceanic sediments using surrogate variables. These variables were selected based on literature descriptions of their environmental distribution and relevance to the investigation. Values used as inputs to the modeling program were based upon the applicability and quality of data from all sources researched.

Correlations

A comparison between the correlation distributions for surrogate variables used in all three areas studied (Narragansett Bay, New York Bight, Philadelphia-Camden dumpsite) demonstrated that the general pattern of relationships was similar in each location (Figure 5a,b,c).

Narragansett Bay had the widest range of correlation coefficients (-.83 to .72) because this estuary represented the most "linear" situation studied. In the bay, the material from the Fields Point facility flowed downstream from a single point source, the outfall pipe. The flow of these contaminants resulted in a gradual downstream dilution of materials. In this instance, distance had the highest negative correlation (-.83) while coliform MPN had the highest positive correlation (.72).

In the New York Bight, the correlations ranged from -.49 to .37 (for sediment color (coded), and lead, respectively). This area did not represent a linear flow. The complex circulation patterns which have been revealed by remotely sensed imagery (Munday, 1983), can be represented as a combination of plumes from the Hudson-Raritan estuary and a meteorologically-forced current regime. In spite of this complexity, there is an area in the New York Bight which resembles, in

some respects, the Narragansett region. The New York Bight site is east of the Hudson Canyon and northeast of the Christiaensen Basin. Both these low lying areas have become depositional centers for sludge related materials deposited at the dumpsite which flow "down hill" into the depressions (Hatcher and McGillivray, 1979). When the New York Bight dumpsite area is divided into four sections (Table 16) the correlation between distance and positive Acanthamoeba cultures in quadrant number four (the westernmost division) becomes $-.69$ as compared to an overall correlation for distance of $-.30$. The correlation between coliform MPN and positive Acanthamoeba cultures for this same quadrant is $.73$ as compared to an overall value of $.02$. These figures compare favorably with those for Narragansett Bay ($-.83$ and $.72$) indicating that, at least in this portion of the New York Bight site, a situation exists which is similar to that found in the more northern study area.

As noted above, the distribution of contaminants was found to follow a general pattern of decreasing concentration from a point source or depositional center. Because salinity and temperature were not subject to the same distributional forces as pollutants, further mention of these environmental variables is necessary. In all cases and for all geographic areas, temperatures and salinities at sample depth were the values used to develop the model.

In Narragansett Bay, the correlation coefficients for temperature and salinity were $-.04$ and $-.35$, respectively. In the bay study, the temperature varied from a high of 9°C to a low of 6°C . This range was spread over a 35 mile track line represented by the distance from the Fields Point facility to offshore, open ocean stations and accounted for the very low correlation value. Salinity, in contrast, ranged from a low of 26 ppt to a high of 34 ppt. Because of the configuration of the

estuarine system, all the lowest salinities were found at the northernmost stations and the salinity values increased downstream toward the ocean. Although the range for salinity was small (8 ppt), the lower salinity values coincided with the highest percentages of positive Acanthamoeba cultures (near the Fields Point facility) and, conversely, the higher salinity values were found in the open ocean at stations for which Acanthamoeba percentages were smallest. Therefore, the distribution of salinity and positive cultures should not be considered a causal relationship but should be seen as the result of a natural, estuarine distribution of the salinity.

In the New York Bight area, the correlation coefficient for salinity was $-.32$ and that for temperature was $.34$. In this location, as noted, much of the sewage-related material had moved away from the shallow depositional center (the 12 mile dumpsite) into deeper waters of the Christiaensen Basin and Hudson Canyon. For this reason, many of the higher Acanthamoeba values were found in relatively deeper, cooler, and less saline waters. Again, there was only a coincidental relationship between the distribution of these environmental factors and the protozoa.

In the Philadelphia-Camden dumpsite, the Acanthamoeba data were found to be positively correlated with salinity ($.41$) and negatively correlated with temperature ($-.13$). Although these values appeared to be valid, the "N" for these variables represented only a small fraction of the samples taken and their significance levels were marginal (see Table 13). For this reason, the numbers were reported but were not used in the stepwise regression procedure which produced the final model for this site.

Although the Philadelphia-Camden dumpsite model did not have the strength of the other two, the correlation range (-.47 to .41) compared favorably with the New York Bight simulation. The Philadelphia-Camden site was divided into an upslope and downslope component. The correlation coefficient for distance increased from -.47 for the entire study area to -.72 for the areas in which materials appeared to be slowly flowing over the shelf break (Table 17). These figures seemed to suggest a similar distributional mechanism for sewage-related materials existed in situations in which there was a downslope or downstream flow from an upslope or upstream depositional center.

Because the Philadelphia-Camden site was an inactive disposal area, there was an opportunity to observe the change in the benthic distribution of the sludge materials. As noted above, there was a high correlation between distance and positive culture of Acanthamoeba southwest of the dumpsite center (-.72). Figure 7 represents the pattern of microbial contamination in and around the depositional center of the area. These patterns were contoured using bacteriological data from the study stations and present strong evidence that dumped materials were migrating away from the central disposal area in a northeast-southwest direction and to a small extent down the continental slope. Thus, the spread of organic contaminants and their associated protozoan components could be tracked using the bacteria and amoebae or surrogate correlative variables as indicators of the presence of sewage-related sediment fractions.

Generally, heavy metals and organics were positively correlated with the percent of positive cultures of Acanthamoeba/station. The other variables showed a less fixed pattern but, in two out of three

cases, were negatively correlated with the percentage of positive cultures.

Models

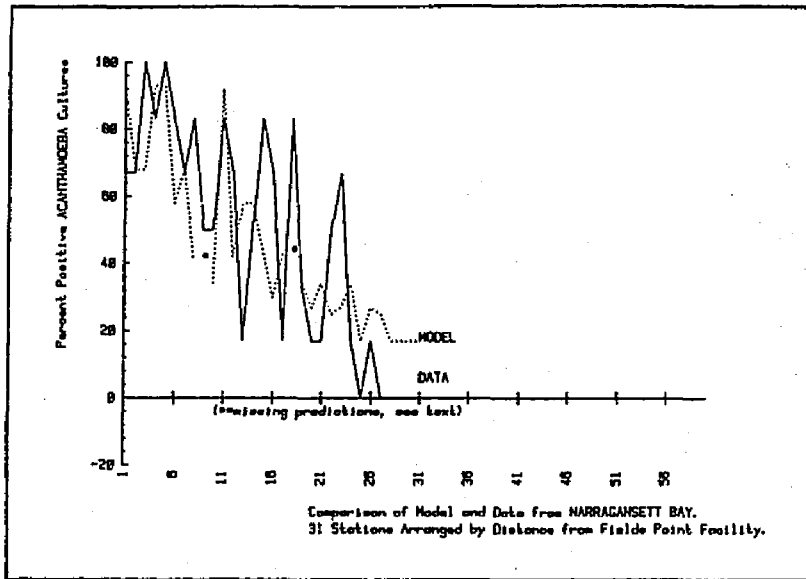
When stations for all areas were arranged from the lowest to the highest predicted values, a linear pattern resulted (Figure 6a,b,c). However, such a presentation may be considered somewhat artificial since the resulting graph is based on the model and not on the actual in situ data. If, however, stations are arranged according to distance from the particular depositional center (Figure 9a,b,c) and the actual data are compared to modeling results, a more valid picture of the comparison between the two curves is produced.

An analysis of the three models using the Durbin-Watson statistic (Table 18) revealed that residuals for the Narragansett Bay and New York Bight models were not autocorrelated. Results for the Philadelphia-Camden model (Figures 5c, 6c, 9c) produced the poorest environmental simulation.

The stepwise regression technique, as has been described, tested all variables to determine those which most significantly increased the R^2 values. In the case of the Philadelphia-Camden dumpsite, there were 59 stations. The "N" for most of the variables used, excluding distance and coliform MPN, ranged from a low of eight to a high of 22. In this instance, the procedure determined that only coliform MPN and distance were valid parameters. The Philadelphia-Camden model, then, was based on these two variables.

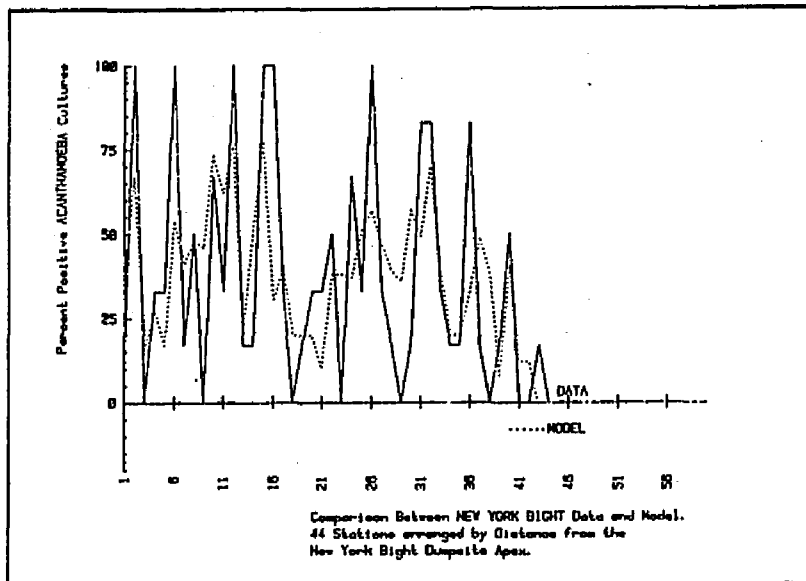
If a highly significant contributing variable was absent from a particular station, the procedure would not make a prediction for that set of data because one or more of the maximizing contributors was

Figure 9. A comparison of models generated for the three geographic areas under study with the actual in situ data. Stations are arranged by distance from the depositional center of the particular disposal/area outfall.

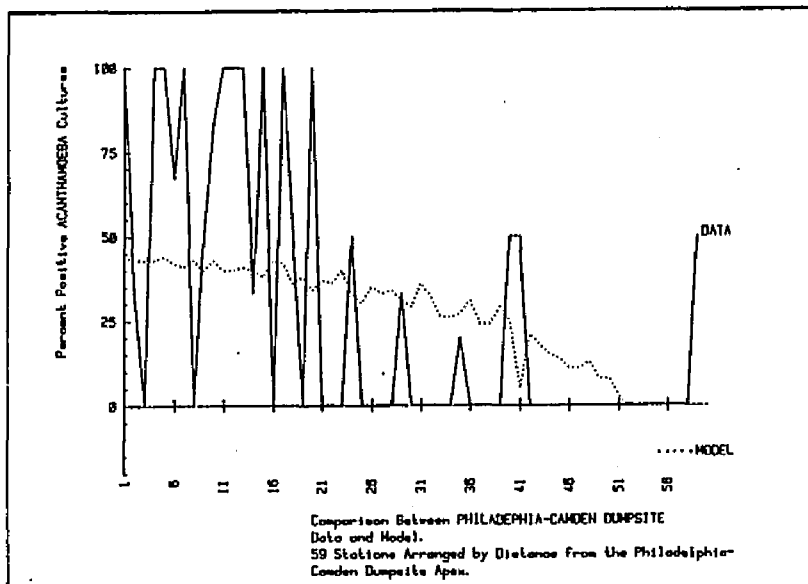


9a.
Model vs. In-Situ
Data
Narragansett Bay

(Stations for all
plots arranged
by increasing
distance from
dumpsite or out-
fall.)



9b.
Model vs. In-situ
Data
New York Bight



9c.
Model vs. In-situ
Data
Philadelphia Dump
Site

missing. The two missing predictions for the Narragansett Bay data (Figures 5a, 6a, 9a) were an example of this situation. There were no temperature, salinity, or total organic carbon values for these stations.

The models generated for the three areas were valid for the prediction of the incidence of positive cultures of Acanthamoeba within the limitations of the stepwise regression and linear model procedures (Tables 6, 11, 12, 15). The validity of any model, however, is dependent upon the number of data points used in the initial construction. The more data points available, the better the simulation will be (Gold, 1977). The present study took advantage of all applicable data sources and computer procedures to produce the best possible models from the widest assemblage of information.

Use of Models

In the simplest situation, models may be used in a straightforward manner using intercept and constant values. These values are generated by the linear model procedures. A constant figure is available for each variable used to obtain the particular model. This number is multiplied by the in situ value for that variable. The intercept value is added or subtracted from the results depending upon the sign of the number (see Microfiche Appendices).

For example, the "tactical" or short-term model for the New York Bight area generated the following constants and intercept value for the variables involved (see Table 11 and microfiche appendix):

| | | |
|-------------------------------|------------------------|---|
| | Intercept value....594 | |
| Sediment color constant...-10 | | Sediment color <u>in situ</u> value...5 |
| Temperature constant.....9 | | Temperature <u>in situ</u> value.....10°C |
| Salinity constant.....-20 | | Salinity <u>in situ</u> value.....28 ppt |
| Coliform MPN constant.....5 | | MPN <u>in situ</u> value.....3.4 |

The constants would be multiplied by the in situ values found at a particular station, these values would be added together, and the intercept would be added to the result, since the sign of this number is positive, as follows:

$$(-10)(5)+(9)(10)+(-20)(28)+(5)(3.4)+594 = 71$$

In this case, the formula predicts, based upon the model used, that there would be 71% positive culture incidence in replicates taken at the station represented by in situ values. The same procedure applied to any of the models generated for any geographic area under study.

The application described above is a simple procedure which could be accomplished using a simple four-function calculator. In this case, the investigator would examine the "tactical" and "strategic" models and determine whether or not the addition of variables requiring more extensive laboratory analysis would be required to significantly increase the multiple R values and select a model accordingly.

The formula approach would work best in areas in which sewage sludge was a known contaminant but for which the ambient population for the Acanthamoeba was unknown. In regions similar to the New York Bight in which Acanthamoeba are known to exist, another approach may be

required. In this case, the use of a prediction table (Figures 10, 11) is required.

The most important consideration to keep in mind when examining these prediction tables is that values are arranged in order from the lowest to highest predicted values for positive Acanthamoeba cultures generated by the model. The actual station values are arranged according to the manner in which they correspond to the model's prediction. The table permits an investigator to ascertain the error rate which can be expected when the prediction table is used and a given background concentration, or population, of Acanthamoeba, represented by the percent of positive cultures/station, is assumed.

An example will serve to illustrate this point. In the Narragansett Bay study, the experimental design called for six culture replicates from each sediment sample. The table (Figure 10) is based on this sampling scheme. If another study were to be undertaken in the Narragansett Bay area using the six sample method and the ambient population was considered to be one out of six cultures/station or 17%, the table would be used in the following manner.

Section AA (Figure 10) would be drawn midway between 17% (one culture) and 33% (two cultures) or 25.3665 on the table. Stations above this line, in the "Actual %" column which represented 17% or less would now be considered the ambient population level and be the new "zero" point. In this case, there are seven values above the line representing zero or 17%. Based on the ordering used to set up the table, these values represent a correct prediction of negative incidence. The one value of 50% above the line represents a prediction of negative incidence when the actual situation is positive. The first two values

Figure 10. A prediction table for Narragansett Bay. Section 'AA' is drawn to delineate an "ambient" population level of one positive culture/station of 17% (one culture out of six). Matrix below the table indicates a possible error rate of one in 29.

Figure 10

*PREDICTION MODEL GENERATED BY THE
 "BEST" MODEL FOR THE NARRAGANSETT
 BAY SITE

| Obs. # | Predicted% | Actual% |
|--------|------------|---------|
| 1 | . | |
| 2 | . | |
| 3 | 13.5995 | 0 |
| 4 | 14.0727 | 0 |
| 5 | 14.0727 | 0 |
| 6 | 14.0727 | 0 |
| 7 | 16.9115 | 0 |
| 8 | 22.9153 | 0 |
| 9 | 24.3347 | 50 |
| 10 | 25.3665 | 17 |
| A 11 | 26.3128 | 67 |
| 12 | 27.7322 | 17 |
| 13 | 31.7740 | 17 |
| 14 | 33.6666 | 17 |
| 15 | 33.7360 | 33 |
| 16 | 36.8768 | 67 |
| 17 | 40.2129 | 17 |
| 18 | 40.6942 | 50 |
| 19 | 41.9827 | 83 |
| 20 | 42.8435 | 83 |
| 21 | 50.0794 | 67 |
| 22 | 53.8514 | 50 |
| 23 | 55.7440 | 17 |
| 24 | 57.6365 | 83 |
| 25 | 65.5490 | 67 |
| 26 | 67.4415 | 100 |
| 27 | 87.2227 | 83 |
| 28 | 87.2227 | 100 |
| 29 | 88.2916 | 83 |
| 30 | 93.4642 | 67 |
| 31 | 94.3197 | 67 |

| | | Actual Values | |
|------------------|------------|--|--|
| | | - cultures | + cultures |
| Predicted values | - cultures | Correct prediction 7 | Incorrect prediction predicting - when + 1 |
| | + cultures | Incorrect prediction predicting - when - 5 | Correct Prediction 16 |

*Values are arranged from the lowest to the highest predicted value for positive Acanthamoeba cultures per station.

of 50% and 83% in the "Actual %" column are ignored since no prediction is available. Below the line AA are 16 values of 33% or more. These values represent correct positive predictions. The five values of 17% below the line represent incorrect positive predictions. All these figures appear in the appropriate box in the matrix below the table.

The most serious error which can be made, from a public health standpoint, is a negative prediction when the actual situation is positive. The matrix indicates that there is a 1 in 29, or 3% chance of making such an error if the ambient population level is considered to be one positive culture/station.

If an ambient level of two positive cultures/station, or 33%, was chosen, section BB would be drawn at the midpoint between 33% (two cultures) and 50% (three cultures) (Figure 11) or about 41%. Using the same rationale as in the previous case, the matrix now indicates that there is a 5/29 or 17% chance of making the error or predicting a negative incidence in a positive incidence situation.

Based on these criteria, the appropriate procedure may be applied to a given environmental situation. For a preliminary investigation in which a sewage sludge impact is known but for which no previous Acanthamoeba data exist, the formula represents the most rapid and economical method. In other areas which have a history of sewage contamination, the table produces the best information.

The preceding examples do not rule out the possibility that a "background level" of one or two cultures may represent a definite public health consideration. Such a decision would be made by the official using the tables based upon his familiarity with area under study and the seriousness of the perceived threat.

In such a case, the tables would be used as presented with the caveat that their success rate is based upon the significance of the model used to generate the values for the table (in the case of Narragansett Bay, the R value for the model is .73 and the probability of a larger "F" is .0001).

Applicability of Procedures

These same procedures may be applied to any variable within the correlation matrices presented (Appendices B, C, D). The programs used to obtain the Acanthamoeba models, tables, and formulae, may be used to produce similar products pertaining to other pollutants. Some modification would be required (e.g., substituting PPM or PPB for percent positive cultures/station), but the results would be equally valid. The stepwise regression procedure or similar package should be used since all variables are evaluated reducing the possibility of invalid predictions based on too few comparisons. The investigator must obtain maximum utility from the available information and insure that the procedures used are designed to produce the most in-depth analysis possible.

Figure 11. A prediction table for Narragansett Bay. Section 'BB' is drawn to delineate an "ambient" population level of two positive cultures/station or 33% (two cultures out of six). Matrix below the table indicates a possible error rate of five in 29.

Figure 11

*PREDICTION MODEL GENERATED BY THE
 "BEST" MODEL FOR THE NARRAGANSETT
 BAY SITE

| <u>Obs. #</u> | <u>Predicted%</u> | <u>Actual%</u> |
|---------------|-------------------|----------------|
| 1 | . | . |
| 2 | . | . |
| 3 | 13.5995 | 0 |
| 4 | 14.0727 | 0 |
| 5 | 14.0727 | 0 |
| 6 | 14.0727 | 0 |
| 7 | 16.9115 | 0 |
| 8 | 22.9153 | 0 |
| 9 | 24.3347 | 50 |
| 10 | 25.3665 | 17 |
| 11 | 26.3128 | 67 |
| 12 | 27.7322 | 17 |
| 13 | 31.7740 | 17 |
| 14 | 33.6666 | 17 |
| 15 | 33.7360 | 33 |
| 16 | 36.8768 | 67 |
| 17 | 40.2129 | 17 |
| 18 | 40.6942 | 50 |
| B- 19 | 41.9827 | 83 |
| 20 | 42.8435 | 83 |
| 21 | 50.0794 | 67 |
| 22 | 53.8514 | 50 |
| 23 | 55.7440 | 17 |
| 24 | 57.6365 | 83 |
| 25 | 65.5490 | 67 |
| 26 | 67.4415 | 100 |
| 27 | 87.2227 | 83 |
| 28 | 87.2227 | 100 |
| 29 | 88.2916 | 83 |
| 30 | 93.4642 | 67 |
| 31 | 94.3197 | 67 |

| | | Actual Values | |
|------------------|------------|--|--|
| | | - cultures | + cultures |
| Predicted values | - cultures | Correct prediction 12 | Incorrect prediction predicting - when + 5 |
| | + cultures | Incorrect prediction predicting + when - 1 | Correct Prediction 11 |

*Values are arranged from the lowest to the highest predicted value for positive Acanthamoeba cultures per station.

CONCLUSIONS

1. Sufficient data exist in the literature, computerized data bases, and other archives to permit the development of a statistically valid method for predicting the incidence of pathogenic protozoans in oceanic sediments. The correlation between surrogate variables and the probability of obtaining positive/negative cultures of Acanthamoeba sp. may be determined using statistical procedures.
2. Use of the stepwise regression technique permits the determination of statistically valid probabilities for the presence/absence of one group of factors (not sampled) based upon correlations between these surrogate variables and other groups of environmental factors (sampled).
3. Tables and formulae may be produced by computer programs which may be used, in a cost effective manner, to evaluate public health situations in which the presence or absence of Acanthamoeba is a contributing factor. Data resulting from such studies may be used to document the spread of sewage-related organic materials from the original depositional centers even after the particular dumpsite is no longer active.
4. These same procedures may be applied to other variables in the data set to produce models similar to those generated for the Acanthamoeba.

Appendix A. Major computerized marine data archives used in this study.

Numerical Data Bases

- *ADP.....In-house computerized data base used by the
Northeast Fisheries Center (NMFS/NOAA).
- *NERFIS.....Northeast Regional Fisheries Information Service,
NOAA/NEFC, Woods Hole, MA.
- *NODC.....National Oceanographic Data Center, Environmental
Data and Information Service, National Oceanic
and Atmospheric Administration, Washington, DC.
The national repository for all oceanographic
data for all disciplines.
- *PIDS.....Parameter Inventory Display System (NODC). This
data base provides listings of cruises, stations,
and data available within a particular geographic
area for a particular discipline.
- *MCCDB.....Marine Core Curators Data Base, National
Geological Survey Data Base providing lists of
authors and principal investigators as well as
raw data printouts.
- *NGSDC.....National Geological Survey Data Center, Boulder,
CO. Marine geology/sedimentology information.
- *STORET.....U. S. Environmental Protection Agency Storage and
Retrieval system. Contains raw data from
pollution studies conducted under the auspices of
EPA.

Appendix A. (continued)

Bibliographic Data Bases

DIALOG/ENVIROLINE.....Computerized information service, accessed through NODC. Provides listings and abstracts of pertinent bibliographic materials using keyword searches.

SDI/NLISD.....Selective Dissemination of Information, NOAA Library and Information Services, WSC-4, Washington Science Center, Rockville, MD. This data base is available to NOAA employees on a subscription basis. Monthly updates on pertinent literature sorted by keyword search for a desired area or discipline are available.

*NOTE: Within these data bases, numerous subfiles were selected in order to access the widest number of sources possible to obtain values and/or references for surrogate variables.

Appendix B. Complete correlation matrix for all 13 variables
(12 surrogate variables and one dependent variable)
used in the Narragansett Bay study.

NARRAGANSETT BAY - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | DIST | PCUL | TA | PB | CU | CR | HGB | CDM |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| DIST | 1.00000 | -0.82735 | -0.77344 | -0.54166 | -0.92117 | -0.72819 | -0.74664 | -0.61426 |
| | 0.0000 | 0.0001 | 0.0001 | 0.0302 | 0.0004 | 0.0072 | 0.0014 | 0.0194 |
| | 31 | 31 | 31 | 16 | 9 | 12 | 15 | 14 |
| PCUL | -0.82735 | 1.00000 | 0.58053 | 0.22901 | 0.71865 | 0.32330 | 0.59253 | 0.36040 |
| | 0.0001 | 0.0000 | 0.0006 | 0.3936 | 0.0292 | 0.3053 | 0.0199 | 0.2056 |
| | 31 | 31 | 31 | 16 | 9 | 12 | 15 | 14 |
| TA | -0.77344 | 0.58053 | 1.00000 | 0.74398 | 0.75921 | 0.64687 | 0.72842 | 0.40478 |
| | 0.0001 | 0.0006 | 0.0000 | 0.0010 | 0.0177 | 0.0230 | 0.0021 | 0.1511 |
| | 31 | 31 | 31 | 16 | 9 | 12 | 15 | 14 |
| PB | -0.54166 | 0.22901 | 0.74398 | 1.00000 | 0.36334 | 0.78653 | 0.40138 | 0.84355 |
| | 0.0302 | 0.3936 | 0.0010 | 0.0000 | 0.5478 | 0.0070 | 0.1959 | 0.0003 |
| | 16 | 16 | 16 | 16 | 5 | 10 | 12 | 13 |
| CU | -0.92117 | 0.71865 | 0.75921 | 0.36334 | 1.00000 | -1.00000 | 0.96014 | 1.00000 |
| | 0.0004 | 0.0292 | 0.0177 | 0.5478 | 0.0000 | 0.0000 | 0.0024 | 0.0001 |
| | 9 | 9 | 9 | 5 | 9 | 2 | 6 | 4 |
| CR | -0.72819 | 0.32330 | 0.64687 | 0.78653 | -1.00000 | 1.00000 | 0.59138 | 0.58420 |
| | 0.0072 | 0.3053 | 0.0230 | 0.0070 | 0.0000 | 0.0000 | 0.0553 | 0.1283 |
| | 12 | 12 | 12 | 10 | 2 | 12 | 11 | 8 |
| HGB | -0.74664 | 0.59253 | 0.72842 | 0.40138 | 0.96014 | 0.59138 | 1.00000 | -0.07182 |
| | 0.0014 | 0.0199 | 0.0021 | 0.1959 | 0.0024 | 0.0553 | 0.0000 | 0.8543 |
| | 15 | 15 | 15 | 12 | 6 | 11 | 15 | 9 |
| CDM | -0.61426 | 0.36040 | 0.40478 | 0.84355 | 1.00000 | 0.58420 | -0.07182 | 1.00000 |
| | 0.0194 | 0.2056 | 0.1511 | 0.0003 | 0.0001 | 0.1283 | 0.8543 | 0.0000 |
| | 14 | 14 | 14 | 13 | 4 | 8 | 9 | 14 |

NARRAGANSETT BAY -- DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | ZNM | LFCC | TC | SPT | SCLR |
|------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| PB | 0.90267 0.0009 9 | 0.52652 0.0361 16 | -0.33884 0.2167 15 | 0.05654 0.8414 15 | -0.57134 0.0208 16 |
| CU | 1.00000 2 | 0.66720 0.0496 9 | -0.54006 0.2108 7 | 0.27517 0.5503 7 | -0.91405 0.0006 9 |
| CR | 0.80498 0.0159 8 | 0.70169 0.0110 12 | -0.49128 0.1249 11 | 0.23754 0.4818 11 | -0.71735 0.0086 12 |
| HGB | 0.30228 0.46668 8 | 0.60964 0.0158 15 | -0.58262 0.0288 14 | 0.35756 0.2094 14 | -0.79058 0.0005 15 |
| CDM | 0.95416 0.0008 7 | 0.62347 0.0172 14 | -0.39126 0.1862 13 | -0.23171 0.4462 13 | -0.61802 0.0185 14 |
| ZNM | 1.00000 0.0000 12 | 0.78151 0.0027 12 | -0.46572 0.1270 12 | 0.06442 0.8423 12 | -0.72408 0.0077 12 |
| LFCC | 0.78151 0.0027 12 | 1.00000 0.0000 31 | -0.32811 0.0823 29 | -0.28491 0.1341 29 | -0.64240 0.0001 31 |
| TC | -0.46572 0.1270 12 | -0.32811 0.0823 29 | 1.00000 0.0000 29 | -0.32818 0.0822 29 | 0.39694 0.0330 29 |

NARRAGANSETT BAY - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | DIST | PCUL | TA | PB | CU | CR | HGB | CDM |
|------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| ZNM | -0.69403 0.0123 12 | 0.35567 0.2565 12 | 0.52888 0.0771 12 | 0.90267 0.0009 9 | 1.00000 2 | 0.80498 0.0159 8 | 0.30228 0.4668 8 | 0.95416 0.0008 7 |
| LFCC | -0.78000 0.0001 31 | 0.68165 0.0001 31 | 0.62800 0.0002 31 | 0.52652 0.0361 16 | 0.66720 0.0496 9 | 0.70169 0.0110 12 | 0.60964 0.0158 15 | 0.62347 0.0172 14 |
| TC | 0.09704 0.6165 29 | -0.03767 0.8462 29 | -0.21287 0.2676 29 | -0.33884 0.2167 15 | -0.54006 0.2108 7 | -0.49128 0.1249 11 | -0.58262 0.0288 14 | -0.39126 0.1862 13 |
| SPT | 0.53287 0.0029 29 | -0.35165 0.0614 29 | -0.24244 0.2051 29 | 0.05654 0.8414 15 | 0.27517 0.5503 7 | 0.23754 0.4818 11 | 0.35756 0.2094 14 | -0.23171 0.4462 13 |
| SCLR | 0.52015 0.0027 31 | -0.42342 0.0176 31 | -0.54396 0.0016 31 | -0.57134 0.0208 16 | -0.91405 0.0006 9 | -0.71735 0.0086 12 | -0.79058 0.0005 15 | -0.61802 0.0185 14 |
| | ZNM | LFCC | TC | SPT | SCLR | | | |
| DIST | -0.69403 0.0123 12 | -0.78000 0.0001 31 | 0.09704 0.6165 29 | 0.53287 0.0029 29 | 0.52015 0.0027 31 | | | |
| PCUL | 0.35567 0.2565 12 | 0.68165 0.0001 31 | -0.03767 0.8462 29 | -0.35165 0.0614 29 | -0.42342 0.0176 31 | | | |
| TA | 0.52888 0.0771 12 | 0.62800 0.0002 31 | -0.21287 0.2676 29 | -0.24244 0.2051 29 | -0.54396 0.0016 31 | -0.57134 0.0208 16 | -0.79058 0.0005 15 | -0.61802 0.0185 14 |

NARRAGANSETT BAY - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | ZNM | LFCC | TC | SPT | SCLR |
|------|----------|----------|----------|----------|----------|
| SPT | 0.06442 | -0.28491 | -0.32818 | 1.00000 | -0.13864 |
| | 0.8423 | 0.1341 | 0.0822 | 0.0000 | 0.4732 |
| | 12 | 29 | 29 | 29 | 29 |
| SCLR | -0.72408 | -0.64240 | 0.39694 | -0.13864 | 1.00000 |
| | 0.0077 | 0.0001 | 0.0330 | 0.4732 | 0.0000 |
| | 12 | 31 | 29 | 29 | 31 |

**Appendix C. Complete correlation matrix for all 21 variables
(20 surrogate variables and one dependent variable)
for the New York Bight apex study.**

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | DIST | BNT | PCUL | TA | PB | HGB | CU | CR |
|------|----------|---------|----------|----------|----------|----------|----------|----------|
| DIST | 1.00000 | 0.23707 | -0.30384 | -0.21436 | -0.45205 | -0.29400 | -0.49577 | -0.33909 |
| | 0.00000 | 0.1213 | 0.0449 | 0.1623 | 0.0021 | 0.0527 | 0.0006 | 0.0243 |
| BNT | 0.23707 | 1.00000 | 0.00080 | 0.34881 | 0.22985 | 0.13973 | 0.15595 | 0.10195 |
| | 0.1213 | 0.00000 | 0.9959 | 0.0203 | 0.1334 | 0.3657 | 0.3121 | 0.5102 |
| PCUL | -0.30384 | 0.00080 | 1.00000 | 0.17259 | 0.37432 | 0.32142 | 0.33564 | 0.07149 |
| | 0.0449 | 0.9959 | 0.00000 | 0.2626 | 0.0123 | 0.0334 | 0.0259 | 0.6447 |
| TA | -0.21436 | 0.34881 | 0.17259 | 1.00000 | 0.76646 | 0.77689 | 0.63942 | 0.59823 |
| | 0.1623 | 0.0203 | 0.2626 | 0.00000 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| PB | -0.45205 | 0.22985 | 0.37432 | 0.76646 | 1.00000 | 0.85114 | 0.82588 | 0.60455 |
| | 0.0021 | 0.1334 | 0.0123 | 0.0001 | 0.00000 | 0.0001 | 0.0001 | 0.0001 |
| HGB | -0.29400 | 0.13973 | 0.32142 | 0.77689 | 0.85114 | 1.00000 | 0.69310 | 0.54041 |
| | 0.0527 | 0.3657 | 0.0334 | 0.0001 | 0.0001 | 0.00000 | 0.0001 | 0.0002 |
| CU | -0.49577 | 0.15595 | 0.33564 | 0.63942 | 0.82588 | 0.69310 | 1.00000 | 0.37433 |
| | 0.0006 | 0.3121 | 0.0259 | 0.0001 | 0.0001 | 0.0001 | 0.00000 | 0.0123 |
| CR | -0.33909 | 0.10195 | 0.07149 | 0.59823 | 0.60455 | 0.54041 | 0.37433 | 1.00000 |
| | 0.0243 | 0.5102 | 0.6447 | 0.0001 | 0.0001 | 0.0002 | 0.0123 | 0.0000 |
| CDM | -0.25990 | 0.21689 | 0.12373 | 0.60348 | 0.72541 | 0.59533 | 0.58200 | 0.36925 |
| | 0.0884 | 0.1573 | 0.4236 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0136 |
| ZNM | -0.46595 | 0.23499 | 0.22313 | 0.77228 | 0.76904 | 0.69873 | 0.73596 | 0.48681 |
| | 0.0014 | 0.1247 | 0.1454 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0008 |
| AGM | -0.36136 | 0.24915 | 0.32786 | 0.73009 | 0.77030 | 0.58903 | 0.70591 | 0.51698 |
| | 0.0159 | 0.1029 | 0.0298 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0003 |

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | DIST | BNT | PCUL | TA | PB | HGB | CU | CR |
|--------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| NIPM | -0.36637 0.0144 | 0.25274 0.0979 | 0.29000 0.0562 | 0.71882 0.0001 | 0.76801 0.0001 | 0.63042 0.0001 | 0.71197 0.0001 | 0.43241 0.0034 |
| LFCC | -0.46872 0.0013 | 0.32955 0.0289 | 0.24175 0.1139 | 0.53566 0.0002 | 0.76265 0.0001 | 0.51547 0.0003 | 0.69545 0.0001 | 0.38623 0.0096 |
| TC | -0.28403 0.0617 | -0.02585 0.8677 | 0.34342 0.0225 | -0.10487 0.4981 | -0.00670 0.9656 | 0.02033 0.8958 | 0.08854 0.5676 | -0.14503 0.3476 |
| SPT | 0.08537 0.5816 | 0.29551 0.0515 | -0.31983 0.0343 | 0.07046 0.6495 | -0.04848 0.7546 | -0.13295 0.3896 | -0.03904 0.8014 | 0.06253 0.6868 |
| SCLR | 0.40690 0.0061 | -0.03445 0.8243 | -0.48880 0.0008 | -0.35304 0.0187 | -0.54299 0.0001 | -0.56458 0.0001 | -0.36043 0.0162 | -0.52749 0.0002 |
| PSILT | -0.35970 0.0165 | 0.08287 0.5928 | 0.26240 0.0853 | 0.80843 0.0001 | 0.87380 0.0001 | 0.87677 0.0001 | 0.69560 0.0001 | 0.64341 0.0001 |
| PCLAY | -0.06096 0.6942 | 0.41952 0.0046 | 0.18986 0.2171 | 0.60000 0.0001 | 0.51582 0.0003 | 0.53377 0.0002 | 0.39658 0.0077 | 0.41432 0.0052 |
| PCB | -0.38961 0.0089 | 0.21054 0.1701 | 0.19424 0.2064 | 0.47017 0.0013 | 0.60333 0.0001 | 0.39531 0.0079 | 0.69716 0.0001 | 0.25509 0.0947 |
| PCOP | -0.37989 0.0110 | 0.16749 0.2772 | 0.23756 0.1205 | 0.44038 0.0028 | 0.58006 0.0001 | 0.55983 0.0001 | 0.54198 0.0001 | 0.41612 0.0050 |
| TCHTOC | -0.42216 0.0043 | -0.15452 0.3166 | 0.05957 0.7009 | 0.14425 0.3502 | 0.04309 0.7812 | 0.07871 0.6115 | -0.05912 0.7030 | 0.25341 0.0970 |
| | CDM | ZNM | AGM | NIPM | LFCC | TC | SPT | SCLR |

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | CDM | ZNM | AGM | NIPM | LFCC | TC | SPT | SCLR |
|------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| DIST | -0.25990 0.0884 | -0.46595 0.0014 | -0.36136 0.0159 | -0.36637 0.0144 | -0.46872 0.0013 | -0.28403 0.0617 | 0.08537 0.5816 | 0.40690 0.0061 |
| BNT | 0.21689 0.1573 | 0.23499 0.1247 | 0.24915 0.1029 | 0.25274 0.0979 | 0.32955 0.0289 | -0.02585 0.8677 | 0.29551 0.0515 | -0.03445 0.8243 |
| PCUL | 0.12373 0.4236 | 0.22313 0.1454 | 0.32786 0.0298 | 0.29000 0.0562 | 0.24175 0.1139 | 0.34342 0.0225 | -0.31983 0.0343 | -0.48880 0.0008 |
| TA | 0.60348 0.0001 | 0.77228 0.0001 | 0.73009 0.0001 | 0.71882 0.0001 | 0.53566 0.0002 | -0.10487 0.4981 | 0.07046 0.6495 | -0.35304 0.0187 |
| PB | 0.72541 0.0001 | 0.76904 0.0001 | 0.77030 0.0001 | 0.76801 0.0001 | 0.76265 0.0001 | -0.00670 0.9656 | -0.04848 0.7546 | -0.54299 0.0001 |
| HGB | 0.59533 0.0001 | 0.69873 0.0001 | 0.58903 0.0001 | 0.63042 0.0001 | 0.51547 0.0003 | 0.02033 0.8958 | -0.13295 0.3896 | -0.56458 0.0001 |
| CU | 0.58200 0.0001 | 0.73596 0.0001 | 0.70591 0.0001 | 0.71197 0.0001 | 0.69545 0.0001 | 0.08854 0.5676 | -0.03904 0.8014 | -0.36043 0.0162 |
| CR | 0.36925 0.0136 | 0.48681 0.0008 | 0.51698 0.0003 | 0.43241 0.0034 | 0.38623 0.0096 | -0.14503 0.3476 | 0.06253 0.6868 | -0.52749 0.0002 |
| CDM | 1.00000 0.0000 | 0.71197 0.0001 | 0.71214 0.0001 | 0.56106 0.0001 | 0.48631 0.0008 | -0.20253 0.1874 | -0.06913 0.6557 | -0.33617 0.0257 |
| ZNM | 0.71197 0.0001 | 1.00000 0.0000 | 0.79329 0.0001 | 0.66241 0.0001 | 0.53712 0.0002 | -0.03795 0.8068 | 0.02116 0.8916 | -0.39683 0.0077 |
| AGM | 0.71214 0.0001 | 0.79329 0.0001 | 1.00000 0.0000 | 0.61825 0.0001 | 0.53101 0.0002 | -0.18000 0.2423 | 0.03159 0.8387 | -0.28973 0.0564 |

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | CDM | ZNM | AGM | NIPM | LFCC | TC | SPT | SCLR |
|--------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| NIPM | 0.56106 0.0001 | 0.66241 0.0001 | 0.61825 0.0001 | 1.00000 0.0000 | 0.68847 0.0001 | -0.09153 0.5546 | 0.03756 0.8087 | -0.36751 0.0141 |
| LFCC | 0.48631 0.0008 | 0.53712 0.0002 | 0.53101 0.0002 | 0.68847 0.0001 | 1.00000 0.0000 | 0.06949 0.6540 | 0.06860 0.6581 | -0.29702 0.0502 |
| TC | -0.20253 0.1874 | -0.03795 0.8068 | -0.18000 0.2423 | -0.09153 0.5546 | 0.06949 0.6540 | 1.00000 0.0000 | -0.12479 0.4196 | -0.14306 0.3542 |
| SPT | -0.06913 0.6557 | 0.02116 0.8916 | 0.03159 0.8387 | 0.03756 0.8087 | 0.06860 0.6581 | -0.12479 0.4196 | 1.00000 0.0000 | 0.22268 0.1463 |
| SCLR | -0.33617 0.0257 | -0.39683 0.0077 | -0.28973 0.0564 | -0.36751 0.0141 | -0.29702 0.0502 | -0.14306 0.3542 | 0.22268 0.1463 | 1.00000 0.0000 |
| PSILT | 0.64175 0.0001 | 0.73253 0.0001 | 0.60961 0.0001 | 0.76901 0.0001 | 0.56668 0.0001 | -0.06069 0.6955 | -0.09346 0.5462 | -0.58639 0.0001 |
| PCLAY | 0.54891 0.0001 | 0.60762 0.0001 | 0.52288 0.0003 | 0.53759 0.0002 | 0.26674 0.0801 | -0.01927 0.9012 | 0.15329 0.3205 | -0.46372 0.0015 |
| PCB | 0.64759 0.0001 | 0.77069 0.0001 | 0.81156 0.0001 | 0.46420 0.0015 | 0.46444 0.0015 | 0.01888 0.9032 | 0.01091 0.9440 | -0.07291 0.6381 |
| PCOP | 0.41746 0.0048 | 0.41934 0.0046 | 0.29039 0.0558 | 0.60153 0.0001 | 0.49069 0.0007 | 0.13675 0.3761 | -0.03099 0.8417 | -0.60732 0.0001 |
| TCHTOC | 0.05269 0.7341 | 0.14474 0.3486 | -0.02519 0.8711 | 0.07979 0.6066 | -0.01104 0.9433 | 0.23497 0.1247 | 0.03370 0.8281 | -0.52686 0.0002 |

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | PSILT | PCLAY | PCB | PCUP | TCHTOC |
|------|--------------------|--------------------|--------------------|--------------------|--------------------|
| DIST | -0.35970 0.0165 | -0.06096 0.6942 | -0.38961 0.0089 | -0.37989 0.0110 | -0.42216 0.0043 |
| BNT | 0.08287 0.5928 | 0.41952 0.0046 | 0.21054 0.1701 | 0.16749 0.2772 | -0.15452 0.3166 |
| PCUL | 0.26240 0.0853 | 0.18986 0.2171 | 0.19424 0.2064 | 0.23756 0.1205 | 0.05957 0.7009 |
| TA | 0.80843 0.0001 | 0.60000 0.0001 | 0.47017 0.0013 | 0.44038 0.0028 | 0.14425 0.3502 |
| PB | 0.87380 0.0001 | 0.51582 0.0003 | 0.60333 0.0001 | 0.58006 0.0001 | 0.04309 0.7812 |
| HGB | 0.87677 0.0001 | 0.53377 0.0002 | 0.39531 0.0079 | 0.55983 0.0001 | 0.07871 0.6115 |
| CU | 0.69560 0.0001 | 0.39658 0.0077 | 0.69716 0.0001 | 0.54198 0.0001 | -0.05912 0.7030 |
| CR | 0.64341 0.0001 | 0.41432 0.0052 | 0.25509 0.0947 | 0.41612 0.0050 | 0.25341 0.0970 |
| CDM | 0.64175 0.0001 | 0.54891 0.0001 | 0.64759 0.0001 | 0.41746 0.0048 | 0.05269 0.7341 |
| ZNM | 0.73253 0.0001 | 0.60762 0.0001 | 0.77069 0.0001 | 0.41934 0.0046 | 0.14474 0.3486 |
| AGM | 0.60961 0.0001 | 0.52288 0.0003 | 0.81156 0.0001 | 0.29039 0.0558 | -0.02519 0.8711 |

NEW YORK SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / N = 44

| | PSILT | PCLAY | PCB | PCOP | TCHTOC |
|--------|---------------------|--------------------|--------------------|--------------------|--------------------|
| NIPM | 0.76901 0.00001 | 0.53759 0.00002 | 0.46420 0.00015 | 0.60153 0.00001 | 0.07979 0.60666 |
| LFCC | 0.56668 0.00001 | 0.26674 0.0801 | 0.46444 0.0015 | 0.49069 0.0007 | -0.01104 0.9433 |
| TC | -0.06069 0.6955 | -0.01927 0.9012 | 0.01888 0.9032 | 0.13675 0.3761 | 0.23497 0.1247 |
| SPT | -0.09346 0.5462 | 0.15329 0.3205 | 0.01091 0.9440 | -0.03099 0.8417 | 0.03370 0.8281 |
| SCLR | -0.58639 0.00001 | -0.46372 0.0015 | -0.07291 0.6381 | -0.60732 0.0001 | -0.52686 0.0002 |
| PSILT | 1.00000 0.0000 | 0.63527 0.0001 | 0.37242 0.0128 | 0.64637 0.0001 | 0.21127 0.1686 |
| PCLAY | 0.63527 0.00001 | 1.00000 0.0000 | 0.34727 0.0209 | 0.55874 0.0001 | 0.26960 0.0768 |
| PCB | 0.37242 0.0128 | 0.34727 0.0209 | 1.00000 0.0000 | 0.11109 0.4728 | -0.15955 0.3009 |
| PCOP | 0.64637 0.00001 | 0.55874 0.0001 | 0.11109 0.4728 | 1.00000 0.0000 | 0.34064 0.0237 |
| TCHTOC | 0.21127 0.1686 | 0.26960 0.0768 | -0.15955 0.3009 | 0.34064 0.0237 | 1.00000 0.0000 |

Appendix D. Complete correlation matrix for 13 variables (12 surrogate variables and one dependent variable) used in the Philadelphia-Camden dumpsite study.

PHILADELPHIA SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | DIST | BNT | PCUL | TA | PB | CU | CR | ZNM |
|------|----------|----------|----------|----------|----------|----------|---------|----------|
| DIST | 1.00000 | 0.37522 | -0.46800 | -0.03341 | -0.23769 | -0.18418 | 0.03224 | -0.02070 |
| | 0.0000 | 0.0034 | 0.0002 | 0.8888 | 0.2868 | 0.4119 | 0.8927 | 0.9271 |
| | 59 | 59 | 59 | 20 | 22 | 22 | 20 | 22 |
| BNT | 0.37522 | 1.00000 | -0.21865 | 0.10865 | -0.22328 | 0.17498 | 0.18158 | 0.10808 |
| | 0.0034 | 0.0000 | 0.0962 | 0.6484 | 0.3179 | 0.4361 | 0.4436 | 0.6321 |
| | 59 | 59 | 59 | 20 | 22 | 22 | 20 | 22 |
| PCUL | -0.46800 | -0.21865 | 1.00000 | -0.07828 | 0.26409 | 0.19352 | 0.02385 | -0.06022 |
| | 0.0002 | 0.0962 | 0.0000 | 0.7429 | 0.2350 | 0.3882 | 0.9205 | 0.7901 |
| | 59 | 59 | 59 | 20 | 22 | 22 | 20 | 22 |
| TA | -0.03341 | 0.10865 | -0.07828 | 1.00000 | 0.59470 | 0.70370 | 0.91859 | 0.90477 |
| | 0.8888 | 0.6484 | 0.7429 | 0.0000 | 0.0057 | 0.0005 | 0.0001 | 0.0001 |
| | 20 | 20 | 20 | 20 | 20 | 20 | 19 | 20 |
| PB | -0.23769 | -0.22328 | 0.26409 | 0.59470 | 1.00000 | 0.70148 | 0.71210 | 0.69166 |
| | 0.2868 | 0.3179 | 0.2350 | 0.0057 | 0.0000 | 0.0003 | 0.0004 | 0.0004 |
| | 22 | 22 | 22 | 20 | 22 | 22 | 20 | 22 |
| CU | -0.18418 | 0.17498 | 0.19352 | 0.70370 | 0.70148 | 1.00000 | 0.77380 | 0.77007 |
| | 0.4119 | 0.4361 | 0.3882 | 0.0005 | 0.0003 | 0.0000 | 0.0001 | 0.0001 |
| | 22 | 22 | 22 | 20 | 22 | 22 | 20 | 22 |
| CR | 0.03224 | 0.18158 | 0.02385 | 0.91859 | 0.71210 | 0.77380 | 1.00000 | 0.90904 |
| | 0.8927 | 0.4436 | 0.9205 | 0.0001 | 0.0004 | 0.0001 | 0.0000 | 0.0001 |
| | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 20 |

PHILADELPHIA SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | DIST | BNT | PCUL | TA | PB | CU | CR | ZNM |
|-------|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| ZNM | -0.02070 0.9271 22 | 0.10808 0.6321 22 | -0.06022 0.7901 22 | 0.90477 0.0001 20 | 0.69166 0.0004 22 | 0.77007 0.0001 22 | 0.90904 0.0001 20 | 1.00000 0.0000 22 |
| NIPM | -0.12972 0.7595 8 | -0.16059 0.7040 8 | 0.12467 0.7687 8 | 0.92546 0.0028 7 | 0.43586 0.2804 8 | -0.09306 0.8265 8 | 0.52709 0.2241 7 | 0.38279 0.3493 8 |
| LFCC | -0.59062 0.0001 58 | -0.38448 0.0029 58 | 0.31362 0.0165 58 | 0.07294 0.7599 20 | 0.38526 0.0766 22 | 0.37287 0.0874 22 | 0.08180 0.7317 20 | 0.00401 0.9859 22 |
| TC | -0.13103 0.7571 8 | 0.19232 0.6482 8 | -0.13135 0.7565 8 | 0.65782 0.5430 3 | 0.95215 0.1977 3 | 0.83947 0.3657 3 | 0.93071 0.2384 3 | 0.98628 0.1056 3 |
| SPT | -0.17541 0.6778 8 | -0.28258 0.4977 8 | 0.40656 0.3175 8 | 0.00000 1.0000 3 | 0.00000 1.0000 3 | 0.00000 1.0000 3 | 0.00000 1.0000 3 | 0.00000 1.0000 3 |
| PSILT | 0.27804 0.2799 17 | 0.30828 0.2286 17 | 0.00575 0.9825 17 | 0.23744 0.3941 15 | 0.31989 0.2107 17 | 0.38689 0.1250 17 | 0.33562 0.2213 15 | 0.26061 0.3124 17 |
| | NIPM | LFCC | TC | SPT | PSILT | | | |
| DIST | -0.12972 0.7595 8 | -0.59062 0.0001 58 | -0.13103 0.7571 8 | -0.17541 0.6778 8 | 0.27804 0.2799 17 | | | |

PHILADELPHIA SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | NIPM | LFCC | TC | SPT | PSILT |
|------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| BNT | -0.16059 0.7040 8 | -0.38448 0.0029 58 | 0.19232 0.6482 8 | -0.28258 0.4977 8 | 0.30828 0.2286 17 |
| PCUL | 0.12467 0.7687 8 | 0.31362 0.0165 58 | -0.13135 0.7565 8 | 0.40656 0.3175 8 | 0.00575 0.9825 17 |
| TA | 0.92546 0.0028 7 | 0.07294 0.7599 20 | 0.65782 0.5430 3 | 0.00000 1.0000 3 | 0.23744 0.3941 15 |
| PB | 0.43586 0.2804 8 | 0.38526 0.0766 22 | 0.95215 0.1977 3 | 0.00000 1.0000 3 | 0.31989 0.2107 17 |
| CU | -0.09306 0.8265 8 | 0.37287 0.0874 22 | 0.83947 0.3657 3 | 0.00000 1.0000 3 | 0.38689 0.1250 17 |
| CR | 0.52709 0.2241 7 | 0.08180 0.7317 20 | 0.93071 0.2384 3 | 0.00000 1.0000 3 | 0.33562 0.2213 15 |
| ZNM | 0.38279 0.3493 8 | 0.00401 0.9859 22 | 0.98628 0.1056 3 | 0.00000 1.0000 3 | 0.26061 0.3124 17 |

PHILADELPHIA SITE - DESCRIPTIVE STATISTICS

CORRELATION COEFFICIENTS / PROB > IRI UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

| | NIPM | LFCC | TC | SPT | PSILT |
|-------|--------------------|--------------------|--------------------|--------------------|--------------------|
| NIPM | 1.00000 0.00000 | 0.11956 0.7780 | 0 0 | -0.52165 0.3673 | 5 |
| LFCC | 0.11956 0.7780 | 1.00000 0.00000 | -0.12623 0.7658 | 0.35839 0.3833 | -0.10753 0.6812 |
| TC | 0 0 | 0.11956 0.7780 | 1.00000 0.00000 | 0.16783 0.6912 | 0.98286 0.1180 |
| SPT | 0 0 | 0.35839 0.3833 | 0.16783 0.6912 | 1.00000 0.00000 | 0.00000 1.00000 |
| PSILT | -0.52165 0.3673 | -0.10753 0.6812 | 0.98286 0.1180 | 1.00000 1.00000 | 1.00000 0.00000 |
| | 8 8 | 8 58 | 8 8 | 8 8 | 3 17 |

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VITA

Carl Robert Berman, Jr.

Born in Ocala, Florida, 6 March 1942. Earned Bachelor of Arts degree, Experimental Psychology, Tulane University of Louisiana, August 1964. Served as a Regular Naval Officer, August 1964 to August 1969. Earned Master of Science degree, Biological Oceanography, Old Dominion University, Norfolk, Virginia, May 1977. Presently serving as Executive Officer, Division of Environmental Assessment, NOAA, Sandy Hook Laboratory, Highlands, New Jersey, with the rank of Commander, NOAA Commissioned Corps.

The author is married and has one daughter.

Carl R. Berman, Jr.
School of Marine Science
College of William and Mary