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BACTERIAL WATER QUALITY OF THE SOUTHERN NEARSHORE ZONE OF LAKE ERIE IN 1978 AND 1979

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BACTERIAL WATER QUALITY OF THE SOUTHERN NEARSHORE ZONE OF LAKE ERIE IN 1978 AND 1979

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PROJECT OBJECTIVES

The microbiological portion of the 1978-1979 Nearshore Studies of the Lake Erie Central Basin involved the enumeration of aerobic heterotrophs, fecal coliforms, fecal streptococci, and Pseudomonas aeruginosa (1978 only). The purpose of the study was to assess present bacteriological water quality and to provide baseline data for future studies.

INTRODUCTION

For the southern nearshore zone of the Lake Erie Central Basin, bacteriological data for aerobic heterotrophs, fecal coliforms, fecal streptococci and Pseudomonas aeruginosa (1978 only) were collected during four cruises each year -- in May, June, September and October in 1978; and in April, July, August, and October in 1979. Each cruise was intended to provide data regarding a specific aspect of the yearly changes occurring in the lake as follows: high flow from the tributaries in the spring; low summer productivity (and the associated low levels of biomass); the probable period for anoxia in the Central Basin during the summer; and the extent of recovery and nutrient regeneration from the sediments in the fall.

Determination of the aerobic heterotroph populations in the water column has been considered useful in the monitoring and surveillance of water quality; and in general, has been used as an indicator of pollution (organic and inorganic) and eutrophication (Rao and Jurkovic 1977, Bowden 1979). In this study, the aerobic heterotroph data was also used to describe the trophic status of Lake Erie's Central Basin, based on criteria employed with the aerobic heterotroph data obtained in the 1976-1977 study of Lake Michigan (Bowden 1979).

The purpose of determining fecal coliform concentrations is to detect the presence of fecal pollution, which could also contain Salmonella, Shigella, or other waterborne pathogens which are present in the fecal material of infected individuals. Geldreich (1970) conducted a study designed to relate concentrations of fecal coliforms with that of Salmonella. Although he was unable to formulate specific relationships between the two, Geldreich's results served to underscore the existence of health hazards in water degraded by fecal contamination.

Fecal streptococcus concentrations were used along with the corresponding fecal coliform data to determine possible sources of fecal pollution using the fecal coliform/fecal streptococcus ratio (FC/FS) employed by Geldreich and his colleagues (Geldreich 1966, Geldreich, et al. 1968, 1969). A ratio of less than 0.7 implies contamination from domestic animals, whereas a ratio greater than 4.0 suggests a human source.

Hoadley (1968) indicated the significance of Pseudomonas aeruginosa as a pathogen of man and animals, a spoilage organism and a slime former. As a human pathogen, Ps. aeruginosa is responsible for fatal septicemias in infants

and adult patients debilitated by burns, malignancies, or old age. In addition, it has been implicated as a possible cause of the high incidence of otitis externa (outer ear infections) during the swimming season (Levin and Cabelli 1972). Sewage represents the major source of Pseudomonas aeruginosa, and for this reason its isolation from surface waters suggests the influence of man.

SUMMARY AND CONCLUSIONS

The following is a summary of the conclusions reached in this study of the bacteria of the Central Basin of Lake Erie:

1. Using the aerobic heterotroph data, the trophic status of the Central Basin can be classified as mostly mesotrophic with eutrophic tendencies near shore, especially in the harbor and river mouth areas.

2. Application of a two-tailed t-test to the aerobic heterotroph split and replicate data shows that given the methods used in this study, it is not possible to measure small scale differences, on the order of meters horizontally, in the bacterial concentrations in the water column.

3. Correlations significant at $p \leq 0.001$ exist between the aerobic heterotroph data and each of the following chemical parameters: ammonia, TOC, DOC, silicate and sulfate. These correlations indicate the common source of the chemical parameters and the aerobic heterotrophs, i.e. the tributaries of the Central Basin.

MATERIALS AND METHODS

SAMPLE COLLECTION

The eighty-nine sampling stations in the Central Basin (Figure 1) were divided into five areas of approximately twenty stations, with each area being sampled on three consecutive days every cruise. The microbiological samples were collected in evacuated standard 300ml BOD bottles which had been autoclaved for thirty minutes (Standard Methods 1975). A JZ-Bacteriological Water Sampler was used. In the 1978 study, sodium thiosulfate solution and/or EDTA were added before evacuation to some sample bottles (100 mg/l and 372 mg/l of sample, respectively) to remove chlorine and heavy metals, respectively, from samples suspected to contain high levels of these substances. However, the levels of chlorine and heavy metals at the sampling locations were found in 1978 to be low enough to allow the elimination of this part of the procedure in the 1979 study. The JZ-Bacteriological Sampler permits the aseptic collection of water samples within the water column, and the use of messengers allows the placement of several samplers on a cable for simultaneous collection at various depths. Upon removal from the sampler frame, the samples were put in an ice water bath until processed. All samples were processed within the eight-hour maximum permissible time, but most

samples were processed within an hour after collection.

Aerobic heterotrophs were sampled at each station on three consecutive days during each cruise, yielding a total of twelve samples per station per year. Fecal coliforms, fecal streptococci and Ps. aeruginosa were sampled on one day out of three for a total of four samples per station per year. These three groups were also sampled whenever half an inch or more of rain had fallen in the previous twenty-four hours, in order to observe the effects of runoff and sewage treatment plant bypassing on fecal coliform, fecal streptococcus and Pseudomonas aeruginosa counts.

MEDIA AND DILUTION WATER PREPARATION

The media preparation was carried out as detailed in Standard Methods (1975) and presented in Table 1. Phosphate buffered dilution water was also prepared as per the instructions in Standard Methods (1975), but with the exclusion of magnesium sulfate in 1979. Properly diluted buffer solution was dispensed into nine liter serum bottles (for use as rinse water) and autoclaved for 90 minutes; ninety -nine ml dilution bottles were also filled and autoclaved for thirty minutes for use as dilution blanks. All autoclaving was carried out as specified in Standard Methods (1975).

SAMPLE PROCESSING AND COUNTING

All samples were filtered on a manifold with Hydrosol filtration units. Before each day of sampling began, the Hydrosol funnels were wrapped in foil and autoclaved for thirty minutes.

The samples were processed as detailed in Standard Methods, using Millipore HA filters for all four bacterial groups in 1978 (Millipore HC filters were used for the fecal colliform samples in 1979). Four or five sample volumes, differing by a factor of ten, were filtered for aerobic heterotrophs; and one to six volumes for fecal coliforms, fecal streptococci and Ps. aeruginosa, depending on the expected water conditions at each sampling location.

Samples from harbor and river mouths with high degrees of turbidity usually yield higher bacterial concentrations, thus requiring more dilution of the samples to obtain accurate counts. The sample volumes filtered ranged from 0.01ul to 100ml and were transferred with 1ml or 10ml serological blow-out pipets, or with 100ml TC graduated cylinders, depending on the volume to be transferred.

After filtration, the filters were incubated on the appropriate media at the temperatures specified for each bacterial type in Standard Methods (1975). The aerobic heterotroph plates were incubated in a growth chamber in the 6-quart food keepers used to store the uninoculated plates, with the addition of several wet paper towels to supply the recommended high humidity. A circulating water bath was used to incubate the fecal coliform plates which were placed in water-tight Whirl-Pak bags before submersion, and dry heat

incubators were used for the fecal streptococcus and Ps. aeruginosa plates.

Plates were selected for enumeration according to Standard Methods (1975). Counting was accomplished at 15X using a Swift Stereo Ninety microscope, a fluorescent illuminator, and a hand tally. When counting aerobic heterotroph colonies, the fluorescent illuminator was placed at a low angle so that the smaller colonies were made more visible by shadow casting. The fecal coliform, fecal streptococcus, and Ps. aeruginosa plates were counted with the illumination nearly perpendicular to the plate. After counting was completed, the bacterial concentration at each sampling location was calculated by converting the plate counts to standard recording units -- bacteria/ml for aerobic heterotrophs and bacteria/100ml for the other three groups.

aerobic heterotrophs:

bacteria/ml = (colony plate count)/(ml sampled)

fecal coliform, fecal streptococcus and Ps. aeruginosa:

bacteria/100ml = (colony plate count/ml sampled) x 100.

The sample processing and counting procedures used in the 1979 study were basically the same as those detailed above for the 1978 study, with some significant exceptions. First of all, the manifold containing the Hydrosols was modified to allow the removal for sterilization of both the funnel and filter receptacle sections of each Hydrosol (instead of just the funnel section); also, two more spaces for Hydrosols were constructed, allowing the simultaneous use of 11 Hydrosols. Millipore HA filters were used for the aerobic heterotroph and fecal streptococcus samples, but Millipore HC filters were used for the fecal coliform samples. The other major difference associated with the sample processing involved the pipetting of sample volumes. For the 1979 study, Eppendorf 1000ul (#22 35 080-3) and 100ul (#22 35 050-1) micro pipets with disposable sterile tips (Brinkmann Instruments, Inc., Westbury, N.Y.) replaced the Corning 1.0ml pipets used in the 1978 study.

QUALITY CONTROL

The quality control procedures utilized in the 1978 and 1979 studies differed from each other in several respects. Both studies included verification testing for the fecal bacteria but the rest of the quality control for 1978 focused on colony-counting accuracy; whereas that of the 1979 study focused on membrane filter contamination. To obtain a measure of reproducibility in the 1978 study, the same analyst recounted arbitrarily selected plates. Calculations were then made to determine if the counts were within 5%. Plates were also randomly chosen and counted by two different analysts and checked for agreement within 10%. The percent variation of these colony counts was calculated using the following relationship:

% variation = (count #2 - count #1 / arithmetic mean) x 100.

Before sampling began each day on the 1979 cruises, an uncovered plate of Plate Count Agar was set out in the microbiology laboratory for fifteen minutes as a means of determining the ambient air quality. At the end of the time period, the plate was placed in the 35 C incubator for 48 hours before counting.

The sterility of the rinse water and Hydrosols was tested by analyzing rinse water samples four times each day--before the first and after the last samples each day, and twice during the day. The sterility tests were accomplished by rinsing each Hydrosol twice with sterile rinse water, and then plating the filters.

Throughout the four cruises in 1979, procedural modifications were made in an effort to obtain the most accurate means of sterility testing. The incubation temperature for the sterility plates was changed from 35 C to 20 C for the Hydrosols used to filter the aerobic heterotrophs and to 44.5 C for those used for the fecal bacteria, in order to correspond to the temperatures used for the actual samples. The same reasoning was employed in the decision to change the plating medium from Plate Count Agar to M-FC agar for the Hydrosols used to filter the fecal bacteria samples.

In an effort to rid the Hydrosols of residual bacteria from samples with high bacterial concentrations a Millipore Ultraviolet Sterilizer was used to irradiate the Hydrosols used for aerobic heterotroph samples. Each Hydrosol was irradiated for three minutes after approximately every four sample bottles. The Hydrosols used for fecal coliform and fecal streptococcus samples were not subjected to irradiation in order to save some time in the processing procedure. The sterility test results (see Appendix) confirmed the lack of carryover within these groups. To help keep sample water (and bacteria) from adhering to the sides of the Hydrosols, the funnels were sprayed with silicone spray and polished before the start of each cruise.

Another aspect of quality control involved the use of split and replicate samples as a means of indicating the precision of the sampling and processing methods. Before the start of each cruise, two stations from each sampling area were designated as replicate stations. These stations remained the same for each of the three successive days of an area, but they were changed for each cruise. For Cruises 1 and 2 in the 1978 study, all sampling depths of each replicate station were replicated; however, only one depth was replicated during Cruises 3 and 4 in 1978 and all cruises in 1979.

Split samples were obtained by processing three identical sets of identical dilution series from a single sample. During the 1978 cruises, the microbiological samples which were split were different from those that were replicated, but in the 1979 study, each replicate sample was also split three ways. Figure 2 shows the relationships between splits and replicates for both years of the study. Due to the relatively small volume of water collected by the JZ-Bacteriological Sampler, only aerobic heterotroph samples were replicated and split; fecal coliform, fecal streptococcus, and Ps. aeruginosa samples were only replicated.

VERIFICATION TESTING

Ten percent of the samples from Cruises 1-4 in 1978 and Cruise 1 in 1979 were verified for fecal coliform and fecal streptococcus bacteria, and five percent from Cruises 2-4 in 1979. Since the Ps. aeruginosa samples were collected only at industrial stations, most of the colonies were verified.

Several criteria were involved in the selection of stations for verification. First of all, whenever possible the stations to be verified were selected from known areas of pollution, such as river mouths, harbor areas, and sewage outfalls. Also, an attempt was made to verify the same stations for both fecal coliforms and fecal streptococci, so that a FC/FS ratio could be calculated and an estimate of the pollution source made.

During each cruise, colonies from the stations selected for verification were transferred from the membrane filters, after counting, to Nutrient Agar slants and allowed to grow at ambient temperature for several days, before refrigeration until the verification testing was performed. The verification procedures are detailed in Standard Methods (1975) for fecal coliform, fecal streptococcus, and Ps. aeruginosa bacteria. Percent verification was calculated as follows:

$$\% \text{ verification} = (\#\text{positive colonies}/\#\text{colonies verified}) \times 100.$$

The results of the verification testing were used to correct the bacterial concentrations only for verified samples.

TREATMENT OF DATA

Splits and replicates

The aerobic heterotroph split and replicate data were divided into three groups based on the average number of bacteria present at each station:

1. stations with $\leq$ 100 bacteria/ml
2. stations with between 100 and 1000 bacteria/ml and
3. stations with $\geq$ 1000 bacteria/ml.

The absolute value of the difference between the two replicates for each replicated station was calculated, and in the same way, the differences between the three pairs of split data were calculated. A two-tailed t-test was then used to determine whether the mean differences between the replicates in each group were equal to the mean differences between splits. The results of the t-test showed if the differences in bacterial concentration in the water column (measured by the replicates) were large enough to be detected given the error in the method (measured by split differences).

Comparison of 1979 Cleveland stations 81, 83 and 89 with stations 80 and 88

In 1978, results from some of the chemistry data suggested that the area off the western end of the Cleveland breakwall might be the site of an outfall of some sort (Richards 1980). Further investigation uncovered the existence of a sewage outfall in the vicinity -- from Cleveland's Westerly Wastewater Treatment Plant -- which led to the relocation of stations 81, 83 and 89 (Figures 1a and 1b), in order to sample the area affected by this outfall.

A two-tailed t-test was utilized in comparing these three stations with two reference stations nearby (80 and 88) which were not affected by sewage effluent. The t-test was used on all three bacterial parameters (fecal coliforms, fecal streptococci and aerobic heterotrophs) to determine the similarity of the bacterial concentrations from the two groups of stations.

Pearson Correlations

An SPSS (Statistical Package for the Social Sciences) computer program for generating Pearson correlation coefficients (Nie, et al. 1975) was used with the aerobic heterotroph data to determine any correlations existing between the heterotroph data and any of the chemical parameters.

FC/FS Ratio

At stations where more than 100 fecal streptococci per 100ml were detected, fecal coliform/fecal streptococcus (FC/FS) ratios were calculated to ascertain the source (human vs. nonhuman) of the fecal pollution at these stations. The following criteria were employed in these source estimations: (Geldreich 1966)

1. A ratio of less than or equal to 0.7 indicates a nonhuman source,
2. the source is undetermined for a ratio between 0.7 and 4.0, and
3. a ratio of 4.0 or greater indicates a human source.

Trophic Status Determination

The aerobic heterotroph data was used to determine the trophic status of Lake Erie's Central Basin by classifying each station in the following manner: (Bowden 1979)

	inshore(bacteria/ml)	offshore(bacteria/ml)
eutrophic (E)	$\geq$ 2000	$\geq$ 200
mesotrophic (M)	120 $\leq$ M < 2000	20 $\leq$ M < 200
oligotrophic (O)	$\leq$ 120	$\leq$ 20

Cruise to Cruise Patterns

As a means of graphically demonstrating the cruise to cruise patterns of aerobic heterotroph, fecal coliform and fecal streptococcus data, the stations were divided into three groups based on their location:

1. offshore: stations $\geq$ 3.3km from shore.

2. onshore: stations $\leq$ 3.3km from shore and not at a river or harbor mouth.

3. harbors and river mouths.

Table 2 contains a list of stations falling into each category and the locations of the stations are shown in Figure 3. For each group of stations for each cruise, the mean, range and standard error of the log transformed data for each of the three bacterial groups were determined and plotted. This procedure was followed for both 1978 and 1979.

RESULTS AND DISCUSSION

QUALITY CONTROL

Much of the quality control data is not directly relevant to interpretation of the environmental data. These quality control data are presented in the Appendix. The results of the split and replicate testing is presented in the statistical analysis section of this report.

SPATIAL AND TEMPORAL VARIATIONS

For each cruise, the data for aerobic heterotrophs, fecal coliforms and fecal streptococci were used to construct isopleth maps (Figures 4 to 9) to show the spatial distribution of each bacterial group. For the aerobic heterotrophs, where each cruise included three measurements at each station, geometric means were used. In Figures 10 - 12 the geometric means of all four cruises for 1978 and for 1979 were used to plot isopleths.

The cruise to cruise changes at the nearshore, offshore and river mouth and harbor areas for each bacterial group are shown in Figures 13 - 15. These plots include the geometric means, the ranges, the numbers of samples and the standard errors. For aerobic heterotrophs the highest concentrations were found during the first cruise each year. The second and third cruises had lower heterotroph concentrations with the first cruise each year showing increased concentrations over the third cruise. This pattern was present in all three areas. A similar pattern was present both years for fecal coliform and in 1979 for fecal streptococci. The fecal streptococci did not show this pattern in 1978 when only small concentration differences existed between the cruise.

The four cruises each year were timed to coincide with the temporal variations in the lake. The first and last cruises would be expected to produce high bacterial concentrations due to the spring runoff and fall nutrient regeneration, respectively. The summer cruises (Cruises 2 and 3) were scheduled to coincide with the periods of low summer productivity and associated low levels of biomass.

Aerobic heterotrophs such as Alcaligenes, Caulobacteria, Chromobacterium, Flavobacterium, Leptospira, Micrococcus, Proteus, Pseudomonas, and others are naturally occurring aquatic bacteria (Scarpino 1971) that are important in aquatic food chains. These bacteria degrade dead algae and organic detritus with the production of carbon dioxide and inorganic nutrients such as ammonia and phosphate. A positive correlation is typically found between the average rates of phytoplankton productivity (i.e. biomass production) and bacterial numbers and production (Wetzel 1975). Menon and his colleagues (1972) found in their studies of Lake Erie phytoplankton and bacteria that the phytoplankton cycle in the Central Basin is of a bimodal structure, with peaks in early May and late October. The heterotrophic bacterial cycle has peaks near the ends of the plankton blooms. In this study aerobic heterotroph concentrations were highest in the spring, with another smaller increase in the fall (Figure 13).

Although fecal bacteria are not endemic to the lake, their natural habitat being primarily the intestinal tract of warm-blooded animals including man, the temporal patterns of the fecal coliforms and fecal streptococci show bimodal peaks similar to those of the aerobic heterotrophs (Figures 14 and 15). High concentrations of fecal coliforms and fecal streptococci are expected during the spring runoff and during storm events. During periods of high flow, many municipal treatment plants must bypass some of their combined sanitary-stormsewer water, thus dumping untreated sewage into the receiving waters. In the summer, flows are lower and the treatment plants can effectively treat their sewage before release; also, for more remote treatment plants, past treatment discharge must travel much farther. Both of these conditions serve to substantially reduce the amount of fecal pollution in the water discharged into the receiving stream or lake.

It is evident from Figures 4 through 15 that the aerobic heterotroph, fecal coliform and fecal streptococcus bacteria are spatially and temporally variable. As expected, the spatial distributions of the fecal coliforms and fecal streptococci are very similar, but slightly different from those of the aerobic heterotrophs, due to their differences in origin. Fecal coliforms and fecal streptococci originate in sewage; whereas aerobic heterotrophs are endemic to water and soil (Scarpino 1971, ReVelle and ReVelle 1974). The stations with the greatest concentrations for all three groups coincide with the areas nearest the shoreline, especially near the river mouths. Municipal discharges contribute dissolved and suspended solids, oxygen-consuming organic matter, oils, toxic substances, bacteria and nutrients to the tributaries and to the lake in general. These substances serve as substrates for heterotrophic bacterial degradation and encourage the growth of these bacteria in discharge areas. A great number of heterotrophs are also brought into the lake via agricultural runoff carried into the lake by the tributaries (IJC 1971).

The spatial distributions of fecal coliforms and fecal streptococci are almost identical to each other, with the concentrations of fecal streptococci usually being less than those of the fecal coliforms by about a factor of ten. The mouths of the Rocky and Cuyahoga Rivers were the sites of the highest concentrations (100 to 1000 bacteria/100ml) of the fecal coliform and fecal streptococcus bacteria; and fecal coliforms were found in the 10 to 100 bacteria/100ml range at the mouths of Euclid Creek and the Chagrin and Ashtabula Rivers, as well as at station 94, which is the discharge site of a pipe of unknown contents below a high-rise apartment complex east of the Cleveland breakwall. Other areas of high concentrations of fecal coliforms and fecal streptococci (10 to 100 bacteria/100ml) were usually found inside the Cleveland breakwall and at the mouths of the Vermilion, Black and Grand Rivers; and sometimes in the vicinity of Arcola, Wheeler and Cowles Creeks.

HISTORICAL TRENDS FOR AEROBIC HETEROTROPHS

Prior to this study, little work had been done with aerobic heterotrophs in Lake Erie with the exception of two studies by Rao and Burnison (1976) in 1967 and 1970. In their work Rao and Burnison used a standard pour plate count incubated at 20 C (Menon, et al. 1967) and a membrane filtration count, also incubated at 20 C, (Van Otterloo, et al. 1968) for the enumeration of aerobic heterotrophs. Because the methods used by Rao and Burnison are different from those used in this study, direct comparisons (i.e. numerical comparisons) with the data collected in this study were not possible. However, it is possible to compare the aerobic heterotroph distribution patterns in general.

Rao and Burnison found a decline in heterotrophs in the offshore regions of the Central Basin from June to October, 1967, and from May to November, 1970. The results from this study show a similar decline from May to August, 1978, and from April to August, 1979 but then an increase from August to October for both years (Figure 13). The difference in the fall concentrations may be due to the difference in station locations between the two studies. The area sampled by Rao and Burnison (1976) was located out in the open lake, whereas the sampling stations in this study were within five miles of shore. The studies from all four years (1967, 1968, 1978, 1979) found consistently high aerobic heterotroph densities in the Central Basin inshore areas, especially in the vicinity of Cleveland.

TROPHIC STATUS

Figures 16 and 17 summarize the trophic status for 1978 and 1979, respectively, using data on aerobic heterotrophs. The Central Basin is mesotrophic offshore with definite eutrophic tendencies near shore. The sets of individual cruise maps from the two years (Figures 18 and 19) support this assessment and serve to show the areas of consistent eutrophy in the areas of the river mouths. The first cruise data from 1979 gives a much more eutrophic picture due to the spring runoff with its resulting high concentrations for

all three of the bacterial groups sampled.

The division of the stations into two groups by their distance from shore and the application of different trophic criteria to these groups resulted in some of the borderline stations being inconsistently classified in relation to nearby stations. For example, station 77 (Cruise 3, 1979, Figure 19c) is classified as oligotrophic (< 3.3km from shore and $\leq$ 120 bacteria/ml). However, the other nearby stations 72, 73 and 76 are mesotrophic due to the fact that they are greater than 3.3km from shore and have concentrations of 20 to 200 bacteria/ml. Similar inconsistencies existed for stations 77, 124 and 130 (Cruise 2, 1978); 100 and 112 (Cruise 2, 1979); and station 112 (Cruise 4, 1979). Aside from the above-mentioned distortion, the Central Basin nearshore region is mesotrophic with eutrophic areas in the vicinity of the tributary mouths and/or harbors. This assessment of the Central Basin trophic status agrees with the overall mesotrophic-eutrophic assessment arrived at by the International Lake Erie Water Pollution Board (1969) using their categories of morphometry, transparency, nutrient concentrations, nutrient loading, oxygen present in the hypolimnion, phytoplankton, zooplankton, bottom fauna and fish production.

STATISTICAL ANALYSES

Splits and Replicates

Most of the statistical tests commonly used with bacterial data assume a normal distribution. However, raw bacterial data is often not normally distributed and must be transformed (Kaper, et al. 1978; Ashby and Rhodes-Roberts 1976; Palmer, et al. 1976; Pipes, et al. 1977). In this study, a log transformation was used for the aerobic heterotroph, fecal coliform and fecal streptococcus data. In addition, 1 was added to all of the fecal coliform and fecal streptococcus data prior to transformation in order to eliminate zero values.

Two-tailed t-tests were used to compare differences between splits with differences between replicates in the aerobic heterotroph data set to determine if small-scale differences in bacterial concentration in the water column (measured by replicate differences) were large enough to be detected, given the error in the method (measured by split differences). The results of the t-tests are shown in Tables 3 and 4 for all three levels of bacterial concentration in 1978 (Table 3), the means of the differences between the replicates are significantly greater than the corresponding means of the differences of the splits, implying that small-scale variations in the water column can be detected. Taking into account the increased accuracy of the microbiological methods employed in 1979, resulting from the refinement of processing methods in general (such as the substitution of Eppendorf 0.1ml and 0.01ml automatic pipets for the Corning pipets), it would be expected that the results of the t-tests would show an even larger difference between the differences of the splits and the differences of the replicates.

That this is not so (Table 4) is probably due in part to the fact that the general sampling techniques were also improved during the 1979 cruises. Improved accuracy in taking the replicate sample, shown by the much lower means of the differences of the replicates in 1979 as compared to 1978, more than balanced the corresponding improvement in the microbiological methods, as measured by the differences of the splits. This combination of factors is responsible for the lower values obtained with the 1979 data. In effect, the 1979 sampling program assessed smaller scale differences than the 1978 program. Samples were drawn in 1979 from a water mass small enough to be considered homogeneous in the statistical sense.

Pearson Correlations

Theoretically there should be some degree of correlation between the aerobic heterotroph data and some chemical data parameters, especially forms of nitrogen and phosphorous (i.e. ammonia, nitrate-nitrite, TKN, TP, TSP, SRP). Tables 5 and 6 indicate the degree of correlation between the 1978 and 1979 aerobic heterotroph data and the corresponding chemical data for the following parameters: pH, conductivity, alkalinity, turbidity, suspended solids, chlorophyll, pheophytin, TSP, TP, SRP, TKN, ammonia, nitrate-nitrite, silicate, chloride, sulfate, cyanide, TOC and DOC. The same calculations were made using the Cruise 1 data from 1979 (Table 7), in an effort to obtain better correlation coefficients by removing an extraneous source of variability (seasonal effects).

The highest correlation coefficients for 1978 data (Table 5) were for nitrate-nitrite, r=0.1752; DOC, r=0.1271; conductivity, r=0.1157; TP, r=0.1130; TKN, r=0.1098; pheophytin, and ammonia, r=0.1010. However, these coefficients are so small that, even though the significance levels (p) are satisfactory, very little correlation exists. The results with the 1979 heterotroph data were somewhat more meaningful, with the highest coefficients being: TOC, r=0.4198; ammonia, r=0.4019; sulfate, r=0.3247; silicate, r=0.3210; TKN, r=0.2888; and chloride, r=0.2818 (Table 6).

Table 7 shows the correlation data for the first cruise in 1979 and the results were by far the highest of the three data sets (1978, 1979 and Cruise 1, 1979), with the highest r values for cyanide, r=0.8034; ammonia, r=0.7480; TOC, r=0.6241; silicate, r=0.5382; sulfate, r=0.5276; and DOC, r=0.3532. To determine the extent of correlation, computer-drawn scatter plots were generated for these parameters (Figures 20 through 25). From the scatter plot for cyanides, in Figure 20, it is apparent that there is no real correlation between cyanide concentrations and aerobic heterotrophs. The scatter plot shows two outlier points which caused the high r value for cyanide. This result is as expected due to the fact that cyanide is generally considered to be detrimental to living organisms. The scatter plots for the other parameters mentioned above (Figures 20 through 25) show that some correlation exists. All of these parameters (ammonia, TOC, silicate, sulfate and DOC) enter the lake via the tributaries, as do a large number of heterotrophs. Thus it appears that the correlations between the aerobic heterotroph data and the data for these chemical parameters are primarily the result of their common sources.

FECAL COLIFORM/FECAL STREPTOCOCCUS RATIO (FC/FS)

When sufficient concentrations of fecal streptococci ($\geq$ 100/100ml) are present, the fecal coliform/fecal streptococcus ratio (FC/FS) can be used to estimate the pollution source. The FC/FS ratio is only an estimate and there are several precautions which should be taken into consideration when using it (Bordner, et al. 1978): 1) Samples should be taken as close as possible to the pollution source in order to minimize the effects of fecal bacteria's low survival rate outside the intestinal tract, and 2) mixed pollution sources are very difficult to analyze via the FC/FS ratio.

In this study, the samples were seldom if ever taken close to a pollution source, and as a result most of them represent mixed pollution sources, which are very difficult to analyze. In spite of these problems, the FC/FS ratio was used to give a general idea of the sources of the fecal pollution found at the stations for which FC/FS ratios could be calculated. Figures 26a and 27a show the locations of the samples in 1978 and 1979 with 100 or more fecal streptococci/100ml, and Tables 8 and 9 give the FC/FS ratio for each of these samples. In 1978 these stations occurred in seven different groups around the mouths of each of the major tributaries (see Figure 26a). When the ratios were calculated, 49% of the samples had a FC/FS ratio $\leq$0.7, implying a nonhuman origin; 33% yielded ratios between 0.7 and 4.0, and were therefore of undetermined origin; and 18% of the samples gave a ratio $\geq$4.0 indicating a probable human pollution source.

In comparison, 53% of the 1979 samples gave ratios $\geq$4.0 and 16% gave ratios $\leq$0.7. However, the percent of samples between 4.0 and 0.7 was very similar for the two years (33% in 1978 and 31% in 1979) (Tables 8 and 9). The 1979 samples with more than 100 fecal streptococci/100ml were primarily from the Cleveland area, especially in the vicinity of the mouth of the Cuyahoga River. Most of the other stations were located at the other tributary mouths (see Figure 27a), and a few were located farther from shore. The much larger percentage of samples with FC/FS ratios of over 4.0 in 1979 was almost entirely due to samples taken on the first cruise (see Table 8), during which all of the bacterial counts were higher than for the other 1979 cruises, due to the spring runoff.

Of the stations with usable ratios ($\leq$0.7 $\geq$4.0), twenty-three have ratios greater than 4.0 (Figures 26b-d and 27b-d), which implies fecal contamination of human origin; and forty-two have ratios less than 0.7, implying nonhuman sources. The rationale for each sampling location (Herdendorf 1978), as given in Table 10 was used to determine possible sources for the fecal pollution found at these stations. A number of stations with ratios $\geq$4.0 (Figure 26d and 27d) are located in the vicinity of discharges from industrial or sewage outfalls, where one would expect to find high FC/FS values. Also, some of the stations are designated as problem pollution areas (Table 10); therefore, a great deal of pollution in general would be expected. Other stations with high FC/FS ratios were located in close proximity to tributary mouths.

Most of the stations with ratios of less than 0.7 (Figures 26b and 27b) are in the vicinity of river or creek mouths, thus these samples probably contain organisms from a variety of locations within the tributaries themselves, as well as from the lake. This results in a great deal of mixing, which makes it difficult to determine the source of the pollution. Another problem involved in the use of the FC/FS ratio is the relative die-off rates of fecal coliforms and fecal streptococci. McFetter and his colleagues (1974) have shown that fecal coliform bacteria as a group tend to die off more quickly than fecal streptococci, which will affect the FC/FS ratio over time. More specifically, the survival times decrease in the following manner: enterococci (streptoccal bacteria from the intestinal material of animals) > fecal coliforms > Streptococcus bovis and Streptococcus equines. Feachem (1974) has expanded this relationship and drawn conclusions concerning the change of the FC/FS ratio over time for human and nonhuman sources. When enterococci are the predominant fecal streptococci, as in human fecal material, the FC/FS ratio will tend to fall over time; whereas, in fecal material where S. bovis and S. equines are the dominant fecal streptococci, as in cattle and pig fecal material, the ratio will tend to increase. In general, the farther from shore a sample is, the lower the FC/FS ratio will be. In nearshore areas, ratios between 4.0 and 0.7 may still be of human origin, due to the phenomenon of die-off in fecal bacteria. Despite the rather substantial limitations of the FC/FS ratio in this particular study, it can still be useful in drawing very general conclusions concerning the origins of fecal pollution at the stations sampled.

A COMPARISON OF SUSPECTED SEWAGE EFFLUENT STATIONS 81, 83 AND 89 WITH STATIONS 80 AND 88 IN 1979.

Stations 81, 83 and 89 in the Cleveland area were moved in 1979 to positions in the vicinity of a sewage outfall from Cleveland's Westerly Wastewater Treatment Plant (Figure 1b) in order to monitor the quality of the water affected by the treatment plant discharge. Two other Cleveland stations (80 and 88) were chosen for comparison with the three new stations in order to show statistically that the bacterial concentrations at 81, 83 and 89 were from a different source than those at other stations located approximately the same distance from shore and in the same general area.

Table 11 gives the t-values for comparison of aerobic heterotroph, fecal coliform and fecal streptococcus concentrations at 81, 83 and 89 with those at 80 and 88. The t-values are high with achieved significance levels of < 0.001. These results lend statistical validity to the conclusions which can be drawn from the raw data itself (Figure 10): a 10- to 100-fold difference exists between the geometric means of the aerobic heterotroph data from stations 81, 83 and 89 and those from stations 80 and 88.

POSSIBLE VIOLATIONS OF WATER QUALITY CRITERIA BASED ON FECAL COLIFORM CONCENTRATIONS

The Ohio EPA has set standards for fecal coliform bacteria in Lake Erie of no more than 200 bacteria per 100ml of sample based on at least five samples in a thirty-day period, and not exceeding 400 per 100ml in over 10% of the samples (OAC 3745-1). However, certain nearshore areas have been exempted from this standard, and all of the stations with $\geq$ 200 fecal coliforms per 100ml are located in these exempted areas. For this reason, and because the stations in this study were not sampled at least five times in a thirty-day period, it cannot be stated that any of the stations in Tables 12 and 13 are definitely in violation of water quality standards.

Nevertheless, Tables 12 and 13 and Figure 28 do serve to indicate the areas of major fecal pollution in the Central Basin of Lake Erie. Table 14 give the stations that showed concentrations of fecal coliforms of over 1,000 per 100ml during one or more of the four cruises each year. The increased number of stations with over 1,000 fecal coliforms per 100ml of sample in 1979 is due in part to the timing of the first cruise of 1979. The 1978 sampling season was begun in late May, after most of the spring runoff had already occurred; however, the 1979 season began about a month earlier, in April, when the runoff was taking place. Despite these differences between the two years, Figure 28 shows the same general trouble spots, the most serious of which is the mouth of the Cuyahoga River in Cleveland Harbor.

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Table 1. Media Preparation. Except where noted, all media were prepared as directed on the container.

Bacteria	Year	Medium	Filter	Comments
Aerobic heterotrophs	both	Plate Count Agar	Millipore HA	
Fecal coliforms	1978	M-FC broth	Millipore HA	
	1979	M-FC agar	Millipore HC	No rosolic acid*
Fecal streptococci	both	KF Streptococcus agar	Millipore HA	
Ps. aeruginosa	1978	M-PA agar	Millipore HA	Synthesized as per Standard Methods (1975)

*Rosolic acid was omitted from the M-FC agar in the belief that its presence was not crucial. Also, this omission permitted the autoclaving of the medium, which in turn prolonged the storage life of the poured plates.

Table 2. Organization of the Central Basin stations used to determine the cruise-to-cruise patterns for the three bacterial groups.

Offshore	Onshore	Harbors and River Mouths
LV 52	LV 51	FP 103	LV 54
63	53	104	55
67	56	106	65
	57	108	66
CW 72	58	109	
73	59	110	CW 75
74	60	111	76
78	61	115	
82	62	116	CE 84
	64	118	85
CE 93	68	119	86
97	69	120	87
100		121	90
101	CW 70	122	91
	71		98
FP 107	77	AS 124	
112	79	127	FP 105
	80	128	113
AS 125	88	129	114
131		130	117
135	CE 92	134	
	94	136	AS 123
	95	137	126
	96	138	132
	99	139	133
	102		

Table 3. Statistical analysis of 1978 aerobic heterotroph splits and replicates. The data has been $\log_{10}$ transformed.

	Number of Cases	Mean of Differences	Standard Deviation of Differences	*T-value	Degrees of Freedom	2-Tail Probability
		For stations with $\leq 10^2$ bacteria/ml				
Splits	25	0.1921	0.222	2.82	44.75	0.007
Replicates	34	0.5032	0.588			
		For stations with between 10^2 and 10^3 bacteria/ml				
Splits	39	0.1634	0.219	3.03	97.15	0.003
Replicates	69	0.03925	0.558			
		For stations with ≥ 10 bacterial/ml				
Splits	35	0.1686	0.258	5.52	52.31	<0.001
Replicates	43	0.8863	0.803			

*SPSS separate variance estimate for use when variances are not equal (Nie, et al. 1975).

Table 4. Statistical analyses for 1979 aerobic heterotroph splits and replicates. The data has been $\log_{10}$ transformed.

	Number of Cases	Mean of Differences	Standard Deviation of Differences	*T-value	Degree of Freedom	2-Tail Probability
		For stations with $\leq 10^2$ bacteria/ml				
Splits	59	0.1320	0.223	0.12	14.03	0.907
Replicates	10	0.1397	0.182			
		For stations with between 10^2 and 10^3 bacteria/ml				
Splits	259	0.1233	0.144	1.40	74.53	0.164
Replicates	62	0.1637	0.215			
		For stations with $\geq 10^3$ bacteria/ml				
Splits	194	0.1267	0.149	0.80	42.18	0.428
Replicates	39	0.1645	0.288			

*SPSS separate variance estimate for use when variances are not equal (Nie, et al. 1975).

Table 5. Pearson correlation coefficients for 1978 heterotrophs and selected chemical data.

Chemical Parameter	Coefficient (r)	Cases	*Significance (p)
TKN	.1098	1456	0.001
NH_3	.1010	1390	0.001
NO_2-NO_3	.1752	1444	0.001
SiO_2	.0709	1410	0.008
Cl	.0841	1415	0.002
SO_4	.0908	1417	0.001
TOC	.0290	267	0.637
DOC	.1271	269	0.037
Conductivity	.1157	1462	0.001
Alkalinity	.0979	1438	0.001
Suspended Solids	-0.0046	262	0.941
Chlorophyll	-0.0195	472	0.672
Pheophytin	.1020	471	0.027
TSP	.0341	1461	0.192
TP	.1130	1461	0.001
SRP	.0125	1443	0.635
pH	-0.0530	1462	0.043

*The closer p is to 0, the better the correlation between the 2 parameters being considered.

Table 6. Pearson correlations for 1979 heterotrophs and selected chemical data.

Chemical Parameter	Coefficient (r)	Cases	*Significance (p)
pH	-0.2387	2049	0.001
Conductivity	.1082	2043	0.001
Alkalinity	.1330	2038	0.001
Turbidity	.1376	2009	0.001
Suspended Solids	.2151	383	0.001
Chlorophyll	-0.0041	1389	0.879
Pheophytin	0.0269	1702	0.268
TSP	.2324	2047	0.001
TP	.2573	2053	0.001
SRP	.2471	2051	0.001
TKN	.2888	2049	0.001
NH_3	.4019	2050	0.001
NO_2-NO_3	.2030	2051	0.001
SiO_2	.3210	2049	0.001
Cl	.2818	2050	0.001
SO_4	.3247	2050	0.001
TOC	.4198	396	0.001
DOC	.3028	396	0.001

*The closer p is to O, the better the correlation between the 2 parameters being considered.

Table 7. Pearson correlations for 1979 Cruise 1 heterotrophs and selected chemical data.

Chemical Parameter	Coefficient (r)	Cases	Significance (p)
pH	-0.3529	522	0.001
Conductivity	0.2473	516	0.001
Alkalinity	0.1701	519	0.001
Turbidity	0.0191	505	0.334
Suspended Solids	0.0728	96	0.240
Chlorophyll	-0.0163	163	0.418
Pheophytin	-0.0837	176	0.135
TSP	0.3362	518	0.001
TP	0.2197	522	0.001
SRP	0.3965	521	0.001
TKN	0.2223	520	0.001
NH_3	0.7480	521	0.001
NO_2-NO_3	0.0481	521	0.137
SiO_2	0.5382	520	0.001
Cl	0.5028	520	0.001
SO_4	0.5276	521	0.001
Cyanide	0.8034	25	0.001
TOC	0.6241	96	0.001
DOC	0.3532	96	0.001

Table 8. FC/FS ratios for 1978 samples containing fecal streptococci at concentrations ≥ 100/100 ml.

Station		Level	Date	Fecal Coliforms Per 100 ml	Fecal Streptococci Per 100 ml	FC/FS	≥ 4.0	4.0 > FC/FS > 0.7	≤ 0.7
LV	51	S	780617	54	190	0.28			X
	54	S	780830	44	120	0.37			X
	60	S	780830	2	140	0.014			X
	62	S	781010	72	160	1.1		X	
	64	B	780830	4	440	0.0091			X
	65	S	780830	78	290	0.27			X
	67	S	780520	1	130	0.0077			X
CW	70	S	780620	43	100	0.43			X
	74	B	780620	0	180	0		X	
	75	S	780524	15,000	22,000	0.68			X
		S	780620	500	270	1.8		X	
	77	B	780902	8	120	0.066			X
	78	S	780524	2	210	0.0095			X
	79	S	780524	370	130	2.8		X	
		S	780620	45	130	0.35			X
	80	S	780524	270	190	1.4		X	
		B	780524	190	100	1.9		X	
	81	B	780524	1	150	0.0067			X
	83	S	780524	35	290	0.12			X
		M	780524	6	130	0.046			X
		B	780524	0	160	0		X	
	89	B	780902	3	650	0.0046			X
CE	85	S	780623	2,700	470	5.7	X		
		S	780905	940	100	9.4	X		
		S	781016	5,600	1,000	5.6	X		
		B	780623	1,100	130	8.5	X		
		B	780905	780	130	6.0	X		
		B	781016	7,800	1,100	7.1	X		
	86	S	780527	700	100	7.0	X		
		S	780623	720	100	7.2	X		
		S	781016	940	330	2.8		X	

Table 8 continued.

Station	Level	Date	Fecal Coliforms Per 100 ml	Fecal Streptococci Per 100 ml	FC/FS	≥ 4.0	4.0 > FC/FS > 0.7	≤ 0.7
CE 86	S	780623	150	100	1.5		X	
	B	780905	1,400	120	12	X		
	B	781016	1,300	260	5.0	X		
87	B	780527	250	120	2.1		X	
	S	780527	410	160	2.6		X	
91	B	780527	110	120	0.92		X	
	S	780623	49	180	0.27			X
94	S	780623	3,600	180	20	X		
	S	780905	710	189	3.0		X	
	S	781016	2,800	200	14	X		
95	S	780527	70	310	0.23			X
	S	781016	460	100	4.6	X		
	S	780527	48	120	0.40			X
96	B	780623	20	540	0.037			X
98	S	780905	34	120	0.28			X
	S	781016	1,400	180	7.6	X		
100	S	780527	15	320	0.047			X
	B	780905	0	130	0		X	
102	S	780905	0	220	0		X	
	B	780623	7	150	0.047			X
103	S	780908	173	140	1.2		X	
104	S	780908	195	240	0.81		X	
105	S	780908	130	160	0.81		X	
106	B	780626	200	280	0.71			X
	B	781019	0	540	0		X	
107	S	780908	0	440	0		X	
	B	780908	2	110	0.018			X
108	S	780908	50	480	0.10			X
111	S	780626	3	240	0.013			X
	B	780908	2	180	0.011			X

Table 8 continued.

Station	Level	Date	Fecal Coliforms Per 100 ml	Fecal Streptococci Per 100 ml	FC/FS	≥ 4.0	4.0 > FC/FS > 0.7	≤ 0.7
FP 111	B	781019	13	110	0.12			X
112	B	780908	1	320	0.0031			X
113	S	780626	460	860	0.54			X
	SR	780626	250	360	0.69			X
	B	781019	400	150	2.7		X	
114	B	780530	2	470	0.0043			X
	B	780626	4	130	0.031			X
115	S	780908	1	140	0.0071			X
	B	780908	28	140	0.20			X
116	B	780626	0	130	0		X	
117	S	780908	69	130	0.53			X
118	S	780626	3	310	0.010			X
119	S	780908	14	430	0.033			X
120	S	780626	0	180	0		X	
121	S	780530	0	250	0		X	
AS 126	S	780911	9	150	0.060			X
128	S	780602	160	140	1.1		X	
	S	781022	120	790	0.15			X
131	B	780911	20	100	0.20			X
	B	781022	2	283	0.0071			X
132	S	780602	780	140	5.6	X		
	SR	780602	680	170	4.0	X		
	S	780911	200	130	1.5		X	
	B	780602	300	130	2.3		X	
	B	780911	310	140	2.2		X	
133	S	780911	4	310	0.019			X
134	B	781022	0	130	0		X	
135	B	780911	3	310	0.010			X
138	S	780911	4	170	0.024			X
	B	780911	1	170	0.0059			X

Table 9. FC/FS ratios for 1979 samples containing fecal streptococci at concentrations ≥ 100/100ml

	Station	Level	Date	Fecal Coliforms Per 100 ml	Fecal Streptococci Per 100 ml	FC/FS	≥ 4.0	4.0 > FC/FS > 0.7	≤ 0.7
LV	65	S	790425	960	150	6.4	X		
		B	790425	1,100	170	6.5	X		
		BR	790425	1,200	130	9.2	X		
CW	71	S	790422	6.7	130	0.05			X
*	72	S	790422	1.3	370	0.004			X
	75	S	790422	320	180	1.8		X	
		S	790829	10,000	1,900	5.3	X		
		S	791015	11,000	150	73.3	X		
	76	S	790422	13	8,700	0.002			X
		SR	790422	13	6,100	0.002			X
		B	790422	19	220	0.09			X
	79	S	790422	3,500	680	5.2	X		
	81	S	790422	1,900	1,300	1.5		X	
		B	790422	9,900	9,100	1.1		X	
		S	790829	5,800	290	20.0	X		
		B	790829	6,900	260	26.5	X		
	82	S	790422	2.7	160	0.02			X
		BR	790422	6.7	210	0.03			X
	83	S	790422	4,800	3,600	1.3		X	
		B	790422	7,500	7,600	0.99		X	
		S	790829	1,500	120	12.5	X		
		BR	790829	1,600	110	14.5	X		
	89	S	790422	3,400	2,400	1.42		X	
		B	790422	6,800	6,200	1.10		X	
		S	790829	4,400	240	18.3	X		
		B	790829	4,500	250	18.0	X		
CE	84	S	790825	3,600	150	24.0	X		
		B	790825	3,600	220	16.4	X		

Table 9 continued.

Station	Level	Date	Fecal Coliforms Per 100 ml	Fecal Streptococci Per 100 ml	FC/FS	≥ 4.0	4.0 > FC/FS > 0.7	≤ 0.7
CE 85	S	790419	34,000	780	43.6	X		
	S	790825	3,900	290	13.4	X		
	B	790825	2,800	220	12.7	X		
	B	791011	1,600	100	16.0	X		
86	S	790419	5,800	260	22.3	X		
	B	790419	8,400	520	16.2	X		
	S	791011	1,700	140	12.1	X		
	B	781011	5,400	190	28.4	X		
87	S	790419	10,000	220	45.5	X		
	B	790419	5,100	220	23.2	X		
94	S	790825	1,200	500	2.4		X	
	S	791011	3,500	110	31.8	X		
95	S	790825	1,300	190	6.84	X		
98	S	790825	14,000	1,000	14.0	X		
	S	791011	7,000	440	15.9	X		
* 100	B	790419	0	140	0		X	
FP 103	S	790416	3,200	1,800	1.78		X	
104	S	790416	8,400	1,400	6.0	X		
105	S	790416	740	340	2.2		X	
113	S	790416	580	250	2.3		X	
	B	790416	580	310	1.9		X	
	S	791007	4,800	1,800	2.7		X	
	B	791007	5,400	1,800	3.0		X	
114	S	791007	3,400	230	14.8	X		
	B	791007	2,100	660	3.2		X	
117	S	790416	810	220	3.7		X	
122	S	790416	17	580	0.03			X
AS 132	S	790413	1,800	950	1.9		X	
	B	790413	730	3,000	0.24			X
	B	790713	1,600	170	9.4	X		

* Offshore stations

Table 10. Central Basin Station Rational (adapted from Herdendorf 1978).

Station	Rationale	Station	Rationale
LV 51	BR, HF, NS, ST, TN	CE 94	HF, NS, TN
52	CF, ND, TN	95	HF, NI, TN
53	BR, HF, IM, NI	96	ND, TN
54	DC, DS, HA, MT, NS, PP, TN	97	ND, TN
55	NI, TN	98	DS, NB, NS, ST, TN
56	NI, TN	99	NI, TN
57	ND, TM, TN	100	ND, TN
58	BR, HF, NS, TN	101	IM, ML, TN
59	ND, TN	102	NB, NI
60	BR, NB, NS, TN	FP 103	BR, NB, NS
61	NI, TN	104	DP, NS, TN
62	ND, TN	105	BR, DC, HA, MT, NS, WL
63	HF, IM, NI	106	NI, TM, TN
64	HF, IM, NI	107	ND, TN
65	DC, DS, HA, NS, TN	108	NB, NS
66	DC, DP, HA, NI, TN	109	BR, HF, NS, TN
67	ND, TM, TN	110	HF, IM, NI, TN
68	HF, NS, TN	111	HF, NI, TN
69	ND, TN	112	CF, NS, TN
CW 70	NB, NS	113	DC, DS, HA, MT, NS, PP, TN
71	HF, NS, TN	114	DC, DP, HA, NI, TN
72	IM, NI, TN	115	ND, TN
73	NI, TN	116	ND, TM, TN
74	ND, TM, TN	117	DC, HA, NI, PP
75	DC, HA, MT, NS, PP, TN	118	HF, NI
76	DC, HA, NI, TN	119	HF, NS, TN
77	NI, TN	120	ND, TM, TN
78	ND, TN	121	DP, II, NB
79	BR, HF, NS, TN	122	IM, NB, NS
80	HF, NI, TN	AS 123	NS, ST, TN
81	ND, TN	124	NI, TN
82	IM, ML, TM, TN	125	ND, TN
83	IM, ML, TM, TN	126	BR, NB, NS, ST
88	ND, TM, TN	127	NB, NS
89	ND, TM, TN	128	BR, HF, NS, TN
*81	DS, HF, NI	129	HE, IM, NI, TN
*83	DS, HF, NI	130	HF, NI, TN
*89	DS, HF, NI	131	ND, TN
CE 84	DS, HA, NS, PP	132	DC, DS, HA, MT, NS, PP, TN
85	DC, DP, HA, MT, NS, PP, TN	133	DC, HA, NI, TN
86	DC, HA, NI, TN	134	ND, TN
87	DC, HA, NS, PP, TN	135	ML, TM, TN
90	DC, HA, NS, PP	136	DP, HF, NS
91	DC, DP, HA, NS, PP	137	NB, NS, TN
92	ND, TN	138	ND, TN
93	ND, TN	139	NB, NS

*1979 positions for these stations.

Rationale Code (Herdendorf 1978)

Code	Rationale
BR	beach, recreational
CF	commercial fishing grounds
DC	dredged channel
DP	discharge, power plant or industrial
DS	discharge, sewage treatment plant
HA	harbor area
HF	harbor flanks
II	intake, industrial
IM	intake, municipal
ML	offshore or main lake
MT	major tributary mouth
NB	nearshore, between major harbor areas
ND	nearshore, deep or outer position
NI	nearshore, intermediate depth
NS	nearshore, shallow or inner position
PP	known pollution problems
ST	small tributary mouth
TM	transect, main lake connection
TN	transect, nearshore

Table 11. A comparison of stations 81, 83 and 89 with stations 80 and 88 using the t-test and $\log_{10}$ transformed data from 1979.

	Number of Cases	Mean	Standard Deviation	*T-value	Degrees of Freedom	2-Tail Probability
			Heterotrophs			
81, 83, 89	115	4.07	0.743	7.14	80.01	<0.001
80, 88	46	3.12	0.774			
			Fecal Streptococci			
81, 83, 89	27	1.66	1.361	3.84	40.04	<0.001
80, 88	16	0.4614	0.672			
			Fecal Coliforms			
81, 83, 89	27	2.67	1.094	5.05	40.29	<0.001
80, 88	16	1.26	0.731			

*SPSS separate variance estimate for use when variances are not equal (Nie, et al. 1975).

Table 12. 1978 stations with concentrations ≥ 200 fecal coliforms/100 ml

Station			Cruise I	Cruise II	Cruise III	Cruise IV
LV	54	S		411		
	65	S		280		240
CW	75	S	15000	500	300	200
	79	S	370			
	80	S	270			
CE	84	S	400			
		B			270	
	85	S	210	2700	940	5600
		B	270	1100	970	7800
	86	S	700	720	1600	940
		B	590		1400	1300
	87	S	250	390		
		B	410	620		
	90	S	310			230
		SR	260			
	91	S				540
		B				580
	92	B				200
	94	S		3600	710	2800
	95	S				460
		B				710
	98	S				1400
FP	106	BR		200		
	113	S		460		
		SR		250		
		B				400
	114	S	280			
		B				470
AS	132	S	780		200	
		SR	680			
		B	300		310	

Table 13. 1979 stations with concentrations ≥ 200 fecal coliforms/100ml

Station			Cruise I	Cruise II	Cruise III	Cruise IV
LV	53	S			240	
	54	S	720		210	
	65	S	960	390	600	
		B	1100			360
		BR	1200			
	66	S	1200			
		B	800			
CW	75	S	320		10000	11000
	76	B				980
	79	S	3500	600		240
	81	S	1900		5800	
		B	9900	300	6900	
	83	S	4800	310	1500	
		B	7500		1600	
		BR			1600	
	88	S	300			
		B	330			
	89	S	3400		4400	
		B	6800	400	4500	
CE	84	S	480		3600	550
		B	550		3600	1100
	85	S	34000		3900	1000
		B	530		2800	1600
	86	S	5800	240	3400	1700
		B	8400		810	5400
	87	S	10000		2200	270
		B	5100		2700	300
	90	S	1800		800	210
		B	1500		990	380
	91	S			550	
		B	540		660	
	94	S			1200	3500
	95	S			1300	
		B		470	900	
		BR			510	
	98	S			14000	7000
	99	B	200			
FP	103	S	3200			
	104	S	8400			
	105	S	740	640		
	108	S	1800			
	109	S	440			
	111	B				460
	113	S	580			4800
		B	580	310		5400
FP	114	S	270			3400
		B	280	200		2100
	115	B				250
	117	S	810			440
AS	123	S	210			
	132	S	1800	500	260	260
		B	730	1600	280	410

Table 14. Stations exhibiting fecal coliform concentrations of more than 1,000 organisms/100ml.

1978	Station		Concentrations exhibited		
	CW 75	S	15,000		
	CE 85	S	2700	5600	
		B	1100	7800	
	86	S	1600		
		B	1400	1300	
	94	S	3600	2800	
1979	LV 65	B	1100		
		BR	1200		
	66	S	1200		
	CW 75	S	10,000	11,000	
	79	S	3500		
	81	S	1900	5800	
		B	9900	6900	
	83	S	4800	1500	
		B	7500	1600	
		BR	1600		
	89	S	3400	4400	
		B	6800	4500	
	CE 84	S	3600		
		B	3600	1100	
	85	S	34,000	3900	1000
		B	2800	1600	
	86	S	5800	3400	1700
		B	8400	5400	
	87	S	10,000	2200	
		B	5100	2700	
	90	S	1800		
		B	1500		
	94	S	1200	3500	
	95	S	1300		
	98	S	14,000	7000	
	FP 103	S	3200		
	104	S	8400		
	108	S	1800		
	113	S	4800		
		B	5400		
	114	S	3400		
		B	2100		
	AS 132	S	1800		
		B	1600		

Figure 1a. Location of sampling stations for the Central Basin Nearshore Zone in 1978.

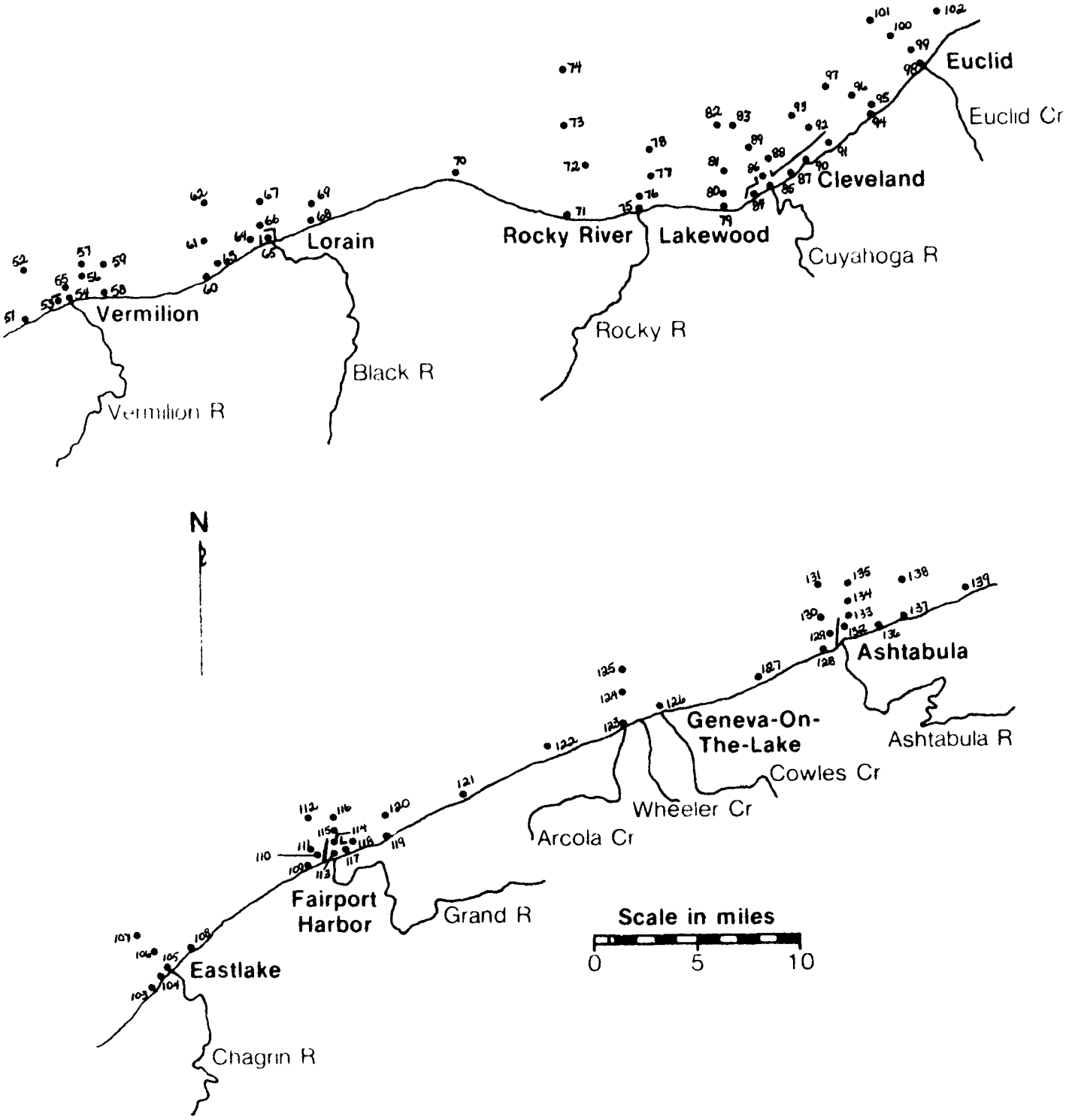


Figure 1b. Location of sampling stations for the Central Basin Nearshore Zone in 1979.

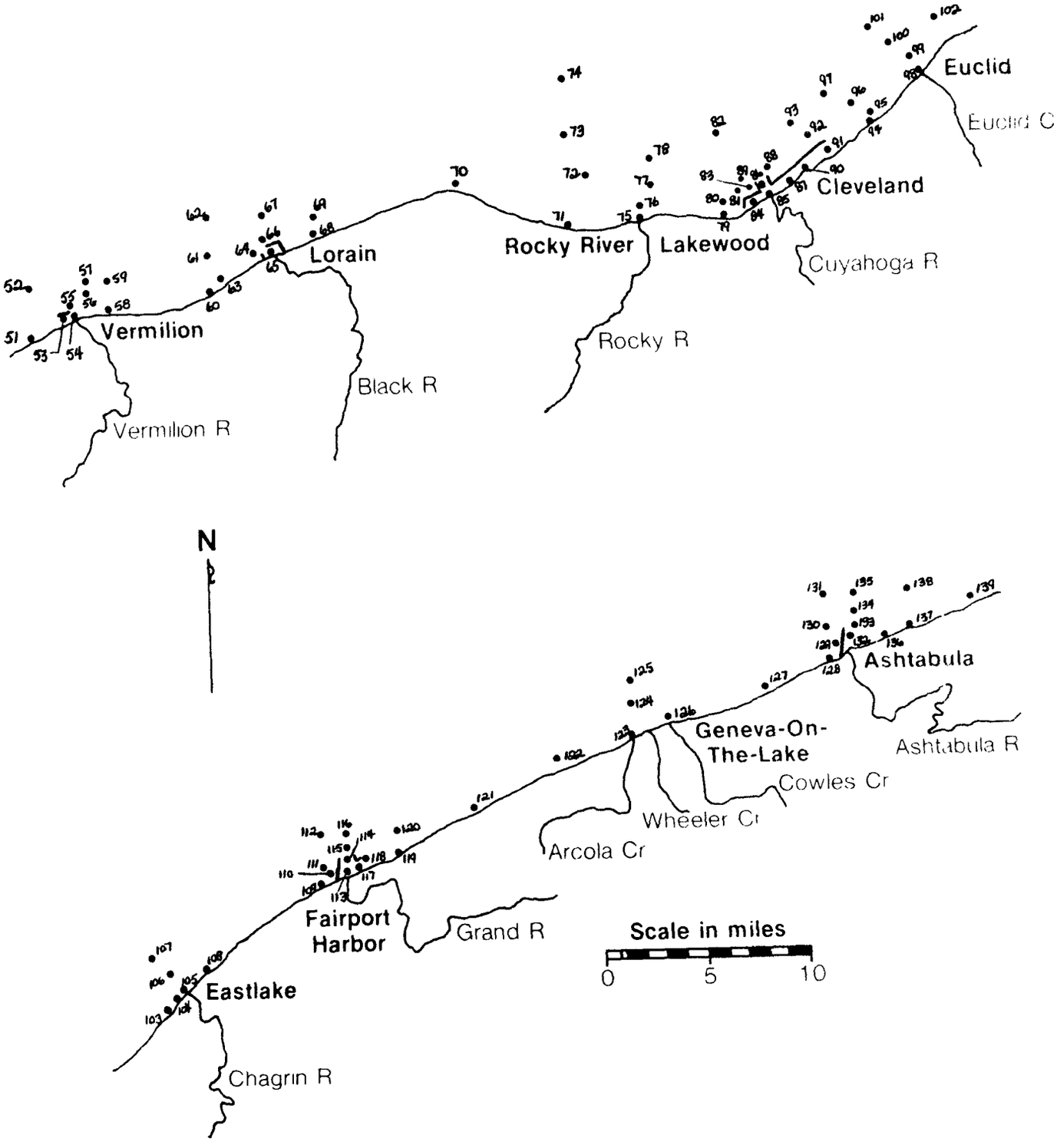


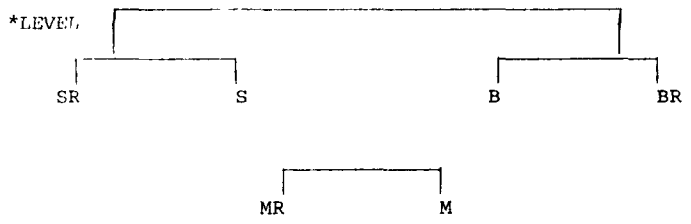
Figure 2. Comparison of the split and replicate sampling programs for 1978 and 1979.

1978

REPLICATES

CRUISES 1 and 2

2 STATIONS EACH DAY



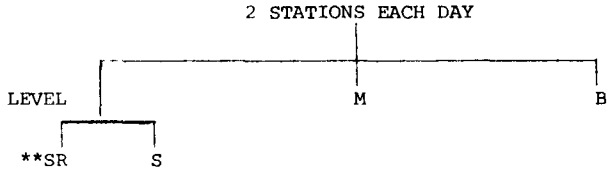
SPLITS

CRUISES 1 - 4

2 STATIONS EACH DAY

*LEVEL THAT WAS SPLIT VARIED.

CRUISES 3 and 4



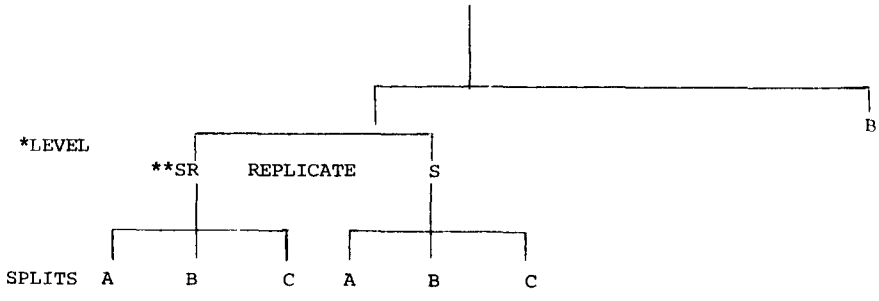
*NUMBER OF LEVELS DEPENDED ON THE DEPTH OF THE PARTICULAR STATION.

**THE LEVEL TO BE REPLICATED WAS SELECTED WHEN THE REPLICATE STATIONS WERE SELECTED - ONE SURFACE AND ONE BOTTOM REPLICATE EACH DAY (FOR CRUISES 3 AND 4).

1979 - ALL CRUISES

REPLICATES AND SPLITS

2 STATIONS EACH DAY



*NUMBER OF LEVELS DEPENDED ON THE DEPTH OF THE PARTICULAR STATION.

**THE LEVEL TO BE REPLICATED WAS SELECTED WHEN THE REPLICATE STATIONS WERE SELECTED -- ONE SURFACE AND ONE BOTTOM REPLICATE EACH DAY.

Figure 3.

Organization of the Central Basin stations used in determining cruise-to-cruise patterns

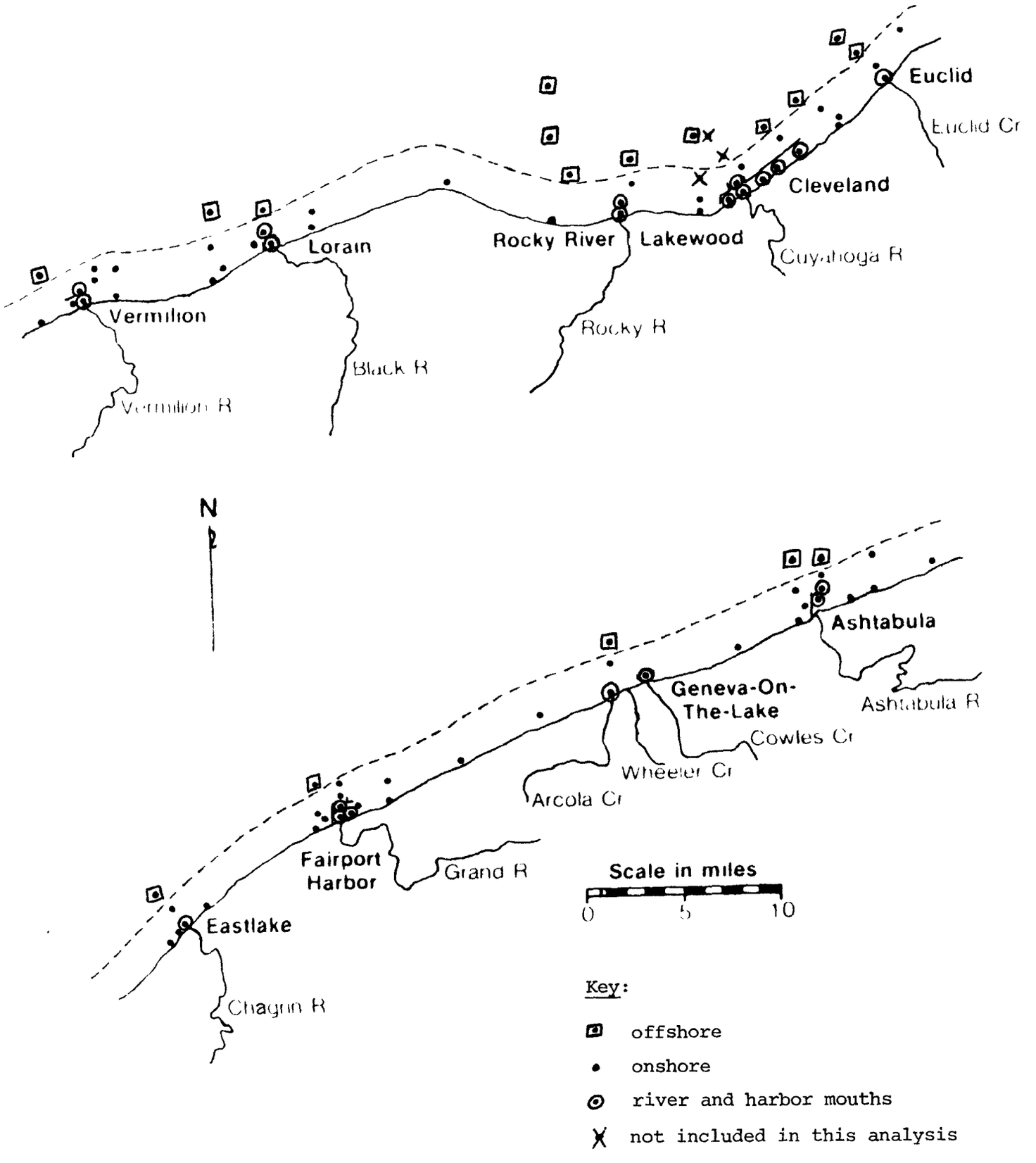


Figure 4a.

Aerobic heterotroph concentration isopleth map for Cruise 1, May, 1978

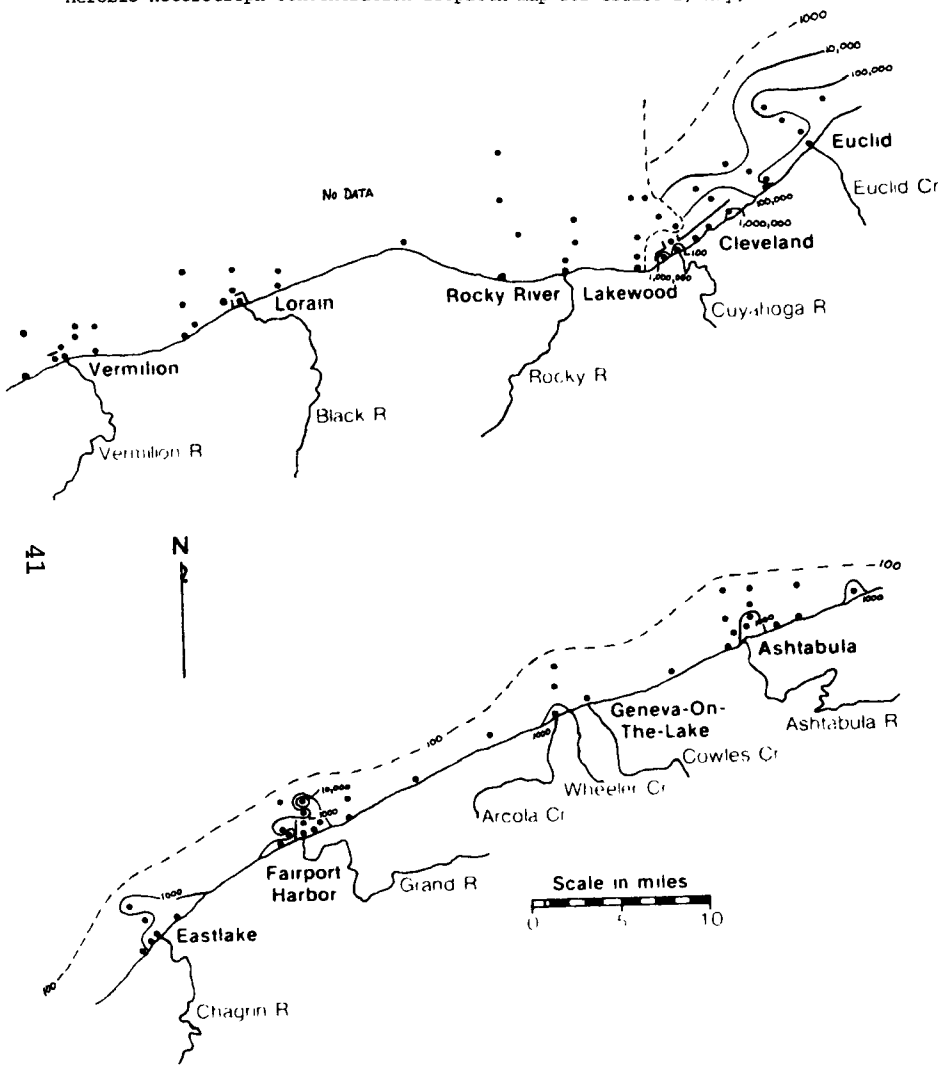


Figure 4b.

Aerobic heterotroph concentration isopleth map for Cruise 2, June, 1978

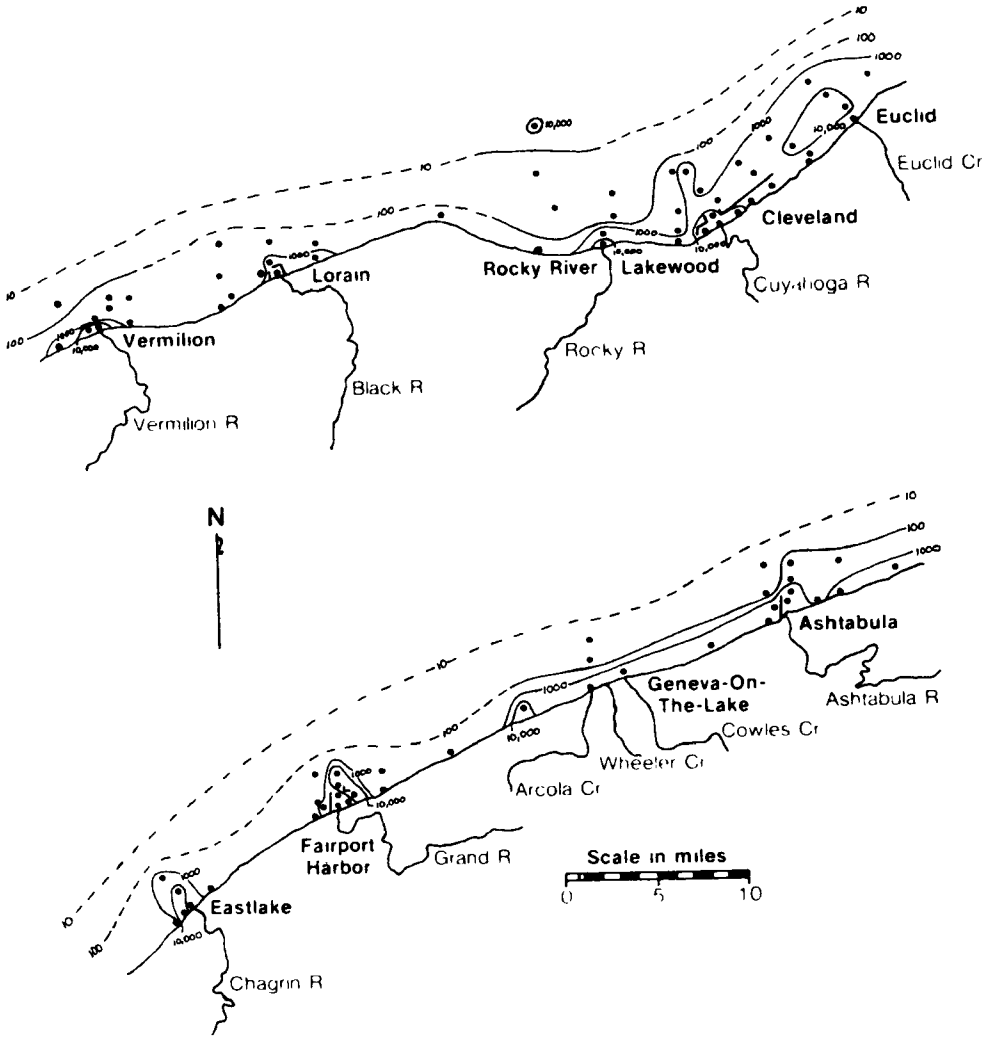


Figure 4c.

Aerobic heterotroph concentration isopleth map for Cruise 3, September, 1978

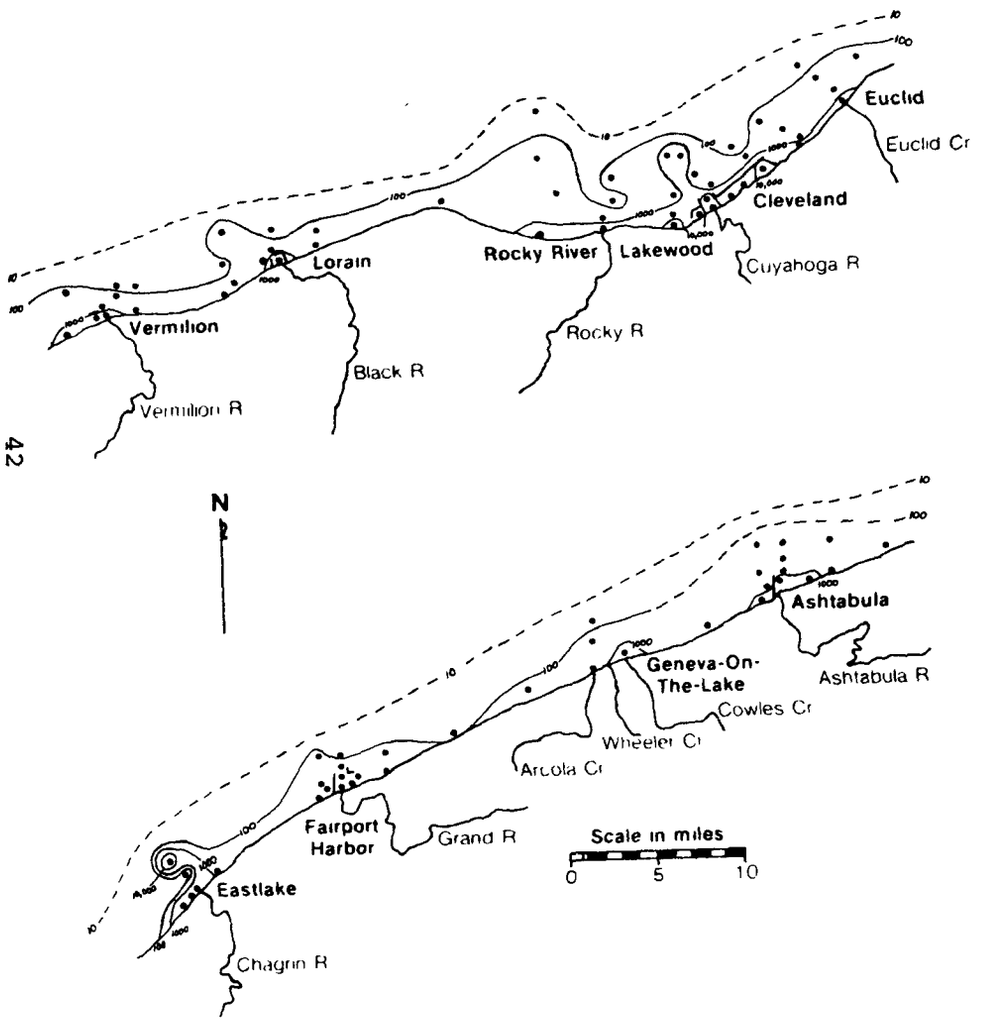


Figure 4d.

Aerobic heterotroph concentration isopleth map for Cruise 4, October, 1978

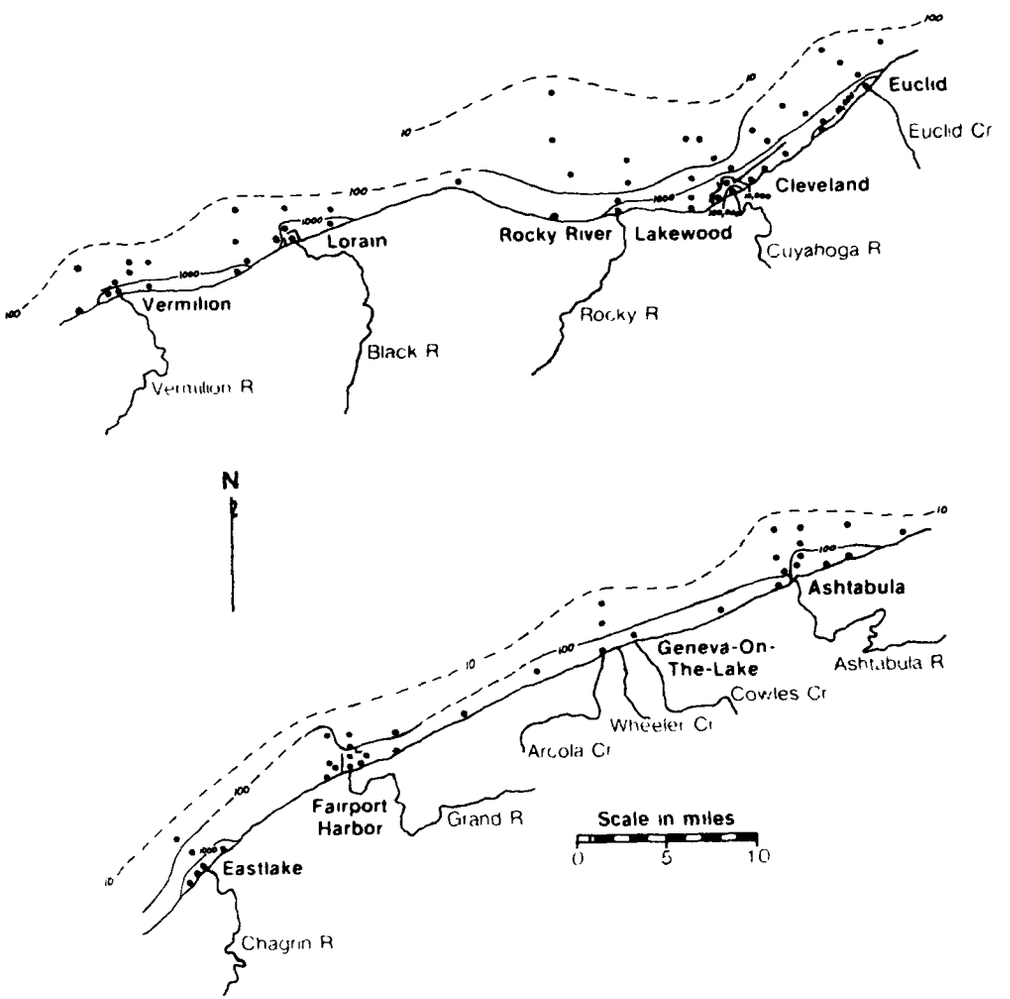


Figure 5a.

Aerobic heterotroph concentration isopleth map for Cruise 1, April, 1979

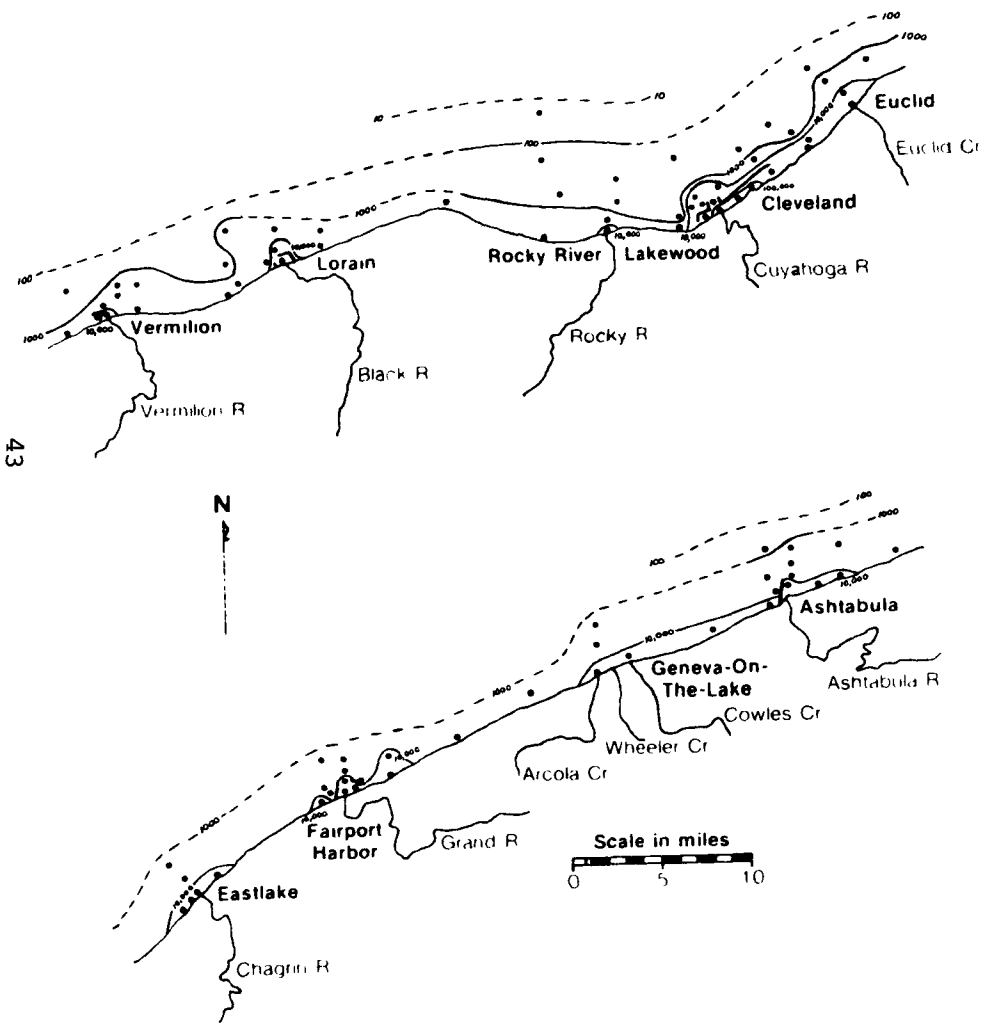


Figure 5b.

Aerobic heterotroph concentration isopleth map for Cruise 2, July, 1979

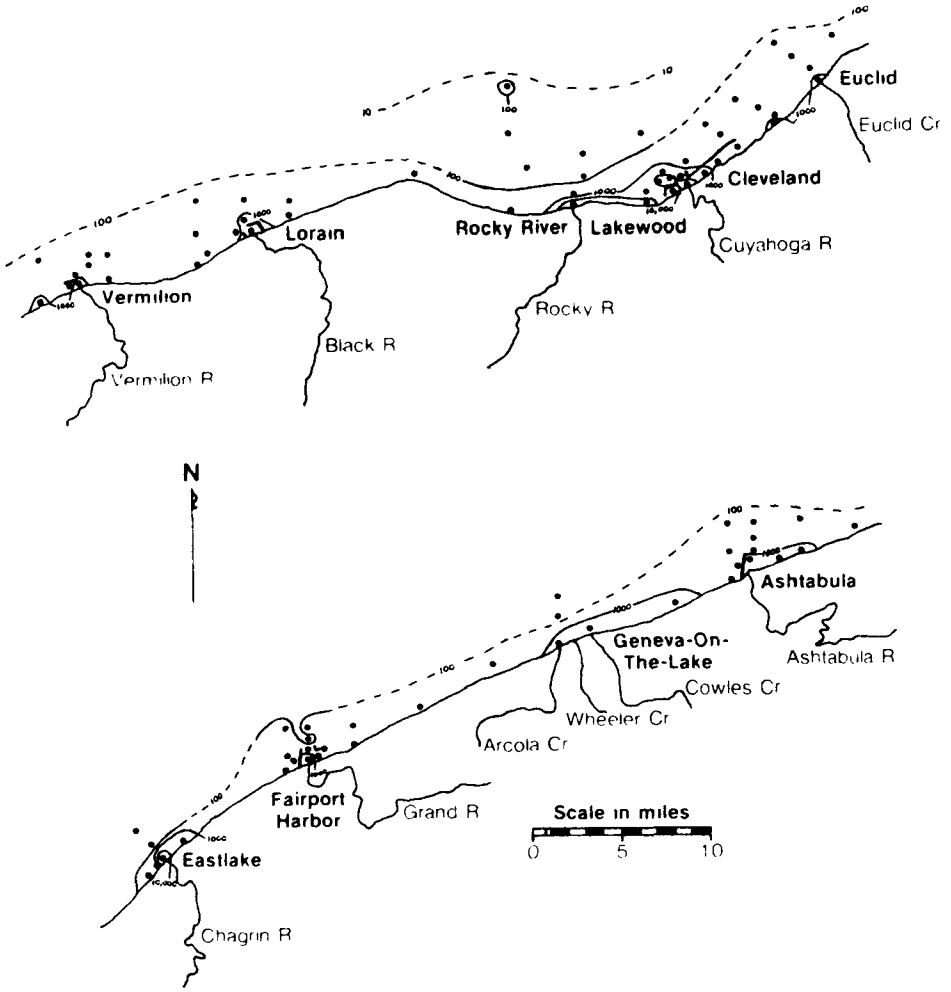


Figure 5c.

Aerobic heterotroph concentration isopleth map for Cruise 3, August, 1979

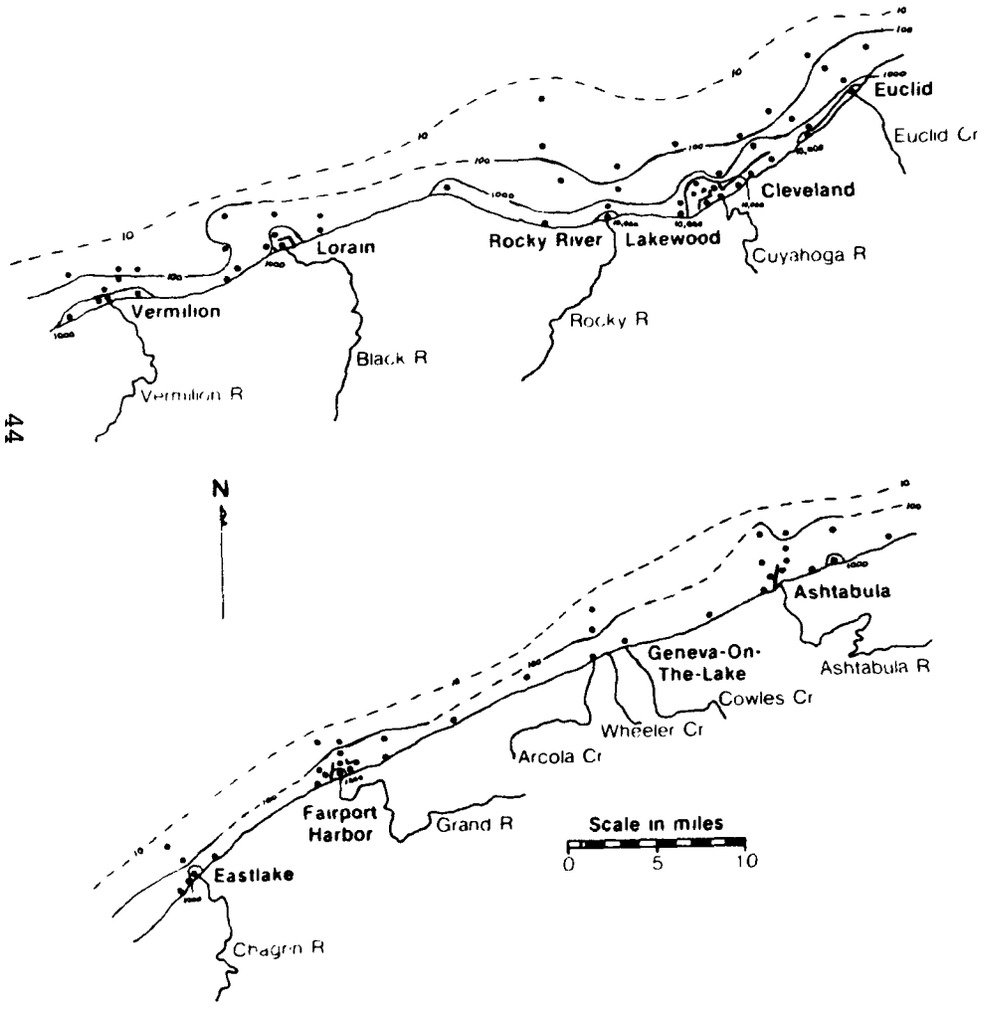


Figure 5d.

Aerobic heterotroph concentration isopleth map for Cruise 4, October, 1979

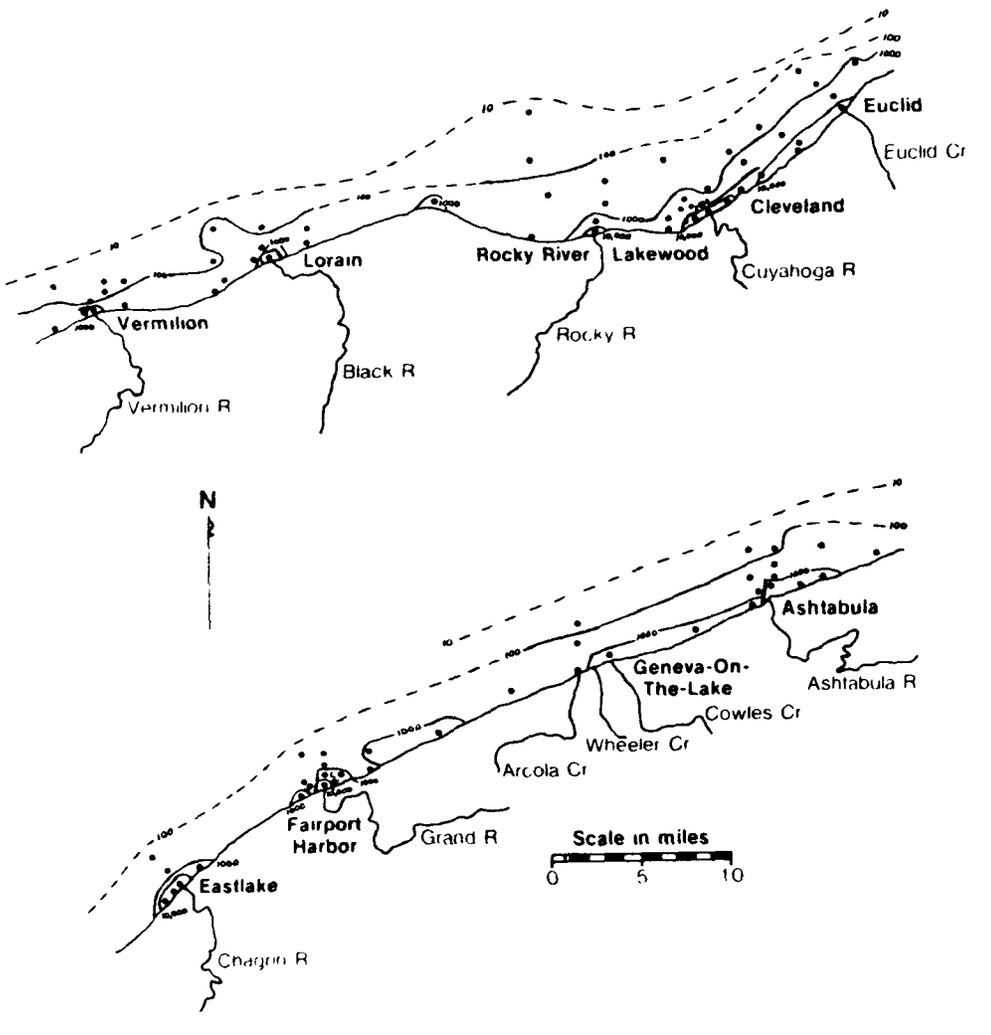


Figure 6a.

Fecal coliform concentration isopleth map for Cruise 1, May, 1978

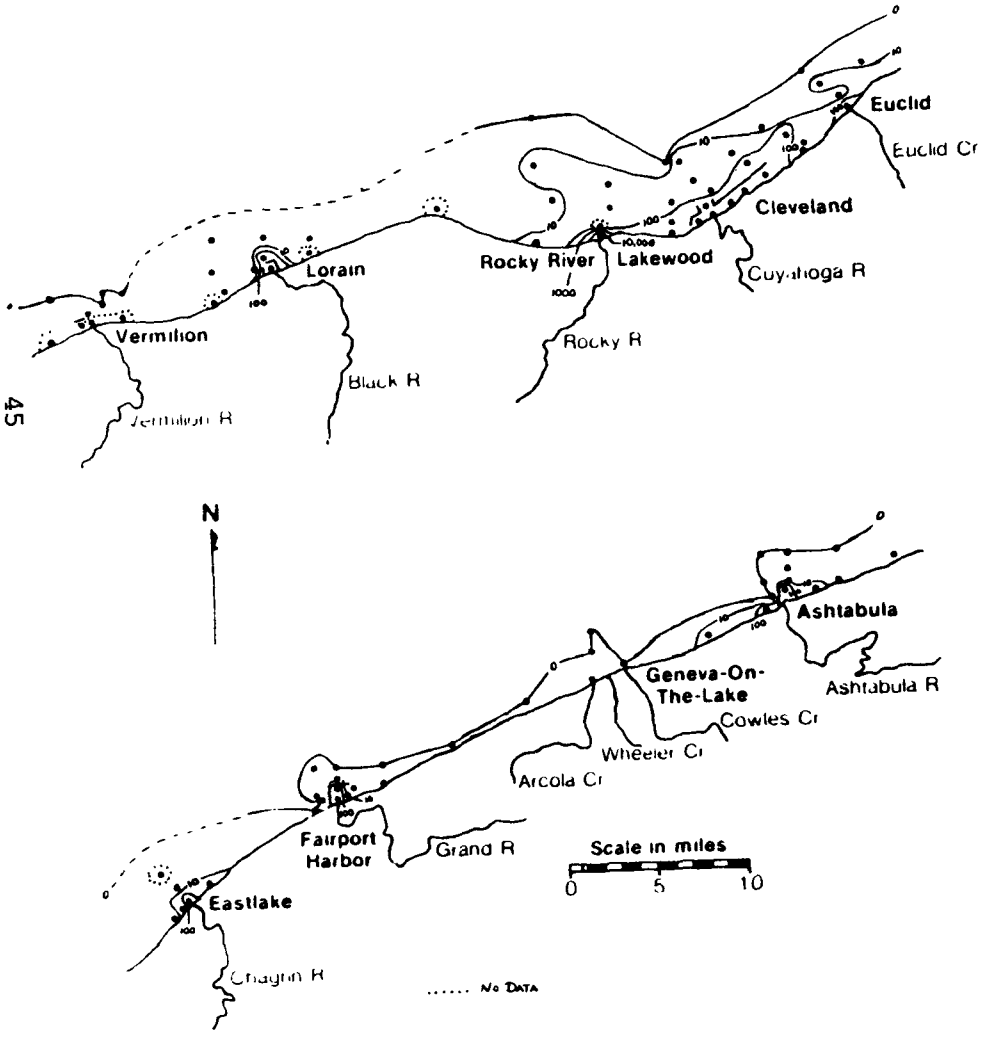


Figure 6b.

Fecal coliform concentration isopleth map for Cruise 2, June, 1978

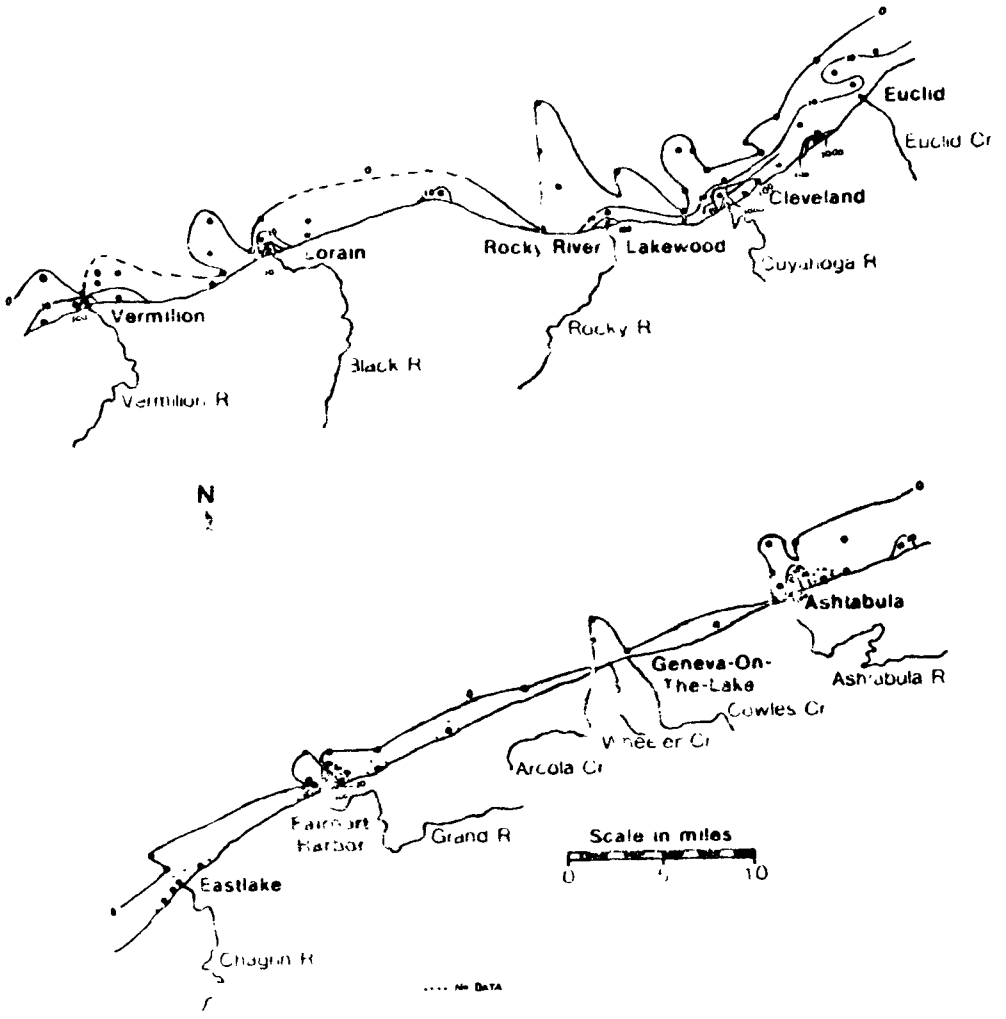


Figure 6c.

Fecal coliform conce tration isopleth map for Cruise 3, September, 1978

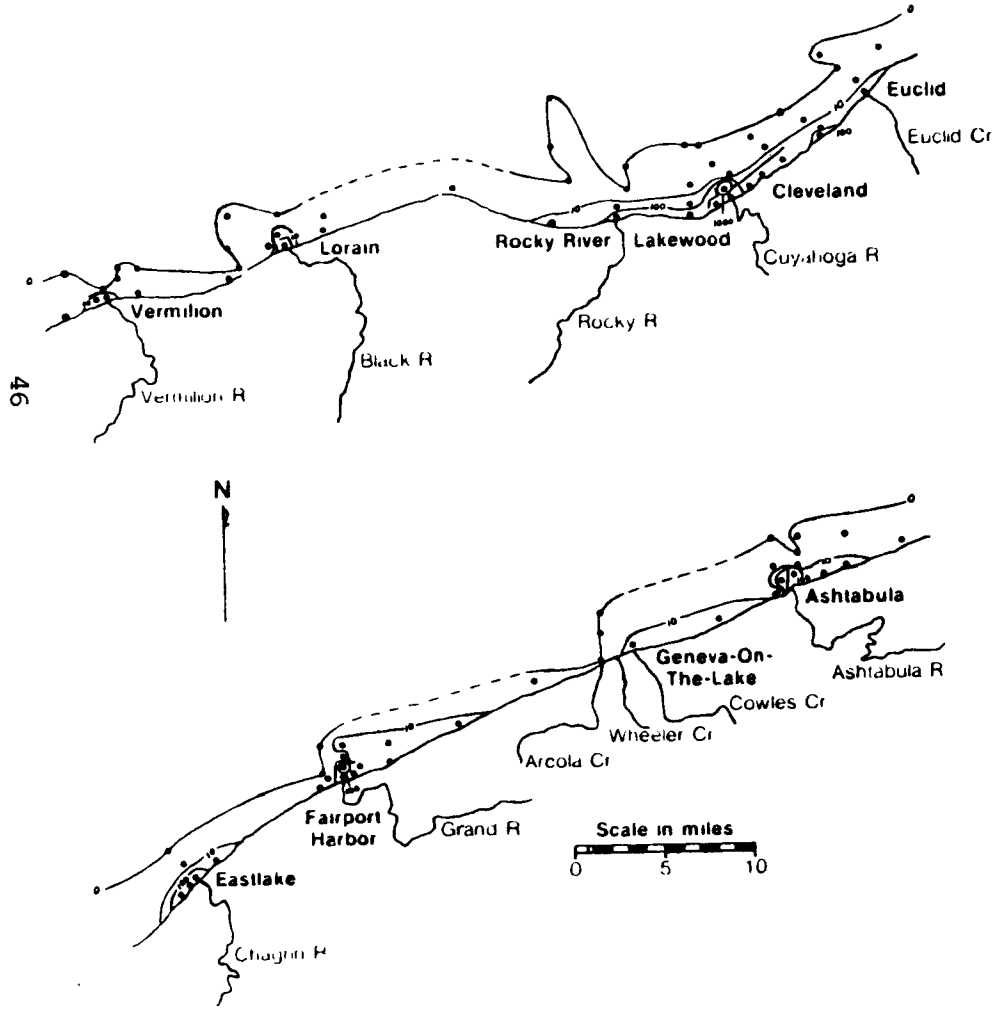


Figure 6d.

Fecal coliform concentration isopleth map for Cruise 4, October, 1978

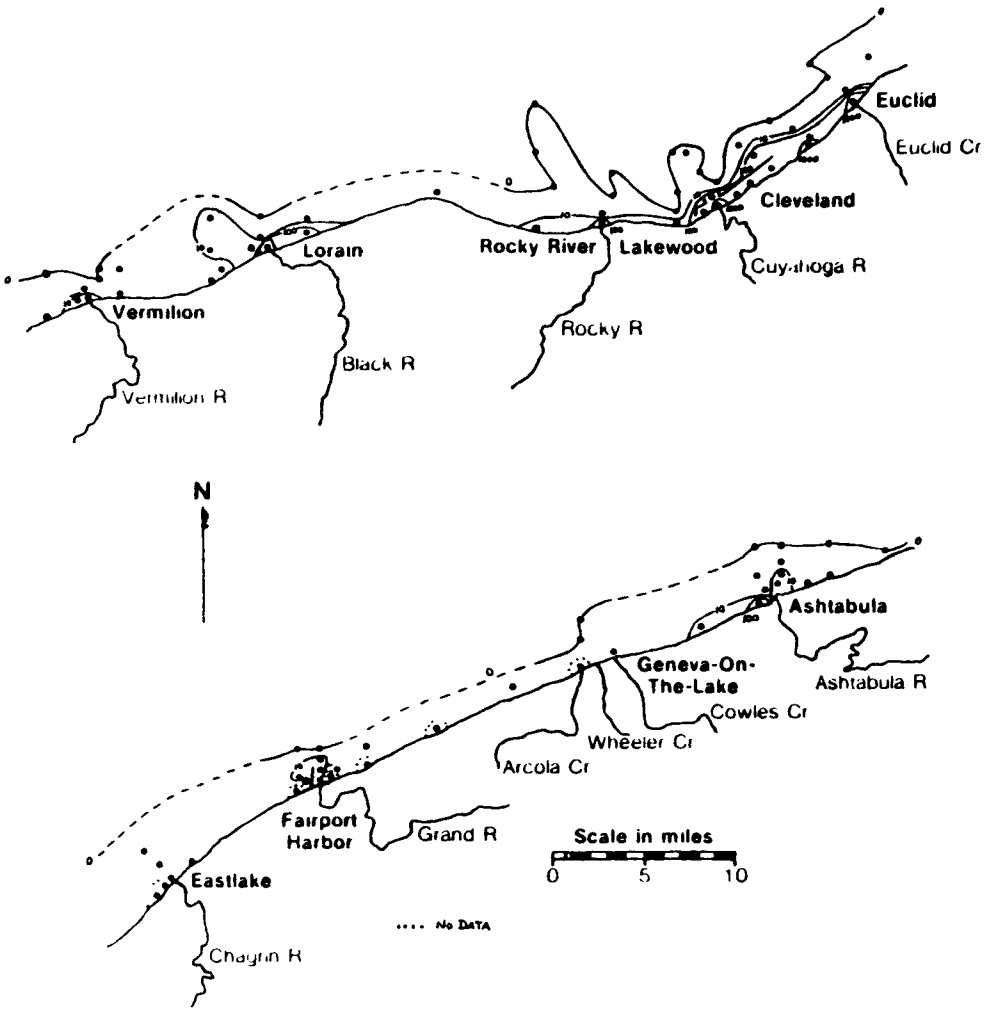


Figure 7a.

Fecal coliform conce tration isopleth map for Cruise 1, April, 1979

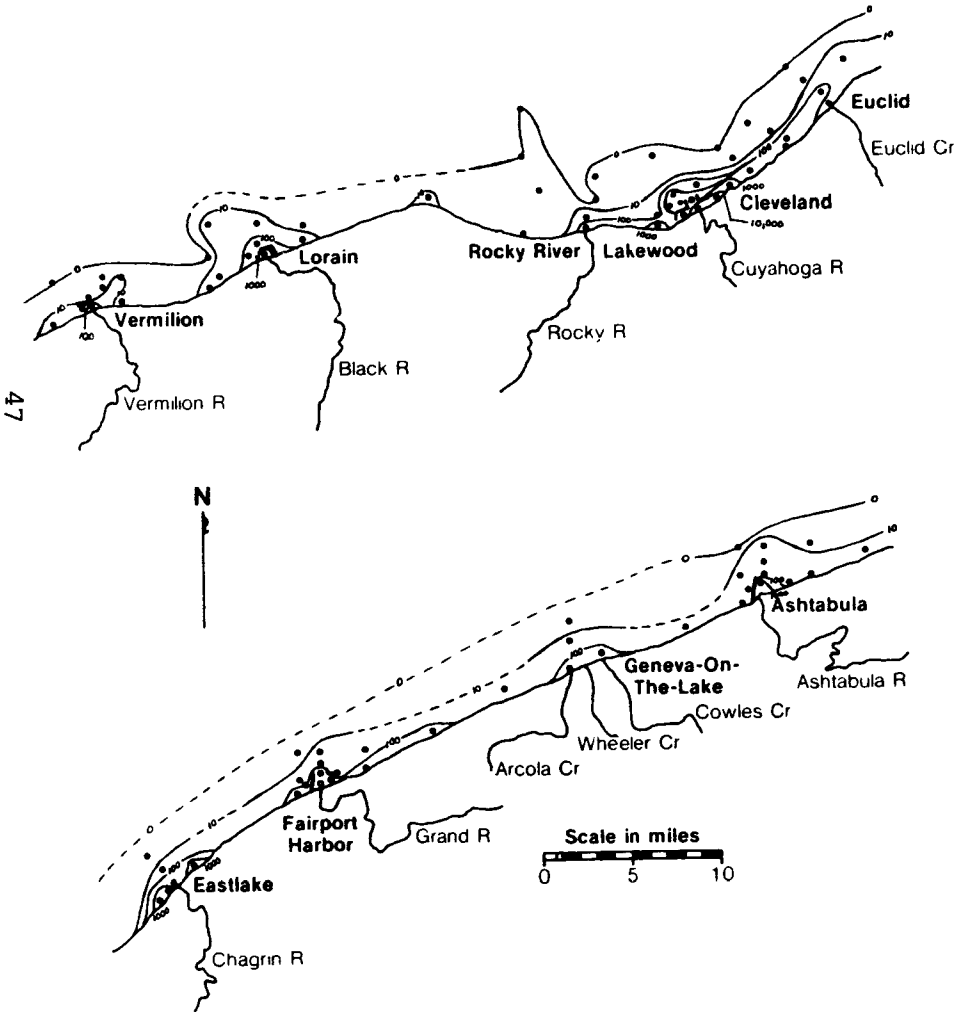


Figure 7b.

Fecal coliform concentration isopleth map for Cruise 2, July, 1979

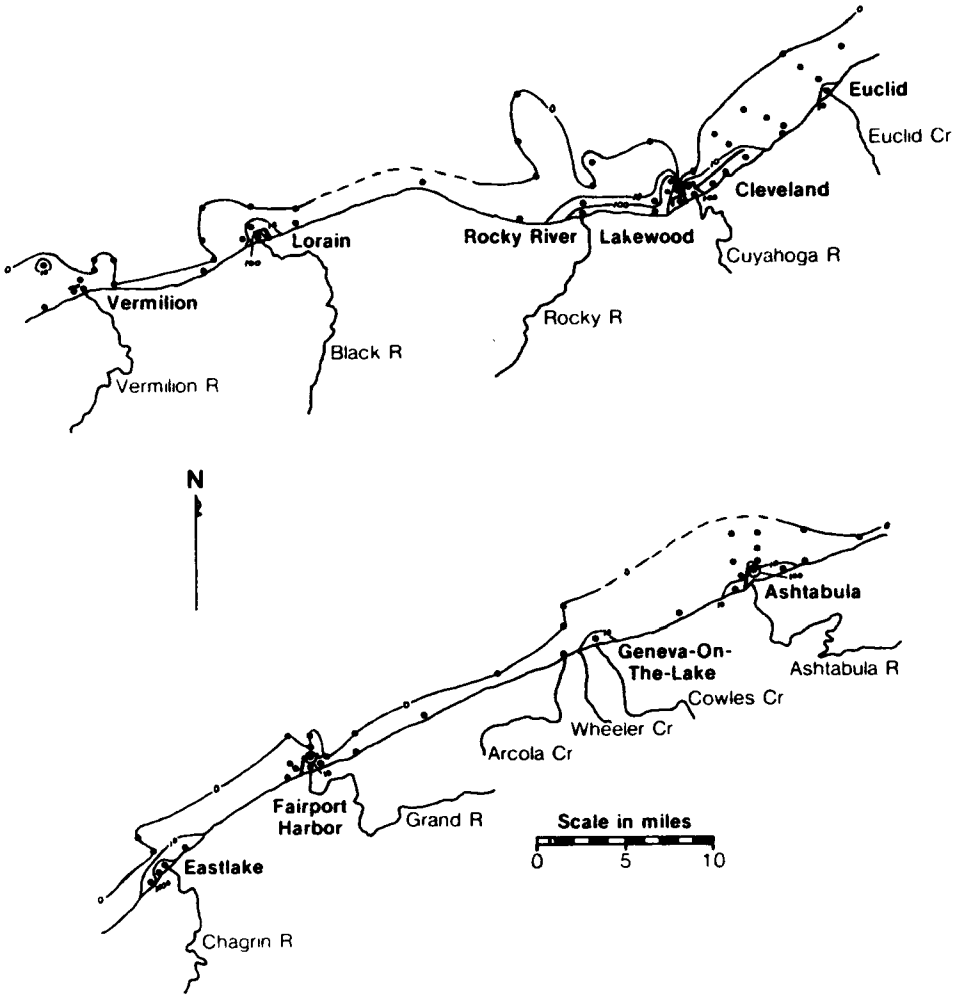


Figure 7c.

Fecal coliform concentration isopleth map for Cruise 3, August, 1979

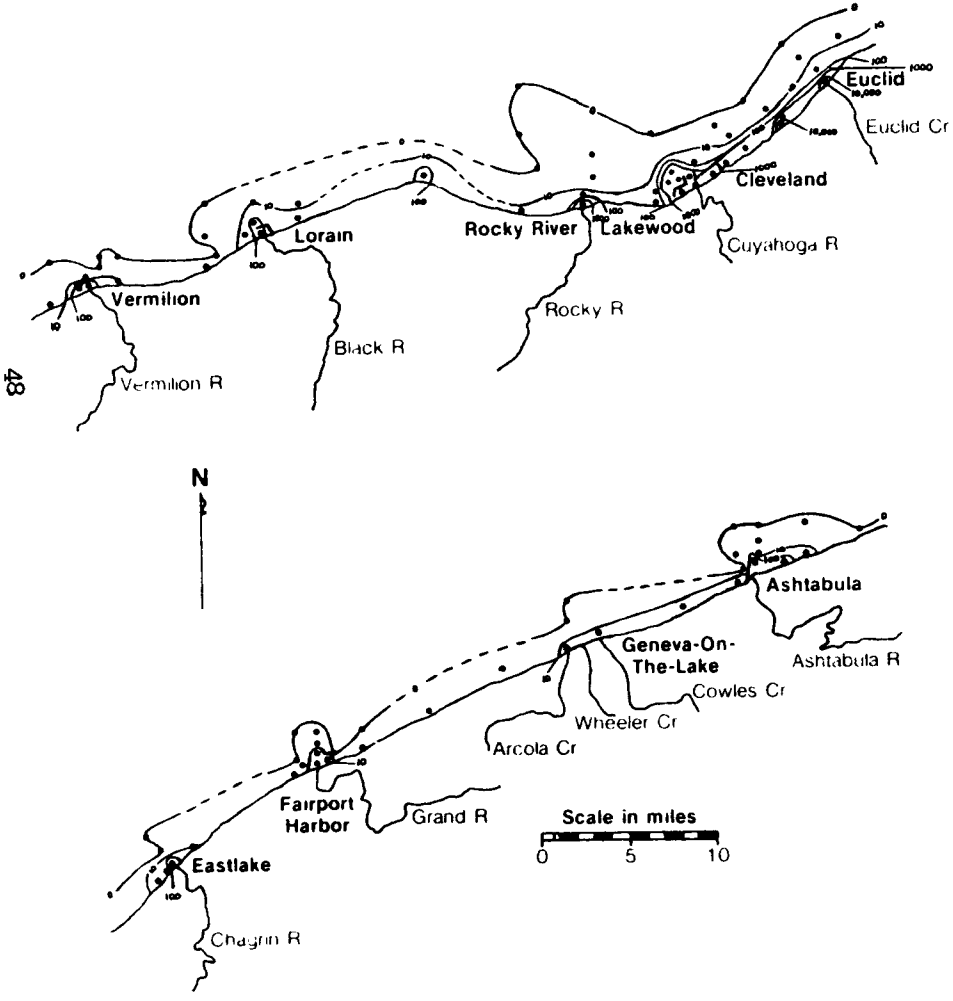


Figure 7d.

Fecal coliform concentration isopleth map for Cruise 4, October, 1979

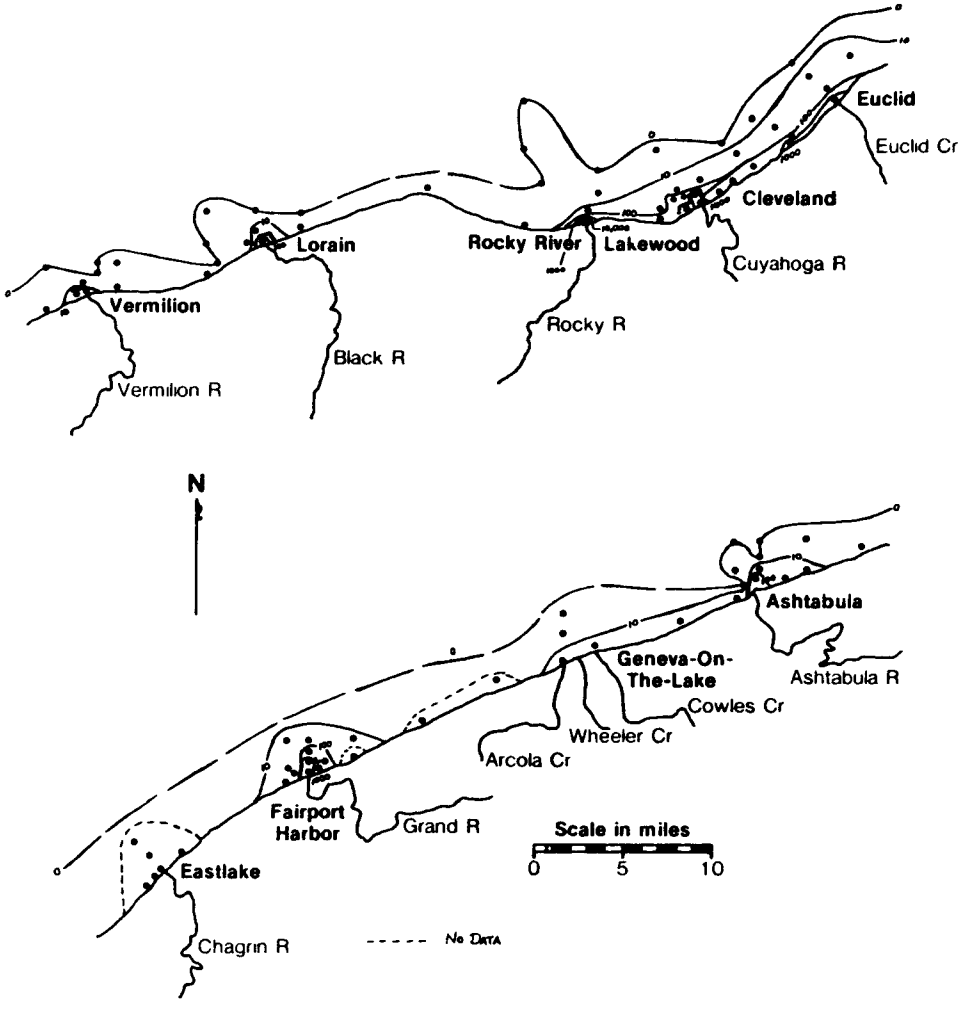


Figure 8a.

Fecal streptococcus concentration isopleth map for Cruise 1, May, 1978

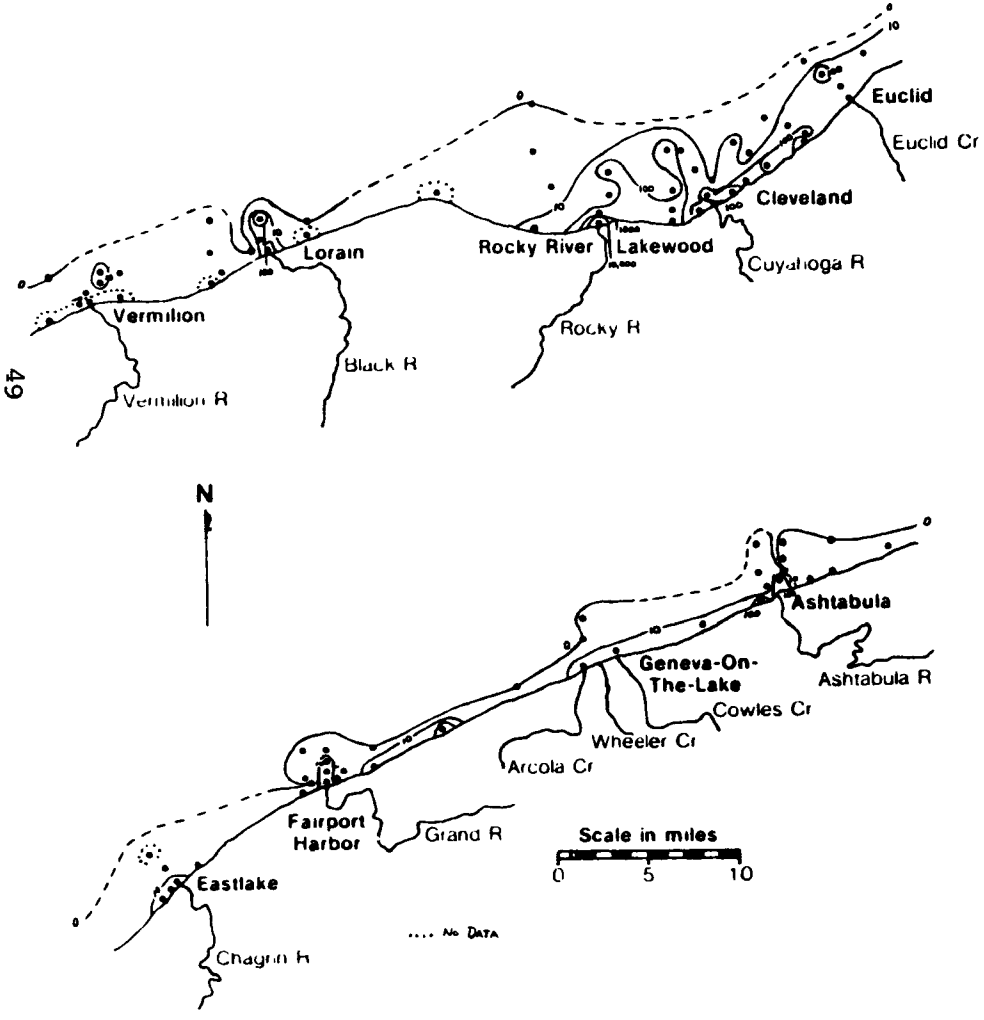


Figure 8b.

Fecal streptococcus concentration isopleth map for Cruise 2, June, 1978

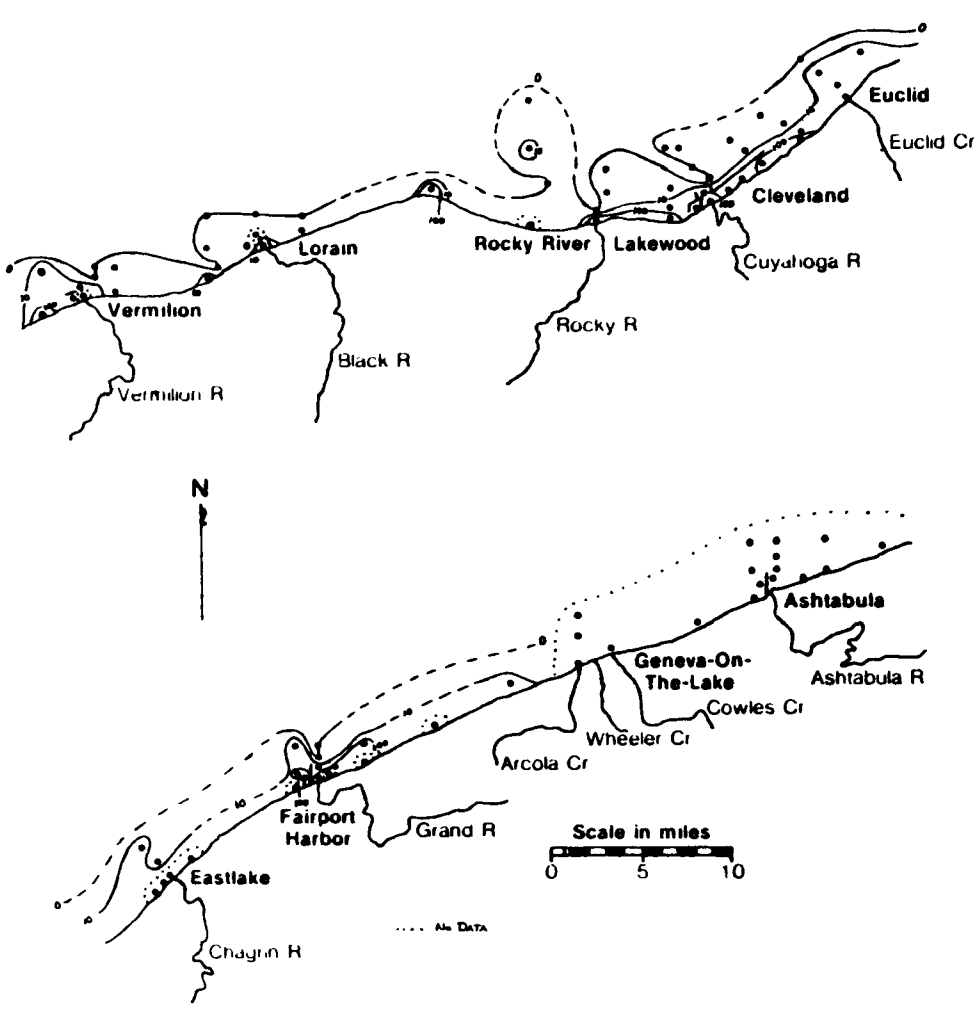


Figure 8c.

Fecal streptococcus concentration isopleth map for Cruise 3, September, 1978

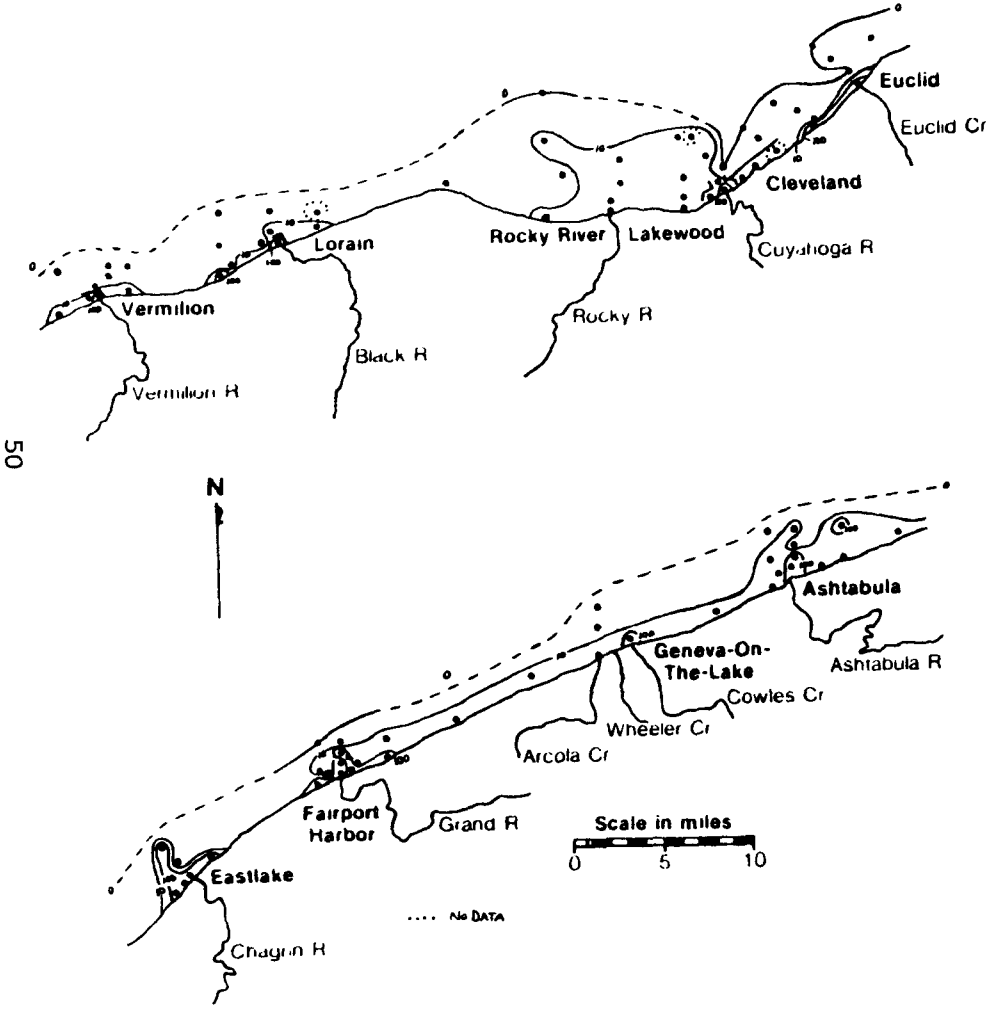


Figure 8d.

Fecal streptococcus concentration isopleth map for Cruise 4, October, 1978

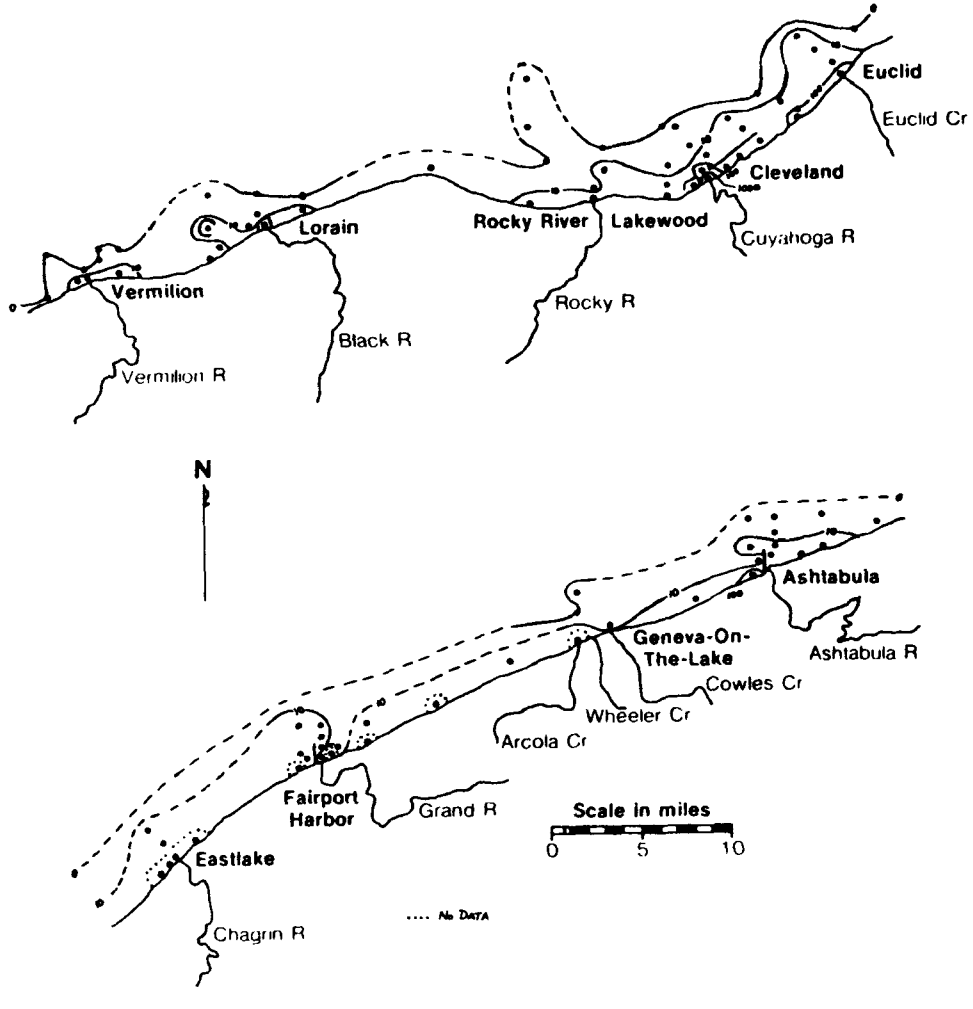


Figure 9a.
Fecal streptococcus concentration isopleth map for Cruise 1, April 1979

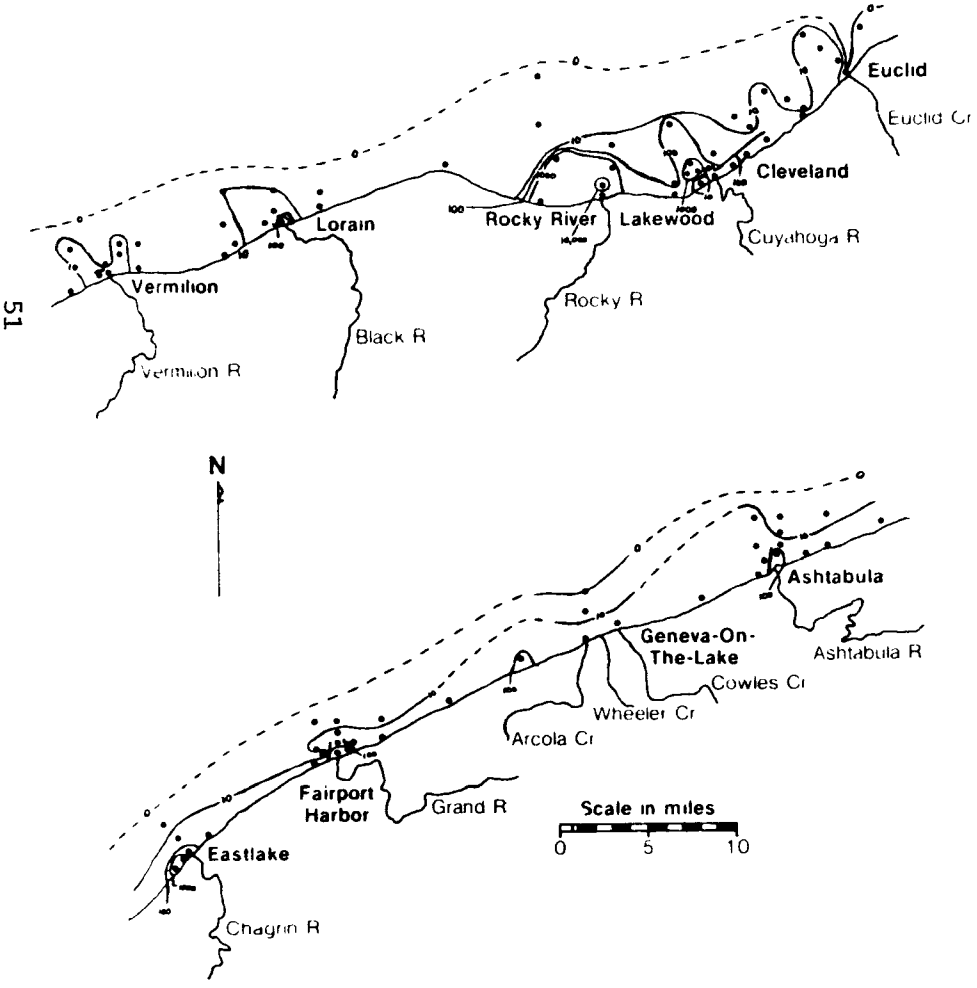


Figure 9b.
Fecal streptococcus concentration isopleth map for Cruise 2, July, 1979

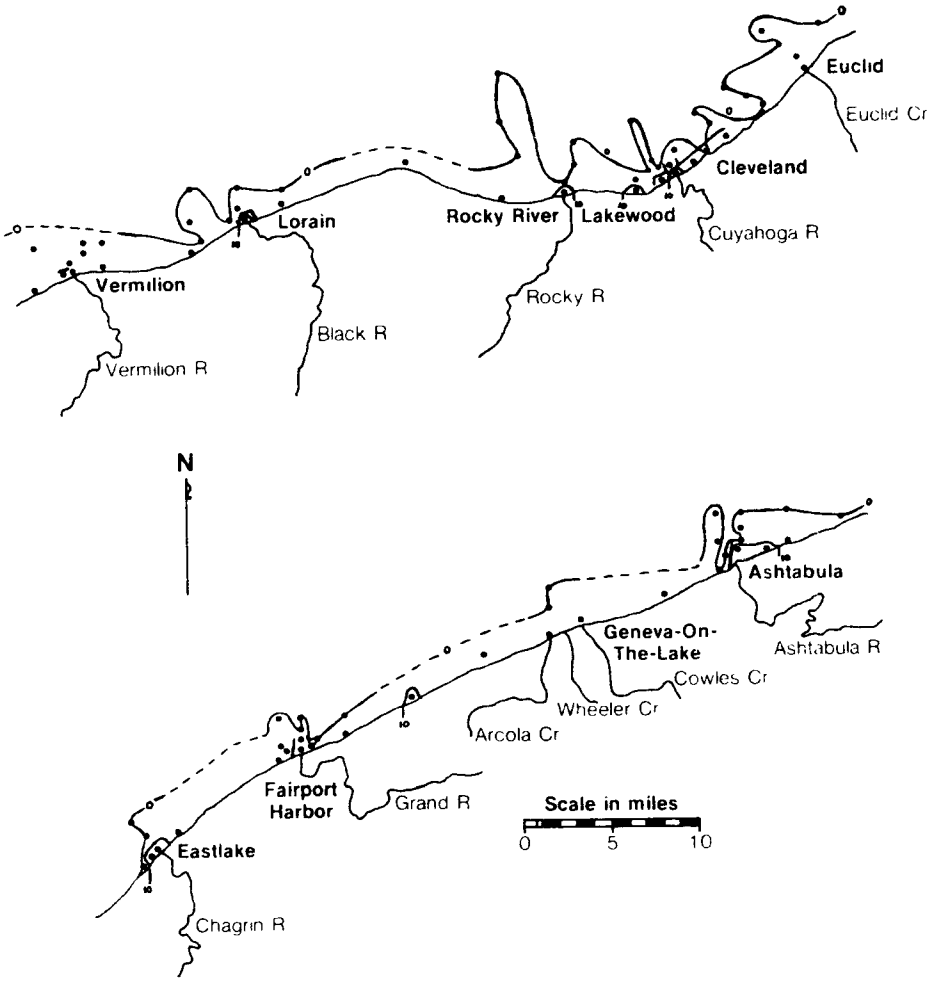


Figure 9c.

Fecal streptococcus concentration isopleth map for Cruise 3, August, 1979

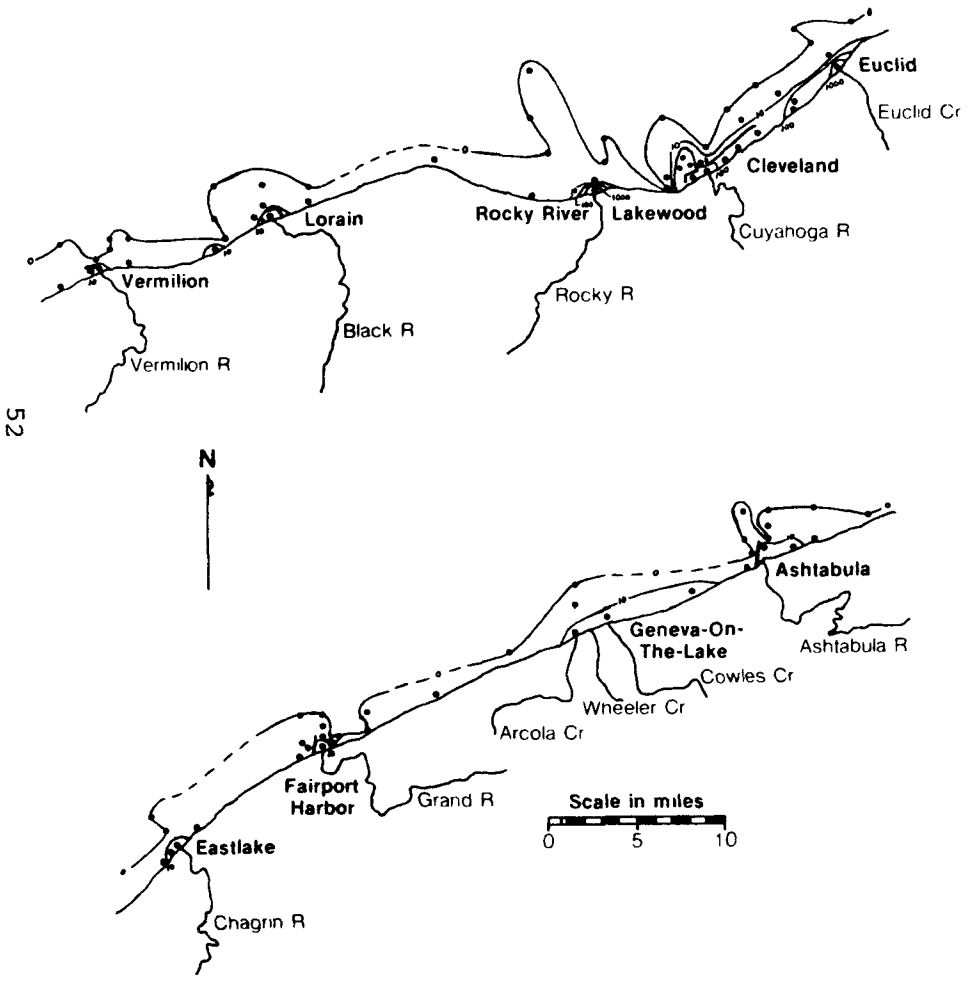


Figure 9d.

Fecal streptococcus concentration isopleth map for Cruise 4, October, 1979

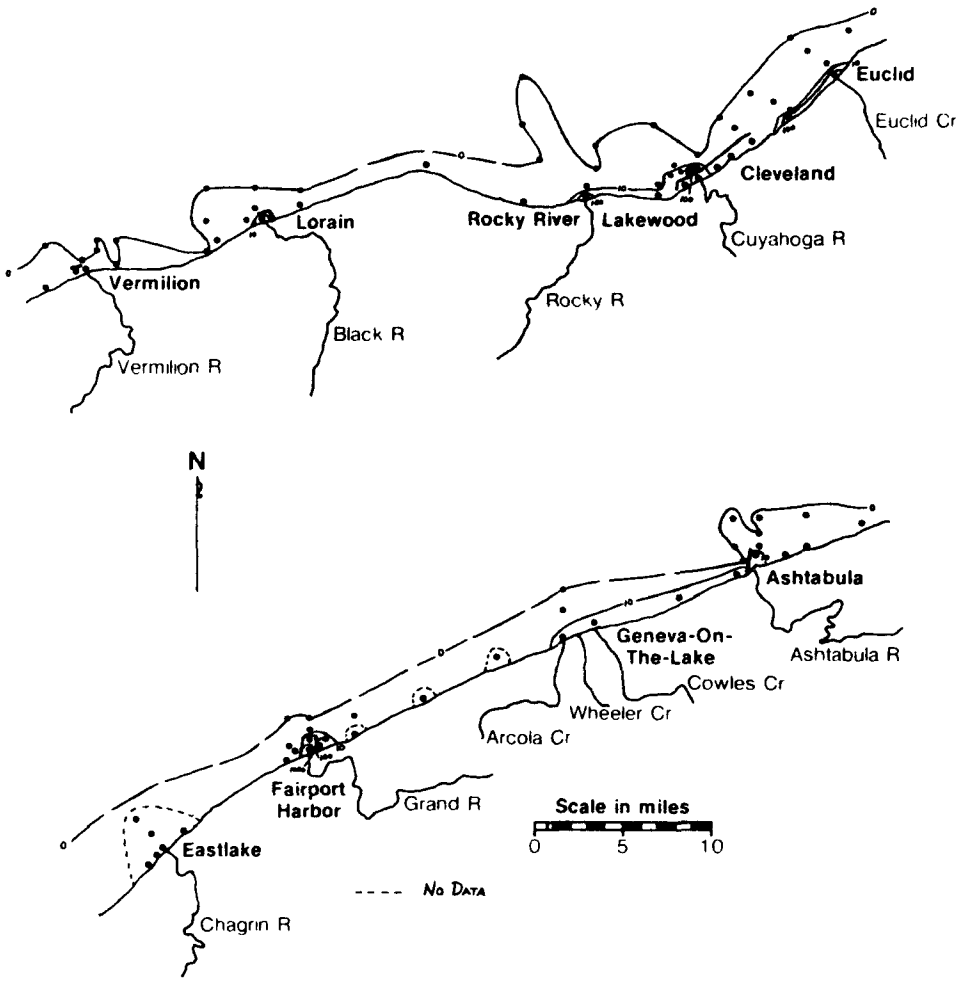


Figure 10a.

Summary: Summary of the 1978 aerobic heterotroph data using geometric means

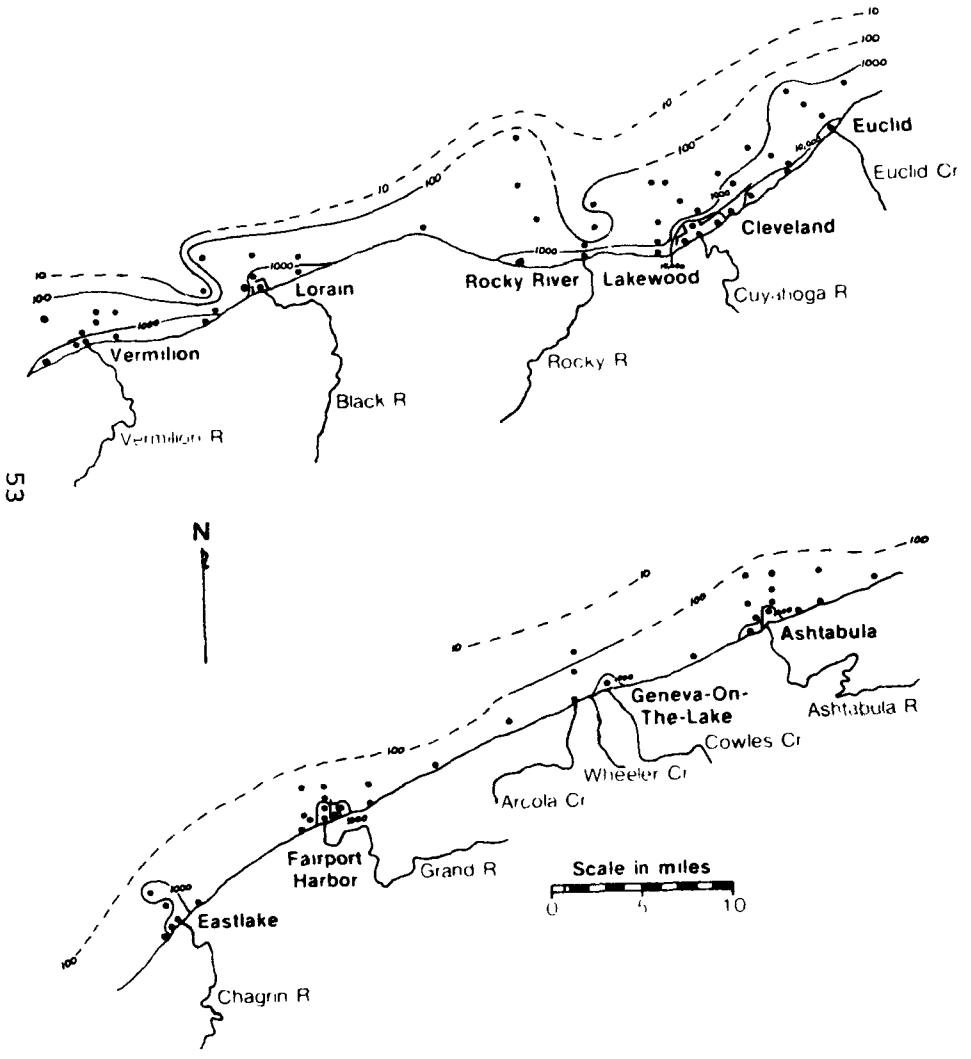


Figure 10b.

Summary: Summary of the 1979 aerobic heterotroph data using geometric means

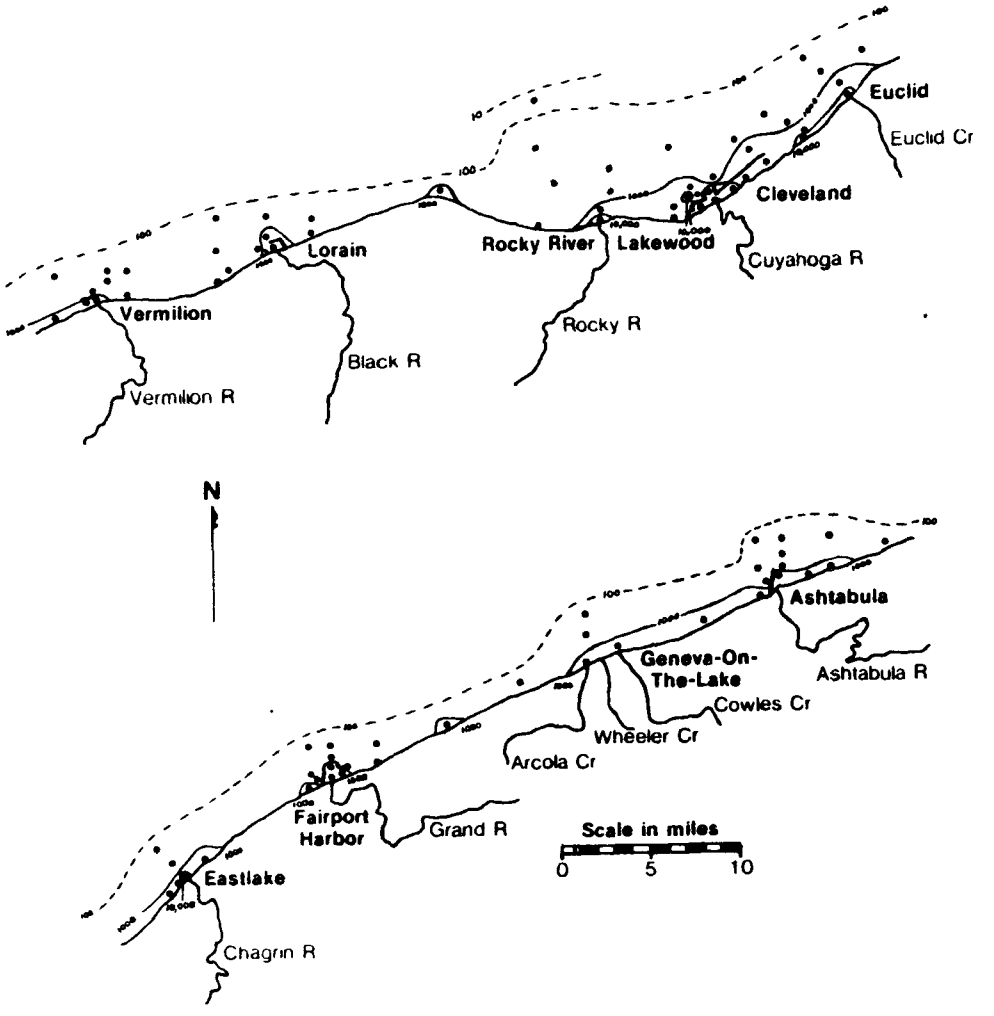


Figure 11a.
Summary of the 1978 fecal coliform data using geometric means

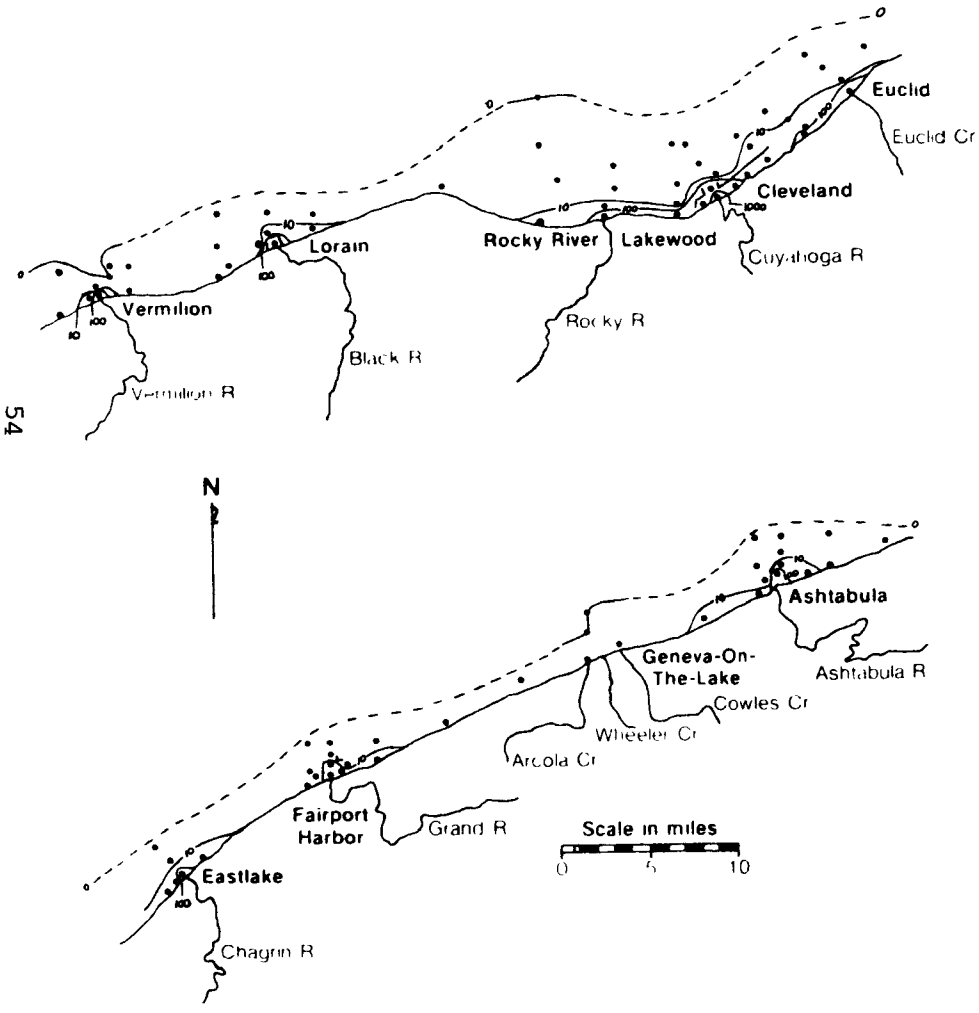


Figure 11b.
Summary of 1979 fecal coliform data using geometric means

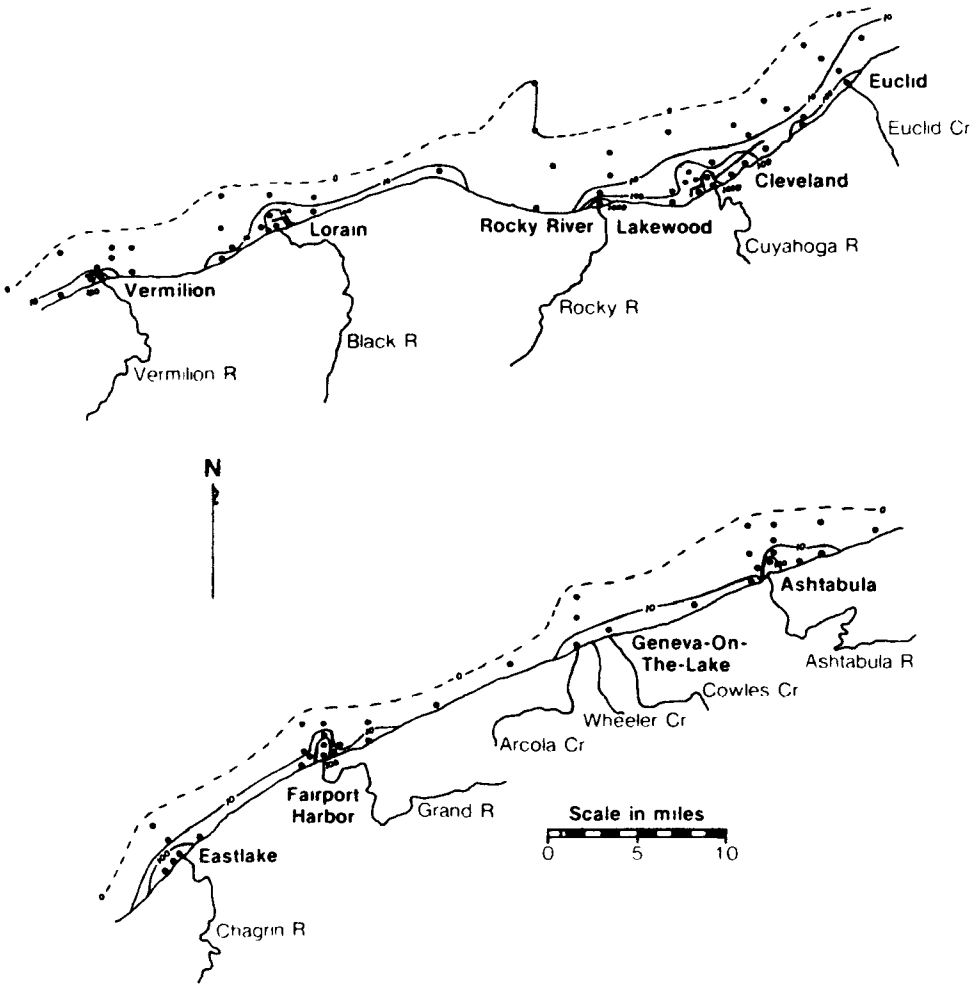


Figure 12a.

Summary: Summary of the 1978 fecal streptococcus data using geometric means

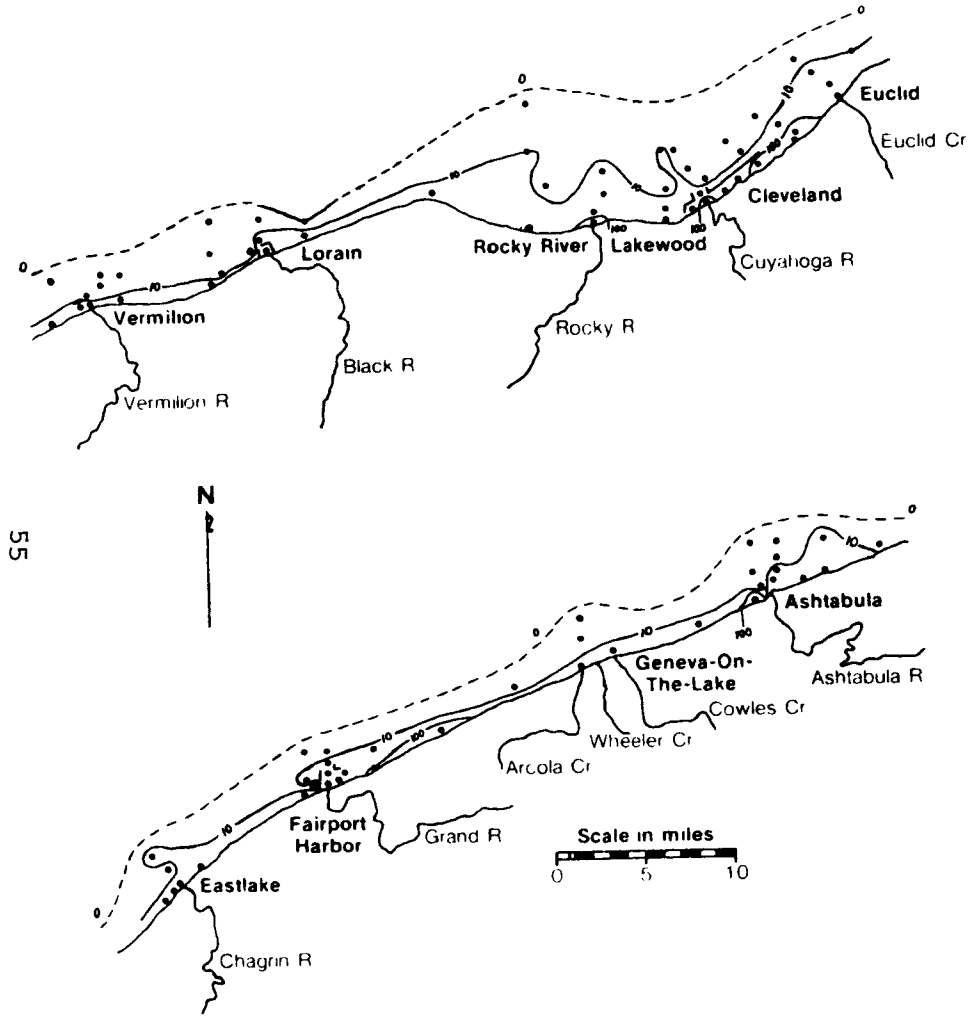


Figure 12b.

Summary: Summary of the 1979 fecal streptococcus data using geometric means

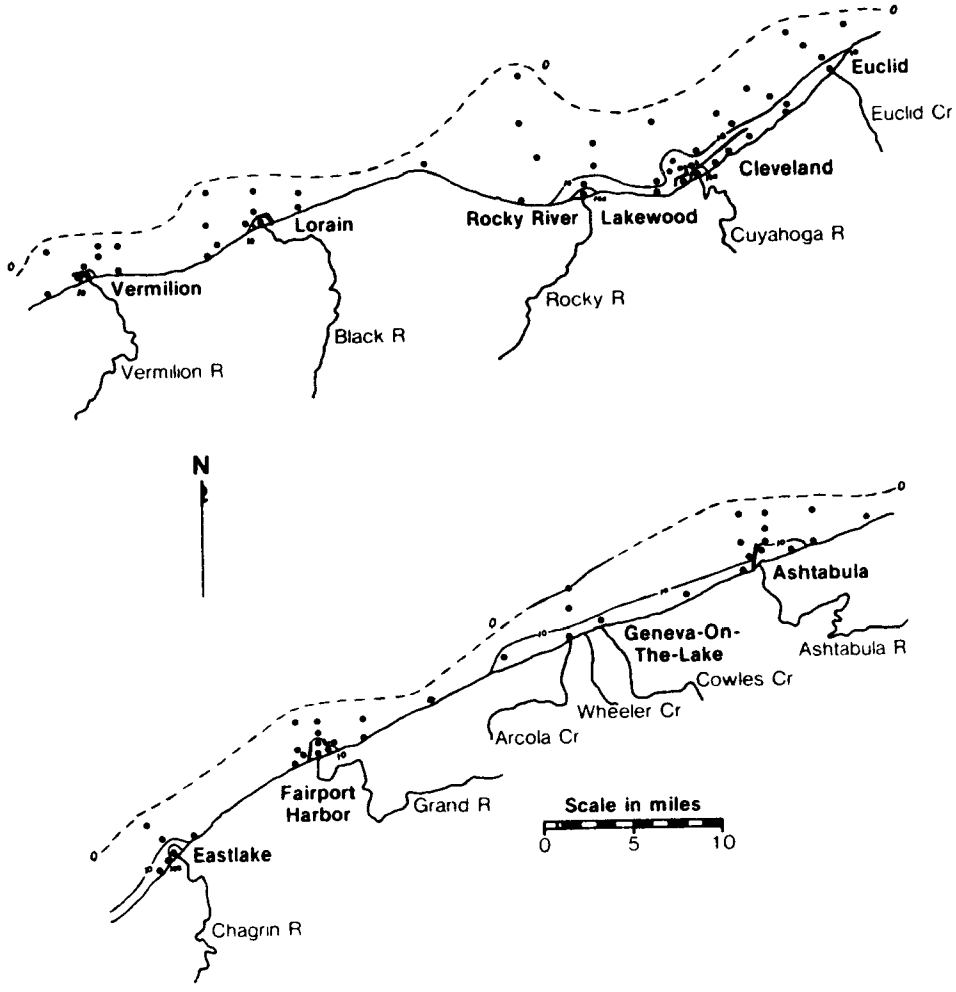
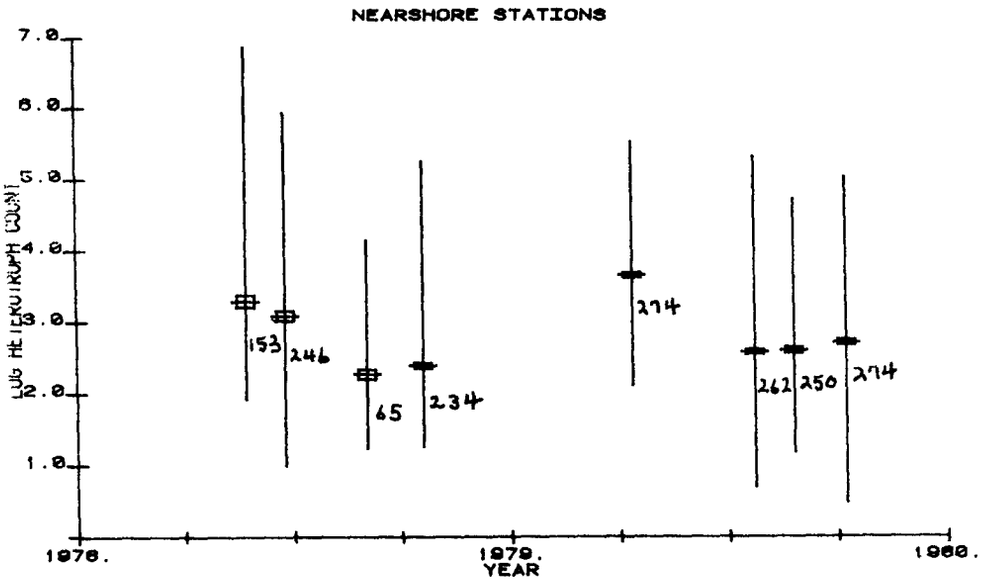
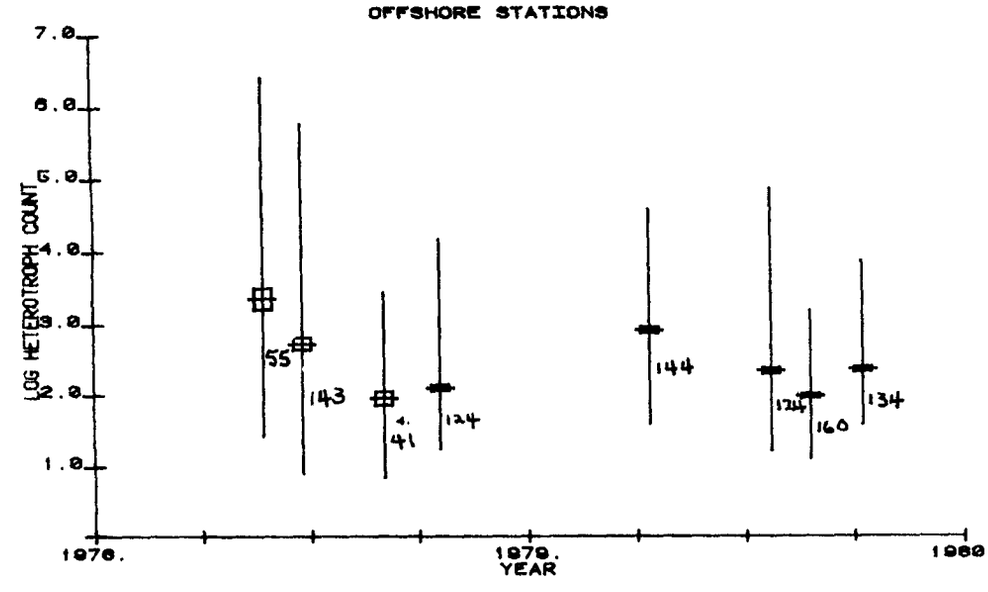


Figure 13. Cruise to cruise patterns for aerobic heterotrophs.





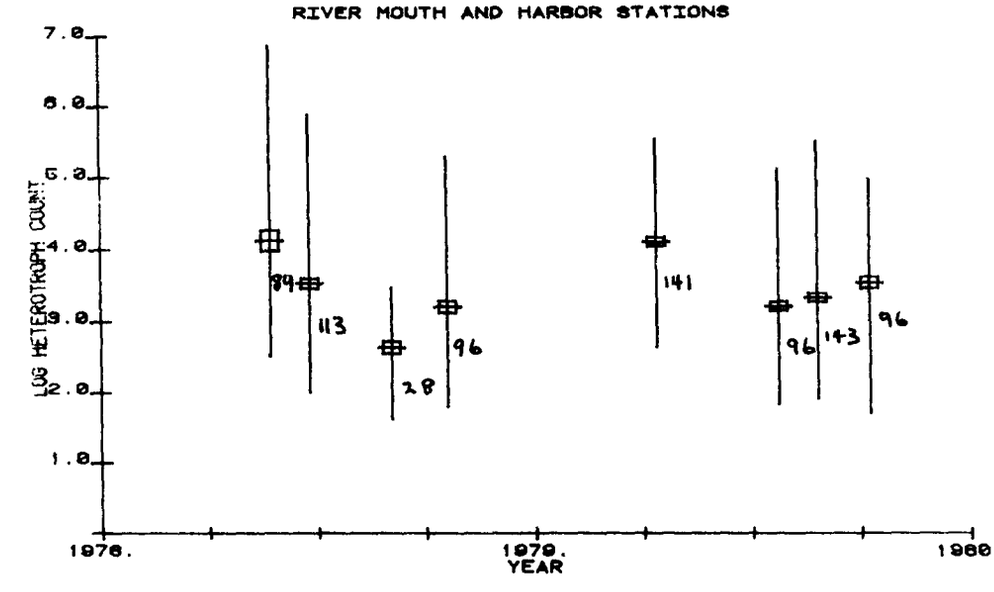
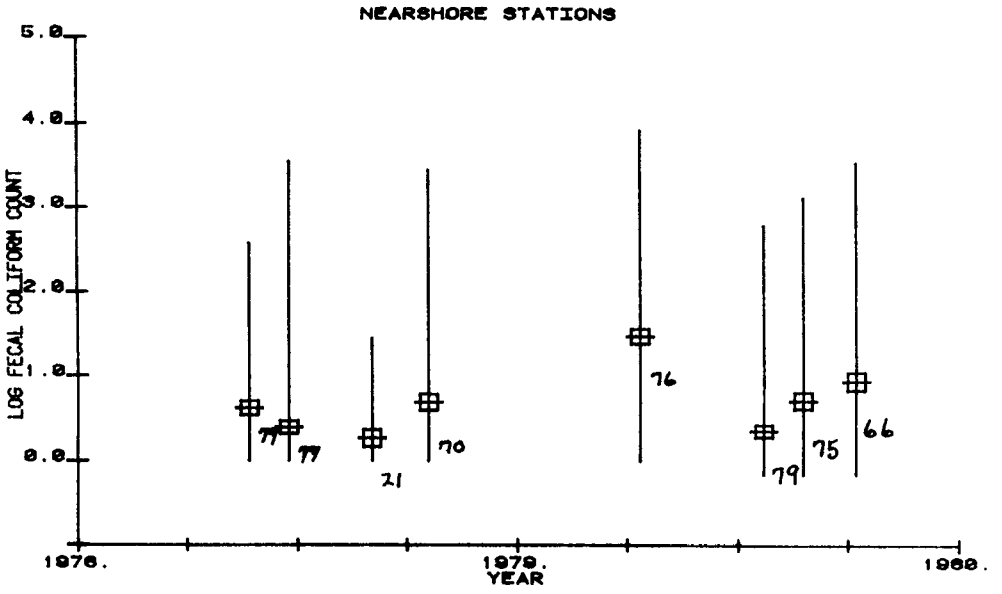
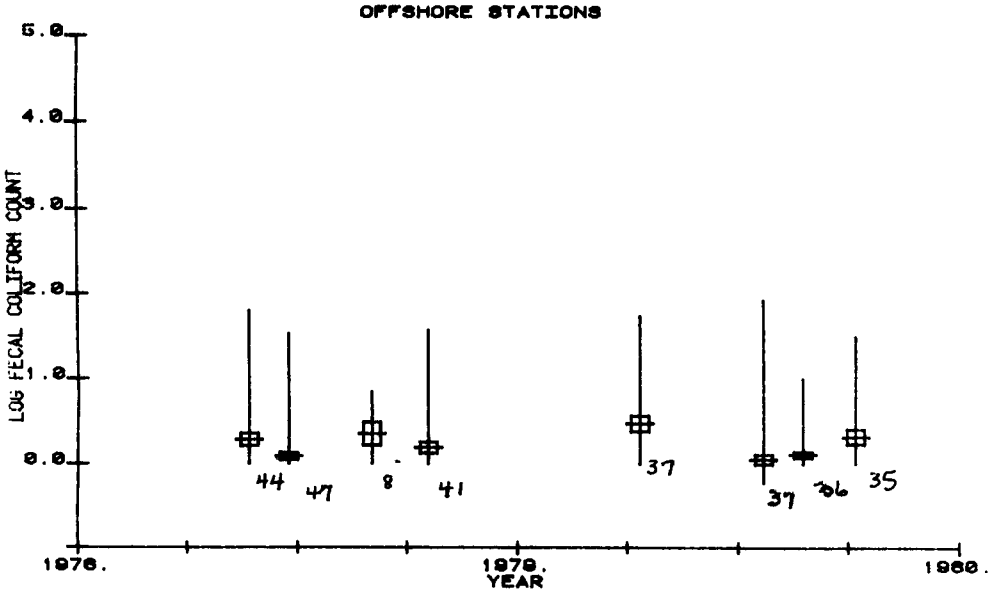


Figure 14. Cruise to cruise patterns for fecal coliforms.





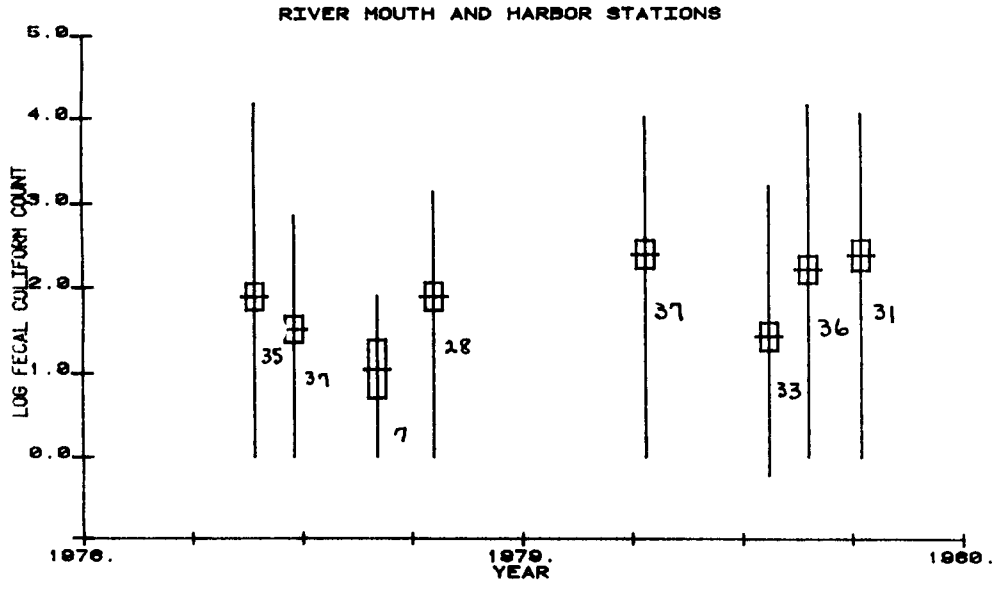
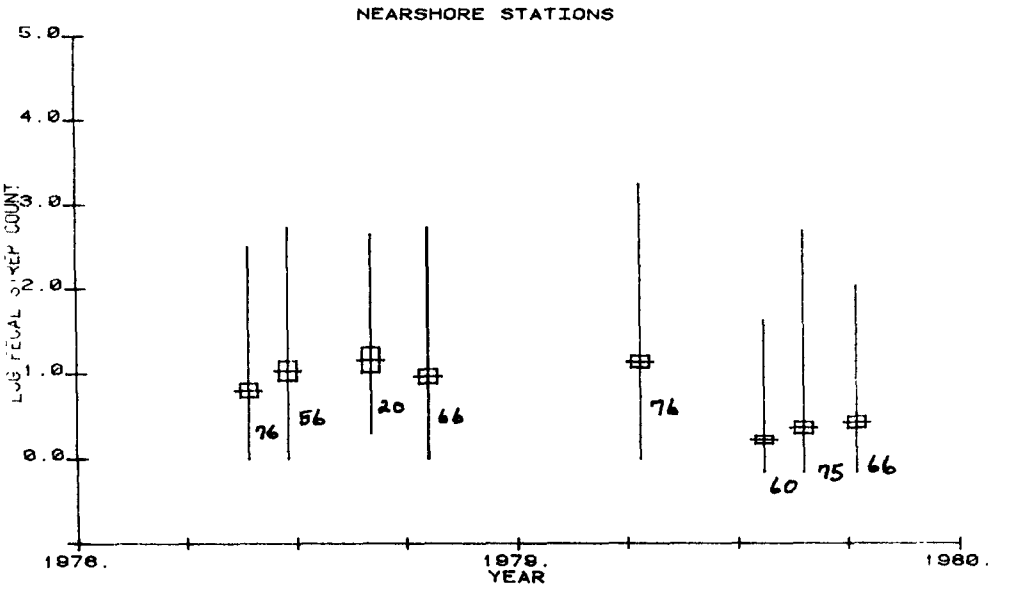
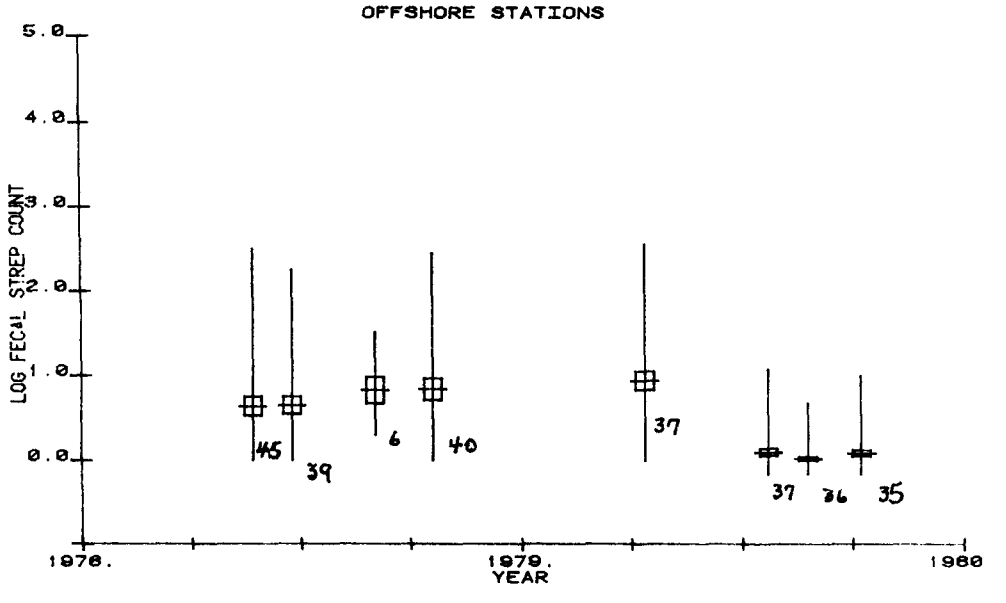


Figure 15. Cruise to cruise patterns for fecal streptococci.





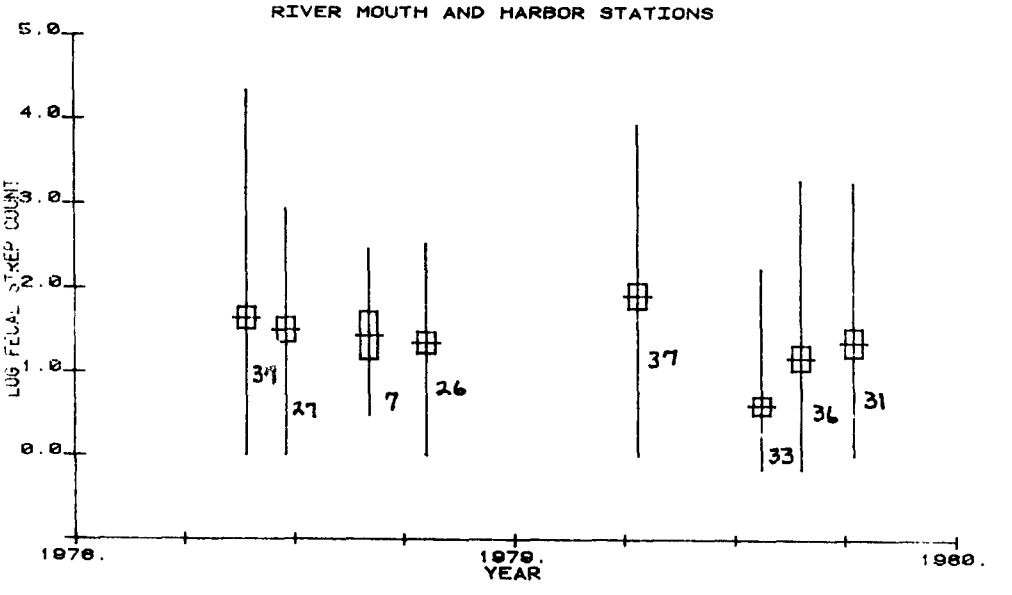
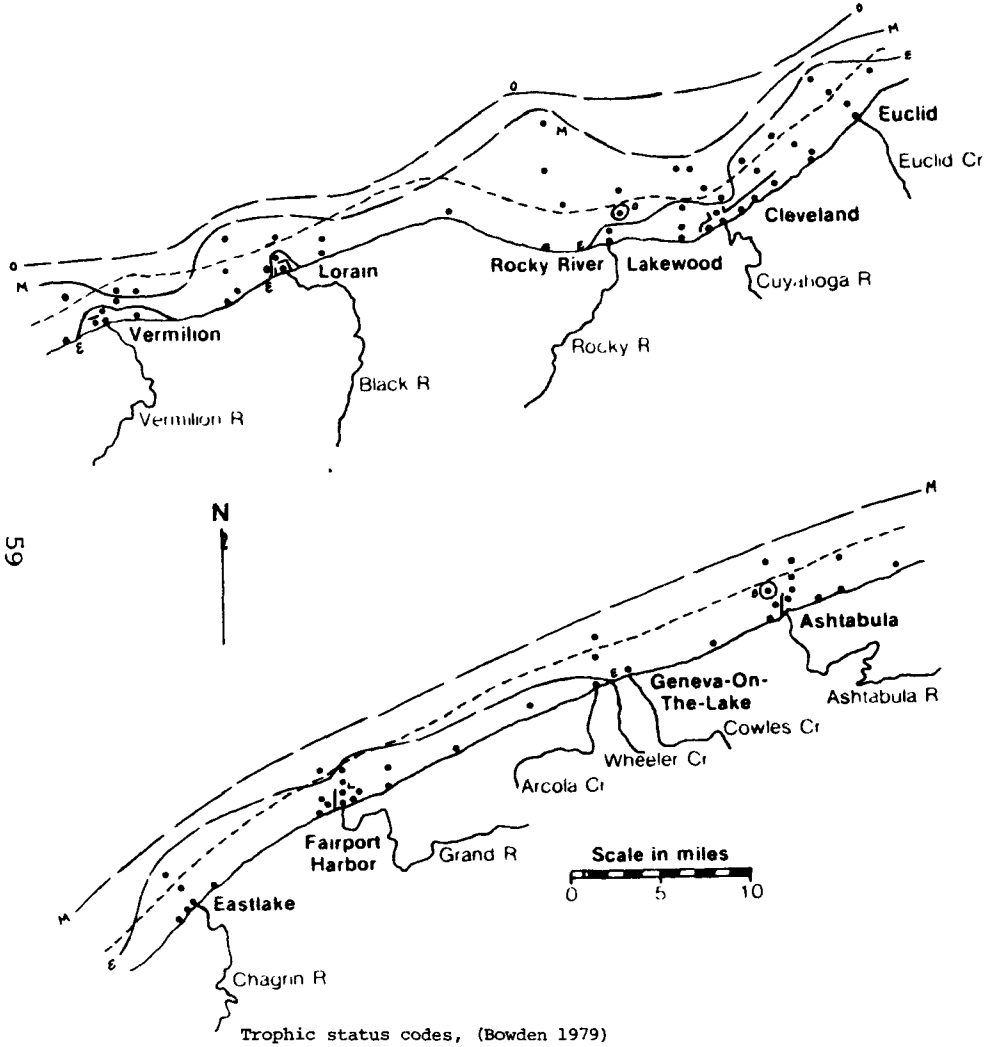


Figure 16.

Summary of the 1978 Central Basin trophic status using geometric means of aerobic heterotroph data



Trophic status codes, (Bowden 1979)

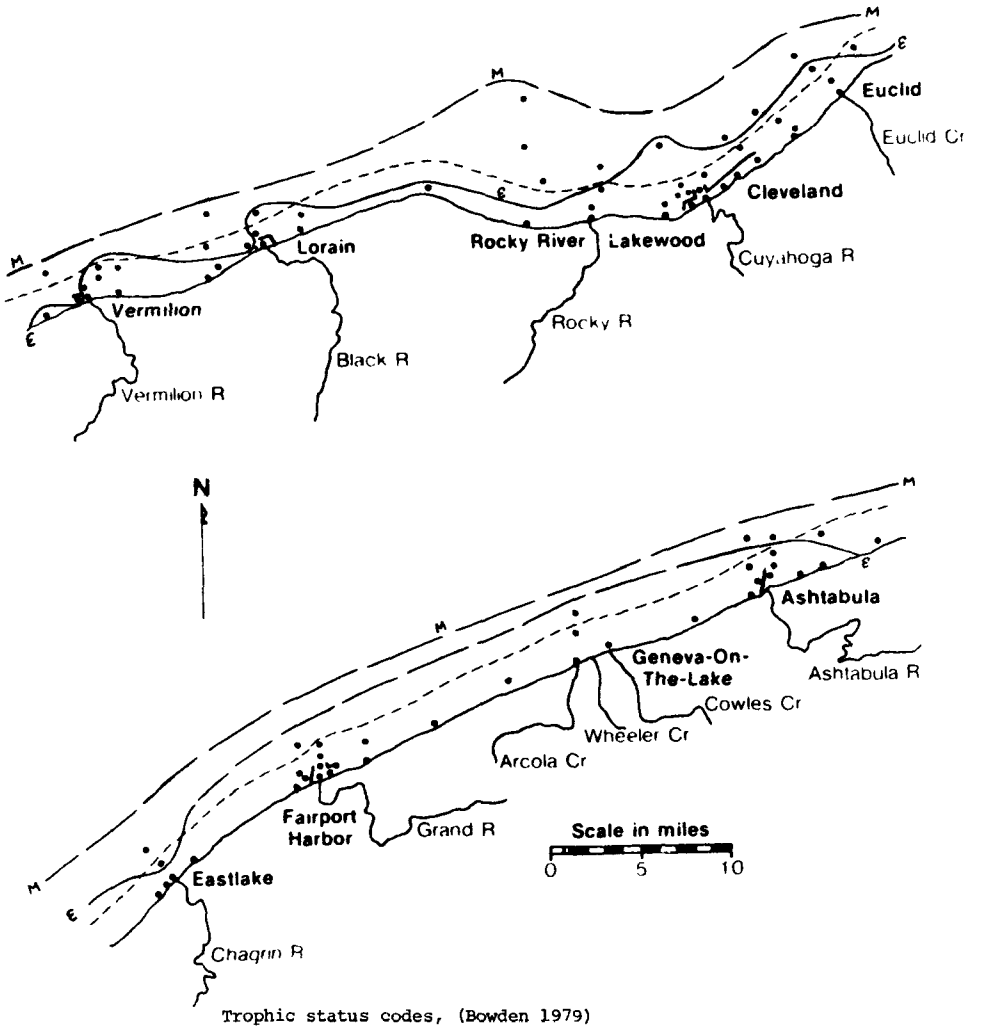
E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 17.

Summary of the 1979 Central Basin trophic status using geometric means of aerobic heterotroph data



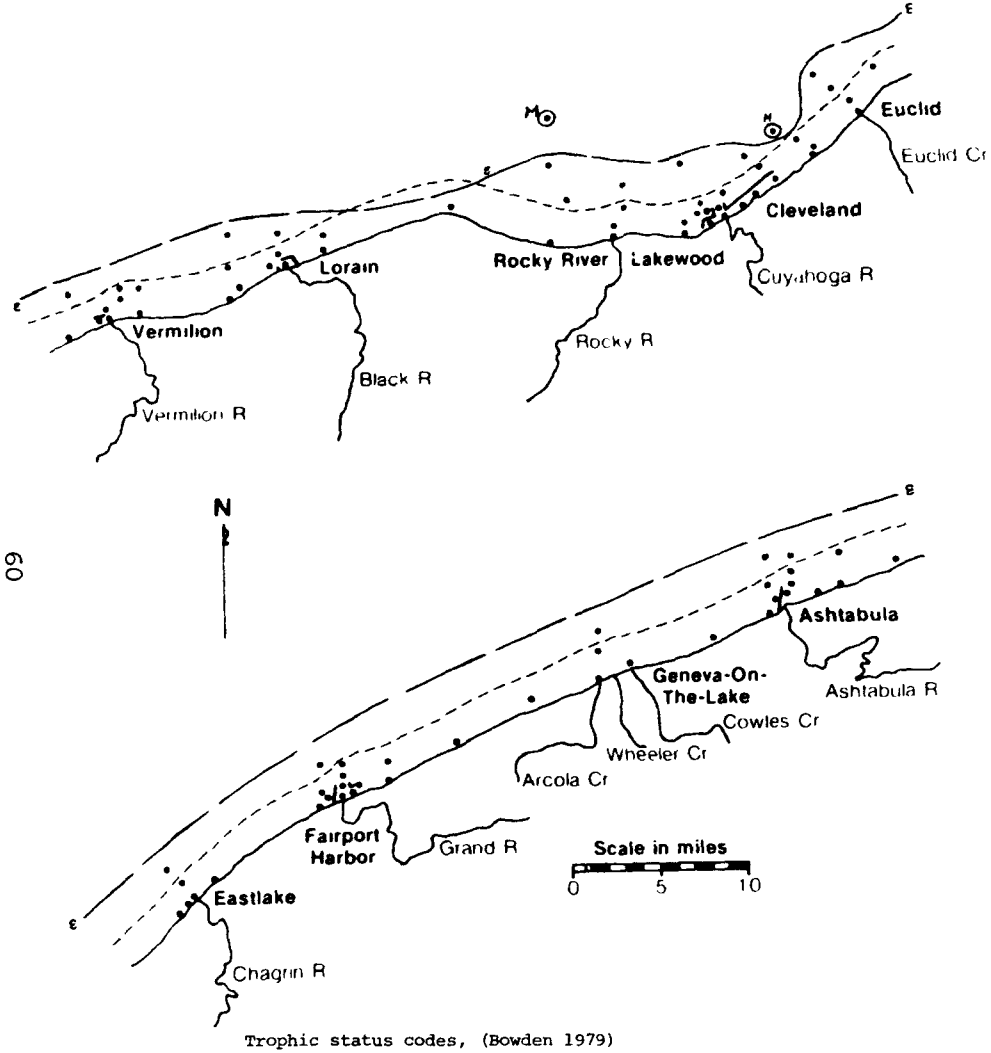
Trophic status codes, (Bowden 1979)

E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 18a.
Cruise 1, 1979, trophic status isopleth map using aerobic heterotroph data



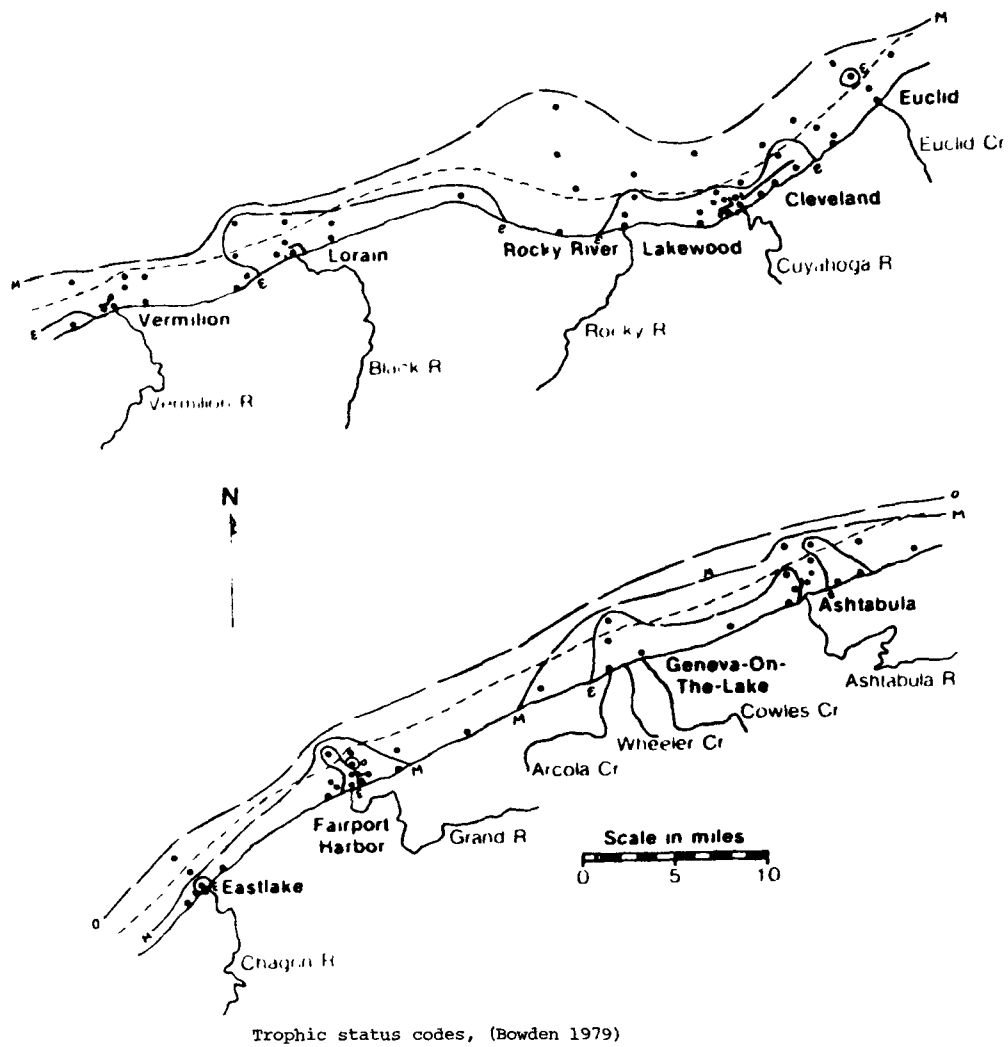
Trophic status codes, (Bowden 1979)

E = eutrophic: stations <3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations <3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 18b.
Cruise 2, 1979, trophic status isopleth map using aerobic heterotroph data



Trophic status codes, (Bowden 1979)

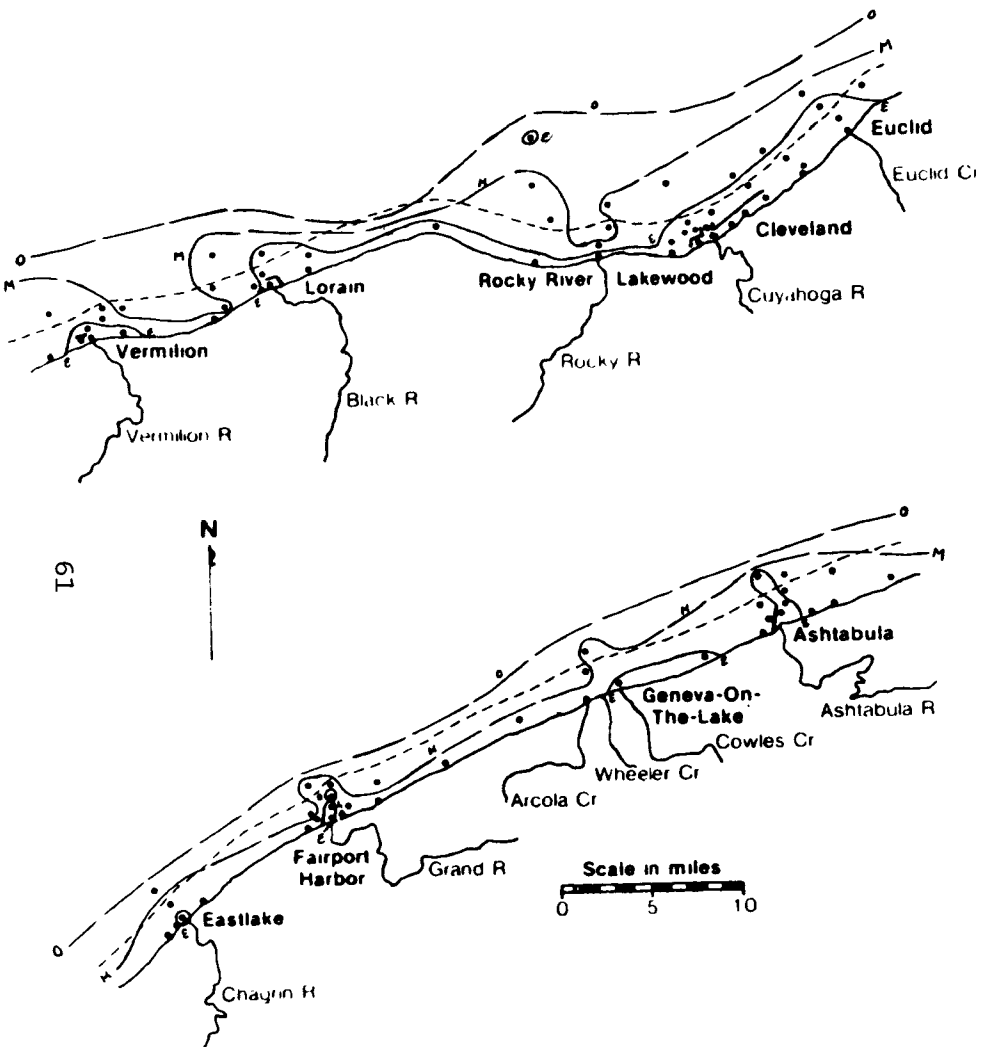
E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, < 20 bacteria/ml.

Figure 18c.

Cruise 3, 1979, trophic status isopleth map using aerobic heterotroph data



Trophic status codes, (Bowden 1979)

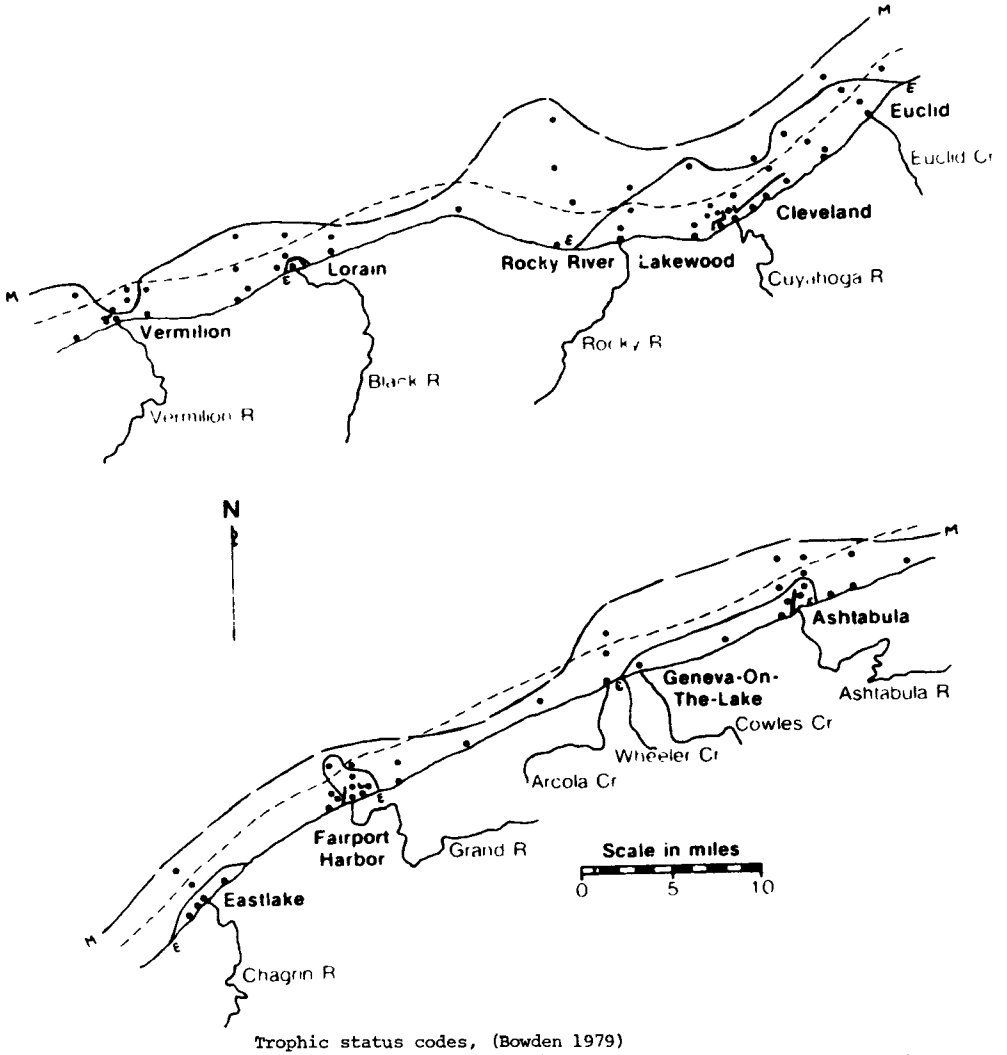
E = eutrophic: stations <3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations <3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥3.3km from shore, 20< M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml

Figure 18d.

Cruise 4, 1979, tropic status isopleth map using aerobic heterotroph data



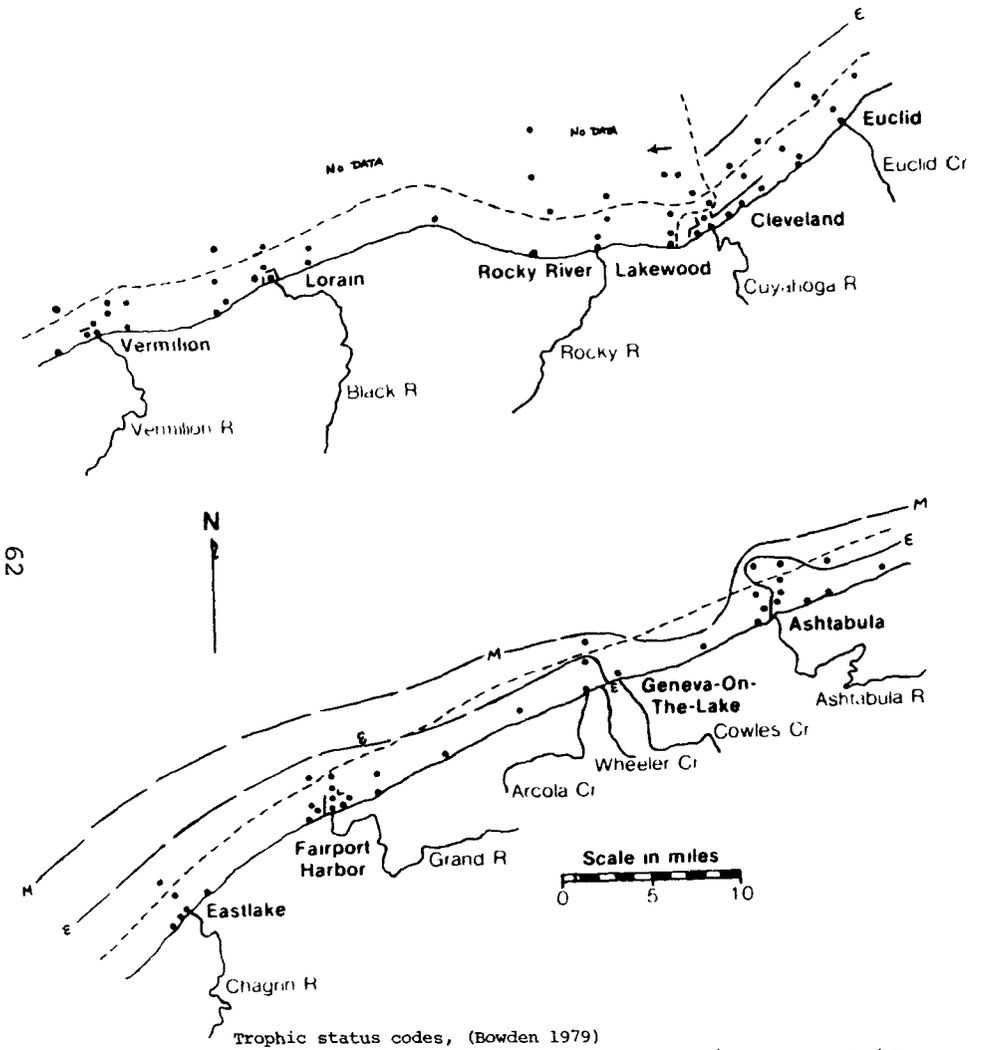
Trophic status codes, (Bowden 1979)

E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120< M < 2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 <M< 200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 19a.
Cruise 1, 1978, trophic status isopleth map using aerobic heterotroph data

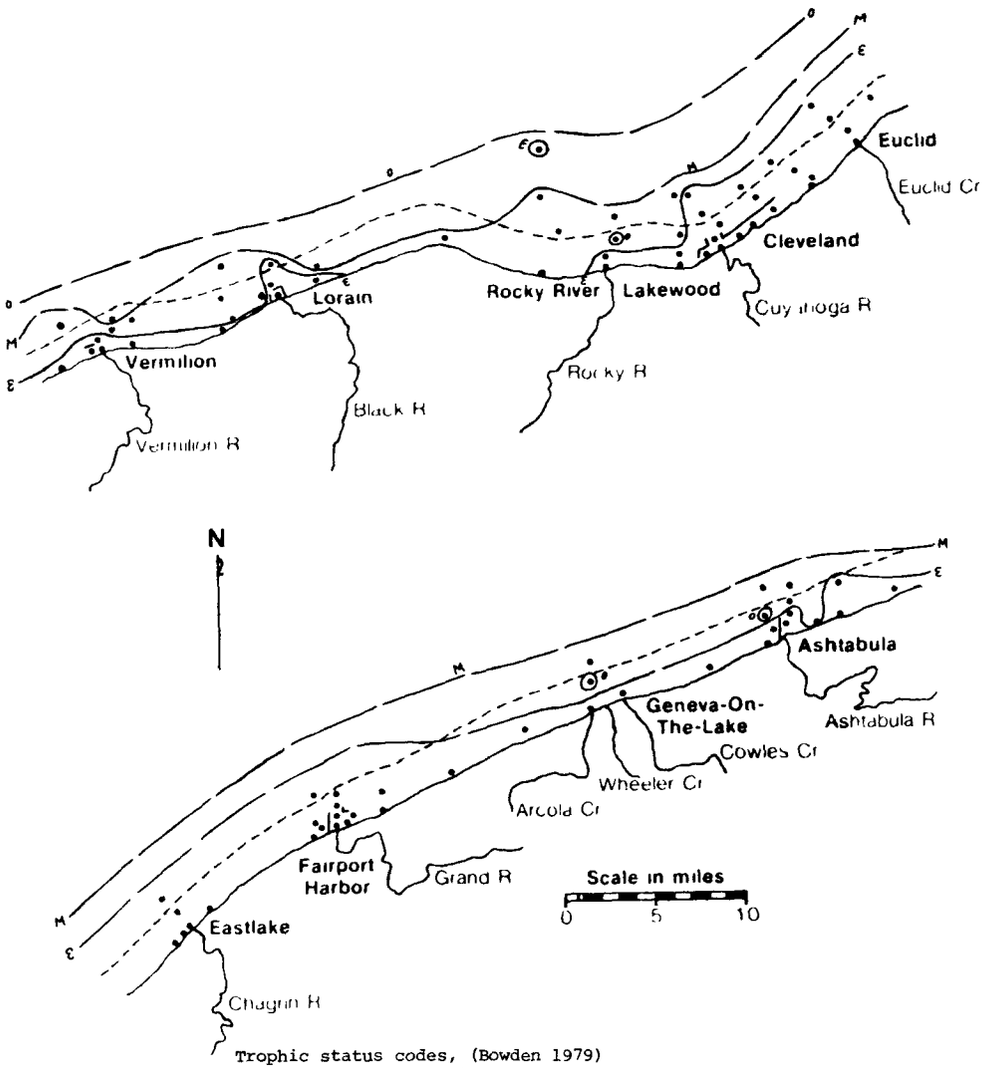


Trophic status codes, (Bowden 1979)
E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml,
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 19b.
Cruise 2, 1978, trophic status isopleth map using aerobic heterotroph data



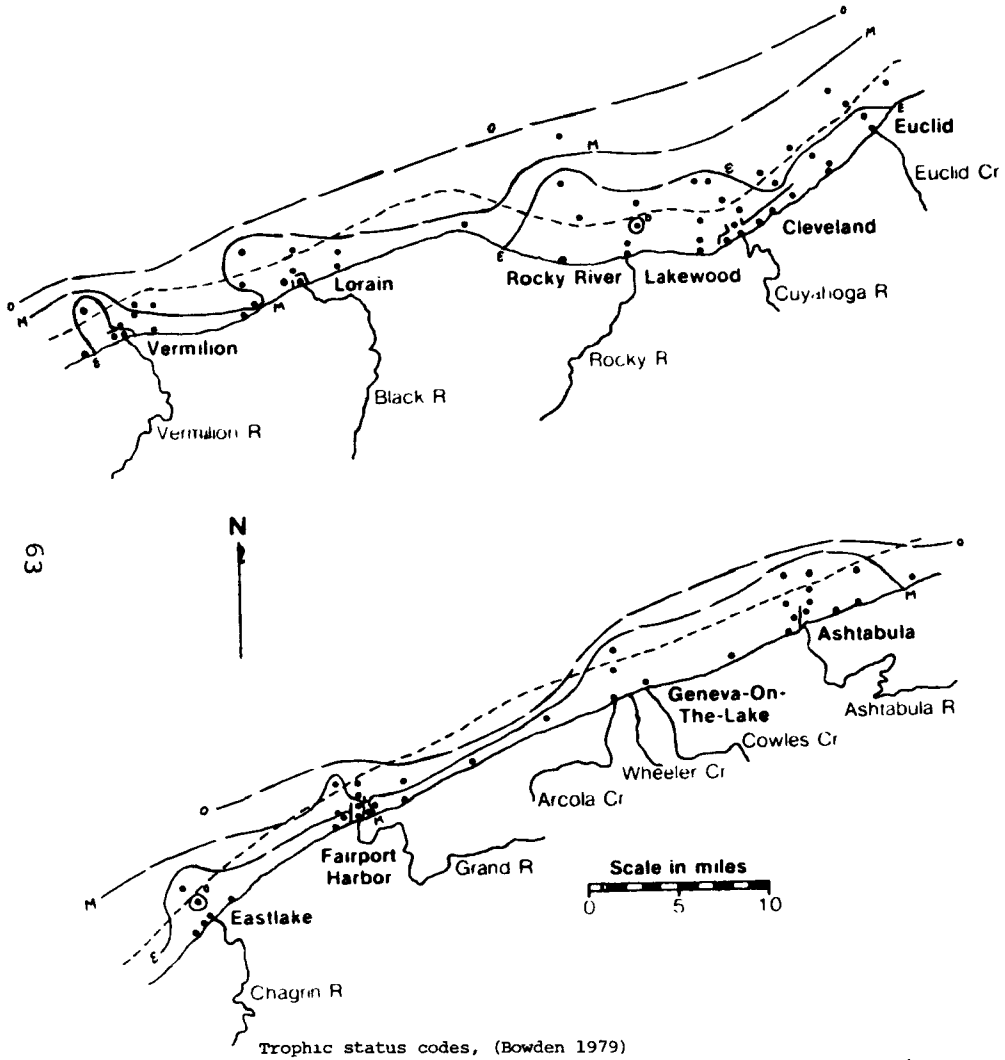
Trophic status codes, (Bowden 1979)
E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M< 2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 19c.

Cruise 3, 1978, trophic status isopleth map using aerobic heterotroph data



Trophic status codes, (Bowden 1979)

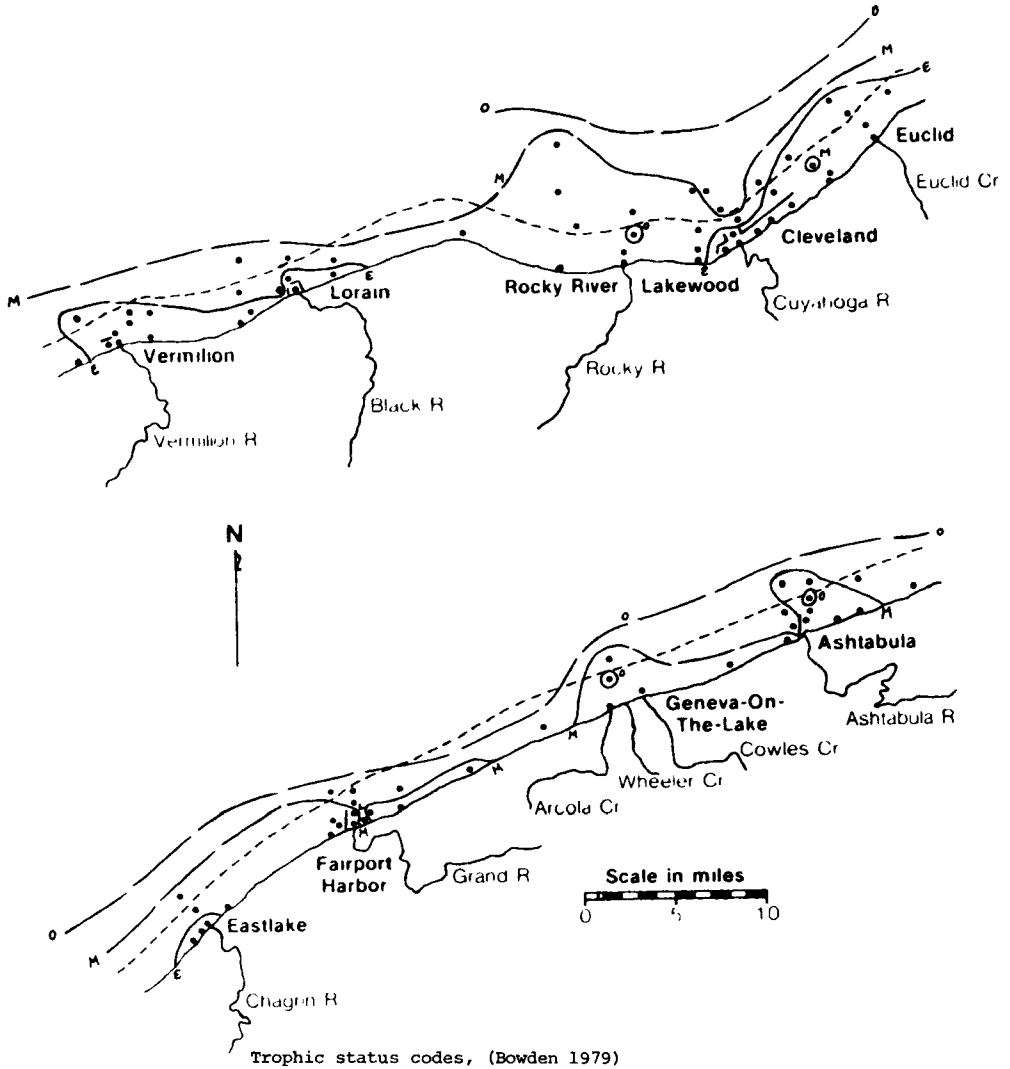
E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 < M <2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 < M <200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

Figure 19d.

Cruise 4, 1978, trophic status isopleth map using aerobic heterotroph data



Trophic status codes, (Bowden 1979)

E = eutrophic: stations < 3.3km from shore, ≥ 2000 bacteria/ml;
stations ≥ 3.3km from shore, ≥ 200 bacteria/ml.

M = mesotrophic: stations < 3.3km from shore, 120 <M< 2000 bacteria/ml;
stations ≥ 3.3km from shore, 20 <M< 200 bacteria/ml.

O = oligotrophic: stations < 3.3km from shore, ≤ 120 bacteria/ml;
stations ≥ 3.3km from shore, ≤ 20 bacteria/ml.

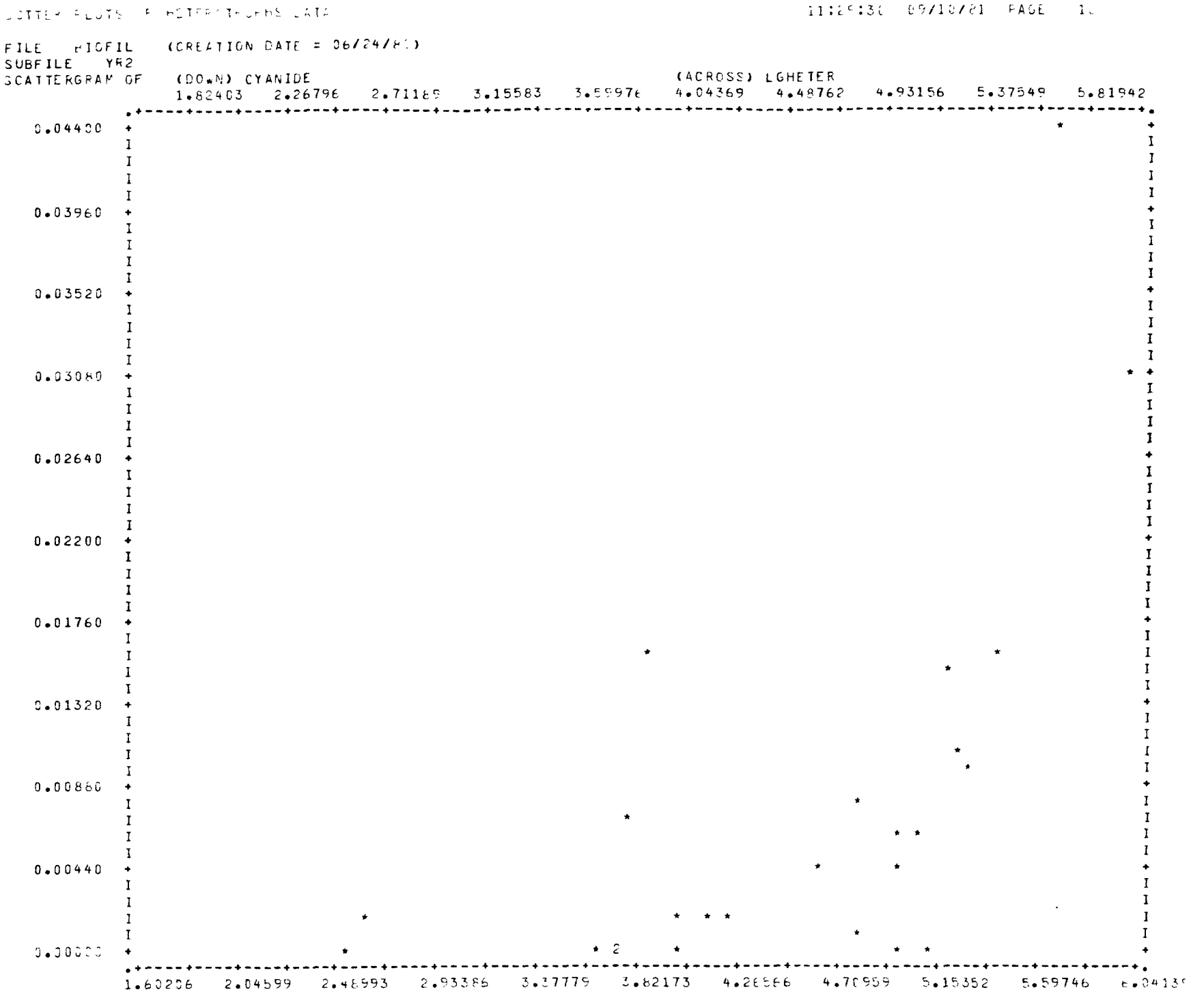


Figure 20. Relationship

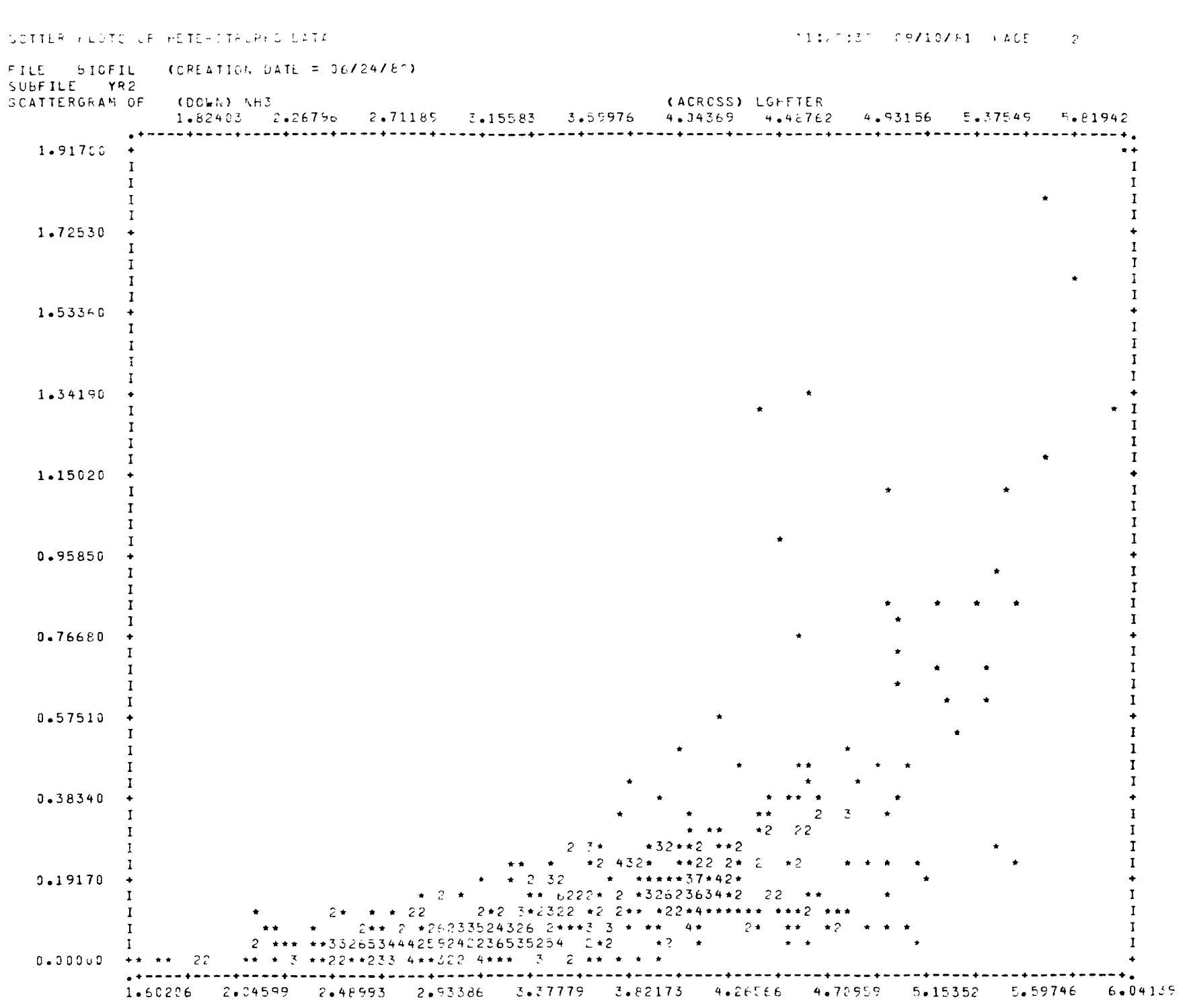
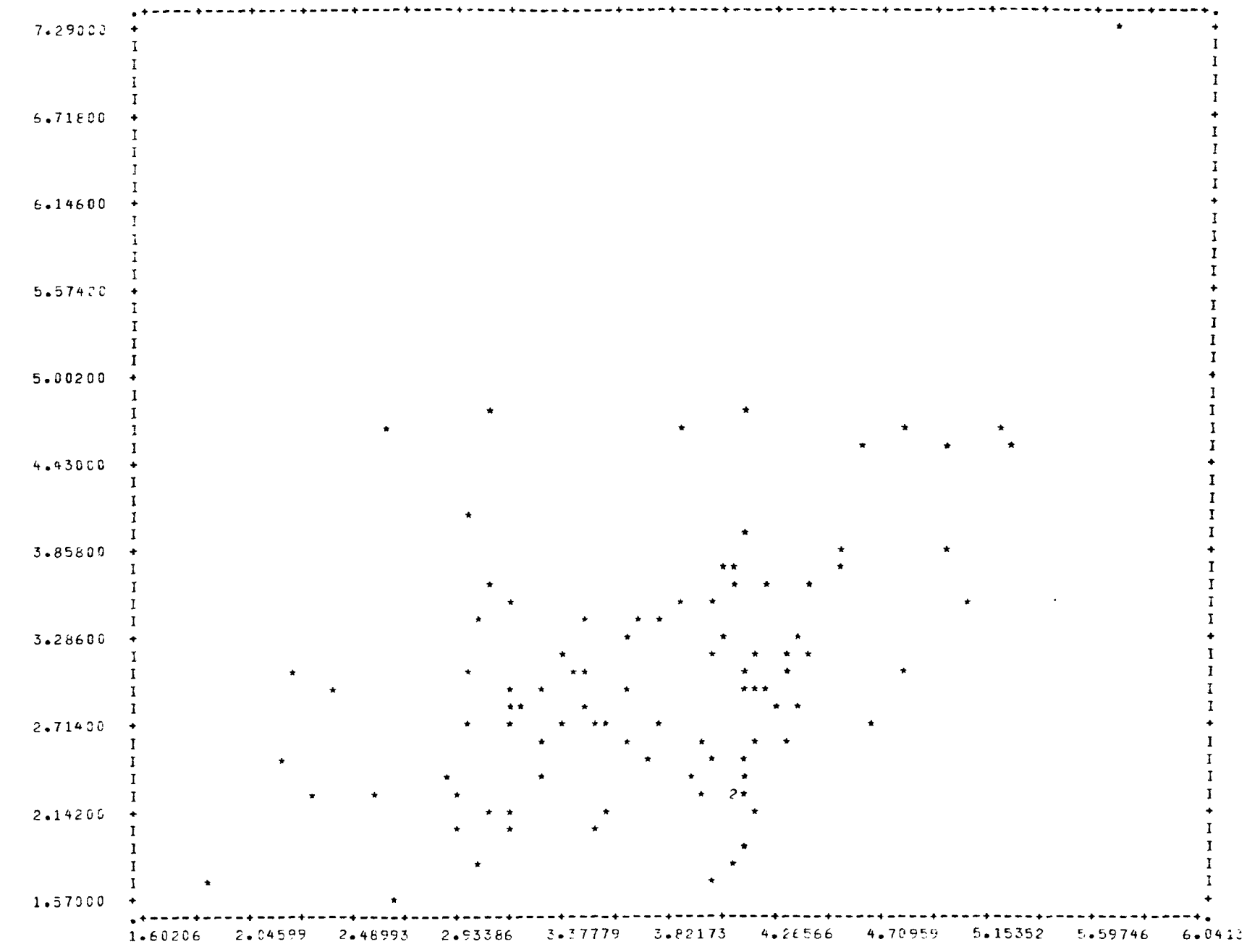


Figure 21. Relationship between log heterotroph concentrations and ammonia concentrations.

FILE BIOFIL (CREATION DATE = 06/24/80)
SUBFILE YR2
SCATTERGRAM OF (DOWN) TOC (ACROSS) LGHETER



FILE BIOFIL (CREATION DATE = 06/24/80)
SUBFILE YR2
SCATTERGRAM OF (DOWN) SIO2 (ACROSS) LGHETER

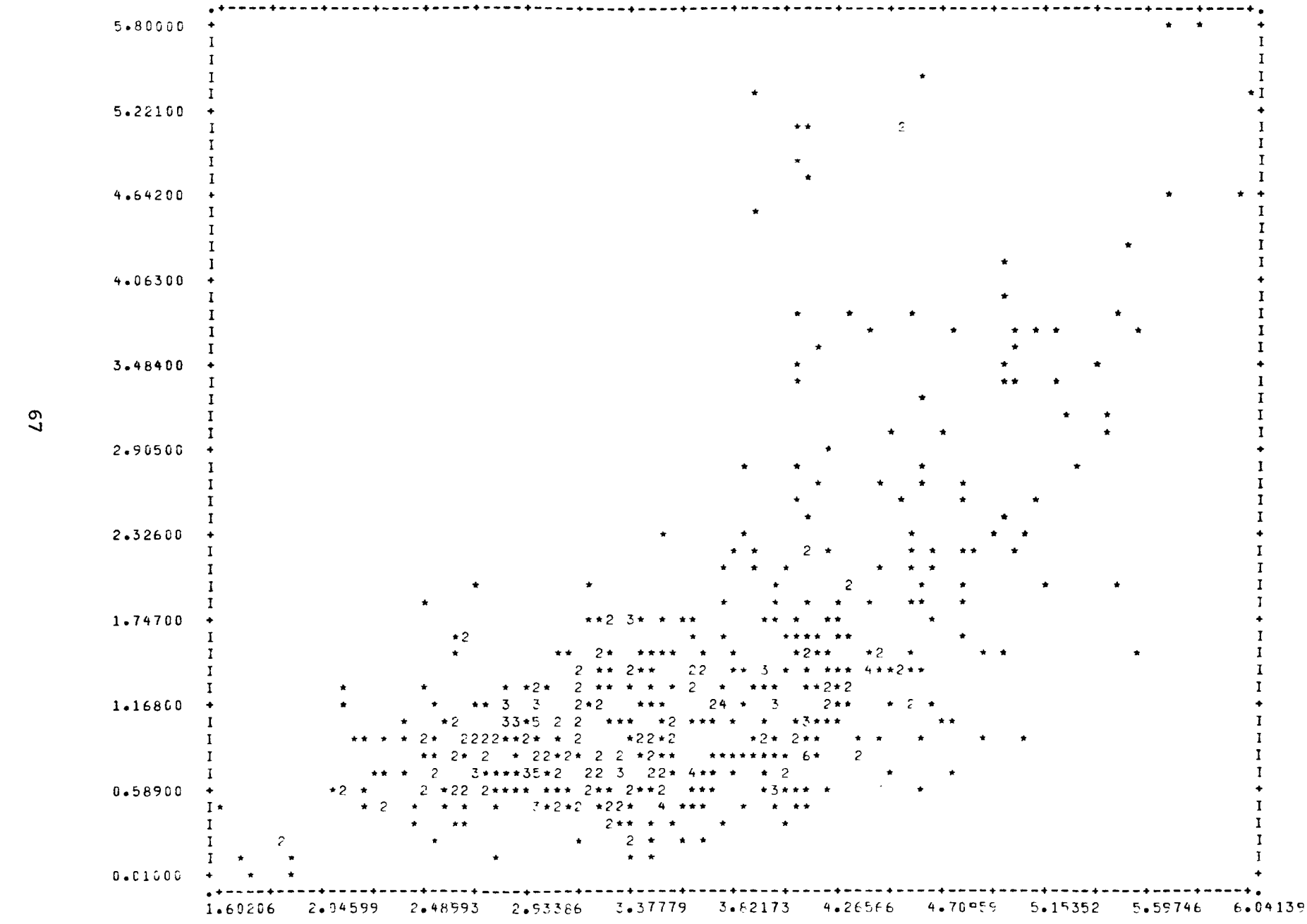
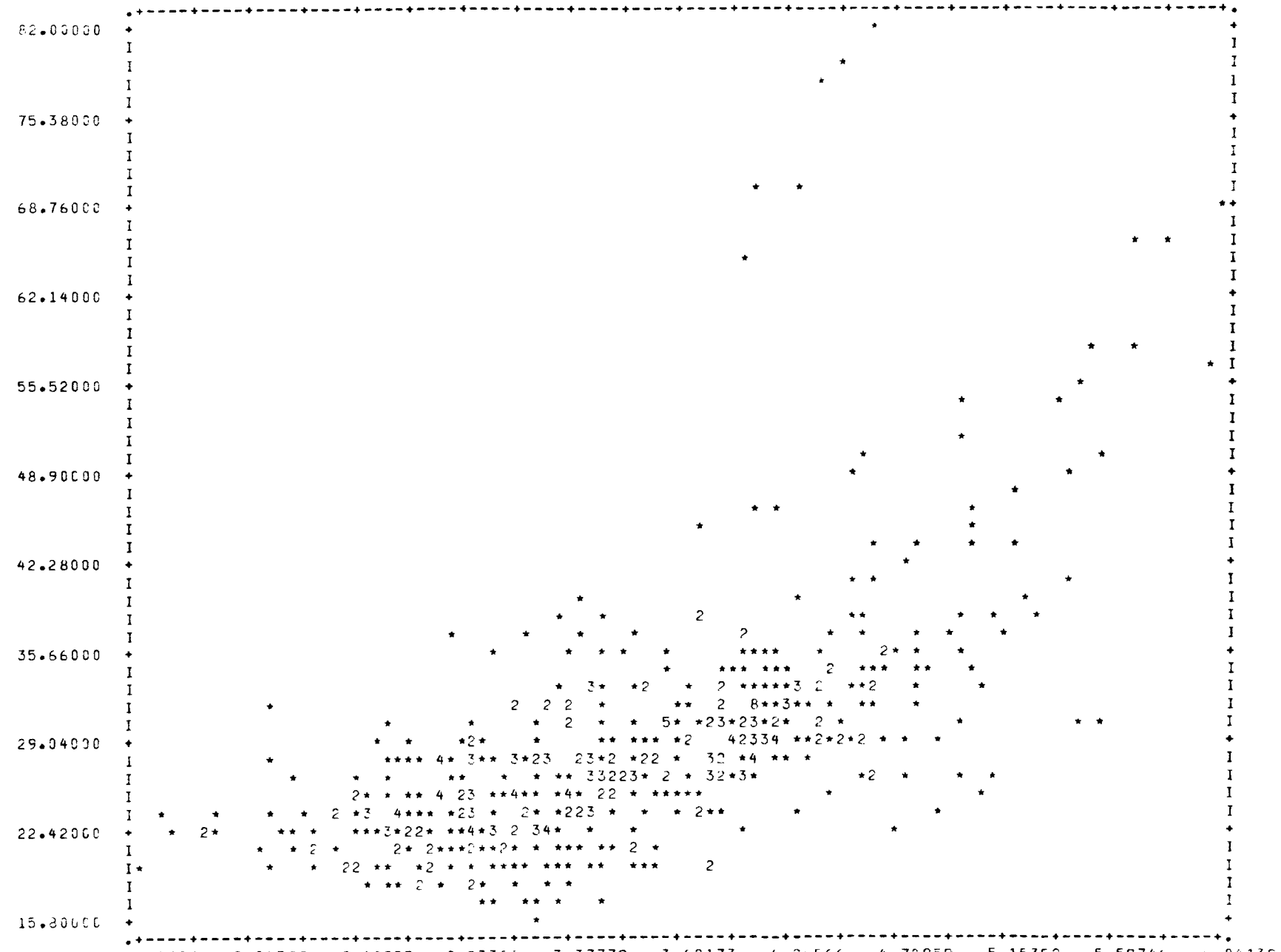


Figure 23. Relationship between log heterotroph concentrations and silicate concentrations.

FILE BIOFIL (CREATION DATE = 06/24/80)
SUBFILE Yr2
SCATTERGRAM OF (DOWN) SO4 (ACROSS) LGHETER



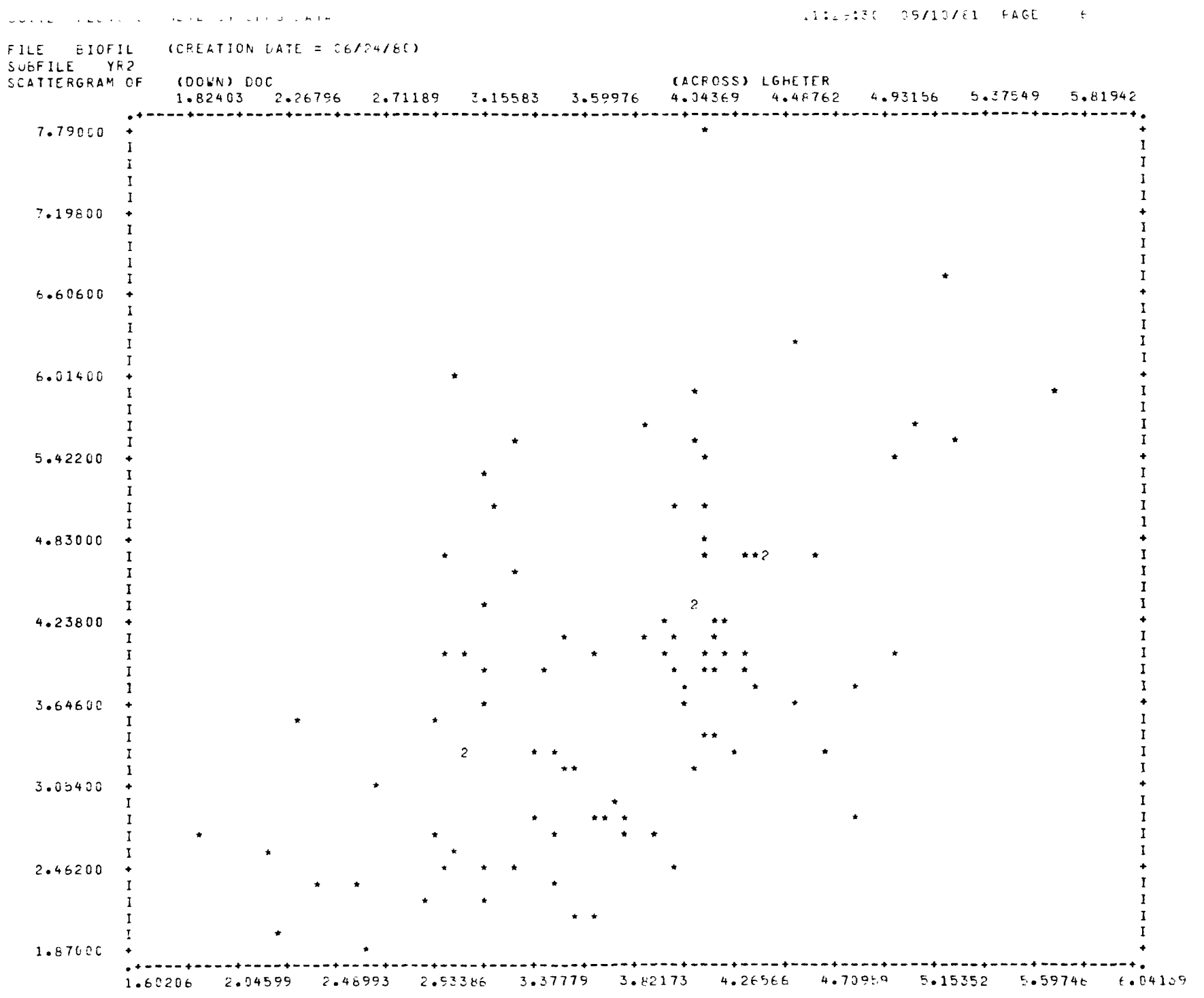
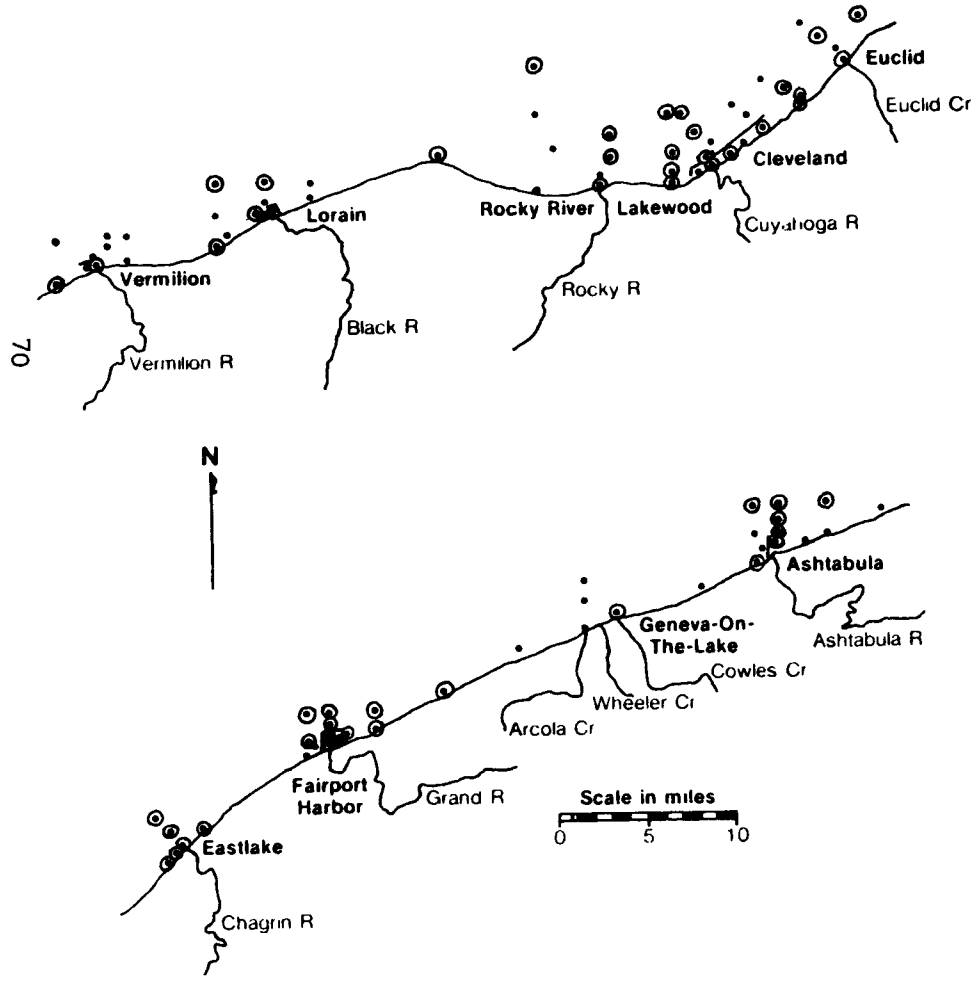


Figure 25. Relationship between log heterotroph concentrations and DOC concentrations.

Figure 26. Application of FC/FS ratio tests to 1978 data.

a. Stations with a fecal streptococcus concentration ≥ 100/100ml, 1978



b. Stations with a fecal coliform/fecal streptococcus ratio ≤ 0.70, 1978

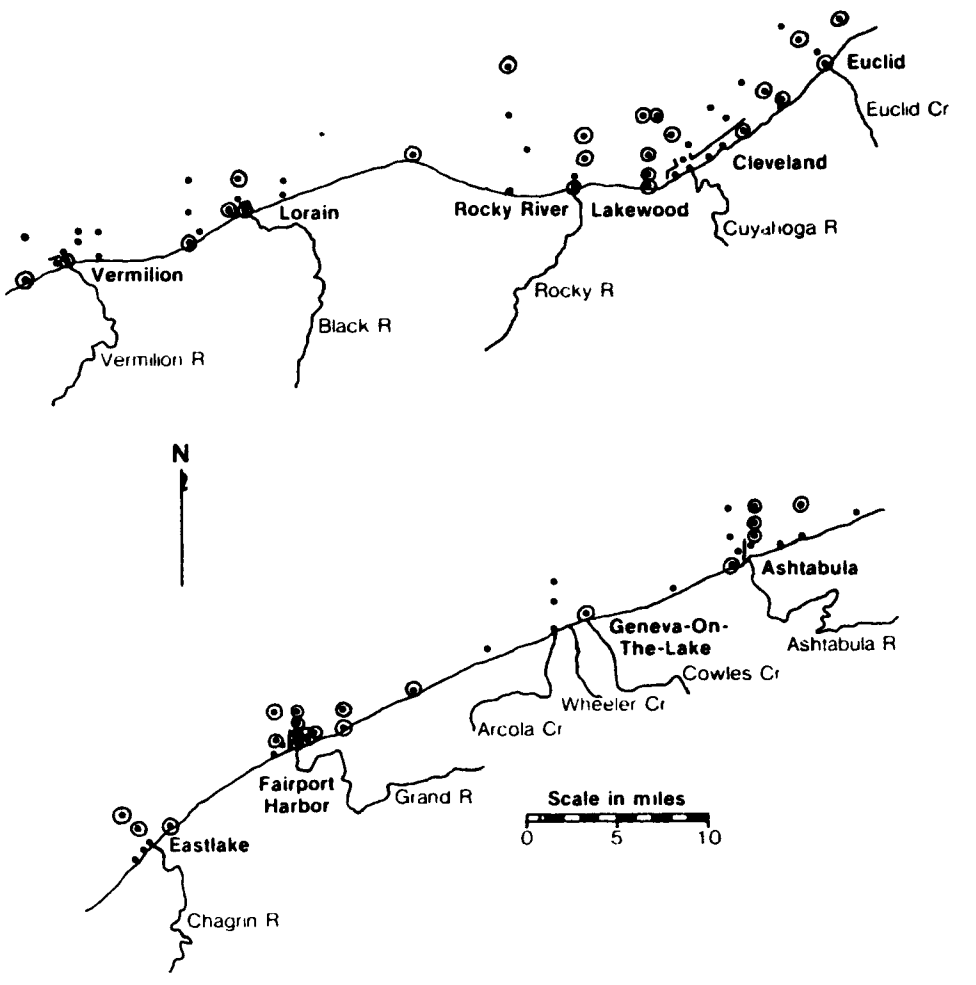
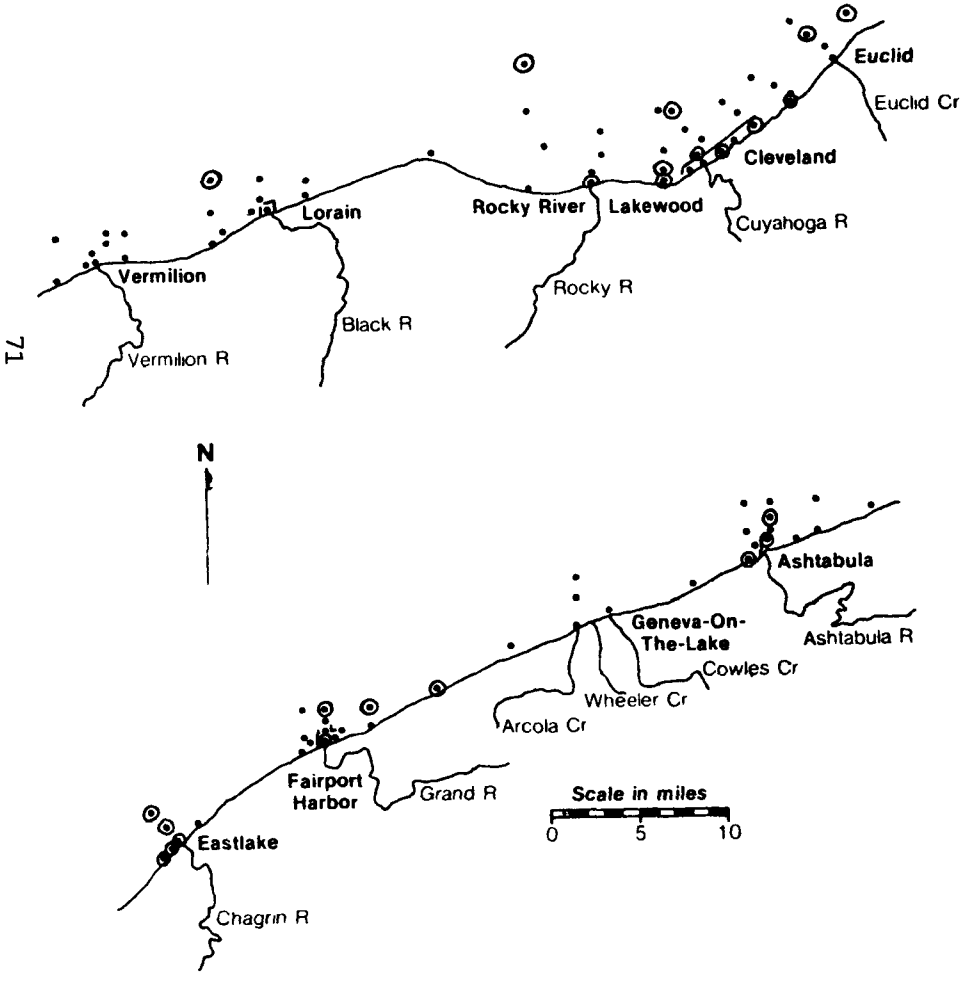


Figure 26. Application of FC/FS ratio tests to 1978 data.

c. Stations with a fecal coliform/fecal streptococcus ratio between 4.0 and 0.70 (4.0 > FC/FS > 0.70), 1978



d. Stations with a fecal coliform/fecal streptococcus ratio ≥ 4.0, 1978

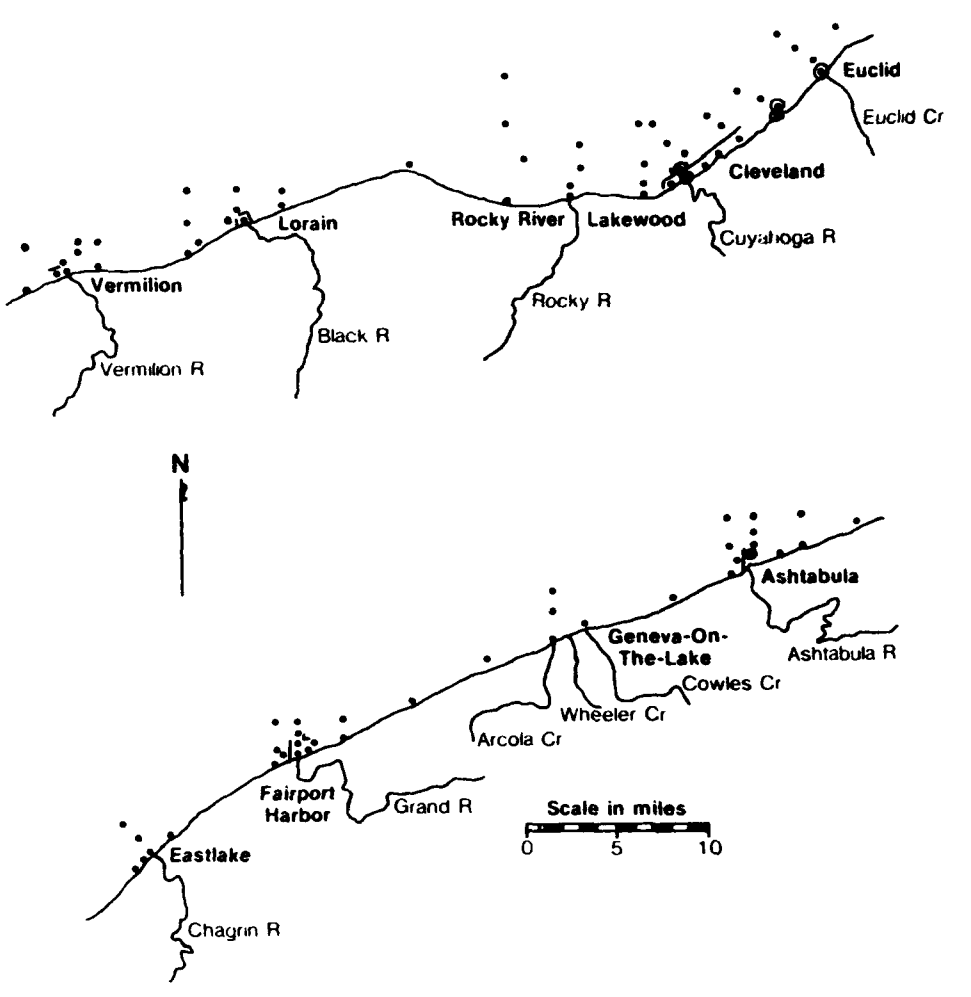
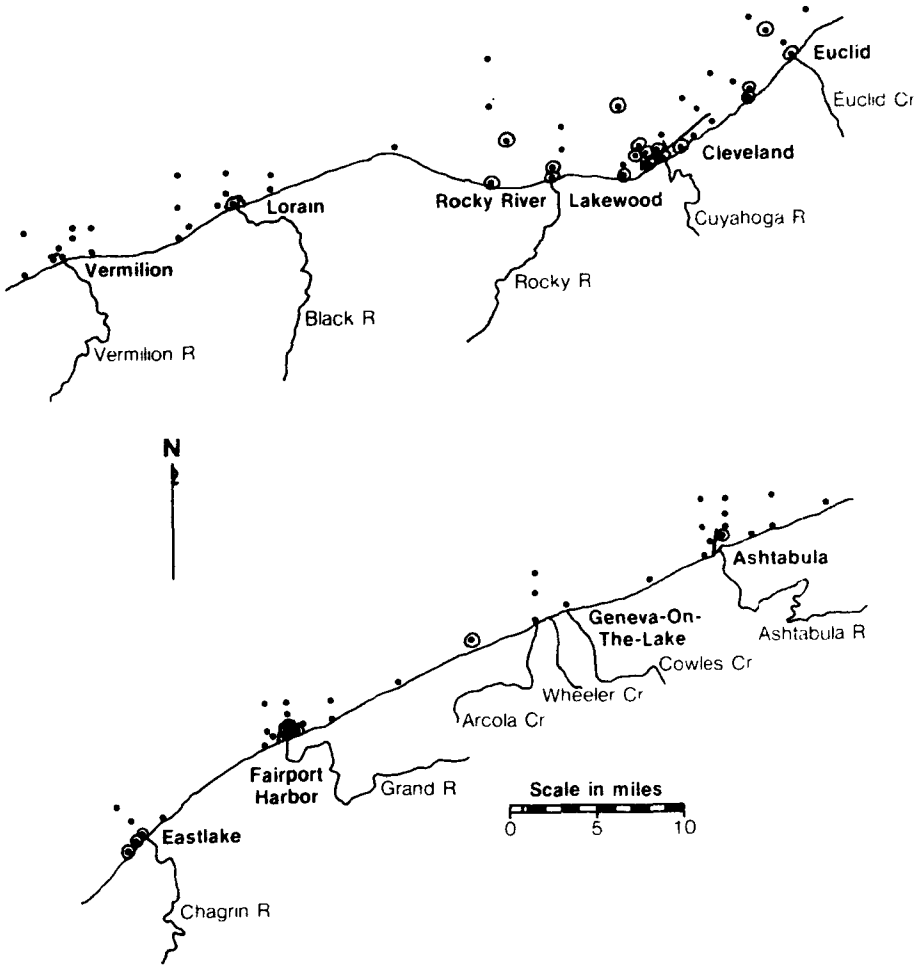


Figure 27. Application of FC/FS ratio tests to 1979 data.

a. Stations with a fecal streptococcus concentration $\geq$ 100/100ml, 1979



b. Stations with a fecal coliform/fecal streptococcus ratio $\leq$ 0.70, 1979

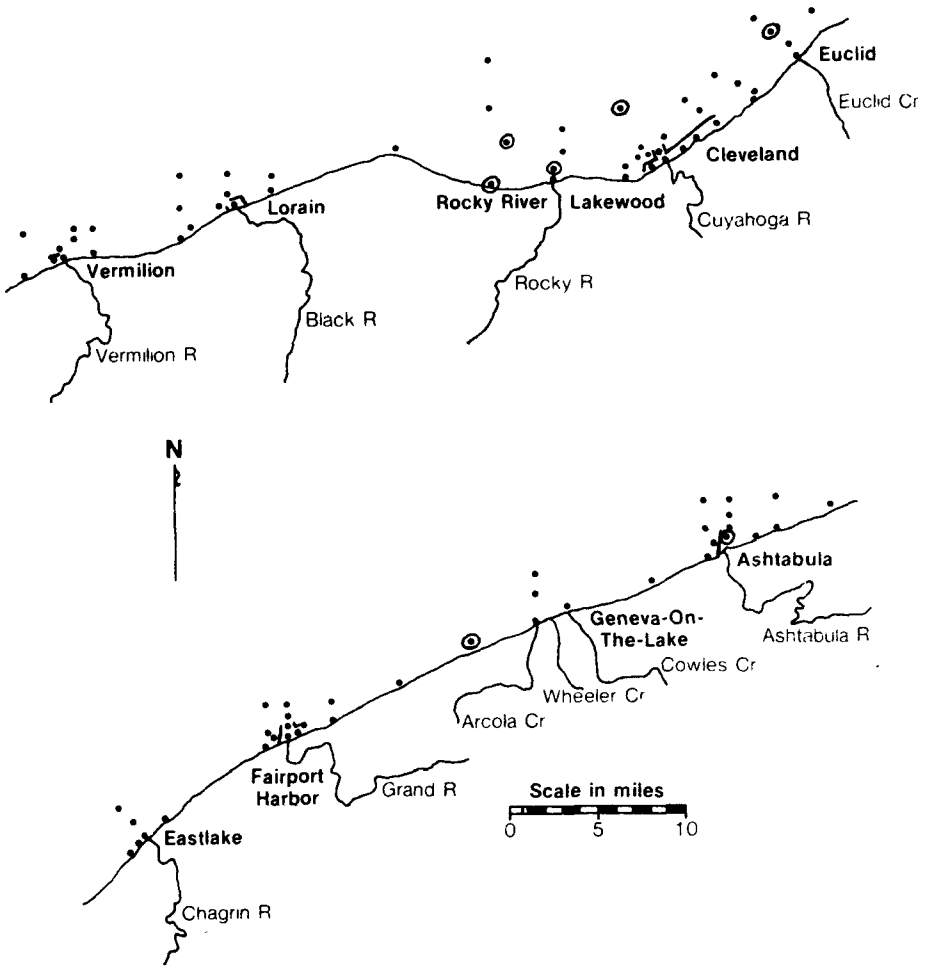
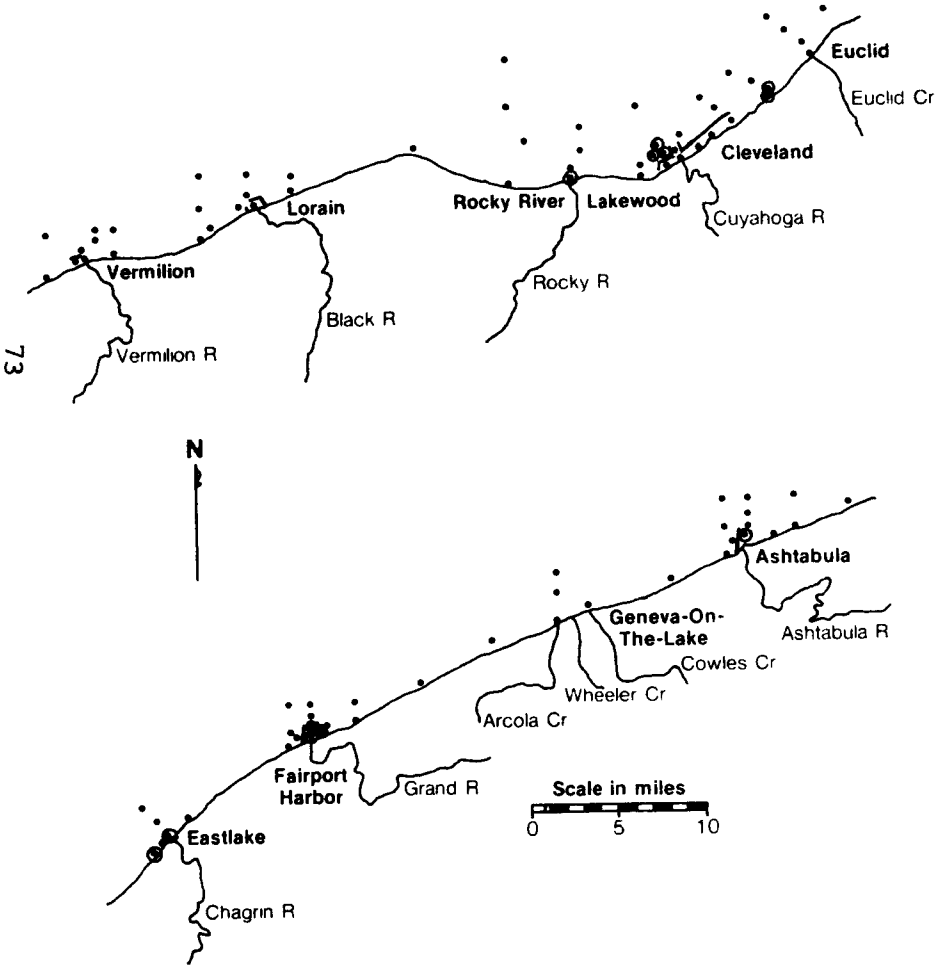


Figure 27. Application of FC/FS ratio tests to 1979 data.

c. Stations with a fecal coliform/fecal streptococcus ratio between 4.0 and 0.70 (4.0 > FC/FS > 0.70, 1979)



d. Stations with a fecal coliform/fecal streptococcus ratio ≥ 4.0, 1979

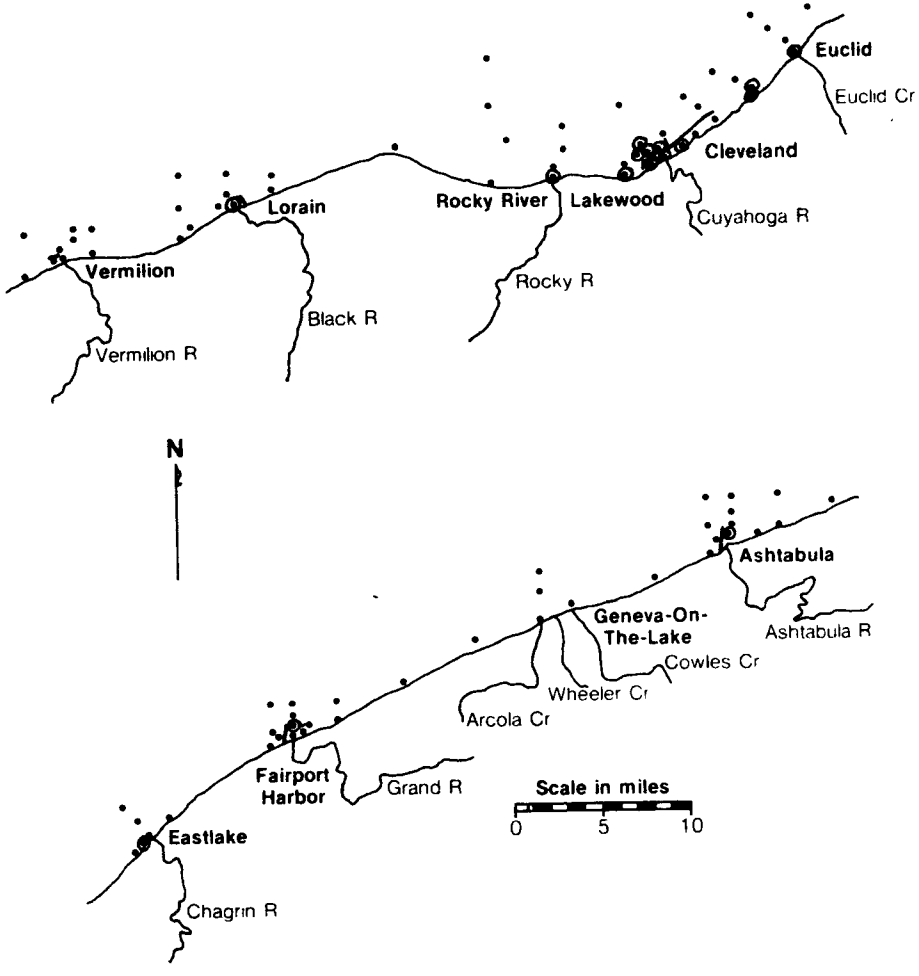
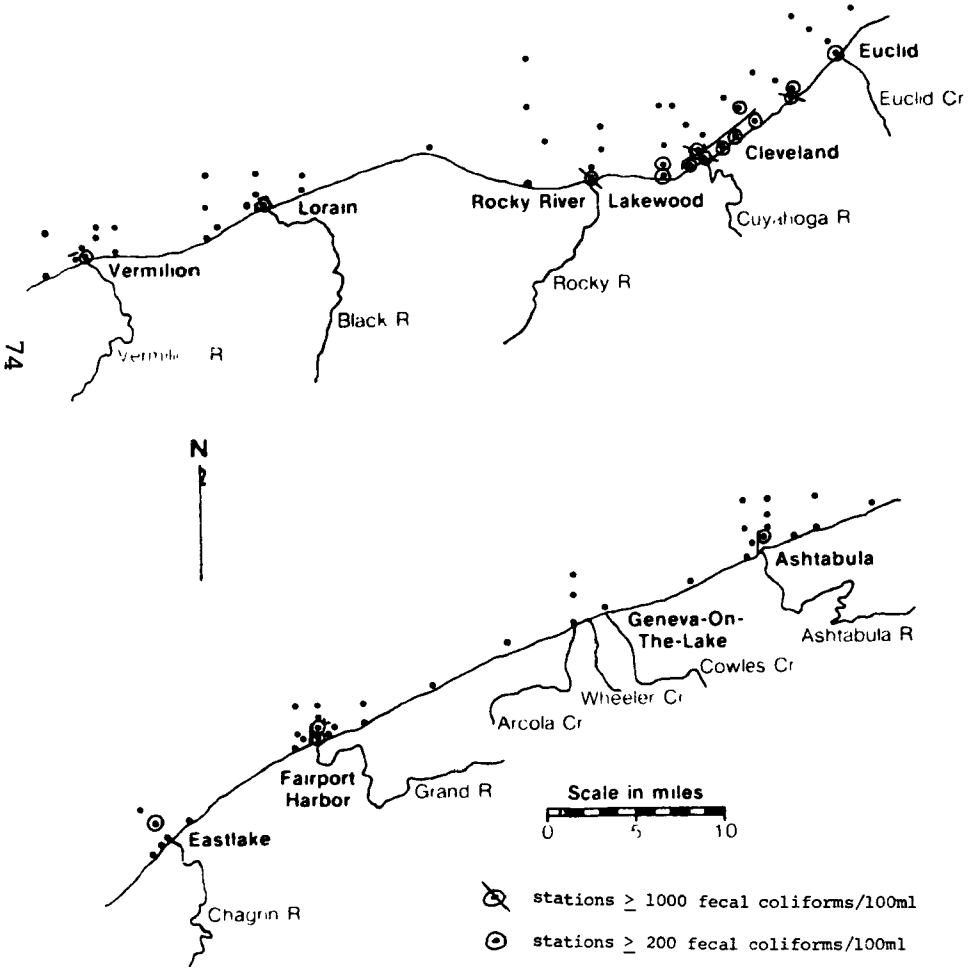
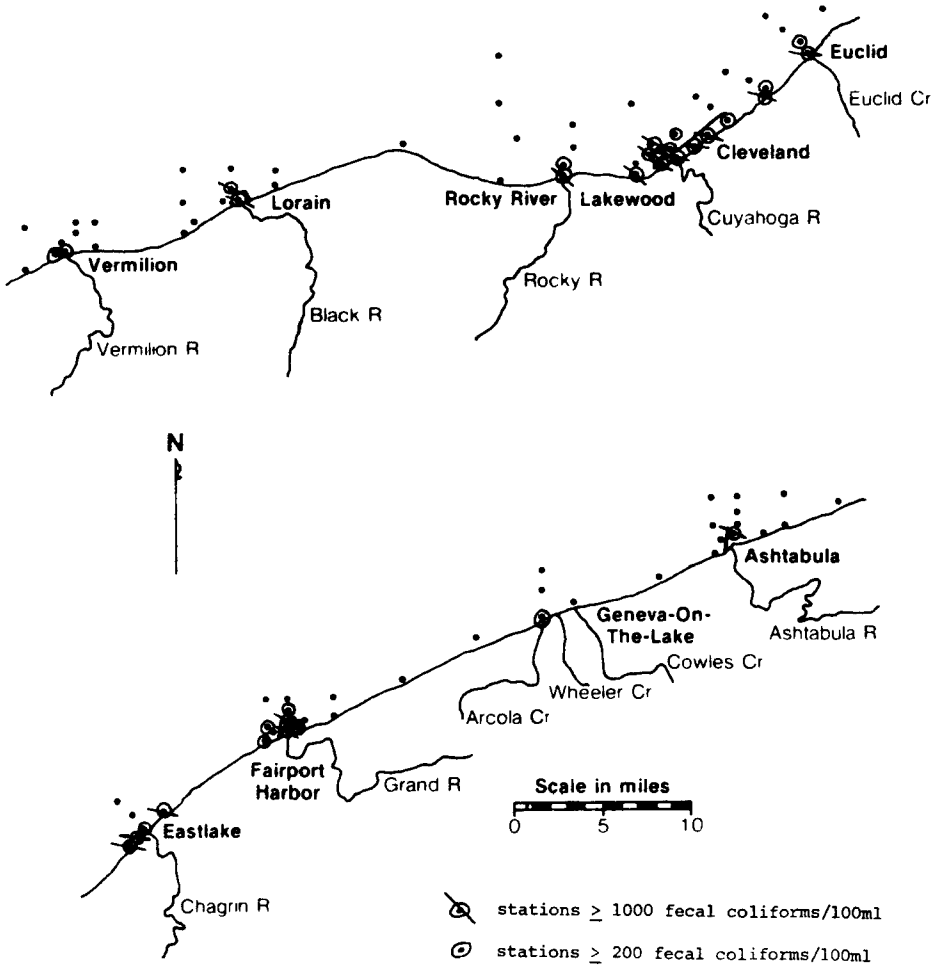


Figure 28. Stations with elevated fecal coliform counts.

a. Stations with a fecal coliform concentration ≥ 200/100ml, 1978



b. Stations with a fecal coliform concentration ≥ 200/100ml, 1979



APPENDIX I

QUALITY CONTROL

APPENDIX I - QUALITY CONTROL

Ambient air quality

The results of the ambient air quality test (Table 1) show that on 72% of the 60 sampling days, the bacterial count was $\leq$ two organisms in 15 minutes, which indicates that there was very little contamination of the aerobic heterotroph samples from airborne organisms in the laboratory. The counts on the other days were between three and eight organisms, with one plate of fifty due to water being dripped on the plate.

Sterility

Tables 2 through 5 shows the results of the 1979 sterility testing, as well as the conditions under which the tests were conducted. The best results were obtained during the last half of Cruise 3 and all of Cruise 4, which represent the effective standardization of the methods used for this quality control procedure.

The M-FC agar used for the fecal coliforms is somewhat less selective than the KF-Streptococcus agar used for the fecal streptococcus samples. The high counts on the aerobic heterotroph control plates in Cruise 1, as compared to mostly zero counts for Cruises 3 and 4, clearly indicate the importance of frequent use of UV sterilization to prevent carryover contamination from one sample to the next.

HC vs. HA Millipore filters

For the 1979 study, fecal coliforms were processed on Millipore HC filters, instead of the Millipore HA filters used previously. Sladek and his colleagues (1975) did a study to determine the optimum membrane structure for enumerating fecal coliforms, and the Millipore HC filter meets their specifications. The pores of the HC filter are funnel-shaped, with a 2.4um surface opening diameter tapering to a pore diameter of 0.7um, which is fecal coliform retentive. A study of the membrane recovery of six different types of membrane filters by Green, et al. (1975) supports the findings of Sladek, et al. (1975) by showing Millipore HC filters to be superior to Gelman, Johns-Manville, Sartorius, Millipore HA and Schleicher and Schuell filters.

The 2.4um surface opening of the Millipore HC filter seems to be the key characteristic that improves the recovery of fecal coliforms on these membrane filters. Sladek and his co-workers (1975) theorized that the larger surface openings of the HC filters allow bacteria to be held below the level of the medium, thus preventing the occurrence of a hypertonic solution around the bacteria (which would result in plasmolysis and death), especially at the elevated incubation temperature (44.5 degrees C) used for fecal coliforms. The larger surface opening also allows an increase in the flow rate through the membrane and an increased diffusion rate of the medium to the membrane surface. In this study the only disadvantage associated with the use of the Millipore HC filters was that the plates could not be successfully transported

before counting, due to the resultant spreading and smearing of the colonies.

Lin (1976) also found the Millipore HC filter to be superior to the HA filter for enumeration of fecal coliforms, and he extended his evaluation of the two types of filters to include total coliforms and fecal streptococci. The HC filters showed no appreciable increase over the HA filters in recovery for total coliforms and fecal streptococci; therefore, the Millipore HC filters were used in this study for only the fecal coliform enumeration, and HA filters were used for the aerobic heterotroph and fecal streptococcus analyses.

VERIFICATION TESTING

The results of the fecal coliform and fecal streptococcus verification testing are presented in Figures 1 through 4 and Tables 6 through 9. The stations chosen for verification were usually river mouth or harbor stations, where the likelihood of obtaining the twenty to twenty-five colonies needed for verification was greatest. These also would be most likely to contain false positive organisms (both fecal coliforms and fecal streptococci) due to the substantial amounts of pollution present in these areas.

In 1978, 164 fecal coliform colonies were tested with 94.6% being positive for fecal coliforms; of the 1016 colonies tested in 1979, 85.2% gave positive results. In 1979 the fecal coliform medium (M-FC agar) was made up without the addition of rosolic acid, allowing the medium to be autoclaved, and it is possible that this omission contributed to the much lower percent verification in 1979, as the purpose of this agent is to inhibit the growth of non-coliforms.

The percent of the organisms verified which gave positive results for fecal streptococci in 1978 was 77.2%, considerably lower than that for fecal coliforms (94.6%); in contrast, the percent verifications for the 1979 fecal coliform and fecal streptococcus samples were almost equal: 85.2% for coliforms and 85.7% for streptococci). Of the eight samples which did not verify 100% in 1978 (Table 8), three had 0% verification due to the presence of very small, poorly developed pink colonies which tested negative for fecal streptococci. These false positive colonies appeared on plates for stations 57, 72 and 73, (bottom, surface and bottom replicate levels, respectively), from the Vermilion, Cleveland West and Cleveland East areas (see Figure 1 for station positions). No further work was done to determine the identity of the small pink colonies, and the only factor all three occurrences seemed to have in common was their presence at stations away from shore. On the basis of the verification tests at these three stations, these very small pink colonies were not counted when they were encountered in other samples.

In 1979 two of the stations verified for fecal streptococci gave 0% verification, but unlike the 1978 samples with 0% verification, these 1979 samples had colonies which appeared to be perfectly normal fecal streptococcus colonies. The two stations where this occurred were 98S (surface samples) at the mouth of Euclid Creek and 106S at the mouth of the Grand River. There is a substantial amount of pollution at the mouth of Euclid Creek at station 98,

which increases the chances of picking up false positive organisms for fecal coliforms as well as for fecal streptococci. However, station 106 is some distance from the mouth of the Grand River, making the above conjecture unlikely for this station.

PSEUDOMONAS AERUGINOSA DATA

Samples for Pseudomonas aeruginosa were processed in 1978 for each of the stations designated as "industrial" stations: stations 65, 66, 84, 85, 86, 87, 88, 90, 91, 92, 132 and 133. These stations were located in all sampling areas where industrial discharges into the lake were present. The Pseudomonas data were not included in any of the data analysis for two major reasons: 1) The M-PA agar (Standard Methods 1975) used for isolation and enumeration was not selective enough to allow reliable identification of Pseudomonas aeruginosa colonies and 2) the number of Pseudomonas isolated (even assuming that all of the Pseudonomas - like colonies were actualy Ps. aeruginosa, was too small to be significant for purposes of analysis.

Figure 1.

Stations verified for fecal coliforms - 1978

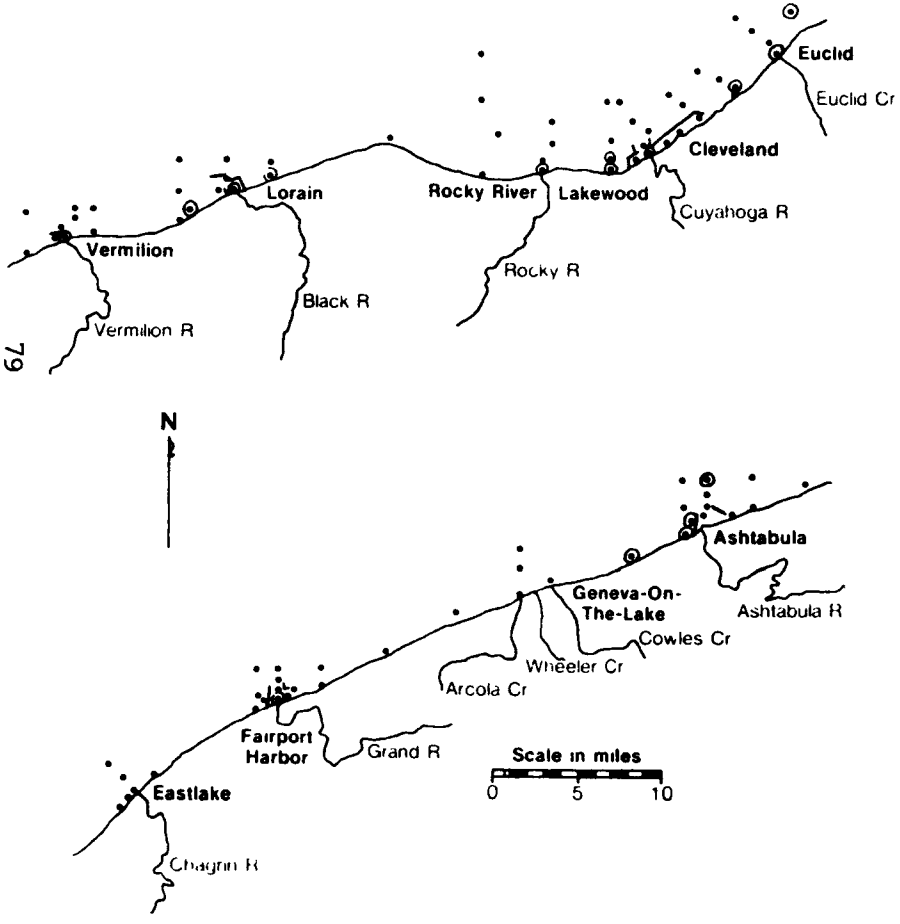


Figure 2.

Stations verified for fecal coliforms - 1979

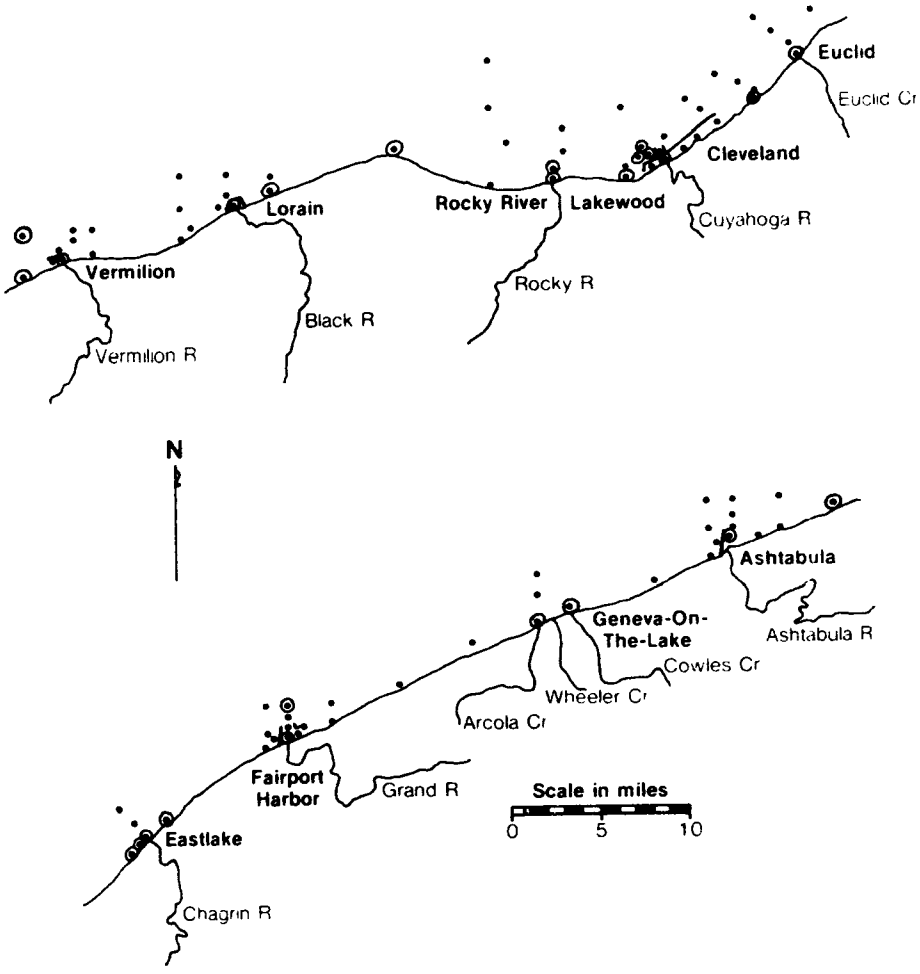


Figure 3.
Stations verified for fecal streptococci - 1978

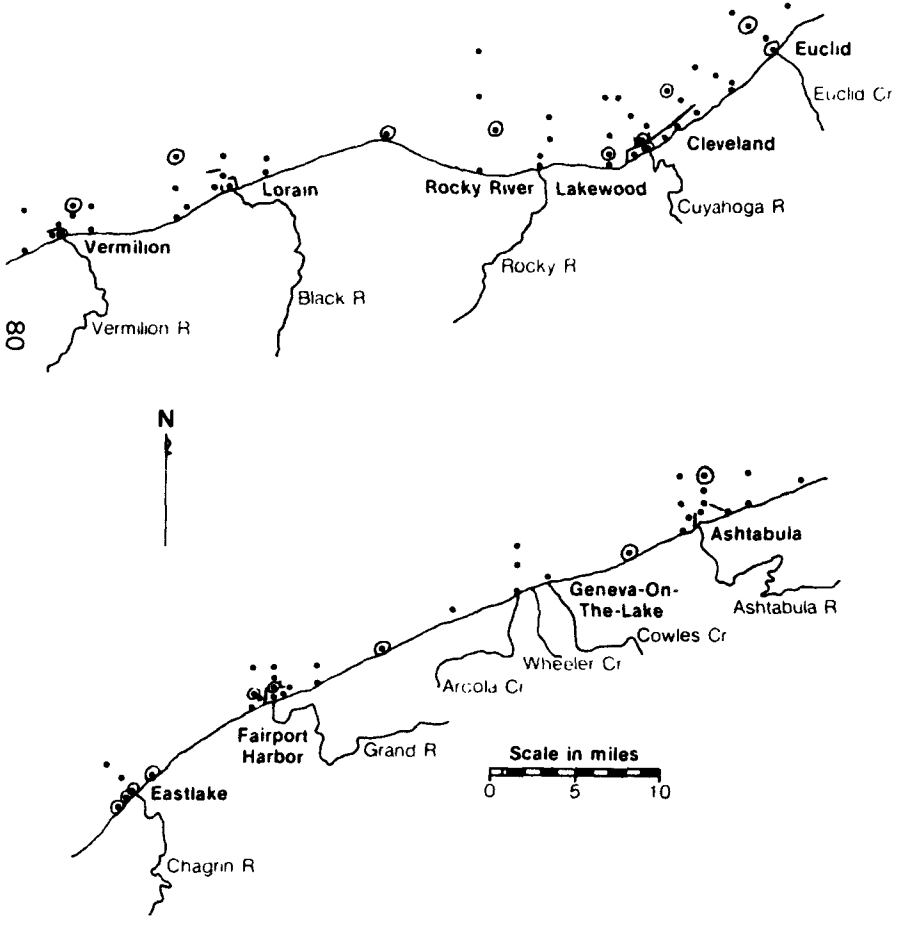


Figure 4.
Stations verified for fecal streptococci - 1979

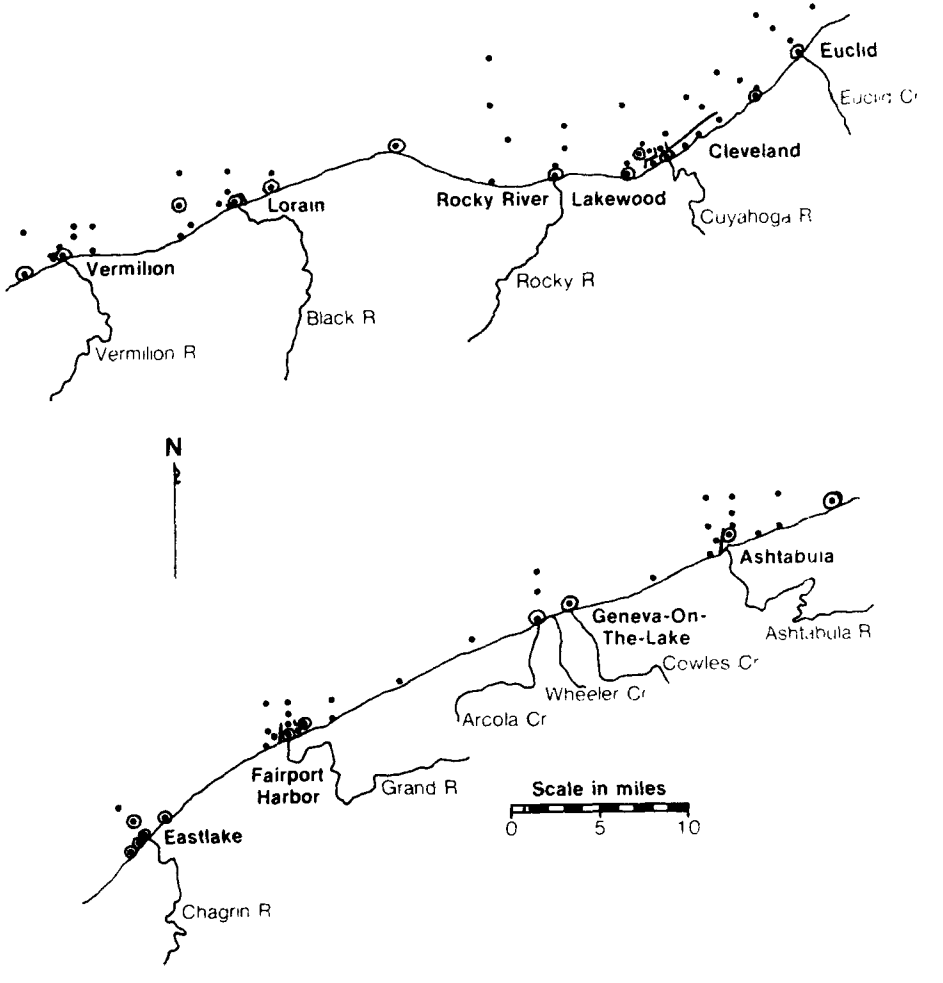


Table 1. 1979 daily ambient air quality test results

Run/Area	Count/15 minutes	Run/Area	Count/15 minutes
	Cruise I		Cruise II
1 LV	0	1 LV	1
2 LV	3	2 LV	0
3 LV	1	3 LV	1
1 CW	1	1 CW	1
2 CW	4	2 CW	0
3 CW	0	3 CW	0
1 CE	1	1 CE	2
2 CE	1	2 CE	3
3 CE	6	3 CE	0
1 FP	0	1 FP	50*
2 FP	0	2 FP	0.71**
3 FP	0	3 FP	3
1 AS	1	1 AS	0
2 AS	0	2 AS	0
3 AS	0	3 AS	2
	Cruise III		Cruise IV
1 LV	2	1 LV	1
2 LV	2	2 LV	5
3 LV	4	3 LV	4
1 CW	2	1 CW	2
2 CW	1	2 CW	0
3 CW	6	3 CW	2
1 CE	3	1 CE	0
2 CE	4	2 CE	2
3 CE	2	3 CE	3
1 FP	8	1 FP	1
2 FP	5	2 FP	1
3 FP	5	3 FP	1
1 AS	1	1 AS	2
2 AS	1	2 AS	0
3 AS	2	3 AS	2

* water dripped on plate.
** length of test = 21 mins.

Table 2 Sterility control - Cruise I. 1979.

Day/Area	Funnel # 1	2	3	4	5	6	7	8	9	10	11
	Colonies/Plate										
1 AS	0	0	4	0							
	1	0	0	0							
	6	0	0	0							
	1	1	0	0	1						
2 AS	0	1	2	0							
	1	0	1	0							
	2	4	1	0							
	0	0	0	0							
3 AS	1	0	0	0	0	0	0	0			
	23	5	60	2	3	57	8	4	12		
	27	7	7	7	12	14	6	0	25		
	48	19	13	14	14	31	29	13	327		
1 FP	0	0	0	0							
	0	0	1	0	0						
	0	6	0	0	0						
	0	0	0	1	0						
2 FP	0	0	0	0	0						
	0	0	1	0	0						
	6	0	0	1	0						
	1	4	2	3	3						
3 FP	1	0	0	0	0	0	0	0	0		
	21	26	12	17	23	15	6	17	14		
	117	57	36	27	108	47	50	51	39		
	15	12	84	40	51	17	12	16	7		
1 CE	1	0	0	1	1						
	8	24	14	6	100						
	50	33	30	100	100						
	100	100	100	100	100						
2 CE	0	1	0	0	0						
	20	13	12	3	10						
	5	0	17	0	0						
	3	2	5	3	1						
+UV 3CE	0	0	1	0	0	1	0	0	4		
	0	0	1	1	1	1	4	3	5		
	50	50	50	23	7	5	40	20	20		
	50	25	50	25	12	25	25	50	50		
+UV 1 CW	5	1	0	0	0						
	0	0	9	1	1						
	0	0	0	0							
	0	2	7	0							
+UV 2 CW	2	0	1	2	0						
	0	0	4	1	1						
	0	0	1	0							
	3	2	0	0							
3 CW	1	0	1	0	7	1	0	5	5		
	3	9	5	3	50	6	50	50	50		
	3	0	1	0	50	3	3	12	12		
	3	1	1	0	30	4	4	30	30		
1 LV	3	5	0	1	0	6	7	4			
	9	4	7	3	14	0*	0*	0*			
	4	0	1	1	7	7	1	2			
	4	9	3	7	50	40	15	40			
2 LV	8	40	35	40	14	25	3	0			
	75	21	1	3	2	0	0	0			
	3	2	0	0	4	0	2	1			
	1	0	1	1	9	1	2	2			
3 LV	0	0	0	0	30	11	19	3	3		
	33	19	9	6	75	75	75	TNTC**	TNTC		
	3	0	6	2	17	100	100	TNTC	100		
	11	23	17	18	100	75	TNTC	TNTC	TNTC		

Incubation temp. - 35°C
Medium - plate count agar; Millipore HA filters.
** Too Numerous to count
* Incubated at 20°C with aerobic heterotrophs.
+ Hydrosols 1 - 5 sterilized with UV light after approximately every 4 sample bottles.

Table 3. Sterility control - Cruise II. 1979.

	Funnel #										
	1	2	3	4	5	6	7	8	9	10	11
Day/Area	Colonies/plate										
1 AS	0	0	0	0	0						
	0	0	0	0							
	0	0	0	0							
	0	0	0	0							
2 AS	0	0	0	0							
	1	2	1	1							
	1	0	0	0							
	1	1	3	1							
3 AS	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	25*	20*	0	10*	22*	3	
	1	0	0	0	10*	66*	3*	93	73	6*	
	400	400	500	200	50	6	16*	30	50*	2	
1 FP	0	0	0	0	0						
	0	0	0	1	0						
	0	0	1	0	1						
	0	0	0	0							
2 FP	0	0	0	0	0						
	0	0	0	0	0						
	5	1	1	1	0						
	0	0	0	0	0						
3 FP	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	17*	2*	2*	29*	30*	2*
	0	0	0	0	---	8*	1*	1*	1*	5*	1*
	0	1*	1*	0	---	23*	25	4*	9*	28*	1*
1 CE	1	0	0	1							
	2	2	7	4							
	67	45	29	24	36						
	0	1	1	1	0						
2 CE	0	0	0	0	1						
	1	10	3	4	13						
	2	1	0	0							
	1	0	0	0	1						
3 CE	0	0	0	0	0	0	0	0	0	0	0
	8	TNTC**	4	0	TNTC	11	7	16	50	40	13
	0	59	71	18	---	30	25	20	40	12	6
	11	10	8	4	5	TNTC	TNTC	100	30	50	100
+UV 1 CW	1	1	0	0	0						
	0	0	0	0							
	37*	1	0	0							
	1	0	0	0							
+UV 2 CW	0	0	1	0	0						
	3	0	0	1	3						
	11*	100*	0	1							
	0	0	2	1							
+UV 3 CW	0	0	0	0	0	0	0	0	1	0	0
	0	0	0	0	---	22*	3*	0	2	0	0
	0	0	0	0	---	40*	TNTC	20*	3	25*	34*
	0	0	1	0	---	7	----	----	1	17*	45*
+UV 1 LV	0	1	0	0	0						
	0	0	0	0							
	1	0	0	0							
	0	0	0	0							
+UV 2 LV	0	0	0	0	0	0	0	0	0	0	0
	0	1	0	0	0	100	20*	15*	20	30	21
	0	0	0	0	---	200	30*	25*	150	100	6*
	0	0	1	0	---	100	4	1	30	19	3
+UV 3 LV	0	0	0	0	0						
	0	0	0	4							
	3	0	0	0							
	0	0	0	0							

Incubation temperature - 20°C.
Medium - plate count agar, Millipore HA filters.
** Too numerous to count.
* Colonies around edge of filter.
+ Hydrosols 1 - 5 sterilized with UV light after approximately every 4th sample bottle.

Table 4. Sterility controls -- Cruise III. 1979.

Day/Area	Funnel # 1	2	3	4	5	6	7	8	9	10	11
	Colonies/plate										
+ 1 AS	0	0	0	0							
	0	0	0	7	0						
	1	0	0	0							
	0	0	1	1							
2 AS	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	50*	24*	2	3	16	2	
	0	0	0	0	---	100	200	1	7	18	0
	0	0	0	0	---	40*	100*	1	6	4	5
3 AS	0	0	0	0							
	0	0	0	0							
	0	0	0	0							
	0	0	0	0							
1 FP	0	0	1	0	0						
	0	0	0	0							
	4*	0	7	0							
	0	0	0	0							
2 FP	0	0	0	0							
	0	0	0	0							
	0	0	0	1							
	0	1	0	0							
3 FP	0	0	0	0							
	0	0	0	0							
	0	0	0	0							
	0	0	0	0							
1 CE	0	0	0	0	0						
	0	0	0	0							
	2	0	0	0							
	15	0	0	0	0						
2 CE	2	0	0	0	0	1	0	0	0	0	
	0	0	0	0	---	30	TNTC**	20*	30*	1	1
	1	0	0	1	---	50	100*	6*	100	15	5
	1	1	0	0	---	TNTC	TNTC*	200	50	50	50*
3 CE	0	0	0	2							
	0	0	0	0							
	1	0	0	0							
	0	0	0	0	0						
1 CW	1	0	0	0	0						
	0	0	0	0							
	16	5	0	0							
	3	4	0	0							
2 CW	0	0	0	0							
	0	0	0	0							
	1	0	0	0							
	10	1	0	0							
3 CW	1	0	0	0	0	0++	0	0	0	0	0
	116	75*	0	0	---	2	2	0	0	0	0
	2	0	0	0	---	0	0	0	0	0	0
	5	1	1	0	---	0	0	0	0	0	0
1 LV	0	1	0	0	0						
	6	2	0	0	0						
	6	0	0	0	0						
	0	0	10	0	0						
2 LV	0	0	2	3	0	0	0	0	0	0	0
	0	1	0	1	0	0	0	0	0	0	0
	0	0	0	0	---	0	1	0	0	0	0
	0	0	0	0	---	0	0	0	0	0	0
3 LV	0	3	0	0	0						
	0	1	0	0	0						
	45	7	0	0	0						
	1	1	1	2							

Incubation temperature - 20°C.
Medium - plate count agar, Millipore HA filters except where otherwise indicated.
** Too numerous to count.
* Colonies around edge of filter.
++ Plates 6 - 11: HC filters on M-FC agar incubated at 44.5°C.
+ Hydrosols 1 - 5 UV sterilized for all areas.

Table 5. Sterility controls -- Cruise IV. 1979.

	Funnel #										
	1	2	3	4	5	6	7	8	9	10	11
Day/Area	Colonies/plate										
+UV 1 AS	0	0	0	0	0						
	0	0	0	1							
	0	0	0	0							
	0	0	0	0	0						
2 AS	0	0	0	0	0						
	0	0	0	0							
	1	1	1	0							
	0	0	0	0							
3 AS	0	0	0	0	0	0++	0	0	0	0	0
	0	0	1	0	---	0	0	0	0	0	0
	1	0	0	0	---	0	0	0	0	0	0
	0	0	0	1	---	0	0	0	0	0	0
1 FP	0	0	0	0	0						
	0	0	0	0							
	5	0	1	0							
	0	0	0	0							
2 FP	0	0	0	0	0						
	0	0	1	0	0						
	1	0	0	0							
	0	2	1	0	0						
3 FP	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	1	---	3	0	0	0	0	0
	---	---	---	---	---	---	---	---	---	---	---
	1	0	0	1	0	0	0	0	0	0	0
1 CE	0	0	0	0	0						
	12*	0	0	0	0						
	12*	1	0	0	0						
	17*	0	1	0	0						
2 CE	0	0	0	0	0						
	8	5	0	0	0						
	0	0	0	0							
	7	1	0	0							
3 CE	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0		0	0	0	0	0	0
	0	1	1	1		2	1	0	0	0	1
	0	5	0	0	0	10	4	1	1	0	1
1 CW	0	0	0	0	0						
	0	0	0	0	0						
	5	0	5	0							
	12	1	1	0							
2 CW	0	0	2	0	1						
	0	0	0	0							
	23	3	0	0							
	25	0	0	0							
3 CW	1	0	0	0	1	0	0	0	0	0	0
	1	0	0	0		0	0	0	0	0	0
	0	0	0	2		0	0	0	0	0	0
	0	0	2	1		0	0	0	0	0	0
1 LV	0	0	0	0	0						
	0	0	0	0	0						
	0	0	0	0							
	0	0	0	0							
2 LV	0	0	0	0	0	0	---	---	---	---	---
	0	0	0	0	0	0	0	0	0	0	0
	1	0	1	0		0	0	0	0	0	0
	0	0	0	0		0	---	---	0	0	---
3 LV	0	0	0	0	0						
	0	0	0	0	0						
	2	0	0	0							
	0	0	0	0							

* Colonies around edge of plate
Medium for plates 1-5 - Plate count agar with Millipore HA filters
Incubation temp. - 20°C
++ Plates 6-11: Millipore HC filters on M-FC agar incubated at 44.5° C.
\+ Funnels 1-5 UV sterilized after approx. every 4 sample bottles for all areas.

Table 6. Verification of fecal coliform colonies, 1978.

Station	Date	FC/100 ml	# of colonies subjected to ver.	% Verification
*LV 54 S	781010	8700	15	100
LV 63 B	781010	37	10	100
LV 65 B	781010	120	10	100
LV 68 S	781010	130	10	100
CW 75 S	781013	200	10	90
CW 79 S	781013	74	9	89
*CW 80 B	781013	34	15	100
*CE 85 B	781016	7800	8	100
CE 95 S	781016	460	8	75
*CE 98 S	781016	1370	10	80
CE 102 B	781016	69	10	80
FP 113 B	781019	400	9	100
*AS 127 SR	781022	28	10	100
AS 128 S	781022	120	10	100
AS 129 B	781022	18	10	100
*AS 135 B	781022	18	10	100

*Fecal streptococci also verified at this station.

Table 7. Verification of 1979 fecal coliform colonies.

Station	Date	FC/100 ml	# Colonies Subjected to Verification	% Verification
		CRUISE I		
LV 51 S	790425	67	15	67
54 S		720	10	80
65 S		960	20	40
68 S		19	20	90
CW 70 S	790422	14	20	80
75 S		320	20	95
79 S		3500	20	95
81 S		1900	20	90
CE 85 S	790419	34000	20	90
94 S		140	20	95
98 S		18	20	85
FP 103 S	790416	3200	20	95
105 S		740	20	100
108 S		1800	20	100
113 S		580	20	80
AS 123 S	790413	210	20	90
126 S		170	20	90
132 S		1800	20	75
139 S		24	20	70
		CRUISE II		
*+LV 52 S	790724	84	5	100
+ 54 S		45	17	100
65 S		390	19	89
CW 75 S	790722	190	20	80
+ 83 S		310	20	100
CE 85 S	790719	110	20	95
98 S		9.4	20	20
FP 105 S	790716	640	20	100
113 S		6.5	22	54
AS 126 S		13	16	94
132 S		500	24	92
		CRUISE III		
LV 65 S	790831	600	25	92
+ 68 S		14	4	100
+ CW 76 S	790829	80	25	96
81 S		5800	25	92
CE 85 S	790825	3900	25	88
94 S		12000	25	100
FP 104 S	790823	92	25	84
113 S		48	25	88
AS 126 S	790819	32	25	56
132 S		260	25	100
		CRUISE IV		
LV 54 S	791017	48	24	83
65 S		360	25	84
CW 75 S	791015	11000	25	92
89 S		130	22	91
CE 85 S	791011	1000	20	85
98 S		7000	21	95
FP 113 S	791007	4800	19	95
+ 116 S		13	13	23
AS 126 S	791004	55	25	100
132 S		260	25	88

*Offshore stations; all others are nearshore.
+Not verified for FS.

Table 8. Verification of fecal streptococcus colonies, 1978.

Station	Date	FS/100 ml	# of colonies subjected to ver.	% verification
*LV 54 S	781010	28	15	100
LV 57 B	781010	451	15	** 0
LV 62 S	781010	155	13	85
CW 70 S	781013	5	10	10
CW 72 S	781013	TNTC	10	** 0
*CW 80 B	781013	28	10	100
CE 86 S	780905	23	3	100
CE 100 B	780905	130	6	67
*CE 85 S	781016	1000	10	100
CE 93 BR	781016	TNTC	10	** 0
*CE 98 S	781016	180	10	100
FP 103 S	780908	140	5	100
FP 104 S	780908	240	5	100
FP 105 S	780908	160	5	80
FP 108 S	780908	480	5	100
FP 114 S	780908	69	5	80
FP 121 S	780908	67	5	100
FP 111 B	781019	110	10	100
FP 114 B	781019	98	10	100
*AS 127 SR	781022	39	10	100
*AS 135 B	781022	60	10	100

** Plate contained numerous, small, poorly-developed pink colonies.
* Fecal coliforms also verified at this station.
TNTC too numerous to count.

Table 9. Verification of 1979 fecal streptococcus colonies.

Station	Date	FS/100 ml	# Colonies Subjected to Verification	% Verification
		CRUISE I		
LV 51 S	790425	2.1	11	9.1
54 S		88	10	80
65 S		150	25	100
68 S		8	23	34.8
CW 70 S	790422	8	2	100
75 S		180	22	86.4
79 S		680	24	100
81 S		1300	23	100
CE 85 S	790419	780	25	100
94 S		20	2	100
98 S		0	17	0
FP 103 S	790416	1800	24	100
105 S		340	24	54.2
108 S		74	24	91.7
113 S		250	22	100
AS 123 S	790413	79	25	92
126 S		71	25	96
132 S		3000	25	100
139 S		17	25	92
		CRUISE II		
+ LV 51 S	790724	7	18	33
65 S		25	25	96
+*CW 73 SR	790722	2.7	23	4.4
75 S		12	12	100
CE 85 S	790719	20	20	100
98 S		2.7	8	38
FP 105 S	790716	95	19	95
+ 106 S		0	6	0
113 S		5	3	100
AS 126 S	790713	4	7	86
132 S		23	23	96
		CRUISE III		
+ LV 54 S	790831	29	22	100
65 S		13	19	95
+ CW 75 S	790829	1900	25	100
81 S		290	25	100
CE 85 S	790825	290	20	100
94 S		500	21	100
FP 104 S	790823	31	23	100
113 S		11	16	94
113 SR		4	4	100
AS 126 S	790819	27	25	100
132 S		25	23	100
		CRUISE IV		
LV 54 S	791017	4	5	100
65 S		24	25	100
CW 75 S	791015	150	23	100
89 S		17	25	100
CE 85 S	791011	91	25	100
98 S		440	25	100
FP 113 S	791007	1800	24	100
+ 118 S		75	25	100
AS 126 S	791004	35	24	96
132 S		35	24	100

*Offshore stations; all others are nearshore.
+Not verified for FC.