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WINTER FIELD SURVEYS AT VOLUNTEER ARMY AMMUNITION PLANT, CHATTA--ETC(U)

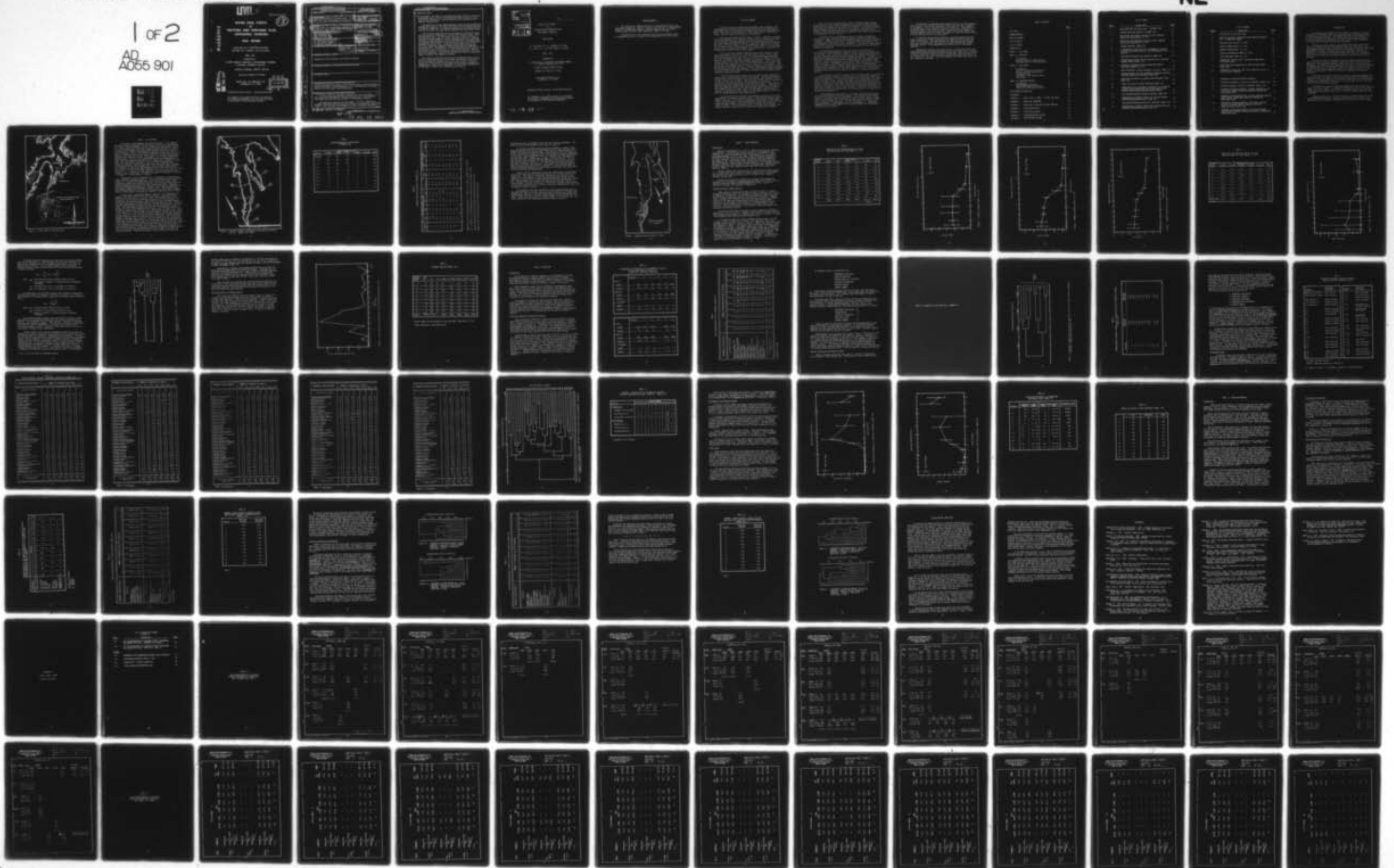
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**WINTER FIELD SURVEYS  
AT  
VOLUNTEER ARMY AMMUNITION PLANT  
CHATTANOOGA, TENNESSEE**

**FINAL REPORT**

**J.H.SULLIVAN, JR., H.D.PUTNAM, M.A.KEIRN,  
B.C.PRUITT, JR., D.R.SWIFT and J.T.Mc CLAVE**

**APRIL, 1978**

**Supported by**

**U.S.ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND  
FT. DETRICK, FREDERICK, MD 21701**

**J.GARETH PEARSON, PROJECT OFFICER**

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**WATER AND AIR RESEARCH, INC.  
GAINESVILLE, FL 32602**

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A winter survey was conducted in Waconda Bay, Lake Chickamauga. The Bay is the receiving water for TNT-manufacturing wastes from Volunteer Army Ammunition Plant located northwest of Chattanooga, Tennessee.  Field work was carried out in two phases. The first, completed in December, determined flow and dispersion patterns in Waconda Bay, munitions levels in the lake water and sediments and other limnological parameters cont.		

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20. Abstract (cont.)

of the system. The Phase I survey determined that munitions levels were sufficiently high to justify an intensive biological study at a later date and provided data for locating sampling sites covering the full range of concentrations.

The second part of the investigation was completed during March and examined the effects of TNT wastes from VAAP on the periphyton and macroinvertebrate communities. A rigorous supplementary study determined the statistical variability occurring in periphyton colonizing glass slides.

Utilizing the population density and community structure of these selected biological compartments, estimates were made of effect levels from munitions waste. In the natural substrate no effects could be discerned in either number of species, population density, or community structure for both periphyton or macroinvertebrates at locations 3,000 feet downbay from the point of waste entry where total TNT concentration averaged less than 25  $\mu\text{g}/\text{l}$ . During the 30 day exposure period for artificial substrates, samples of VAAP effluent were collected and analyzed for TNT. Concentration of 5 munitions compounds averaged 575  $\mu\text{g}/\text{l}$  and ranged as high as 2,370  $\mu\text{g}/\text{l}$ . In the 0-3,000 foot zone, definite effects were noted in the periphyton and macroinvertebrates colonizing artificial substrates. Effects were manifested by shifts in population, number of species, and community structure.

The results of the study indicate no environmental effect on periphyton or macroinvertebrates from a complex TNT manufacturing effluent at a total munitions concentration of less than 25  $\mu\text{g}/\text{l}$ . Definite effects were noted during a period when total munitions averaged 500-600  $\mu\text{g}/\text{l}$  with a peak of over 2,000  $\mu\text{g}/\text{l}$ . Concentrations believed to be in the range of 50-100  $\mu\text{g}/\text{l}$  produced minimal effects.

*microgram/l*

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An appreciation is also extended to the Ecological Research Office at Edgewood Arsenal for assistance in the completion of the field study.

## EXECUTIVE SUMMARY

Field surveys were conducted during December, 1976, and March, 1977, in Waconda Bay to determine the distribution and effect on periphyton and macroinvertebrates of TNT effluent discharged from Volunteer Army Ammunition Plant. The study was an extension of a 1975 summer effort to determine no-effects levels for munitions compounds in fresh water.

The study was divided into two phases. Phase I, completed in December, defined the movement of the effluent in the bay to aid in selection of chemical and biologic sampling sites for the more extensive Phase II work. A dye tracer study utilizing Rhodamine B, in situ, and total munitions measurements were utilized in Phase I. Results showed that dispersion of effluent into the Bay was most influenced by reservoir stage changes. A rising stage had the effect of holding the effluent in the upper part of the Bay while a drawdown period moved effluent rapidly downbay.

During a one-week intensive sampling period, total munitions concentration was highest in the first 3,000 feet from the waste outfall. Mean total levels were as high as 397  $\mu\text{g}/\text{l}$ . The principal munitions compounds near the discharge were 2,4-DNT, 2,6-DNT, and 2,4,6-TNT. Analysis of data from this first phase shows that pollutant concentration in the initial 3,000 feet was about 0.5 to 1 times the effluent concentration. Levels diminished rapidly the next 1,600 feet to 0.01 to 0.05 times that of the effluent. Based on results of the December study, stations for Phase II were grouped in high, medium, and low concentration zones. Chemical and biological characterizations were determined for water and sediments. Low stage conditions which continued to prevail during March (Phase II) caused a channel to form, extending about 3,000 feet beyond the outfall.

Daily munitions analysis during the first week in March showed that highest levels occurred consistently in the channel. Average total munitions concentration in this area was 78  $\mu\text{g}/\text{l}$  with the principal components being 2,4-DNT, 2,6-DNT, and 2,4,6-TNT. Total munitions concentration in the effluent during this time based on average 24 hour composites, was 89  $\mu\text{g}/\text{l}$ . It is apparent that little dilution was occurring in the channel. Concentrations of munitions compounds dropped significantly beyond the channel. Relative to other compounds only three of the individual Bay samples showed 2,4-DNT and five samples contained 1,3-DNB. Only 1,3,5-TNB showed increased levels downbay. It appears that this compound is a breakdown product of 2,4,6-TNT. No 1,3,5-TNB was detected in the plant effluent.

In early March total nitrogen in the channel averaged 14.2 mg/l with approximately 79 percent occurring as  $\text{NO}_3\text{-N}$ . Concentrations downbay showed a gradual reduction. The N levels in the medium zone averaged 5.6 mg/l with nitrate occurring again in the same proportion. Outer bay stations reflected a mean nitrogen content of 4 mg/l with  $\text{NO}_3\text{-N}$  concentration as the most significant form. Other constituents such as chloride, total hardness, and sulfate followed a similar pattern of decreasing concentrations downbay.

For a period of one month while artificial substrates were exposed in Waconda Bay, 24-hour composite samples were collected of VAAP effluent. Results show that average levels during the incubation of biological samplers were higher than during the early March field survey. Total munitions in the channel probably were near 2,000  $\mu\text{g/l}$  at a maximum and averaged 500  $\mu\text{g/l}$ . Organisms in the intermediate zone just beyond the channel were likely exposed to a concentration of 50 - 100  $\mu\text{g/l}$ .

Periphyton and macroinvertebrates were the biological components selected for study. Of these, periphyton analysis provided the most useful information. Community structure was examined following a 30-day colonization of standard glass microscope slides. Suspension units containing up to 8 periphytometers were utilized during incubation. A correlative study examined between slide and within slide variability using population size and species diversity as variables.

The periphyton population density was highly correlated with concentrations of VAAP effluent. Population sizes expressed as  $\text{no/mm}^2$  were all less than 1,000 in the narrow channel. As the channel widened where down-bay water mixed and diluted the waste, populations increased two orders of magnitude and remained at this level throughout Waconda Bay. Treatment of the data by the Pinkham-Pearson Similarity Index shows this relationship among stations. Levels of chlorophyll *a* and organic biomass add support to this latter conclusion. Chlorophyll *a* on colonized slides at the various stations show a distribution consistent with population counts. Organic biomass follows a similar pattern. However, Shannon-Weaver diversity did not correlate with munitions concentrations. This index appears to be of limited value in situations where the main environmental response is a change in population density.

The statistical analysis indicates that a principal source of variability in estimating either diversity or population size of periphyton colonizing artificial substrates is the difference among direct microscopic field counts within individual slides. Unequal distribution of organisms which occurred during the drying of the aliquot on the glass coverslip produced this variability. Locations near the edge of the coverslip had lower populations than those near the center. The result of an uneven distribution of diatoms is a masking of aliquot and slide to slide variation. The error due to drying on the coverslip must be reduced before a full understanding of periphyton variability can be attained.

Macroinvertebrate distribution in sediments did not reveal clearly defined relationships to TNT waste products. Low populations encountered because of the cold season are considered as the principal reason. Midges which are an abundant form in Waconda Bay colonize mainly during the summer but are sparse during the winter. Species dominating in the March survey were *Procladius* sp., *Coelotanytus* spp. and a *Chironomus* sp. *Procladius* dominated in the channel nearest to the outfall and may have represented a form tolerant to the waste.



Chironomids also dominated Hester-Dendy artificial substrate samplers. The data show a more defined relationship among stations than is apparent with sediments. Even so the definition is not clear cut. There appears to be maximum impact in the 3,000 foot channel section with a suggestion of transition zones in the outer bay areas. Overall the results suggest that macroinvertebrates are not as useful a parameter to study TNT effects during a winter sampling effort as are periphyton.

It should be noted that the observed correlations between munitions concentrations and biologic responses do not establish precise cause and effect relationships. The complex effluent at VAAP contains many compounds that vary in concentration with regard to the specific munitions-unique compounds measured. These compounds may be factors also in the effects observed.

Nevertheless, based on the winter survey, no biological effects were discerned when total munitions concentration was less than 25  $\mu\text{g}/\text{l}$ . Definite effects were noted when munitions levels averaged 500 - 600  $\mu\text{g}/\text{l}$  with a peak of around 2,000  $\mu\text{g}/\text{l}$ . Minimal effects were observed at munitions levels in the range of 50 - 100  $\mu\text{g}/\text{l}$ . These data suggest a no-effects level of 25  $\mu\text{g}/\text{l}$  for a complex TNT effluent.

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## INTRODUCTION

The Volunteer Army Ammunition Plant (VAAP) is situated on 7,300 acres northwest of Chattanooga, Tennessee and manufactures trinitrotoluene (TNT) on a contractor-operated basis. Wastewater from VAAP drains northward into a series of treatment lagoons and is discharged into the head of Waconda Bay after undergoing pH adjustment with lime. Figure 1 shows Waconda Bay in relationship to Harrison Bay and Lake Chickamauga.

The purpose of this study was to determine the effects of munitions wastes on periphyton and macroinvertebrates during the cold season. The investigation was dual-phased initiated by a dye study coupled with abbreviated environmental sampling. The main objective of the Phase I study was to determine if sufficient concentrations of munitions wastes were present in Waconda Bay to justify more intensive biologic work and, if so, to select sampling sites so that the full range of munitions concentrations could be evaluated.

The Phase II work included more intensive biologic sampling of periphyton and macroinvertebrate communities utilizing both artificial and natural substrates. This work was originally scheduled for early February but was postponed to early March because Waconda Bay was frozen in January and February. A supplementary study was included in Phase II to determine the statistical variability occurring in population density and community structure of periphyton colonizing glass slides as a function of sampling and processing technique.

Wastewater and sediment characterization was conducted throughout both segments of the field study. Plant effluent samples were also collected and analyzed for TNT and associated transformation products for a one-month period following the second phase field survey. This coincided with the incubation of the artificial substrate samplers.

Differences in reservoir conditions between this work and the prior summer 1975 work included: 1) lower water temperatures, and 2) lower reservoir stage. This latter factor significantly restricted mixing in the upper end of Waconda Bay by confining the flow to a relatively narrow channel for about 3,000 feet.

Midway through this second field survey the Volunteer Plant was closed by the Department of Defense. Although TNT production was stopped, wastes continued to be released into Waconda Bay during the entire period of study.

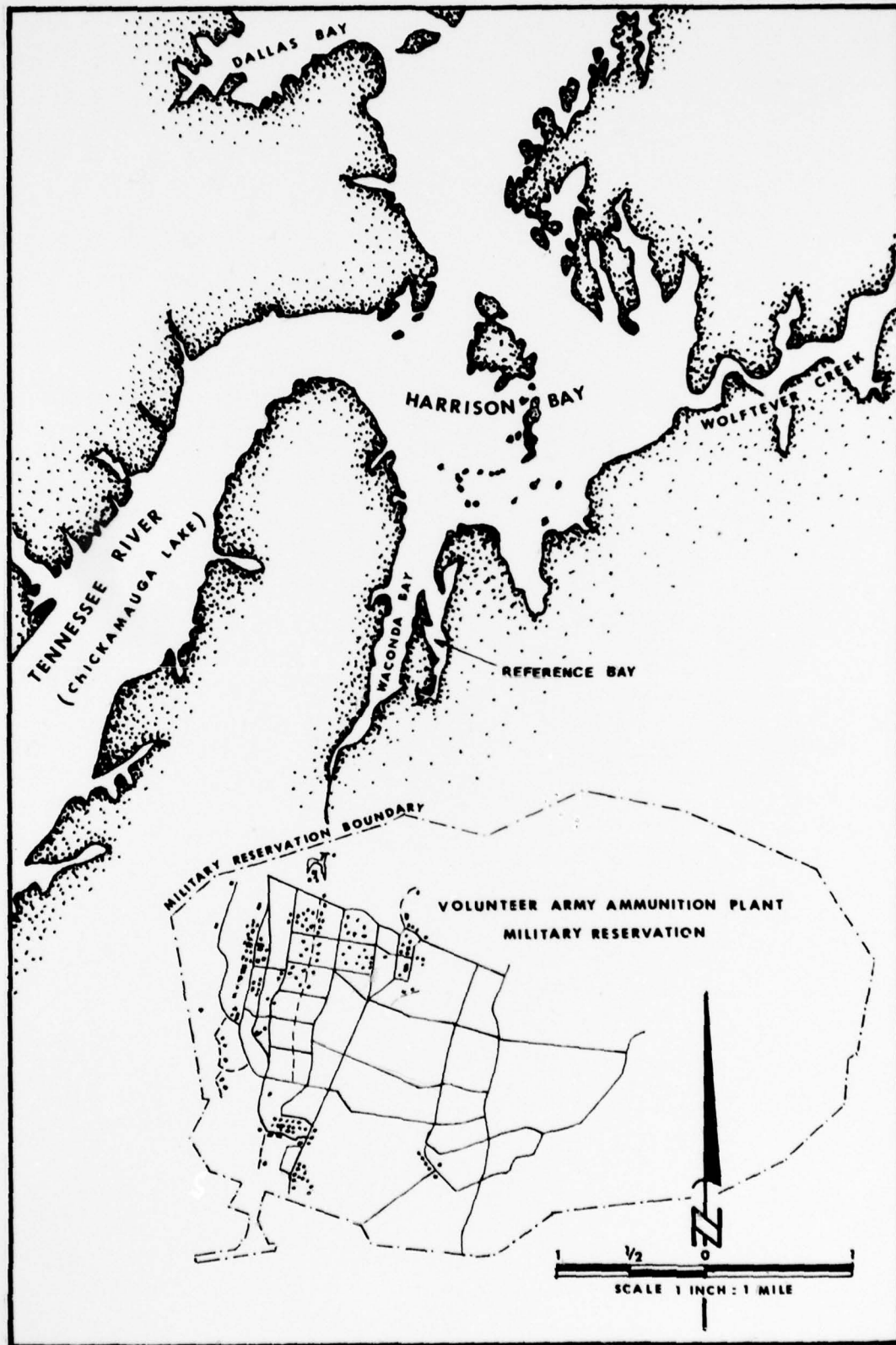


FIGURE 1. VICINITY MAP OF VAAP STUDY AREA.

## PHASE I - FIELD SURVEY

The purpose of the December, 1976 field survey was: 1) to conduct a dye tracer study to determine flow and dispersion patterns in Waconda Bay; 2) to make on-site measurements of D.O., temperature, conductivity, and pH; 3) to collect samples of water and sediment for munitions analysis; and 4) to obtain limited samples of benthic and periphyton organisms for familiarization purposes. Based on the information obtained, a decision could be made as to whether or not a sufficient concentration of munitions existed to justify a more intensive biologic survey, and if so, further determine the sampling sites locations in order to encounter the full range of munitions concentrations. The survey was conducted during the period December 6-14, 1976. Daily measurements were made of D.O., temperature, conductivity, and pH for the 5 day period, December 7-11 at nine sites in Waconda Bay. Samples for munitions analysis were taken daily from these nine sites plus the effluent from the last treatment pond. Each munitions sample consisted of a composite of three grabs taken during the day. The sites utilized were the same as used in the summer 1975 work (see Figure 2) with the following exceptions. Site B-1 was not sampled as it was dry land at the low reservoir levels encountered. One sample was taken in the middle of Transect F; Transects S, T, and U were not sampled.

Tabulations of the field-measured D.O., temperature, and conductivity values are shown in Appendix Table A-1. Dissolved oxygen was near saturation values at all stations and temperatures were in the 5-10°C range. High conductivity values were observed at stations near the waste outfall and this parameter appeared to be a good indicator of munitions waste. Equipment failure precluded measurement of pH. Munitions concentrations found in water and sediment samples are tabulated in Tables 1 and 2. The concentrations found exceeded those which in prior field studies have been shown to produce effects in both benthic and periphyton communities (Sullivan, et al., 1977).

The dye tracer study began December 7 when 153 ml of Rhodamine B was placed in the plant effluent from 1030 to 1300 hours. Based on previous experience in other waters, it was felt that a dye concentration of near 50 ppb could be utilized with minimum discoloration of the lake water. Unfortunately, the lake water had a light green tint that contrasted sharply with the red dye making concentrations of 10-20 ppb highly visible. State of Tennessee environmental personnel had been informed and approved of the tracer study. They had requested however, that obvious discoloration be kept to a minimum to limit citizen complaint and/or alarm. Dye feed began at about 32 ppb but was reduced after 20 minutes to 15 ppb because of excess color. Even at the reduced rate, the dye plume in the upper end of Waconda Bay was clearly visible initially. By the following morning, however, maximum dye concentration was down to 2.5 ppb, and was not visually detectable. By mid-afternoon, maximum concentration was 2.0 ppb and most of the dye was within the narrow channel at the upper end of the bay. The rapid decrease in dye concentration observed in one day suggested that the concentration would be below detection limits when it moved further down into a much wider portion of the bay. Since it was desirable to observe the dye further down bay, an additional 116 ml of dye was released into the center of the plume at 1600 hours on December 8. Even with this addition, the maximum concentration found the following morning was 2.0 ppb and the peak concentration was upbay of where it had been the previous afternoon. Transects were made of the dye plume vertically and

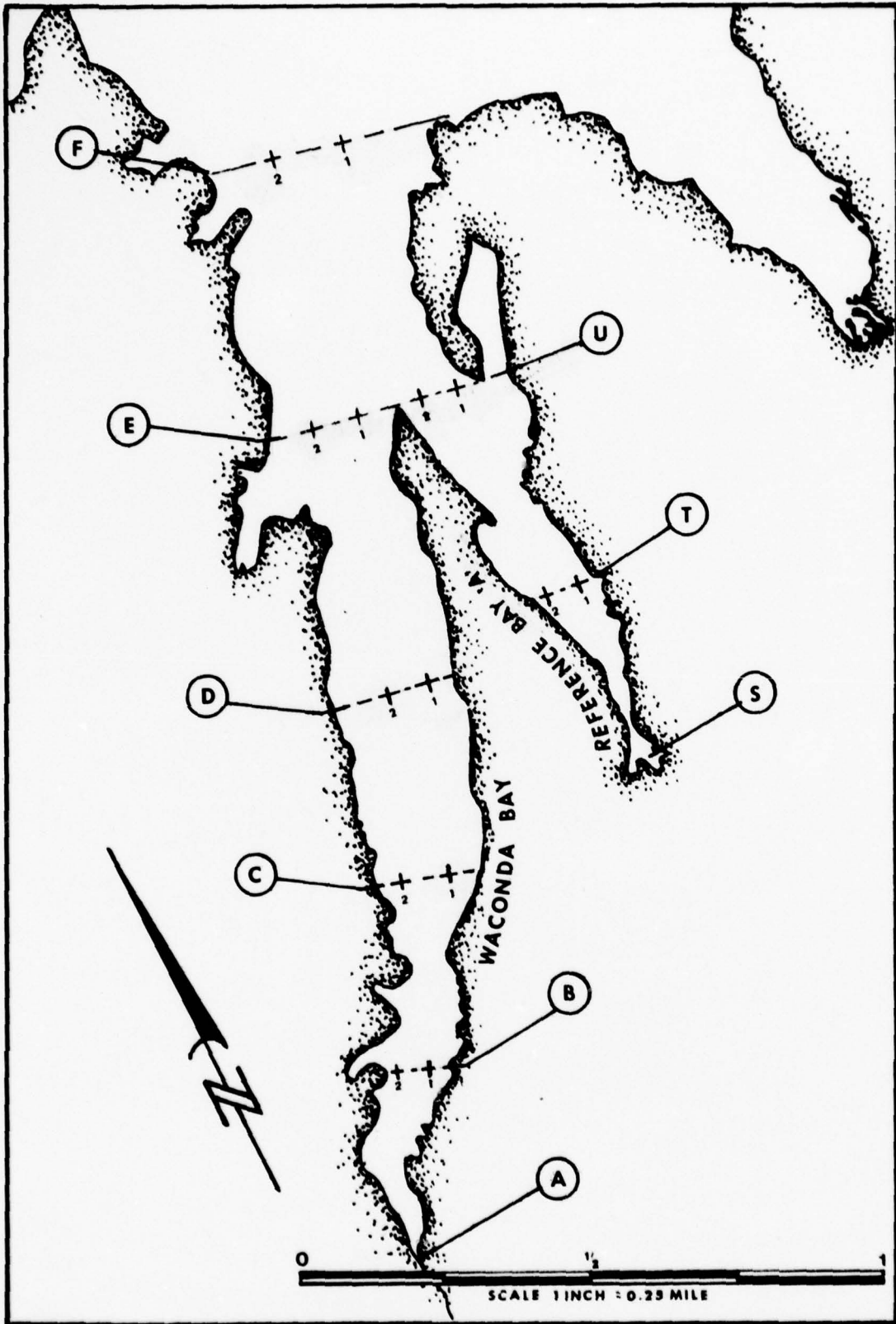


FIGURE 2. SAMPLING STATIONS IN WACONDA BAY AND ADJACENT REFERENCE BAY 'A'. SUMMER, 1975 SURVEY



TABLE 1  
 WATER MUNITIONS DATA, MEAN VALUES  
 DECEMBER 1976

STATION	VALUES REPORTED IN $\mu\text{g/l}$					TOTAL
	2,4-DNT	2,6-DNT	TNT	1,3-DNB	1,3,5-TNB	
EFFLUENT	93	53	47	4	<1	198
A	173	89	128	6	<1	397
B	156	88	110	5	<1	360
C-1	23	18	5	<1	<1	48
C-2	78	40	34	6	<1	159
D-1	9	4	<1	1	2	17
D-2	14	11	<1	<1	2	29
E-1	6	3	<1	<1	<1	$\leq 12$
E-2	2	<1	<1	<1	<1	$\leq 6$
F	2	<1	<1	<1	<1	$\leq 6$

TABLE 2  
 SEDIMENT MUNITIONS ANALYSES, DECEMBER 1976

STATION NO.	As Received Basis, ppm			% Moisture	Dry Weight Basis, ppm			TOTAL
	2,4 DNT	2,6 DNT	2,4,6 TNT		2,4 DNT	2,6 DNT	2,4,6 TNT	
A	0.2	0.1	0.4	77.7	0.9	0.4	1.8	3.1
B	0.15	0.1	0.25	76.2	0.6	0.4	1.1	2.1
C-1	<0.1	<0.1	<0.1	68.5	<0.3	<0.3	<0.3	<0.3
C-2	<0.1	<0.1	0.15	44.3	<0.2	<0.2	0.3	0.3
D-1	0.1	<0.1	0.15	27.8	0.1	<0.1	0.2	0.3
D-2	<0.1	<0.1	0.1	64.6	<0.3	<0.3	0.3	0.3
E-1	<0.1	<0.1	<0.1	58.5	<0.2	<0.2	<0.2	<0.2
E-2	<0.1	<0.1	<0.1	27.7	<0.1	<0.1	<0.1	<0.1
F	<0.1	<0.1	<0.1	66.0	<0.3	<0.3	<0.3	<0.3

Detection limit on As Received Basis - 0.1 ppm for 2,4 DNT, 2,6 DNT, and 2,4,6 TNT

No 1,3 DNB or 1,3,5 TNB detected with a detection limit of about 0.3 ppm

Overall recovery efficiency through extraction and clean-up ~ 80%

Above values are not corrected for recovery efficiency.

horizontally using a continuously measuring and recording fluorometer. The general pattern of dye movement can be seen in Figure A-1.

In an effort to better understand the dye movement, reservoir stage data were obtained from TVA and isoconcentration plots were prepared of the conductivity and total munitions data. These data are shown in Figures A-2 to A-4. Examination of stage data shows that the dye was initially introduced at the end of a rapid drawdown period. From December 7 through December 10, the reservoir stage increased by 1.15 feet. From Transect E upstream this increase represents a volume change of about 43 million gallons. This is more than the total effluent flow from VAAP during this period so net flow was actually upbay at Transect E. This would have the effect of holding effluent in the upper end of the bay. The reservoir level dropped approximately 0.9 feet during the morning of December 11, which had the effect of moving contaminants downbay at an accelerated rate and which the conductivity and total munitions data demonstrate.

Analysis of the combined data shows that concentrations of pollutants in the first 3,000 feet of the bay were roughly 0.5-1.0 times effluent concentrations. This appears to be due to the limited cross-section of the channel at the low reservoir stages seen during the winter months (channel width 100 ft. depth 5-6 ft.) which minimizes mixing with cleaner water in the bay. Concentrations then drop off very rapidly over the next 1600 ft. to levels around 1-5 percent of effluent. Variations in absolute concentrations can be significantly affected by changes in effluent concentrations, flow, and by changes in reservoir stage.

Four sampling sites were selected for "high" concentration ranges in the upper 2500 feet of the bay; three sites were selected in "middle to low" ranges within 1500 feet of the point where the bay significantly widens and; two additional sites were selected further downbay for the extreme low level range. Three sites were chosen as reference points in the adjacent bay. All sites selected for the second phase study are shown in Figure 3.

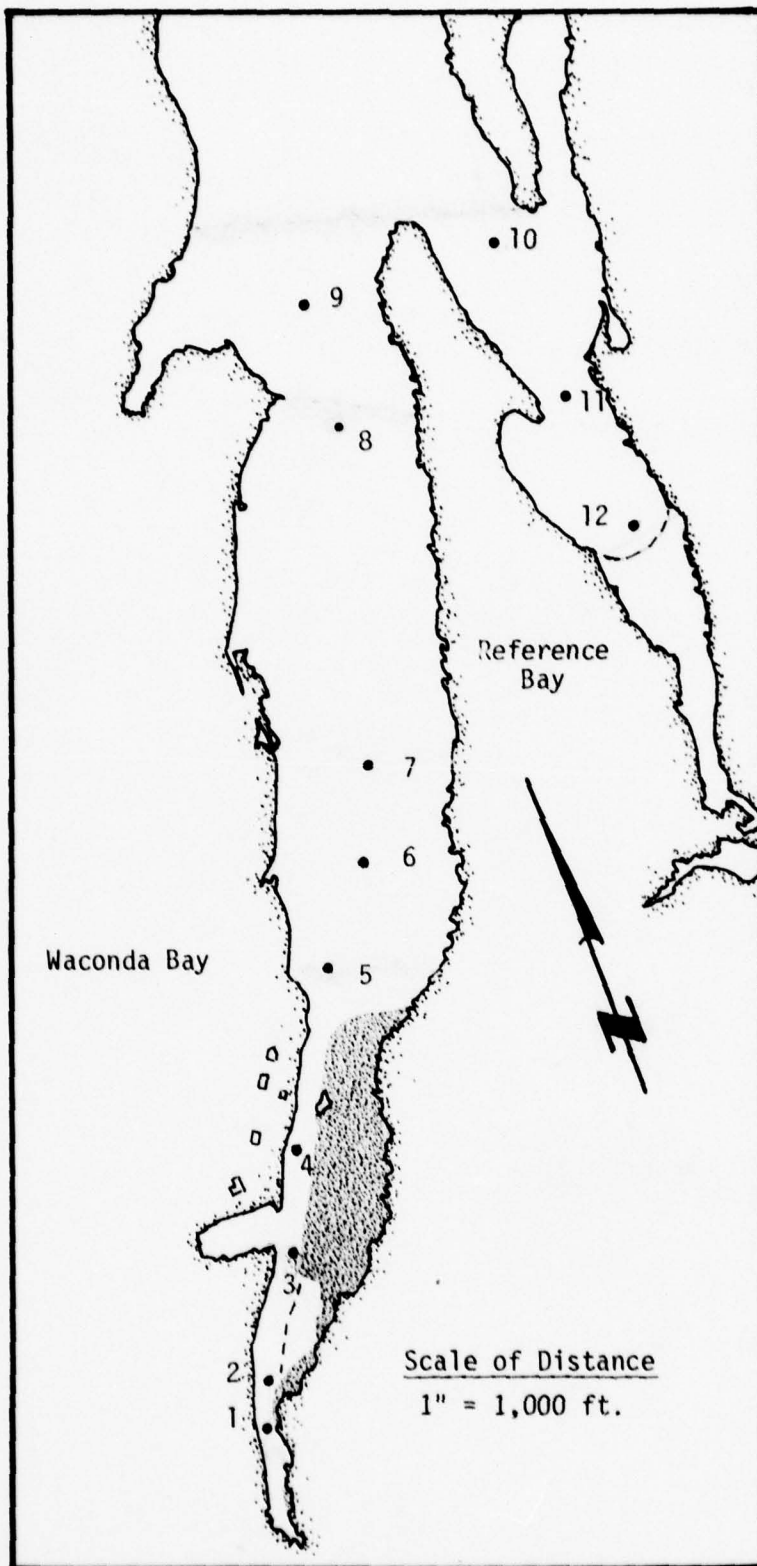


FIGURE 3. SAMPLING SITES FOR PHASE II STUDY

## PHASE II - WATER CHEMISTRY

### Introduction

During the period March 1-5, 1977, a second field survey was conducted in Waconda Bay. Field data were taken daily for D.O., temperature, and conductivity. Dissolved oxygen was near saturation values at all stations and temperatures were in the 7-13°C range. High conductivity values were observed at stations near the waste outfall and this parameter appeared to be a good indicator of munitions waste. Equipment failure precluded the measurement of pH. Water samples were taken at each site three times per day and composited. Analyses were made for ammonia-N, total Kjeldahl nitrogen, nitrate-N, chloride, total hardness, sulfate, and munitions. Additionally, effluent samples (24 hour composites) were taken daily during March for munitions analysis.

Sediment samples were taken at each site in Waconda Bay (three samples per site where each sample consisted of three dredge grabs). These samples were analyzed for total and volatile solids, nitrate-N, total Kjeldahl nitrogen, and munitions.

Except for the munitions samples, all analyses were performed in accordance with procedures of Standards Methods (APHA, 1975), (EPA, 1974), or Chemistry Laboratory Manual Bottom Sediments (EPA, 1969). Details of analytical procedures are presented in Appendix B.

### Characterization of Water Quality

Mean values for water quality data are shown in Table 3. Typical concentration-distance profiles are shown in Figures 4 through 6 for nitrate-N, ammonia-N, and chloride. For non-munitions parameters, these data show nearly constant average concentrations for stations 1 through 5. Sampling sites 1 through 4 are located in the relatively narrow and shallow channel existing in the upper end of Waconda Bay during low reservoir stages. Station 5 is at the mouth of this channel. The channel geometry in this area limits mixing and dispersion of effluent with other water in the reservoir. Down bay from station 5 the reservoir widens considerably and concentrations decrease rapidly.

Nitrate nitrogen levels were about 11 mg/l at stations 1 through 5 dropping to 1 mg/l at station 9. Ammonia, total Kjeldahl nitrogen, chloride, total hardness, and sulfate also showed elevated levels at stations 1 through 5 with concentrations decreasing from stations 5 to 9.

Five specific munitions related compounds were analyzed: 2,4,6 TNT; 2,4 DNT, 2,6 DNT; 1,3 DNB, and 1,3,5 TNB. The summation of these five compounds versus distance is shown in Figure 7. Mean values for these samples are presented in Table 4. Only three of the individual lake samples showed the presence of 2,4 DNT. Five samples contained 1,3 DNB. This compound was not detected at stations 6 through 9. Most samples contained 2,6 DNT and 2,4,6 TNT and the concentration appeared to decrease from station 5 through 9. By contrast, 1,3,5 TNB was more often found at down bay locations. On only two occasions was this compound detected at stations 1 through 5 compared to thirteen occurrences at stations 6 through 9. It appears that 1,3,5 TNB is a breakdown product, probably of 2,4,6 TNT. Plant effluent samples contained no detectable 1,3,5 TNB or 1,3 DNB.

TABLE 3

MEAN VALUES FOR CHEMICAL ANALYSES OF DAILY  
SAMPLING DURING THE PERIOD MARCH 1-5, 1977

STATION NUMBER	Mean Values					
	NH <sub>3</sub> -N	TKN	NO <sub>3</sub> -N	Cl	T. Hard	SO <sub>4</sub>
1	1.23	1.91	11.2	17.2	156	155
2	1.21	2.01	11.3	16.9	157	162
3	1.11	1.84	11.5	16.3	161	157
4	1.09	1.87	11.3	15.9	159	163
5	0.95	1.59	11.1	15.2	161	206
6	0.44	0.94	5.59	12.3	125	85.5
7	0.23	0.68	3.24	9.9	107	69.7
8	0.14	0.50	1.89	8.7	92.0	44.2
9	0.07	0.39	1.04	7.8	82.0	21.9
EFF	1.27	1.97	14.1	18.3	162	164
	mgN/l	mgN/l	mgN/l	mgCl/l	mg/l as CaCO <sub>3</sub>	mgSO <sub>4</sub> /l

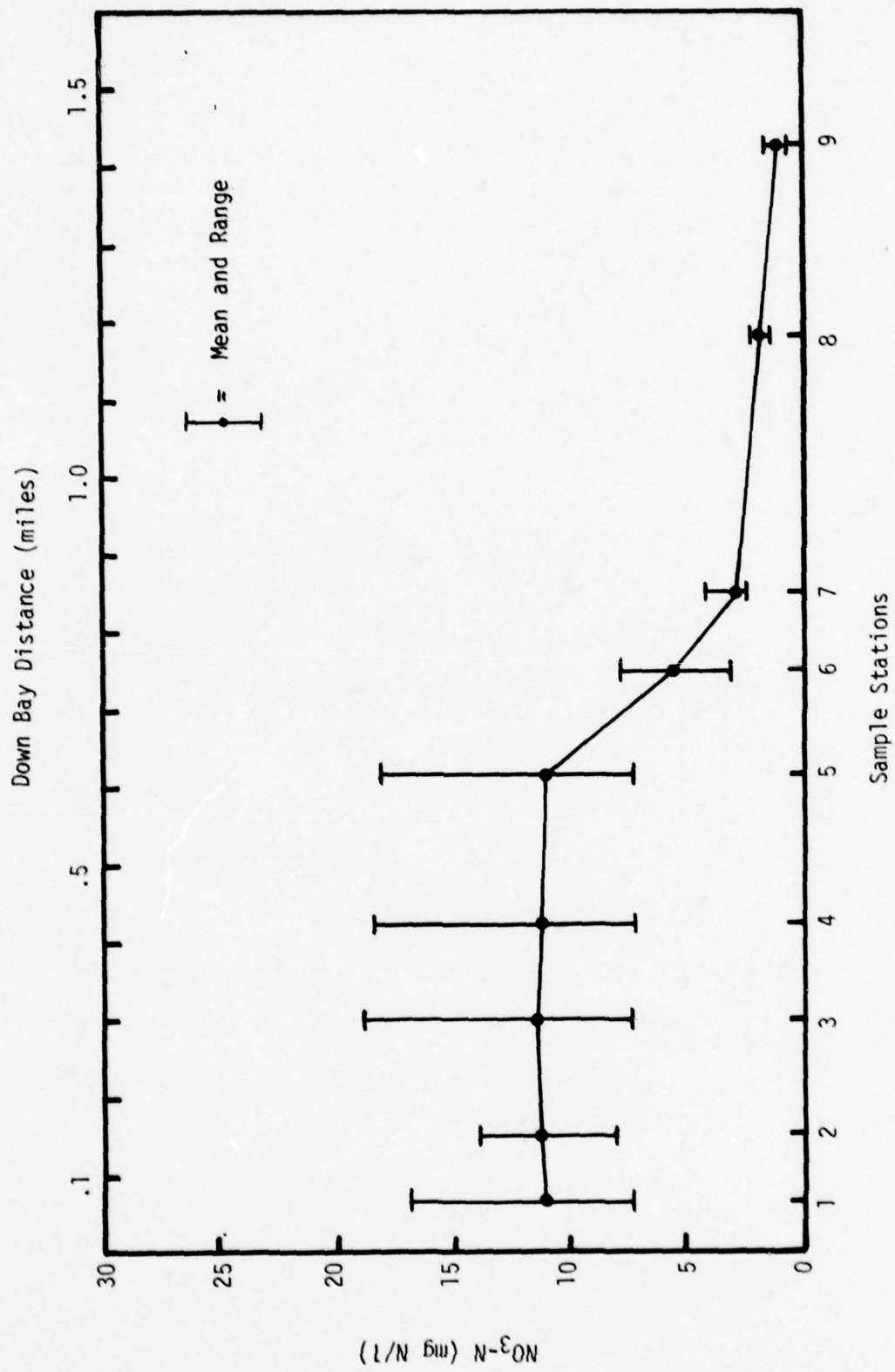


FIGURE 4. NITRATE NITROGEN, MARCH 1-5, 1977

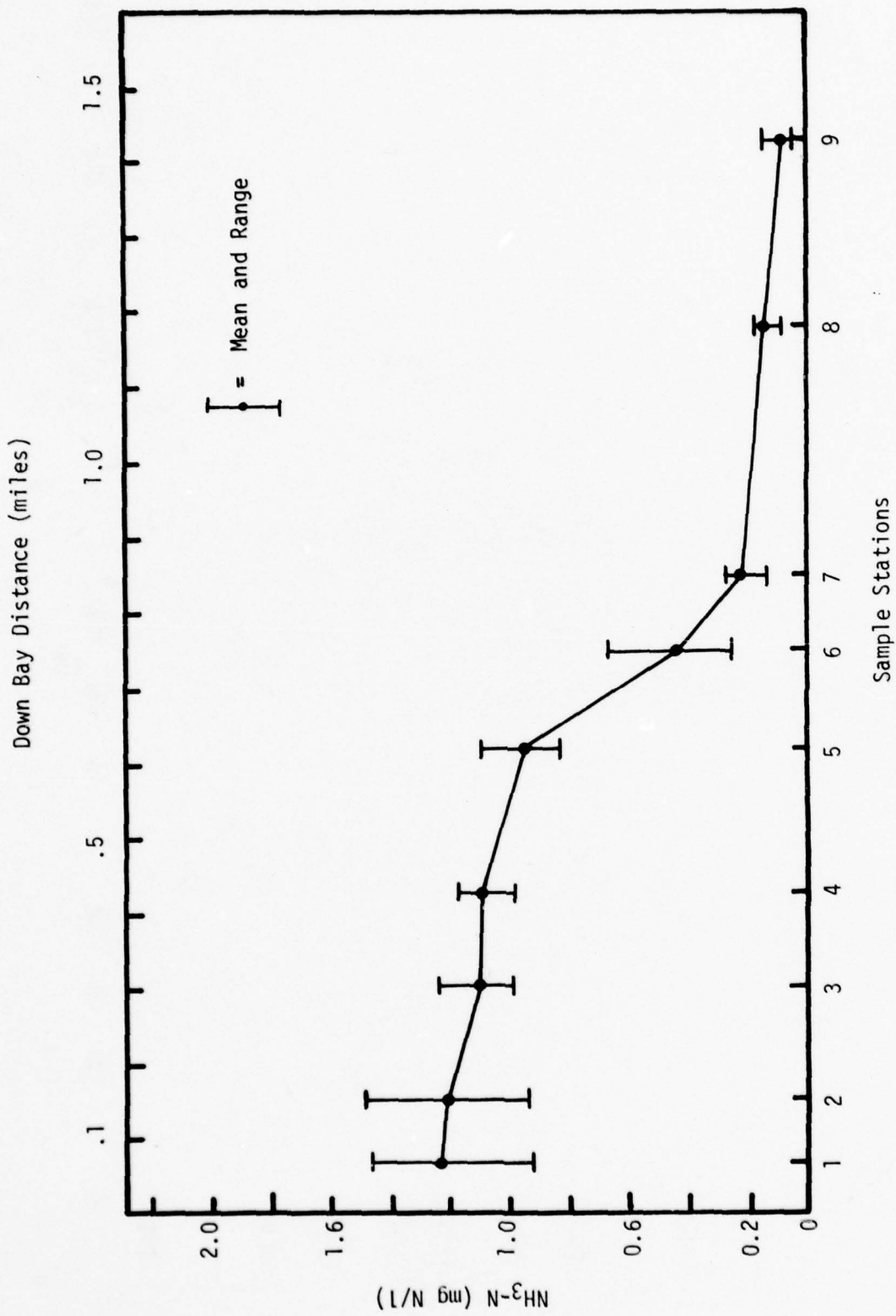


FIGURE 5. AMMONIA NITROGEN, MARCH 1-5, 1977



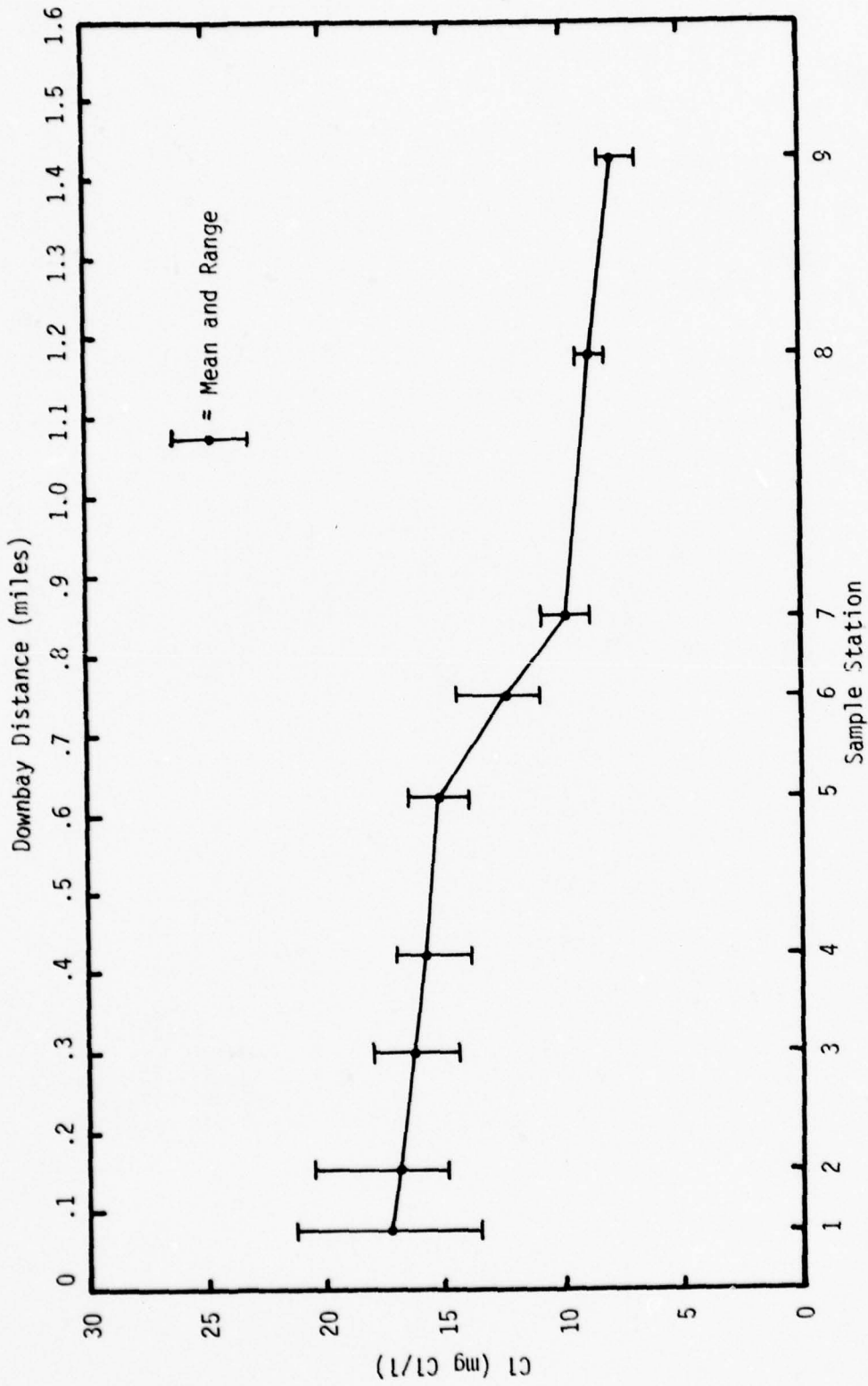


FIGURE 6. CHLORIDE CONCENTRATION, MARCH 1-5, 1977.

TABLE 4

MEAN VALUES FOR MUNITIONS ANALYSES OF DAILY  
SAMPLING DURING THE PERIOD MARCH 1-5, 1977

STATION NUMBER	MEAN Values in $\mu\text{g}/\text{l}$					TOTAL
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT	
1	4.1	2.9	< 0.10	17.9	48.0	73.0
2	<0.25	<0.75	22.1	38.7	40.3	102
3	<0.25	<0.75	< 0.10	32.0	43.7	76.7
4	0.4	<0.75	17.8	37.2	13.3	69.5
5	0.5	17.1	< 0.10	25.7	26.2	69.7
6	<0.25	1.2	< 0.10	12.1	1.8	$\leq 15.6$
7	<0.25	1.6	< 0.10	7.6	0.2	$\leq 9.8$
8	<0.25	10.6	< 0.10	1.3	0.1	$\leq 12.4$
9	<0.25	20.6	1.3	2.3	0.3	24.8
24 hr comp EFF	<0.25	<0.75	28.3	15.5	44.5	89.4

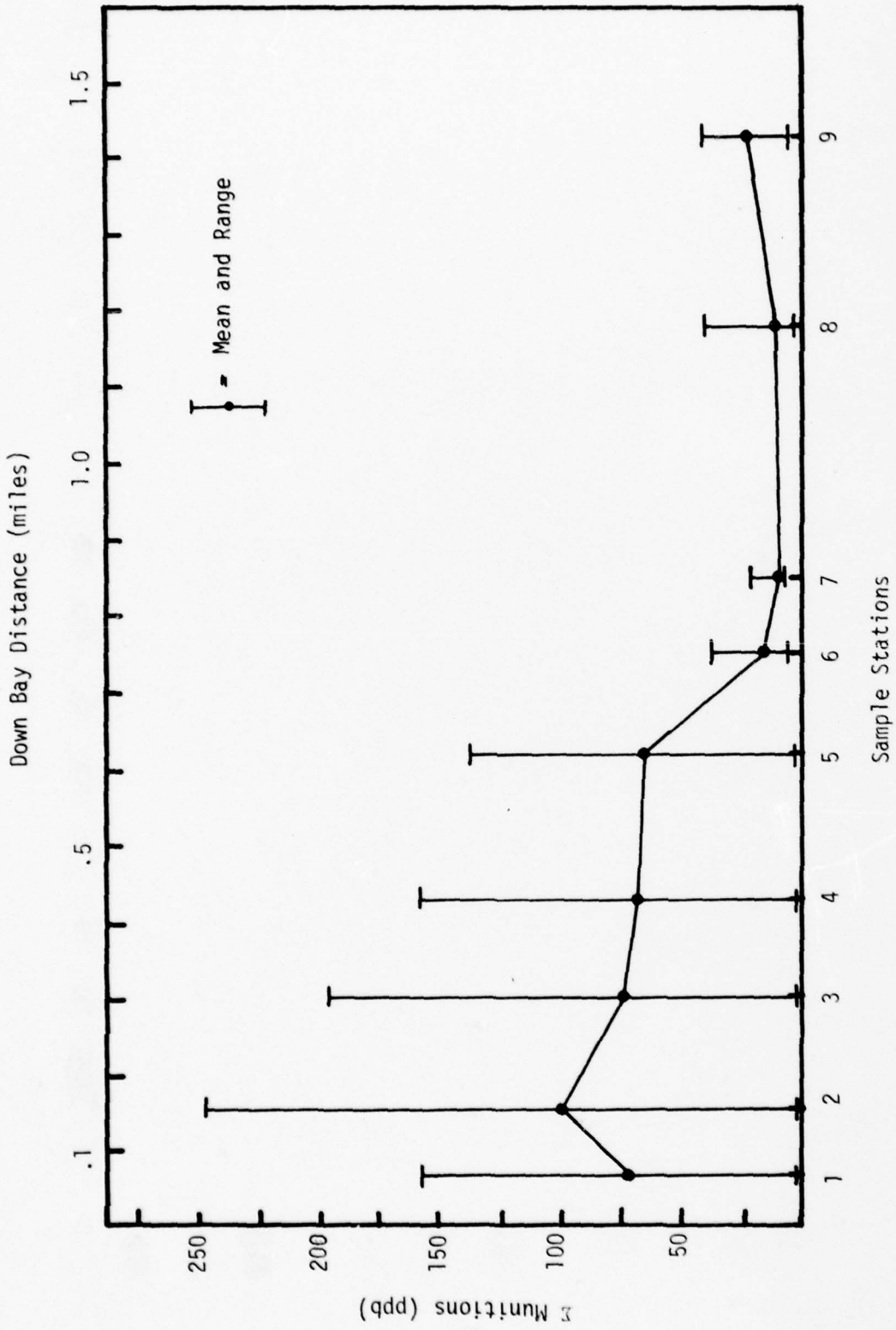


FIGURE 7. TOTAL MUNITIONS, MARCH 1-5, 1977

A station-to-station comparison was made using the following chemical data:  $\text{NH}_3\text{-N}$ , TKN,  $\text{NO}_3\text{-N}$ , Cl, total hardness,  $\text{SO}_4$ , and total munitions. The data for each parameter was first converted by dividing all values by the maximum value in the set. This puts data between a zero-to-one range. Station-to-station comparisons were made by calculating an average Euclidean distance as follows:

$$\Delta_{AB} = \left[ \sum_{i=1}^n (x_{iA} - x_{iB})^2 \right]^{1/2}$$

- where  $\Delta_{AB}$  = the Euclidean distance between Stations A and B  
 $n$  = the number of chemical or chemical/biologic parameters considered  
 $x_{iA}$  = the magnitude of the  $i$  th parameter at Station A  
 $x_{iB}$  = the magnitude of the  $i$  th parameter at Station B

The magnitude of the Euclidean distance,  $\Delta_{AB}$ , increases for any pair of stations as the number of parameters considered is increased. To eliminate this dependence, an average distance,  $d_{AB}$ , may be calculated (Sokal and Sneath, 1963):

$$d_{AB} = \sqrt{(\Delta_{AB})^2/n}$$

- where  $d_{AB}$  = average distance between Stations A and B  
 $\Delta_{AB}$  = Euclidean distance between Stations A and B  
 $n$  = number of chemical or chemical/biologic parameters considered

The average Euclidean distance values were used to construct a phenogram showing the relationships between the nine stations (see Appendix for details). This phenogram, Figure 8, shows stations 1 through 5 as decidedly different from stations 6 through 9. The cophenetic correlation coefficient of 0.951 indicates that the phenogram is an accurate representation of the relationship between stations. Generally, coefficients greater than 0.8 are considered to indicate accurate phenograms.

Biologic samplers were placed in Waconda Bay during this survey and remained for a month. During this period samples of plant effluent were taken by VAAP personnel and retained for subsequent munitions analysis. This was done so that the actual munitions concentrations existing during the incubation period could be estimated. The results are shown in Figure 9, and Appendix Table C-1. During the field survey period (March 1-5) the effluent munitions\* averaged 89  $\mu\text{g/l}$ . During the following six days effluent munitions concentration generally increased to a maximum of about 2,400  $\mu\text{g/l}$  on March 11. It then decreased to 157  $\mu\text{g/l}$  on March 17. From then until April 1, average effluent concentration was 217  $\mu\text{g/l}$ . With regard to the specific compounds, 1,3 DNB and 1,3,5 TNB were usually below detection limits. For the twenty

\* Total of the five specific compounds measured.

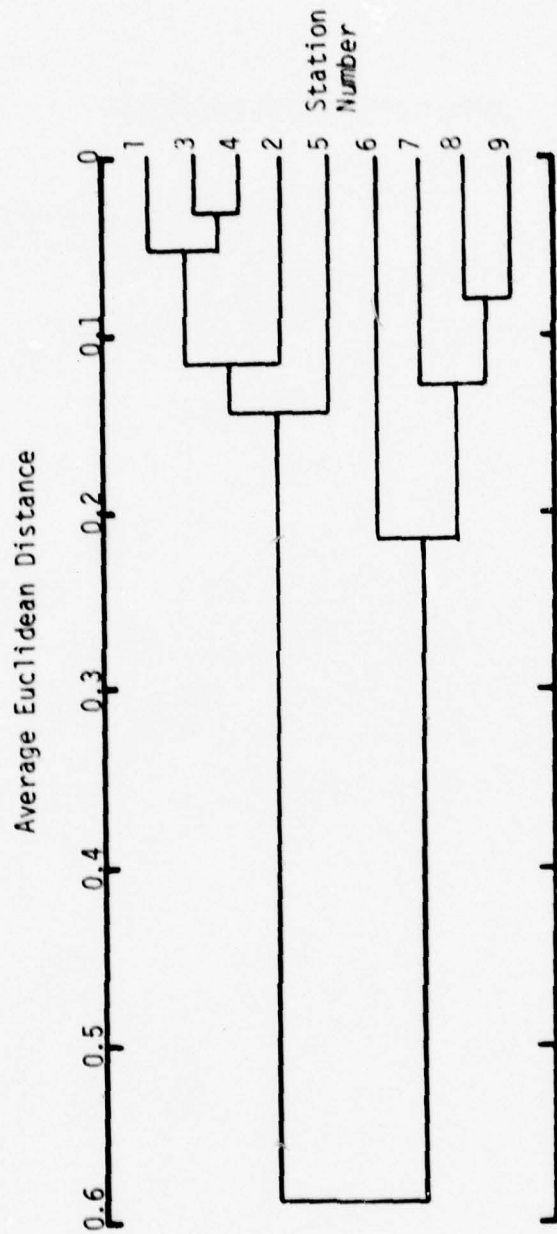


FIGURE 8. PHENOGRAM OF CHEMICAL DATA. COPHENETIC CORRELATION COEFFICIENT 0.951

effluent samples taken from March 5 through April 1, 1977 the concentrations of 2,4 DNT, 2,6 DNT, and 2,4,6 TNT averaged 323, 98, and 151  $\mu\text{g/l}$  respectively. Effluent flow rates remained relatively constant throughout this period averaging 5.9 MGD (see Appendix Table C-2).

Consequently, it appears that average concentrations during the incubation period exceeded those during the field studies. Total munitions in the channel extending down bay to and including station 5 probably approached 2,000  $\mu\text{g/l}$  and averaged near 500  $\mu\text{g/l}$ . Based on the relative munitions concentrations found at station 6 through 9 during the early March survey, total concentrations of munitions may have risen to 50 - 100  $\mu\text{g/l}$  at these sites later in March.

Thus, the munitions concentrations encountered by the organisms colonizing the artificial substrates may have been significantly higher than the concentrations encountered by the natural substrate organisms prior to the early March sampling. No detailed munitions analyses are available for the period preceding the March sampling.

#### Characterization of Sediment Quality

Mean values for sediment quality are shown in Table 5. Results for nitrate, total kjeldahl nitrogen, and total solids reveal little except slightly lower TKN values at the first three stations. The munitions results show that 1,3,5 TNB and 2,4,6 TNT clearly predominate 1,3 DNB, 2,4 DNT, and 2,6 DNT. The former compounds were found at all stations and in all except one replicate. For both compounds concentrations at stations 6 through 9 were higher than at stations 1 through 5; 260 and 109  $\mu\text{g/kg}$  for 1,3,5 TNB and 97 and 133  $\mu\text{g/kg}$  for 2,4,6 TNT. At stations 1 and 2 only, 2,4 DNT was found at  $<10$   $\mu\text{g/kg}$  at stations 1 through 5 only, 1,3 DNB and 2,6 DNT were found at  $<20$   $\mu\text{g/kg}$ .

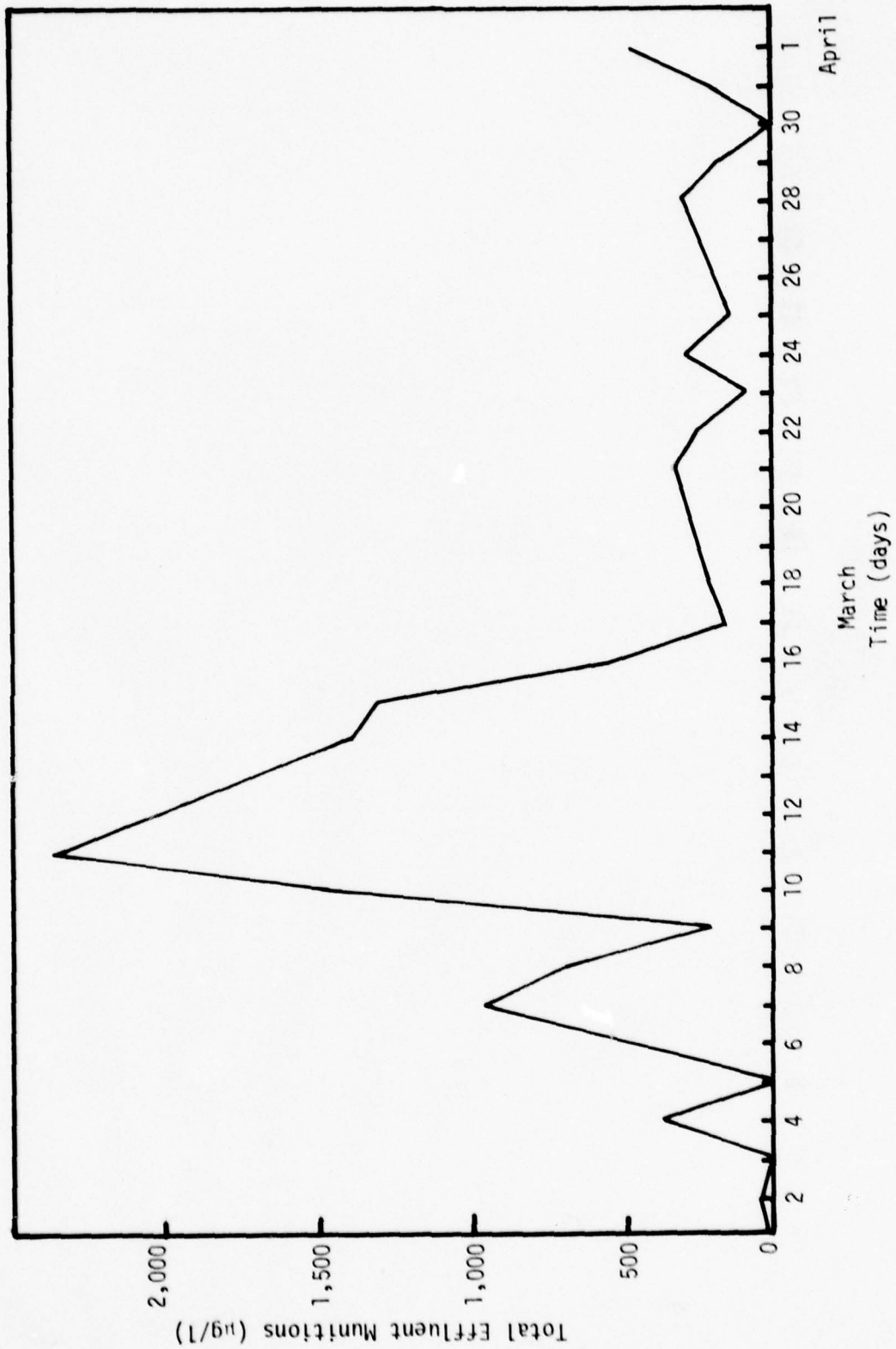


FIGURE 9. TOTAL MUNITIONS CONCENTRATION IN VAAP EFFLUENT DURING MARCH, 1977

TABLE 5  
 SEDIMENT ANALYSES, MARCH, 1977

STATION NUMBER	NO <sub>2</sub> - NO <sub>3</sub>	TKN	TS	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT
1	≤2	350	14	≤13	73	≤3.4	10	91
2	4	333	12	≤12	≤179	≤7.9	17	119
3	≤2	352	17	≤14	76	<2.5	17	119
4	<2	644	32	11	135	<2.5	≤4.3	86
5	≤5	495	27	14	80	<2.5	11	70
6	<2	628	23	<6.3	221	<2.5	<1.3	138
7	≤11	529	22	<6.3	250	<2.5	<1.3	105
8	<2	466	9	<6.3	304	<2.5	<1.3	142
9	≤2	687	35	<6.3	263	<2.5	<1.3	148
UNITS	mgN/kg	mgN/kg	%	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg

Figures shown are the average of three replicates taken March 3, 1977.  
 Values reported on a wet weight basis.



## PHASE II PERIPHYTON

### Introduction

The periphyton or "Aufwuchs" community is an assemblage of attached microorganisms (primarily algae) growing on the surface of submerged substrates and forming a coating which is commonly a green or brown color. This community consists of both autotrophic (i.e. unicellular or filamentous algae) and heterotrophic (bacteria, protozoa, rotifers, ect.) forms.

These organisms are well suited to biological investigations since they remain at fixed locations and are sensitive to environmental alterations. Their populations and biomass are relatively easy to quantify using standard laboratory procedures and are adaptable to a variety of statistical analyses. The results of the survey conducted at VAAP during the winter of 1977 consist of two parts: 1) data pertaining to the field survey and 2) treatment of this information via statistical inference.

The community structure was examined using diatoms and filamentous algae, chlorophyll a, biomass and vital staining. Use of the staining technique showed the live-dead relationship among the periphyton population. The number of diatom frustules that are inactive in a community colonizing a glass slide cannot be estimated using standard techniques. Since the inactive portion may be substantial, an estimate was made on selected stations using tetrazolium violet. The methodology for the various procedures is appended.

### Artificial Substrate Colonization Studies

Glass microscope slides with a surface area of 3871 mm<sup>2</sup> were exposed and allowed to colonize for 30 days at the 12 selected stations. Periphytometers containing 8 slide-unit-cartridges were placed together in suspension units as shown in Appendix D. Suspension units containing both 6 and 8 periphytometer units were used in the study. The suspension units with the lower number of periphytometers were used to provide additional slides for the statistical portion of the investigation and were added at those stations predicted to reflect high, medium and low stress from VAAP effluent.

Organism density ranged from 27 per mm<sup>2</sup> at Station 2 to 37,267 at Station 11. Numbers of species per station ranged from 22 to 52 in Waconda Bay and from 76 to 83 in the reference bay. To simplify data presentation and eliminate rare occurring species, a culling routine was completed and all species whose numbers did not constitute at least one percent of the total population at any one station were eliminated. This procedure resulted in a reduction of species from 124 to 30. However, the maximum drop in population density at any one station was 7 percent. Table 6 shows the differences in density and number of species before and after culling. The culled data are shown in Table 7. Organism density is significantly different at stations 1 through 5 as compared to stations 6 through 12 (see statistical report in Appendix).

TABLE 6

A COMPARISON OF POPULATION SIZE AND NUMBERS OF SPECIES  
IN CULLED AND UNCULLED DATA AT 1%.  
PERIPHYTON - ARTIFICIAL SUBSTRATE

	STATION 1	2	3	4	5	6
CULLED						
no/mm <sup>2</sup>	135	27	76	165	727	14721
species	24	22	25	25	25	18
UNCULLED						
no/mm <sup>2</sup>	141	27	78	169	745	14916
species	39	22	46	46	52	37
% DIFFERENCE						
no/mm <sup>2</sup>	4	0	3	2	2	1
species	38	0	46	46	52	51

	STATION 7	8	9	10	11	12
CULLED						
no/mm <sup>2</sup>	18714	27021	21970	-	35158	18354
species	21	25	22	-	22	24
UNCULLED						
no/mm <sup>2</sup>	18941	27785	22458	-	37267	19696
species	40	52	39	-	76	83
% DIFFERENCE						
no/mm <sup>2</sup>	1	3	2	-	6	7
species	48	52	44	-	71	71

TABLE 7

VAAP PERIPHYTON, ARTIFICIAL SUBSTRATE, MARCH 1977

Taxonomic Classification	Number of Organisms per mm <sup>2</sup> at Station:											
	1	2	3	4	5	6	7	8	9	11	12	
BACILLARIOPHYTA (DIATOMS)												
ACHNANTHES LANCEOLATA V DUBIA	P	2	P	5	P	291	621	2198	3668	7172	42	123
ACHNANTHES MINUTISSIMA	15	P	-	P	P	-	-	-	-	-	-	3612
AMPHORA VENETA	-	-	-	-	-	-	-	-	-	-	-	-
COCCONEIS PLACENTULA V EUGLYPTA	4	-	12	72	8	12987	14357	11211	6108	2828	-	1926
DIATOMA TENUE V ELONGATUM	53	2	18	12	8	511	222	1911	1108	2828	-	1926
EUGLIA CUMVATA	3	P	1	P	7	77	161	592	415	4325	-	1870
FRIGILARIA CAPSINA	14	1	4	P	24	330	1311	5170	4952	9327	-	2909
FRIGILARIA VAUCERIAE	-	-	-	-	-	-	-	-	-	-	-	-
GOMPHONEMA ANGUSTATUM	9	2	2	2	P	19	15	17	61	1511	-	2571
GOMPHONEMA BREVULUM	7	2	2	17	18	107	130	187	61	1300	-	209
MELDSTIA GRANULATA	-	-	-	-	11	8	27	68	15	197	-	98
MEDUSIA ITALICA	3	3	3	4	11	69	276	1085	1477	379	-	153
MEDUSIA PAPILLATA	3	2	2	P	8	31	31	102	-	417	-	158
NALICULA CRYPTOCEPHALA	-	-	-	-	-	-	-	-	-	-	-	-
NITZSCHIA ACUMINATA	P	P	P	P	3	5	38	366	497	511	-	454
NITZSCHIA CRISTATA	1	P	2	3	3	5	31	34	21	400	-	370
NITZSCHIA FRUSTULUM V SUBSALINA	2	P	P	2	19	38	48	83	81	-	-	-
NITZSCHIA GUEZZINGIANA	2	P	P	2	19	38	48	83	81	-	-	-
NITZSCHIA PALLIDA	-	-	-	-	19	38	48	83	81	-	-	-
PINACULA OBSCURA	-	-	-	-	P	-	-	-	-	-	-	-
PINACULARIA RUPESTRIS	-	-	-	-	P	-	-	-	-	-	-	-
STEPHANODISCUS ASTRIFEA V MINUTULA	1	P	2	2	P	25	123	251	261	493	-	115
STEPHANODISCUS INVISITATUS	1	P	2	2	11	46	123	251	261	493	-	115
SURELLA OVALA	4	2	11	13	19	8	8	25	15	68	-	268
SURELLA QUADRATA	1	2	11	13	19	8	8	25	15	68	-	268
SYNEURA DELICATISSIMA	1	2	11	13	19	46	253	155	168	17	-	21
SYNEURA RUPENS V FAMILIARIS	9	P	4	P	11	560	989	3595	2223	2087	-	975
SYNEURA RUPENS	2	1	5	19	43	23	92	680	307	1914	-	1491
SYNEURA ULNA	2	1	5	19	43	23	130	281	307	307	-	115
TOTAL NUMBER OF ORGANISMS	135	27	76	165	727	14721	18714	27021	21970	35158	-	16354
NUMBER OF TAXA	24	22	25	25	25	18	21	25	22	22	-	24

Culled at 1% Level

The dominant species in Waconda Bay are:

Achnanthes minutissima  
Cymbella prostrata  
Diatoma tenue var. elongatum  
Fragilaria vaucheriae  
Melosira varians  
Synedra rumpens

These species reached population levels of at least 1,000 individuals/ $\text{mm}^2$  at one or more stations in Waconda Bay. Diatom populations were reduced in the impact area from stations 1 to 5 although the actual number of species was approximately the same as down bay.

The marked population increase at station 6 can be correlated with the water chemistry, which, during the March 1-5 survey, showed a significant drop in concentration of several components between stations 5 and 6. (See chemistry section.) Considering the dominants, the ratio of density for dominant organisms between stations 6 and 5 where the Bay widens is as follows:

<u>Achnanthes minutissima</u>	- 48
<u>Diatoma tenue</u>	- 25
<u>Fragilaria vaucheriae</u>	- 14
<u>Melosira varians</u>	- 9
<u>Synedra rumpens</u>	- 51

Figure 10 shows the clustering of stations by the Pinkham-Pearson Index using culled data with mutual absence important. The phenogram shows that stations 1 through 5 are decidedly dissimilar from stations 6 through 12. Additionally, the reference bay stations, 11 and 12, form a sub-group somewhat different from Waconda Bay stations 6 through 9.

Shannon-Weaver diversity values are shown in Table 8. Theoretically, the higher the diversity the "healthier" the community. However, examination of the results shows that diversity in the area of maximum stress, stations 1 to 5, is higher than at the downbay stations 6 to 9. This anomalous condition is caused by the severe drop in population density of all dominant species at stations 1 to 5 which results in a condition of better balance between the surviving species. Since diversity considers only relative proportions of species and ignores absolute population density, it seems to be of limited value in a situation where the main input is seen as reduction in population density.

#### Natural Substrate Colonization Studies

Natural substrate collections were made at 9 stations in Waconda Bay and 3 stations in the reference bay. A description of these samples and the

PAGE 33 - FIGURE 10 is also PAGE 167 - FIGURE G-1

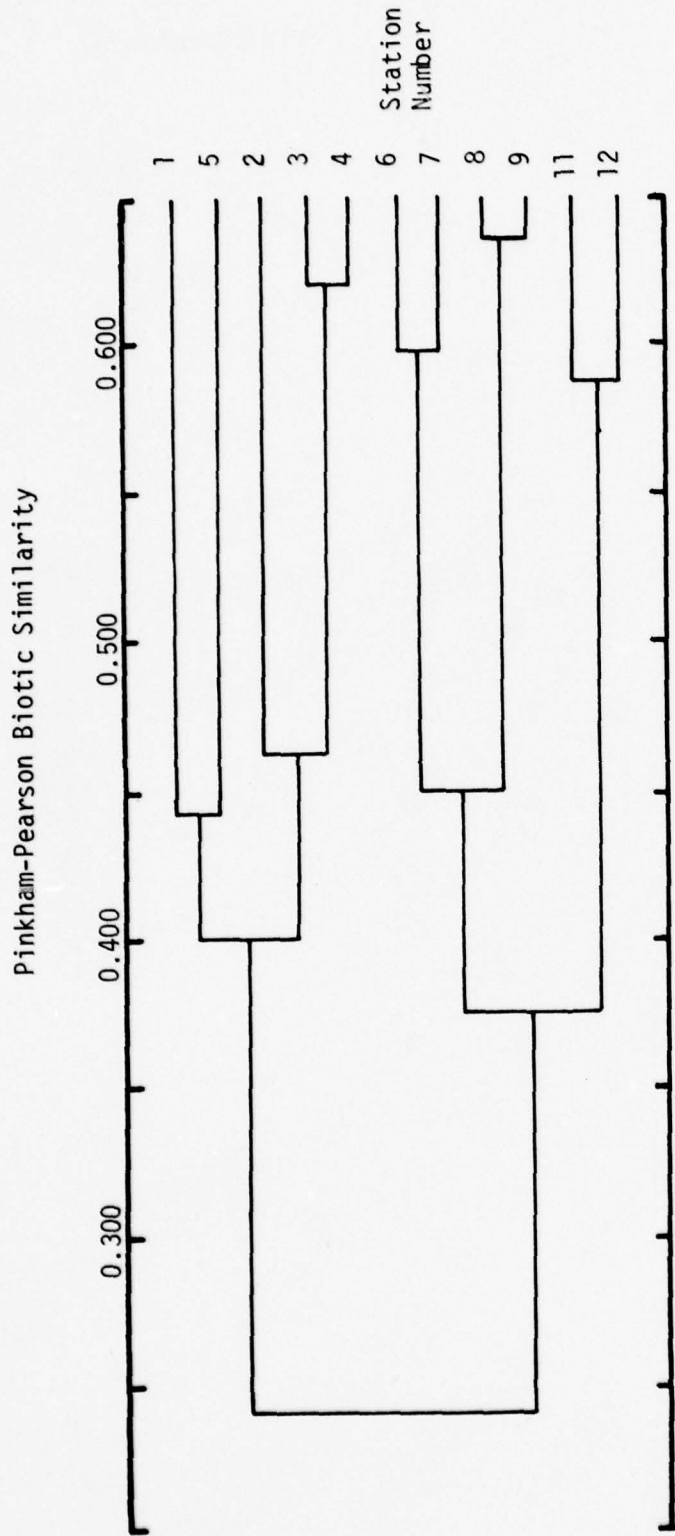


FIGURE 10. PHENOGRAM OF PERIPHYTON, ARTIFICIAL SUBSTRATE, CULLED 1%, MUTUAL ABSENCE IMPORTANT, COPHENETIC CORRELATION COEFFICIENT .871.

TABLE 8  
 SHANNON-WEAVER DIVERSITY INDICES (H) FOR VAAP ARTIFICIAL SUBSTRATE DIATOMS,  
 MARCH, 1977

STATIONS	MAGNITUDE culled	MAGNITUDE unculled
1	2.22	2.37
2	2.49	2.49
3	2.49	2.56
4	2.12	2.21
5	1.40	1.54
6	0.60	0.70
7	1.10	1.18
8	1.88	2.03
9	2.04	2.16
10	-	-
11	2.27	2.54
12	2.46	2.78

Base e

resulting data are shown in Table 9 and 10. Generally scrapings were made from submerged branches, rocks, and styrofoam floats. Three substrates were sampled at each location except stations 4 and 8. Counts were made to approximate 500 diatom valves. Comparisons among stations can only be made relative to numbers of individual species colonizing these substrates. The overall total number of species ranged from 11 to 77 with no marked differences related to station locations. In fact, the two extreme values both occurred at station 3. The data are culled to eliminate species that don't make up at least one percent of the total at at least one station. The five most commonly occurring species were:

- 1) Achnanthes minutissima
- 2) Fragilaria capucina
- 3) Fragilaria vaucheriae
- 4) Stephanodiscus invisitatus
- 5) Synedra rumpens

In considering the distribution of individual species it is interesting to note that Achnanthes minutissima was as common an occurrence in the impact zone as in other areas of Waconda Bay and the reference bay. This suggests that Achnanthes is not as susceptible to munitions waste as might be expected. In an earlier study conducted during the summer of 1975, Achnanthes minutissima showed a similar response and accounted for 80 percent of the diatom population colonizing artificial substrates in areas of severe stress from VAAP waste. The two Fragilaria sp. reached a maximum of approximately one fifth the population. These levels were attained only at downbay stations. With the exception of Synedra rumpens at station 4, the minor dominants were severely growth restricted in the high stress zone between stations 1 through 5.

Pinkham-Pearson comparisons among the various samples are shown in Figure 11. Clustering of stations using natural substrates results in a grouping based primarily on substrate type rather than location relative to the waste discharge. For example, the first station cluster, with one exception, has a common denominator of wood scrapings as a substrate type. The second, beginning with 4AR includes predominately rock scrapings. Styrofoam substrates principally compose the next group and include samples from stations 6 through 8, 11 and 12. Treatment of the data by the Pinkham-Pearson analysis is especially useful in this case to point out the substrate dependence of periphyton diatoms. The phenographic display in this instance further demonstrates the utility of uniform substrates for periphyton investigations. The cophenetic correlation coefficient of 0.734, however, indicates that caution should be exercised in interpreting this phenogram.

#### Filamentous Algae

Filamentous forms colonizing glass slides were restricted to green and blue-green species. The data are shown in Table 11. Six genera predominated with the blue-green, Schizothrix calcicola, the most abundant organism at all stations. The second dominant, Oscillatoria sp., was observed at all sampling locations but occurred only sporadically between stations 1 through 5. An Anabaena species was recorded only once from station 12 in the reference area.



TABLE 9  
 INVENTORY OF NATURAL PERIPHYTON SUBSTRATE  
 SAMPLES COLLECTED FEBRUARY 28, 1977

<u>Station</u>	<u>Substrate Description</u>	<u>Station</u>	<u>Substrate Description</u>
Waste Pond Spillway	Rock Scraping	S-7	Dock Styrofoam <sup>S</sup>
Waste Outfall Stream	Rock Scraping	S-7	Dock Styrofoam <sup>S</sup>
"Old Station A"*	Branch Scraping	S-7	Branch Scraping <sup>W</sup>
"Old Station A"	Branch Scraping	S-7	Stump Scraping <sup>W</sup>
"Old Station A"	Branch Scraping	S-8	Styrofoam from "No Wake" Marker <sup>S</sup>
S-1	Branch Scraping <sup>W</sup>	S-8	Scraping from Metal Buoy <sup>M</sup>
S-1	Branch Scraping <sup>W</sup>	S-9	Scraping of Metal Buoy <sup>M</sup>
S-1	Log Scraping <sup>W</sup>	S-9	Rock Scraping <sup>R</sup>
S-2	Branch Scraping <sup>W</sup>	S-9	Rock Scraping <sup>R</sup>
S-2	Branch Scraping <sup>W</sup>	S-10	Dock Styrofoam <sup>S</sup>
S-2	Branch Scraping <sup>W</sup>	S-10	Rock + Old Bottle Scraping <sup>R</sup>
S-3	Rock Scraping <sup>R</sup>	S-10	Rock Scraping <sup>R</sup>
S-3	Branch Scraping <sup>W</sup>	S-10	Surface Sediment <sup>B</sup>
S-3	Rock Scraping <sup>R</sup>	S-11	Wood Dock <sup>W</sup>
S-4**	Dock Scraping <sup>W</sup>	S-11	Dock Styrofoam <sup>S</sup>
S-5	Rock Scraping <sup>R</sup>	S-11	Rock Scraping <sup>R</sup>
S-5	Rock Scraping <sup>R</sup>	S-12	Rock Scraping <sup>R</sup>
S-5	Rock Scraping <sup>R</sup>	S-12	Dock Styrofoam <sup>S</sup>
S-6	Stump Scraping <sup>W</sup>	S-12	Branch Scraping <sup>W</sup>
S-6	Rock Scraping <sup>R</sup>		
S-6	Dock Styrofoam <sup>S</sup>		

\*20 yards from VAAP outfall

\*\*Only 1 substrate available at Station S-4

W = Wood; R = Rock; S = Styrofoam; M = Metal; B = Surface Sediment

TABLE 10  
VAAP PERIPHYTON, NATURAL SUBSTRATE, CULLED AT 1% MARCH 1977

Taxonomic Classification	Number of Organisms at station:						
	1AH	1BH	1CH	2AH	2BH	2CH	3AH
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V. METEOROVALVE							
ACHNANTHES LANCEOLATA V. DURIA							
ACHNANTHES RINTOSSINA	193	210	402	361	370	401	69
ACHNANTHES NULLII							
ANGULONEIS VITREA			1	2		1	1
ASTERIONELLA FORMOSA	1						
CALONEIS NYALINA	1		1				
COCCONEIS PLEACENTULA V. EUGLYPTA							
CYCLOTELLA ATOMUS		3			1	2	7
CYCLOTELLA HNEGHEMINIANA	1						10
CYCLOTELLA STELLIGENA	2		1	1		2	10
CYMBELLA AFFINIS							
CYMBELLA DELICATULA						2	2
CYMBELLA LAEVIS							2
CYMBELLA RHYNOCEPHALA							2
CYMBELLA PROSTRATA V. AUERSHALDII	5	1	1	2	12	7	16
CYMBELLA TUMIDA							
DIATOMA TENUE V. ELONGATUM			1				
FUNOTIA PECTINALIS V. MINOR		1			7	1	1
FRAGILARIA CAPUCINA V. RESOLEPTA			1		1	2	1
FRAGILARIA CAPUCINA							
FRAGILARIA CONSTRUENS N							
FRAGILARIA CONSTRUENS V. VENTER		1			1		3
FRAGILARIA PINNATA							
FRAGILARIA VAUCHERIAE	1						
GOMPHONEMA ANGUSTATUM		6					1
GOMPHONEMA PARVULUM	9	6	6	4	12	6	1
MELOSIRA GISTANS	18	11	1	6	3		2
MELOSIRA GRANULATA	10	6	2	1	2	2	2
MELOSIRA ITALICA	28	64					2
MELOSIRA VARIANS	2		1				1
NAVICULA CAPITATA V. HUNGARICA							1
NAVICULA CINCTA					1	6	5
NAVICULA CONIFERACEA V. PEREGRINA	1	1					7
NAVICULA CONTENTA V. RICEPS		4		4	3	2	6
NAVICULA CRYPTOCEPHALA	12		3				
NAVICULA MENISCULUS V. UPSALIENSIS	2						
NAVICULA MINIMA	4	3	3	1	1		6
NAVICULA NOTICA							1
NAVICULA PELLICULOSA	2						
NAVICULA POPULA V. NOTATA	1				1	1	1
NAVICULA RADIOSA N							
NAVICULA RADIOSA V. TENELLA			2				
NAVICULA RHYNOCEPHALA V. GENNANI	4	1		1		1	15
NAVICULA SALINARIUM V. INTERMEDIA							
NAVICULA SECRETA V. APICULATA	20	16		3	3	2	12
NAVICULA SEMICULUM							
NAVICULA CF. SINOLA							
NAVICULA SUBHAMULATA	1			1			
NAVICULA SUBTILISSIMA					1	1	2
NAVICULA THIESENHANNIT	3	2					2
NAVICULA SP. 6							
NITZSCHIA ACCOMMODATA	1		1	2			2
NITZSCHIA ACCOMMODATA	23	40	1	2			2
NITZSCHIA AMPHIBIA						1	3
NITZSCHIA CISTA	3	2	3	1	1		3
NITZSCHIA DISSIPATA							
NITZSCHIA DENTICULA	7		3				7
NITZSCHIA FILICORNIS				2	3		
NITZSCHIA FRUSTULUM V. SUBSALINA	7	10	2	2	2	4	15
NITZSCHIA KUTZINGIANA	24	37	13	13	7	9	12
NITZSCHIA PALLA		12					12
NITZSCHIA PECTA							
NITZSCHIA SINUATA V. TABELLARIA	2	1			6	1	2
NITZSCHIA SP. 6							
STEPHANODISCUS ASTRAEA							
STEPHANODISCUS ASTRAEA V. MINUTULA	3	7					
STEPHANODISCUS INVISITATUS	14	25	5	20	1	3	38
SURIRELLA OVATA	7	2		1			9
SURIRELLA SUEVICA		1					1
SYNDRA BELICATAISSIMA	3	10	1				10
SYNDRA RUPPENS					20	13	
SYNDRA RUPPENS V. FAMILIARIS	3	2		1	1	4	1
SYNDRA ULNA							
TABELLARIA FLUCCULOSA							
TOTAL NUMBER OF ORGANISMS	441	483	476	492	491	490	460
NUMBER OF TAXA	40	30	26	34	29	29	44

Taxonomic Classification	Number of Organisms at station:						
	3BR	3CR	4AR	5AR	5BR	5CR	6AR
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V. HETEROVALVE					4	12	1
ACHNANTHES LANCEOLATA V. DURIA					12		
ACHNANTHES MINUTISSIMA	425	477	203	322	105	57	169
ACHNANTHES NULLI							
ACROGONEIS VITATA							
ASTERIONELLA FORMOSA			1	1		1	2
CALONEIS HYALINA	1		1		20	11	
COCCONEIS PLACENTULA V. EUGLYPTA					12		
CYCLOTELLA ATOMUS							5
CYCLOTELLA MENEGHINIANA							
CYCLOTELLA STELLIGEMA						3	7
CYMBELLA AFFINIS							
CYMBELLA DELICATULA					1		
CYMBELLA LAEVIS				1	1		2
CYMBELLA RHYNOCEPHALA							
CYMBELLA PROSTRATA V. AUERSWALDII		1	3	3	1	1	13
CYMBELLA TUMIDA							
DIATOMA TENUE V. ELONGATUM			25	35	2	27	16
EUNDITIA PECTINALIS V. MINOR	6	15	1	1	27	17	14
FRAGILARIA CAPUCINA V. MESOLEPTA							
FRAGILARIA CAPUCINA			1	4	10	8	14
FRAGILARIA CONSTRUENS N							
FRAGILARIA CONSTRUENS V. VENTER				1		1	
FRAGILARIA PINNATA							
FRAGILARIA VAUCHERIAE			14	12	1	14	14
GOMPHONEMA ANGUSTATUM			5	3		17	1
GOMPHONEMA PARVULUM		7			14	17	1
MELOSIRA DISTANS							
MELOSIRA GRANULATA		1	9	10	12	5	5
MELOSIRA ITALICA	3	2	11	20	2	5	14
MELOSIRA VARIANS							
NAVICULA CAPITATA V. HUNGARICA	1			1	1		
NAVICULA CINCTA					2		5
NAVICULA CONFERRACEA V. PEREGRINA			2	2			
NAVICULA CONTENTA V. RICEPS							
NAVICULA CRYPTOCEPHALA	3		7	14	17	1	16
NAVICULA MENISCULUS V. UPSALIENSIS				5	3		
NAVICULA MINIMA	6				13	19	
NAVICULA MUTICA							
NAVICULA PELLICULOSA							
NAVICULA POPULA V. MUTATA					1	1	
NAVICULA RADIOSA N							
NAVICULA RADIOSA V. TENELLA							
NAVICULA RHYNOCEPHALA V. GERMANI			2	2			2
NAVICULA SALINARIUM V. INTERMEDIA							
NAVICULA SECURATA V. APICULATA	18		7	3	18	14	13
NAVICULA SEMINULUM	1				2	10	
NAVICULA CF. SIMULA							
NAVICULA SUBHAMULATA							
NAVICULA SUTILISSIMA		1	1	8			
NAVICULA THENEHANNII	2	2		8			
NAVICULA SP. 6							
NITZSCHIA ACCUMUDATA			1				
NITZSCHIA ACCUMUDATA							
NITZSCHIA AMPHIBIA			1		1		
NITZSCHIA BITA			1				
NITZSCHIA DISSIPATA							
NITZSCHIA DENTICULA	1		1		36		
NITZSCHIA FILIFORMIS							
NITZSCHIA FRUSTULUM V. SUBSALINA	5		1	10	2		
NITZSCHIA KUETZINGIANA	1						
NITZSCHIA PALEA							
NITZSCHIA NECTA							
NITZSCHIA SINUATA V. TABELLARIA	1						
NITZSCHIA SP. 8							
STEPHANODISCUS ASTRAEA							
STEPHANODISCUS ASTRAEA V. MINUTULA	1		1	8	18		5
STEPHANODISCUS INVISITATUS			25	45			2
SUMIRELLA OVATA							
SYMPLELLA SUETICA							
SYNEDRA DEPRESSISSIMA							
SYNEDRA RHODENS	19		12	15			56
SYNEDRA RUMPENS V. FAMILIARIS			8				
SYNEDRA ULNA							
TABELLARIA FLUCCULOSA							
TOTAL NUMBER OF ORGANISMS	494	507	491	454	495	484	462
NUMBER OF TAXA	19	9	37	46	36	42	43

TABLE 10. (Continued)

Taxonomic Classification	Number of Organisms at station:						
	6B5	6C4	7A8	7B8	7C4	8A8	8B4
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V. HETEROUALVE	32	1	2	2	2	1	1
ACHNANTHES LANCEOLATA V. DUBIA	131	103	190	104	22	340	326
ACHNANTHES MINUTISSIMA	86	10	76	42	1	8	29
ACHNANTHES NULLII	2	3	1	1	1	1	1
ANDROGONEIS VITREA	-	-	-	-	-	-	-
ASTERIONELLA FORMOSA	-	-	-	-	-	-	-
CALONEIS HYALINA	-	-	-	-	-	-	-
COCconeis PLACENTULA V. EUGLYPTA	-	-	-	-	-	-	-
CYCLOTELLA ATOMUS	-	3	-	-	-	-	-
CYCLOTELLA HENEGBINIANA	1	-	-	-	-	-	-
CYCLOTELLA STELLIUMNA	1	-	2	2	1	1	1
CYMBELLA AFFINIS	-	-	-	-	-	-	-
CYMBELLA DELICATULA	44	-	-	-	-	-	-
CYMBELLA LAEVIS	4	-	2	2	-	-	-
CYMBELLA MICROCEPHALA	4	-	2	2	-	-	-
CYMBELLA PROSTRATA V. AUERSWALDII	13	13	2	17	18	8	12
CYMBELLA TURIDA	3	7	12	8	10	8	16
DIATOMA TENUE V. ELONGATUM	-	-	-	-	-	-	-
EUNOTIA PECTINALIS V. MINOR	-	-	-	-	-	-	-
FRAGILARIA CAPUCINA V. MESOLEPTA	1	-	-	-	-	-	-
FRAGILARIA CAPUCINA	-	17	13	16	23	17	16
FRAGILARIA CONSTRUENS N	-	-	-	-	-	-	-
FRAGILARIA CONSTRUENS V. VENTER	-	-	-	-	-	-	-
FRAGILARIA PINNATA	-	-	-	-	-	-	-
FRAGILARIA VAUCHERIAE	15	7	27	76	27	10	32
GOMPHONEMA ANGUSTATUM	1	-	-	1	2	-	-
GOMPHONEMA PARVULUM	-	-	-	-	-	-	-
MELOSIRA DISIANS	2	7	1	1	2	1	1
MELOSIRA GRANULATA	1	1	1	1	1	1	1
MELOSIRA ITALICA	3	19	2	2	2	1	1
MELOSIRA VARIANS	-	1	2	1	1	2	2
NAVICULA CAPITATA V. HUNGARICA	-	6	1	1	1	4	2
NAVICULA CINCTA	17	-	12	22	1	-	12
NAVICULA CONFERACEA V. PEREGRINA	-	-	-	-	-	-	-
NAVICULA CONTENTA V. BICEPS	-	-	-	-	-	-	-
NAVICULA CRYPTOCEPHALA	6	11	-	6	32	-	1
NAVICULA MENISCVLUS V. UPSALIENSIS	-	6	1	1	1	1	1
NAVICULA MINIDA	10	-	1	1	1	1	1
NAVICULA MUTICA	-	-	-	-	-	-	-
NAVICULA PELLICULOSA	-	1	-	-	-	-	-
NAVICULA POPULA V. MUTATA	-	1	-	-	1	-	-
NAVICULA RADIOSA N	1	-	2	-	1	-	-
NAVICULA RADIOSA V. TENELLA	67	22	23	35	1	8	-
NAVICULA RHYNCHOCEPHALA V. GERMANI	-	-	-	-	-	-	-
NAVICULA SALINARIUM V. INTERMEDIA	-	-	-	-	-	-	-
NAVICULA SECRETA V. APICULATA	4	18	-	9	11	-	-
NAVICULA SEMINULUM	-	-	-	-	-	-	-
NAVICULA SP. SIMULA	-	-	-	-	-	-	-
NAVICULA SUBHAMULATA	-	-	-	-	-	-	-
NAVICULA SURTILISSIMA	-	1	-	1	1	8	-
NAVICULA TITENEMANNII	3	4	-	-	-	-	-
NAVICULA SP. 6	-	-	-	-	-	-	-
NITZSCHIA ACCUMUDATA	-	-	-	-	-	-	-
NITZSCHIA ACCUMUDATA	-	-	-	-	-	-	-
NITZSCHIA AMPHIBIA	-	-	-	-	-	-	-
NITZSCHIA BITA	1	2	-	-	-	-	-
NITZSCHIA DISSIPATA	1	2	-	1	1	1	1
NITZSCHIA DENTICULA	-	-	1	-	-	-	-
NITZSCHIA CALIFORNIA	1	1	2	1	1	1	-
NITZSCHIA CRUSTULUM V. SUBSALINA	1	1	2	1	2	1	-
NITZSCHIA KUETZINGIANA	3	15	1	-	1	-	1
NITZSCHIA PALLA	1	14	1	1	1	2	-
NITZSCHIA RECTA	-	-	-	1	1	1	-
NITZSCHIA SINUATA V. TARELLARIA	2	10	-	1	1	1	-
NITZSCHIA SP. 8	-	2	-	-	-	-	-
STEPHANODISCUS ASTRAEA	-	2	1	-	1	-	-
STEPHANODISCUS ASTRAEA V. MINUTULA	2	3	1	7	2	2	6
STEPHANODISCUS INVISITATUS	18	37	38	11	21	12	17
SUMIRELLA OVATA	-	-	-	-	-	-	1
SUMIRELLA SUECICA	1	-	-	-	1	-	-
SYNDRA DELICATISSIMA	-	-	-	-	-	-	-
SYNDRA HUNGARICA	19	68	36	63	37	21	17
SYNDRA RUPENS V. FAMILIARIS	4	-	1	9	3	4	6
SYNDRA ULNA	1	-	1	1	1	1	1
TABELLARIA FLUCCULOSA	-	-	1	-	-	-	-
TOTAL NUMBER OF ORGANISMS	502	492	496	496	501	497	499
NUMBER OF TAXA	38	40	30	40	46	32	26

TABLE 10. (Continued)

Taxonomic Classification	Number of Organisms at station:						
	9AM	98R	9CR	10AB	108H	10CN	11AS
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V. HETEROVALE	-	1	2	-	4	1	-
ACHNANTHES LANCEOLATA V. DUBIA	1	3	8	1	7	1	-
ACHNANTHES MINUTISSIMA	206	41	47	8	61	91	253
ACHNANTHES NULLII	16	13	9	3	27	45	11
ANDROLOONEIS VITREA	5	-	-	-	3	-	1
ASTERIONELLA FORMOSA	-	2	1	0	-	-	1
CALONEIS HYALINA	-	-	-	-	-	-	-
COCCONEIS PLACENTULA V. EUGLYPTA	-	1	-	-	1	2	1
CYCLOTELLA ATOMUS	-	-	-	-	-	-	-
CYCLOTELLA HENEGHINIANA	-	-	-	-	-	-	-
CYCLOTELLA STELLIGERA	-	-	1	-	1	1	-
CYMBELLA AFFINIS	20	-	8	-	1	3	-
CYMBELLA DELICATULA	1	-	-	-	-	-	1
CYMBELLA DELVISA	7	2	-	4	2	3	8
CYMBELLA RHYNOCEPHALA	3	-	7	1	2	2	0
CYMBELLA PROSTRATA V. AUERSWALDII	26	10	9	104	40	10	10
CYMBELLA TUMIDA	1	-	-	1	-	-	-
DIATOMA TENUE V. ELONGATUM	29	23	22	55	19	12	7
EUNOTIA PECTINALIS V. MINOR	-	-	2	-	-	-	-
FRAGILARIA CAPUCINA V. MESOLEPTA	1	-	2	4	-	3	-
FRAGILARIA CAPUCINA	27	31	108	70	6	18	19
FRAGILARIA CONSTRUENS N.	1	-	15	-	-	-	-
FRAGILARIA CONSTRUENS V. VENTER	-	-	14	-	2	-	-
FRAGILARIA PINNATA	1	-	7	-	-	-	-
FRAGILARIA VAUCHERIAE	32	79	26	168	77	31	10
GOMPHONEMA ANGUSTATUM	-	-	1	-	2	-	7
GOMPHONEMA PARVULUM	-	-	6	-	-	-	-
HELOSIRA DISTANS	-	2	3	-	2	2	3
HELOSIRA GRANULATA	2	10	1	6	3	6	11
HELOSIRA ITALICA	-	-	-	-	-	-	-
HELOSIRA VARIANS	5	7	3	5	12	3	1
NAVICULA CAPITATA V. HUNGARICA	-	-	4	-	3	-	-
NAVICULA CINCTA	2	2	2	-	-	2	20
NAVICULA CONFERACEA V. PEREGRINA	-	-	1	-	-	-	-
NAVICULA CONTENTA V. BICEPS	-	-	15	3	-	11	1
NAVICULA CRYPTOCEPHALA	-	10	-	-	-	-	-
NAVICULA MENISCULUS V. UPSALIENSIS	3	1	3	1	10	1	1
NAVICULA MINIMA	-	7	3	1	18	13	1
NAVICULA MUTICA	-	-	-	-	-	-	-
NAVICULA PELLICULOSA	-	-	1	-	-	-	-
NAVICULA PUPILA V. MUTATA	-	-	-	-	1	1	1
NAVICULA RADIOSA N.	-	-	-	-	1	-	1
NAVICULA RADIOSA V. TENELLA	-	1	-	1	3	1	4
NAVICULA RHYNOCEPHALA V. GERMANI	-	1	-	-	-	1	-
NAVICULA SALINARIUM V. INTERMEDIA	-	6	4	3	12	6	6
NAVICULA SECRETA V. APICULATA	-	3	3	1	9	2	6
NAVICULA SP. MINULUM	-	-	-	-	2	-	1
NAVICULA SP. SIMULA	-	-	-	-	-	-	-
NAVICULA SUBHAMULATA	-	-	-	-	1	-	3
NAVICULA SUBTILISSIMA	-	1	-	-	2	2	3
NAVICULA THIENEMANNII	-	-	-	1	-	2	1
NAVICULA SP. 6	-	-	-	-	-	-	-
NITZSCHIA ACCUMODATA	-	-	-	-	-	-	-
NITZSCHIA ACCUMODATA	-	-	-	-	-	-	-
NITZSCHIA AMPHIBIA	-	-	-	-	1	-	-
NITZSCHIA BETA	-	-	-	-	-	-	-
NITZSCHIA DISSIPATA	6	67	12	6	11	32	3
NITZSCHIA DENTICULA	-	-	3	-	2	-	6
NITZSCHIA FILICEPHALIS	-	-	1	-	2	1	1
NITZSCHIA FROSTULUM V. SUBSALINA	1	1	7	-	2	1	3
NITZSCHIA KUETZINGIANA	-	4	9	-	2	6	1
NITZSCHIA MALLI	-	-	1	-	-	1	1
NITZSCHIA RECTA	-	5	-	-	-	1	-
NITZSCHIA SINUATA V. TARELLARIA	1	4	6	2	1	12	13
NITZSCHIA SP. 6	-	-	-	-	-	-	-
STEPHANODISCUS ASTRAEA	-	1	1	-	12	2	1
STEPHANODISCUS ASTRAEA V. MINUTULA	32	25	34	6	20	27	19
STEPHANODISCUS INVISITATUS	70	96	64	26	46	66	18
SUMMELLA OVATA	1	-	3	-	2	1	-
SUMMELLA SUECICA	-	1	2	4	6	-	3
SYNECHA DELICATISSIMA	-	-	-	-	-	-	-
SYNECHA RUPPENS	13	23	1	17	4	28	6
SYNECHA RUPPENS V. FAMILIARIS	-	6	-	2	1	-	-
SYNECHA ULVA	-	1	-	-	-	-	-
TARELLARIA FLOCCULOSA	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	514	480	496	518	369	489	486
NUMBER OF TAXA	28	38	44	30	46	43	41

TABLE 1Q (Continued)

Taxonomic Classification	Number of Organisms at station:				
	118W	11CM	12AR	1288	12CH
BACILLARIOPHYTA (DIATOMS)					
ACHNANTHES EXIGUA V. HETEROVALVE	=	2	1	18	=
ACHNANTHES LANCEOLATA V. DUBIA	=	2	=	=	2
ACHNANTHES MINUTISSIMA	22	48	48	69	50
ACHNANTHES NOLLII	22	24	=	38	1
ANDROEDONEIS VITREA	2	2	1	1	1
ASTERIONELLA FORMOSA	=	=	=	=	=
CALONEIS HYALINA	=	=	1	1	1
COCCONEIS PLACENTULA V. EUGLYPTA	=	=	=	=	=
CYCLOTELLA ATOMUS	=	=	=	=	=
CYCLOTELLA MENECHINIANA	=	=	=	=	=
CYCLOTELLA STELLIGERA	=	2	2	2	=
CYMBELLA AFFINIS	1	1	=	=	=
CYMBELLA DELICATULA	17	=	=	98	=
CYMBELLA LAEVIS	3	1	=	2	=
CYMBELLA MICROCEPHALA	=	16	=	10	=
CYMBELLA PROSTRATA V. AUERSWALDII	13	3	1	20	17
CYMBELLA TUMIDA	=	=	=	=	=
DIATOMA TENUE V. ELONGATUM	53	4	11	22	17
EUNDTIA PECTINALIS V. MINOR	=	=	=	=	=
FRAGILARIA CAPUCINA V. MESOLEPTA	1	=	=	=	1
FRAGILARIA CAPUCINA	241	8	31	4	120
FRAGILARIA CONSTRUENS N	=	=	=	=	=
FRAGILARIA CONSTRUENS V. VENTER	=	=	=	=	=
FRAGILARIA PINNATA	=	=	=	=	=
FRAGILARIA VAUCHERIAE	51	6	17	18	10
GOMPHONEMA ANNUSTATUM	1	=	=	=	2
GOMPHONEMA PARVULUM	5	2	7	2	4
MELOSIRA DISTANS	=	5	=	=	1
MELOSIRA GRANULATA	1	2	2	1	1
MELOSIRA ITALICA	1	2	2	1	1
MELOSIRA VARIANS	=	=	=	=	=
NAVICULA CAPITATA V. HUNGARICA	5	2	1	6	1
NAVICULA CINCTA	5	2	1	28	1
NAVICULA CONFERACEA V. PEREGRINA	=	=	=	=	=
NAVICULA CONTENTA V. RICEPS	=	=	=	1	1
NAVICULA CRYPTOCEPHALA	=	7	5	=	14
NAVICULA MENISCVLUS V. UPSALIENSIS	=	4	6	=	1
NAVICULA MINIMA	1	11	10	10	1
NAVICULA MUTICA	=	=	=	=	=
NAVICULA PELLICULOSA	=	=	=	=	=
NAVICULA POPULA V. MUTATA	=	1	1	=	1
NAVICULA RADIOSA N	=	=	=	8	1
NAVICULA RADIOSA V. TENELLA	12	=	=	=	=
NAVICULA RHYNDOCEPHALA V. GENMANNI	1	3	1	6	1
NAVICULA SALINARIUM V. INTERMEDIA	1	3	1	1	1
NAVICULA SECTATA V. APICULATA	1	2	7	2	1
NAVICULA SEMINULUM	1	2	=	10	1
NAVICULA CF. SIMULA	1	2	=	10	1
NAVICULA SURHAMULATA	=	=	=	=	=
NAVICULA SUBTILISSIMA	=	7	2	10	1
NAVICULA THIENERMANNII	=	2	2	=	=
NAVICULA SP. 6	=	=	=	=	=
NITZSCHIA ACCUMODATA	=	2	=	=	=
NITZSCHIA ACCUMODATA	=	2	=	=	=
NITZSCHIA AMPHIBIA	=	=	=	=	=
NITZSCHIA BITA	=	=	=	=	=
NITZSCHIA DISSIPATA	5	11	6	8	1
NITZSCHIA DENTICULA	=	=	=	=	=
NITZSCHIA FILIFORMIS	=	=	=	=	=
NITZSCHIA FRUSTULUM V. SUBSALINA	1	1	3	3	1
NITZSCHIA KUETZINGIANA	=	10	19	=	20
NITZSCHIA PALSA	=	=	=	1	1
NITZSCHIA RECTA	=	=	=	=	=
NITZSCHIA SINUATA V. TABELLARIA	1	8	2	3	1
NITZSCHIA SP. 6	=	=	=	=	=
STEPHANODISCUS ASTRAEA	1	=	=	=	=
STEPHANODISCUS ASTRAEA V. MINUTULA	1	2	12	10	1
STEPHANODISCUS INVISITATUS	1	2	11	17	1
SURINELLA OVATA	=	=	65	=	=
SURIRELLA SUECICA	=	7	6	=	=
SYNEDRA DELICATISSIMA	41	2	6	28	1
SYNEDRA RUPENS	=	=	=	=	=
SYNEDRA RUPENS V. FAMILIARIS	3	1	21	3	1
SYNEDRA ULNA	=	=	=	=	=
TABELLARIA FLUCCULOSA	=	=	8	1	=
TOTAL NUMBER OF ORGANISMS	499	499	478	520	501
NUMBER OF TAXA	35	51	46	41	46

TABLE 10. (Continued)

Pinkham-Pearson Biotic Similarity

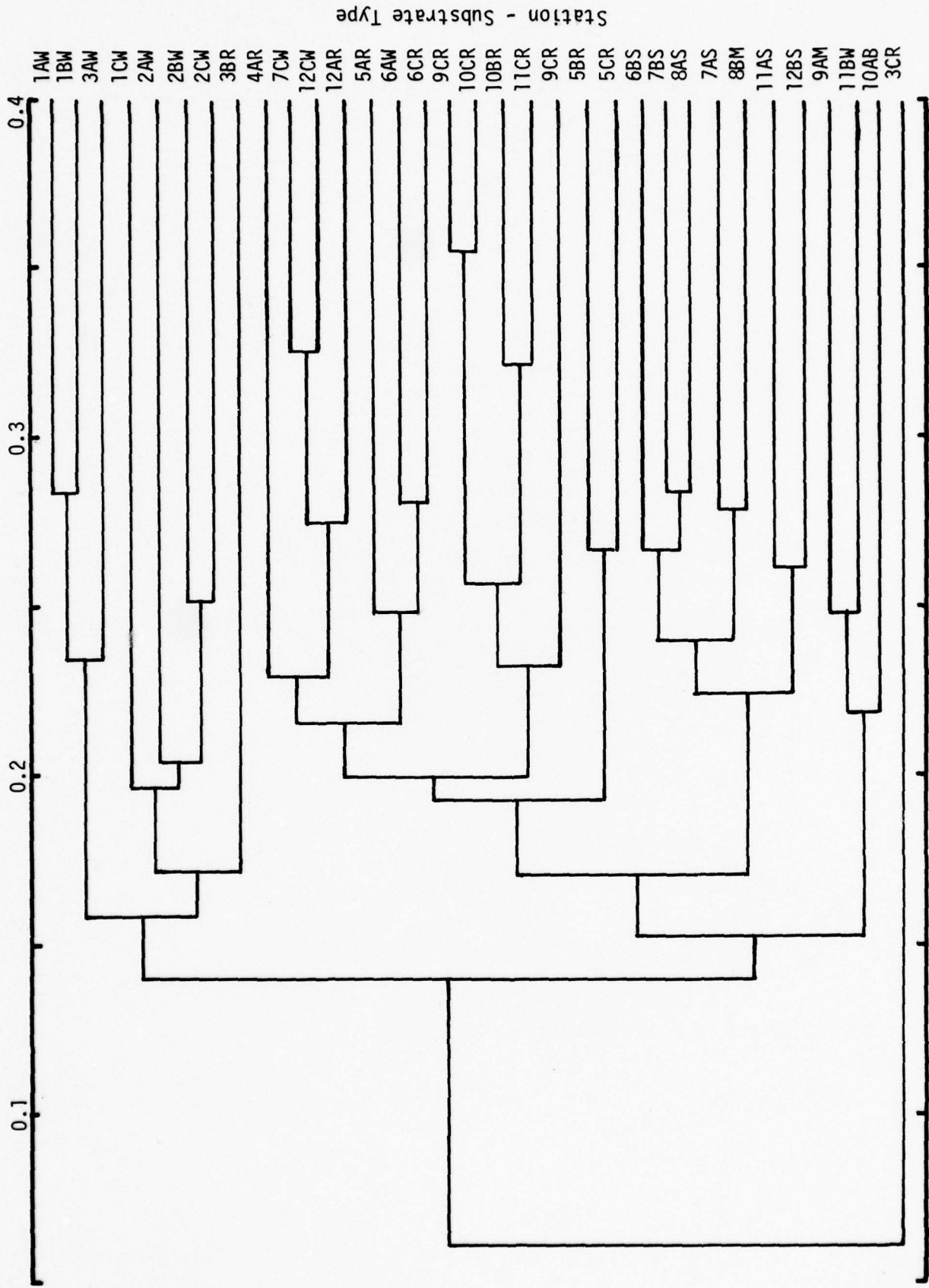


FIGURE 11. PHENOGRAM OF PERIPHYTON, NATURAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UNIMPORTANT, COPENHETIC CORRELATION COEFFICIENT 0.734.

TABLE 11

PRESENCE - ABSENCE DATA FOR FILAMENTOUS ORGANISMS  
COLLECTED FROM VAAP ARTIFICIAL SUBSTRATES - MARCH, 1977

SPECIES	STATION NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
<u>Cyanophyceae</u>												
Anabaena cf articulata												X
Oscillatoria sp 1		X		X		X	X	X	X		X	X
Schizothrix calcicola*	X	X	X	X	X	X	X	X	X		X	X
<u>Chlorophyceae</u>												
Mougeotia sp 1											X	X
Rhizoclonium sp 1											X	X
Stigeoclonium sp1				X		X			X		X	X

\* dominant at all stations



Three species of Chlorophyta were present. Of these, only Stigeoclonium occurred in the impact zone between stations 1 through 5. Green algae represented by Stigeoclonium, Rhizoclonium and Mougeotia were found more frequently in the reference bay. Only Stigeoclonium was present in Waconda Bay samples.

#### Chlorophyll a and Organic Biomass

Estimates of plant pigment and biomass levels were completed at all stations except S-10, where the periphytometer suspension unit was lost. Sixteen replicate slides for each of the two analyses were collected from the suspension units. These data are presented for each station in Figures 12 and 13. Table 12 contains summary information for means and ranges as well as the calculated Autotrophic Index based on mean values per station.

Chlorophyll a concentration on colonized slides at the various stations revealed a pattern consistent with population counts. Stations 1 through 5 had virtually no chlorophyll but a sharp increase occurred at S-6. A maximum average concentration of 40 mg/m<sup>2</sup> was observed at Station 7. Pigment levels gradually declined at downbay stations to 17 mg/m<sup>2</sup> at S-9. The reference bay samples showed means of 14 and 28 and could be considered comparable to outer Waconda Bay.

Organic biomass shows a similar pattern. Some heterotrophic growth occurs in the high stress zone between stations 1 through 5. Further downbay periphyton biomass increases with a maximum of 4.8 gms/m<sup>2</sup> at S-8. A maximum mean level of 6.2 mg/m<sup>2</sup> was observed at S-11 in the Reference Bay.

Calculation of the autotrophic index suggests an imbalanced environment among all stations except S-7. According to Weber (1973), ratios greater than 100 indicate organic pollution. Autotrophic index values for the winter survey ranged from 90 to 55,000. Maximum ratios were observed at stations 1 through 3.

#### Vital Stain

Three replicate slides from the periphytometer units were exposed to tetrazolium violet to estimate the physiological activity of diatoms. Cells were separated into live, senescent, or dead, based on the uptake of dye and the presence of chlorophyll. Cells completely filled with stain were classified as live. Those which contained chlorophyll but no stain were considered senescent. Those with neither dye nor chlorophyll were classified as dead. The technique offers some insight into growth characteristics of diatoms. Present methods of diatom enumeration using cleaned frustules probably leads to overestimates in that all cells are considered to be viable.

The data have been expressed as fractions using each category as it is related to the total number of individuals observed. This information as shown in Table 13 reveals that conditions existing in the channel between stations 1 and 4 resulted in nonviable populations that ranged up to 46 percent. A sharp demarcation occurred at station 5 where the percent dead organisms dropped to 7 percent. A further reduction to 1 percent was noted at station 6 where 93 percent of the organisms were vital staining.

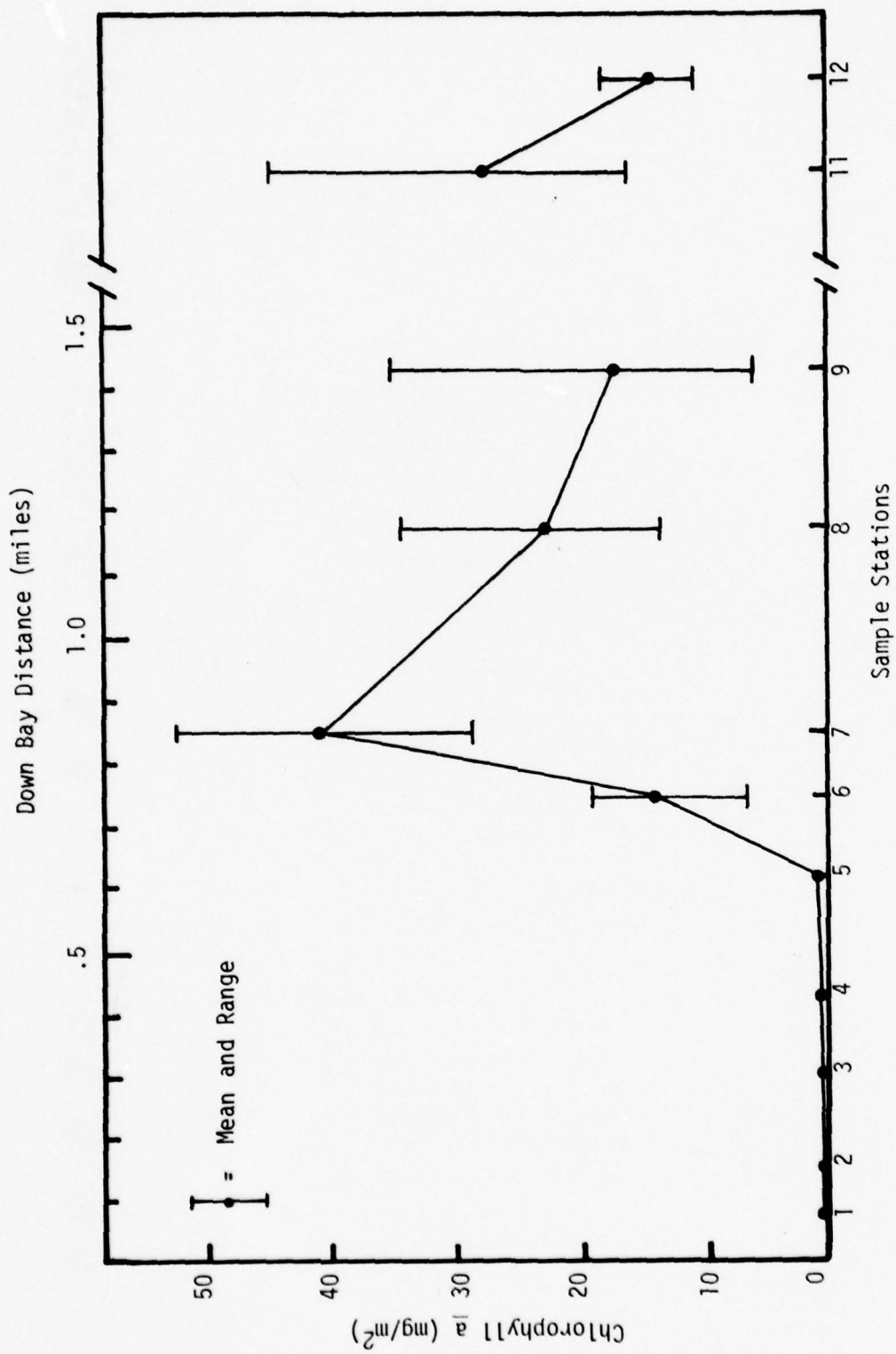


FIGURE 12. PERIPHYTON CHLOROPHYLL a, ARTIFICIAL SUBSTRATE, MARCH 1977

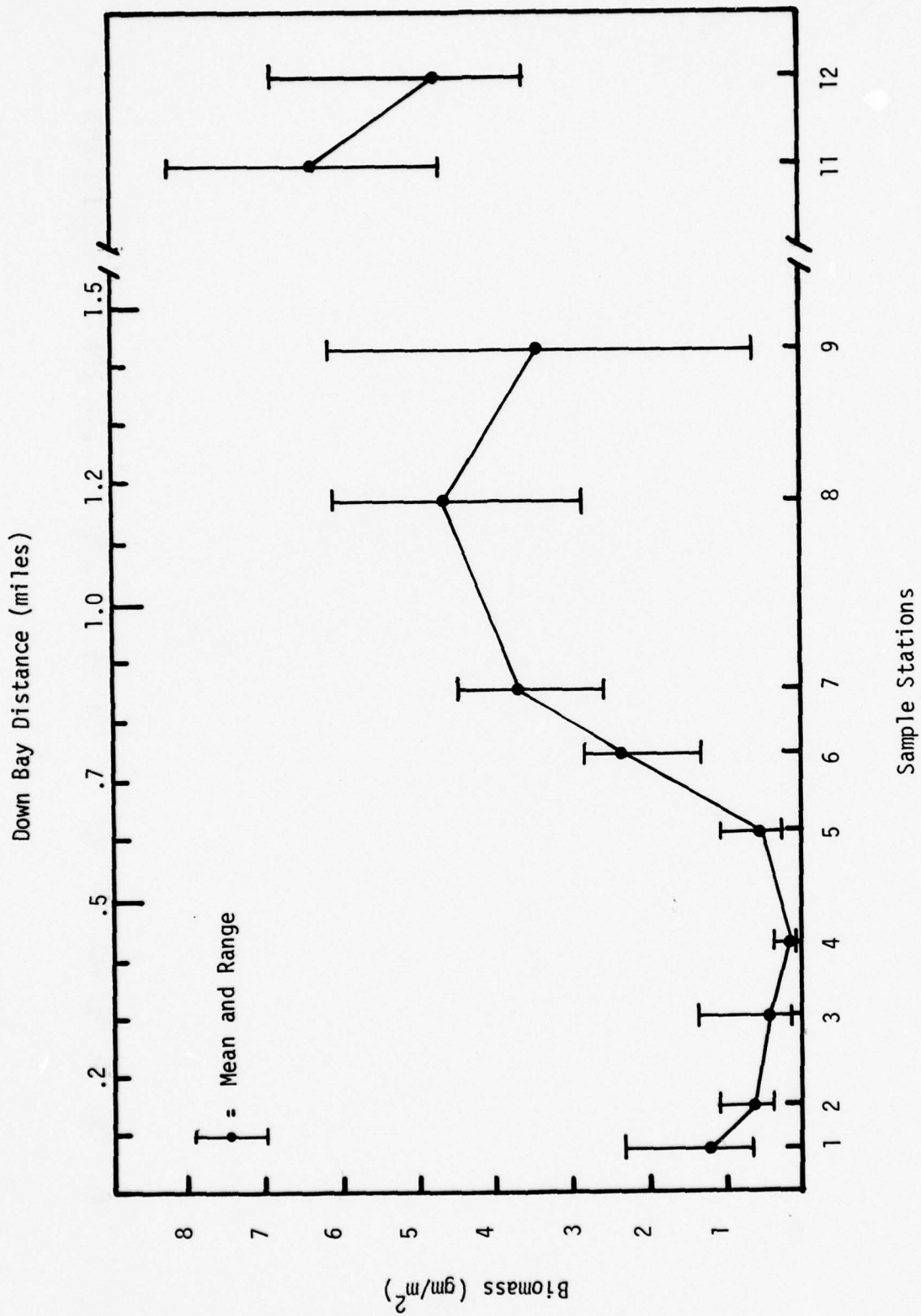


FIGURE 13. PERIPHYTON BIOMASS, ARTIFICIAL SUBSTRATE, MARCH 1977

TABLE 12

PERIPHYTON CHLOROPHYLL a, BIOMASS AND  
AUTOTROPHIC INDEX, MARCH 1977

Station	Chlorophyll a mg/m <sup>2</sup>		Organic Biomass gm/m <sup>2</sup>		Autotrophic Index
	Mean	Range	Mean	Range	
1	.02	.01-.06	1.1	.61-2.2	55,000
2	.02	.01-.04	.53	.27-1.0	26,500
3	.08	.02-.1	.34	.02-1.3	4,250
4	.2	.1 -.4	.10	.02- .26	500
5	.5	.2 -.8	.50	.22-1.0	1,000
6	14	7 - 19	2.3	1.2 -2.8	164
7	41	29 - 52	3.7	2.6 -4.4	90
8	23	13 - 34	4.8	2.9 -6.0	209
9	17	5 - 35	3.4	.57-6.1	200
10	-	-	-	-	-
11	28	16 - 44	6.2	4.7 -8.2	221
12	14	11 - 18	4.7	3.6 -6.8	336

TABLE 13

VIABLE CELL RATIOS IN VAAP PERIPHYTON, MARCH, 1977

	Live	Senescent	Dead
1	.25	.63	.13
2	.08	.46	.46
3	.48	.29	.24
4	.47	.20	.33
5	.48	.45	.07
6	.93	.06	.01
7	.89	.09	.01
8	.71	.25	.04
9	.72	.24	.03
10	---	---	---
11	.62	.27	.12
12	.70	.19	.11

## PHASE II - MACROINVERTEBRATES

### Introduction

Aquatic macroinvertebrates are a diverse group of small aquatic animals comprised of snails, clams, arthropods, annelids (segmented worms and leeches), planarians, and coelenterates. Of these, oligochaetes and chironomid (midge fly) larvae account for the majority of the organisms in this study.

Aquatic macroinvertebrates are a major biological component of aquatic systems and form an important part of the food chain. They feed on detritus and microscopic plants and animals. They, in turn, are eaten by small fish which support the larger and more economically and recreationally important species. Macroinvertebrates are of special importance in stream environments because of their role in recycling large amounts of organic detritus introduced from uplands.

Macroinvertebrates were selected as a parameter for this study because they are sensitive to environmental changes and thus, are important indicators of water quality. Natural or man-induced fluctuations in the physical-chemical characteristics of a lentic system are reflected by shifts in benthic community structure. They tend to remain at fixed locations and have a relatively short life span of usually a year or less and therefore, reflect both the present and recent past environmental conditions.

Macroinvertebrate species composition (population size, number of taxa, and diversity) is primarily dependent on three factors -- water quantity, water quality, and substrate composition.

Water quantity limits species within a site. For example, some organisms prefer large, deep lakes while others are found in small, shallow lakes. Water quality is also a significant factor in determining the assemblage of macroinvertebrates. Principal parameters include oxygen, temperature, hardness, related cations, and dissolved solids. The most important of these is oxygen. Increasing evidence shows that adverse effects can be expected on macroinvertebrates in environments supersaturated with oxygen as well as those that are undersaturated. Aquatic macroinvertebrates are also affected by temperature extremes. The Aquatic Life Advisory Committee (1956) indicates that benthic communities in temperature zones are adapted to seasonal fluctuations of temperature between 0 and 32°C (32 - 90°F).

Substrate is the most important determinant in species composition (Hynes, 1960). There is a direct relationship between the amount of available surface area and species abundance and diversity. That is to say, there are more hiding and foraging places in a rock or pebble bottom than in a sand or mud bottom. Contribution of organic matter, particularly from plants, is also an important consideration. Aquatic plants increase the abundance and diversity of benthic organisms viz. there is more surface area, periphytic food organisms, food from the plants themselves, and detritus from which to feed. Beck (1954) states, "...after careful examination of many streams, diversity of fauna was primarily the result of one factor -- the diversity of habitat."

## Environmental Conditions

The winter of 1976-1977 was severe and unusually low temperatures were recorded throughout the season. Ice cover on Waconda Bay prevented the commencement of Phase II until late February. These conditions influenced the composition of the macroinvertebrate community by reducing populations. Low lake levels caused a significant reduction in water surface area in the upper end of Waconda Bay. Effluent from VAAP was confined to a relatively narrow, shallow channel for some 3,000 feet downbay after reaching reservoir pool elevation. Lack of mixing in this channel caused significant concentrations of effluent to extend further downbay than would be expected at higher pool elevations.

Detritus was present at all stations, but was abundant only at Station 6. A mat of filamentous algae grading from heavy to sparse between stations 3-6 was also observed. The slender spikerush (Eleocharis acicularis) was found in small quantities at Station 12.

Table 14 presents the characteristics of the bay sediments at the various stations. Except for Stations 8, 9, and 10, all samples were obtained at comparable depths. During Phase II, 15 replicate grabs were collected at each station. In addition, 14 Hester-Dendy 5-plate units were placed at each station location for colonization over a 4-week period.

## Natural Substrate Colonization

The results for this part of the study are shown in Table 15 and are expressed as numbers of organisms/square meter. The various taxa are grouped into two categories representing chironomids and other forms. Except at Station 6, midges composed 50 percent or more of the total population. Three forms were most abundant. These were Procladius sp., Coelotanytus spp. and Chironomus sp. Procladius sp. dominated at Stations 1-5 and may represent forms more tolerant to VAAP waste.

Total population/m<sup>2</sup> ranged from 586 to 1,318. Numbers of species were generally uniformly distributed with 9 stations showing 10 - 15 species each. The lowest number of taxa (7) was noted at Station 2.

Other forms such as oligochaetes were not abundant. The largest population amounted to 793 organisms/m<sup>2</sup> at Station 6. Populations of fingernail clams were rather sparse between Stations 1 and 4. Further downbay where effluent effects would be less, these fingernail clams were observed ranging from 60 to 190/m<sup>2</sup>. Other organisms demonstrated similar patterns. Hexagenia mayflies were found at Station 3 indicating, at least, some tolerance to the waste in this location. A large increase in these insects was observed at Stations 4, 5, and 6. Shannon-Weaver Diversity is expressed both for culled and uncultured data in Table 16. For natural substrates values range to slightly above 2 for each of the culling routines. Station 3 represents the area of highest diversity followed by Station 8. Stations 1 and 2 in the maximum zone of impact were the least diverse. In general, there seemed to be no relationship between diversity and station location vis-a-vis the waste outfall.

TABLE 14

CHARACTERISTICS OF THE BENTHIC SUBSTRATE AT THE SAMPLING STATIONS WITHIN WACONDA BAY AND THE REFERENCE BAY, AS OBSERVED FROM DREDGED MACROINVERTEBRATE SAMPLES.

Substrate Characteristics	Stations											
	1	2	3	4	5	6	7	8	9	10	11	12
Depth (meters)	1.5	1.5	1.5	1.5	1.5	1.3	2	3	4	4	1.5	.75
Silt & clay*												
(a) orange	1-2	1-2	2-4	2-4	2-4	V	P	P	P	P	P	P
(b) gray	P	P	P	P	P	P	P	P	P	P	P	P
(c) brown												
(d) oil						P			P			
Gravel												
Detritus												
Fil. Algae	P	P	P	P	P	A	P	P	P	P	P	P
<u>Eleocharis</u>						V						
<u>Hexagenia burrows</u>												
<u>Decaying fish</u>	A	A	A	P	P		A	A	A	P	P	

\* Depth in centimeters

P = Present  
 A = Abundant  
 V = Veneer





TABLE 16

SHANNON - WEAVER DIVERSITY INDICES ( $\bar{H}$ ) FOR  
VAAP NATURAL SUBSTRATES, MACROINVERTEBRATES  
MARCH, 1977

Station	Magnitude Culled	Magnitude Unculled
1	0.73	0.82
2	0.67	0.74
3	2.26	2.35
4	1.84	1.88
5	1.84	1.90
6	1.43	1.43
7	1.87	1.87
8	1.98	2.02
9	1.92	1.96
10	1.74	1.74
11	1.73	1.76
12	1.83	1.95

Base e

The data as analyzed by cluster analysis are presented in Figures 14 and 15. The phenograms are representative of two options in the analysis procedure. In Figure 14, organisms which were present at 4 percent or less of the total population at any station were culled and no longer considered in the analysis procedure. Using the Pinkham-Pearson Similarity Index, the option of mutual absence important was employed. Figure 15 shows the clustering pattern based on all organisms that were collected and where mutual absence was considered unimportant. These two routines were run to examine the impact on station clustering of organisms occurring on a seldom or rare basis. As the figures show, the clustering is relatively similar. In both cases stations 1 and 2 are highly unrelated to the remaining stations. Stations 3, 4, and 5 are somewhat similar. No really consistent pattern exists for the remaining stations indicating that stations 6 through 9 in Waconda Bay are not distinguishable from stations 10 through 12 in the reference bay.

#### Artificial Substrate Colonization

Overall, colonization of the Hester-Dendy units was sparse and densities were greatly reduced over those in the benthos. For example, chironomids were 2 to 3 orders of magnitude less than those populating sediments in Waconda Bay. Combined data from five Hester-Dendy units per station are presented in Table 17.

Chironomids dominated artificial substrates at Stations 7 through 9 in Waconda Bay, and Stations 10 and 11 in the reference bay. Cricotopus spp. accounted for about two-thirds of the Chironomidae. Several species of Cricotopus are involved; however, presently there are no effective means to separate these species. Four species of Dicrotendipes and two species of Glyptotendipes comprised most of the remaining chironomids. These six feed upon planktonic materials. Cricotopus was present throughout Waconda Bay, and was abundant in the reference bay. Dicrotendipes and Glyptotendipes were relatively common in the reference bay and were frequently found in the open bay stations (6 through 9) of Waconda Bay. Two other genera, Ablabesmyia and Tanytarsus, were frequently found in the reference bay, but rarely found in Waconda Bay.

Of the non-chironomid fauna, only two of the nine found on artificial substrates were present in significant numbers. The larvae of Caenis, a small herbivorous mayfly, reached its greatest population at Station 6 where it formed 61 percent of the population. Densities fell steeply either upbay or downbay. It was not collected at Stations 1, 2, 9, and 10. Oligochaetes occurred sporadically in Waconda Bay, but were the dominant taxa on reference bay artificial substrates. The larvae of Argia sp., a predaceous damselfly, were found only at Stations 1 and 2. This genus has previously been reported only from unpolluted situations (Beck, 1954) in Florida, but Beck (personal communication) believes this may not hold true for some non-Florida species.

The highest densities and numbers of species occurred in the reference bay stations which suggests some inhibition in all Waconda Bay samples. Considering total number of species per station the stations seem to group as follows: stations 1 to 5 averaged 3.3 species; stations 6 to 9 averaged 9.5

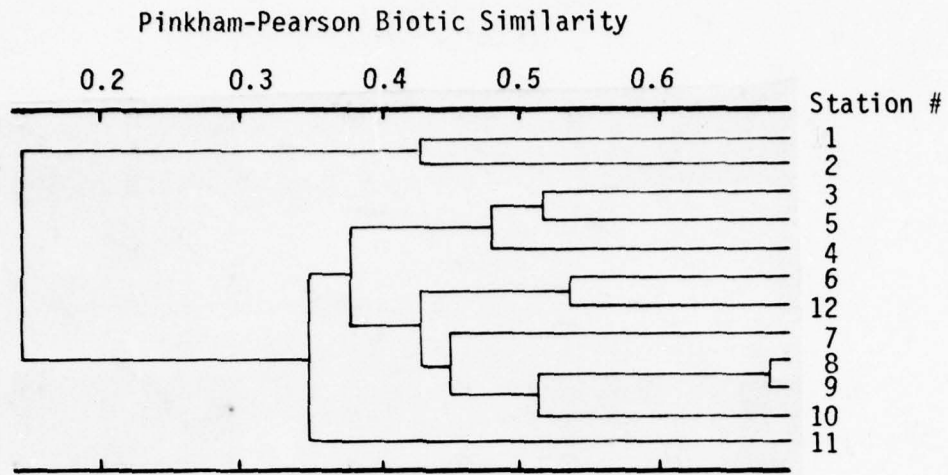


FIGURE 14 . PHENOGRAM OF MACROINVERTEBRATES, NATURAL SUBSTRATE, CULLED AT 4%, MUTUAL ABSENCE IMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.917.

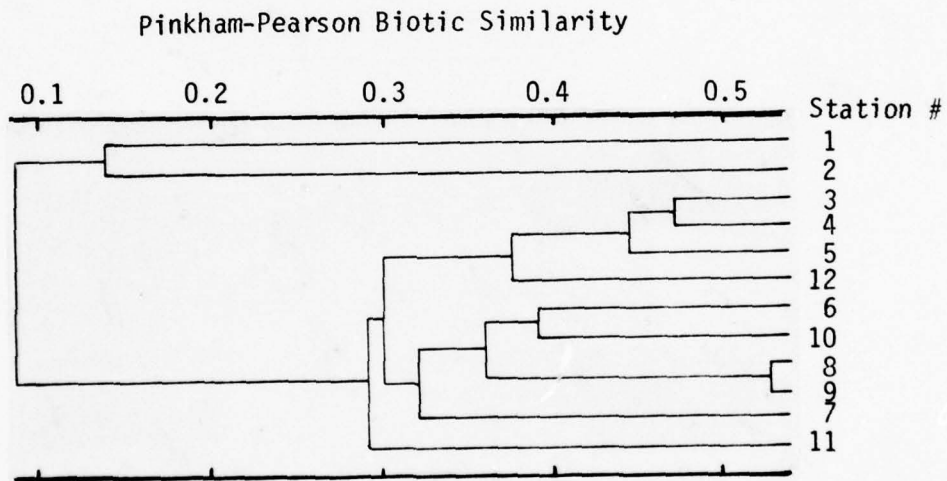


FIGURE 15 . PHENOGRAM OF MACROINVERTEBRATES, NATURAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UNIMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.900.

TABLE 17

VAAP MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, UNCULLED, MARCH 1977

Taxonomic Classification	Number of Organisms per m <sup>2</sup> at Station:											
	1	2	3	4	5	6	7	8	9	10	11	12
<b>CHIRONOMIDAE</b>												
ALABESHTIA HALLOMIA	-	-	-	-	-	7	-	-	3	19	29	19
ALABESHTIA TABELLA	-	-	-	-	-	-	-	-	-	-	23	19
CHIRONOMUS SP	-	-	-	-	3	10	20	23	17	90	210	1227
CHIRONOMUS SP	-	-	-	-	-	-	-	-	-	-	3	7
CHIRONOMUS FULVUS	-	-	-	-	-	-	-	7	-	10	37	120
CHIRONOMUS RUBROSCISS	-	-	-	-	3	17	3	3	-	17	30	30
CHIRONOMUS RUBROSCISS	-	-	-	-	-	-	3	10	-	17	10	13
DICROTENDIPES NEGROQUESTUS	-	-	-	-	-	-	-	-	-	-	33	20
DICROTENDIPES NERVOSUS	-	-	-	-	-	-	-	-	-	-	37	40
GAMMARUS SP	-	-	-	-	-	-	3	7	13	29	37	13
GLYPTOTENDIPES LOBIFERUS	-	-	-	-	-	3	-	-	3	3	-	3
GLYPTOTENDIPES SP	-	-	-	-	-	-	-	-	-	-	-	3
MICROSESTIA SP A	-	-	-	-	-	-	-	-	-	-	-	3
PARATANYTANUS SP A	-	-	-	-	-	-	-	-	-	-	-	3
POLYPEDILUM FALLAX	-	-	-	3	-	3	-	-	-	-	-	3
POLYPEDILUM NEAR ILLINOENSE TYPE B	-	-	-	-	-	-	-	-	-	-	-	3
PSECTROCLADUS SP A	-	-	-	-	-	3	-	7	-	33	53	31
TANYTANUS SP	-	-	-	-	-	3	-	-	-	-	-	3
THISSOCLADUS SP	-	-	-	-	-	3	-	-	-	-	-	3
UNIDENTIFIED ORTHOCLADIAN	-	-	-	-	-	-	-	-	3	-	-	-
<b>NON-CHIRONOMIDAE</b>												
ARZIA SP	-	-	-	-	-	-	-	-	-	-	-	7
CAMIS SP	3	10	13	50	63	106	10	1	-	-	13	30
CERATOPRONIDAE (NO LARVAL KEY)	-	-	-	3	-	-	-	3	-	3	-	-
CORICOLA MANILENSIS	-	-	-	7	-	3	-	-	-	-	-	7
ENALLERMA SP	-	-	-	-	-	-	-	-	-	-	-	3
HYALINELLA AZTECA	-	-	-	-	-	7	-	-	20	157	287	1693
OPHONETIA	-	-	-	-	-	7	-	-	-	-	-	-
SPERMONEA SP	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	5	13	23	69	69	175	68	77	69	372	718	3271
NUMBER OF TAXA	2	2	3	6	3	13	6	9	8	14	17	22

species; stations 10 and 11 averaged 15.5 species. Station 12 was excluded because the sampling rack had been overturned in the field resulting in the Hester-Dendy units being suspended at about one half the depth of the units at other stations.

Using the five replicates from each station, an analysis of variance was performed on the organism density data. These data were first transformed to  $\ln(N + 1)$ . The calculated F value (degrees of Freedom 11, 48) was 47.3 which is significant at less than 0.0001. Using Duncan's multiple range test (5% criteria) to compare treatment means it was found that significant differences in population density are as follows:

1, 2, 3 < 7, 5, 4, 9, 8 < 6 < 10 < 11 < 12

Table 18 shows diversity values for the community colonizing the Hester-Dendy units. The data are expressed using the culled-unculled format. Treatment of the data by either option shows Station 8 to be the most diverse with a maximum of 2.02. The lowest diversities were observed between Stations 1 through 5, suggesting that these stations were under greater stress.

Cluster analysis using the Pinkham-Pearson approach was performed on data from the Hester-Dendy units (Figures 16&17). The unculled data were analyzed on a mutual absence unimportant basis whereas when the data were culled, mutual absence was considered important. In the culled data, Stations 1 to 5 group separately as do Stations 11 and 12. In the unculled data, at Stations 1, 3, 4 and 5 are grouped together, Station 2 is unlike any other station and Stations 11 and 12 are highly similar. Thus, in both cases, Stations 1 to 5 seem to separate from the remaining group.

TABLE 18

SHANNON - WEAVER DIVERSITY INDICES ( $\bar{H}$ ) FOR  
VAAP ARTIFICIAL SUBSTRATES MACROINVERTEBRATES  
MARCH, 1977

Station	Magnitude Culled	Magnitude Unculled
1	0.69	0.69
2	0.54	0.54
3	0.95	0.95
4	1.01	1.01
5	0.36	0.36
6	1.39	1.57
7	1.55	1.55
8	2.02	2.02
9	1.80	1.80
10	1.69	1.81
11	1.66	1.80
12	1.11	1.20

Base e

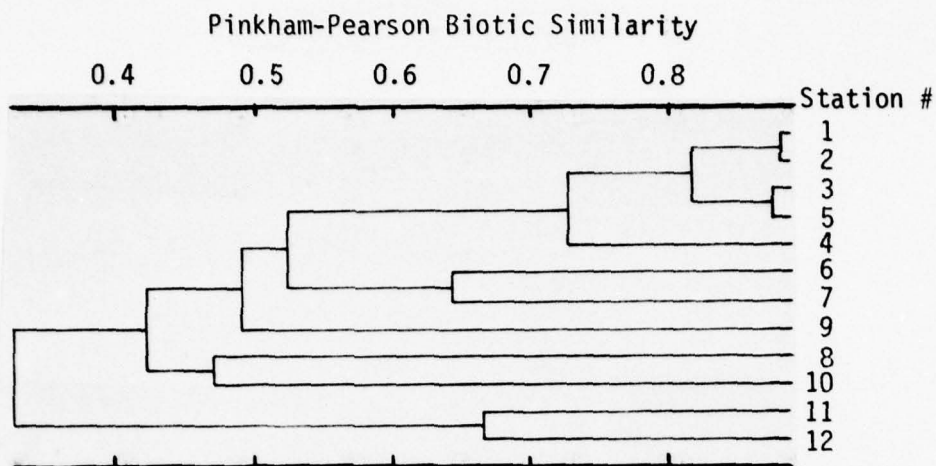


FIGURE 16 . PHENOGRAM OF MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, CULLED AT 4%, MUTUAL ABSENCE IMPORTANT. COPENETIC CORRELATION COEFFICIENT, 0.902

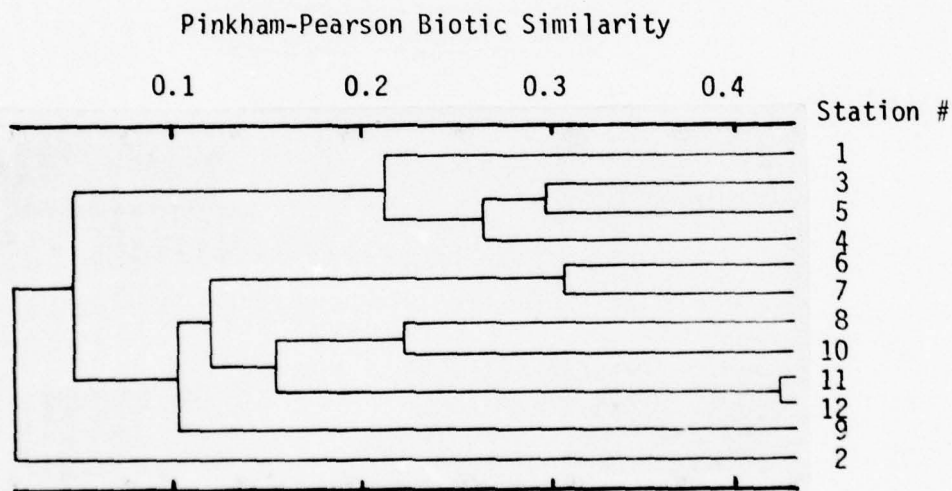


FIGURE 17 . PHENOGRAM OF MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UN-IMPORTANT. COPENETIC CORRELATION COEFFICIENT, 0.882.



## DISCUSSION AND CONCLUSION

In relating environmental effects to munitions concentrations in a field study such as this, one should understand the several assumptions and/or limitations that are inherent. First, the concentrations at any site vary with time. For the natural substrate sampling, the concentrations measured from March 1-5 are presumed to be "normal" or "average" and the biologic communities are assumed to be in equilibrium with these concentrations. For the artificial substrates, measurements were made of munitions in the effluent during the incubation period but no bay samples were taken. However, from the effluent concentrations and an understanding of how the effluent mixes with bay waters, one can predict possible resultant bay concentrations. Also, the plant effluent contains a complex mixture of munitions-related organic compounds plus significant concentrations of other ions, particularly nitrate and sulfate. Thus the observed effects may not be due to only the five specific munitions compounds measured. Even with these limitations, certain conclusions can be relatively firm. Other conclusions can only be tentative due to the factors noted above and because the effects indicated are subtle.

Overall, the data appear to be very consistent. The water chemistry, artificial substrate periphyton population density, chlorophyll *a*, biomass and viable cell ratios, and artificial and natural substrate macroinvertebrate community structure all indicate that Stations 1-5 are affected by the VAAP discharge. The only data that do not support this conclusion are sediment chemistry and natural substrate periphyton. Given that the effluent does appear to be having an effect, the problem thus becomes one of relating the effects to specific causes.

First, at what concentration levels were no measurable effects observed? In the natural substrate no-effect could be discerned in either number of species, density, or community structure for both benthic and periphyton organisms at stations 6 through 9 as compared to reference bay stations 10 through 12. During the sampling period total concentration of the five munitions compounds averaged less than 25  $\mu\text{g/l}$  at stations 6 through 9. In the December sampling average munitions levels in this area ranged as high as 27  $\mu\text{g/l}$ . Hence, it appears that for total munitions concentration less than 25  $\mu\text{g/l}$  no effects would be expected.

At the opposite end of this range, effluent munitions concentrations during the incubation period for the artificial substrate samples averaged about 575  $\mu\text{g/l}$  and reached a peak of 2370  $\mu\text{g/l}$ . Concentrations at stations 1 through 5 should not have exceeded these values. At this level, definite effects were noted in periphyton population density and community structure and in macroinvertebrate numbers of species and community structure. It cannot be determined whether the peak or average concentration was most influential in the result.

Between these extremes, effects were subtle and less pronounced. In the five day period prior to the incubation of the artificial substrate, munitions concentrations in the upper channel, stations 1 through 5, were

averaging about 80  $\mu\text{g}/\text{l}$ . Data from the December survey and results of prior studies suggest that the long term average munitions concentration in this area might actually be somewhat higher. Even so, the only observed effect on macroinvertebrate infauna was a slight shift in community structure. Substrate variation in periphyton samples may have masked slight shifts in community structure in that compartment also.

During the incubation of the artificial substrate samples, it is predicted that the concentration of munitions compounds at stations 6 - 9 could have gone up to 50 - 100  $\mu\text{g}/\text{l}$ . For macroinvertebrates, density, number of species, and community structure at stations 6 - 9 were different than at stations in the reference bay. For periphyton, the numbers of species seemed to be slightly lower at stations 6 to 9 as compared to the reference bay stations. These differences were not as substantial as those observed between the upbay and reference areas. However, they were of such magnitude as to eliminate them from the definite no-effect category.

In the 1975 study (Sullivan, *et al.*, 1977), biostimulation was observed in certain portions of Waconda Bay. In this winter survey no such biostimulation was observed. Possibly the low temperature encountered prevented this.

In summary, the work reported herein indicates no environmental effects on periphyton or macroinvertebrates from a complex TNT manufacturing effluent at a total munitions concentration of less than 25  $\mu\text{g}/\text{l}$ . Definite effects were noted during a period when total munitions averaged 500-600  $\mu\text{g}/\text{l}$  with a peak of over 2000  $\mu\text{g}/\text{l}$ . The data further suggests the tentative conclusion that concentrations in the range of 50-100  $\mu\text{g}/\text{l}$  produce minimal effects. Again it should be noted that the concentrations of other non-munitions unique compounds changed as the munitions concentrations changed. These changes probably were factors also in the biologic changes observed.

These results are in good agreement with the conclusions of the 1975 study (Sullivan *et al.* 1977). In that work, toxicity was indicated in the range of 50-350  $\mu\text{g}/\text{l}$  total munitions and no effects were observed at concentrations of 20  $\mu\text{g}/\text{l}$  and less.

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APPENDIX A  
PHASE I AND II SURVEY  
Figures and Tables

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APPENDIX A

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TABLE A-1

IN SITU MEASUREMENTS OF DISSOLVED  
OXYGEN, TEMPERATURE AND CONDUCTIVITY  
VAAP, DECEMBER 1976, PHASE I



Water and Air Research, Inc.  
 6821 S.W. Archer Road, P. O. Box 1121  
 GAINESVILLE, FLORIDA 32602  
 (904) 372-1500

JOB \_\_\_\_\_  
 SHEET NO. 1 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. A

DEPTH	PARAMETER	DATE					AVERAGE VALUE	RANGE
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC		
0.5ft	TEMP, °C	9.0	8.0	7.0	6.0	7.0	7.4	6.0 - 9.0
	D.O., mg/l	11.4	11.7	11.6	11.5	11.2	11.5	11.2 - 11.7
	COND, $\mu\text{mho}/\text{cm}$	370	455	565	380	560	466	370 - 565
2.0ft	TEMP, °C	9.0	8.0				8.5	8.0 - 9.0
	D.O., mg/l	10.9	11.3				11.1	10.9 - 11.3
	COND, $\mu\text{mho}/\text{cm}$	410	455				433	410 - 455
3.0ft	TEMP, °C	9.0	8.0		6.0		7.7	6.0 - 9.0
	D.O., mg/l	10.8	11.1		11.5		11.1	10.8 - 11.5
	COND, $\mu\text{mho}/\text{cm}$	500	455		920		625	455 - 920
4.0ft	TEMP, °C	9.0	<del>8.0</del>			9.5		
	D.O., mg/l	10.7	11.0			11.0		
	COND, $\mu\text{mho}/\text{cm}$	460	455			1130		
		* <input type="checkbox"/>				<input type="checkbox"/>		
5.0ft	TEMP, °C				8.0			
	D.O., mg/l				11.4			
	COND, $\mu\text{mho}/\text{cm}$				1700			
					<input type="checkbox"/>			
6.0ft	TEMP, °C			9.0				
	D.O., mg/l			11.6				
	COND, $\mu\text{mho}/\text{cm}$			660				
				<input type="checkbox"/>				

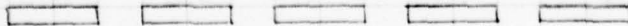
\* DOUBLE LINE INDICATES  
 BOTTOM DEPTH

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 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. B<sub>2</sub>

DEPTH	PARAMETER	DATE					AVERAGE	
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	VALUE	RANGE
0.5ft	TEMP, °C	8.5	8.0	7.0	6.0	7.0	7.3	6.0 - 8.5
	D.O., mg/l	11.2	10.7	11.4	11.1	11.1	11.1	10.7 - 11.4
	COND, µmho/cm	500	440	510	490	480	484	440 - 510
2.0ft	TEMP, °C	8.5	8.0				8.3	8.0 - 8.5
	D.O., mg/l	10.9	10.6				10.8	10.6 - 10.9
	COND, µmho/cm	520	440				480	440 - 520
3.0ft	TEMP, °C	8.5	8.0			7.0	7.8	7.0 - 8.5
	D.O., mg/l	10.6	10.6			11.0	10.7	10.6 - 11.0
	COND, µmho/cm	550	440			605	532	440 - 605
4.0ft	TEMP, °C	8.3	8.0		7.0		7.8	7.0 - 8.3
	D.O., mg/l	10.4	10.6		11.2		10.7	10.4 - 11.2
	COND, µmho/cm	645	460		1080		728	460 - 1080
5.0ft	TEMP, °C	8.3	8.0				8.2	8.0 - 8.3
	D.O., mg/l	10.3	10.6				10.4	10.3 - 10.6
	COND, µmho/cm	655	600				628	600 - 655
5.5ft	TEMP, °C	8.3	8.0	7ft 6.5	7ft 8.3	6ft 9.0		<u>DEPTH OF BOTTOM</u>
	D.O., mg/l	10.2	10.2	11.4	10.4	10.6		
	COND, µmho/cm	670	605	605	1900	1160		



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 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. C1

DEPTH	PARAMETER	DATE				
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC
0.5ft	TEMP, °C		6.0	6.5		6.5
	D.O., mg/l		12.6	13.8		12.6
	COND, $\frac{\mu\text{mho}}{\text{cm}}$		200	160		180
			<input type="text"/>	<input type="text"/>		<input type="text"/>
1.0ft	TEMP, °C	10.5			6.5	
	D.O., mg/l	8.0			11.0	
	COND, $\frac{\mu\text{mho}}{\text{cm}}$	400			390	
		<input type="text"/>			<input type="text"/>	

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 SHEET NO. 4 OF 12  
 CALCULATED BY DPC DATE 21 DEC 96  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. C2

DEPTH	PARAMETER	DATE					AVERAGE	
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	VALUE	RANGE
0.5ft	TEMP, °C	8.5	6.5	6.5	5.5	6.5	6.7	5.5-8.5
	D.O., mg/l	11.3	12.6	12.2	13.2	12.3	12.3	11.3-13.2
	COND, $\mu\text{mho}/\text{cm}$	510	185	305	235	260	299	185-510
2.0ft	TEMP, °C	8.5	6.5					
	D.O., mg/l	10.9	12.4					
	COND, $\mu\text{mho}/\text{cm}$	540	180					
3.0ft	TEMP, °C	8.5						
	D.O., mg/l	10.7						
	COND, $\mu\text{mho}/\text{cm}$	545						
4.0ft	TEMP, °C	8.5			5.8			
	D.O., mg/l	10.6			13.0			
	COND, $\mu\text{mho}/\text{cm}$	550			750			
5.0ft	TEMP, °C	8.5		<del>6.5</del> 6.5	5.5	9.5	<sup>no depth</sup> 7.5	
	D.O., mg/l	10.5		11.4		10.4	10.4	
	COND, $\mu\text{mho}/\text{cm}$	550		400		~1000	460	

DEPTH OF BOTTOM

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JOB \_\_\_\_\_  
 SHEET NO. 5 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. D1

DEPTH	PARAMETER	DATE					AVERAGE VALUE	RANGE
		7DEC	8DEC	9DEC	10DEC	11DEC		
0.5ft	TEMP, °C	7.5	6.5	6.0	5.5	6.0	6.3	5.5 - 7.5
	D.O., mg/l	13.4	12.4	13.2	13.3	12.2	12.9	12.2 - 13.4
	COND, $\mu\text{mho}/\text{cm}$	50	170	190	210	230	170	50 - 230
2.0ft	TEMP, °C	7.5	6.5		5.5			
	D.O., mg/l	13.0	12.3		13.1			
	COND, $\mu\text{mho}/\text{cm}$	80	175		210			
2.5ft	TEMP, °C					6.0		
	D.O., mg/l					12.2		
	COND, $\mu\text{mho}/\text{cm}$					230		
5.0ft	TEMP, °C			6.0				
	D.O., mg/l			13.2				
	COND, $\mu\text{mho}/\text{cm}$			210				

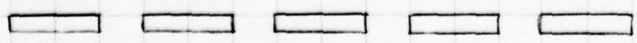
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JOB \_\_\_\_\_  
 SHEET NO. 6 OF 12  
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 SCALE \_\_\_\_\_

STATION NO. D2

DEPTH	PARAMETER	DATE					AVERAGE	
		7DEC	8DEC	9DEC	10DEC	11DEC	VALUE	RANGE
0.5ft	TEMP, °C	7.5	7.0	6.0	5.5	6.0	6.4	5.5 - 7.5
	D.O., mg/l	13.4	12.6	13.2	13.2	12.4	13.0	12.4 - 13.4
	COND, µmho/cm	155	180	225	190	220	194	155 - 225
2.0ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.1	12.5				12.8	12.5 - 13.1
	COND, µmho/cm	165	180				173	165 - 180
3.0ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.0	12.4				12.7	12.4 - 13.0
	COND, µmho/cm	170	175				173	170 - 175
4.0ft	TEMP, °C	7.3	7.0	6.0	5.5	6.0	6.4	5.5 - 7.3
	D.O., mg/l	12.6	12.2	13.0	13.1	12.4	12.7	12.2 - 13.1
	COND, µmho/cm	205	180	230	230	220	213	180 - 230
5.0ft	TEMP, °C	7.3	7.0				7.2	7.0 - 7.3
	D.O., mg/l	12.4	12.0				12.2	12.0 - 12.4
	COND, µmho/cm	290	190				240	190 - 290
5.5ft	TEMP, °C	7.8	7.0	6.0	5.5	6.0		
	D.O., mg/l	10.8	11.6	12.8	12.8	12.6		
	COND, µmho/cm	500	225	340	350	460		

DEPTH OF BOTTOM



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 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

DEPTH	PARAMETER	STATION NO. E.					AVERAGE	
		DATE	7DEC	8DEC	9DEC	10DEC	11DEC	VALUE
0.5ft	TEMP, °C	7.5	7.0	6.5	6.0	6.5	6.7	6.0 - 7.5
	D.O., mg/l	13.6	12.4	13.0	12.7	11.8	12.7	11.8 - 13.6
	COND., μmho/cm	140	150	185	170	170	163	140 - 185
2.0ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.2	12.3				12.8	12.3 - 13.2
	COND., μmho/cm	140	155				148	140 - 155
3.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.7	12.1				12.4	12.1 - 12.7
	COND., μmho/cm	145	155				150	145 - 155
4.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.6	12.0				12.3	12.0 - 12.6
	COND., μmho/cm	150	155				153	150 - 155
5.0ft	TEMP, °C	7.3	7.5					
	D.O., mg/l	12.4	11.9					
	COND., μmho/cm	150	155					
6.0ft	TEMP, °C	7.5	<u>7.5ft</u> 6.5	<u>8.0ft</u> 6.0	<u>8.0ft</u> 6.5			<u>MID DEPTH</u>
	D.O., mg/l	11.8	12.8	12.4	11.8			
	COND., μmho/cm	160	185	180	175			
7.0ft	TEMP, °C	7.5	<u>14ft</u> 6.0	<u>16ft</u> 6.0	<u>16ft</u> 6.5			<u>DEPTH OF BOTTOM</u>
	D.O., mg/l	11.8	12.4	12.4	11.2			
	COND., μmho/cm	180	230	240	370			

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JOB \_\_\_\_\_  
 SHEET NO. 8 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

DEPTH	PARAMETER	STATION NO. E2					AVERAGE VALUE	RANGE
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC		
0.5ft	TEMP, °C	8.0	7.0	7.0	6.3	7.0	7.1	6.3 - 8.0
	D.O., mg/l	13.1	12.5	13.0	12.4	11.6	12.5	11.6 - 13.1
	COND., μmho/cm	65	115	150	160	160	130	65 - 160
2.0ft	TEMP, °C	7.8	7.0				7.4	7.0 - 7.8
	D.O., mg/l	13.0	12.2				12.6	12.2 - 13.0
	COND., μmho/cm	85	120				103	85 - 120
3.0ft	TEMP, °C	7.5	7.0			7.0	7.2	7.0 - 7.5
	D.O., mg/l	12.7	12.2			11.5	12.1	11.5 - 12.7
	COND., μmho/cm	90	120			160	123	90 - 160
4.0ft	TEMP, °C	7.5	7.0		<u>4.5ft</u> 6.3		6.9	6.3 - 7.5
	D.O., mg/l	12.4	12.1		12.3		12.3	12.1 - 12.4
	COND., μmho/cm	95	130		160		128	95 - 160
5.0ft	TEMP, °C	7.5	7.0	7.0				
	D.O., mg/l	12.2	12.0	12.6				
	COND., μmho/cm	100	135	160				
6.0ft	TEMP, °C		7.0					
	D.O., mg/l		12.0					
	COND., μmho/cm		145					
7.0ft	TEMP, °C		7.0			6.5		
	D.O., mg/l		11.9			11.4		
	COND., μmho/cm		150			160		



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 SHEET NO. 9 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. E2

DEPTH	PARAMETER	DATE					AVERAGE VALUE	RANGE
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC		
8.0ft	TEMP, °C		7.0					
	D.O., mg/l		11.9					
	COND, $\mu$ mho/cm		155					
9.0ft	TEMP, °C		7.0	6.5	6.3			
	D.O., mg/l		10.8	12.4	12.2			
	COND, $\mu$ mho/cm		160	170	160			
10.0ft	TEMP, °C		7.5					
	D.O., mg/l		11.2					
	COND, $\mu$ mho/cm		175					

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 SHEET NO. 10 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
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 SCALE \_\_\_\_\_

STATION NO. F

DEPTH	PARAMETER	DATE					AVERAGE	
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	VALUE	RANGE
0.5ft	TEMP, °C	7.8	7.5	7.0	6.5	6.5	7.1	6.5 - 7.8
	D.O., mg/l	13.0	12.4	12.7	11.9	11.3	12.3	11.3 - 13.0
	COND, $\mu$ mho/cm	130	110	160	160	155	143	110 - 160
2.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.7	11.8				12.3	11.8 - 12.7
	COND, $\mu$ mho/cm	130	120				125	120 - 130
3.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.4	11.6				12.0	11.6 - 12.4
	COND, $\mu$ mho/cm	135	125				130	125 - 135
4.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.2	15.4				13.8	12.2 - 15.4
	COND, $\mu$ mho/cm	140	130				135	130 - 140
5.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.1	15.4				13.8	12.1 - 15.4
	COND, $\mu$ mho/cm	140	140				140	—
6.0ft	TEMP, °C	7.3					7.3	—
	D.O., mg/l	12.1					12.1	—
	COND, $\mu$ mho/cm	140					140	—

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 SHEET NO. 11 OF 12  
 CALCULATED BY DPC DATE 21 DEC 76  
 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. F

DEPTH	PARAMETER	DATE					AVERAGE	
		7DEC	8DEC	9DEC	10DEC	11DEC	VALUE	RANGE
7.0ft	TEMP, °C	7.3					7.3	—
	D.O., mg/l	11.9					11.9	—
	COND, $\mu\text{mho}/\text{cm}$	140					140	—
8.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.9					11.9	—
	COND, $\mu\text{mho}/\text{cm}$	140					140	—
9.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.8					11.8	—
	COND, $\mu\text{mho}/\text{cm}$	140					140	—
10.0ft	TEMP, °C	7.5	7.5	6.5	6.5		7.0	6.5 - 7.5
	D.O., mg/l	11.8	15.4	12.0	11.7		12.7	11.7 - 15.4
	COND, $\mu\text{mho}/\text{cm}$	140	145	160	160		151	140 - 160
11.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.9					11.9	—
	COND, $\mu\text{mho}/\text{cm}$	140					140	—
12.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.8					11.8	—
	COND, $\mu\text{mho}/\text{cm}$	140					140	—

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 SHEET NO. 12 OF 12  
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 CHECKED BY \_\_\_\_\_ DATE \_\_\_\_\_  
 SCALE \_\_\_\_\_

STATION NO. F

DEPTH	PARAMETER	DATE					AVERAGE	
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	VALUE	RANGE
13.0ft	TEMP, °C	7.5				6.5	7.0	6.5 - 7.5
	D.O., mg/l	11.9				11.2	11.6	11.2 - 11.9
	COND, $\mu\text{mho}/\text{cm}$	165				160	163	160 - 165
14.0ft	TEMP, °C	7.5						
	D.O., mg/l	12.2						
	COND, $\mu\text{mho}/\text{cm}$	220						
15.0ft	TEMP, °C		7.5					
	D.O., mg/l		15.4					
	COND, $\mu\text{mho}/\text{cm}$		150					
20.0ft	TEMP, °C		7.5					
	D.O., mg/l		15.3					
	COND, $\mu\text{mho}/\text{cm}$		155					
22.6ft	TEMP, °C				6.5			
	D.O., mg/l				11.5			
	COND, $\mu\text{mho}/\text{cm}$				160			
25.6ft	TEMP, °C			6.5		26ft 6.5		
	D.O., mg/l			11.8		11.4		
	COND, $\mu\text{mho}/\text{cm}$			210		205		

DEPTH OF BOTTOM

TABLE A-2

IN SITU MEASUREMENTS OF DISSOLVED  
OXYGEN, TEMPERATURE AND CONDUCTIVITY  
VAAP, MARCH 1977, PHASE II

STATION NUMBER: 1

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	9.1	8.0	9.5	10.0	13.0	11.0	8.0-13.0	
	DISSOLVED OXYGEN mg/l	10.4	10.4	10.8	11.0	10.0	9.4	9.4-11.0	
	CONDUCTIVITY µmho/cm	590	480	565	515	585	560	480-590	
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—	
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	
BOTTOM ~ 3 ft	TEMPERATURE °C	8.5	8.5	10.5	10.0	12.5	11.0	8.5-12.5	
	DISSOLVED OXYGEN mg/l	10.6	10.8	10.8	10.8	10.4	9.6	9.6-10.8	
	CONDUCTIVITY µmho/cm	605	480	625	515	630	570	480-630	
DEPTH	ft	4 1/2	3	3	2	3	4	3	2-4 1/2

STATION NUMBER: 2

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	11.3	8.5	9.5	10.0	12.0	12.0	8.5-12.0	
	DISSOLVED OXYGEN mg/l	9.8	10.0	10.0	10.8	10.0	9.2	9.2-10.8	
	CONDUCTIVITY µmho/cm	588	475	545	510	690	580	475-690	
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—	
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	
BOTTOM ~3 ft	TEMPERATURE °C	10.2	8.5	10.0	10.8	11.5	11.5	8.5-11.5	
	DISSOLVED OXYGEN mg/l	10.0	10.4	10.4	10.4	10.4	9.4	9.4-10.4	
	CONDUCTIVITY µmho/cm	579	480	600	515	700	570	480-700	
DEPTH	ft	4	3	3	3	2	4	3	2-4

STATION NUMBER: 3

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	12.0	10.0	9.5	10.3	11.5	12.5	9.5-12.5	
	DISSOLVED OXYGEN mg/l	9.5	9.4	10.0	10.6	9.4	9.2	9.2-10.6	
	CONDUCTIVITY µmho/cm	522	545	555	530	550	570	522-570	
MID-DEPTH 3 ft	TEMPERATURE °C	—	10.0	—	—	—	—	—	
	DISSOLVED OXYGEN mg/l	—	9.6	—	—	—	—	—	
	CONDUCTIVITY µmho/cm	—	550	—	—	—	—	—	
BOTTOM ~6 ft	TEMPERATURE °C	10.0	10.0	10.5	10.3	11.0	12.0	10.0-12.0	
	DISSOLVED OXYGEN mg/l	9.8	9.6	10.8	10.4	9.8	9.2	9.2-10.8	
	CONDUCTIVITY µmho/cm	588	540	595	530	570	560	530-595	
DEPTH	ft	5	6	6	5	5	7	6	5-7



STATION NUMBER: 4

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR		
SURFACE 1/2 ft	TEMPERATURE °C	12.5	10.0	10.0	10.0	11.5	12.0	11.0 10.0-12.5
	DISSOLVED OXYGEN mg/l	9.4	9.2	10.0	10.6	9.4	9.2	9.6 9.2-10.6
	CONDUCTIVITY umho/cm	550	535	560	530	525	580	547 525-580
MID-DEPTH 3 ft	TEMPERATURE °C	—	10.0	—	—	—	—	10.0
	DISSOLVED OXYGEN mg/l	—	9.6	—	—	—	—	9.6
	CONDUCTIVITY umho/cm	—	540	—	—	—	—	540
BOTTOM ~5 ft	TEMPERATURE °C	11.0	10.0	10.5	10.3	11.0	12.0	10.8 10.0-12.0
	DISSOLVED OXYGEN mg/l	9.6	9.6	10.8	10.2	9.6	9.4	9.9 9.4-10.8
	CONDUCTIVITY umho/cm	559	540	560	530	530	580	550 530-580
DEPTH	ft	6	6	3	5	6	6	5 3-6

STATION NUMBER: 5

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	13.0	10.0	9.5	10.0	10.5	11.5	9.5-13.0	
	DISSOLVED OXYGEN mg/l	10.6	9.0	10.4	10.4	9.8	9.0	9.0-10.6	
	CONDUCTIVITY µmho/cm	400	540	590	550	530	540	400-590	
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—	
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	
BOTTOM ~ 5 ft	TEMPERATURE °C	12.8	10.0	11.0	10.0	11.0	11.0	10.0-12.8	
	DISSOLVED OXYGEN mg/l	10.6	9.2	10.5	9.8	9.8	9.0	9.0-10.6	
	CONDUCTIVITY µmho/cm	410	540	610	550	525	525	410-610	
DEPTH	ft	4	5	5	3	5	6	5	3-6

STATION NUMBER: 6

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR		
SURFACE 1/2 ft	TEMPERATURE °C	10.0	10.0	9.5	9.5	10.5	10.0	9.5-10.5
	DISSOLVED OXYGEN mg/l	11.2	10.0	11.4	10.8	10.0	10.0	10.0-11.4
	CONDUCTIVITY µmho/cm	230	400	350	415	360	300	230-415
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—
BOTTOM ~ 2 ft	TEMPERATURE °C	10.0	10.0	11.0	10.0	—	10.0	10.0-11.0
	DISSOLVED OXYGEN mg/l	10.8	10.4	11.6	10.6	—	10.4	10.4-11.6
	CONDUCTIVITY µmho/cm	330	420	425	465	—	330	330-465
DEPTH	ft	2	2	2	2	< 2	2	—

STATION NUMBER: 7

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	10.1	10.0	9.5	9.5	10.3	10.0	9.5-10.3	
	DISSOLVED OXYGEN mg/l	10.8	11.0	11.0	11.4	10.0	10.4	10.0-11.4	
	CONDUCTIVITY umho/cm	246	260	330	330	345	310	246-345	
MID-DEPTH 3 ft	TEMPERATURE °C	—	9.5	—	9.5	10.0	—	9.5-10.0	
	DISSOLVED OXYGEN mg/l	—	11.0	—	11.2	10.8	—	10.8-11.2	
	CONDUCTIVITY umho/cm	—	290	330	340	340	—	290-340	
BOTTOM ~6 ft	TEMPERATURE °C	9.2	9.0	10.0	9.5	10.0	10.0	9.0-10.0	
	DISSOLVED OXYGEN mg/l	10.8	10.0	11.4	11.2	10.4	10.6	10.0-11.4	
	CONDUCTIVITY umho/cm	265	440	350	350	340	300	265-440	
DEPTH	ft	6	7	5	6	6	7	6	5-7

STATION NUMBER: 8

DEPTH	PARAMETER	DATE				AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR			4 MAR
SURFACE 1/2 ft	TEMPERATURE °C	10.0	9.5	9.5	9.5	9.5	9.5-10.5	
	DISSOLVED OXYGEN mg/l	11.2	11.2	11.2	10.4	10.4	10.4-11.2	
	CONDUCTIVITY umho/cm	250	235	290	280	270	235-310	
MID-DEPTH ~5 ft	TEMPERATURE °C	—	9.5	9.5	9.5	9.5	9.5-10.0	
	DISSOLVED OXYGEN mg/l	—	11.6	12.2	11.4	11.0	11.0-12.2	
	CONDUCTIVITY umho/cm	—	240	235	280	280	235-310	
BOTTOM ~10 ft	TEMPERATURE °C	9.0	9.0	9.0	9.5	9.5	9.0-9.5	
	DISSOLVED OXYGEN mg/l	11.1	10.8	11.6	11.2	10.8	10.0-11.6	
	CONDUCTIVITY umho/cm	240	260	290	265	270	210-290	
DEPTH		10	11	10	11	9	10	9-11

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ARMY MEDICAL CORPS V PHASE II  
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STATION NUMBER: 9

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE	
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR			5 MAR
SURFACE 1/2 ft	TEMPERATURE °C	9.5	9.0	8.5	9.5	9.5	10.0	8.5-10.0	
	DISSOLVED OXYGEN mg/l	11.8	11.4	12.0	10.0	10.4	10.4	10.0-12.0	
	CONDUCTIVITY umho/cm	179	195	215	205	210	285	179-285	
MID-DEPTH ~6 ft	TEMPERATURE °C	—	8.5	8.5	9.0	9.5	9.0	8.5-9.5	
	DISSOLVED OXYGEN mg/l	—	12.0	12.0	11.4	11.0	11.0	11.0-12.0	
	CONDUCTIVITY umho/cm	—	190	230	205	210	210	190-230	
BOTTOM ~12 ft	TEMPERATURE °C	8.5	8.0	8.5	8.5	9.0	8.5	8.0-9.0	
	DISSOLVED OXYGEN mg/l	12.0	11.6	11.6	11.0	10.6	10.4	10.4-12.0	
	CONDUCTIVITY umho/cm	180	190	240	200	210	210	180-240	
DEPTH		12	12	13	11	11	15	12	11-15

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

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STATION NUMBER: 10

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR		
SURFACE 1/2 ft	TEMPERATURE °C	8.5	—	—	—	—	8.5	—
	DISSOLVED OXYGEN mg/l	11.0	—	—	—	—	11.0	—
	CONDUCTIVITY µmho/cm	190	—	—	—	—	190	—
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—
BOTTOM 12 ft	TEMPERATURE °C	7.5	—	—	—	—	7.5	—
	DISSOLVED OXYGEN mg/l	11.0	—	—	—	—	11.0	—
	CONDUCTIVITY µmho/cm	200	—	—	—	—	200	—
	DEPTH ft	12	—	—	—	—	12	—

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

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STATION NUMBER: 11

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR		
SURFACE 1/2 ft	TEMPERATURE °C	10.5	10.0	10.0	9.0	11.0	10.1	9.0-11.0
	DISSOLVED OXYGEN mg/l	11.0	11.2	11.6	11.6	10.0	11.1	10.0-11.6
	CONDUCTIVITY µmho/cm	180	200	230	220	240	214	180-240
MED- DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—
BOTTOM ~ 7 ft	TEMPERATURE °C	8.5	—	9.5	9.0	9.0	9.0	8.5-9.5
	DISSOLVED OXYGEN mg/l	10.8	—	11.8	11.6	10.6	11.2	10.6-11.8
	CONDUCTIVITY µmho/cm	199	—	235	225	230	222	199-235
DEPTH	ft.	8	—	8	8	5	7	5-8



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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

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STATION NUMBER: 12

DEPTH	PARAMETER	DATE					AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR		
SURFACE	TEMPERATURE °C	12.0	—	—	—	—	12.0	—
	DISSOLVED OXYGEN mg/l	12.0	—	—	—	—	12.0	—
	CONDUCTIVITY µmho/cm	215	—	—	—	—	215	—
MID-DEPTH	TEMPERATURE °C	—	—	—	—	—	—	—
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—
BOTTOM	TEMPERATURE °C	—	—	—	—	—	—	—
< 1 ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—
	DEPTH ft	< 1	—	—	—	—	< 1	—

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WATER AND AIR RESEARCH INC GAINESVILLE FLA  
WINTER FIELD SURVEYS AT VOLUNTEER ARMY AMMUNITION PLANT, CHATTA--ETC(U)  
APR 78 J H SULLIVAN, H D PUTNAM, M A KEIRN DAMD17-75-C-5049

F/G 6/6

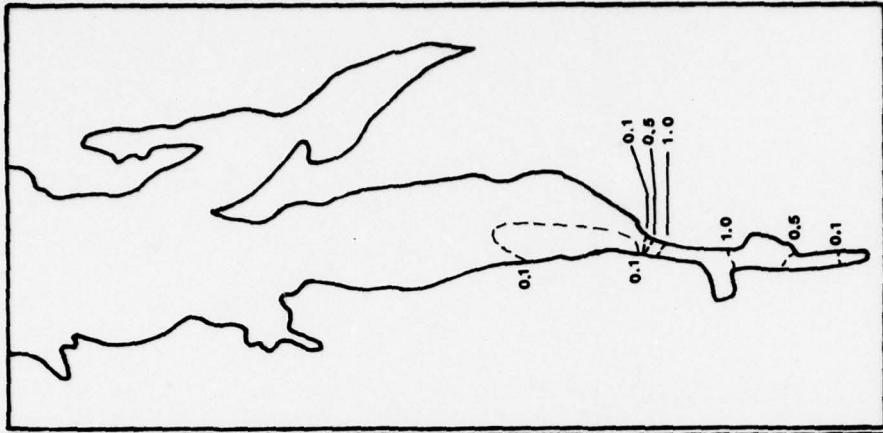
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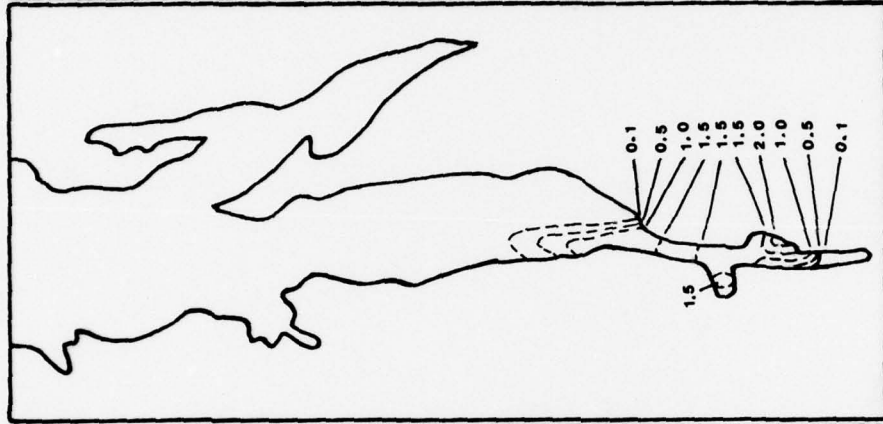
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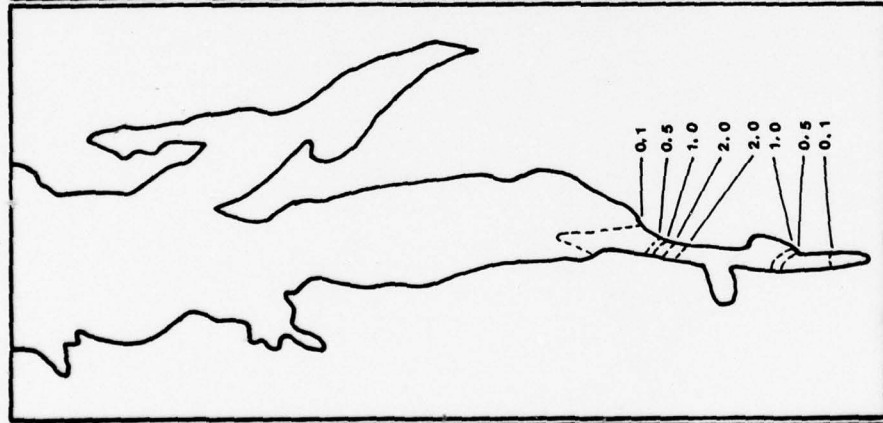
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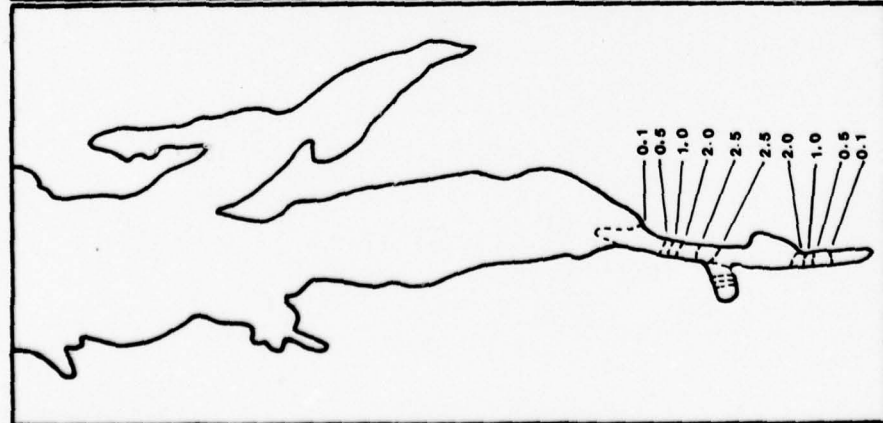
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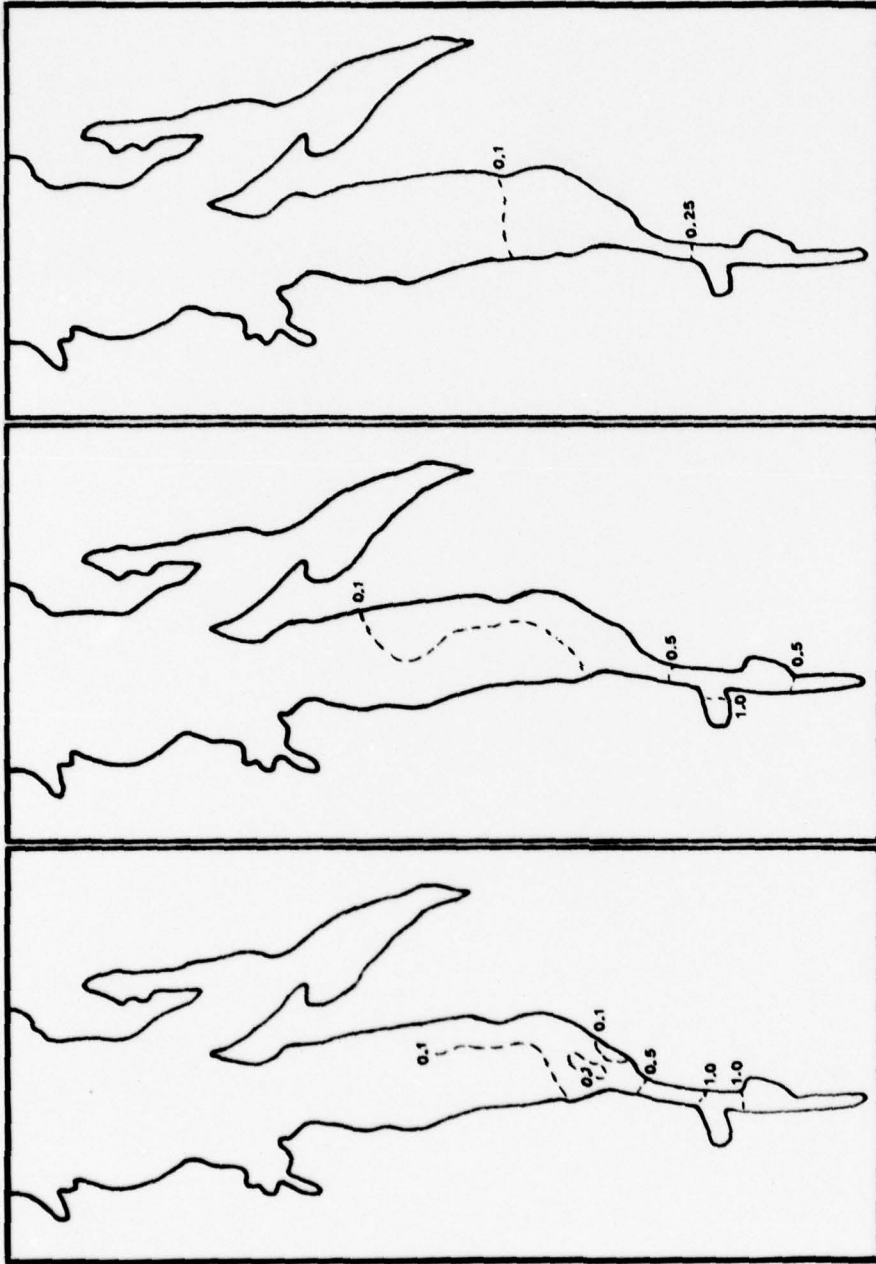
12/8/76  
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0916-1025

RHODAMINE B DYE CONCENTRATION - SURFACE  
(ppb by weight)

FIGURE A-1

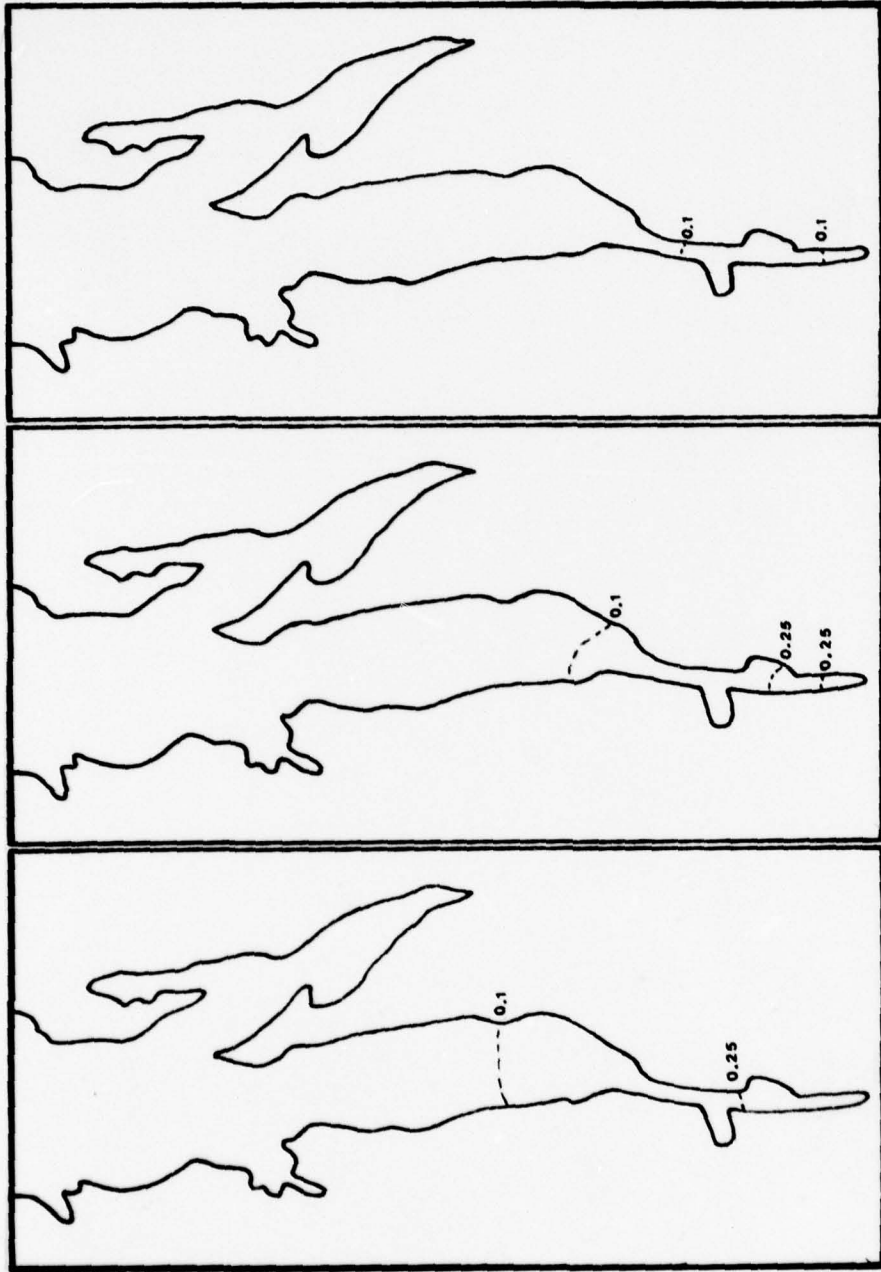


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0838-1009

12/10/76  
1530-1636

12/10/76  
0916-1148

FIGURE A-1. RHODAMINE B DYE CONCENTRATION-SURFACE  
(ppb by weight)



12/13/76

12/12/76

12/11/76  
1216-1320

FIGURE A-1 RHODAMINE B DYE CONCENTRATIONS-SURFACE  
(ppb by weight)

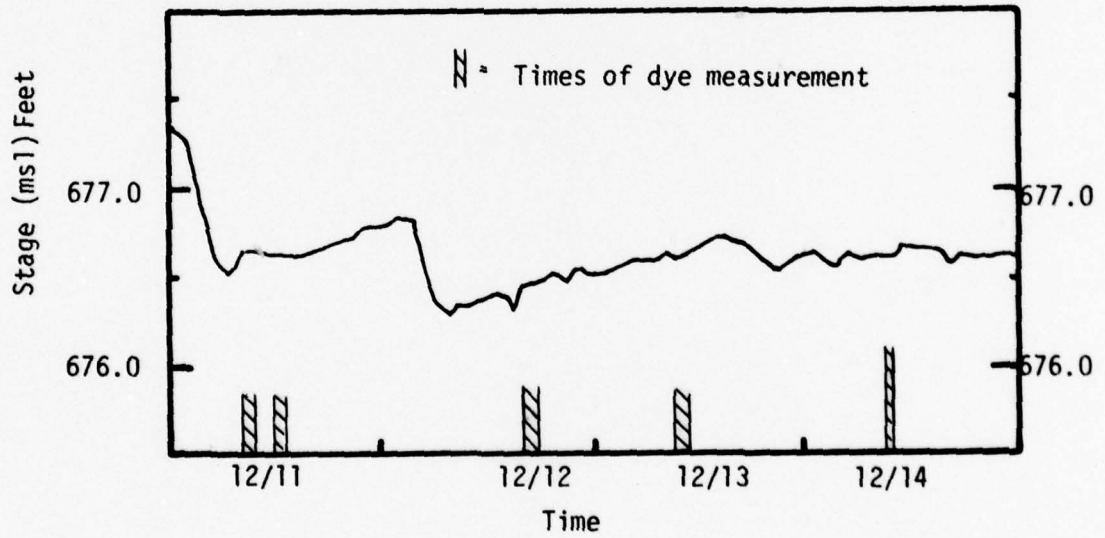
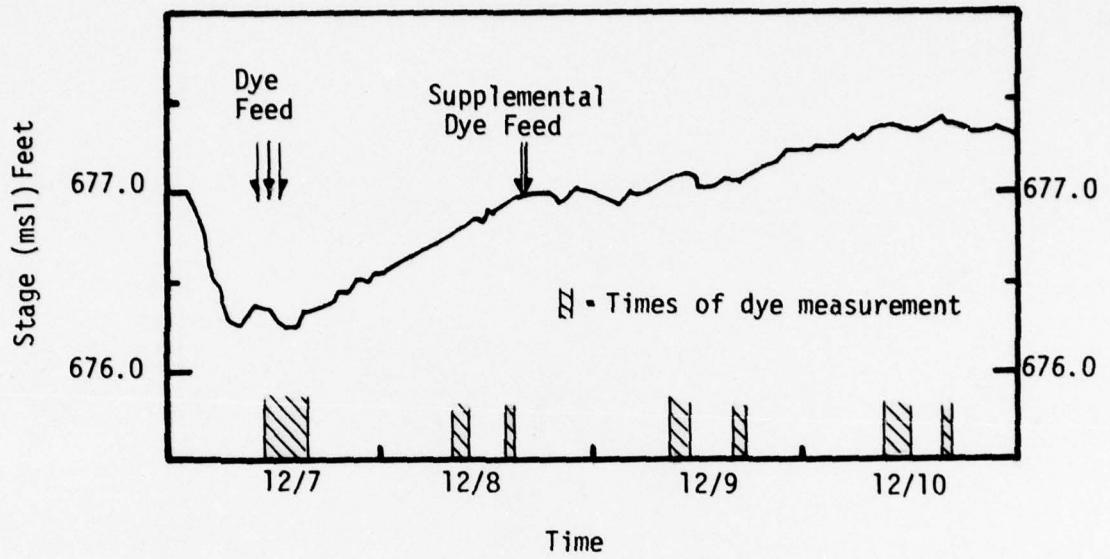
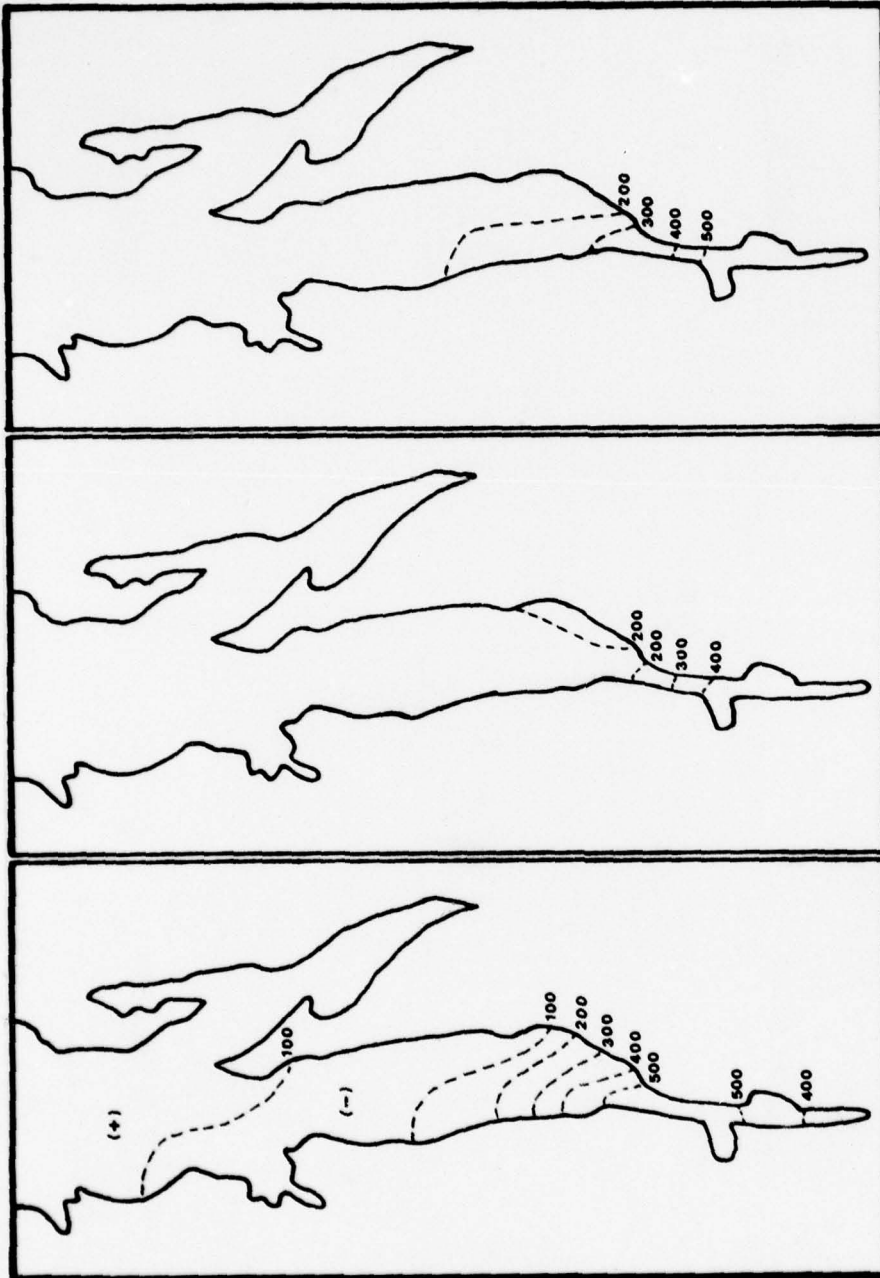


FIGURE A-2. CHICKAMAUGA RESERVOIR STAGE VS. TIME.

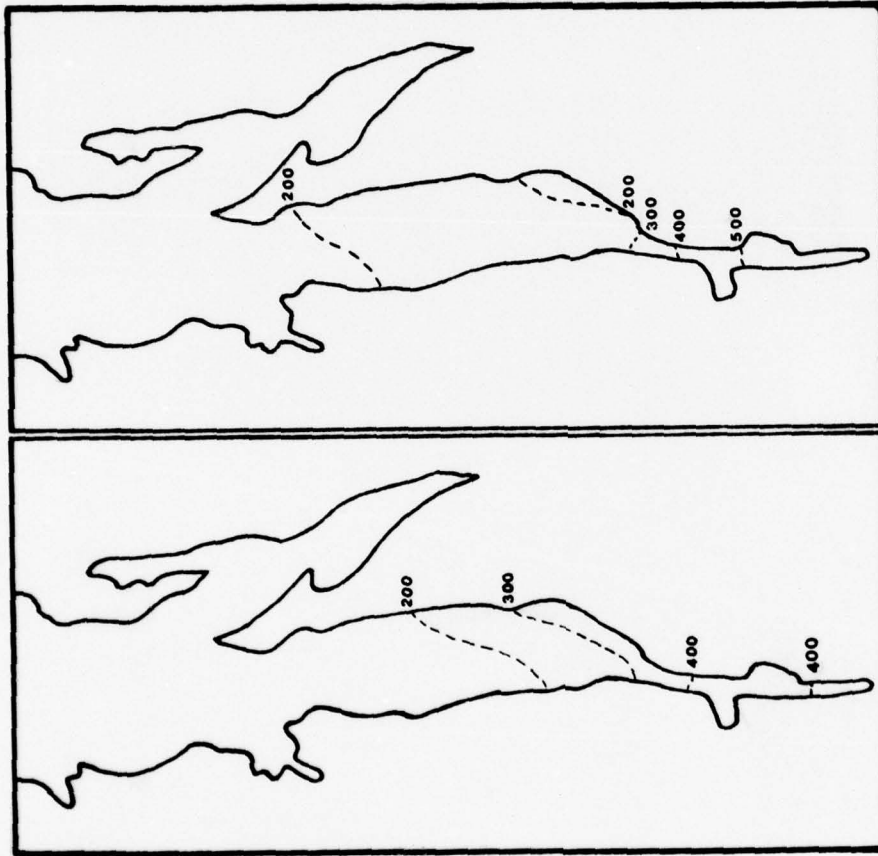


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FIGURE A-3. CONDUCTIVITY-SURFACE ( $\mu\text{mhos/cm}$ )

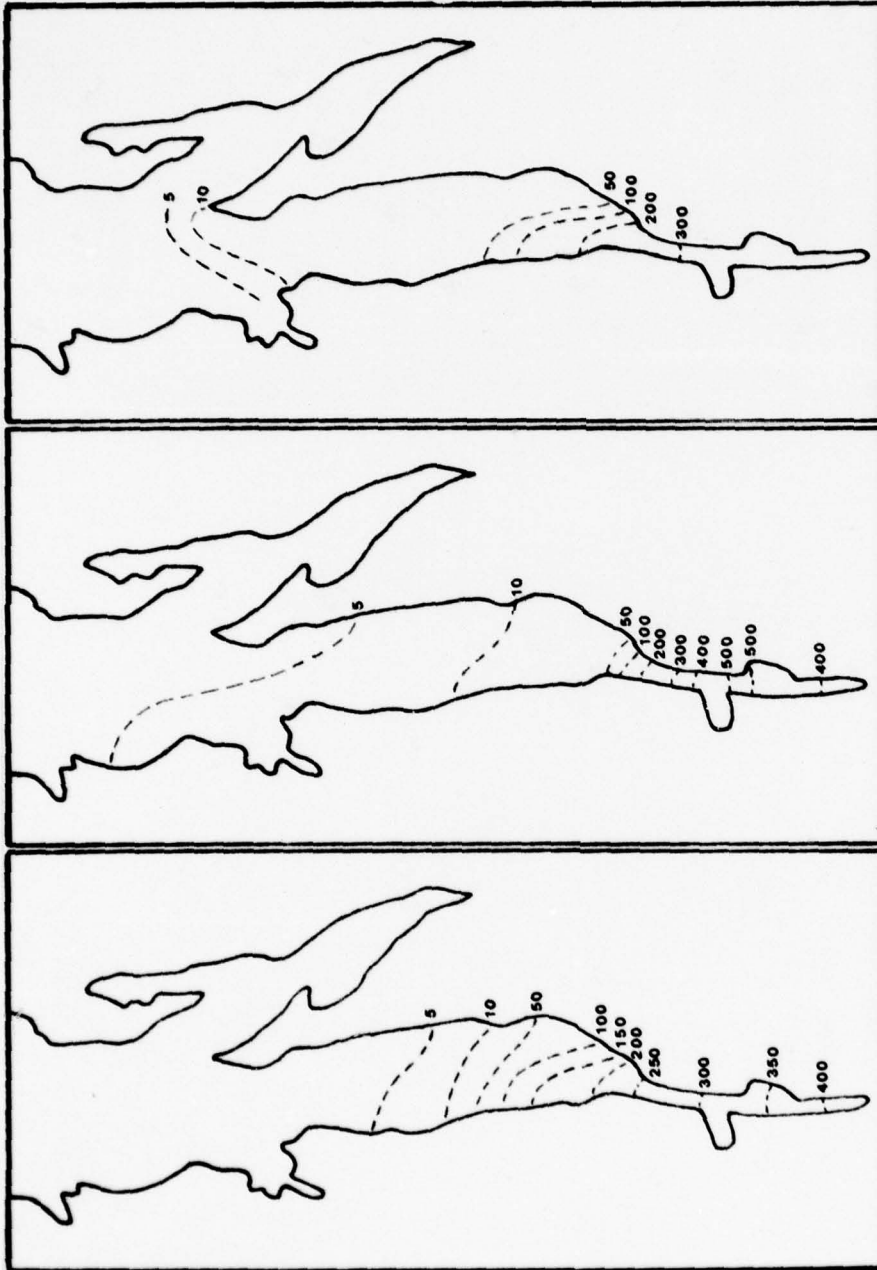


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12/10/76

FIGURE A-3. CONDUCTIVITY-SURFACE ( $\mu\text{hos/cm}$ )



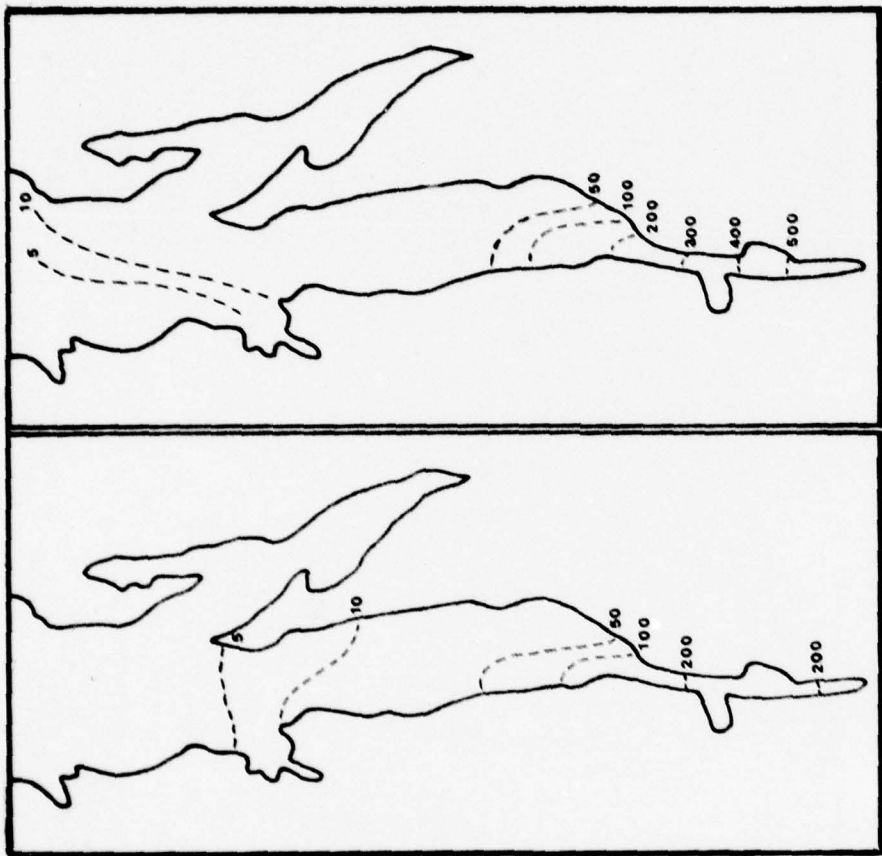


12/9/76

12/8/76

12/7/76

FIGURE A-4. TOTAL MUNITIONS CONCENTRATION,  $\mu\text{g/l}$ .



12/11/76

12/10/76

FIGURE A-4. TOTAL MUNITIONS CONCENTRATION, µg/l.

APPENDIX A - ANALYTICAL PROCEDURES

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APPENDIX B  
ANALYTICAL PROCEDURES

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APPENDIX B - ANALYTICAL PROCEDURES

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## ANALYTICAL PROCEDURES

Standard Methods or EPA-approved procedures were employed to characterize the background water quality (see Table B-1 for specific procedures). Ammonia nitrogen and total Kjeldahl nitrogen were analyzed on a Technicon Autoanalyzer II using the manufacturer's specified methodology. The block digester and the salicylate-nitroprusside colorimetric methods are currently being researched by the EPA and are approved for use in NPDES permit monitoring (Gales, EPA, 1977 personal communication). Analytical procedures (Table B-2) utilized on sediment samples were primarily from Chemistry Laboratory Manual Bottom Sediments (EPA, 1969).

### Munitions Analysis

The water samples were collected in amber glass reagent bottles that were pre-rinsed in acetone. The samples were refrigerated until analysis, which consisted of extraction, concentration and gas-liquid chromatography. Sediment samples were collected in glass jars that had been pre-rinsed with acetone. Saran wrap was used as a cap liner.

Extraction of Water Samples, December 1976. A sample of 250 ml was measured into a clean 500 ml separatory funnel equipped with a Teflon stop-cock. Seventy-five ml of ethyl acetate (pesticide grade) was added, the flask stoppered, and shaken for 2 to 3 minutes. The layers were allowed to separate and the lower (water) layer drained into a second 500 ml separatory funnel and again extracted with 50 ml ethyl acetate. The water layer was discarded. The extracts were combined and filtered through a plug of cotton previously wetted with ethyl acetate. The separatory funnels were rinsed with an additional 10 ml of ethyl acetate and filtered through the cotton plug. The ethyl acetate was evaporated to a volume of 2.5 ml under reduced pressure with the flask temperature not exceeding 40°C.

Extraction of Water Samples, March 1977. Same procedure except 500 ml samples were used.

Extraction of Sediment Samples. In order to dry the wet sediments, 80 gm of sodium sulfate was added to 20 gm of wet sediment. This was then packed into a chromatographic column and extracted for one hour with ethyl acetate. The extraction was followed by evaporation of the ethyl acetate extract to a volume of 5.0 ml under the same conditions as described earlier. Clean-up techniques are discussed in the recovery studies section of this procedure.

Chromatography of Extracts, December 1976. Samples were chromatographed on a 5 1/2 ft. x 1/8 in. silanized glass column packed with 2% OV 101/3% QF1 on 100/120 mesh Gas Chrom Q. A Varian Model 1840 Gas Chromatograph with electron capture (EC) and Thermionic (Alkali Flame Ionization Detector) (AFID) detectors was chosen. The readout was obtained by using a Varian Model 285 Electronic Integrator which was recorded permanently by a Beckman 1 mv, 10 inch scale recorder. Peak areas were automatically printed by integrator. Electron capture was chosen as the prime detector with AFID as back-up and confirmation detector.

TABLE B-1

SUMMARY OF ROUTINE LABORATORY ANALYTICAL PROCEDURES  
FOR WATER SAMPLES

<u>Parameter</u>	<u>Procedure</u>
Chloride	Standard Methods, 408B: Mercuric Nitrate Method, p. 304.
Total Hardness	Standard Methods, 309B: EDTA Titrimetric Method, p. 202.
Sulfate	Standard Methods, 427C: Turbidimetric Method, BaCl <sub>2</sub> , p. 496.
Ammonia Nitrogen	Technicon Corp. Industrial Method #239-74 W/A: Automated colorimetric salicylate-nitroprusside method, 1976.
Total Kjeldahl Nitrogen	EPA, STORET #00625: Acid Digestion, Distillation, Nesslerization, p. 175. or Technicon Corp. Industrial Method #376-75-W/A: Acid digestion on BD-20, 1975. Technicon Corp. Industrial Method #329-74-W/A: Automated colorimetric salicylate-nitroprusside method, 1976.
Nitrate-Nitrite Nitrogen	EPA, STORET #00630: Automated Cadmium Reduction Method, p. 207.

EPA 1974, Manual of Methods for Chemical Analysis of Water and Wastes.

Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975, APHA, AWWA, WPCF.

Technicon Industrial Systems; Tarrytown, New York.

TABLE B-2

SUMMARY OF ROUTINE LABORATORY ANALYTICAL PROCEDURES  
FOR SEDIMENT SAMPLES

<u>Parameter</u>	<u>Procedure</u>
Total Kjeldahl Nitrogen	<u>Bottom Sediments - Great Lakes; Acid Digestion, Distillation, and Titration with 0.02N H<sub>2</sub>SO<sub>4</sub>, p. 38.</u>
Nitrate-Nitrite Nitrogen	<u>Bottom Sediments - Great Lakes: Acid Digestion, p. 32.</u> <u>EPA, STORET #00630: Automated Cadmium Reduction Method, p. 207.</u>
Total Solids	<u>Bottom Sediments - Great Lakes: Gravimetric Method, p. 85.</u>
Total Volatile Solids	<u>Bottom Sediments - Great Lakes: Gravimetric Method, p. 85.</u>

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Chemistry Laboratory Manual Bottom Sediments, EPA 1969, compiled by Great Lakes Region Committee on Analytical Methods.

EPA 1974, Manual of Methods for Chemical Analysis of Water and Wastes.

An alternate column used for confirmatory information was a 4 ft. x 1/7 in. glass column packed with 8 percent UCW 98 on 80/100 mesh Gas Chrom Q. Instrument conditions for both columns and detectors were:

Column temperature:	175°C. isothermally
Injector temperature:	220°C
Detector temperature:	220°C
Carrier gas:	Nitrogen @ 70 ml/min.
Electrometer setting:	10-10 afs at 1 x attenuation into integrator with appropriate attenuation setting for recorder.

Five  $\mu$ l injections of sample extracts and standards were first injected onto the 2% OV 101/3% AF1 column using the EC Detector. Peaks corresponding to standards were noted and the areas compared. Samples and standards were next injected onto USW-98 column and like comparisons were made. Likewise, samples and standards were injected onto the 2% OV 101/3% QF1 column using the Thermionic or Alkali Flame Ionization Detector. Again, peaks corresponding to the standards were noted and the areas were compared. Sample peaks which did not elute at the same times as the standards on both sets of columns and detectors were rejected, and only those that were peaks confirmed on both sets were quantitated. Quantification was with the 2% OV 101/3% QF1 column with the EC detector.

Chromatography of Extracts, March 1977. Same procedure and conditions as above with the following exceptions:

Electron capture detector with a standing current of 100% at  $16 \times 10^{-10}$   
Attenuation set at  $8 \times 10^{-10}$   
Injection volume of 4.0  $\mu$ l  
Column temperature, 165°C  
Injector temperature, 200°C

Recovery Studies, December 1976. Recovery studies for water samples were made by addition of standards to tap water and carrying through entire extraction, concentration and GC procedures. Standards were prepared in acetone solution prior to addition to water. Previous recovery studies had been made by addition of standards prepared in ethyl acetate solution, however, it was thought that this might bias the study since ethyl acetate would be used for subsequent extraction. Hence, the selection of water soluble acetone as solvent would negate any positive effect which the standard solvent may have had.

Since previous studies had shown levels of less than 1 ppm TNT and analogs in water samples, spiking was made at and below this level as follows:

500 ml water was used in all cases.



<u>COMPONENT</u>	<u>QUANTITY ADDED</u>	<u>QUANTITY RECOVERED</u>	<u>% RECOVERY</u>
2, 4 DNT	500 µg	480	96
	250 µg	230	92
	100 µg	90	90
2, 6 DNT	200 µg	470	94
	250 µg	240	96
	100 µg	92	92
1, 3 DNB	500 µg	480	96
	250 µg	225	90
	100 µg	94	94
2, 4, 6 TNT	500 µg	460	92
	250 µg	230	92
	100 µg	95	95

SPIKING STUDIES, WATER SAMPLES

250 ml sample used

<u>SAMPLE NO.</u>	<u>COMPONENT</u>	<u>µg PRESENT</u>	<u>µg ADDED</u>	<u>TOTAL RECOVERY</u>	<u>% RECOVERY</u>
AB 21	2,4 DNT	0	25	24	96
	2,6 DNT	0	25	21	84
	TNT	0	25	25	100
	1,3 DNB	0	25	22	88
AB 110	2,4 DNT	55	25	70	88
	2,6 DNT	24	25	45	92
	TNT	69	25	95	101
	1,3 DNB	1	25	22	85
AB 114	2,4 DNT	6	25	30	97
	2,6 DNT	3	25	25	89
	TNT	3	25	24	86
	1,3 DNB	0	25	23	92
AB 37	2,4 DNT	0	62	59	95
	2,6 DNT	0	62	63	102
	TNT	0	62	60	97
	1,3 DNB	0	62	58	94
AB 103	2,4 DNT	51	62	105	93
	2,6 DNT	26	62	81	92
	TNT	19	62	75	93
	1,3 DNB	1.5	62	60	94
AB 204	2,4 DNT	28	62	82	91
	2,5 DNT	15	62	73	95
	TNT	15	62	75	97
	1,3 DNB	1.5	62	58	91

Sediment samples were column extracted with ethyl acetate after adding sufficient anhydrous sodium sulfate to absorb moisture and yield apparently dry product. Samples thus extracted contained too many interfering substances to be run directly on GLC and, therefore, were subjected to florisol column cleanup.

Elution of florisol with ethyl acetate not only removed the TNT compounds, but most of the interfering substances as well. It was found that elution of florisol column with 6% ethyl ether in hexane gave quantitative recovery of the compounds of interest while leaving most of the extraneous materials on the column. This procedure was adopted for cleanup.

Overall recovery averaging approximately 80% was not as good as with water samples. A more efficient extraction procedure would improve recovery.

Recovery of Standards added to Anhydrous Sodium Sulfate

<u>COMPOUND</u>	<u>QUANTITY ADDED</u>	<u>QUANTITY RECOVERED</u>	<u>% RECOVERY</u>
2,4 DNT	100 µg	94	94
2,6 DNT	100 µg	90	90
TNT	100 µg	95	95
2,4 DNT	250 µg	230	92
2,6 DNT	250 µg	225	90
TNT	250 µg	235	94

Indicated recovery ≈ 92%.

Recovery of Standards from Florisol Cleanup Procedure

<u>COMPOUND</u>	<u>QUANTITY ADDED</u>	<u>QUANTITY RECOVERED</u>	<u>% RECOVERY</u>
2,4 DNT	100 µg	96	96
2,6 DNT	100 µg	95	95
TNT	100 µg	98	98
2,4 DNT	250 µg	240	96
2,6 DNT	250 µg	230	92
TNT	250 µg	240	96

Recovery from Spiked Samples of Sediment

SAMPLE NO.	COMPONENT	$\mu\text{g}$ PRESENT	$\mu\text{g}$ ADDED	TOTAL RECOVERY	% RECOVERY
W4-1	2,4 DNT	0	25	21	84
	2,6 DNT	0	25	20	80
	TNT	0	25	22	88
W4-10	2,4 DNT	3	25	22	79
	2,6 DNT	2	25	21	78
	TNT	5	25	26	87
W4-4	2,4 DNT	0	50	40	80
	2,6 DNT	0	50	38	76
	TNT	0	50	42	84
W4-6	2,4 DNT	0	50	43	86
	2,6 DNT	0	50	42	84
	TNT	3	50	42	79

Recovery of standards added to samples and then put through extraction and cleanup procedures was not as good as extraction and cleanup without the presence of sample, thus indicating that the TNT compounds present some difficulty in extraction from sediment samples. Overall recovery indicated recovery is approximately 80%.

Recovery Studies, March 1977. Standards were prepared in acetone solution by diluting stock standard (1 mg/ml) with acetone to 100  $\mu\text{g}/\text{ml}$  or 0.1  $\mu\text{g}$  per  $\mu\text{l}$ . The combination standard then contained 0.1  $\mu\text{g}/\mu\text{l}$  of each of the following:

- 1, 3 DNB
- 1, 3, 5 TNB
- 2, 4 DNT
- 2, 6 DNT
- 2, 4, 6 TNT

Five hundred ml of distilled water was added to each of three separatory funnels and combination standard was added.

- 100 l (10  $\mu\text{g}$ ) to No. 1
- 500 l (50  $\mu\text{g}$ ) to No. 2
- 2.5 ml (250  $\mu\text{g}$ ) to No. 3

Contents of each funnel was mixed, the funnel stoppered and allowed to stand 1 hour. Contents of each funnel was then extracted and taken through same cleanup concentration and chromatography as were samples.

Recoveries were:

Component	Quantity Added μg	Quantity Recovered μg	Percent Recovered
1,3 DNB	10	9.1	91
	50	46.8	94
	250	240	96
1,3,5 TNB	10	9.0	90
	50	47.0	94
	250	235.0	94
2,4 DNT	10	9.5	95
	50	43.0	86
	250	230.0	92
2,6 DNT	10	9.3	93
	50	48.0	96
	250	232.0	93
2,4,6 TNT	10	9.2	92
	50	51.1	102
	250	242.0	97

Studies were also made with spiked samples similar to above except that known increments of standards were added to actual samples. Two hundred ml samples were spiked with varying amounts of standards using same procedures as with distilled water. These were extracted, concentrated, run through cleanup and chromatographed same as original samples with the following results:

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (μg)	3.1	12.5	-	1.7	14.1
Amount added (μg)	10.0	10.0	10.0	10.0	10.0
Total present (μg)	13.1	12.5	10.0	11.7	24.1
Assay (μg)	12.3	12.3	9.1	10.5	22.2
% Recovery	93.9	98.4	91.0	89.7	92.1
Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (μg)	-	8.8	1.1	2.2	22.6
Amount added (μg)	10.0	10.0	10.0	10.0	10.0
Total present (μg)	10.0	18.8	11.1	12.2	32.6
Assay (μg)	9.3	18.0	10.3	11.1	31.2
% Recovery	93.0	95.7	92.8	91.0	95.7
Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (μg)	-	0.2	-	6.5	1.0
Amount added (μg)	5.0	5.0	5.0	5.0	5.0
Total present (μg)	5.0	5.2	5.0	11.5	6.0
Assay (μg)	5.1	4.8	4.7	10.7	5.8
% Recovery	102.0	92.3	94.0	93.0	96.7

Component	1,3 DNB	1,3,5 TNB	3,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found ( $\mu\text{g}$ )	-	-	1.5	0.7	1.5
Amount added ( $\mu\text{g}$ )	5.0	5.0	5.0	5.0	5.0
Total present ( $\mu\text{g}$ )	5.0	5.0	6.5	5.7	6.5
Assay ( $\mu\text{g}$ )	4.9	4.8	6.2	5.6	6.6
% Recovery	98.0	96.0	95.4	98.2	101.5
Component	1,3 DNB	1,3,5 TNB	3,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found ( $\mu\text{g}$ )	-	-	20.8	15.0	5.9
Amount added ( $\mu\text{g}$ )	10.0	10.0	10.0	10.0	10.0
Total present ( $\mu\text{g}$ )	10.0	10.0	30.8	25.0	15.9
Assay ( $\mu\text{g}$ )	9.7	9.5	27.9	23.2	15.1
% Recovery	97.0	95.0	90.6	92.8	95.0

Sediment samples were column extracted with ethyl acetate after adding sufficient anhydrous sodium sulfate to absorb moisture and yield an apparently dry product. Samples thus extracted contained too many interfering substances to be run directly on GLC and, therefore, were subjected to florisil cleanup, eluting with 6% ether in hexane. The areas of the plate containing the compounds of interest were then removed, extracted with ethyl acetate. This cleaned up sample was then adjusted in volume and quantitated using GLC.

Results of spiking studies are given below:

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally present ( $\mu\text{g}$ )	1.1	6.4	-	0.8	4.7
Amount added ( $\mu\text{g}$ )	2.5	2.5	2.5	2.5	2.5
Total present ( $\mu\text{g}$ )	3.6	8.9	2.5	3.3	7.2
Assay ( $\mu\text{g}$ )	3.0	7.0	2.0	2.5	6.0
% Recovery	83.3	78.7	80.0	75.8	83.3
Component		1,3,5 TNB			2,4,6 TNT
Amount originally present ( $\mu\text{g}$ )		15.5			8.6
Amount added ( $\mu\text{g}$ )		5.0			5.0
Total present ( $\mu\text{g}$ )		20.5			13.6
Assay ( $\mu\text{g}$ )		16.5			11.0
% Recovery		80.5			80.9
Component		1,3,5 TNB			2,4,6 TNT
Amount originally present ( $\mu\text{g}$ )		6.5			6.2
Amount added ( $\mu\text{g}$ )		5.0			5.0
Total present ( $\mu\text{g}$ )		11.5			11.2
Assay ( $\mu\text{g}$ )		9.0			9.5
% Recovery		78.3			84.8

Because of several factors such as extraction efficiency, additional cleanup steps and handling losses, overall recovery from sediment samples averages about 80% whereas recovery from water samples was considerably better.

APPENDIX C  
WATER QUALITY AND VAAP  
EFFLUENT ANALYSES

LIST OF TABLES  
APPENDIX C

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C-1	MUNITIONS LEVELS VAAP EFFLUENT, MARCH 1977	115
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MUNITIONS LEVELS VAAP EFFLUENT  
MARCH 1977

Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V  
Project

PHASE II  
Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 5-25, 1977

SAMPLING DATE	Value in µg/l					TOTAL
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT	
3/5-7/77	<0.25	<0.75	580	269	106	956
3/8/77	<0.25	<0.75	529	53.3	92.8	676
3/9/77	<0.25	8.2	<0.10	42.9	151	202
3/10/77	<0.25	<0.75	1040	223	198	1460
3/11/77	<0.25	<0.75	1520	416	426	2370
3/12-14/77	<0.25	<0.75	866	188	323	1380
3/15/77	<0.25	<0.75	758	153	387	1300
3/16/77	<0.25	<0.75	261	28.8	234	525
3/17/77	<0.25	<0.75	90.7	10.6	55.4	158
3/18/77	<0.25	9.8	111	46.6	45.7	213
3/19-21/77	<0.25	<0.75	167	69.3	83.4	321
3/22/77	<0.25	<0.75	113	38.5	98.1	251
3/23/77	<0.25	<0.75	9.4	7.8	69.8	88.0
3/24/77	<0.25	<0.75	161	55.4	70.9	288
3/25/77	<0.25	<0.75	<0.10	86.8	62.3	150

Comments *Composite effluent samples taken by VAAP; 24 hour composites excepting the weekend composites.*

TABLE C-1 (CONT.)



Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V  
Project

PHASE II  
Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 28, 1977  
TO APRIL 1, 1977

SAMPLING DATE	Value in $\mu\text{g/l}$					TOTAL
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT	
3/28/77	<0.25	<0.75	121	85.8	90.4	298
3/29/77	<0.25	<0.75	<0.10	53.4	121	176
3/30/77	<0.25	<0.75	<0.10	0.6	1.1	≤2.80
3/31/77	<0.25	<0.75	<0.10	47.8	152	201
4/1/77	<0.25	<0.75	137	87.2	245	470

Comments *Composite effluent samples taken by VAAP; 24 hour composites excepting the weekend composites.*

TABLE C-2  
VAAP EFFLUENT FLOW, MARCH 1977

Date	Flow, mgd	Date	Flow, mgd	Date	Flow, mgd
1	7.0	11	3.5	21	5.0
2	6.5	12	7.7	22	6.6
3	3.2	13	7.7	23	7.3
4	6.2	14	7.7	24	6.0
5	7.8	15	7.9	25	6.0
6	7.8	16	4.9	26	5.6
7	7.8	17	4.9	27	5.5
8	5.3	18	5.2	28	5.5
9	4.2	19	5.0	29	4.6
10	3.7	20	5.0	30	---
				31	5.6

TABLE C-3

VAAP MUNITIONS CHEMISTRY  
RANGE OF VALUES



Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V

PHASE II

Project

Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED March 1-5, 1977

STATION NUMBER	RANGE of Values in $\mu\text{g/l}$						TOTAL
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT		
1	<0.25 - 17.2	<0.75 - 11.5	<0.10	<0.05 - 50.9	<0.10 - 146		$\leq 1.70 - 158$
2	<0.25	<0.75	<0.10 - 88.1	<0.05 - 113	<0.10 - 118		<1.35 - 249
3	<0.25	<0.75	<0.10	<0.05 - 58.0	<0.10 - 139		<1.35 - 198
4	<0.25 - 1.2	<0.75	<0.10 - 88.7	<0.05 - 109	<0.10 - 49.4		<1.35 - 160
5	<0.25 - 1.0	<0.75 - 66.3	<0.10	0.3 - 53.7	<0.10 - 95.5		$\leq 1.60 - 140$
6	<0.25	<0.75 - 2.0	<0.10	1.9 - 32.5	<0.10 - 4.8		$\leq 4.90 - 38.5$
7	<0.25	0.9 - 2.2	<0.10	4.5 - 17.5	<0.10 - 0.3		$\leq 5.85 - 20.4$
8	<0.25	<0.75 - 39.3	<0.10	<0.05 - 3.9	<0.10 - 0.2		<1.35 - 40.6
9	<0.25	<0.75 - 38.4	<0.10 - 6.0	1.1 - 3.4	<0.10 - 0.4		$\leq 4.00 - 40.9$
EFF	<0.25	<0.75	<0.10 - 104	<0.05 - 60.0	<0.10 - 29.6		<1.35 - 195
24 hr comp EFF	<0.25	<0.75	<0.10 - 111	<0.05 - 62.0	<0.10 - 187		<1.35 - 361

Comments \_\_\_\_\_



TABLE C-4  
 VAAP WATER CHEMISTRY  
 RANGE OF VALUES  
 Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V  
 Project

PHASE II  
 Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 1-5, 1977

STATION NUMBER	Range of Values							
	NH <sub>3</sub> -N	TKN	NO <sub>3</sub> -N	Cl	T.Hard	SO <sub>4</sub>		
1	0.94-	1.57-	7.31-	13.5-	118-	132-		
	1.47	2.11	16.9	21.2	212	198		
2	0.95	1.53-	8.16-	15.0-	122-	139-		
	1.49	2.64	13.9	20.5	202	198		
3	1.00-	1.69-	7.49-	14.5-	132-	144-		
	1.24	1.99	13.9	18.0	192	177		
4	0.99-	1.31-	7.24-	13.8-	128-	153-		
	1.17	1.99	13.3	17.0	190	175		
5	0.84-	1.52-	7.28-	14.0-	125-	153-		
	1.09	1.72	18.1	16.5	188	290		
6	0.26-	0.57-	3.15-	11.0-	112-	56.0-		
	0.68	1.22	7.71	14.5	147	122		
7	0.12-	0.48-	2.55-	9.0-	98.0-	61.0-		
	0.26	0.96	4.11	10.8	117	77.0		
8	0.08-	0.40-	1.40-	8.3-	88.0-	32.3-		
	0.16	0.71	2.25	9.3	94.0	48.7		
9	0.04-	0.23-	0.63-	7.0-	78.0	12.5-		
	0.13	0.52	1.55	8.5	88.0	27.3		
EFF	1.02-	1.63-	7.25-	14.0-	118-	146-		
	1.57	2.25	21.8	24.0	230	193		
	mgN/l	mgN/l	mgN/l	mgCl/l	mg/l as	mgSO <sub>4</sub> /l		
					CaCO <sub>3</sub>			

Comments \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

APPENDIX D  
PERIPHYTON METHODS

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## METHODOLOGY FOR PERIPHYTON COLLECTION

Within the study area periphyton samples were collected on natural and artificial substrates. Forms colonizing natural substrates were collected at the end of February, while those on exposed glass slides were not collected until the end of March. For this reason, the data are not comparable except in a general qualitative sense.

Three samples representative of natural substrates were obtained at each site and preserved in 5 percent formalin. These were oxidized with hydrogen peroxide and potassium dichromate to remove organic matter. The treated sample was dried on an 18 x 18 coverslip, mounted in Hyrax (refractive index 1.7), and examined under oil immersion (1000X). For qualitative comparisons, a minimum of 500 diatom valves were identified per sample. Raw-count data were transferred to a coding sheet. This information was key punched prior to computer analysis. Several routines were utilized including culling the data to eliminate species which occurred only rarely. The collection of periphyton on glass slides is considered to give more reliable station-to-station comparisons than natural substrates because of influence by substrate specificity. Standard 1 x 3 inch microscope glass slides were placed in periphyton samplers (Periphytomer II)<sup>TM</sup> one inch below the water surface at nine stations located in Waconda Bay, and three stations located in the reference bay (Figure 3). Periphytometers were placed in suspension units as shown in Figure D-1. Samplers were placed according to recommended procedures outlined in Standard Methods for the Examination of Water and Wastewater 14th Edition, (APHA, 1975), and Biological Field and Laboratory Methods for Measuring the Quality of Surface Water and Effluents, (Weber, 1973).

Periphytometers were anchored in a northerly direction utilizing the suspension unit designed for this study. A total of 8 racks (64 slides) were placed in the suspension units at each station. As part of a supplemental statistical study to measure variability between replicate slides and to determine the best practical method for processing periphyton slides, additional 6-rack (48 slides) suspension units were placed at Stations 1, 3, 8, 9, 11 and 12. Anchorline adjustments were made for anticipated lake level fluctuations of 2 feet or less. All slides were collected over a 2-day period from March 31 - April 1, 1977, following an incubation period of 30 days. Sampling devices were recovered at all stations except 9 and 10, where periphytometer units had been overturned exposing slides to the air. However, at station 9, the statistical 6-rack unit of slides was still intact and was used as a substitute for the vandalized 8-rack units.

At the end of the 30-day incubation period, slides from both the 8-rack and 6-rack units were recovered utilizing the following collection procedures. End slides (Positions 1 and 8) from the 8-rack units were removed, dried, and placed into labeled glass bottles. Slides for biomass, chlorophyll a, diatom, filamentous algae, and vital stain counts were collected from slide positions 2 - 7 using the random number generator of a Texas Instruments SR-51A.



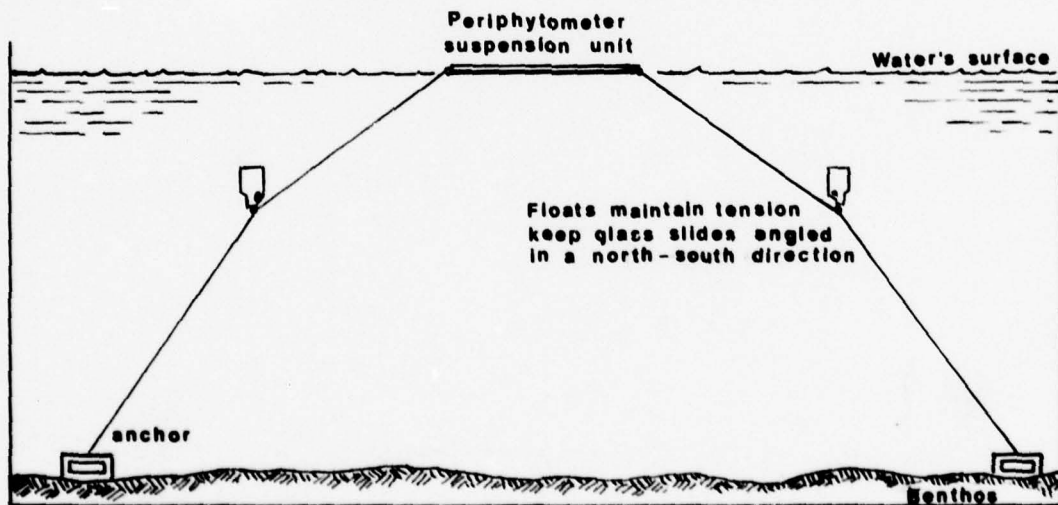
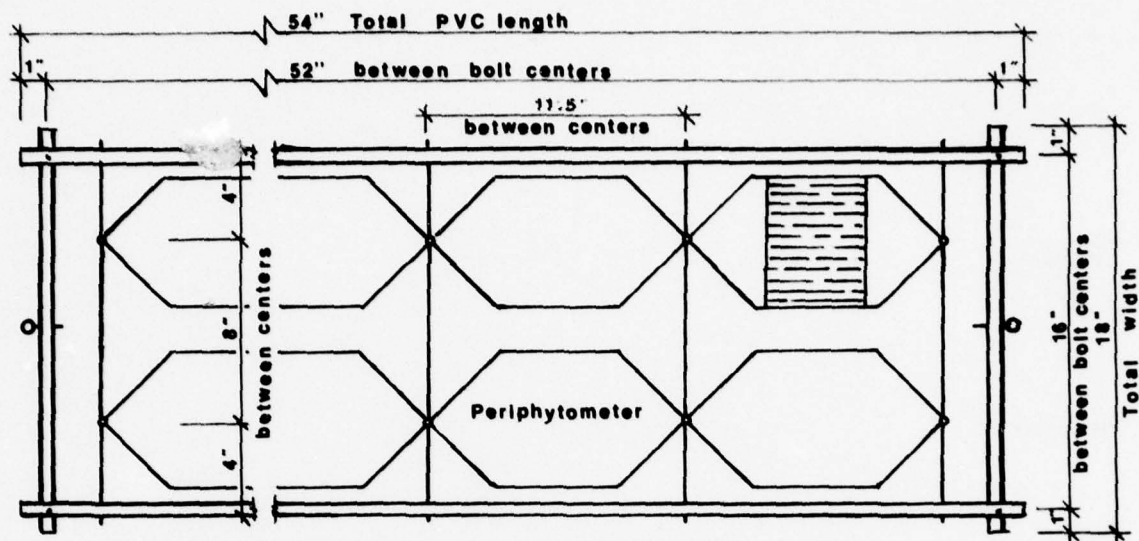


FIGURE D-1. PERIPHYTOMETER SUSPENSION UNIT

calculator. The priority for selecting slides from each rack was:

2 end slides	- Air-dried
2 slides	- Biomass determination
2 slides	- Chlorophyll <u>a</u>
1 slide	- Diatom counts
1 slide	- Filamentous algae or vital stain counts
<u>8 slides</u>	- Total

For the 8-rack periphyton suspension units the minimum numbers of slides for analyses were:

<u>Analyses</u>	<u>Minimum Number of Replicates per Station</u>
Chlorophyll <u>a</u>	16
Biomass	16
Diatom counts	5
Filamentous algae	2
Vital stain counts	3
Total	<u>42</u>

Slides from the 6-rack suspension units were all air-dried for diatom counts.

#### Chlorophyll a Determination

Except for Station 10, a total of 16 replicate periphyton slides were collected at each station for chlorophyll a analysis. While in the field, periphyton slides were placed in 50 ml of a 90 percent acetone (v/v), 10 percent of a saturated  $MgCO_3$  solution and immediately stored in the dark on dry ice. Prior to analysis, chlorophyll was extracted for 24 hours in the dark at 4°C. To facilitate extraction, slides were scraped and the acetone suspension ground 30 seconds at 500 rpm in a Potter-type tissue homogenizer.

Following extraction chlorophyll a, corrected for phaeophytin, was determined fluorometrically after the methods of Yentsch and Menzel (1963), Holm-Hansen, et al. (1965), Lorenzen (1967), and Moss (1968), using a Turner Design Model 10 fluorometer. Fluorometric determination of chlorophyll depends on red fluorescence emitted by the chlorophyll a molecule when excited by ultraviolet light and is 100 times more sensitive than spectrophotometric analysis. The method is limited to chlorophyll a only; chlorophyll b and c cannot be determined.

The chlorophyll a reference solution was a purified spinach chlorophyll standard (Product No. C5753, Sigma Chemicals, St. Louis, MO) calibrated by spectrophotometric chlorophyll analysis.

Acidification of chlorophyll a converts it quantitatively to phaeophytin. Reading the fluorescence before and after adding one drop of 1N HCl to the sample cuvette allows calculation of an acid factor related to the interference.

Periphytic chlorophyll a was calculated as follows:

$$\text{Chlorophyll } \underline{a} \text{ (mg/m}^2\text{)} = \frac{(F)(r)(Ca)(\text{ml extract})}{(r-1)[\text{substrate area (mm)} 10^{-3}]}$$

where: Ca = fluorometer reading before-fluorometer reading after acidification

$$r = \frac{\text{standard before acidification}}{\text{standard after acidification}}$$

$$F = \left[ \frac{Ca}{\text{fluorometer reading}} \right] \left[ \frac{\text{dilution rasion fluorometer}}{\text{dilution ratio spectrophotometer}} \right]$$

#### Organic Biomass.

Sixteen replicate biomass slides were collected from each station. In the laboratory each slide was rehydrated for 15 minutes, accumulated material scraped from the slide into a graduated cylinder, and then resuspended in a total volume of 50 ml distilled water. An aliquot of the suspension was filtered on a tared, fired-glass filter (Gelman, GFA), the ash-free dry weight determined (APHA, 1975) and converted to grams of organic matter per square meter.

#### Diatom Cell Densities.

At least five replicate slides per station were examined for diatom community structure and cell density estimates. Periphyton growth was scraped from glass slides into tall labeled beakers using razor blades and a rubber policeman. Samples were oxidized with 20 ml of 50 percent hydrogen peroxide and approximately 50 mg of potassium dichromate. The solution was cooled, allowed to settle for 24 hours, decanted, and brought to a volume of 50 ml. Preliminary examination of oxidized material from Stations 6 - 12 indicated the samples were too concentrated to count accurately. Therefore, 4- and 8-fold dilution was necessary to permit observations of 10 - 15 organisms per microscope field. In contrast, samples at Stations 1 - 5 were too sparse and had to be concentrated by a factor of 5 to permit accurate counting.

Permanent slides were prepared by pipetting 0.4 ml of the "cleaned" material onto an 18 x 18 coverslip (324 mm<sup>2</sup>) and allowing the sample to dry at 65°C (150°F) on a laboratory hot plate. The dried coverslip was placed on a standard microscope slide containing one drop of Hyrax mounting medium (refractive index 1.7) and the slide gently heated to drive off the toluene solvent. When cooled, the permanent slide was labeled with station number, date, location, and dilution factor. Under an oil immersion lens (Zeiss microscope, 1000X) diatoms were identified and enumerated to the species level, where possible, utilizing the following standard taxonomic references: Hustedt, 1930, 1962; Schmidt, et al., 1974-1959; Huber-Pestalozzi, and F. Hustedt, 1949; and Patrick and Reimer, 1966, 1975. Voucher diatom slides were sent to Dr. Charles Reimer, Academy of Natural Sciences of Philadelphia, for taxonomic verification.

Cell densities were estimated by performing field counts at 6-slide coordinates randomly selected on the coverslip. A total of 10 microscope fields were examined at each of the 6 selected coordinates for a total of 60 fields. At 1000X, each microscope field represented an area of 0.0182 mm<sup>2</sup>

with a total area examined of 1.092 mm<sup>2</sup> (i.e. 0.0182 mm<sup>2</sup> x 60 fields). Therefore, cell densities were determined as follows:

$$\text{Cells/mm}^2 = \text{Diatom Counts} \times \frac{\text{Total Area of Coverslip (324 mm}^2\text{)}}{\text{Total Area Examined (1.092 mm}^2\text{)}} \times \frac{\text{Original Volume of Periphyton Suspension (50 ml)}}{\text{Volume of Sample dried on coverslip (0.4 ml)}} \times \frac{\text{Dilution Factor}}{\text{Original Surface Area of Slide (3,871 mm}^2\text{)}}$$

Filamentous Algae. Two replicate periphyton slides per station were collected from artificial substrate units located in Waconda Bay and the reference bay. Slides were mechanically scraped with razor blades and preserved in 5 percent formalin. Clumped periphyton material was dispersed by blending samples in a Waring blender. Algal filaments were identified using a 50 ml plankton sedimentation chamber and a Zeiss invertoscope D. Identifications were carried to species level, where possible, utilizing the following standard references: Drouet (1968); Prescott (1962); and Desikachary (1956).

Vital Stain Counts. Recent studies have shown that natural and artificial substrate periphyton populations contain significant numbers of diatom frustules that are either empty or have less-than-robust chloroplasts (Pryfogle and Lowe, 1976). Standard diatom cleaning procedures using ammonium persulfate, acid or hydrogen peroxide oxidation do not distinguish between diatoms having metabolically active chloroplasts and those having empty frustules. Under certain conditions an investigator may wish to determine the metabolic state of diatoms in response to a pollution source. The following experimental vital stain procedure was conducted at 11 stations located in Waconda Bay and the reference bay using tetrazolium violet. Tetrazolium salts have been used to determine the dehydrogenase activities of bacterial populations in activated sludge and to a limited extent in the analysis of plankton populations (Armitage, 1977). The enzymatic reduction of the tetrazolium salt (yellow) to triphenyl formazin precipitate (violet stain) visually tags diatom cells with active cytochrome systems. As a result, cell counts can be qualitatively differentiated into one of three metabolic categories:

<u>Metabolic Activity</u>	<u>Cell Description</u>
Viable or active	Violet precipitate present throughout cell, or concentrated in mitochondria.
Senescent	Chlorophyll present, no violet precipitate formed.
Dead or fossil forms	No violet precipitate or chlorophyll present in frustule.

Three replicate periphyton slides per station were removed from the samplers and placed in glass bottles (75 ml) containing lake water collected at the site. These samples were then placed in the dark and 1 ml of a 0.2 percent (w/w) solution of paraiodotetrazolium was added to the sample bottle.

The slides were incubated in the light for a period of 12 hours after which they were preserved with 3 percent formalin. Vital stain counts were made utilizing a 50 ml plankton sedimentation chamber with a Zeiss inverted microscope equipped with an oil immersion 1000X lens. Diatom populations were differentiated into metabolically active, senescent, or dead (fossil).

APPENDIX E  
STATISTICAL METHODS

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## STATISTICAL METHODS

### I. Introduction

The statistical analyses of the artificial substrate periphyton data considered the following conditions:

- comparison of variability in density and diversity estimates between fields on a single slide with variability between aliquots.
- comparison of variability in density and diversity estimates between fields and aliquots with variability between slides.
- comparison of variability in density and diversity estimates when one slide is used with that when a composite of five slides is used.
- comparison of the eleven sampled stations with respect to diversity and density.
- at one station a procedure for determining dominant species to a specified degree of precision was determined.

Shannon's measure of diversity

$$\hat{H} = -\sum_{i=1}^S \hat{p}_i \ln \hat{p}_i,$$

was used where  $\hat{p}_i$  is the proportion of the  $i^{\text{th}}$  species in the sample ( $\hat{p}_i = \frac{n_i}{N}$ ),  $s$  is the number of observed species, and we use natural logarithms.

Basharin (1959) has shown that  $\hat{H}$  is a biased estimate of the population diversity

$$H = -\sum_{i=1}^S p_i \ln p_i$$

where  $p_i$  is the true proportion of species  $i$  in the population, and  $S$  is the true number of species present in the population. The bias of  $\hat{H}$  is negative, implying that  $\hat{H}$  tends to underestimate  $H$ . Basharin shows that

$$\text{Bias} = -\frac{s-1}{2N}$$

where  $N$  is the total number of organisms observed. Thus a bias-adjusted estimate of  $H$

$$\hat{H}_B = \hat{H} + \frac{s-1}{2N}$$

can be obtained. Basharin also derives the standard error of  $\hat{H}_B$  showing that

$$\text{S.E.}(\hat{H}_B) = \frac{1}{N} \left[ \sum_{i=1}^S p_i \ln^2 p_i - H^2 \right]$$

which can be estimated by

$$S.E.(\hat{H}_B) = \frac{1}{N} \left[ \sum_{i=1}^S \hat{p}_i \ln^2 \hat{p}_i - \hat{H}_B^2 \right]$$

This allows one to measure the reliability of the diversity estimate and to compare the station diversities statistically.

Turning to density, the logarithmic transformation

$$L_i = \ln(N_i + 1)$$

was used to transform  $N_i$ , the total number of organisms per observational unit (usually a group of fields) to a variable  $L_i$  which more nearly satisfies the assumptions necessary for an analysis of variance. Specifically, the variable  $L_i$  will often have a distribution which is approximately normal when  $N_i$  is skewed to the right. This transformed density measure can now be used to make statistical comparisons of the various sampling procedures, and of the stations.

## II. Comparison of Diversity and Density Variability Between Fields With That Between Aliquots

### A. Description of Experiment

One slide was selected from each of four stations, 3, 8, 11, and 12. Ten aliquots were taken from each slide, and 120 fields or 500 organisms, whichever came first (with a 60 field minimum), were counted on the microscope slide made from each aliquot. Counts were made in units of 10 fields. This experiment enables one to compare the variability between fields to that between aliquots at each station.

### B. Diversity

As shown in Section VIIIA, one may analyze the bias-adjusted diversities using a nested analysis of variance model. Table E-1 presents the results of the analysis for the four stations.

### C. Density

The same nested analysis of variance model which was used for diversity was used to analyze  $L$ , the logarithmic transform of density. The results are shown in Table E-2.

### D. Conclusion

Whether the replicates consist of groups of fields from one microscope slide, or of fields from different aliquots, one can pool the results when estimating diversity and density. There is no evidence that the aliquot variability and the field within aliquot variability differ.

TABLE E-1  
 COMPARISON OF DIVERSITY VARIABILITY BETWEEN ALIQUOTS  
 TO THAT BETWEEN FIELDS FOR 4 STATIONS

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
3	Aliquot	9	3.4132	.3792	1.00
	Fields(Aliquot)	110	41.826	.3802	
8	Aliquot	9	0.1648	.01831	0.46
	Fields(Aliquot)	60	2.4041	.04007	
11	Aliquot	9	0.1731	.01923	0.86
	Fields(Aliquot)	50	1.1240	.02248	
12	Aliquot	9	0.3569	.03966	1.06
	Fields(Aliquot)	70	2.6068	.03724	

None of the F values are significant at even the .10 level. There is no evidence that aliquoting introduces variability into the estimate of diversity.

TABLE E-2  
 COMPARISON OF DENSITY VARIABILITY BETWEEN ALIQUOTS  
 TO THAT BETWEEN FIELDS FOR 4 STATIONS

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
3	Aliquot	9	1.9445	.2161	.81
	Fields(Aliquot)	110	29.3589	.2669	
8	Aliquot	9	1.1033	.1226	.70
	Fields(Aliquot)	60	10.5679	.1761	
11	Aliquot	9	1.4106	.1567	1.01
	Fields(Aliquot)	50	7.7954	.1559	
12	Aliquot	9	1.7829	.1981	.98
	Fields(Aliquot)	70	14.1604	.2023	

As with diversity, there is no evidence to suggest that aliquoting contributes variability to the estimate of density.

### III. Comparison of Diversity and Density Variability Between Slides To That Within Slides

#### A. Experiment

Nine slides were selected from each of the stations 3, 8, 11, and 12, and a count was obtained for 60 fields (10 fields at each of 6 random locations) on a single microscope slide prepared from each experimental slide. Thus the variability between slides can be determined, and then compared to that within slides.

#### B. Diversity

The variance estimate of diversity between slides is based on the 60 field counts of the single microscope slide; while the within slide variability is estimated from the 10 field counts from the previous experiment. Since the same stations are sampled, one would expect approximately six times more organisms in 60 fields than in 10, which theoretically increases the diversity variance by a factor of six. An appropriate test statistic to compare these variance components is therefore

$$F = \frac{6(\text{Variance Between Slides})}{(\text{Variance Within Slides})}$$

The results of this test are summarized in Table E-3.

The five percent level of F is about two in each case. It appears that the between slide variability is larger than the within slide variability at least at stations 8 and 11. That is, there is evidence that the diversity variance between slides may be slightly larger than that within slides.

#### C. Density

The assumption that the logarithmic transform L of density is approximately normal plays a key role in the comparison of density variability between and within slides. Using standard results for the lognormal distribution, one can find an estimate of within slide variance to be

$$S_W^2 = e^{2\bar{y}_L + S_L^2} (e^{S_L^2} - 1)$$

where  $\bar{y}_L$  is the mean of the log transform L and  $S_L^2$  is the variance of L. The same formula can be used with the between slide data to calculate  $S_B^2$ , the estimate of between slide variability. Then one can test that the between slide variability exceeds the within slide variability by computing

$$F = \frac{S_B^2}{6S_W^2}$$

TABLE E-3  
 COMPARISON OF DIVERSITY VARIABILITY  
 BETWEEN SLIDES TO THAT WITHIN SLIDES

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
3	Between Slides	8	00.6010	0.07152	1.13
	Within Slides	119	45.2396	0.3802	
8	Between Slides	8	0.1385	0.01731	2.79
	Within Slides	69	2.5688	0.03723	
11	Between Slides	8	0.08544	0.01068	2.91
	Within Slides	59	1.2971	0.02199	
12	Between Slides	8	0.07891	0.009864	1.58
	Within Slides	79	2.9638	0.03751	

where the factor of 6 is present because 6 times more fields are involved in each density count for the between slide calculations. The results of the analysis are given in Table E-4. Note that this F test is only approximate, since the distributions are log-normal rather than normal.

#### D. Conclusion

There appears to be slightly more variability between slides than within slides for both diversity and density. The implication is that one should obtain replication by sampling several slides rather than aliquoting a single slide. This will protect against the possibility that the sampled station has more variability between than within slides, as some of the experimental ones did. The number of slides which must be sampled will be considered in Section VI.

### IV. Comparison of Diversity and Density Variability When One Slide is Used With That When Five Slides Are Composited

#### A. Experiment

Five slides were composited and one microscope slide formed, on which 60 fields were counted. Five replicates of this experiment were performed at each of four stations, 3, 8, 11 and 12.

#### B. Diversity

In order to determine whether the compositing reduces variability, one can compare the composited variance to the single slide variance from the previous experiment (see III-A). The results are summarized in Table E-5.

#### C. Density

The same comparison can now be made on the logarithmic transforms of the densities (see Table E-6).

#### D. Conclusion

The compositing of five slides does not seem to reduce the variability of either diversity or density estimates. Since more replication (and thus better variance estimates) can be obtained by analyzing the slides separately, the single slide method is probably preferable to compositing slides.

### V. Station Comparisons

#### A. Experiment

Since the previous results indicated that a replicated, single slide experiment at each station was preferred, this experiment was performed at seven other stations, five replicates at each station.

#### B. Diversity

The stations, their bias-adjusted diversity, and the standard error (see Basharin, 1959) are given in Table E-7.

TABLE E-4  
COMPARISON OF DENSITY VARIABILITY BETWEEN  
SLIDES TO THAT WITHIN SLIDES

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>Variance</u>	<u>F</u>
3	Between Slides	8	250.05	2.99
	Within Slides	119	13.95	
8	Between Slides	8	17,489.1	3.42
	Within Slides	69	853.06	
11	Between Slides	8	9,293.4	1.10
	Within Slides	59	1,409.7	
12	Between Slides	8	12,394.2	2.35
	Within Slides	79	880.88	

There is ample statistical evidence to conclude that the density variability between slides exceeds that within slides at stations 3, 8, and 12.



TABLE E-5  
COMPARISON OF COMPOSITED DIVERSITY VARIANCE  
TO SINGLE SLIDE VARIANCE

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>Variance</u>	<u>F</u>
3	Single	8	.07152	1.21
	Composite	4	.05920	
8	Single	8	.01731	1.46
	Composite	4	.01185	
11	Single	8	.01068	3.29
	Composite	4	.00325	
12	Single	8	.009864	0.84
	Composite	4	.01170	

Since the F value for significance at the 0.10 level is  $F = 3.94$ , no evidence that composite diversity variance exceeds single slide variance is found.

TABLE E-6  
 COMPARISON OF COMPOSITED DENSITY VARIANCE  
 TO SINGLE SLIDE VARIANCE

<u>Station</u>	<u>Source</u>	<u>DF</u>	<u>Variance</u>	<u>F</u>
3	Single	8	.1129	15.6
	Composite	4	.007224	
8	Single	8	.1236	2.85
	Composite	4	.04330	
11	Single	8	.03815	0.38
	Composite	4	.1017	
12	Single	8	.04572	0.62
	Composite	4	.07419	

No consistent pattern in these variance comparisons is found. There seems to be significantly more variance in the single slide experiment than the composite slide experiment at Station 3, but somewhat less at Station 11.

TABLE E-8  
POPULATION DENSITIES FOR ALL STATIONS

<u>Station</u>	<u>Density (number/mm<sup>2</sup>)</u>
11	37,293
8	27,803
9	22,462
12	19,698
7	18,936
6	14,911
5	750
4	174
1	144
3	85
2	28

Thus the conclusion is that the station densities are aligned as follows:

11 > 9, 12, 7, 6, 5, 4, 1, 3, 2

8 > 6, 5, 4, 1, 3, 2

9, 12, 7, 6 > 5 > 4 > 1, 3 > 2

TABLE E-7  
DIVERSITIES FOR ALL STATIONS

<u>Station</u>	<u>Diversity</u>	<u>Standard Error</u>
6	0.716	0.035
7	1.189	0.033
5	1.608	0.043
8	2.045	0.025
9	2.175	0.032
4	2.440	0.074
11	2.557	0.020
1	2.573	0.074
12	2.790	0.019
2	2.918	0.089
3	2.929	0.061

An analysis of variance comparison was deemed inappropriate due to the probable inequality of the diversity variances. Thus the stations were compared in a pairwise fashion. The lines on the left of the station numbers reflect the results of comparing the diversities by using the two sample Z statistic, connecting stations which cannot be declared significantly different. The formula for Z is

$$Z = \frac{(\text{Station } i \text{ Diversity} - \text{Station } j \text{ Diversity})}{\sqrt{(\text{Standard Error } i)^2 + (\text{Standard Error } j)^2}}$$

Since Basharin (1959) shows that Shannon's diversity estimate is asymptotically normal, this statistic should approximate a standard normal random variable when the true population diversities are the same, and it does not necessitate equal variances. When the value of Z is large (negative or positive), the implication is that the true station diversities differ. A conservative .01 significance level,  $Z = 2.58$ , was used since so many comparisons were made. Table E-7 shows that the stations' true diversities are arranged as follows:

$$6 < 7 < 5 < 8 < 9 < 4, 11, 1 < 12, 2, 3$$

### C. Density

The logarithmic density transforms were subjected to a standard analysis of variance. The station comparison yielded a highly significant F value of 319.81, leaving no doubt that the station densities differ. The transformed densities were then subjected to Duncan's (1975) multiple comparison procedure to determine which stations differ. The estimated densities (untransformed) are shown in Table E-8, with the solid lines on the left of the station numbers connecting those stations which one cannot conclude as different at the .05 significance level.

## VI. Sample Size Determination

Perhaps the most crucial decision which must be made in planning an experiment is the sample size, or number of replicates to be processed. One can use information gleaned from previous experiments to plan more carefully.

In Section III it was concluded that replication of slides was more desirable than replication of aliquots from a single slide. Hence, it was decided that the effect on a single slide consist of counting ten adjacent fields at each of six randomly selected locations (60 total fields). The question then becomes: how many slides should be sampled?

### A. Diversity

Since diversity is analyzed on an untransformed scale, it is easier to work in absolute rather than percentage differences. The following equation (Steel and Torrie 1960) is for the absolute difference between two stations' diversities which can be detected with 90 percent confidence for n replicate slides:

$$D = 2(1.645)\sigma_1 \sqrt{\frac{2}{n}} = 4.653\sigma_1 / \sqrt{n}$$

TABLE E-8  
POPULATION DENSITIES FOR ALL STATIONS

<u>Station</u>	<u>Density (number/mm<sup>2</sup>)</u>
11	37,293
8	27,803
9	22,462
12	19,698
7	18,936
6	14,911
5	750
4	174
1	144
3	85
2	28

Thus the conclusion is that the station densities are aligned as follows:

11 > 9, 12, 7, 6, 5, 4, 1, 3, 2  
 8 > 6, 5, 4, 1, 3, 2  
 9, 12, 7, 6 > 5 > 4 > 1, 3 > 2

where  $\sigma_1$  is the standard deviation of the diversity based on single slide estimates. This assumes that the stations being compared have the same standard deviation  $\sigma_1$ . The probability of falsely concluding a difference exists is set at  $\alpha = .10$ .

A plot of D versus n for the four experimental stations is shown in Figure E-1. Note that for all but Station 3, where the density of organisms was very low, one can detect a diversity difference of slightly less than 0.3 with 90% confidence using five replicate slides.

#### B. Density

Since the statistical analysis of density is performed on a logarithmic scale, the differences one can detect between station densities will be expressed in percentages. The absolute percentage difference which can be detected with 90% confidence, with  $\alpha = .10$ , is given by

$$P = 100\{\exp[4.653\sigma_1/\sqrt{n}] - 1\}$$

where  $\sigma_1$  is the standard deviation of the transformed density for single slide estimates. A plot of p versus n for the four experimental stations is shown in Figure E-2. Note that one must have at least five replicate slides before he can be 90% sure of detecting density differences of 100%. It is obvious that density comparisons require more replication than do diversity comparisons to achieve similar reliability.

#### C. Conclusions

In order to determine the necessary sample size for estimating and comparing station diversities or station densities, a preliminary estimate of the variance,  $\sigma_1^2$ , must be obtained. One procedure is to count approximately 60 fields on a single slide from the station of interest, and then to estimate  $\sigma_1^2$  by the following.

Diversity:

$$\sigma_1^2 = \frac{1}{N} \left[ \sum_{i=1}^S \hat{p}_i \ln^2 \hat{p}_i - \hat{H}_B^2 \right] \quad (\text{See Section I for notation definitions})$$

Then the number of slides necessary to detect a difference, D, in diversities with 90 percent confidence for two stations having approximately the same standard deviation is given by

$$n = 21.65 \sigma_1^2 / D^2$$

If the stations of interest have very different variances, compute the average variance to use in the formula for n,

Density: The computation of an estimated variance for the density is more difficult. Perhaps the best method is to sample 3 slides from each station, count 60 fields on each slide, and then calculate the sample variance,  $s_1^2$ , for the 3 log-transformed density observations. Then the total number of slides which need to be sampled in order to detect a

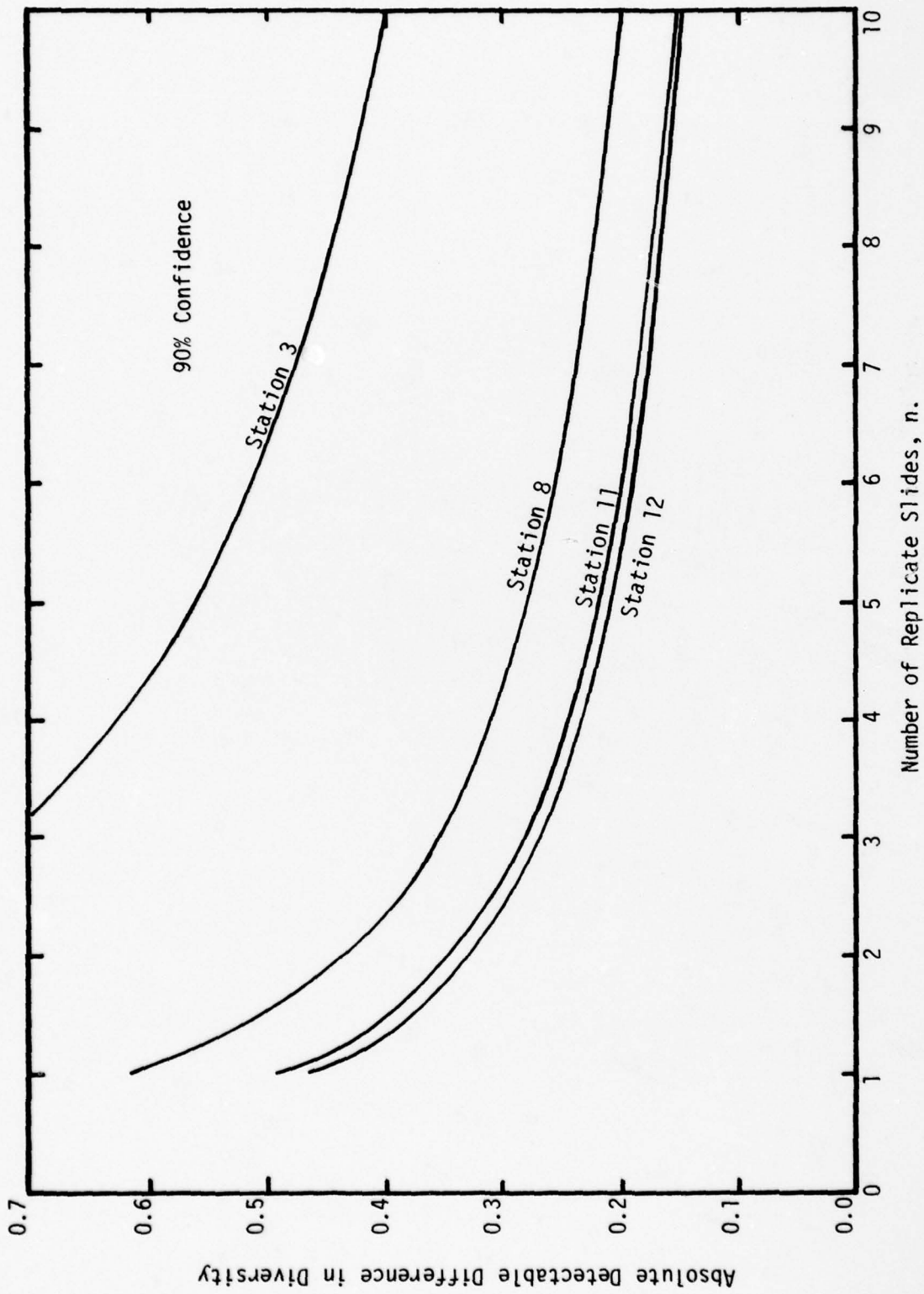


FIGURE E-1. SAMPLE SIZE TO MEET DIVERSITY CRITERIA



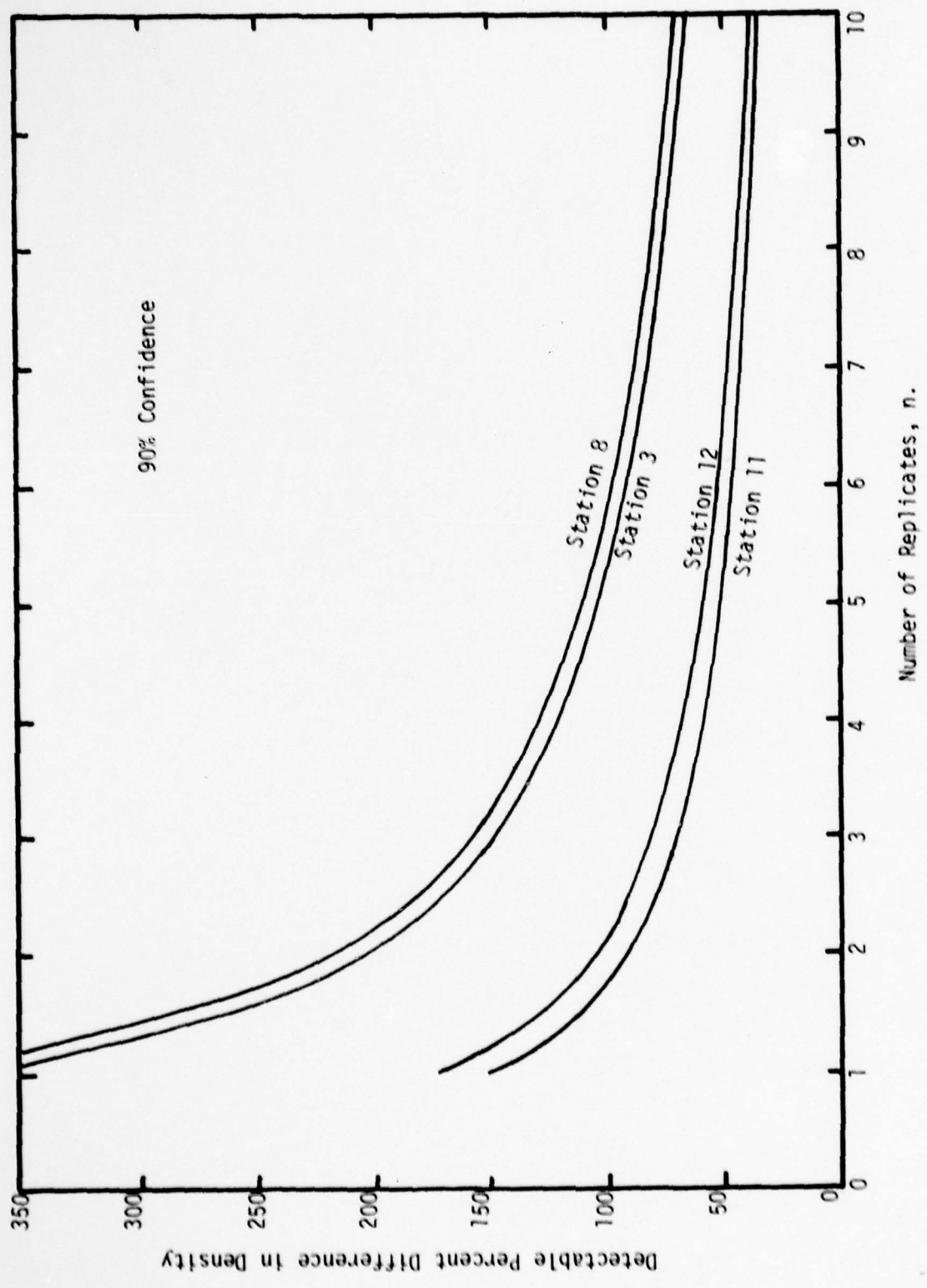


FIGURE E-2. SAMPLE SIZE TO MEET DENSITY CRITERIA

TABLE E-9  
 DOMINANT SPECIES FOR STATION 8, PERIPHYTON,  
 ARTIFICIAL SUBSTRATE

Organism	Slide No.									9- Slide Total
	1	2	3	4	5	6	7	8	9	
<i>Achnanthes minutissima</i>	No. 56 8.0	35 7.9	12 5.1	27 7.5	27 8.1	40 12.5	27 7.2	13 6.1	21 7.4	258 7.9
<i>Cymbella prostrata v. auerswaldii</i>	No. 23 3.3	19 4.3	6 2.6	12 3.3	16 4.8	5 1.6	10 2.7	10 4.7	6 2.1	107 3.3
<i>Diatoma tenue v. elongatum</i>	No. 284 40.8	179 40.1	120 51.1	131 36.2	150 44.9	95 29.7	145 38.8	83 39.0	130 45.9	1317 40.4
<i>Fragilaria vaucheriae</i>	No. 138 19.8	77 17.3	40 17.0	100 27.6	54 16.2	64 20.0	51 13.6	36 16.9	47 16.6	607 18.6
<i>Melosira varians</i>	No. 33 4.7	23 5.2	8 3.4	8 2.2	7 2.1	15 4.7	13 3.5	10 4.7	8 2.8	125 3.8
<i>Synedra rumpens</i>	No. 81 11.6	56 12.6	26 11.1	47 13.0	38 11.4	44 13.8	75 20.1	26 12.2	29 10.3	422 12.9
All other species	No. 82 11.8	57 12.8	23 9.8	37 10.2	42 12.6	57 17.8	53 14.2	35 16.4	42 14.8	428 13.1
Total	697	446	235	362	334	320	374	213	283	3264

percentage difference P between two stations is given by

$$n = 21.65 s_1^2 / [\ln(1+P/100)]^2$$

Again,  $s_1^2$  may be obtained by averaging the various station variances if preliminary samples are obtained for several stations.

#### VII. Determination of Dominant Species

An analysis of dominant species was conducted using 9 replicate slides at Station 8. The basis for dominant species selection was that each species make up at least 3% of the pooled sample at the station. Six species satisfied this criteria at Station 8. The data are shown in Table E-9.

The first question to be answered was: Can one pool all nine replicates and treat each species composition estimate as a binomial estimate. For example, the total count of species 1 in all replicates was 258, which is 7.9% of the total count of 3264. If one can use binomial theory, the 95% confidence interval for species 1 percent composition is

$$7.90 \pm 2 \times \sqrt{\frac{(7.9)(100 - 7.9)}{3264}}$$

or

$$7.90 \pm 0.94$$

Thus, one would estimate with 95% confidence that between 6.96 and 8.84 percent of the population consists of species 1.

A  $\chi^2$  contingency table test was conducted to determine whether there is significant difference between replicates. The table consisted of 7 rows corresponding to the 6 dominant species and all others combined and 9 columns corresponding to the 9 replicate slides. The  $\chi^2$  statistic of 110.8 is significant at the .0001 level when compared to the  $\chi^2$  distribution with 48 degrees of freedom. Thus, it is concluded that a significant difference exists between the replicate estimates of percent composition for the 6 species. Since the replicates are all taken from the same population (station), the percent composition of each replicate estimates the true composition at the station. The fact that the  $\chi^2$  test shows a significant difference in replicate percentages warns that the pooling and use of binomial theory is inadvisable.

Using the theory given in Section VIII-B, we can obtain a sample estimate of variability without assuming the binomial distribution applies. Both the binomial and sample estimates are shown in Table E-10.

It is obvious that if one were to use the binomial formula for variance, the result would be an underestimate. Thus, in determining the sample size necessary to estimate the percent composition for a species with a specified accuracy, the binomial formula will underestimate the necessary sample size.

Several methods can be employed to more realistically determine the sample size necessary. For example, suppose one wanted to estimate the percent composition attributable to species 3, Diatoma tenue v. elongatum (from Table E-9), the most dominant, to within 2% with 95% confidence. Although the binomial bound is already less than 2% (1.72%) with 3,264

TABLE E-10  
 VARIANCE AND BOUND BASED ON BINOMIAL THEORY AND ACTUAL SAMPLING RESULTS

Species	Estimate	Variance		95% Confidence Bound	
		Binomial	Sample	Binomial	Sample
1	7.90	.0727	.1203	0.94	1.21
2	3.28	.0317	.0401	0.62	0.70
3	40.35	.2407	1.0856	1.72	3.64
4	18.60	.1514	.7033	1.36	2.94
5	3.83	.0368	.0509	0.67	0.79
6	12.93	.1126	.3205	1.17	1.93

organisms counted, the more realistic sample estimate is 3.64%. The number of organisms necessary to reduce this bound to 2% is found by solving:

$$n = \frac{4 (\text{Estimated Variance})}{(\text{Bound})^2} \times 10^4$$

$$= \frac{4(1.0856)}{(2)^2} \times 10^4 = 10,856$$

This works out to about 21 more replicate slides at the present rate of approximately 363 organisms per slide (60 fields).

The main disadvantage of the above method is that one must have an estimated sample variance before  $n$  can be calculated. Thus, several replicates (at least three would be advisable) must be analyzed before the necessary sample size can be estimated. A cruder alternative is available when preliminary sampling cannot be conducted. First, one can estimate the sample size using the binomial formula, then multiply by a "safety factor" to adjust for replicate variability. The appropriate factor is the ratio of the true variance to the sample variance. In our example, Table E-10, the factor ranges from about 1.5 for the species at the lower end of the dominant scale to about 4 for the species with high percent composition.

For example, if one were to use the binomial variance to estimate the sample size necessary to estimate the species 3 percent composition to within 2%, with 95% confidence, one would get:

$$n = \frac{4 (\text{Binomial Variance})}{(\text{Bound})^2} \times 10^4$$

$$= \frac{4(.2407)}{(2)^2} \times 10^4 = 2407$$

Using the safety factor of 4, since species 3 is at the upper end of the dominant percentages, one finds that about 9600 organisms should be counted. This is not too far from the estimate of 10,856 obtained using the sample results.

In summary, if slide replicates are to be used for estimating percent compositions, the variability of the estimates may be considerably more than is expected assuming a binomial distribution. More realistic estimates of variability can be calculated from the actual sample results, and these in turn can be used to estimate sample sizes necessary for a specified accuracy of estimation. If the binomial distribution is used to determine sample size, a safety factor should be used to adjust for replicate variability. To be safe, this factor should be at least 2 for species at lower percentages (5-10%), and 3 to 4 for species above 10%.

### VIII. Detailed Procedures

#### A. Analysis of variance for Shannon's Diversity

Suppose one is estimating a station's true diversity by selecting a single slide, preparing  $n_A$  aliquots (and thus  $n_A$  microscope slides), and then counting  $n_C$  sets of 10 fields each on each slide (see section II of this report for the analysis of such an experiment). A statistical model for such an experiment would be:

$$H_{jk} = H + A_j + \epsilon_{jk}$$

where  $H_{jk}$  is the Shannon diversity estimate for the  $k^{\text{th}}$  group of 10 fields in aliquot  $j$

$H$  is the true Shannon diversity,

$A_j$  is a random effect associated with aliquot  $j$

$\epsilon_{jk}$  is a random effect associated with the  $k^{\text{th}}$  group of 10 fields on aliquot  $j$ .

Assume:

$$E(A_j) = 0, \text{Var}(A_j) = \sigma_A^2 / N_j$$

$$E(\epsilon_{jk}) = 0, \text{Var}(\epsilon_{jk}) = \sigma_\epsilon^2 / N_{jk}$$

$$\text{Cov}(A_j, \epsilon_{jk}) = 0$$

where  $N_{jk}$  = number of organisms in  $k^{\text{th}}$  group of 10 fields in aliquot  $j$

$$N_j = \sum_{k=1}^{n_C} N_{jk} = \text{number of organisms in aliquot } j$$

Thus

$$E(H_{jk}) = H$$

$$\text{Var}(H_{jk}) = \sigma_A^2 / N_j + \sigma_\epsilon^2 / N_{jk}$$

Basharin (1959) shows that,

$$\text{Var}(H_{jk}) = \left[ \sum_{i=1}^S p_i \ln^2 p_i - H^2 \right] / N_{jk}$$

so that this model is in agreement to the order of  $N_{jk}$ .

Now one can apply the usual analysis of variance methods to this nested model for  $H_{jk}$ . First calculate

$$\bar{H}_j = \sum_{k=1}^{n_c} H_{jk}/n_c \quad j = 1, 2, \dots, n_A$$

$$\bar{H} = \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} H_{jk}/n_A n_c$$

In terms of the model,

$$\bar{H}_j = H + A_j + \sum_{k=1}^{n_c} \epsilon_{jk}/n_c$$

$$\bar{H} = H + \sum_{j=1}^{n_A} A_j/n_A + \sum_j \sum_k \epsilon_{jk}/n_A n_c$$

so that

$$\begin{aligned} \text{Var}(\bar{H}_j) &= \frac{\sigma_A^2}{N_j} + \frac{\sigma_\epsilon^2}{n_c^2} \sum_{k=1}^{n_c} \frac{1}{N_{jk}} \\ &= \frac{\sigma_A^2}{N_j} + \frac{\sigma_\epsilon^2}{n_c^2 N_j} \end{aligned}$$

$$\text{where } N_j = \left( \sum_{k=1}^{n_c} \frac{1}{N_{jk}} \right)^{-1}$$

$$\begin{aligned} \text{Var}(\bar{H}) &= \frac{\sigma_A^2}{n_A^2} \sum_{j=1}^{n_A} \frac{1}{N_j} + \frac{\sigma_\epsilon^2}{n_A^2 n_c^2} \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} \frac{1}{N_{jk}} \\ &= \frac{\sigma_A^2}{n_A^2 N} + \frac{\sigma_\epsilon^2}{n_A^2 n_c^2 \underline{N}} \end{aligned}$$

$$\text{where } N = \left( \sum_{j=1}^{n_A} \frac{1}{N_j} \right)^{-1}, \quad \underline{N} = \left( \sum_j \sum_k \frac{1}{N_{jk}} \right)^{-1}$$

Define

$$\begin{aligned}
 \text{SSA} &= n_c \sum_{j=1}^{n_A} (\bar{H}_j - \bar{H})^2 \\
 &= n_c \sum_{j=1}^{n_A} [(\bar{H}_j - H) - (\bar{H} - H)]^2 \\
 &= n_c \sum_{j=1}^{n_A} (\bar{H}_j - H)^2 - n_c n_A (\bar{H} - H)^2
 \end{aligned}$$

Thus

$$\begin{aligned}
 E\{\text{SSA}\} &= \frac{n_c \sigma_A^2}{N} + \frac{\sigma_\epsilon^2}{n_c N} \\
 &\quad - \frac{n_c \sigma_A^2}{n_A} - \frac{\sigma_\epsilon^2}{n_A n_c N} \\
 &= (n_A - 1) \left[ \frac{n_c \sigma_A^2}{N} + \frac{\sigma_\epsilon^2}{N} \right] \\
 E\{\text{MSA}\} &= E\left\{ \frac{1}{n_A - 1} \text{SSA} \right\} = \frac{n_c \sigma_A^2}{n_A N} + \frac{\sigma_\epsilon^2}{N n_A n_c}
 \end{aligned}$$

Secondly,

$$\begin{aligned}
 \text{SSC} &= \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} (H_{jk} - \bar{H}_j)^2 \\
 &= \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} [(H_{jk} - H) - (\bar{H}_j - H)]^2 \\
 &= \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} (H_{jk} - H)^2 - n_c \sum_{j=1}^{n_A} (\bar{H}_j - H)^2
 \end{aligned}$$

$$E(\text{SSC}) = \frac{(n_c - 1) \sigma_\epsilon^2}{n_c N}$$

$$E(\text{MSC}) = E\left\{ \frac{1}{(n_c - 1) n_A} \text{SSC} \right\} = \frac{\sigma_\epsilon^2}{n_A n_c N}$$



If one forms the approximate\* F ratio,

$$F = \frac{MSA}{MSC}$$

he gets a test of the hypothesis  $H_0: \sigma_A^2 = 0$ , or that the aliquoting contributes no extra variability (beyond that of the sets of fields within aliquots) to the diversity estimate. Large values of F would imply that  $\sigma_A^2 > 0$ , or that the aliquoting does contribute additional variability. These tests are conducted in section II. B.

Note that this same reasoning could be extended to additional hierarchies. For example, one could similarly analyze an experiment involving stations, slides within stations, and fields within slides within stations. In this way one can determine which variance components seem to contribute most to the variability of the diversity estimate. These tests were conducted in sections II to IV of this report.

#### B. Procedure for Estimation of Percentage of Specific Organism

Assume  $r$  replicate slides, with  $n_1, n_2, \dots, n_r$  organisms identified on each slide. Let  $y_1, y_2, \dots, y_r$  represent the number of a particular species in each replicate. If  $p$  is the true fraction of the population which is the species of interest, then one may estimate  $p$  by the  $r$  fractions

$$\hat{p}_1 = \frac{y_1}{n_1}, \hat{p}_2 = \frac{y_2}{n_2}, \dots, \hat{p}_r = \frac{y_r}{n_r}$$

Central limit theory implies that these sample fractions will be approximately normally distributed, with mean  $p$ . If the binomial assumption is made, the variance of  $\hat{p}_i$  is  $\frac{p(1-p)}{n_i}$ . In the periphyton

sampling experiment, there is evidence that this value underestimates the actual variance, hence it is proposed that the assumption

$$\text{Variance } (\hat{p}_i) = \frac{\sigma^2}{n_i} \text{ be used}$$

where  $\sigma^2$  is an unknown constant, exceeding  $p(1-p)$ . One can estimate  $\sigma^2$  by the following sample statistic:

$$s^2 = \frac{\sum_{i=1}^r n_i (\hat{p}_i - \hat{p})^2}{r - 1}$$

where  $\hat{p}$  is the pooled estimate of  $p$ , i.e.

$$\hat{p} = \frac{\sum_{i=1}^r y_i}{\sum_{i=1}^r n_i} = \frac{\sum_{i=1}^r y_i}{N}$$

\*Basharin (1959) proves that the diversity estimate is asymptotically normal, which lends credence to the assumption that this ratio possesses an F distribution.

Then use  $\hat{p}$  to estimate  $p$ , but instead of estimating the variance of  $\hat{p}$  by  $\frac{\hat{p}(1-\hat{p})}{N}$ , use

$$\text{Var}(\hat{p}) \approx \frac{s^2}{N}$$

To estimate a percentage composition to within a specified bound  $B$  and with a confidence level of  $C$  percent, first find the  $z$  value from the normal tables corresponding to  $C$ , i.e.  $z = 2$  for  $C = 95$  percent,  $z = 1.65$  for  $C = 90$  percent, etc. Then solve

$$n = \frac{z^2 s^2}{B^2} \times 10^4$$

where the  $10^4$  factor is used if  $B$  is given as a percent, but eliminated if  $B$  is given as a decimal fraction.

#### IX. Biologic Interpretation of Results

The statistical analysis has shown that a significant source of variability in determining either diversity or density on artificial substrate periphyton samples is the difference between fields on an individual slide. In the procedure utilized here, the slides were prepared by placing a 0.4 ml aliquot on an 18 x 18 mm coverslip. A random number procedure was used to determine the location on the coverslip to conduct the actual count. Ten adjacent fields were counted at each selected coordinate, i.e. if 60 total fields were counted, six locations on the coverslip were utilized.

The data for the separate 10 field units shows a considerable variation in total numbers. Locations near the edge of the coverslip tend to have much lower organism density than locations near the center. The reason for this is that when the 0.4 ml aliquot dries, it does so from the edge to the center. This has the effect of concentrating organisms at the center of the coverslip. Hence, there is a very uneven distribution of diatoms that is particularly apparent when a random number procedure is utilized to determine the counting position on the coverslip.

Given the variation described above, it is possible that the significance of aliquot to aliquot or slide to slide variation may have been masked. Hence, if the variation due to drying of the aliquot on the coverslip could be reduced then the aliquot to aliquot and slide to slide variation could be assessed in a more precise manner. A re-examination of these variables would be appropriate.

An obvious needed improvement in processing technique is the development of a procedure that does not concentrate organisms near the coverslip center. Lacking this, it is important that a randomized procedure be used to select coverslip locations which counteracts the natural tendency to count near the center where most of the organisms are concentrated.

APPENDIX F  
MACROINVERTEBRATE METHODS

LIST OF FIGURES

<u>FIGURE</u>	<u>DESCRIPTION</u>	<u>PAGE</u>
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## METHODOLOGY FOR MACROINVERTEBRATE COLLECTION

Aquatic macroinvertebrates were collected from natural and artificial substrates in March, 1977, at 12 stations. Natural substrates were sampled with a petite Ponar dredge. Hester-Dendy artificial substrates were suspended approximately 1.5 - 3.0 feet below the surface. Fifteen replicates of the natural substrate, and fourteen units of artificial substrates, were collected at each station to minimize natural variability.

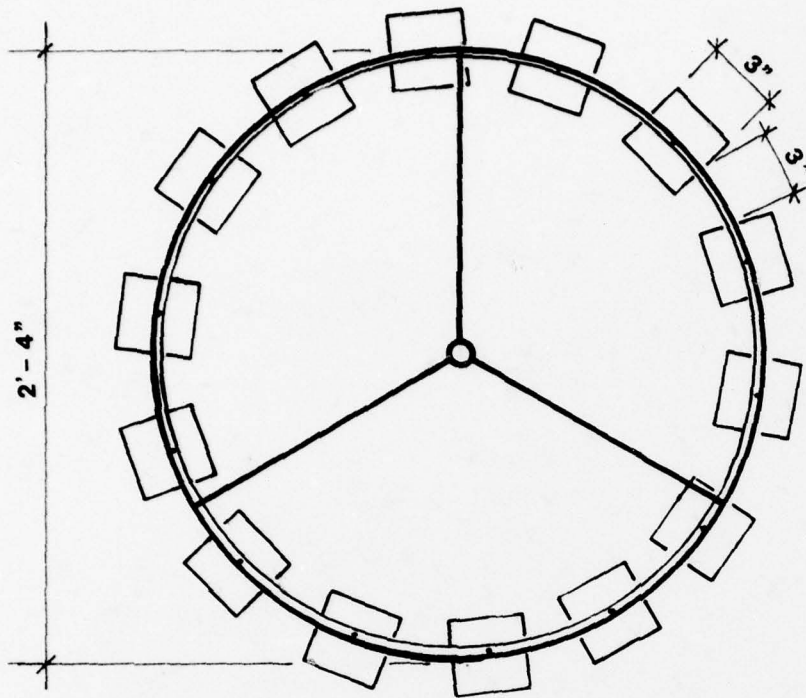
To decrease variability a special unit was fabricated for Hester-Dendy plate suspension (Figure F-1). This sampling device was designed and built by Mr. B.C. Pruitt of Water and Air Research, Inc. The unit consists of PVC pipe molded into a circle with a circumference of approximately 2-1/2 feet. Fourteen 5 plate units are attached to the suspension frame by wing nuts. Floats are attached to a line of predetermined length. The entire apparatus is anchored at selected station locations. For purpose of this survey, units were suspended 18 inches below the surface.

In the field, dredge samples of the natural substrate were washed in a bucket sieve (U.S. Standard No. 30 mesh) and bottled. Rose Bengal dye was then added to facilitate laboratory sorting. Samples were preserved in 10 percent formalin. Natural substrate samples were rewashed in the laboratory and picked in a white enamel pan partially filled with water. After sorting, organisms were placed in vials containing 95 percent ethanol. Chironomid larvae were mounted in polyvinyl-lactophenol for microscopic identifications.

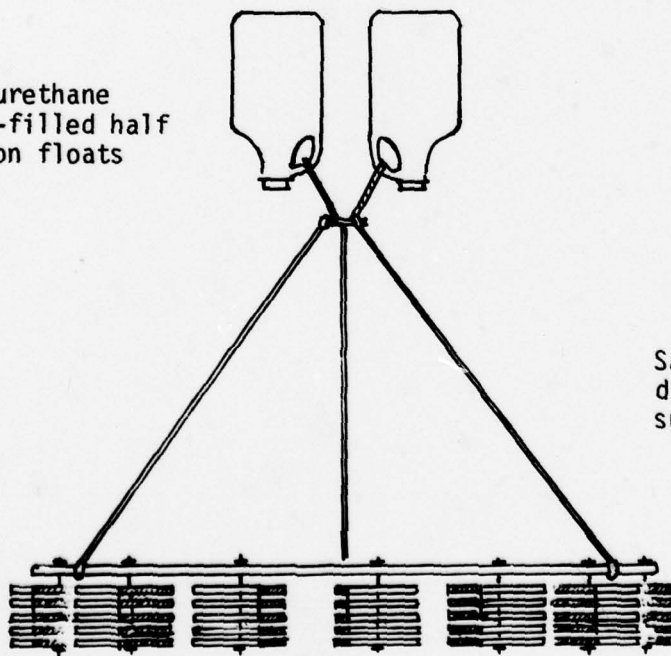
Samples from all stations were analyzed and identifications were made to the lowest practical taxonomic level. Fifteen natural substrate replicates were processed for stations 1, 5, and 11, and five were processed for the remaining nine stations. Five of the 14 artificial substrate replicates (whole units) were processed for all twelve stations. The community structure indices which were computed include the Shannon-Weaver diversity index and the Pinkham-Pearson index of biotic similarity.

### Organism Loss From Processing

A problem encountered with the quantification of benthic macroinvertebrates was the loss of organisms during laboratory processing. This occurs when benthic samples are rinsed in a 30-mesh sieve to remove formalin, dye, and the finer sediments. Therefore, we saved the rinse water and examined it for macroinvertebrates for the five replicates at Station 1 in order to estimate losses from this technique. This experiment was unnecessary for artificial substrates since they were not rinsed in a sieve.



Polyurethane  
foam-filled half  
gallon floats



Samplers bolted  
directly to  
suspension unit

FIGURE F-1. HESTER DENDY ARTIFICIAL SUBSTRATE SUSPENSION UNIT.

The table below, with the five replicates pooled, enumerates the results:

Taxa	Number of Organisms Retained By:	
	30-mesh Sieve	Rinse Pan
<u>Procladius</u> sp.	88	3
<u>Tanytus neopunctipennis</u>	7	3
Oligochaeta	1.5	0.5
Nematoda	1	0
Unidentified Coleopteran	1	0
Totals	98.5 (93.8%)	6.5 (6.2%)

$\Sigma=105$

A similar experiment was conducted on a leaf pack sample. Of an estimated 400-600 organisms within the sample, approximately 100-200 passed through the sieve. Almost all those lost were chironomids and we feel that a 6.2 percent loss is acceptable. The loss is apparently only partially related to organism size when considering those with small diameters. Experience has shown that there is very little loss when samples contain moderate to large amounts of filamentous algae or sand. Apparently, sand plugs many of the sieve openings and filamentous algae entangle the organisms which results in higher sieve retention.

The sediment in the study area was composed almost entirely of clay and silt. This matrix was of a pudding-like consistency. It was typically orange or brown (oxidized) on top, becoming gray (reduced) underneath. The top orange layer, as observed from dredged benthic grabs, was about 1-2 cm at Stations 1 and 2. It was 2-4 cm, at Stations 3, 4 and 5, but reduced to a thin veneer at Station 6. The orange coloration was replaced by brown at Stations 7 through 11. It was a brownish-orange at Station 12, grading to medium brown below. Some gravel remains of an old dam were found at Station 9. Small amounts of shell were observed at Stations 6 through 11.

Detritus was present at all stations, but was abundant only at Station 6. Oil was also found only at Station 6 and formed a film on the water surface when sieving macroinvertebrate samples. A mat of filamentous algae covered the bottom at Stations 3 through 6 where it was relatively dense. However, it was very thin at Station 6. A slender spikerush (Eleocharis acicularis?) was found in small quantities on the benthos at Station 12.

Dead fish were present in the sediment at Stations 1 through 5. They were partially decomposed and almost unidentifiable but were probably gizzard shad and appeared most abundant at Stations 1 through 3. Burrows of the mayfly larvae Hexagenia sp. were abundant at Stations 7 through 9 while only a few were found at Stations 10 and 11.

An effort was made to sample benthic macroinvertebrates at comparable depths. This was possible at all stations except 8, 9, 10 and 12. Stations 8, 9, and 10 were 3-4 meters deep, and Station 12 was about 0.75 meters deep. The remaining stations were 1.3-2 meters deep.



APPENDIX G  
COMPUTATIONAL METHODS

LIST OF FIGURES

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G-1	PHENOGRAM OF PERIPHYTON, ARTIFICIAL SUBSTRATE, CULLED 1%, MUTUAL ABSENCE IMPORTANT, COPENETIC CORRELATION COEFFICIENT .871.	167

## COMPUTATIONAL METHODS\*

### Community Analysis

#### Introduction

Biotic components of water quality are generally quantified by one-dimensional diversity indices when single samples or stations are examined, or two-dimensional coefficients of biotic similarity when sample/sample, station/station, or species/species comparisons are undertaken.

Diversity indices are mathematical expressions that describe the distribution of individuals within the community. There are a number of diversity expressions in use. In general, maximum diversity exists if each individual belongs to a different species. An environmental parameter that influences community structure will also modify the diversity index. In cases where environmental stress may occur (such as competition among species, physiochemical limiting factors, or pollution), the community is reduced in the number of species present. Frequently, this reduction in the number of species is accompanied by an increase in the number of individuals of the remaining species, especially in the case of organic pollution. Environmental stress, therefore, tends to reduce the magnitude of diversity indices. One-dimensional diversity indices include the Shannon-Weaver Species Diversity, Evenness, and Simpson's Index of Dominance.

Coefficients of biotic similarity quantify the taxonomic overlap between two samples or stations. Most of these coefficients assume values between 0 and 1, where a value of 0 indicates no species overlap, and a value of 1 implies identical species composition. Morisita's Index of Faunal Affinity and the Pinkham-Pearson Index of Biotic Similarity are measures of biotic similarity.

In this study, data processing subsequent to manual taxonomic identification/confirmation was executed through the IBM 370/OS system at the Northeast Regional Data Center of the State University System of Florida (NERDC). Diversity indices and coefficients of similarity were calculated by proprietary FORTRAN IV routines. The phenograms were generated through application of the NT-SYS Numerical Taxonomy System developed by Rohlf, Kishpaugh and Kirk at Stony Brook (1974).

#### Shannon-Weaver Species Diversity Index (H)

The Shannon Weaver Species Diversity Index,  $\bar{H}_e$  (Odum, 1971) is defined as:

$$\bar{H}_e \equiv \sum_{i=1}^t \frac{n_i}{N} \ln \frac{N}{n_i}$$

\*Procedures outlined and examples cited were part of a study conducted for the U.S. Army Medical Research and Development Command.

where  $n_i$  = total number of organisms present as species  $i$

$$N = \sum_{i=1}^t n_i = \text{total number of organisms present in the sample}$$

$t$  - number of taxa present in the sample.

$\bar{H}_e$  ranges from a minimum of 0.0, occurring when all organisms belong to the same taxon (no diversity), to a maximum of  $\ln N$ , occurring where each organism present belongs to a unique taxon (maximum diversity).

The Shannon-Weaver Index is commonly expressed to other logarithmic bases, especially base 2 and base 10, and is easily converted by the following expression:

$$\bar{H}_{\text{base}_x} = \frac{\bar{H}_e}{\ln x}$$

#### Pinkham-Pearson Index of Biotic Similarity (B)

The previously discussed index ( $\bar{H}$ ) quantifies community structure with a sacrifice of taxonomic integrity important to paired comparisons between samples or stations. Such an index is incapable of distinguishing samples of similar gross community structure, but unlike taxonomic composition. That is, in computation, the  $i$ th species of one sample is not necessarily the same  $i$ th species of another sample.

This insensitivity to taxonomic overlap is surmounted by the Pinkham-Pearson Index of Biotic Similarity, B (Pinkham and Pearson, 1974) defined as:

$$B = \frac{1}{t} \sum_{i=1}^t \frac{\text{Min}(n_{iA}, n_{iB})}{\text{Max}(n_{iA}, n_{iB})}$$

where  $t$  = number of taxa considered

$n_{iA}$  = number of organisms of species  $i$  present at Station A

$n_{iB}$  = number of organisms of species  $i$  present at Station B

$\text{Min}(n_{iA}, n_{iB})$  = the minimum value of the pair:  $n_{iA}, n_{iB}$

$\text{Max}(n_{iA}, n_{iB})$  = the maximum value of the pair:  $n_{iA}, n_{iB}$

Biotic similarity is defined only for a paired comparison between two samples or stations. If two samples are characterized by identical taxonomic overlap (all species occur in identical abundance), the calculated index assumes a value of 1.0 (maximum similarity). Two samples possessing no species in common share an index of 0.0 (minimum or no similarity). The number of species considered,  $t$ , may include only those species observed in either or both of

the two samples, or, if mutual absence is deemed important, may include species not necessarily present in either sample. If mutual absence is considered important,  $\text{Min}(0,0) \equiv 1$  and  $\text{Max}(0,0) \equiv 1$  in the computation of biotic similarity.

A biotic similarity index,  $B'$ , between species may be defined on spatial and numerical occurrence by transposition of the axes in the preceding expression of station similarity:

$$B' \equiv \frac{1}{k} \sum_{j=1}^k \frac{\text{Min}(n_{j1}, n_{j2})}{\text{Max}(n_{j1}, n_{j2})}$$

where:  $k$  = number of samples or stations considered

$n_{j1}$  = number of organisms of species 1 at Station  $j$

$n_{j2}$  = number of organisms of species 2 at Station  $j$

$\text{Min}(n_{j1}, n_{j2})$  = the minimum value of the pair:  $n_{j1}, n_{j2}$

$\text{Max}(n_{j1}, n_{j2})$  = the maximum value of the pair:  $n_{j1}, n_{j2}$

This index likewise ranges from 0.0 (minimum similarity) to 1.0 (maximum similarity).  $B'$  may possess utility for grouping species according to environmental preference or pollution tolerance -- that is, it may delineate "indicator organisms".

### Phenograms

The quantification of similarity between paired stations, samples, or species by any of the previously-defined coefficients of similarity generates a diagonal matrix containing PC unique elements, where PC is calculated from the expression (Pinkham and Pearson, 1974):

$$PC = \frac{S(S-1)}{2}$$

where: PC = number of unique paired comparisons

$S$  = number of stations, samples, or species being compared.

For a study comprising only 25 stations, a similarity matrix of 300 unique elements is produced. Evaluation and presentation of such a voluminous matrix is impractical without computer-aided analysis and graphic models.

Algorithms for clustering similarity matrices into two-dimensional, hierarchic relationships have been developed by numerical taxonomists (Sokal and Sneath, 1963). A technique frequently invoked by ecologists and generally regarded as introducing the least distortion into similarity relationships is the sequential, agglomerative, hierarchic, nonoverlapping clustering method (SAHN) using unweighted pair-groups with arithmetic averaging (UPGMA), described by Sokal and Sneath (1963). The product of this procedure is a branched diagram termed a phenogram (or dendrogram), illustrated in Figure G-1.

Figure G-1.

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Use Figure 10

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This phenogram was generated by computer using the NT-SYS Numerical Taxonomy Package (Rohlf, Kishpaugh, and Kirk, 1974).

The horizontal scale or abscissa of the phenogram is graduated in the units of the similarity measure upon which the clustering was based -- in this case, the Pinkham-Pearson Biotic Similarity Index. Points of furcation (branching) between the horizontal stems, representing stations or groups of stations imply that the similarity between the two streams is at the coefficient value shown above the branch on the abscissa.

The magnitude of similarity between any two stations represented on the phenogram will, in general, differ from the corresponding magnitude given in the original similarity matrix. This arises as a consequence of the averaging necessary to recursively agglomerate the separate stations into a single, structured set containing all the stations.

The degree of distortion resulting from the cluster analysis may be quantified by the cophenetic correlation coefficient,  $r_{coph}$ , defined as the product moment correlation coefficient computed between the elements of the original similarity matrix and the corresponding indices implied by the phenogram (Sokal and Sneath, 1963). High values of  $r_{coph}$  ( $r_{coph} > 0.8$  for fewer than 10 stations) indicate that the distortion introduced by the clustering procedure and depicted by the phenogram has not significantly masked the informational content of the original similarity matrix.

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