A COMPARISON BETWEEN A POLLUTED AND A NON-POLLUTED STREAM IN PITT COUNTY, NORTH CAROLINA

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ABSTRACT

Owen Guilford Foster. A COMPARISON BETWEEN A POLLUTED AND A NON-POLLUTED STREAM IN PITT COUNTY, NORTH CAROLINA. (under the direction of Dr. Clifford B. Knight) Department of Biology, May 1972.

Physical, biological and chemical comparisons of a stream system receiving primary treated sewage effluent and a stream receiving only agricultural run-off were made by determining the relative numbers of pollution tolerant and pollution sensitive macroinvertebrates in each stream system and by using chemical and physical data, a total ecological picture was obtained.

Bartsch (1948) and Gaufin and Tarzwell (1952) confirmed the concept of stream ecosystems supporting only certain macroinvertebrate populations under certain conditions of chemical and physical environment.

Dredge samples, chemical and physical data were collected semi-monthly at several stations on each stream from January to June, 1971.

The data showed that the creek polluted by treated sewage was able to recover from this overload within a few miles of the effluent outflow. Agricultural run-off during the latter part of the study caused a noticeable increase in the chemical and biological factors linked with increased pollution levels. This increase was noted in both creeks but the creek lacking sewage effluent reflected the presence of increased run-off to a greater extent since this was the predominant pollution source in the stream system.

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DEDICATION

This thesis is dedicated to my wife Toni and daughter Betsy.

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INTRODUCTION

Stream systems are a valuable natural resource in Eastern North Carolina as they are in all parts of the world. The water is used for industrial, agricultural and recreational purposes. Overuse and misuse have placed stress on the delicately balanced stream ecosystem.

Stream pollution has concerned conservationists for the past century, but major technical advancements in water pollution analysis have been developed only within the past 25 years. The investigation of macroinvertebrate populations in relation to organic pollution is one of the primary innovations. Bartsch (1948) integrated chemical, physical and biological factors to provide a total analysis of a pollutant's effect on the ecosystem. He linked the chemical environment with the macroinvertebrates present; this concept was expanded by Gaufin and Tarzwell (1952). The correlation of pollution zones and invertebrate populations was accomplished by Wurtz (1955), Gaufin and Tarzwell (1955, 1956), Mackenthun and Ingram (1964) and Mackenthun (1966). This substantiated the use of benthic macroinvertebrates as dependable indicators of organic pollution. Sensitive or tolerant status is determined by body form and oxygen requirements (Bartsch and Ingram, 1959; Hawkes, 1963). The greater the degree of pollution tolerance the lower the need for dissolved oxygen and the smaller the appendages.

In this 6 month study physical, chemical and biological factors in a stream receiving sewage effluent and agricultural run-off were compared with a stream receiving only agricultural run-off. Grindle

Creek, a channelized permanent 28.1 mile stream, flows southeastward from Bethel to Grimesland where it enters the Tar River. It serves as the receiving stream for sewage effluent from the town of Bethel with a population of 1,534. The total drainage area is 78.1 square miles and the average flow between January and June, 1971, was 70.47 cubic feet per second. Water sources for the stream come primarily from rural run-off and secondarily from the Bethel Sanitation Lagoon which contributes 150,000 gallons of primary treated sewage per day.

Johnson's Mill Run, a channelized permanent 13.1 mile stream, emanates from the Grindle pocosin and flows southward to the Tar River where it enters approximately 1.5 miles west of Greenville. The total drainage area of this stream is 25.5 square miles with an average flow of 46.3 cubic feet per second.

REVIEW OF LITERATURE

Biological Analysis

Benthic macroinvertebrates are reliable indicators of organic pollution by virtue of their preferred habitat and their inability to move great distances by self-locomotion (Mackenthun, Ingram and Bartsch, 1966). A knowledge of the organism's life cycle is advantageous because it substantiates the seasonal variation in species number and population size (Gaufin and Tarzwell, 1955).

Bartsch (1948) analyzed the catalytic activity of micro- and macro-invertebrates on organic pollution and equated biological action with the chemical environment. The concept of a particular species establishing populations in certain aquatic zones was amplified by Gaufin and Tarzwell (1952). Rat tailed maggots (*Eristalis tenax*) indicated heavy pollution, sludgeworms (*Tubifex* sp.) are next in tolerance, followed by bloodworms (*Chironomus* sp.). Young (1970) categorized the gastropod, *Helisoma*, as a tolerant form also. The determination of sensitive and tolerant classes was based primarily on oxygen requirements. The rat tailed maggot (*E. tenax*) requires no dissolved oxygen but other tolerant forms require certain amounts of dissolved oxygen for survival. The total requirement for any tolerant form does not exceed one part per million (Bartsch and Ingram, 1959; Hawkes, 1963). Pollution sensitive forms require a higher dissolved oxygen concentration for survival. Mayflies (*Pentagenia* sp.), Caddisflies (*Leptocella* sp.), and Dragonflies (*Macromia* sp.) exemplify this group. Organic sludge can foul the filamentous gills and long appendages ultimately causing death (Bartsch and Ingram, 1959; Hawkes, 1963).

The correlation of the general pollution zones (Kolkwitz and Marsson, 1909) with indicator organisms (Gaufin and Tarzwell, 1952) was graphically shown by Wurtz (1955). The graphs showed statistically significant correlation between indicator organisms and the pollution zone schematic. Interpretation of stream conditions was derived from the relationship between the sensitive and tolerant organisms. Sensitive forms were found both above and a few miles below the sewage effluent outflow. The effluent greatly reduced the number of species present but resulted in an increase in pollution tolerant forms (Bartsch and Ingram, 1959; Wurtz, 1955).

Gaufin and Tarzwell (1955, 1956) correlated seasonal variation with indicator populations. They concluded that a species change was apparent but the ratio of sensitive to tolerant forms remained relatively constant. The septic zone contained 40% Diptera, 20% Coleoptera, 20% segmented worms, 10% Hemiptera, and 10% Mollusca throughout the study.

Keup (1966) found that a quantitative algal assay by chlorophyll extraction and spectrographic analysis provided accurate results. Decreased flow and increased light intensity during the warm weather causes the greatest eutrophication in a polluted stream (Anon, 1969). Artificial eutrophication was attributed to an increase and imbalance of phosphate-nitrate ratio as a result of agricultural run-off or sewage effluent (Owens and Wood, 1968; Fruh, 1968). An algal bloom depletes the oxygen content in a lagoon and receiving stream. When the large masses of algae die decomposition imposes an immense biological oxygen demand (B.O.D.) on both systems. Diurnal rhythms alter the photosynthetic rate causing a variation in the dissolved oxygen level because of the nightly cessation of oxygen production. Continuous addition of effluent high in B.O.D. and respiration of fauna and flora rapidly deplete the dissolved oxygen reserves (Bartsch and Ingram, 1959).

Collection procedures for the macroinvertebrates were discussed by Mackenthun (1966, 1969), Mackenthun and Ingram (1964) and Anon (1965). In addition, the authors detailed sample analyses, data presentation, and necessary equipment for this type of pollution study.

Pennak (1953), Usinger (1968), Chu (1949), and Pratt (1927) wrote keys to the invertebrates or the Insecta. The proper identification of macroinvertebrates is essential to the evaluation of water quality.

Statistical analysis provides an acceptable documentation of theorized relationships. Wilhm (1967) employed the coefficient of correlation between pollution sensitive and pollution tolerant organisms to establish the zones of pollution. Burlington (1962) applied a percent similarity index to correlate the stations sampled over a period of six months. Ingram and Bartsch (1960) illustrated research results graphically while Ingram, Mackenthun and Bartsch (1966) utilized both graphic and tabular forms of data expression. The proper methods of organization and presentation of the results in a water quality report were discussed by Mackenthun (1969).

Chemical and Physical Analyses

The measurements of chemical and physical factors in water analysis are standard scientific procedures. Standard Methods for the Examination of Water and Waste Water is a textbook for the total analysis of water and waste water. Golterman (1970) presents the principles as well as the methods for chemical analysis of fresh waters. The various methods of analysis for dissolved oxygen in natural and waste waters is presented by Mancy and Jaffe (1966). They concluded that the membrane electrode determination of dissolved oxygen was superior to all other methods with two provisions. First, the electrode must be calibrated chemically by the Winkler Test and secondly, the electrode, membrane, and electrolyte must be serviced as needed. Operational precision and accuracy will be maintained if the author's suggestions are followed.

Flow was the primary physical factor affecting benthic populations of macroinvertebrates (Austin and Engelbrecht, 1969). They stated that benthic nematodes subjected to sewage effluent were greatest in number when the flow was sluggish and lowest in concentrations in the main channel. Flood flows accompanied by high velocities scoured out appreciable quantities of the benthic community.

Moore (1968) presented water quality criteria which have been adopted by the state of North Carolina (Taylor, North Carolina Department of Water and Air Resources, personal conversation). A water encyclopedia by Todd (1968) contains water quality standards for most water systems. Anon (1961) provided total drainage area, stream length and flow data for Grindle Creek. The general physical characteristics for Johnson's Mill Run were provided by the American Soil Conservation Service (unpublished data).

Sewage Treatment

Hawkes (1963) studied the composition and function of bacterial beds. Algal growth supplies oxygen for the aerobic conversion of ammonia to nitrates and nitrites by nitrifying bacteria. These processes reduce the biological oxygen demand of sewage in the lagoon. If oxygen utilization is greater than reaeration resulting from surface atmospheric interaction and photosynthesis, dissolved oxygen becomes depleted. If the D.O. is insufficient to stimulate aerobic bacterial degradation, anaerobic degradation occurs yielding toxic products such as methane and hydrogen sulfide. Because of a time lag, the oxygen lag appears some distance below the site of effluent discharge. B.O.D. places a stress on the receiving stream that must be removed to replenish adequate dissolved oxygen levels. Klein (1962) stated that degradation of ammonia to nitrates and nitrites is the primary function of sewage treatment.

According to Taylor (personal conversation, 1971), the Bethel Sanitation Lagoon provides primary treatment for 150,000 gallons of raw sewage daily. The biological oxygen demand (B.O.D.) is 28.5 pounds per acre per day. Chemical analysis of the liquor at the inflow and outflow showed an 85% reduction in B.O.D. during the 60 to 120 day retention within the lagoon.

MATERIALS AND METHODS

Biological Analysis

Twelve stations were established on Grindle Creek and four on Johnson's Mill Run. Each station was located upstream of any structure in the water, preventing the sampling of an atypical environment. Twice each month four stations on Grindle Creek and two stations on Johnson's Mill Run were randomly selected for analysis.

Macroinvertebrate samples were collected with a 6" x 6" messenger type Ekman dredge. The samples were taken from three sites transecting the creek bed at each station. Site one was approximately one-half meter from the right bank, site two was located at mid-stream and site three was about one-half meter from the left bank. The dredge contents were emptied into a five gallon bucket, poured onto a 30 mesh sieve and concentrated by emersing the sieve in the stream and gently agitating the contents. The organisms, debris and gravel collected in the sieve were poured into an appropriately labeled plastic container for transportation to the laboratory.

A 12" x 8" x 2" enamel pan was used as a sorting chamber. The pan was partially filled with water and a trowel of sample was placed in the center. Forceps were used to separate the bottom sample and remove the larger organisms. The smaller macroinvertebrates were removed with a small pipette.

A monocular and a dissecting microscope were used to identify the genera in the samples. After identification and tabulation the organisms were stored in labeled glass vials containing 10% formalin. The number of organisms present in a 6" x 6" bottom sample was multiplied by four to estimate the number of organisms per square foot (Anon, 1965; Mackenthun, 1966, 1969a).

The quantitative algal assay was accomplished by chlorophyll extraction and spectrographic analysis. A 250 ml water sample was filtered in the field using a 50 ml B-D Plastipac syringe and a Gelman filtration unit. The fiberglass filter was removed, placed in a vial containing 10 ml of methanol and stored in an ice chest to reduce chlorophyll activity and decomposition. The samples were either assayed upon return to the laboratory or frozen for later analysis. The samples were boiled two minutes releasing the cell bound chlorophyll. A second filtration removed foreign material yielding a pure chlorophyll extract.

The sample extract was placed in a Bausch and Lomb Spectronic 20 at 423 mu to determine the percent absorbance. The instrument was calibrated with a methanol blank before analyzing each sample (Keup, 1966).

Chemical Analysis

Measurement of temperature, pH, dissolved oxygen and total dissolved solids were made in the field to decrease value changes between sampling and laboratory analysis. A Yellow Springs model 51 oxygen meter with thermister gave immediate dissolved oxygen and temperature readings. A modified Winkler test was used to calibrate the dissolved oxygen electrode (Mancy and Jaffe, 1966; Golterman, 1970).

To a 50 ml syringe filled with a water sample 1 ml manganese sulfate, 2 ml sodium azide, 2 ml sulfuric acid and 2 ml starch were added. The dark blue mixture was titrated to clearness with sodium thiosulfate. The milligrams (mg) of oxygen per liter was calculated by the following formula

$$\text{mg } O_2/L = \frac{(\text{ml of titrant})\ (8)\ (1000)\ (\text{normality of titrant})}{\text{ml of sample}}$$

The Beckman model RB-3 Solu-bridge provided an indication of the total dissolved solids by measuring the conductivity in umho/cm.

The remainder of the chemical tests were performed in the laboratory according to Golterman (1970). Opaque plastic sampling bottles (250 ml) were used to collect the water samples from each station. The filtered water was acquired during the process used for algae collection. The samples were cooled immediately and analyzed within 12 hours of collection. Each sample was refrigerated between tests to prevent contamination and degradation.

Total alkalinity was determined potentiametrically with .1 N HCL on a Fisher Accumet model 220 pH meter. The end point was found graphically and converted to milliequivalents (meq) per liter as follows.

$$\text{Total Alkalinity meq/l} = \frac{(\text{normality of HCL})\ (\text{ml of HCL})\ (1000)}{\text{ml of sample}}$$

Calcium and calcium plus magnesium were determined volumetrically with .01 molar sodium ethylene diamine tetra acetate (Na EDTA) as the titrant, sodium hydroxide and borate as the respective buffers and Calgon and Eriochrome Black T as the respective indicators. Calculations were as follows:

$$\frac{\text{mg } Ca^{++}/l}{\text{mg } Ca^{++}\ Mg^{++}/l} = \frac{(\text{molarity of titrant})\ (\text{ml of titrant})\ (1000)}{\text{ml of sample}}$$

Determination of nitrate-nitrite was accomplished with a Corning model 101 Digital Electrometer with a selective nitrate ion and calomel reference electrode. A series of nitrate standards were determined and graphed with millivolts (ordinate) against moles per liter (abscissa). The sample value was converted from millivolts to moles per liter graphically and milligrams per liter mathematically.

$$\text{mg } NO_3/l = \frac{(\text{moles per liter})\ (\text{molecular weight of } NO_3)}{1000}$$

Ortho-phosphate was analyzed colorimetrically at 720 mu with molybdate, ascorbic acid and antimony as reagents. The spectrographic assay employed the Bausch and Lomb Spectronic 20. The unknown was compared to a standard curve with optical density (ordinate) against milligrams of phosphate per liter (abscissa).

Physical Analysis

A portable, six foot, metal pole was used to establish a working site at each station. An extended cloth tape measure attached to the pole provided the width measure and a block with a six foot string attached determined the flow. The time used to travel six feet was converted first to feet per second and finally to cubic feet per second as follows (Needham, 1962).

$$\text{flow in feet/second} = \frac{6}{\text{time}}$$

$$\text{flow in cubic feet/second} = \frac{(\text{width})\ (\text{depth})\ (.9)\ (\text{feet covered})}{\text{time in seconds}}$$

A Secchi disc with calibrated chain provided both depth and turbidity readings.

A substrate analysis required 4, 12, 50, and 105 mesh sieves and a Blue M single wall transite oven model SW-11-TA. The bottom samples were gathered with a 6" x 6" messenger type Ekman dredge and placed directly into labeled metal soil cans. The cans were returned to the laboratory, opened and placed in an oven at $105^{o}C$ for three days. One hundred grams of each sample were weighed out using a Fisher triple beam balance tared to the contained weight (33.5 g). The substrate was poured into the sieve nest and agitated. The process separated the bottom sample into coarse and fine gravel, coarse and fine sand and a silt-clay mixture. The mass of sample retained by each screen was determined and divided by the total sample weight to provide the percent composition (Knight, 1965).

The percent similarity was acquired by using the I.B.M. model 360 computer. A single genus was compared for similarity with all other genera at all stations in both creeks. Data for all stations on Johnson's Mill Run were combined and compared to each station on Grindle Creek. Each station on Johnson's Mill Run was similarly compared to the combined control data providing an index of similarity (Burlington, 1962).

The basic statistical analyses were performed after Schefler (1969).

RESULTS

The data were analyzed for seasonal variation as well as for comprehensive creek variation. Graphic expression was chosen because of the simplicity of data comparison (Ingram and Bartsch, 1960), but the tabular expression is included in Appendix A.

Flow was a major physical factor in seasonal variation (Fig. 1). A marked increase occurred on the 54th and the 142nd days in both creeks due to rainfall. The flow rate generally decreased from a high on the 54th day to a low on the 174th day. Table VI, Appendix A, shows a negative correlation coefficient when stream flow is compared with pollution tolerant and sensitive forms in both creeks. This fact indicates that increased flow was detrimental to both forms but the sensitive organisms were more severely affected (Austin and Engelbrecht, 1968).

Grindle Creek and Johnson's Mill Run had similar patterns for total alkalinity and percent absorbance of chlorophyll throughout the six month study period (Fig. 2). Total alkalinity peaked at the 42nd and the 174th days. The sharp decline to the 54th day was possibly due to the increased flow rate. No data was collected between the 74th and the 142nd days due to a malfunction in instrumentation. The concentration of algae increased in both creeks during the study with the greatest increase appearing in Grindle Creek on the 174th day. The interaction of flow, total alkalinity, phosphate-nitrate and total dissolved solids were all contributing factors in the increase.

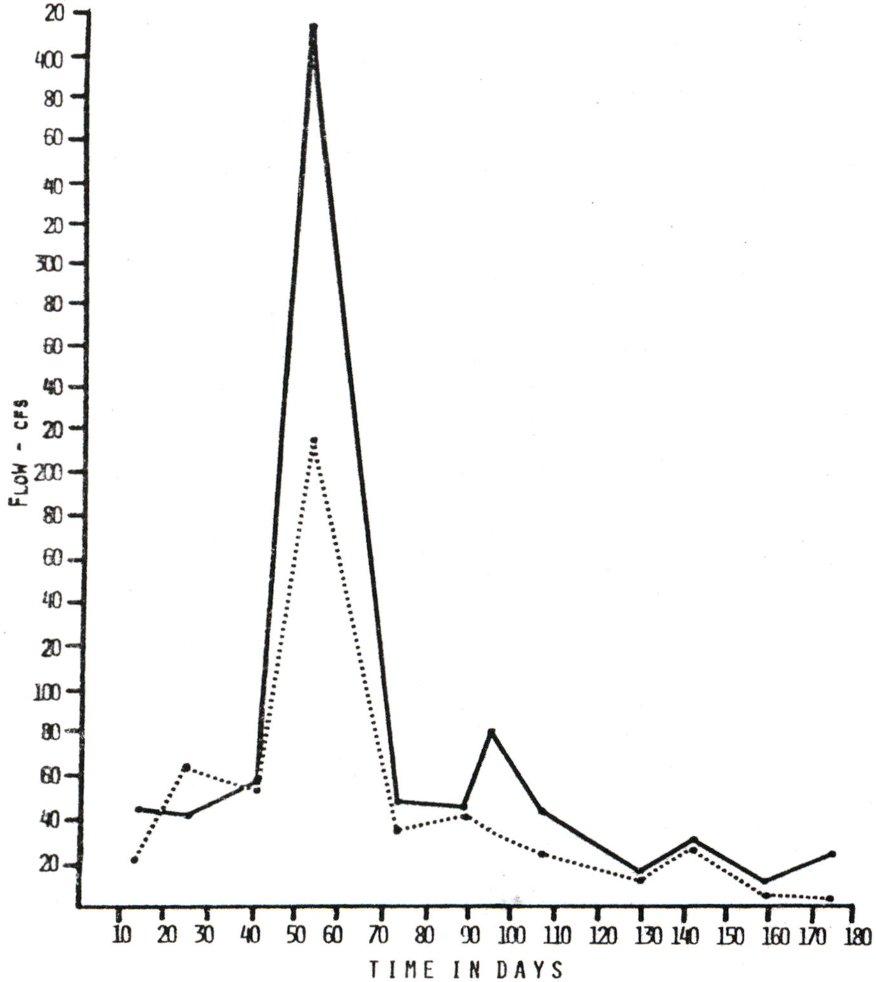

FIGURE 1. SEASONAL VARIATION IN FLOW. GRINDLE CREEK IS REPRESENTED BY THE SOLID LINE AND JOHNSON'S MILL RUN BY THE DOTTED LINE. THIS CODE IS MAINTAINED THROUGHOUT THE THESIS.

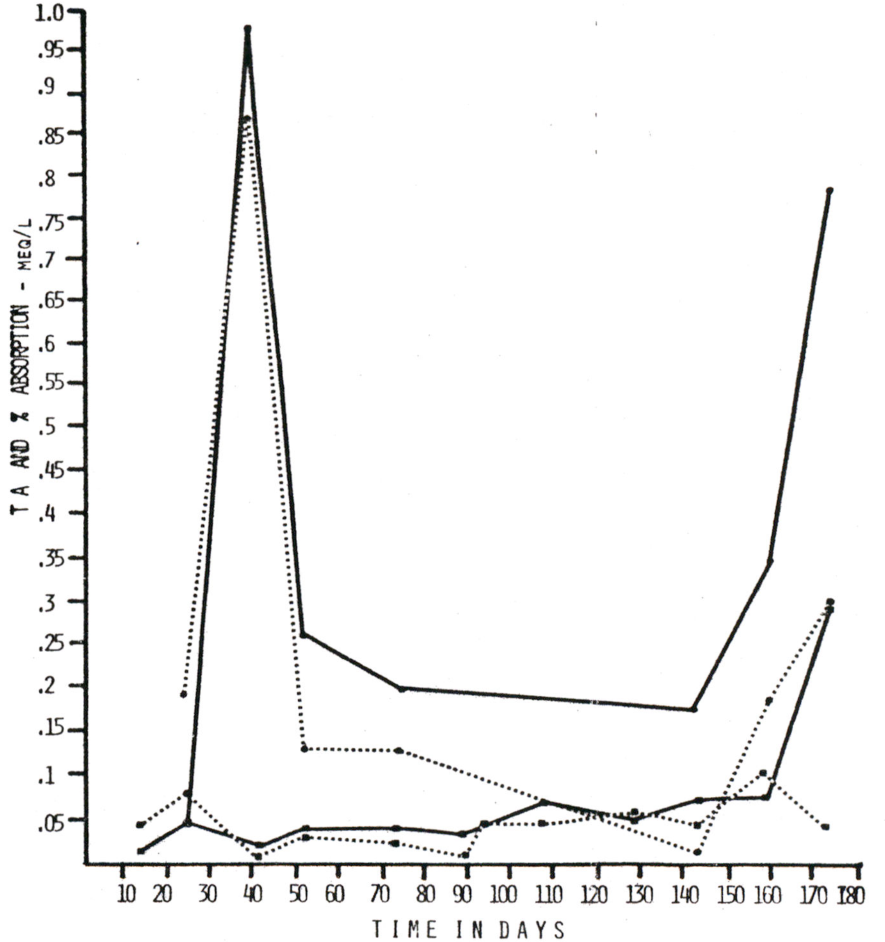

FIGURE 2. SEASONAL VARIATION OF TOTAL ALKALINITY (DOTS) AND PERCENT ABSORBANCE OF CHLOROPHYLL (SQUARES).

Dissolved oxygen and pH had no uniform pattern as seen in Figure 3. The dissolved oxygen was highest during the winter months with a marked decline as the temperature increased during the spring and summer; pH values were more stable, showing only minor fluctuations throughout the 174 day study period. The diurnal variation in dissolved oxygen and pH values was compensated for by sampling any one station at approximately the same time (Mackenthun, 1969). There was no significant correlation between these factors and the organisms present (Table VI, Appendix A).

Data collection for total dissolved solids (TDS) was expressed graphically from the 109th day because of an alteration in data collection. Both Grindle Creek and Johnson's Mill Run posted a net gain in TDS as seen in Figure 4. The coefficient of correlation between this factor and the organisms was +.77 and +.92 for tolerant organisms and +.12 and -.42 for sensitive forms in Grindle Creek and Johnson's Mill Run, respectively. High total dissolved solids benefited pollution tolerant forms while inhibiting the sensitive group.

Total hardness peaked at the 42nd and 109th days. A gradual decrease is evident from the 109th to the 174th day in both creeks. This factor had no significant effect on organism distribution.

Variations in phosphate and nitrate in Grindle Creek paralleled throughout the winter months but after the 90th day variations were in opposite directions (Fig. 5). The amount of nitrate increased as the phosphate levels decreased.

Johnson's Mill Run demonstrated no regular pattern for nitrate or phosphate values. Nitrate levels fluctuated continuously with a

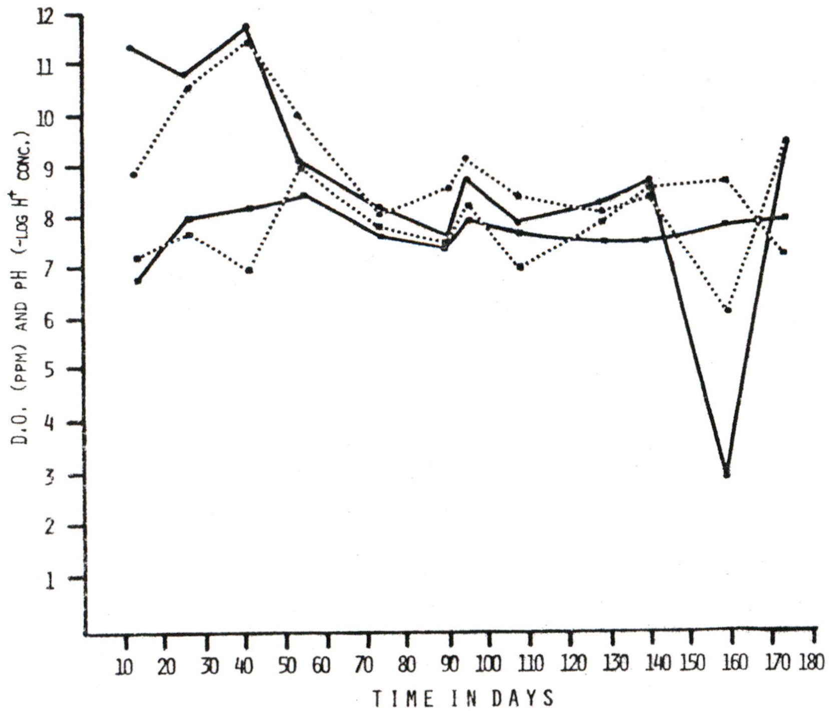

FIGURE 3. SEASONAL VARIATION OF DISSOLVED OXYGEN (DOTS) AND pH (SQUARES).

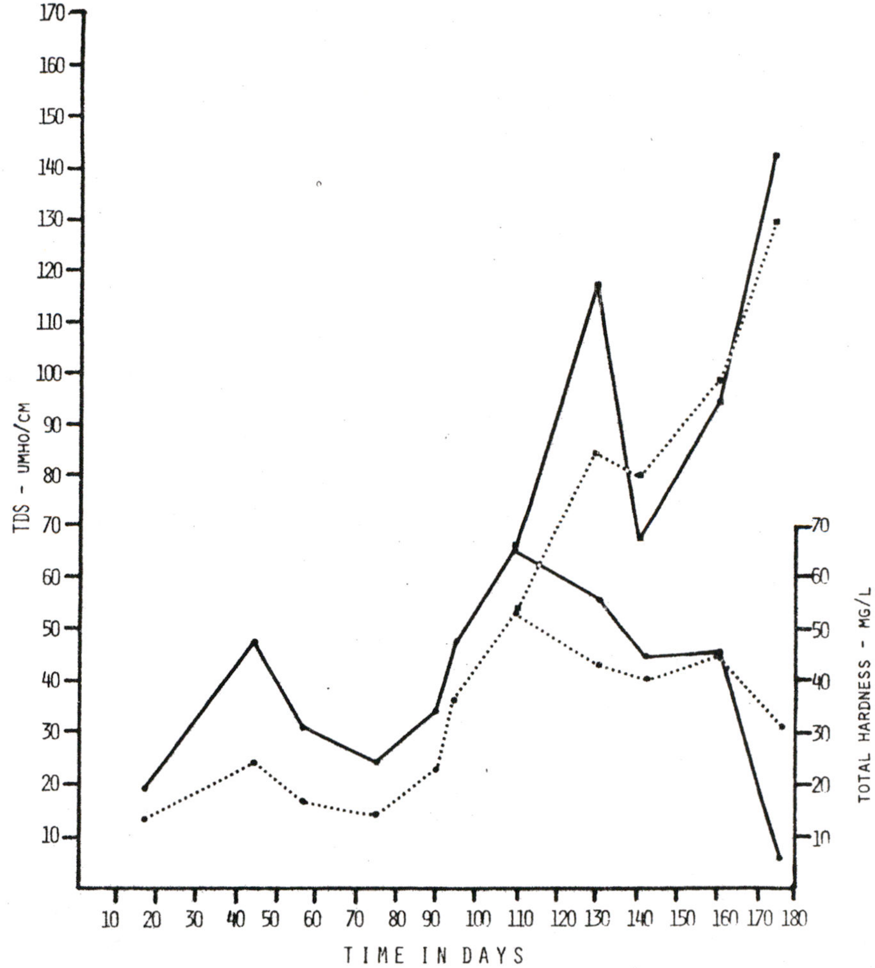

FIGURE 4. SEASONAL VARIATION OF TOTAL HARDNESS (DOTS) AND TOTAL DISSOLVED SOLIDS (SQUARES)

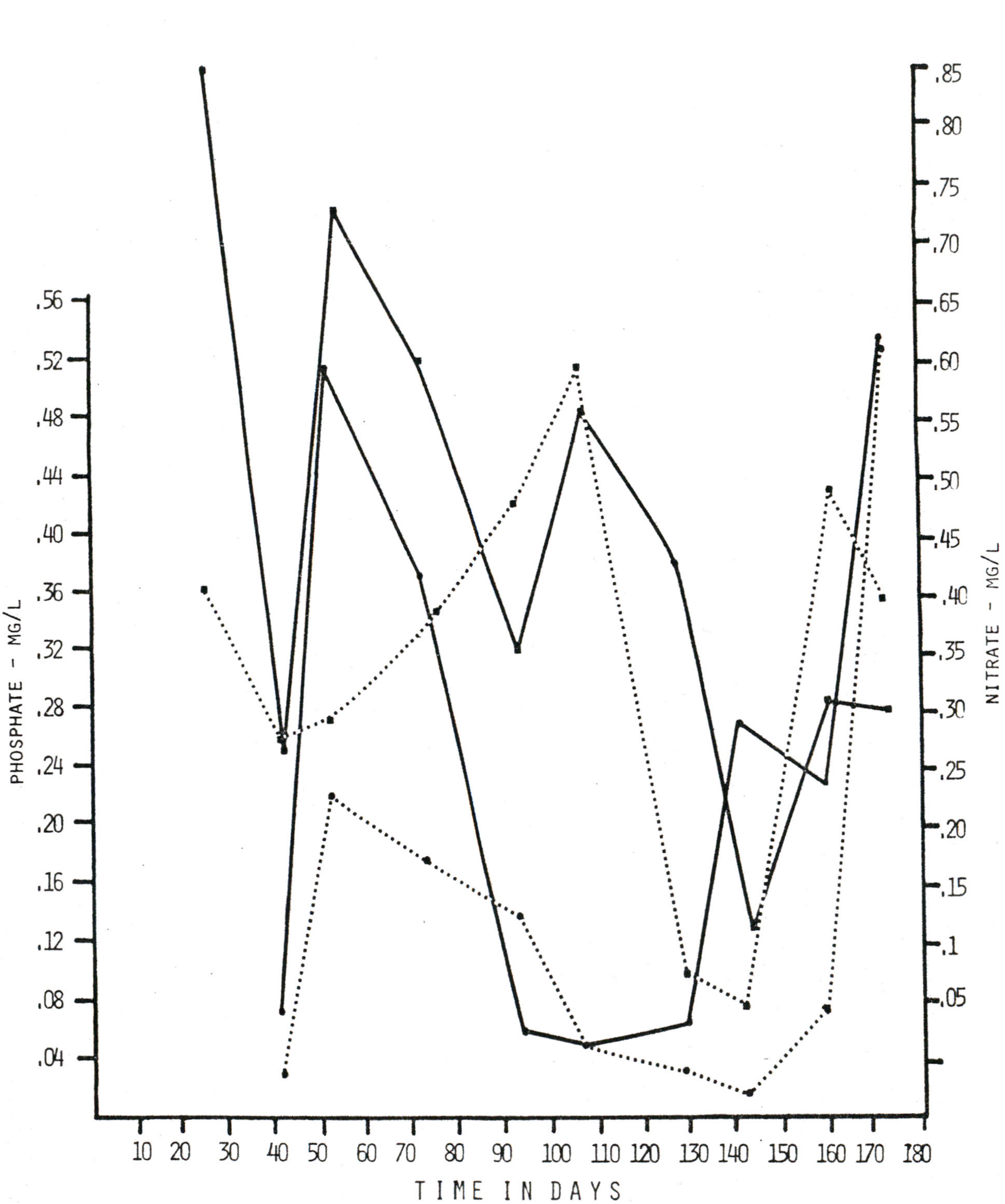

FIGURE 5. SEASONAL VARIATION OF PHOSPHATE (DOTS) AND NITRATE (SQUARES).

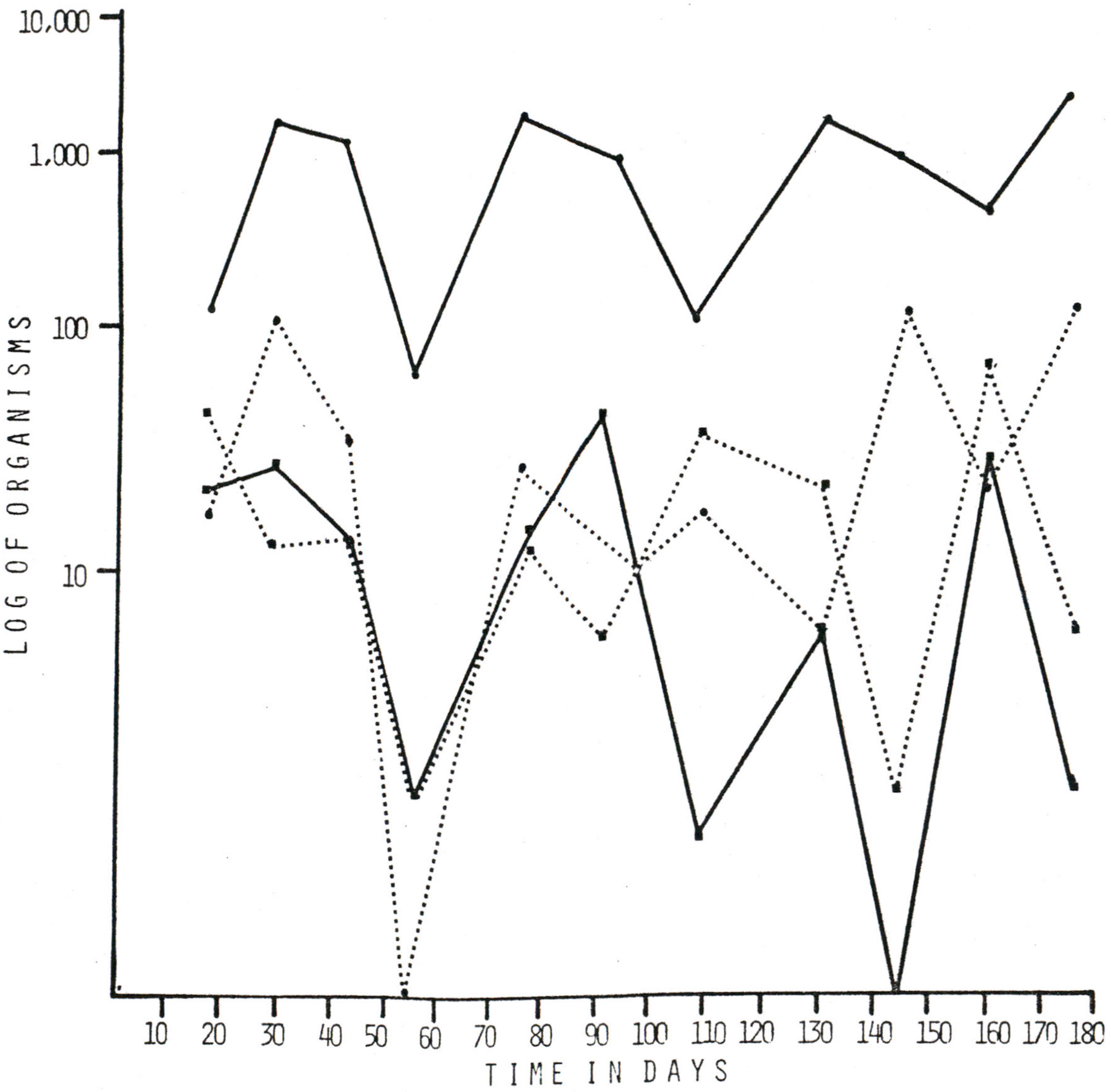

FIGURE 6. SEASONAL VARIATION OF POLLUTION TOLERANT (DOTS) AND POLLUTION SENSITIVE (SQUARES) MACROINVERTEBRATES.

maximum of .85 on the 27th day. Phosphate concentrations increased from the 142nd day to the termination of the study. The phosphate increase yielded a +.66 correlation with tolerant forms and a -.41 for sensitive organisms (Table VI, Appendix A). Agricultural run-off was considered to be a factor in this increase. Grindle Creek contained more pollution tolerant forms than Johnson's Mill Run, indicating that seasonal variation was not a limiting factor in organism distribution. There were definite fluctuations in both sensitive and tolerant forms possibly due to changes in flow, total dissolved solids and phosphate levels.

Station analysis of both creeks clearly illustrated the effects of sewage effluent on the stream ecosystem. There was a direct correlation between dissolved oxygen and pH in Grindle Creek (Fig. 7). Dissolved oxygen was lowest at stations 3, 5, 10, and 12 with peaks at the remaining stations. The pH coincides closely with the above pattern. There was an inverse correlation between dissolved oxygen and pH in Johnson's Mill Run. Dissolved oxygen was a limiting factor for sensitive forms in Grindle Creek only. An increase in D.O. had a +.93 correlation coefficient for sensitive forms and only a +.21 for tolerant macroinvertebrates (Table V, Appendix A). The correlation coefficient for these chemical factors in Johnson's Mill Run was not statistically significant (Table V, Appendix A).

Figure 8 shows that both phosphate and nitrate peaked at station 3 on Grindle Creek. There was a net decrease in both values as the effluent traveled down-stream. Johnson's Mill Run posted an increase

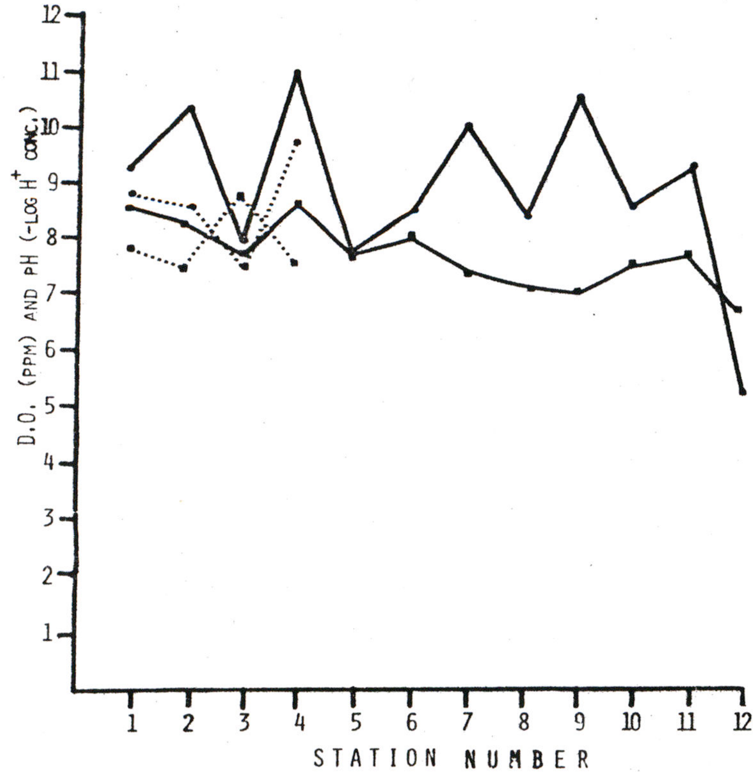

FIGURE 7. STATION ANALYSIS OF DISSOLVED OXYGEN (DOTS) AND pH (SQUARES).

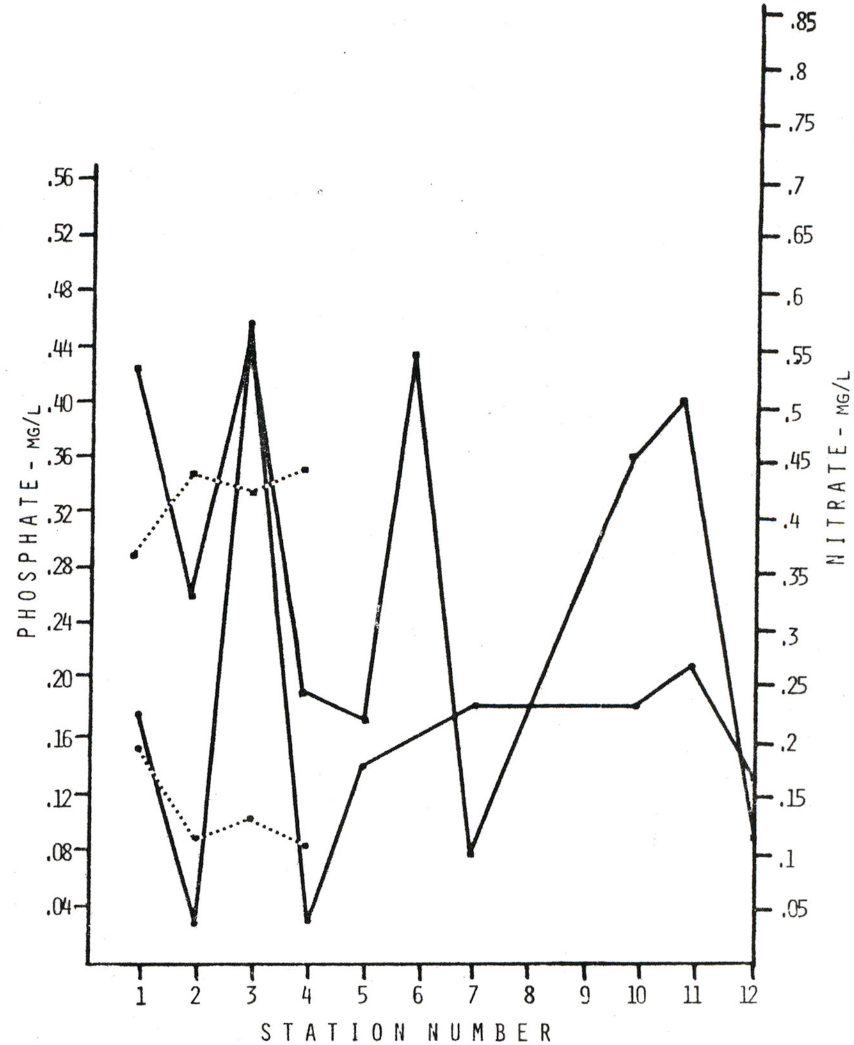

FIGURE 8. STATION ANALYSIS OF PHOSPHATES (DOTS) AND NITRATES (SQUARES).

in phosphate and a decrease in nitrate values. The probable cause was agricultural run-off. The coefficient of correlation for these two factors was not significant (Table V, Appendix A).

Total alkalinity and percent absorbance of chlorophyll both peaked at Grindle Creek station 2, the site of the sewage outflow. The factors do not reach this level at any other station which indicates peak pollution at this point. Johnson's Mill Run total alkalinity decreased during its flow to the Tar River but the percent absorbance increased to a peak of .07 at station 3 (Fig. 9).

The highest values for total dissolved solids were found at station 2 on Grindle Creek and station 1 on Johnson's Mill Run (Fig. 10). The concentrations in Grindle Creek sharply decreased at stations further from the outflow. Total hardness declined to station 2 and then gradually increased to station 6. Beyond this station a fluctuating decline then occurred to a low of 20.2 at station 9. The control stream (Johnson's Mill Run) had a decrease in the total dissolved solids and an increase in total hardness in all four stations. There was no significant coefficient of correlation between total hardness and organism distribution.

The relationship between the pollution tolerant and sensitive forms in both creeks is seen in Figure 11. Grindle Creek had greater numbers of pollution tolerant organisms at all the stations with a peak value of 1,057 at station 2. After a small increase at station 5 there was a marked decrease in tolerant forms coupled with an increase in sensitive organisms and this fact is important for the establishment of the pollution zones (Fig. 12).

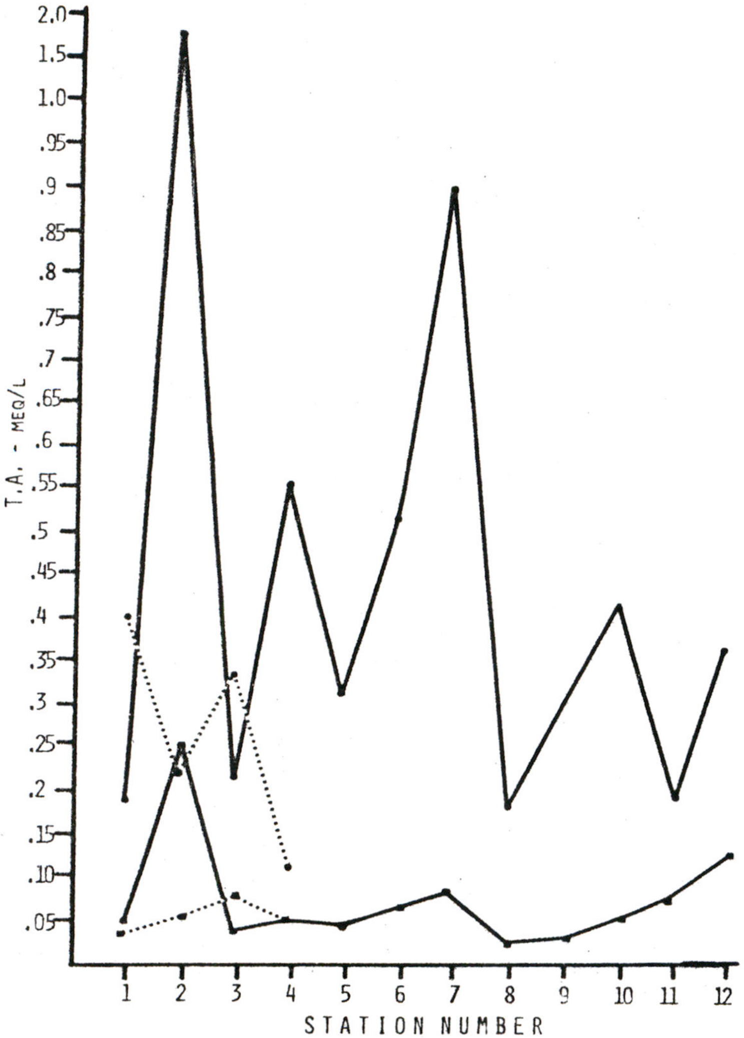

FIGURE 9. STATION ANALYSIS OF TOTAL ALKALINITY (DOTS) AND PERCENT ABSORBANCE OF CHLOROPHYLL (SQUARES).

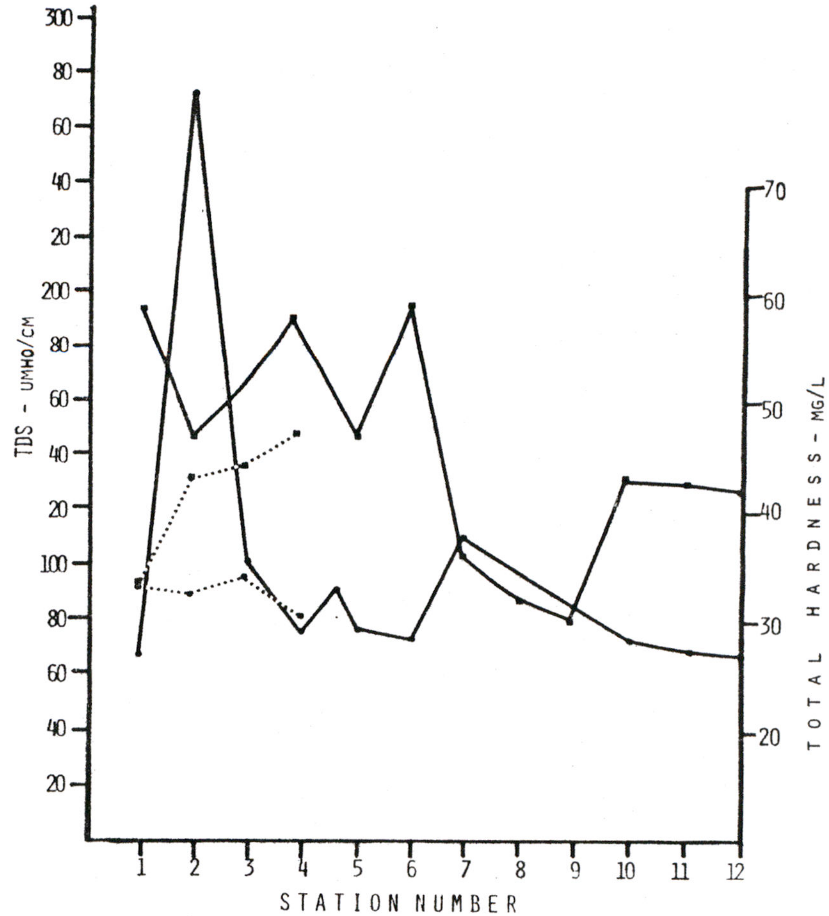

FIGURE 10. STATION ANALYSIS OF TOTAL DISSOLVED SOLIDS (DOTS) AND TOTAL HARDNESS (SQUARES).

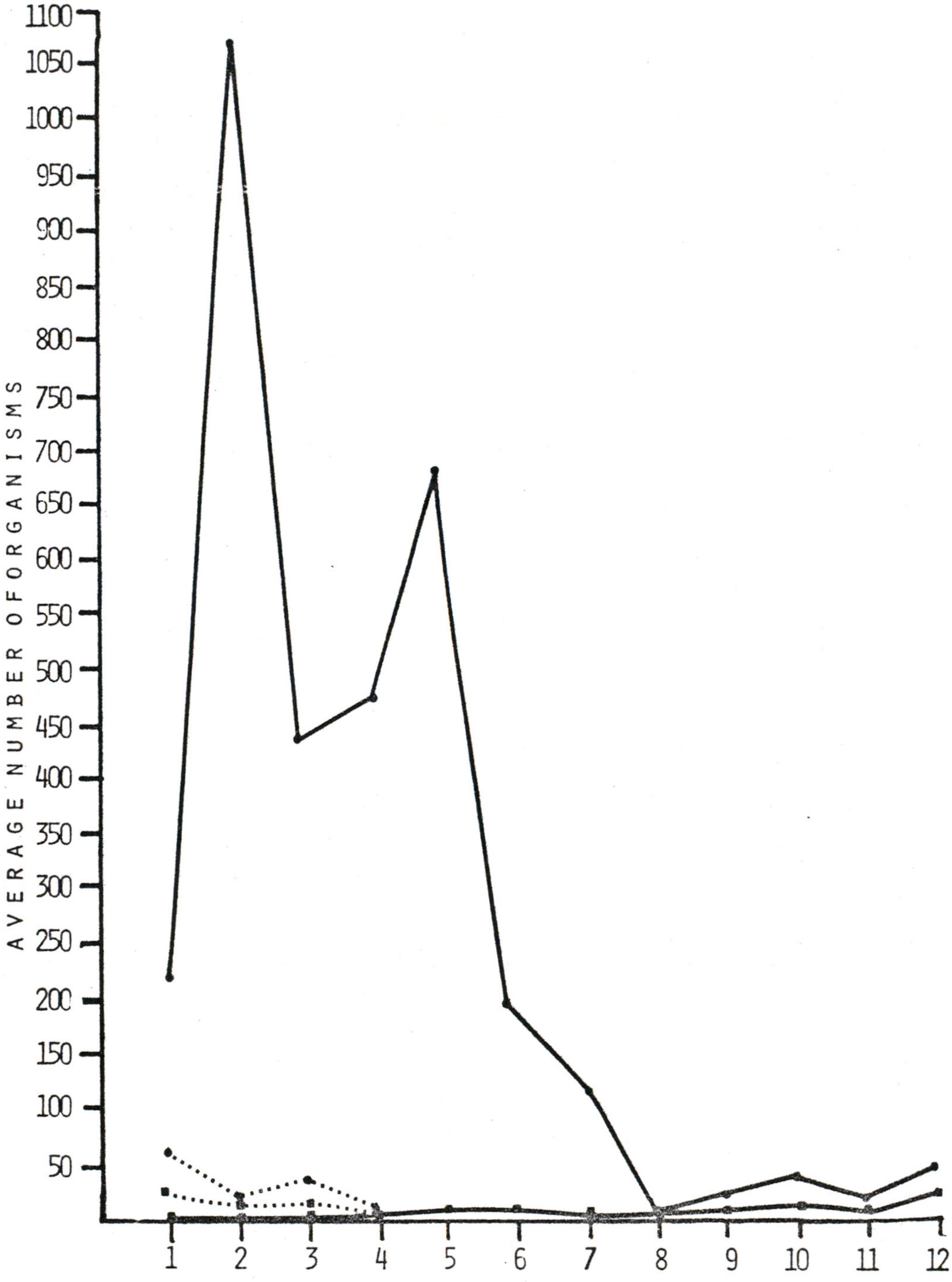

FIGURE 11. STATION ANALYSIS OF POLLUTION TOLERANT (DOTS) AND POLLUTION SENSITIVE (SQUARES) MACROINVERTEBRATES.

Johnson's Mill Run had more tolerant than sensitive forms but the peak value was only 56.6 at station 1 compared to the 1,057 in Grindle Creek. The difference between the numbers of sensitive and tolerant forms in Johnson's Mill Run was minimal when compared to that of Grindle Creek (Table XI, Appendix A). The percent similarity based on benthic macroinvertebrates between Grindle Creek stations 2 through 4 and all stations on Johnson's Mill Run is low. A greater similarity was apparent between Grindle Creek station 5 through 12 and the control stream. Johnson's Mill Run maintained an extremely consistent percent similarity between the 4 stations (Table VIII, Appendix A).

DISCUSSION

Comparison of the benthic macroinvertebrates of a polluted stream and a non-polluted stream was one of the main objectives of the study. Analysis of physical and chemical factors and their relationship to the distribution of the organisms further clarified the sewage effluent's effect on stream life.

There was definite seasonal variation in the macroinvertebrate populations. It is important to note that Grindle Creek always had a greater number of pollution tolerant forms and usually had less sensitive forms than Johnson's Mill Run. Pollution greatly increases the numbers of tolerant organisms and reduces the variety of species as well as the number of sensitive forms (Bartsch and Ingram, 1959; Wurtz, 1955). The presence of large numbers of the genera _Tubifex_ and _Chironomus_ were directly related to the degree of organic pollution at that site. Data in Table IX, Appendix A, illustrate this fact perfectly. An increase in the genera _Pentagenia_, _Leptocella_, _Enallagma_, _Progomphus_ and _Macromia_ were indicative of relatively pollution free water. Seasonal variation did not affect the overall pattern stated above since it occurred in both streams (Gaufin and Tarzwell, 1955; Bartsch, 1948).

The increased flow on day 54 caused a sharp decline in both invertebrate forms. The material carried into the stream system during this period could have further polluted the water causing the rise on day 74.

Total dissolved solids were an important consideration in organism distribution. The positive correlation of tolerant forms and negative correlation of sensitive groups verified this fact. Grindle Creek had higher total dissolved solid values than Johnson's Mill Run; effluent from the Bethel Sanitation Lagoon increased this value which is substantiated by Figure 10. Grindle Creek station 2 had the highest total dissolved solid value, which is directly related to the sewage outflow there.

A high positive correlation between phosphate and tolerant organisms in both creeks is evident. The seasonal comparison showed an increase in phosphate levels during the spring and summer months. Decreased flow and increased fertilizer in agricultural run-off are thought to be the causative agents. The effect of the increase is most evident in Johnson's Mill Run (Fig. 5) because agricultural run-off is the major source of pollution in the stream system.

Station analysis showed that Grindle Creek had peak phosphate levels at station 3. This was due to sewage effluent which enters the stream approximately 3/4 mile up-stream. Johnson's Mill Run had the highest values at station 1. A poultry farm up-stream from this station probably contributed the fertilizer elevating levels of phosphate and tolerant organisms.

Percent absorbance of chlorophyll also correlated directly with phosphate levels. The same factors mentioned previously are thought to be the cause. Grindle Creek had a peak percent absorbance, .25, at the sewage outflow. Chlorophyll absorption for Johnson's Mill Run deviated from this pattern by posting the peak value at station 3, .07., while the peak phosphate level was at station 1. Only two chemical factors peak at this station, total hardness and pH (Table 2, Appendix A). An algal bloom would be the result of a pH increase but would not be initiated by one (Owens and Wood, 1968). Total hardness might be the controlling factor but no literature confirmed this concept.

Acceptable levels of phosphate and nitrate are .1 ppm and 1 ppm respectively (Moore, 1968). Grindle Creek water exceeded the phosphate limit at all but two stations and the 1:10 phosphate-nitrate ratio was not found at any of the stations. This indicates that the sewage lagoon was not effectively reducing the raw sewage during treatment (Klein, 1962). Owens and Wood (1968) stated that artificial eutrophication was attributed to an imbalance in the phosphate-nitrate ratio as a result of sewage effluent or agricultural run-off.

Dissolved oxygen was designated as a major factor in the distribution of benthic macroinvertebrates by Mackenthun and Ingram (1964), Bartsch (1948), Gaufin and Tarzwell (1952, 1956) and Mackenthun (1966, 1969a). This study did not support the above statement. Gaufin and Tarzwell (1955) stated that during the winter, oxygen depletion was not a limiting factor in the distribution of aquatic invertebrates in Lytle Creek, Ohio. This fact explained the lack of correlation between dissolved oxygen and the distribution of benthic macroinvertebrates in this study during the winter months. In June the flow velocity decreased, total dissolved solids and phosphates increased, and the dissolved oxygen was beginning to decline. Both streams were entering the summer pattern as described previously by Mackenthun and Ingram (1964), Bartsch (1948), Gaufin and Tarzwell (1952, 1956) and Mackenthun (1966, 1969a). Dissolved oxygen began influencing the macroinvertebrate populations but the extent of this influence had not reached statistically significant levels at the termination of the study.

Hawkes (1963) stated that because of a time lag the oxygen sag appeared some distance below the effluent discharge. This statement explains the high dissolved oxygen value at station 2 and the sharp decline in oxygen levels by station 3. The dissolved oxygen graph for station study showed great fluctuation below station 3. The fluctuations could be due to periodic algal blooms up-stream that were not detected in the sampling. Oxygen levels were elevated by the bloom and further down-stream they were diminished due to the added biological oxygen demand from decomposing algae (Taylor, North Carolina Department of Water and Air Resources, personal conversation, 1971). No confirmation of this theory was found in the literature.

A primary purpose for the station analysis was to determine the pollution zones in Grindle Creek (Fig. 12). The indicators used to establish the zones were the relative numbers of pollution tolerant to pollution sensitive forms, the percent similarity, percent absorbance, total dissolved solid levels, phosphate levels and total alkalinity. The values for station 1 indicate that it was not a clean water zone by strict definition, but it matched the description of a recovery area (Gaufin and Tarzwell, 1952; Bartsch, 1948; Mackenthun and Ingram, 1964; Mackenthun, 1966, 1969a; Wurtz, 1955; Kolkwitz and Marsson, 1909).

Run-off from the town dump and a large farm possibly caused this small degree of pollution. The zone of degradation is classically established from the point of sewage outflow to a point a short distance down-stream. The absence of rat tailed maggots (E. tenax), indicator organism for this zone, indicated that the sewage treatment lagoon eliminated this zone. The zone of active decomposition is defined as an area where waste products are decomposed and those products that are not settled as sludge are assimilated by benthic macroinvertebrates in life processes (Mackenthun, 1969a). The sludgeworms (Tubifex sp.) and bloodworms (Chironomus sp.) are principle inhabitants of this zone (Bartsch, 1948). Data indicate that Grindle Creek station 2 to station 5 exemplify the active decomposition zone. The recovery zone is described as a stream section where water quality is gradually returned to conditions existing prior to the entrance of pollutants. This zone exists between station 5 and station 7 because the pollution tolerant forms are declining while the pollution sensitive organisms began to appear in dredge samples. At a point between station 7 and 8 biological, chemical and physical values of Grindle Creek approach those of the control stream (Johnson's Mill Run) initiating the clean water zone which continues to the Tar River (Mackenthun, 1969a).

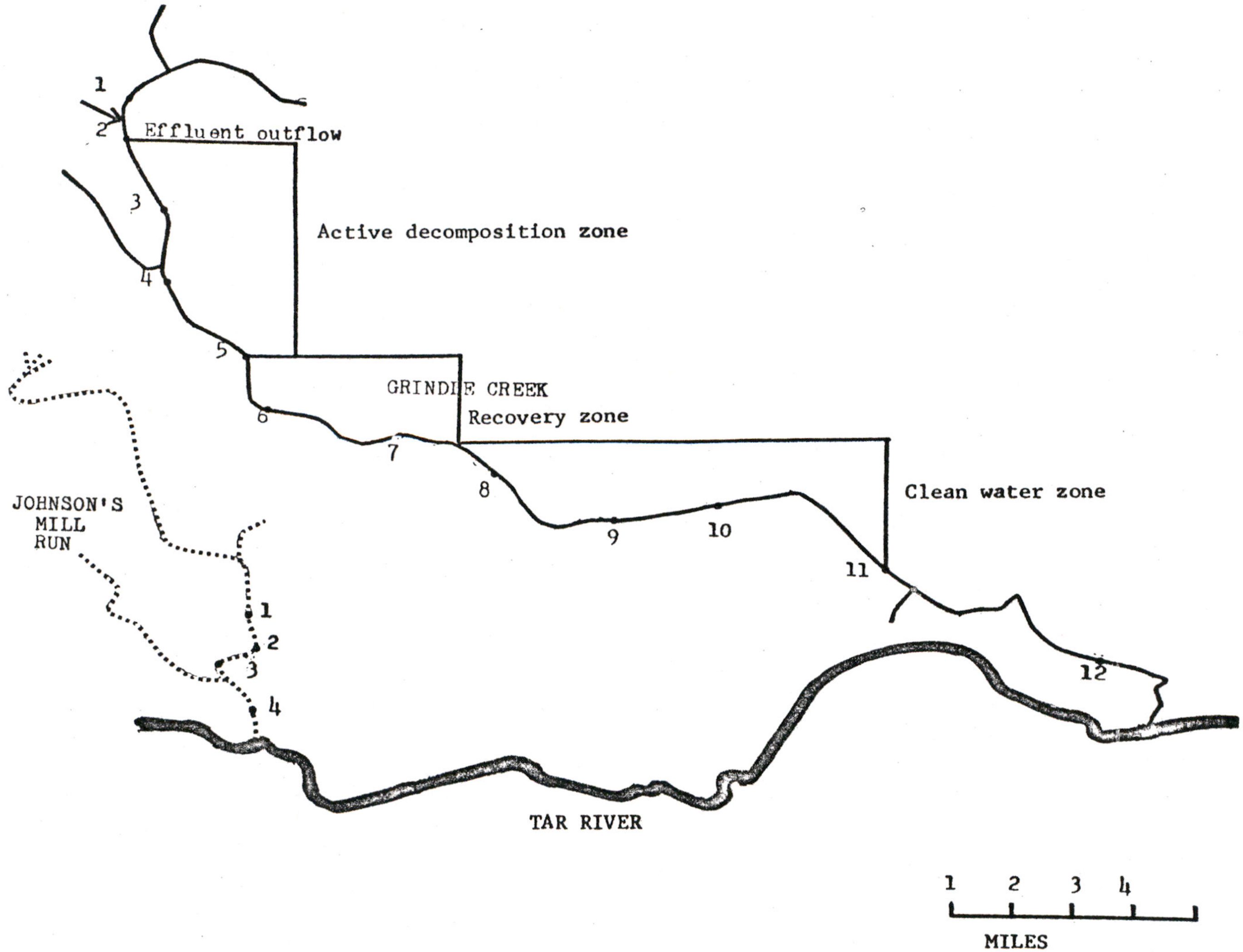

Figure 12. Composite map including pollution zones

SUMMARY

Benthic macroinvertebrate distributions and numbers provided an adequate method for pollution analysis in Grindle Creek and Johnson's Mill Run. The integration of physical, chemical and biological data allowed the investigator to establish pollution zones in Grindle Creek as well as determine the effects of agricultural run-off in both stream ecosystems. Grindle Creek was not considered to be heavily polluted for two reasons. First, a true septic zone was not apparent and secondly, the recovery zone was established within a relatively short distance of the effluent outflow. Sewage discharge from the Bethel Sanitation Lagoon was definitely detrimental to the stream ecology of Grindle Creek. Secondary sewage treatment should be instituted as soon as possible in order to return Grindle Creek to its original unpolluted condition.

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APPENDIX A

TABULAR DATA EXPRESSION

Table I. Chemical Characteristics of Stations on Grindle Creek

Station	TA_1	$Hard_2$	DO_3	pH_4	TDS_5	$Algae_6$	$NO3_7$	$Phos_8$
1	.19	45.88	9.12	8.32	67.60	.05	.53	.17
2	1.70	36.78	10.38	8.25	273.75	.25	.33	.03
3	.22	40.88	8.04	7.72	102.00	.04	.55	.45
4	.55	47.88	11.04	8.44	75.00	.05	.24	.03
5	.31	36.93	7.80	7.80	76.70	.05	.22	.14
6	.51	48.69	8.47	7.97	75.00	.07	.53	.16
7	.90	27.70	10.00	7.45	110.00	.09	.10	.18
8	.19	21.84	7.37	7.15	--	.03	--	--
9	--	20.20	10.60	7.00	--	.03	--	--
10	.41	33.40	8.68	7.55	72.50	.05	.45	.19
11	.19	33.70	9.28	7.62	70.00	.07	.50	.23
12	.36	32.74	5.80	6.80	67.60	.12	.12	.14

Table II. Chemical Characteristics of Stations on Johnson's Mill Run

Station	TA_1	$Hard_2$	DO_3	pH_4	TDS_5	$Algae_6$	$NO3_7$	$Phos_8$
1	.40	23.57	8.85	7.70	95.42	.04	.30	.15
2	.15	32.81	8.58	7.44	90.42	.05	.35	.10
3	.34	34.56	7.58	8.85	95.00	.07	.33	.11
4	.11	36.60	9.26	7.65	80.12	.05	.34	.10

1. TA - Total alkalinity measured in milliequivalents per liter.
2. Hard - Total hardness measured in milligrams per liter.
3. DO - Dissolved oxygen measured in parts per million.
4. pH - minus the log of the hydrogen ion concentration.
5. TDS - Total dissolved solids measured in umho per liter.
6. Algae - measured in percent absorbance at 423 mu.
7. NO_3 - Nitrate measured in milligrams per liter.
8. Phos - Phosphate measured in milligrams per liter.

Table III. Seasonal Chemical Characteristics for Grindle Creek

Days	DO_1	pH_2	$Algae_3$	$Flow_4$	TA_5	$Ca\text{-}Hard_6$	$Phos_7$	NO_{3_8}	TDS_9
13	8.83	6.73	.02	44.03	.00	18.5	.00	.00	.0
27	10.33	8.0	.05	42.42	.05	0.0	.00	.85	188.0
41	11.38	8.1	.02	57.95	.98	47.04	.07	.32	107.4
54	9.93	8.6	.04	409.93	.26	30.78	.51	.77	244.0
74	7.63	7.7	.04	47.74	.20	22.89	.37	.58	206.4
90	8.35	7.6	.035	43.00	.00	34.23	.00	.00	.0
95	8.65	8.0	.045	79.71	.00	47.60	.06	.35	.0
109	8.15	7.8	.06	41.97	.00	64.19	.05	.58	63.5
130	8.13	7.7	.05	19.46	.00	56.14	.05	.42	117.5
142	8.35	7.7	.07	26.46	.19	47.01	.27	.11	63.75
160	6.13	7.8	.07	10.87	.35	29.30	.23	.31	95.0
174	9.55	8.0	.32	22.44	.76	13.78	.53	.30	141.9

Table IV. Seasonal Chemical Characteristics for Johnson's Mill Run

Days	DO_1	pH_2	$Algae_3$	$Flow_4$	TA_5	$Ca\text{-}Hard_6$	$Phos_7$	NO_{3_8}	TDS_9
13	11.15	7.20	.045	23.58	.00	9.63	.00	.00	.00
27	10.75	7.85	.070	62.20	.20	.00	.00	.40	.40
41	11.60	7.00	.015	54.54	.82	21.70	.03	.27	145.00
54	9.00	9.05	.035	203.13	.14	16.20	.23	.34	209.50
74	7.75	7.80	.025	36.18	.12	14.00	.20	.36	55.50
90	7.50	7.80	.015	40.35	.00	23.80	.00	.00	.00
95	8.30	8.30	.045	35.63	.00	36.40	.13	.48	.00
109	8.00	7.00	.045	22.26	.00	51.80	.05	.59	52.50
130	8.75	8.00	.055	15.66	.00	42.74	.03	.09	77.50
142	8.60	8.40	.040	24.66	.01	39.93	.01	.05	90.25
160	3.45	8.80	.105	3.00	.18	46.06	.07	.49	98.10
174	9.50	6.75	.040	1.83	.25	28.00	.53	.40	128.10

1. DO - Dissolved oxygen measured in parts per million.
2. pH - minus the log of the hydrogen ion concentration.
3. Algae measured in percent absorbance at 423 um.
4. Flow measured in cubic feet per second.
5. TA - Total alkalinity measured in milliequivalents.
6. Ca-Hard--Calcium - Total hardness measured in milligrams per liter.
7. Phos - Phosphate measured in milligrams per liter.
8. NO_3 - Nitrate measured in milligrams per liter.
9. TDS - Total dissolved solids measured in umho per liter.

Table V. Coefficient of Correlation for Station Analysis

Creek	TDS*		PO_4*		NO_3*		DO*	
	S_1	T_2	S	T	S	T	S	T
JMR_3	-.61	+.51	+.27	-.25	+.27	-.30	-.09	-.25
GC_4	-.51	+.50	+.19	-.32	+.30	-.12	+.93	-.21

1. Sensitive organisms
2. Tolerant organisms
3. Johnson's Mill Run
4. Grindle Creek

*see footnote on previous page for units and definition of symbols.

Table VI. Coefficient of Correlation Seasonal Analysis

Creek	TDS*		PO_4*		NO_3*		DO*		Flow*	
	S_1	T_2	S	T	S	T	S	T	S	T
JMR_3	-.42	+.77	-.41	+.66	+.51	-.14	-.35	+.29	-.42	-.28
GC_4	+.12	+.92	-.07	+.17	+.52	-.02	-.26	+.19	-.74	-.41

1. Sensitive organisms
2. Tolerant organisms
3. Johnson's Mill Run
4. Grindle Creek

*see footnote on previous page for units and definition of symbols.

Table VII. Bottom Type Analysis for Grindle Creek

Soil	Station												
	1	2	3	4	5	6	7	8	9	10	11	12	Av
Gravel	12.4	20.0	8.8	4.8	7.5	9.0	25.7	8.2	15.1	9.8	4.6	59.0	15.4%
Sand	87.3	79.7	90.7	95.0	93.1	90.8	74.1	83.2	83.8	90.3	95.0	38.0	83.2%
Silt-Clay	.3	.3	.4	.3	.8	.2	.3	.1	.3	.2	.3	2.0	.46%

Table VII[a]. Bottom Type Analysis for Johnson's Mill Run

Soil	Station				
	1	2	3	4	Av
Gravel	6.9	4.2	7.0	16.2	8.57%
Sand	93.5	95.2	92.9	83.2	91.2%
Silt-Clay	.1	.4	.2	.5	.3%

Table VIII. Percent Similarity Between Grindle Creek and Johnson's Mill Run

	Station											
	1	2	3	4	5	6	7	8	9	10	11	12
% Similarity	37.49	32.77	34.60	34.05	42.91	40.24	39.4	19.4	35.0	38.37	49.99	20.24

Percent Similarity Between Each Station on Johnson's Mill Run

	Station			
	1	2	3	4
% Similarity	36.58	42.93	41.71	36.14

Table IX. Average number of individuals of each genus in Grindle Creek

	Station											
Genus	1	2	3	4	5	6	7	8	9	10	11	12
	25	125	97	49	96	42	13	6	2	8	5	3
Tubifex												
	10	254	11	34	94	17	35	0	1	12	2	0
Chironomus												
	12	6	14	34	3	3	0	2	3	0	0	1
Helisoma												
	0	0	3	7	0	0	0	1	0	0	0	5
Physa												
	0	0	0	2	0	2	0	1	0	1	0	0
Prostoma												
	0	0	0	0	0	0	0	0	0	1	0	0
Ophidonais												
	0	0	1	0	0	0	0	0	0	0	0	0
Dugesnia												
	0	0	0	0	2	0	1	1	1	2	0	0
Pentagenia												
	0	0	0	0	0	2	0	3	0	0	0	0
Progomphus												
	0	0	0	0	0	0	0	1	0	0	0	0
Macromia												
	0	0	0	0	0	0	0	1	0	0	0	0
Helobdella												
	0	0	0	0	0	0	0	0	0	1	0	0
Berosus												
	0	0	1	0	2	0	0	2	0	1	7	0
Leptocella												
	0	0	0	0	3	1	0	0	0	0	0	0
Sialis												
	1	0	0	0	0	0	0	0	0	0	0	0

Table X. Average number of individuals of each genus in Johnson's Mill Run

Genus	Station 1	2	3	4
Tubifex	58	11	30	8
Chironomus	55	15	3	9
Helisoma	1	0	0	0
Physa	5	0	1	0
Prostoma	4	0	4	0
Ophidonais	0	0	0	0
Pentagenia	3	1	1	1
Progomphus	0	1	0	0
Berosus	0	0	0	1
Leptocella	37	22	9	2
Sialis	2	0	0	0
Dineutus	1	0	0	0
Gammarus	0	0	0	1
Necturus	0	0	0	1
Enallagma	0	0	0	2
Syncaris	0	0	1	0

Table XI. Average Number of Organisms per Station on Grindle Creek

Organism	Station 1	2	3	4	5	6	7	8	9	10	11	12
Sensitive	2	0	2.4	2.67	6.4	4.0	2.0	14.67	4	5.1	5.6	0
Tolerant	226	1057	428.8	477.3	688.0	199.2	120.0	14.67	24	36.0	21.6	60

Average Number of Organisms per Station on Johnson's Mill Run

Organism	Station 1	2	3	4
Sensitive	28	17.7	14.0	5.7
Tolerant	56.6	19.2	35.0	10.86

Table XII. Pollution Tolerant and Sensitive Genera

Tolerant Forms	Sensitive Forms
Tubifex	Ophidonais
Chironomus	Pentagenia
Helisoma	Progomphus
Physa	Macromia
Prostoma	Berosus
Dugenia	Leptocella
Helobdella	Sialis
Syncaris	Prostoma
	Dineutus
	Gammarus
	Necturus
	Enallagma
	Strophitus