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A COMPARATIVE STUDY OF THE PHYSICAL AND CHEMICAL
PARAMETERS AND BENTHIC ALGAL COMMUNITIES
ABOVE AND BELOW THE SEWAGE TREATMENT
PLANT ON THE SOUTH FORK OF THE
NEW RIVER

A Thesis

by

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ABSTRACT

A COMPARATIVE STUDY OF THE PHYSICAL AND CHEMICAL PARAMETERS AND BENTHIC ALGAL COMMUNITIES ABOVE AND BELOW THE SEWAGE TREATMENT PLANT ON THE SOUTH FORK OF THE NEW RIVER. (DECEMBER 1985).

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Nutrient loading and its effect on the composition and structure of benthic algal communities were examined on the South Fork of the New River where this shallow mountain stream receives the effluent from the Boone Sewage Treatment Plant. Results of water analyses indicated that the river is receiving large quantities of inorganic nutrients in the form of ammonia nitrogen and orthophosphate. Overall the concentration of ammonia nitrogen was three and a half times greater at the downstream site, however at times, the downstream station showed an increase of as much as eleven times that found at the control station. Orthophosphate concentrations were generally ten times greater at the downstream station but at times PO_4

levels would be as much as 23 times greater than at the control site. Nitrate nitrogen concentrations showed the least difference between the two sites. Both temperature and dissolved oxygen concentrations fluctuated together throughout the study period with the dissolved oxygen concentrations being slightly higher upstream where temperatures were consistently lower.

The benthic algal community's composition and structure were consistently different between the two stations. Data from the upstream site showed community composition and structure characteristic of an unpolluted stream. The composition of the algal community at the control site included representatives from five separate divisions. The benthic algal community at the downstream station included organisms found from only three divisions of producers.

Standing crop biomass and species diversity showed differences between study sites and fluctuations seasonally. However, results showed that the benthic algal community at the downstream station experienced a decrease in species diversity along with an increase in biomass that corresponded to an increase in nutrient concentrations from the sewage effluent outfall.

ACKNOWLEDGEMENTS

I would like to express my great appreciation to the head of my advisory committee, Dr. Mary U. Connell, and to the other members of my advisory committee. I would also like to thank my other professors and fellow students for their support.



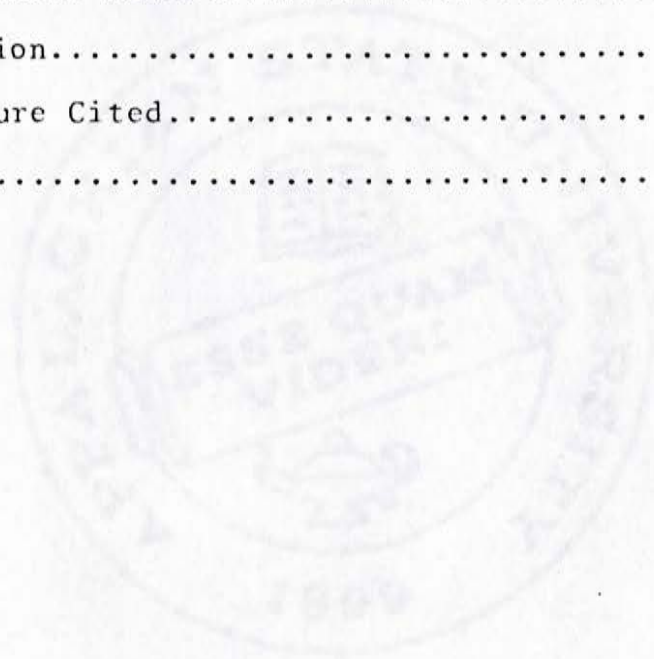
DEDICATION

This work is dedicated to my wife, Marsha H. Canterbury, whose love, understanding, and undying support made this work possible.



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INTRODUCTION

Rivers and streams are net consumers of both organic and inorganic matter. Natural enrichment of these systems is usually through the input of allochthonous material such as leaf litter, erosion of substrate, runoff from watersheds, decomposition of organic material, and the natural fixation of nitrogen by certain plants and bacteria. Additional nutrients may be introduced into a stream due to human activities which greatly adds to the nutrient load of the stream.

A major source of nutrient enrichment in rivers and streams is the outfall of effluent from sewage treatment plants (Reid, 1961; Martin and Goff, 1972). This effluent contains plant nutrient salts in the form of nitrogen and phosphorus. Drainage from agricultural areas and the use of synthetic detergents are other sources of these inorganic pollutants (Lee, 1973).

Phosphorus and nitrogen are the two most critical elements limiting plant growth in aquatic systems (Reid, 1961; Smith, 1974). These nutrients occur in

small quantities naturally, however excessive input by humans has resulted in the eutrophication of aquatic systems.

Phosphorus is perhaps the most important element in the eutrophication of aquatic systems. Nitrogen is fixed naturally by certain plants and bacteria, therefore it is present in larger quantities than phosphorus (Smith, 1974). Phosphorus, which is vital to the cell's energy transport systems, normally occurs in very small quantities. A deficiency of this nutrient leads to a reduction in the number of primary producers and increased productivity. A major source of phosphorus enrichment is the input from sewage effluent due to the use of synthetic detergents (Jenkins *et al*, 1973).

Nitrogen is found in three forms in aquatic ecosystems, nitrate nitrogen, ammonia nitrogen and nitrite nitrogen. Ammonia nitrogen and nitrate nitrogen are the two forms most important in stream eutrophication. Nitrate nitrogen is the form that is most efficiently used in protein synthesis by plants. This nutrient normally occurs in relatively small quantities in unpolluted fresh waters, however

levels increase significantly when introduced into aquatic systems through the input of organic or inorganic pollution.

Ammonia nitrogen is another form of nitrogen utilized by certain plants. This form of nitrogen is produced through the oxidative decomposition of plant and animal protein. Sewage treatment processes involve the biological degradation of organic wastes through the activity of bacteria. Since primary and secondary sewage treatment does not include the removal of inorganic nutrients, sewage effluent is a major cause of ammonia nitrogen enrichment (Goldman *et al*, 1973).

Another important indicator of the condition of an aquatic ecosystem is the amount of dissolved oxygen present in the water. The presence of dissolved oxygen in water is a result of diffusion across the air-water interface, turbulence, and photosynthesis. Several factors influence the concentration of dissolved oxygen with temperature and photosynthesis being the most important (Reid, 1961). Natural fluctuations in temperature and the duration and intensity of sunlight effect the natural concentration of dissolved oxygen in aquatic systems. Reid (1961)

states that the entry of an important sewage outfall tends to reduce concentrations of dissolved oxygen especially in areas which lack mixing. This would be most apparent if the sewage contained large quantities of organic materials.

Blum (1956) states that the flora downstream from a sewage outfall changes depending on the respective amounts of stream flow, quantity of effluent discharged and degree to which it has been decomposed. Water depth, bottom type and current speed are other important factors which must be considered (Tarzwell and Gaufin, 1953). A massive dose of sewage outfall into a stream results in the reduction or even total destruction of the normal biotic community along a portion of the stream's course (Butcher, 1940; Brinley, 1942a, 1942b, 1942c).

Hooper (1969) and Lack (1972) have attempted to correlate changes in nutrient levels with changes in community composition and structure. From a biological standpoint, the most significant effect of nutrient enrichment in a stream is a reduction in the number of producer species. This corresponds to an increase in productivity due to an explosion of certain specific populations which are better able to

utilize the high concentration of nutrients (Blum, 1956; Gaufin, 1958; Ingram, and Towne, 1959; Williams, 1964; Woodburn, 1972; Kann, 1982; Steinberg and Arzet, 1984). The longitudinal distribution of aquatic organisms can be directly linked to nutrient enrichment with various organisms becoming dominant as successively distant stretches of the stream become affected (Blum, 1953; Hynes, 1969; Potter *et al*, 1983).

The presence of pollution can be detected by the use of qualitative and quantitative biological methods (Arumugam and Furtado, 1982; Findlay, 1982). These methods can be used in conjunction with water quality data to evaluate the condition of a body of water (Dean and Burlington, 1963). Aquatic organisms have been recognized as a useful tool to evaluate the condition of the ecosystem (Patrick, 1950; Gaufin and Tarzwell, 1958; Grimes *et al*, 1984). If found growing in abundant numbers algae are trustworthy indicators of inorganic pollution (Wiebe, 1928; Fjerdingstad, 1950; Gunale and Chaugule, 1980). Lists and discussions pertaining to the species characteristic of the different zones in a polluted

stream are available in Wiebe, 1928; Kehr *et al.*, 1941; Butcher, 1947; Fjerdinstad, 1950; and Blum, 1953.

The use of both natural and artificial substrates have been employed by many researchers to analyze the communities in lotic and lentic habitats. Butcher (1947) studied the ecology of the Trent River in England by collecting algae on glass slides. Authur and Horning (1968) used masonite boards as a substrate to study pollution in the Minnesota and Mississippi Rivers where they found progressive increases in the periphyton crop below major sources of organic pollution. Hoagland (1983) collected diatoms on artificial substrate to measure the effects of wavelengths on standing crop and diversity. Methods for the use of artificial substrates to assess the effects of pollution on aquatic pollution are discussed by Dean and Burlington (1963), and Sladeckova (1962).

The purpose of this study was to examine nutrient loading and its effects on the composition and structure of the benthic algal communities in the South Fork of the New River, near the outfall of the Boone Sewage Treatment Plant. This area, near

the headwaters of the New River, is in the early stages of industrial growth and therefore in a unique position to study the effects of nutrient enrichment on stream communities in an area where the natural waters are relatively unpolluted.



MATERIALS AND METHODS

Nutrient loading and its effect on the composition and structure of benthic algal communities was examined on the South Fork of the New River near Boone, North Carolina. This shallow mountain stream receives the effluent from the Boone Sewage Treatment Plant, a secondary treatment plant located about two miles east of Boone. Nitrate nitrogen, ammonia nitrogen, and orthophosphate concentrations of this stream were determined above and below the effluent outfall. The composition and structure of the benthic algal communities were examined in order to assess the effect of nutrient loading on these producer organisms.

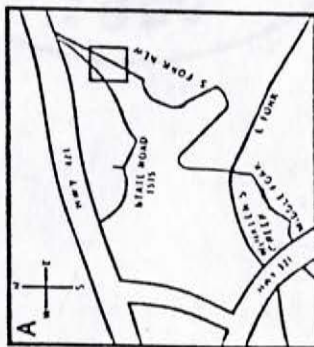
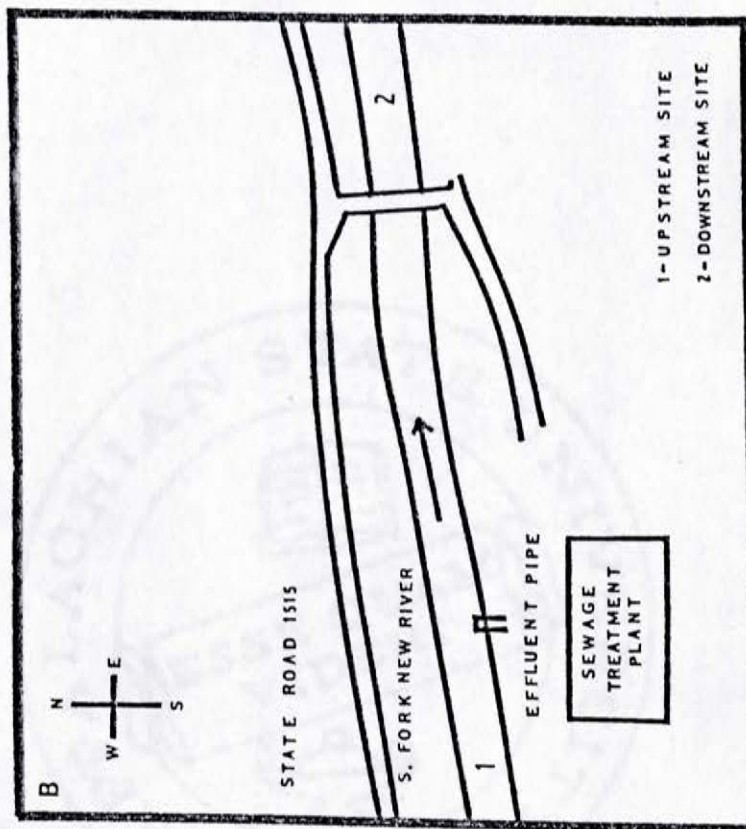
Field Collections

Water samples and benthic algae were collected bi-weekly from May 9, 1984 through November 10, 1984 from two sites on the South Fork of the New River near the treatment plant's outfall with the exception of August 11 when water levels were too high. Site 1 was located approximately 75 meters upstream from the effluent pipe while site 2 was placed 150 meters downstream from the effluent outfall (Fig. 1). Site



Figure 1. Map of Collection Stations

- (a) The location of the Boone Sewage Treatment Plant on the South Fork of the New River, Watauga County, North Carolina.
- (b) Expanded view of the insert showing the location of the study sites in relation to the sewage effluent pipe on the South Fork of the New River.



selections were determined from Standard Methods for the Examination of Water and Wastewater (*Standard Methods for the Examination of Water and Wastewater*, A. P. H. A., 16th edition, 1985).

Stream width, depth, and rate of flow were relatively equivalent at each site. Average depth at the upstream or control site was .38 meters with an average width of 12.3 meters. Average depth at the experimental site was .43 meters with an average width of 13.7 meters. The current was swift with an average rate of flow of .8 meters/second and a volume of 3.49 cubic meters/second (Table 1). The stream bottom consisted of small rocks and sand.

Water samples for chemical and dissolved oxygen analyses were collected in three separate 300 ml B.O.D. bottles at each site. Samples used in determining dissolved oxygen concentrations were chemically fixed in the field. Water temperature at each site was recorded using a standard celsius thermometer.

Algae samples were collected from each site to determine the composition and structure of the benthic algal communities. At each station, five building bricks were placed evenly across the stream,

TABLE 1

STREAM DESCRIPTION AND STUDY SITE CHARACTERISTICS

	Site 1	Site 2
Location	75 meters upstream from the effluent outfall	150 meters downstream from the effluent outfall
Depth		
\bar{x}	0.38 meters	0.43 meters
sd	0.09	0.04
Width	12.3 meters	13.7 meters
Flow rate		
\bar{x}	0.73 meters/second	
sd	0.02	
Volume	3.49 cubic meters/second	

perpendicular to stream flow. These provided an artificial substrate for the attachment of benthic organisms and an even surface for more accurate quantification of the algal community.

At each site algae were collected by scraping three 4 cm² areas of attached material from the smooth surface of three bricks chosen in a non-bias manner. The samples from each brick were washed into separate containers and filled to 50 ml with river water for a total of three 50 ml samples per site. Each brick was then thoroughly scraped to insure even growth for the next collection date.

Water and algae samples were returned directly to the laboratory. Chemical analyses were performed immediately upon return and algae samples were placed in the refrigerator for later identification and quantification.

Laboratory Procedures

Dissolved oxygen concentrations at each site were determined using the azide modification of the Winkler Method (*Standard Methods*, 1985). Sulfamic acid was added to each chemically fixed sample bottle and a 200 ml sample was titrated with PAO (Phenylarsine

Oxide standard solution). A 1 ml starch solution was added to help determine the end point (Strickland and Parson, 1968). Three separate samples were analyzed from both the control and experimental station.

Water samples from each site were analyzed to determine the concentrations of nitrate nitrogen, ammonia nitrogen, and orthophosphate. Concentrations were determined colorimetrically using Hach's reagents (Hach Chemical Company, Ames, Iowa) and a Bausch and Lomb Spectronic 20 spectrophotometer. Procedures were based on those in *Standard Methods for the Examination of Water and Wastewater*, (A. P. H. A.). All tests were done in triplicate for samples from each collection site.

Algae were identified using keys by Whitford and Schumacher (1969). Replicate counts of these organisms were made using an A & O (Series 150) binocular microscope equipped with a Whipple disc and 20X eyepieces. Four separate 1 ml aliquats from each algae sample were suspended on a Sedgewick rafter cell and the organisms counted in 25 random fields of view for a total of 100 counts per sample (*Standard Methods*, 1985).

Biomass was determined by removing a 25 ml aliquot from each algae sample and placing it in an analytically clean, ashed, tared crucible. All crucibles were placed in a Fisher drying oven at 100 degrees centigrade for 48 hours (Fisher Isotemp Oven, 100 Series, Model 106G). The samples were then weighed to obtain dry weight and then ashed for 1 hour at 600 degrees celsius in a Thermolyne 1500 Muffle Furnace. The crucibles were then cooled in an anhydrous calcium carbonate dessicator for 15 minutes and then reweighed to determine biomass. Weights were recorded to the nearest 0.00001 of a gram on a Mettler H₂O balance.

Species diversity was determined using the Shannon-Weaver Index of Species Diversity (Poole, 1974). All data were statistically compared using two-tailed t-tests and procedures from Sokal and Rohlf (1981).

RESULTS

Temperature, nutrient concentration, and benthic algal community composition and structure were consistently different in the South Fork of the New River above and below the Boone Sewage Treatment Plant's effluent outfall throughout the course of this study. From May 15, 1984, to November 10, 1984, the nutrient concentrations were higher at the downstream site. The benthic algal community composition and structure also differed between the two sites.

Physical-Chemical Results

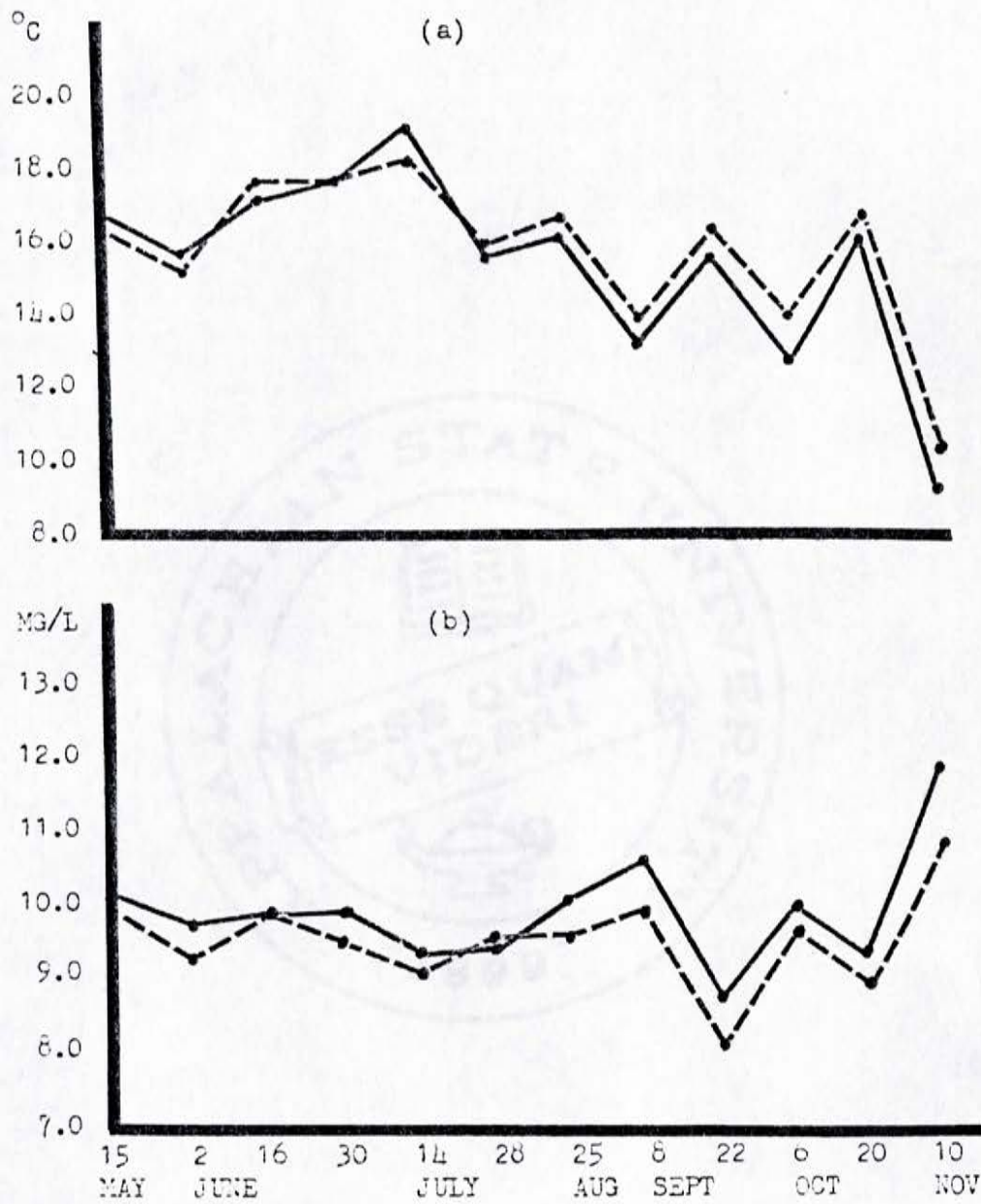
Water temperature showed a seasonal fluctuation at both stations throughout the sampling period. However, temperature differences of at least 0.5°C existed between the control and experimental sites except on June 30 and July 28 (Table 2). From May 15 throughout July 14 temperature was not consistently higher at either station; but, from July 28 to the end of the study period, the water temperature was always higher at the downstream site (Fig. 2). Highest temperatures at each station were recorded on July 14 with a reading of 19.0°C at the control site and 18.0 at the experimental site. After July 14, the

TABLE 2

WATER TEMPERATURE IN THE SOUTH FORK OF THE NEW RIVER,
UPSTREAM AND DOWNSTREAM FROM THE
BOONE SEWAGE TREATMENT PLANT

<u>DATE</u>	<u>UPSTREAM SITE</u>	<u>DOWNSTREAM SITE</u>
5/19	16.5°C	16.0°C
6/2	15.5	15.0
6/16	17.0	17.5
6/30	17.5	17.5
7/14	19.0	18.0
7/28	15.5	15.8
8/25	16.0	16.5
9/8	13.0	13.5
9/22	15.15	16.0
10/6	12.5	13.5
10/20	16.0	16.5
11/10	9.0	10.0

FIGURE 2. TEMPERATURE (a) AND DISSOLVED OXYGEN
CONCENTRATIONS (b) UPSTREAM (————))
AND DOWNSTREAM (-----) FROM THE
BOONE SEWAGE TREATMENT PLANT.



temperature steadily dropped at both stations until it reached its lowest value of 9.0°C upstream and 10.0°C downstream on November 10.

The concentration of dissolved oxygen fluctuated along with temperature throughout the study period (Fig. 2). Differences in dissolved oxygen concentrations between sites were apparent with the upstream site consistently showing slightly higher levels (Table 3). The concentration of dissolved oxygen reached its lowest level of 8.6mg/l at the upstream site and 8.0mg/l downstream on November 10. The highest concentration at the upstream site was recorded on September 22 at 11.8mg/l while downstream the highest concentration reached was 10.7mg/l on November 10. T-test comparisons for dissolved oxygen concentrations gave significant values for all collecting sites.

The concentration of nitrate nitrogen (NO_3), ammonia nitrogen (NH_4), and orthophosphate (PO_4), fluctuated seasonally throughout the study and was highest at the downstream station (Fig. 3). Of the three nutrients, nitrate nitrogen (NO_3), concentrations showed the lowest increase downstream (Table 4). Both NH_4 and PO_4 showed great differences in concentration between the two sites as well as over time.

TABLE 3

DISSOLVED OXYGEN CONCENTRATIONS (mg/l)
UPSTREAM AND DOWNSTREAM FROM THE
BOONE SEWAGE TREATMENT PLANT

Date	UPSTREAM SITE			DOWNSTREAM SITE			t	P
	\bar{x}	S^2	$S\bar{x}$	\bar{x}	S^2	$S\bar{x}$		
5/19	10.0	0.0000	0.0000	9.73	0.0033	0.3332	8.14	0.001
6/2	9.67	0.0033	0.0332	9.37	0.0033	0.0332	6.40	0.01
6/16	9.90	0.0100	0.0577	9.87	0.0033	0.0332	0.451	0.20*
6/30	9.87	0.0033	0.0332	9.53	0.0233	0.0881	3.611	0.05
7/14	9.30	0.0300	0.1000	9.00	0.0000	0.0000	3.000	0.05
7/28	9.47	0.0033	0.0332	9.50	0.0000	0.0000	0.905	0.20*
8/25	10.03	0.0033	0.0332	9.53	0.0033	0.0332	10.670	0.001
9/8	10.50	0.0000	0.0000	9.87	0.0133	0.0667	9.46	0.001
9/22	8.63	0.1033	0.1853	8.00	0.0000	0.0000	3.40	0.05
10/6	9.97	0.0033	0.0332	9.67	0.0033	0.0332	6.40	0.01
10/20	9.33	0.0133	0.0667	8.87	0.0133	0.0667	4.89	0.01
11/10	11.87	0.0233	0.0881	10.73	0.0133	0.0667	10.32	0.001

*Not Significantly Different

FIGURE 3. THE CONCENTRATION OF NITRATE NITROGEN (a), AMMONIA NITROGEN (b), AND ORTHOPHOSPHATE (c) UPSTREAM (————) AND DOWNSTREAM (-----) FROM THE BOONE SEWAGE TREATMENT PLANT.

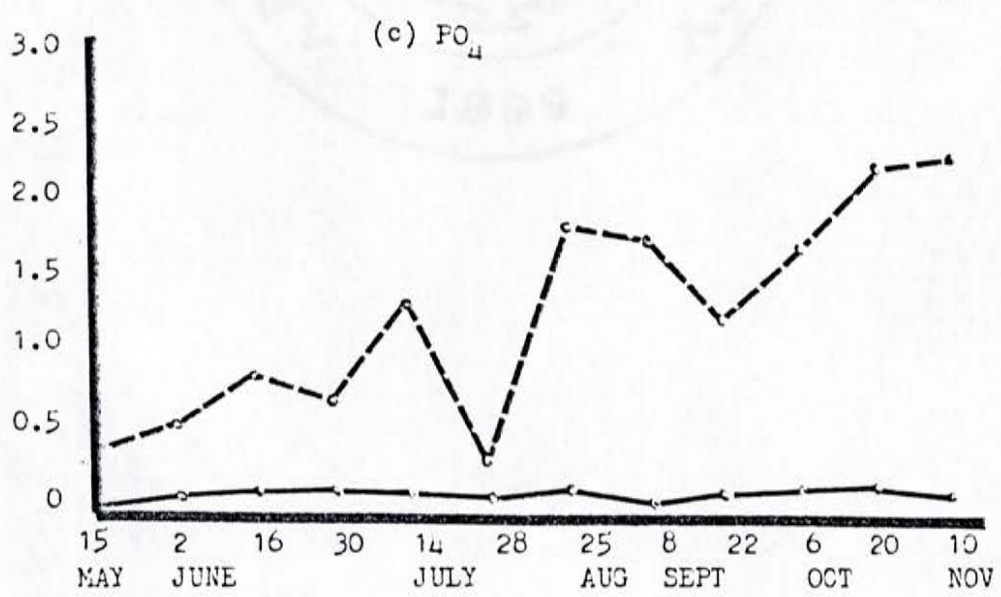
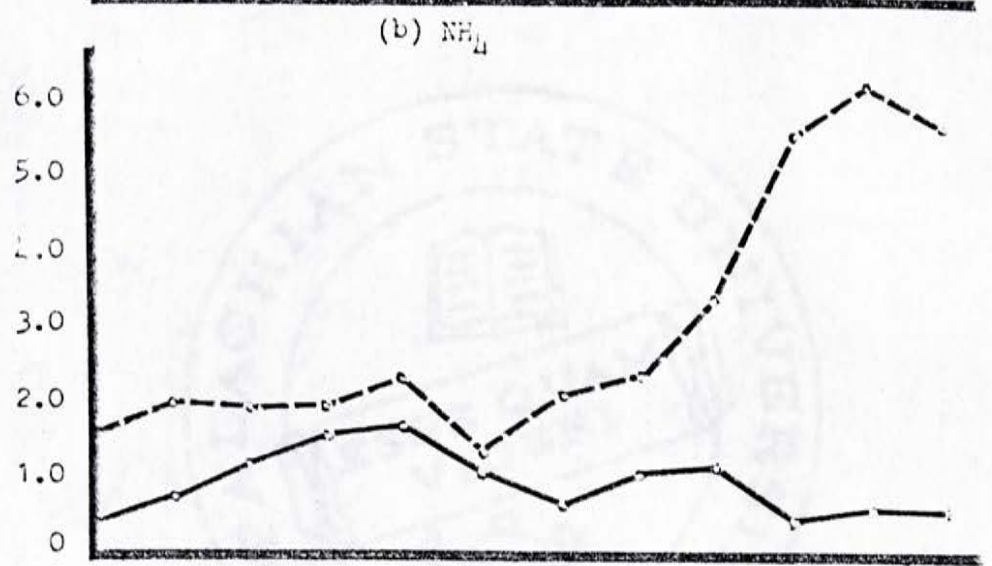
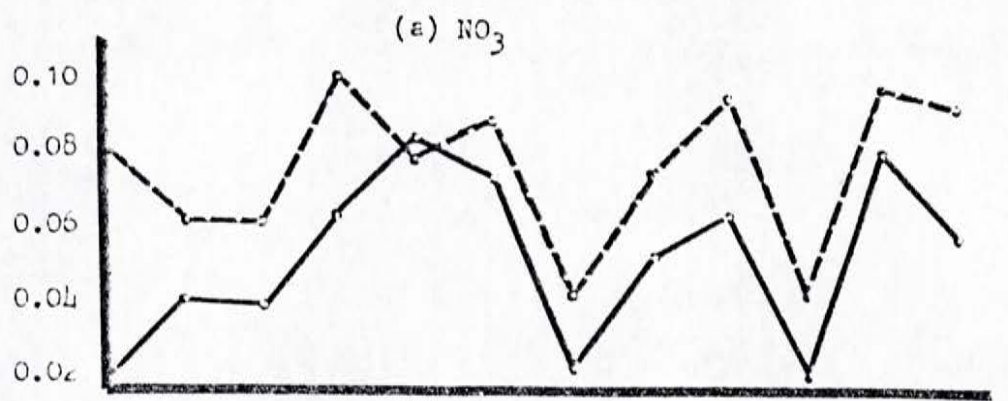


TABLE 4

CONCENTRATION OF NITRATE NITROGEN (mg/l)
UPSTREAM AND DOWNSTREAM FROM THE
BOONE SEWAGE TREATMENT PLANT

DATE	<u>UPSTREAM SITE</u>			<u>DOWNSTREAM SITE</u>			t	P
	\bar{x}	S^2	$S\bar{x}$	\bar{x}	S^2	$S\bar{x}$		
5/19	0.02	0.0003	0.0100	0.08	0.0000	0.0000	-6.00	0.01
6/2	0.04	0.00003	0.0030	0.06	0.00003	0.0030	-4.47	0.02
6/16	0.04	0.0001	0.0060	0.06	0.0002	0.0090	-3.49	0.05
6/30	0.07	0.00003	0.0030	0.10	0.0002	0.0090	-3.87	0.02
7/14	0.09	0.00003	0.0030	0.08	0.0000	0.0000	2.21	0.1*
7/28	0.08	0.00003	0.0030	0.09	0.0000	0.0000	-4.41	0.02
8/25	0.03	0.00003	0.0030	0.04	0.00003	0.0030	-3.58	0.05
9/8	0.05	0.00003	0.0030	0.08	0.00003	0.0030	-5.37	0.01
9/22	0.07	0.0004	0.0120	0.097	0.00003	0.0030	-2.42	0.1*
10/6	0.02	0.0000	0.0000	0.04	0.00003	0.0030	-7.27	0.01
10/20	0.08	0.00003	0.0030	0.10	0.0000	0.0000	-5.38	0.01
11/10	0.06	0.0000	0.0000	0.097	0.00003	0.0030	-11.70	0.001

*Not significantly different

While the concentration of these two nutrients was found to be higher at the downstream site at the beginning of this study period, both greatly increased at this station as the study progressed.

Nitrate nitrogen concentrations showed a seasonal fluctuation pattern that was similar at both stations even though the concentration of NO_3 was higher at the downstream site except on July 14 when the upstream site recorded slightly higher levels (Fig. 3a). The mean maximum concentration of NO_3 recorded at the control site was .09mg/l on July 14 with a minimum value of 0.02mg/l recorded at this site on May 19 (Table 4). At the downstream station, the level of NO_3 reached its highest concentration of 0.1mg/l on June 30 and its lowest of 0.04mg/l on both August 25 and October 26. T-test comparisons of NO_3 concentrations show the higher concentrations at the downstream site to be significant.

Ammonia nitrogen concentration showed some seasonal fluctuations at the control site from May 15 to November 10. A similar pattern developed at the experimental station from May 15 only until August 25 after which levels began to climb abruptly (Fig. 3b).

The lowest concentration of NH_4 , 0.04mg/l, was recorded at the control site on May 19 with the highest concentration, 1.8mg/l, reached on July 14. At the downstream station, the lowest concentration of 1.45mg/l was recorded on July 28 with a maximum of 6.5mg/l recorded on October 20 (Table 5). The overall mean concentration of NH_4 at the experimental site was 3.1mg/l. This was approximately three and a half times greater than that found at the control site. However, at times, the downstream station showed an increase of as much as eleven times that found at the control station on the same date. Comparison of the NH_4 levels at the two sites gave t-values that were highly significant.

Orthophosphate concentrations showed a pattern in that the differences in concentration of PO_4 between the two sites increased as the study period progressed. Although PO_4 levels showed a drastic increase over time at the experimental site, the concentration at the control site remained consistently low (Fig. 3c). The mean concentration of PO_4 was lowest at the upstream site on May 19 with a value of 0.09mg/l. The highest concentration

TABLE 5

CONCENTRATION OF AMMONIA NITROGEN (mg/l)
UPSTREAM AND DOWNSTREAM FROM THE
BOONE SEWAGE TREATMENT PLANT

DATE	UPSTREAM SITE			DOWNSTREAM SITE			t	P
	\bar{x}	S ²	S \bar{x}	\bar{x}	S ²	S \bar{x}		
5/19	0.55	0.0008	0.0166	1.72	0.0027	0.0300	-34.19	0.001
6/2	0.92	0.1185	0.1988	2.18	0.0272	0.0952	- 5.72	0.01
6/16	1.35	0.0210	0.0837	2.07	0.1865	0.2493	- 2.71	0.1*
6/30	1.66	0.0752	0.1583	2.06	0.0065	0.0467	- 2.42	0.1*
7/14	1.84	0.4003	0.3652	2.46	0.0027	0.0300	- 1.69	0.2*
7/28	1.17	0.1008	0.1833	1.45	0.3484	0.3408	- 0.73	0.2*
8/25	0.72	0.1209	0.2008	2.27	0.0520	0.1316	- 6.47	0.01
9/8	1.14	0.0033	0.0332	2.49	0.0027	0.0300	-30.25	0.001
9/22	1.28	0.0033	0.0332	3.57	0.0133	0.0666	-30.79	0.001
10/6	0.53	0.0021	0.0266	5.67	0.0133	0.0666	-71.67	0.001
10/20	0.65	0.0258	0.0927	6.47	0.0133	0.0665	-50.98	0.001
11/10	0.58	0.0462	0.1241	5.83	0.2133	0.2666	-17.85	0.001

* Not significantly different

upstream was recorded on October 20 at 1.7mg/l. The mean minimum concentration at the downstream site of 0.04mg/l occurred on May 19. The concentration climbed to its maximum of 2.4mg/l on November 10 (Table 6). The overall mean of 1.2mg/l downstream was ten times greater than that found upstream, 0.12mg/l. However, at times, levels of PO₄ were as much as twenty-three times higher downstream. T-test comparison of PO₄ data showed significant differences between the sites.

Biological Results

Twenty-eight genera of algae representing five separate divisions were identified from samples obtained from both sites during the study period. The division Chlorophycophyta (green algae) was represented by the highest number of genera (14) while diatoms of the division Chrysophycophyta were present in the greatest individual numbers. Representatives from the divisions Cyanochloronta (blue-green organisms), Pyrrophyphyta (dinoflagellates), Euglenophycophyta (Euglenoids), and other members of the Chrysophycophyta (golden-brown algae) were also identified.

TABLE 6

CONCENTRATION OF ORTHOPHOSPHATE (mg/l)
UPSTREAM AND DOWNSTREAM OF THE
BOONE SEWAGE TREATMENT PLANT

DATE	UPSTREAM SITE			DOWNSTREAM SITE			t	P
	\bar{x}	s^2	$S\bar{x}$	\bar{x}	s^2	$S\bar{x}$		
5/19	00.09	0.0000	0.0000	0.41	0.0005	0.0133	-24.30	0.001
6/2	0.10	0.0007	0.0153	0.54	0.0100	0.0577	- 7.30	0.01
6/16	0.12	0.0000	0.0000	0.89	0.0176	0.0766	-10.00	0.001
6/30	0.13	0.0003	0.0100	0.69	0.0033	0.0332	-16.26	0.001
7/14	0.15	0.0003	0.0100	1.35	0.0031	0.0321	-35.05	0.001
7/28	0.12	0.00003	0.0032	0.27	0.0003	0.0100	-14.59	0.001
8/25	0.12	0.0002	0.0082	1.81	0.0000	0.0000	-206.61	0.001
9/8	0.096	0.0001	0.0066	1.79	0.0008	0.0166	-94.83	0.001
9/22	0.13	0.0001	0.0066	1.24	0.0005	0.0133	-75.27	0.001
10/6	0.13	0.0001	0.0066	1.71	0.0000	0.0000	-239.51	0.001
10/20	0.17	0.00003	0.0032	2.26	0.0645	0.1466	-14.30	0.001
11/10	0.10	0.0001	0.0066	2.39	0.0016	0.0233	-94.29	0.001

Composition of the algae community at the control site included representatives from all five divisions mentioned above (Table 7). While the division Cyanochloronta was represented by two genera, *Oscillatoria* and *Anabaena*, *Oscillatoria* was by far the most numerous representative of this division. Fourteen genera belonging to the Chlorophycophyta were collected at the upstream station. Members of this division were consistently present in relatively few numbers during the study period with two genera, *Stigeoclonium* and *Ulothrix* being the dominant green algae at the upstream station. The division Chrysophycophyta was represented by ten genera at the control site. Two genera, *Cymbella* and *Navicula* were not only the dominant members of this division but the dominant organisms in the upstream flora. One genus of dinoflagellate, *Gymnodinium*, and one of Euglenophycophyta, *Euglena*, were also collected at the upstream station.

The benthic algae community downstream included only three divisions of producers: Cyanochloronta, Chlorophycophyta, and Chrysophycophyta (Table 8). The Cyanochloronta were again represented by the two genera *Oscillatoria* and *Anabaena* with *Oscillatoria*

TABLE 7

DIVISIONS AND GENERA OF ALGAE COLLECTED IN THE SOUTH FORK OF THE NEW RIVER,
UPSTREAM FROM THE BOONE SEWAGE TREATMENT PLANT

UPSTREAM SITE	5/19	6/2	6/16	6/30	7/14	7/28	8/25	9/8	9/22	10/6	10/20
<i>Cyanochloronta</i>	2	-	-	-	-	2	-	-	1	13	1
<i>Anabaena</i>	391	141	162	123	190	1951	940	41	82	123	28
<i>Oscillatoria</i>											80
Chlorophycophyta											
<i>Actinastrum</i>	-	4	-	-	-	-	-	-	-	-	-
<i>Ankistrodesmus</i>	6	-	-	-	-	-	-	-	-	-	-
<i>Chorella</i>	-	-	1	-	-	-	-	-	-	63	-
<i>Closterium</i>	-	2	3	2	-	-	1	3	3	22	1
<i>Cosmarium</i>	2	-	-	-	-	-	-	-	-	-	-
<i>Microcystium</i>	-	-	1	-	-	-	-	-	-	-	-
<i>Microspora</i>	5	-	2	-	-	-	-	-	-	-	-
<i>Scenedesmus</i>	-	1	3	8	1	6	-	-	-	1	-
<i>Spirogyra</i>	-	-	-	3	-	-	-	-	-	-	-
<i>Staurastrum</i>	-	-	2	7	7	2	5	-	-	5	18
<i>Stigeoclonium</i>	36	12	2	-	23	33	4	-	-	10	-
<i>Tetraedron</i>	-	-	-	-	-	-	2	-	-	-	-
<i>Pediastrum</i>	-	-	-	-	-	-	2	-	-	-	-

TABLE 7 (cont.)

	5/19	6/1	6/16	6/30	7/14	7/28	8/25	9/8	9/22	10/6	10/20	11/10
Chlorophycophyta	18	95	3	11	33	8	17	-	1	1	2	2
<i>Ulothrix</i>												
Chrysophycophyta												
<i>Asterionella</i>	11	33	-	-	-	-	-	-	-	-	-	-
<i>Cymbella</i>	766	182	2572	403	1539	2026	8545	6851	2575	1459	193	462
<i>Fragillaria</i>	4	-	-	4	-	2	-	-	-	-	-	-
<i>Gomphonema</i>	1	1	-	3	17	-	27	27	40	146	27	66
<i>Melosira</i>	3	-	64	62	31	61	39	52	76	88	62	47
<i>Meridon</i>	-	-	-	1	-	-	-	-	-	-	-	-
<i>Navicula</i>	5133	960	2688	987	985	870	2740	1288	1380	1975	426	1153
<i>Stephanodiscus</i>	-	7	-	-	-	-	-	-	-	-	-	-
<i>Syncdra</i>	1	-	-	-	11	46	3	5	7	22	23	-
<i>Tabellaria</i>	2	-	-	1	-	-	-	-	-	-	-	-
Dinoflagellates												
<i>Gymnodinium</i>	1	-	-	-	-	40	-	-	-	-	-	-
Euglenophycophyta												
<i>Euglena</i>	-	-	-	-	1	-	-	-	-	-	-	-
<u>SPECIES DIVERSITY</u>	0.707	1.119	0.889	1.131	1.229	1.226	0.919	0.546	0.882	1.200	1.247	1.092

TABLE 8 (cont.)

Chrysophycophyta	5/19	6/2	6/16	6/30	7/14	7/28	8/25	9/8	9/22	10/6	10/20	11/10
<i>Asterionella</i>	7	-	-	-	-	-	-	-	-	-	-	-
<i>Cymbella</i>	395	91	1565	473	40	183	2294	444	697	57	32	83
<i>Fragillaria</i>	2	-	3	-	-	1	-	-	-	-	-	-
<i>Gomphonema</i>	-	-	-	-	3	-	3	-	-	-	1	4
<i>Melosira</i>	-	-	16	5	1	10	1	-	6	-	5	10
<i>Meridon</i>	-	-	1	-	-	-	-	-	-	-	-	-
<i>Navicula</i>	3695	309	182	671	115	117	399	155	700	127	261	496
<i>Stephanodiscus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Synedra</i>	3	-	-	-	-	-	4	2	-	-	8	-
<i>Tabellaria</i>	2	8	-	-	-	-	-	-	-	-	-	-
Dinoflagellates												
<i>Gymnodinium</i>	-	-	-	-	-	-	-	-	-	-	-	-
Euglenophycophyta												
<i>Euglena</i>	-	-	-	-	-	-	-	-	-	-	-	-
SPECIES DIVERSITY	0.968	0.773	0.972	1.154	1.375	0.817	1.004	1.096	0.213	0.029	0.099	1.092

dominating. Only seven genera of green algae were collected at the downstream site with *Stigeoclonium* and *Chlorella* the dominant members. Nine genera of Chrysophycophyta were collected at this site. Again the diatoms, *Navicula* and *Cymbella*, were dominant but in fewer numbers than found upstream. No dinoflagellates or euglenoids were collected from the downstream flora.

Seasonal fluctuations of the dominant genera at each station were examined to compare differences in community composition and structure (Table 9). Of the green algae, *Stigeoclonium* shared co-dominance with *Ulothrix* at the control site from May 15 until August 25 when their numbers dwindled. At the downstream site, *Stigeoclonium* alone dominated the Chlorophycophyta and showed a similar seasonal pattern as that found in the green algae upstream. However, *Stigeoclonium* consistently occurred in higher numbers at the downstream site. *Ulothrix* disappeared totally from the downstream collections after June 16. Another green algae, *Chlorella* was found in small quantities at both the control and experimental sites from May 15 until September 22.

TABLE 9

COMPARISON OF THE DOMINANT ALGAL GENERA COLLECTED UPSTREAM AND DOWNSTREAM FROM THE BOONE SEWAGE TREATMENT PLANT

UPSTREAM SITE	5/19	6/2	6/16	6/30	7/14	7/28	8/25	9/8	9/22	10/6	10/20	11/10
Cyanochloronta	391	141	162	123	190	1951	940	41	82	123	28	80
<i>Oscillatoria</i>												
Chlorophycophyta												
<i>Chorella</i>	-	-	-	1	-	-	-	-	-	63	-	-
<i>Stigeoclonium</i>	36	12	2	-	23	33	4	-	-	10	-	-
<i>Ulothrix</i>	18	93	3	11	33	8	17	-	1	1	2	2
Chrysophycophyta												
<i>Cymbella</i>	766	182	2572	403	1539	2026	8545	6851	2575	1459	193	462
<i>Navicula</i>	5133	960	2688	987	985	870	2749	1288	1380	1985	426	1153
DOWNSTREAM SITE												
Cyanochloronta												
<i>Oscillatoria</i>	751	6	120	777	90	1241	1788	328	123	8	84	12
Chlorophycophyta												
<i>Chorella</i>	1	-	1	-	-	-	-	-	40124	50257	23894	269
<i>Stigeoclonium</i>	382	-	82	22	62	68	64	4	-	8	3	1
<i>Ulothrix</i>	2	5	39	-	-	-	-	-	-	-	-	-
Chrysophycophyta												
<i>Cymbella</i>	395	91	1565	473	40	183	2294	444	697	57	32	83
<i>Navicula</i>	3695	309	182	671	115	117	399	155	700	127	261	496

The *Chlorella* dominated the downstream flora and depressed the other populations in the community. The diatoms, *Cymbella* and *Navicula*, were by far the most significant members of the algae community throughout the study period at both stations. These diatoms dominated the upstream community from May 15 until September 22 at which time the *Chlorella* bloom occurred. The population of the dominant Cyanochloronta, *Oscillatoria*, fluctuated at both sites until the *Chlorella* bloom on September 22 when their abundance declined at the downstream station.

Standing crop biomass showed differences between study sites and fluctuations seasonally (Table 10). A relative increase in biomass occurred at both stations from May 15 until August 25. The control site consistently showed a higher biomass than that at the experimental station until September 9 when biomass at the control site decreased to a point below that found downstream. Biomass downstream continued to outweigh that found upstream until November 10 when they converged to relatively equal amounts. Patterns seen in biomass data from September 22 to November 10 corresponds with the increase in nutrient levels seen from August 25 to November 10 at the downstream

TABLE 10
 STANDING CROP BIOMASS OF THE BENTHIC ALGAL COMMUNITY UPSTREAM AND DOWNSTREAM
 FROM THE BOONE SEWAGE TREATMENT PLANT

DATE	UPSTREAM SITE		DOWNSTREAM SITE		t	P	
	\bar{x}	S ²	\bar{x}	S ²			
5/19	0.00342	3.71 x 10 ⁻⁷	3.52 x 10 ⁻⁴	2.74 x 10 ⁻⁷	3.02 x 10 ⁻⁴	4.16	0.02
6/2	0.00346	1.11 x 10 ⁻⁶	6.08 x 10 ⁻⁴	1.18 x 10 ⁻⁷	1.98 x 10 ⁻⁴	4.05	0.02
6/16	0.00797	1.21 x 10 ⁻⁵	2.01 x 10 ⁻³	3.79 x 10 ⁻⁶	1.12 x 10 ⁻³	0.89	0.20*
6/30	0.00707	2.49 x 10 ⁻⁵	2.88 x 10 ⁻³	5.08 x 10 ⁻⁷	4.12 x 10 ⁻⁴	1.45	0.20*
7/14	0.00838	1.49 x 10 ⁻⁵	2.23 x 10 ⁻³	1.36 x 10 ⁻⁶	6.73 x 10 ⁻⁴	2.36	0.10*
7/28	0.00774	4.47 x 10 ⁻⁶	1.22 x 10 ⁻³	5.99 x 10 ⁻⁷	5.47 x 10 ⁻⁴	1.15	0.20*
8/25	0.01080	6.65 x 10 ⁻⁷	4.71 x 10 ⁻⁴	4.51 x 10 ⁻⁷	3.88 x 10 ⁻⁴	8.95	0.001
9/8	0.00887	3.16 x 10 ⁻⁶	1.03 x 10 ⁻³	1.03 x 10 ⁻⁶	5.86 x 10 ⁻⁴	2.98	0.05
9/22	0.00123	1.24 x 10 ⁻⁶	6.43 x 10 ⁻⁴	1.63 x 10 ⁻⁹	2.33 x 10 ⁻⁵	5.33	0.01
10/6	0.00174	5.18 x 10 ⁻⁷	4.16 x 10 ⁻⁴	4.43 x 10 ⁻⁹	3.84 x 10 ⁻⁵	4.91	0.01
10/20	0.00562	4.31 x 10 ⁻⁷	3.79 x 10 ⁻⁴	1.41 x 10 ⁻⁶	6.86 x 10 ⁻⁴	2.58	0.10*
11/10	0.00410	3.22 x 10 ⁻⁷	3.28 x 10 ⁻⁴	1.41 x 10 ⁻⁷	2.17 x 10 ⁻⁴	0.15	0.20*

*Not significantly different

station (Fig. 4). T-test comparison showed differences in biomass at the two sites to be significant.

Species diversity showed no specific pattern from May 15 until September 8 as it fluctuated between sites and over time (Table 11). Species diversity reached its highest of 1.246 at the upstream site on October 20, while the downstream site fell to its lowest point of 0.0294 on the same date. The following collection date showed the species diversities converging back to similar values. The pattern seen in species diversity from September 22 until November 10 corresponds to the increases seen in nutrient levels from August 25 until November 10 (Fig. 5). This pattern also corresponds to the pattern seen in the biomass data during the same period is accompanied by a plunge in species diversity (Fig. 6). These changes in community composition also occurred during that part of the study period, August 25 to November 10, when temperatures were beginning to decrease. The convergence of the biomass and species diversity values for both sites on November 10 corresponded to the time when temperatures at both sites were their lowest.

FIGURE 4. A COMPARISON OF THE STANDING CROP BIOMASS (IN GRAMS) OF THE BENTHIC ALGAL COMMUNITY AND THE CONCENTRATIONS OF ORTHOPHOSHPATE AND AMMONIA NITROGEN UPSTREAM (————) AND DOWNSTREAM (----) FROM THE BOONE SEWAGE TREATMENT PLANT.

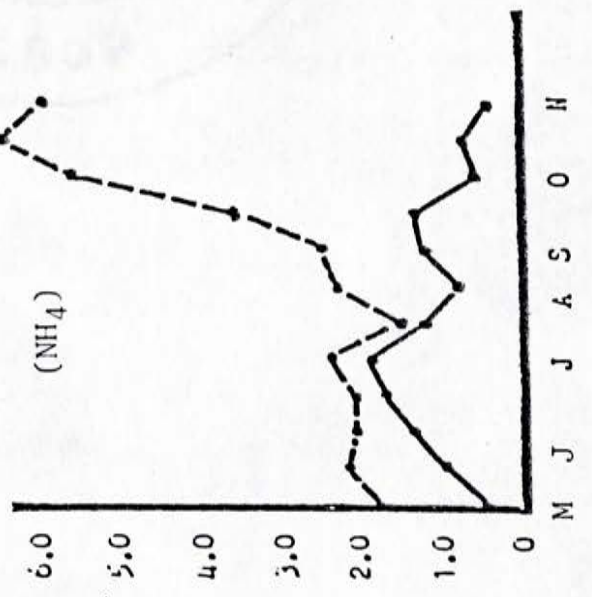
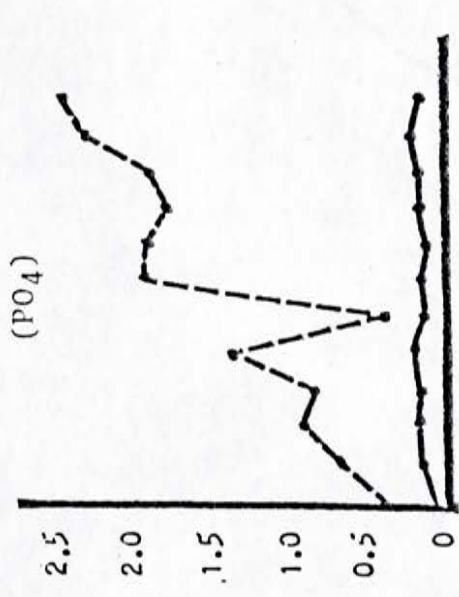
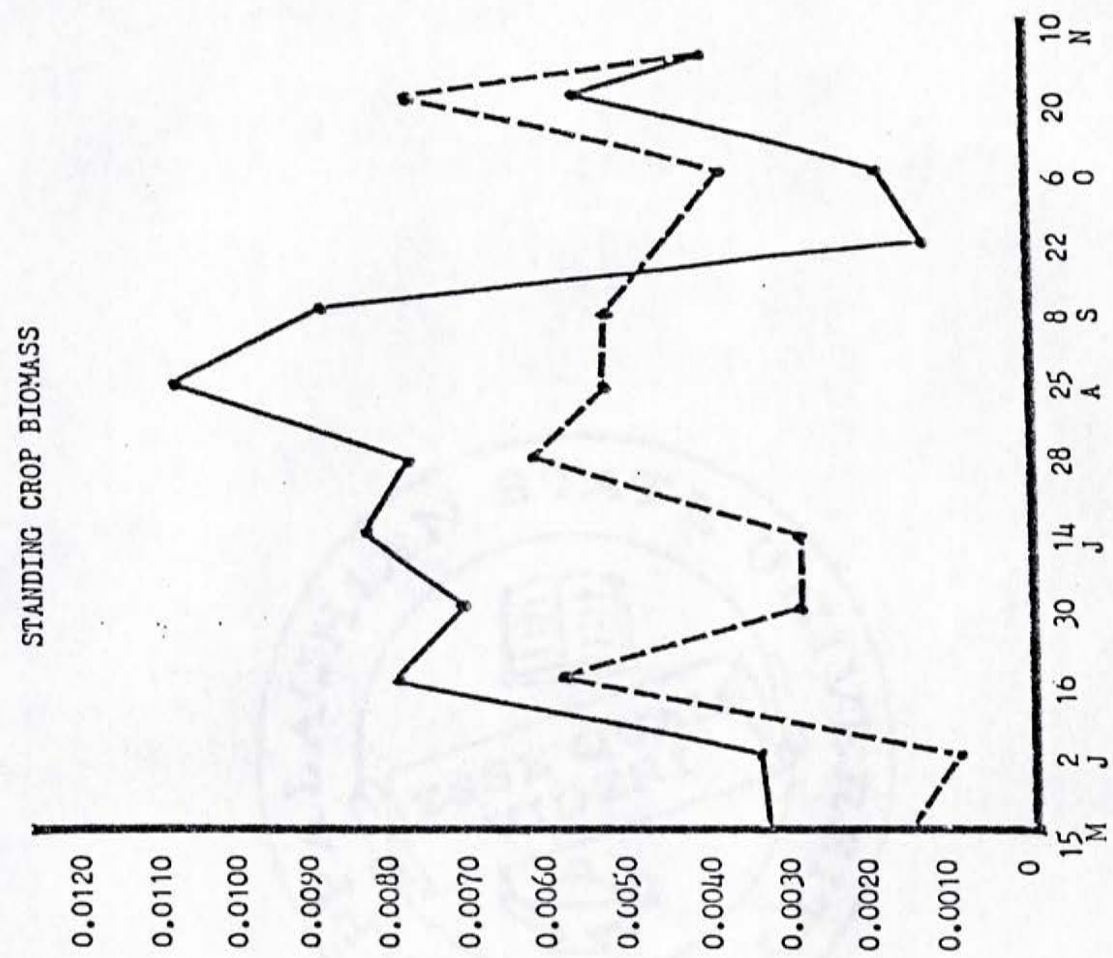


TABLE 11

SPECIES DIVERSITY OF THE BENTHIC ALGAL COMMUNITY
IN THE SOUTH FORK OF THE NEW RIVER,
UPSTREAM AND DOWNSTREAM FROM THE
BOONE SEWAGE TREATMENT PLANT

<u>DATE</u>	<u>CONTROL SITE</u>	<u>EXPERIMENTAL SITE</u>
5/19	0.70677	0.96759
6/2	1.11923	0.77307
6/16	0.88934	0.97239
6/30	1.13141	1.15410
7/14	1.22882	1.37464
7/28	1.22573	0.81744
8/25	0.91931	1.00370
9/8	0.54581	1.09588
9/22	0.88152	0.21297
10/6	1.20041	0.02947
10/22	1.24678	0.09951
11/10	1.09160	1.09209

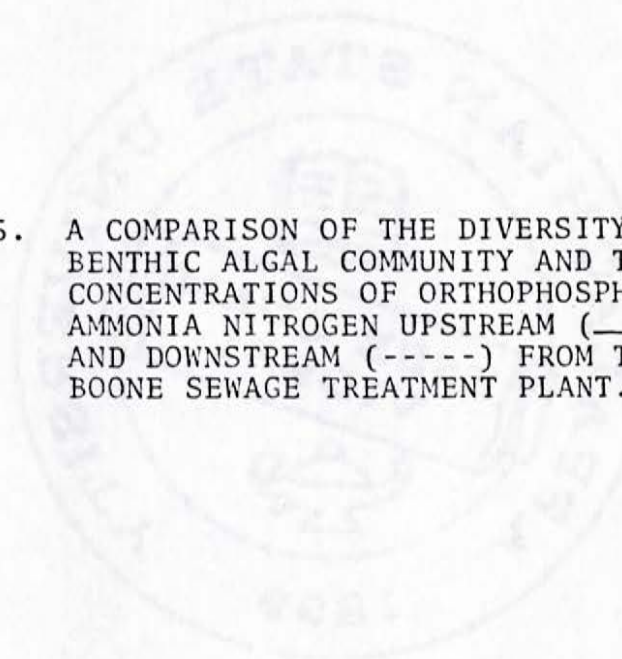


FIGURE 5. A COMPARISON OF THE DIVERSITY OF THE BENTHIC ALGAL COMMUNITY AND THE CONCENTRATIONS OF ORTHOPHOSPHATE AND AMMONIA NITROGEN UPSTREAM (————) AND DOWNSTREAM (-----) FROM THE BOONE SEWAGE TREATMENT PLANT.

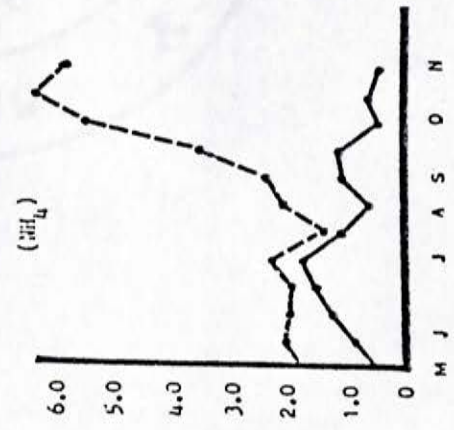
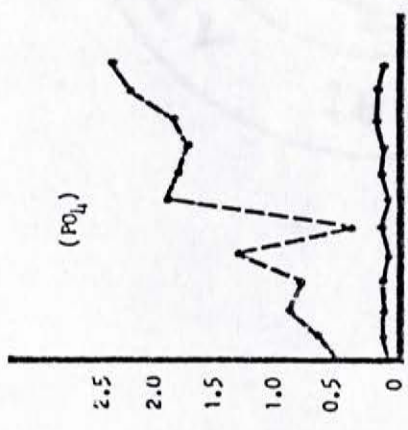
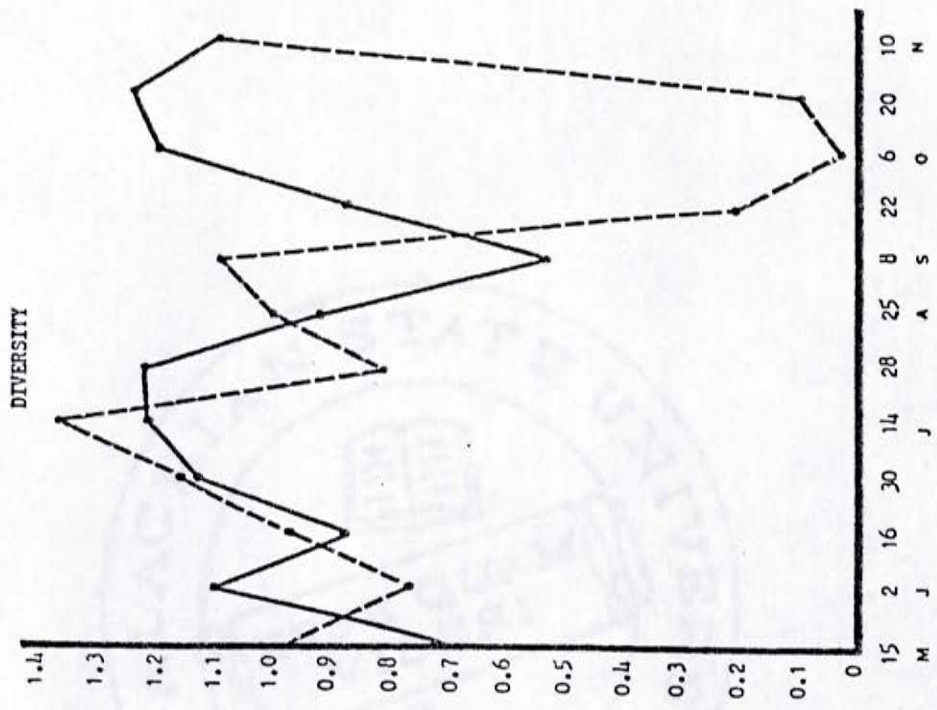
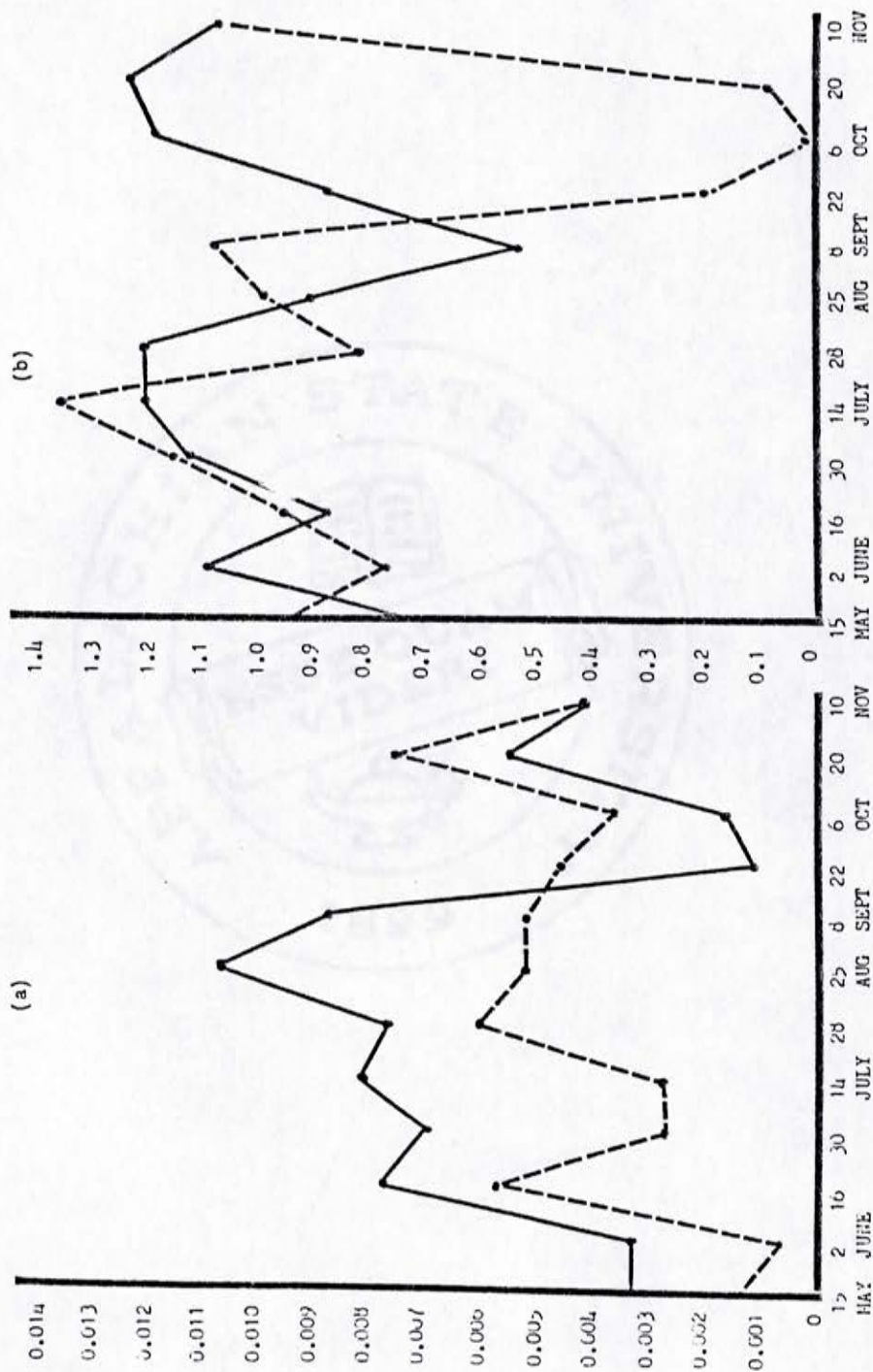


FIGURE 6. A COMPARISON OF THE STANDING CROP BIOMASS (a) AND SPECIES DIVERSITY (b) OF THE BENTHIC ALGAL COMMUNITY UPSTREAM (_____) AND DOWNSTREAM (-----) FROM THE BOONE SEWAGE TREATMENT PLANT.



DISCUSSION

This study has shown that although the concentration of the major nutrients in the South Fork of the New River fluctuated seasonally, the concentration of the inorganic nutrients, orthophosphate and ammonia nitrogen, were consistently higher at the experimental site located downstream from the sewage effluent outfall. The composition and structure of the benthic algal community were also shown to be different between the control and experimental sites and these differences correspond to the nutrient increases at the downstream site.

PHYSICAL-CHEMICAL

Water temperatures in the South Fork of the New River showed a seasonal fluctuation at both study sites throughout the study period. This followed seasonal fluctuations in atmospheric temperatures. Seasonal temperature changes tend to increase in a downstream fashion (Reid, 1961; Hynes, 1969), however, the distance between sites in this study was not great enough to exert a significant influence. A temperature difference of 0.5°C existed between sites throughout the study. This difference fluctuated

between the sites from the beginning of the study until 7/28 when the temperature remained consistently higher at the experimental site for the remainder of the study. This was accompanied by an increase in levels of PO_4 and NH_4 during the same period and may be attributed to an increase in activity at the treatment plant.

This study showed that at both sites the concentration of dissolved oxygen fluctuated along with temperature throughout the study period and that the dissolved oxygen concentration at the upstream station was only slightly higher than levels found at the downstream site. The annual cycle of oxygen in a stream is expected to fluctuate along with temperature and thus the concentration of dissolved oxygen displays an inverse relationship to temperature (Hutchinson, 1957; Reid, 1961; Ruttner, 1968). However, Reid (1961) states that the entry of organic sewage into an aquatic system will drastically reduce the concentration of dissolved oxygen. Since the control site in this study consistently showed only slightly higher levels of dissolved oxygen, apparently the river is not receiving a large input

of raw sewage. These small differences in dissolved oxygen at the two sites are probably therefore only due to the slight temperature differences between sites.

Most aquatic systems experience seasonal fluctuations in nutrient concentrations (Hutchinson, 1957; Reid, 1961; Ruttner, 1968). Nitrate nitrogen concentrations exhibited such a seasonal fluctuation at both collecting sites. According to Reid (1961) inorganic nutrients normally occur in very small amounts in unpolluted freshwater with the concentration of NO_3 averaging 0.30 mg/l worldwide. Levels of this nutrient were found to be well below this average at both sites throughout the study period. Although there was a slight increase of NO_3 at the experimental site consistently, only on three dates, 6/16, 8/25, and 11/10, were the differences great enough to be significant. This suggests that the sewage effluent contributes only small amounts of nitrogen in the form of NO_3 . Subsequent examination of NO_3 concentration of the sewage outfall support this conclusion (Connell, personal communication).

Seasonal fluctuations in the concentrations of both ammonia nitrogen and orthophosphate were evident

throughout this study only at the control site. The experimental site showed normal seasonal fluctuations in these two nutrients only from May 19, 1984 through August 8, 1984 after which the levels of both NH_4 and PO_4 began to climb dramatically. While the concentration of dissolved substances in streams can be expected to increase progressively downstream due to runoff and input from feeder streams (Reid, 1961; Hynes, 1969), the distance involved between sites and the absence of any other input besides the sewage effluent suggests that differences found in nutrient levels between the sites are due to the outfall from the sewage treatment plant. The increase in nutrient levels at the downstream site after August 8, 1984 corresponds with the beginning of the fall semester at Appalachian State University located in Boone, N. C. The influx of new and returning students greatly increases the population of this small mountain town. The resultant extra input into the sewage treatment plant decreases holding time allowed and thus the efficiency with which the sewage is decomposed and treated. This suggests that the University community may be exerting a rather unexpected influence on the aquatic systems of this

area. This is further supported by Connell's findings that NH_4 and PO_4 levels in the New River below the sewage treatment plant are high in the spring and decrease abruptly after the University completes its spring semester (Connell, personal communication).

The concentration of NH_4 and PO_4 in an aquatic system are used to assess the degree of nutrient pollution (Dufour *et al*, 1981). Reid (1961) states that unpolluted freshwaters contain 1 mg/l or less NH_4 . Results of this study have shown that NH_4 levels in the South Fork of the New River are consistently low only at the control site. However, at this site on six of the sampling dates NH_4 levels surpassed the quantity with the highest concentration recorded at 1.8 mg/l. These levels at the control site may be linked to the runoff of fertilizer from the Boone Golf Course and a neighborhood located upstream from this site. Also since the town of Blowing Rock, N. C. has its sewage treatment plant on the New River approximately eight miles upstream from Boone, it may contribute to NH_4 levels. The experimental site, on the other hand, consistently showed levels of NH_4 characteristic of polluted water. The concentration never dropped below 1.45 mg/l and

climbed to a high of 6.47 mg/l on 10/20. These findings suggest that the Boone Sewage Treatment Plant contributes large quantities of NH_4 to the New River's ecosystem. Subsequent examination of the NH_4 levels of the effluent show ammonia concentrations eight times higher than upstream waters (Connell, personal communication).

Phosphorus is the most critical factor involved in the process of eutrophication based on the fact that it is present only in very small quantities in unpolluted freshwater (Reid, 1961; Smith, 1974; Prasad, 1983). While the control site in this study had levels of PO_4 characteristic of an unpolluted stream with a grand mean of 0.12 mg/l over the study period, the grand mean of 1.2 mg/l at the experimental site shows evidence of phosphorus enrichment from the sewage treatment plant. Orthophosphate levels of the effluent itself show very high levels of this nutrient with the high of 18.6 mg/l being recorded to date (Connell, personal communication). Since the input of nitrogen and phosphorus into an aquatic system results in the eutrophication of the system, it can be concluded that the sewage effluent from

the Boone Sewage Treatment Plant is significantly contributing to the nutrient enrichment of the South Fork of the New River.

COMMUNITY COMPOSITION AND STRUCTURE

Producers in aquatic systems vary seasonally due to fluctuations in temperature, duration and intensity of sunlight, and nutrient levels (Smith, 1950; Moore, 1977). The input of nitrogen and phosphorus into an aquatic system resulting in the eutrophication or enrichment of the system can disrupt the normal community and when coupled with conditions of adequate light and temperature, such enrichment may result in rapid algal growth (Lund, 1969; Jaag, 1973; Sheppard and Hoyle, 1977). These producer communities, therefore can be used as biological indicators of stream enrichment (Dean and Burlington, 1963). Although temperature differences between sites varied only slightly in this study, results of the nutrient tests showed a large increase in both PO_4 and NH_4 at the experimental site. Since the ability of an organism to survive in an altered environment is a function of its physiological and morphological adaptations (Gaufin, 1958) in general, polluted areas contain fewer number of species while certain individuals become exceedingly abundant (Gaufin, 1958; Ingram

and Towne, 1959; Plinski, 1983; Revelante *et al.*, 1984). This was evident based on the results of this study. The control site showed a greater number of different divisions and genera of benthic algae than found at the experimental site. Also quite apparent is the rapid and sustained bloom of *Chlorella* at the downstream site that followed the large increase in PO_4 and NH_4 in the fall of 1984.

Cooke, Beyers, and Odum (1968) describe the structure of ecosystems in terms of successional maturity and they along with Margalef (1957, 1960, 1963) have used Information Theory to interpret community dynamics in both terrestrial and aquatic environments. A mature ecosystem can be defined as one having a high species diversity, low productivity to biomass ratio, more complete use of food, and a relatively poor nutrient load. A disturbance such as the input of excess nutrients, can effect an ecosystem by reducing community complexity and stability. This is manifested by a decrease in species diversity, increase in productivity to biomass ratio, and increase in the availability of nutrients. The ecosystem has been reduced to a state of immaturity. As time passes, the system will gradually reach its former state of maturity provided no further disturbance occurs (Edmonson and Lehman, 1981).

However, a continuous input of excess nutrients will render the community in a constant state of immaturity until the input is halted. Results of this study have shown community structure to be affected by the nutrient input from the sewage treatment plant's outfall. Nutrient levels of the stream were consistently higher at the downstream site with the numbers of genera of the benthic algal community correspondingly lower compared to the control site although the diversity index used did not consistently express this finding. During the early part of the summer when difference in nutrient levels between the two sites were at their lowest, the upstream station consistently had a higher standing crop biomass. This, along with the greater species richness, would imply a more stable community than that which occurred downstream even though the species diversity index proved unpredictable in separating these two communities at this time. However, with the marked increase in nutrient levels from 8/25 to 11/10 at the downstream site a further decrease in species richness and equibility occurred at the downstream site concurrent with the bloom of *Chlorella*. This was quantitatively expressed by a plunge in species

diversity and a sharp rise in biomass which implies a marked reduction in community stability and maturity. Based on this we can conclude that the higher nutrient load downstream exerts an effect on the benthic algae community at all times but that there is a critical threshold above which nutrient increases will result in a predictable community response consistent with the interpretation of Cooke, Beyers, and Odum (1968) and Margalef (1957, 1960, and 1963).

Based upon these results this aquatic system might be easily modeled and could lend itself to research examining community responses to predictable periodicities in nutrient concentrations. Other studies should try to isolate additional sources of pollution in the area and should examine the length of the river relative to removal of the nutrient load and stream recovery.

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