# SPATIAL AND TEMPORAL VARIATION IN PRODUCTIVITY, SPECIES DIVERSITY, AND PIGMENT DIVERSITY OF PERIPHYTON IN A STREAM RECEIVING DOMESTIC

AND OIL REFINERY EFFLUENTS

Bу

# JAMES MICKEY COOPER

Bachelor of Science East Central State College Ada, Oklahoma 1962

Master of Science Oklahoma State University Stillwater, Oklahoma 1965

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Thesis Approved; ファ 2 1 Major Adviser Thesis Adviser Toold C. Dean of the Graduate College

#### PREFACE

The objectives of the present study were to measure spatial and temporal variation in productivity of the periphyton community in a stream receiving domestic and oil refinery effluents, to relate species diversity to levels of productivity and degrees of pollution, and to evaluate the autotrophic index as a means of characterizing water quality.

Dr. Jerry L. Wilhm directed the research. Dr. John E. Thomas served as major adviser, Drs. Troy C. Dorris, Glenn W. Todd and the late U. T. Waterfall served on the advisory committee. Dr. Robert D. Morrison and Mrs. Iris McPherson provided statistical and computer assistance. The help and encouragement of all these people are greatly appreciated. Special thanks are due my family, Earlene, James, and Mark for their sacrifice and encouragement during this study.

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#### CHAPTER I

#### INTRODUCTION

Attached algae, periphyton, are the most important primary producers in small, flowing streams. Periphyton have been analyzed in terms of standing crop (Young, 1945; Blum, 1956; Cooke, 1956), species assemblages (Yount, 1956; Castenholz, 1961; Sladeckova, 1962; Round, 1964), chlorophyll <u>a</u> content (McConnell and Sigler, 1959; Grezenda and Brehmer, 1960; Waters, 1961), and productivity (Wetzel, 1964, 1965; Kevern, Wilhm, and Van Dyne, 1966).

Methods for measuring productivity of phytoplankton are not usually suitable for stream periphyton. Carbon-14 and light and dark bottle techniques involve enclosure of the periphyton, and the restriction of water movement has been shown to affect metabolism greatly in rheophilic periphyton (Whitford, 1960; Whitford and Shumacher, 1961). McConnell and Sigler (1959) found the method of measuring productivity by diurnal oxygen curves (Odum, 1956) to be unsuitable in Logan River, Utah. The most widely used method of estimating productivity of periphyton has been to place artificial substrata in a stream for a single extended time period (Wetzel and Westlake, 1969). This technique provides a measure of the average rate of biomass accumulation resulting from the process of colonization and ensuing growth of the attached periphyton (Wetzel, 1969). Low estimates of productivity of a well-established mat are obtained

when a single time period is used because accumulation and growth is low in the colonization phase. In addition, a single exposure period may include part of the asymptotic level of the growth curve when increases in biomass are balanced by losses due to grazing and sloughing. Thus, as the time period increases, the estimated productivity decreases (Cooper and Wilhm, 1970).

Kevern, Wilhm, and Van Dyne (1966) measured biomass accumulation on Plexiglas plates in a series of exposures of increasing duration (e.g., 3, 6, 9, 12 days, etc.) and plotted a growth curve of the standing crop of periphyton. They recognized two components in the growth curve and used a discontinuous linear regression model to distinguish between colonization and acceleration phases. The growth rate following the colonization phase was found to provide a good estimate of the productivity of a well-established periphyton mat. Their study was terminated before the growth curve reached an asymptote.

Primary productivity in streams is increased by nutrient enrichment through organic pollution (Hynes, 1966). A stream receiving organic enrichment in the headwaters generally has high levels of nutrients and productivity upstream and lower levels downstream. Nutrient levels downstream are decreased by dilution and incorporation by algae.

Various numerical and graphic methods have been used to summarize the structure of populations of periphyton. Reporting numbers of individuals of each species per unit area (Castenholz, 1961) or relative abundance of each species (Dickman, 1968) does not provide a single numerical value and comparisons among different studies are difficult.

Observing total numbers of individuals per unit area (Foerster and Schlichting, 1965) does not include the relative importance of each species. Graphic methods have included plotting the number of species versus logarithm of number of specimens (Patrick, Hohn, and Wallace, 1954) and cumulative species against the log of cumulative individuals (Yount, 1956). Graphic methods do not provide a single numerical value and comparisons among studies are difficult.

A single numerical value can be obtained by using mathematical equations called diversity indices. Several diversity indices have been proposed (Gleason, 1922; Fisher, Corbet, and Williams, 1943; Preston, 1948; Patten, 1962; Menhinick, 1964). Wilhm and Dorris (1968) have evaluated different diversity indices and concluded that the approach discussed by Margalef (1956) best summarizes species assemblages. The equation given as a measure of species diversity by Patten (1962) is,

$$\overline{d} = - \ge \left(\frac{n}{1}\right) \log_2 \left(\frac{n}{1}\right),$$

where  $n_i$  is the sample estimate of number of individuals in the ith species and n is the total number of individuals sampled. The index  $\overline{d}$  possesses features which make it a reasonable measure of community structure (Wilhm and Dorris, 1968). The relative importance of each species in the community is expressed in a ratio representing the contribution of each species to the total diversity. With increasing sample size, values of  $\overline{d}$  increase rapidly at first and then reach an asymptote. Many rare species may not be taken in a sampling procedure or be overlooked in enumeration. The contribution of rare species to d is small when compared to their influence on other diversity indices. The equation is dimensionless and numbers or biomass in any units can be used.

Diversity indices have been measured on benthic macroinvertebrates (Wilhm and Dorris, 1966), phytoplankton (Patten, 1962; Staub, et al., 1970), zooplankton (Kochsiek, 1970), and diatoms (Patrick, 1968). However, values of  $\overline{d}$  for stream periphyton have not been determined. The expected trend of periphyton species diversity in a stream receiving organic enrichment in the headwaters would be low values upstream with an increase downstream as water quality improves.

Pigment diversity of phytoplankton communities has been related to nutrients and aging (Margalef, 1958). Pigment diversity is the ratio of the absorbance of 90% acetone extract at 430 nm and 665 nm. Lowest values are usually found in young, growing populations and highest values in old, stable populations (Margalef, 1968). A stream having a longitudinal gradient of nutrients would be expected to exhibit a gradient of pigment diversity. However, Wilhm and Long (1969), working with laboratory microcosms were unable to establish a relationship of pigment diversity to nutrient levels or time.

Weber and McFarland (1969) have proposed an autotrophic index of periphyton to reflect the nature and severity of water pollution. The autotrophic index is defined as the ratio of ash-free weight to chlorophyll <u>a</u> concentration. Periphyton in unpolluted or slightly polluted waters are mostly algae and the autotrophic index is usually less than 100. Increased organic pollution may result in the algae being replaced by non-chlorophyllous organisms and an increase in the

autotrophic index. This index has not been widely evaluated with periphyton communities in enriched streams.

Productivity, species diversity, and pigment diversity have been studied in many different types of communities, but few studies have attempted to investigate relationships among these parameters in periphyton communities of streams. The objectives of the present study were to measure spatial and temporal variation in productivity of the periphyton community in a stream receiving domestic and oil refinery effluents, to relate species diversity and pigment diversity to levels of productivity and degrees of pollution, and to evaluate the autotrophic index as a method of characterizing water quality.

#### CHAPTER II

#### DESCRIPTION OF STUDY AREA

#### General Description

Skeleton Creek originates near Enid, Garfield County, Oklahoma, flows southeasterly for 113 km through Kingfisher and Logan Counties and empties into the Cimarron River 8 km north of Guthrie, Oklahoma (Fig. 1). Stream elevation is 387 m at Enid and 227 m at the mouth. Average gradient is  $0.9 \text{ m km}^{-1}$ . Width increases to 15 m near the mouth with a mean annual flow of  $1.4 \text{ m}^3 \text{ sec}^{-1}$  (Wilhm and Dorris, 1966). Riffles are abundant upstream and pools are more common in the downstream course. Depth in the riffles is often only a few cm while some pools are more than 1.5 m. Low flow occurs throughout most of the year. The stream bottom is composed of rocks, gravel, sand, and silt. Based on the degree of branching (Horton, 1945) Skeleton Creek passes from a third order stream at the uppermost station to a sixth order stream at the lowermost station.

The Skeleton Creek drainage basin lies in a mixed-grass prairie association. Topography is undulating prairies dissected by wooded valleys. The basin is approximately 106,230 ha (U. S. Dept. of Interior, 1968). Primary land uses are cultivation and pasture.

Exposed rocks in the drainage basin are sandstone and shales belonging to the Enid group of the Permian Red Bed (Galloway, 1960).



- Figure 1. Skeleton Creek with Sampling Stations Indicated by Distance in Kilometers Downstream from the Confluence with Boggy Creek. A = Sewage Lagoons, B = Enid State School Outfall, C = Air Base Outfall, D = Oil Refinery Outfall, E = Enid Municipal Sewage Outfall

Soils of the area belong to the Renfrow-Zaneis-Vernon association and are brown to reddish-brown clay or sandy loam (Gray and Galloway, 1959).

Climate of the area is continental with distinct seasonal variations in temperature. Summers are warm with temperatures often exceeding 40 C. Winters are mild with numerous short, cold periods. Temperature range for the 61-year period through 1955 was -31 C to 46 C and averaged 15 C. Mean annual rainfall was 76 cm for the 61-year period through 1958, minimum annual rainfall was 30.5 cm during 1936 and maximum was 132 cm for 1908 (Galloway, 1960).

#### Source of Waste Effluents

Five sources of municipal and industrial wastes enter the headwaters of Skeleton Creek. Approximately  $340 \text{ m}^3 \text{ day}^{-1}$  of domestic sewage effluent from two lagoons enter Skeleton Creek 10 km above its confluence with Boggy Creek. Sewage effluent from Enid State School enters Skeleton Creek 3 km downstream from the sewage lagoons. Treatment facilities at the school consist of an Imhoff Tank, a trickling filter, and a final settling basin. About 450 m<sup>3</sup> of sewage is treated daily.

Effluents from three sources are discharged into Boggy Creek, a small intermittent stream. Domestic wastes from an Air Force Base enter Boggy Creek 16 km above its confluence with Skeleton Creek. The waste treatment plant at the Air Force Base has a primary settling basin, trickling filter, sludge-drying beds, and a final settling basin. Average outfall in May, 1964, was 700 m<sup>3</sup> day<sup>-1</sup>. The Enid municipal sewage effluent enters Boggy Creek about 1.5 km above the

confluence with Skeleton Creek. Treatment includes preaeration, activated sludge, and sludge drying. Approximately 15,000 m<sup>3</sup> day<sup>-1</sup> of sewage are treated. About 70% of the final effluent from the sewage treatment plant is pumped to an oil refinery for use in the refinery process and the rest is discharged into Boggy Creek. Waste water from the oil refinery is routed through an API trap for removal of oil and into a holding pond. The water is pumped into a series of six settling pits where it is mixed with boiler blow-down and lime slurry. The waste water then passes through five biological oxidation ponds, enters a small ditch and flows into Boggy Creek 90 m above the Enid sewage treatment outfall. Final effluent is 750 m<sup>3</sup> day<sup>-1</sup>.

#### Sampling Stations

Four stations were selected for study and were numbered according to their distance in km downstream from the confluence of Boggy and Skeleton Creek (Fig. 1). An attempt was made to select stations with similar physical properties so any observed differences might be attributed to the effect of organic enrichment.

#### CHAPTER III

#### METHODS

#### Sampling Times

This study was based on collections made from October, 1967 through October, 1968. Five exposure periods of approximately two months each were used. The exposure periods were divided into five seasons. Fall included the period from October 26 to December 28, 1967. Winter was from January 23 to March 23, 1968. Spring extended from March 23 to June 27, 1968. Summer was from July 5 to August 12, 1968. The late summer period was from September 7 to October 22, 1968. Collections were made at 3 to 7 day intervals during each exposure period.

#### Physicochemical

Measurements of temperature, pH, conductivity, light transmission, alkalinity, dissolved oxygen, and current were made at each station during each sampling time. Water temperature was determined with a mercury centigrade thermometer. Hydrogen-ion concentration, expressed as pH, was measured with a Hellige Comparator. Conductivity was determined with an Industrial Instruments Conductivity Bridge. Light transmission was measured with a Bausch and Lomb Spectronic 20 colorimeter. Phenolphthalein and methyl purple alkalinity were measured by titration with 0.02 N sulfuric acid. Duplicate dissolved

oxygen samples were fixed by the Alsterberg (Azide) Modification of the Winkler Method (A. P. H. A., 1960). Current was estimated following the method of Robins and Crawford (1954).

## Biological

Plexiglas plates mounted vertically on a raft were used at each station to sample periphyton. Rafts were a 50 x 125 cm rectangle of  $2.5 \times 5$  cm redwood slats with an additional slat inserted in the middle of the long axis. A pointed extension of 6 mm hardware cloth was added to the front to divert debris. Twelve Plexiglas plates, each  $5 \times 12$  cm, were attached with bolts and wing nuts to each of the three slats on the long axis. A pilot study was conducted during September, 1967, to test the durability of the raft and to provide information on exposure times.

Three plates were removed from each raft on each sampling day. During the latter part of the study an additional plate was removed on alternate dates for pigment analysis. Each side of a plate was considered a sample and was specified by drawing numbered cards.

Biomass as ash-free weight was determined from three samples at each station for each sampling date. Periphyton was scraped with a glass microscope slide into a crucible. Ash-free weight was determined by drying the material in an oven at 105 C for 24 hours and ashing in a muffle furnace at 550 C for 1 hour.

The data were graphed to determine the colonization, acceleration, and asymptotic phase of growth. Three linear components were suggested in the growth curves and the following discontinuous linear regression model was developed to analyze the data:

$$Y = d(b_1t) + e[a + b_2(t - t_1)] + f[c + b_3(t - t_1)],$$

where Y is the standing crop,  $b_1$  is the slope of the colonization phase,  $b_2$  is the slope of the acceleration phase,  $b_3$  is the slope of the asymptotic phase, t is time,  $t_1$  is the time at the intersection of  $b_1$  and  $b_2$ ,  $t_j$  is the time at the intersection of  $b_2$  and  $b_3$ , d, e, and f are coefficients determined such that if

$$t < t_{i}, d = 1, e = 0, f = 0$$
  
 $t_{i} < t < t_{j}, e = 1, d = 0, f = 0$   
 $t > t_{i}, f = 1, e = 0, d = 0$ 

a is the value of Y at time  $t_i$ , c is the value of Y at time  $t_i$ .

Regression lines were determined by the least squares method. Differences in  $b_2$  slopes were compared using an analysis of variance and a multiple t-test (Steele and Torrie, 1960). A t-test was conducted to determine if  $b_3$  slopes differed significantly from zero.

Samples to be used for determining species diversity were scraped from the plates and preserved in 3% formalin. Two subsamples from each of two samples were used for counting the number of individuals in each species. Third samples were held in reserve.

The number of individuals necessary to obtain a species diversity value independent of sample size was evaluated by graphic and statistical methods from collections made during the fall and spring. One thousand individuals in units of 100 were counted from each of the four subsamples for each station. A diversity index was calculated for each progressively increasing sample size of 100. Values of diversity from sample size 100 to 1,000 were plotted to determine if  $\overline{d}$  reached an asymptote. No analysis of variance could be performed with this method since samples were not independent.

Independent samples were obtained by dividing the 1,000 individuals into groups of size 100, 200, 300, and 400. Estimates of  $\overline{d}$  were calculated for each sample size and an analysis of variance was performed to test for significant differences among  $\overline{d}$  values obtained from the independent samples.

Periphyton for pigment analysis was scraped from plates into test tubes containing 90% acetone. Pigments were extracted in the dark at 4 C for 24 hr. Samples were then centrifuged for 5 min, the supernatant was transferred to a 1 cm cuvette, and the sample was scanned from 430 nm to 750 nm in a Perkin-Elmer spectrophotometer. Pigment concentrations were calculated according to the equations of Parsons and Strickland (1963). Readings were taken relative to the chlorophyll a peak even if displaced laterally (Banse and Anderson, 1967).

#### CHAPTER IV

#### RESULTS AND DISCUSSION

#### Physicochemical

Mean summer water temperature at the four stations ranged from 27.1 C to 29.6 C (Table I). Maximum temperature recorded was 33 C at Station 4 on July 14, 1968. Spring and late summer temperatures were similar as were fall and winter. Ice covered all stations during late December. Highest temperatures occurred at Station 4 probably due to the shallow, unshaded condition of the stream at this station. The stream banks were shaded downstream and pools were deeper.

The mean seasonal pH ranged from 7.6 to 8.3 (Table I). The pH at Station 4 and 97 was generally higher than at Station 17 and 44, but differences were small. Little seasonal variation was evident among the four stations.

Daytime dissolved oxygen concentration ranged from 2.2 ppm to 17.2 ppm (Table I). Highest values were measured at Station 4 for all seasons. During the winter and spring dissolved oxygen values generally decreased downstream. During fall, summer, and late summer highest values occurred at Station 4 followed by Station 97. Lowest values occurred at Station 17. Low oxygen concentration during late summer possibly resulted from heavy runoff carrying oxygen-demanding wastes further downstream.

Season	Station	Temperature (C)	рН	Dissolved Oxygen (ppm)	Alkalinity (ppm)	Light Trans. (%)	Conductivity (µ mhos)
			<u></u>			· · · · · · · · · · · · · · · · · · ·	······································
Fall	4	8.6	8.1	10.7	308	76	1557
	17	7.2	7.8	6.5	300	84	1730
	44	7.0	7.6	6.6	267	52	1427
	97	7.2	8.0	10.2	234	56	1289
Winter	4	11.0	8.2	14.9	289	66	1793
	17	8.0	8.1	12.5	272	73	2054
	44	10.3	8.0	9.2	264	81	1893
	97	7.7	8.1	11.5	236	89	1763
Spring	4	20.8	8.2	17.2	306	65	1916
	17	19.3	8.3	11.4	330	54	2129
	44	18.0	.8.0	10.8	281	62	1608
	97	17.3	8.0 -	8.2	314	33	1085
Summer	4	29.6	8.3	10.7	285	60	1904
	17	27.1	8.0	6.1	202	57	1896
	44	27.5	8.1	8.6	213	53	1585
	97	28.8	8.3	8.4	234	58	1301
Late Summer	4	22.4	8.2	12.8	315	64	1828
	17	19.7	7.7	2.2	306	66	2097
	44	20.8	7.8	5.2	268	37	1512
1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1	97	21.2	8.1	9.4	238	28	1091

#### MEAN SEASONAL PHYSICOCHEMICAL CONDITIONS

TABLE I

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Alkalinity varied between 202 ppm and 315 ppm (Table I). Alkalinity was primarily due to bicarbonate since phenophthalein alkalinity was detected on only two occasions. In general, alkalinity decreased downstream (Table I). The higher levels upstream possibly resulted from the lime slurry method of treating waste water at the oil refinery to remove emulsified oil particles. Reduction downstream was due to precipitation and dilution. The seasonal trend was highest values during the spring followed by late summer, fall, winter, and summer. Interference in the colorimetric method for determining alkalinity occurred during periods of low light transmission. Filtering the sample before adding methyl purple helped remove the interference.

In general, light transmission decreased downstream except in winter (Table I). Values varied from a minimum of 33% to 84%. Decreased light transmission downstream was due to erosion of adjacent cultivated land, inflow from tributaries, and resuspension of silt from the streambed (Baumgardner, 1966). High light transmission occurred during the winter. Rainfall totaled only 4.1 cm during this period and stream flow was low allowing for settling of suspended particles.

Conductivity ranged from 1085 u mhos to 2129 u mhos (Table I). The general pattern was for the highest values to occur at the two upstream stations. Lowest values occurred at Station 97. The decrease in conductivity downstream was due to dilution and incorporation of ions by algae (Hynes, 1966).

Precipitation was highest during the spring and summer (Fig. 2). The stream overflowed the banks several times during these two seasons. The lowest amount of precipitation occurred during the winter followed by late summer and fall.



Figure 2. Precipitation Amounts Recorded at Enid, Oklahoma, by the U. S. Weather Bureau

#### Community Composition

Diatoms dominated the algal flora of Skeleton Creek. Fourteen of the 22 observed taxa belong to the Bacillariophyceae (Table II). <u>Euglena</u> <u>viridis</u> was the only other alga to occur in large numbers during the study. The two upstream stations were completely dominated by <u>Euglena</u> viridis during the winter.

Photomicrographs of the algae collected during the study are deposited in the herbarium of Oklahoma State University.

## Biomass Accumulation

The use of a discontinuous linear regression model permitted separating a growth curve into a colonization, acceleration, and asymptotic phase. Slope b<sub>1</sub> represents the initial colonization phase and was considerably lower than the acceleration phase, slope b<sub>2</sub> (Table III). The time required for colonization during the fall at all stations and during the summer for Station 44 and 97 ranged from 10 to 18 days. Colonization time was less than 10 days for other stations and seasons. Colonization slopes were not computed if the acceleration phase started before the second sampling time.

The rate of colonization is influenced by the kinds of organisms present, substrata, and the number of available propagules. Diatoms were the major component of the periphyton community in Skeleton Creek. Glass slides collected representative samples of species of diatoms in a river (Patrick et al., 1954). No significant difference was found in relative abundance of epilithic and epiphytic diatom species and most other ephermal epilithic algae on smooth glass, frosted glass, smooth Plexiglas, and frosted Plexiglas (Castenholz, 1961). Propagules for the colonization

## TABLE II

#### ALGAE IDENTIFIED DURING THE STUDY

#### Chlorophyta

<u>Cosmarium sp.</u> <u>Pediastrum duplex</u> <u>Scenedesmus quadrangulata</u> <u>Stigeoclonium tenue</u>

Euglenophyta

Euglena viridis Phacus sp.

Chrysophyta

Amphiprora paludosa <u>Cocconeis sp.</u> <u>Cyclotella meneghiniana</u> <u>Cymbella affinis</u> <u>Cymbella sp.</u> <u>Fragilaria crotonensis</u> <u>Frustulina vulgaris</u> <u>Gomphonema olivacium</u> <u>Nitzschia acicularis</u> <u>Nitzschia hungarica</u> <u>Nitzschia palea</u> <u>Pleurosigma delicatulum</u> <u>Suriella angustata</u> <u>Synedra ulna</u>

Cyanophyta

Oscillatoria sp.

à

## TABLE III

SLOPES FOR THE THREE GROWTH PHASES AT STATIONS IN SKELETON CREEK FOR THE FIVE SAMPLING PERIODS

Slope	Station 4	Station 17	Station 44	Station 97
·····	· · · · · · · · · · · · · · · · · · ·			· <u> · · · · · · · · · · · · · · · · ·</u>
		Fall		
<b>b</b> <sub>1</sub>	0.05	0.17	0.01	0.04
ь b <sub>2</sub>	1.98	1.28	0.80	0.67
b_3	-0.23	0.04	-0.19	0.21
	•			
		Winter		
<sup>b</sup> 1	· <u> </u>	<del>.</del> '		<del>.</del>
b <sub>2</sub>	2.70	1.38	0.87	0.54
ъ <sub>3</sub>	0.60	-0.96	-1.48*	-
		Spring		
b.	· · -	_ ·	<b></b>	<b>-</b> ·
<sup>b</sup> 2	2.28	3.73	2.16	0.53
<b>b</b> <sub>3</sub>	-	<b>_</b>	-	-
	· · · · · · ·	Summer		
b.	—	-	0.30	0.08
ь b <sub>2</sub>	0.96	1.61	0.80	0.64
<sup>b</sup> 3	-	-0.52	-0.40	-0.25
		Late Sum	mer	
b.	· · · · ·	· -	<u>_</u>	· <b>-</b>
b <sub>o</sub>	0.86	0.75	0.62	1.17
. 4	0.10	0.00	0.07	0.044

- indicates insufficient data to calculate a slope value.

\*b, slopes significantly different from zero.

of new substrata in Skeleton Creek are provided by detached periphyton mats. The upstream stations had a higher colonization rate except when a large amount of detergent foam was present. It is possible that detergents interfere with the adhesion of organisms to a substrate, thus reducing the colonization rate.

The slope of the regression line fitted to the acceleration phase of growth,  $b_2$ , was used to estimate periphyton productivity for each station and season (Table III). Slope values correspond to periphyton productivity in g m<sup>-2</sup> day<sup>-1</sup>. Analysis of variance coupled with a multiple t-test was used to test for differences among stations for each season (Table IV).

Productivity as measured by the slope of the acceleration phase of growth, b<sub>2</sub>, exhibited distinct longitudinal variation in Skeleton Creek. Longitudinal variation in productivity was similar for the fall and winter (Figs. 3 and 4). Greatest productivity occurred at Station 4 and decreased progressively downstream. The multiple t-test indicated that the two upstream stations were significantly greater in rate of ' accumulation than the two downstream stations (Table IV).

Productivity at the three upstream stations during spring was higher than at Station 97 (Table IV and Fig. 5). Station 17 had the maximum productivity recorded during the entire study,  $3.73 \text{ gm}^{-2} \text{ day}^{-1}$ , and was statistically greater than the other three stations. The minimum value of productivity,  $0.053 \text{ gm}^{-2} \text{ day}^{-1}$ , was also recorded during the spring at Station 97. Station 4 and 44 were not significantly different from each other.

During summer, productivity at Station 17 was significiantly greater than the other three stations (Table IV and Fig. 6). Station 4, 44, and 97 were not significantly different from each other.

T	AB	LE	IV

COMPARISON OF b<sub>2</sub> SLOPES WITH STATIONS RANKED FROM LOWEST TO HIGHEST PRODUCTIVITY VALUES\*

			но се	
	Fall			
97	44	17	4	
	Winter			
97	44	17	4	
الإغراد باريونية	nin den ten Biskington,			
	Spring			
97	44	4	17	
, -				
	Summer			
97	44	4	17	
	<u>,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,</u>			
	Late Summe	er		
44	17	4	97	
	97 97 97 97 44	Fall      97    44      Winter      97    44      97    44      97    44      97    44      97    44      Summer    97      97    44      Late Summer      44    17	Fall      97    44    17      Winter    17      97    44    17      97    44    4      97    44    4      Spring      97    44    4      Late Summer      44    17    4	Fall9744174Winter9744174974441717974441717Summer9744417Late Summer4417497

\*Stations underscored by the same line are not judged to be significantly different ( $\mathcal{O} = 0.05$ ).

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Figure 3. Discontinuous Linear Regression Model Fitted to Growth of Periphyton for the Fall



Figure 4. Discontinuous Linear Regression Model Fitted to Growth of Periphyton for the Winter



Figure 5. Discontinuous Linear Regression Model Fitted to Growth of Periphyton for the Spring



Figure 6. Discontinuous Linear Regression Model Fitted to Growth of Periphyton for the Summer

Although no significant difference in rate of accumulation among stations for late summer was found (Table IV and Fig. 7), productivity was highest at Station 97. Dense phytoplankton blooms were observed in the downstream pools during this period and possibly contributed to productivity at Station 97.

Considerable seasonal variation in productivity was measured during the study (Table III). Values were generally higher in spring followed by winter, fall and summer. Lowest seasonal productivity occurred during the late summer. The high productivity recorded during the spring was due to the spring maxima of algal growth and abundance of available nutrients (Blum, 1956).

Nutrients such as nitrates, phosphates, and potassium which stimulate plant growth are products of the breakdown of organic matter, particularly sewage. Below zones of heavy organic pollution the amounts of these substances are often greatly in excess of their normal concentrations in natural waters (Hynes, 1966). Four sources of domestic sewage and an oil refinery effluent enter Skeleton Creek above Station 4. The effluent undoubtedly carries a large amount of nutrients. A progressive decrease in productivity downstream from the effluent outfalls is attributed to uptake of nutrients by large numbers of periphyton upstream, dilution by inflow from tributaries, and lower light transmission (Baumgardner, 1965).

Estimates of primary productivity from various studies and the mean annual values from Skeleton Creek are given in Table V. All values were determined by biomass accumulation. Productivity at the two upstream stations in Skeleton Creek greatly exceeds the estimates from other studies. Station 44 has a considerably lower value than the upstream



Figure 7. Discontinuous Linear Regression Model Fitted to Growth of Periphyton for the Late Summer

#### TABLE V

#### Productivity Treatment Habitat Reference $(g m^{-2} day^{-1})$ Skeleton Creek 4 km below 1.75 Present Study outfalls 17 km below Skeleton Creek 1.75 Present Study outfalls 44 km below Skeleton Creek 1.05 Present Study outfalls High levels of 0.75 Microcosm Wilhm and Long (1969) N and P Skeleton Creek 97 km below 0.71 Present Study outfalls Red Cedar River 0.58 Warm-water Grezenda et al. (1968) stream Artificial Stream Grazing 0.58 Kehde (1969) Artificial Stream No grazing 0.51 Kehde (1969) Artificial Stream None 0.51 Kevern et al. (1966) Intermediate 0.40 Wilhm and Long (1969) Microcosm levels of N and P Ponds Fertilizer 0.30-0.44 Knight and Ball (1962) Spring 0.20 Kevern et al. (1966) Low levels of 0.14 Wilhm and Long (1969) Microcosm N and P

### COMPARISON OF PERIPHYTON PRODUCTIVITY ESTIMATES FROM VARIOUS HABITATS

stations but is still higher than the other studies. Productivity at Station 97, fartherest downstream from the outfalls, is comparable to microcosms with high levels of nitrogen and phosphorus, artificial streams, and a warm water stream. The value for Station 97 exceeds that of microcosms with intermediate and low levels of nitrogen and phosphorus, ponds treated with fertilizer, and a spring.

Slope  $b_3$ , the asymptotic level was not significantly different from zero ( $\propto = 0.05$ ) in 12 of 14 measured intervals (Table III). Zero slope is maintained when increases in biomass are balanced by sloughing and grazing.

Mean seasonal biomass maintained at the asymptotic level ranged from 4.5 g m<sup>-2</sup> to 40.7 g m<sup>-2</sup> (Figs. 3 to 7). Biomass was similar for the two upstream stations with lower values measured at the two downstream stations. Stations with the highest productivity generally had the largest biomass. Estimates of both productivity and biomass are necessary to distinguish between a large biomass metabolizing at a slow rate and a small biomass metabolizing at a high rate.

#### Ratio of Net Productivity to Biomass

Margalef (1958) found that as succession proceeded in phytoplankton communities in the ria of Vigo the ratio of net productivity to biomass decreased. He recognized three stages of succession. Stage one was characterized by small-celled organisms with a  $P_n/B$  ratio of 0.5 to 2.0. This type of community developed in turbulent waters with a high nutrient concentration. Stage two was composed of medium-sized diatoms and had a  $P_n/B$  ratio of 0.2 to 0.5. Stage three was large free-swimming forms with a  $P_n/B$  of less than 0.2. In the present study no definite trend in spatial or temporal variation in the  $P_n/B$  ratio was observed (Table VI). Values ranged from 0.03 to 2.40. Only one value was in the range of stage two and all others were in stage three. All values would have been much less had dry-weight instead of ash-free weight been used for biomass. Wilhm and Long (1969) used dry weight and found the range of values proposed by Margalef not to be applicable to their study of microcosms. All their values were characteristic of the third stage of succession.

#### Sample Size

Wilhm and Dorris (1968) have shown that as sample size of benthic macroinvertebrates is increased, values of species diversity,  $\overline{d}$ , increases rapidly at first and then reaches an asymptote. Kochsiek (1970) found  $\overline{d}$  of zooplankton to reach an asymptote as sample size was increased. The number of individuals in the present study necessary to obtain a  $\overline{d}$  value independent of sample size was determined from collections made during the fall and spring. One thousand individuals in units of 100 were counted from each of four subsamples for each station. A diversity value was calculated for each progressively increasing sample size of 100. Mean values of diversity from 100 to 1,000 were plotted to determine if  $\overline{d}$  reached an asymptote (Table VII). Graphical evaluation did not indicate a difference in  $\overline{d}$  among sample size of 100 to 1,000. No analysis of variance could be performed with this method as the pooled samples were not independent.

Independent samples for statistical analysis were obtained by dividing the 1,000 individuals into groups of size 100, 200, 300, and 400. Values of  $\overline{d}$  were calculated for each of the groups (Table VIII).

## RATIO OF NET PRODUCTIVITY TO BIOMASS IN SKELETON CREEK

Gaagan	Station						
Season ———	4	17	44	97			
Fall	0.06	0.17	0.08	0.07			
Winter	0.10	0.03	0.05	0.03			
Spring	-	-	-	-			
Summer	-	0.12	0.11	0.11			
Late Summer	0.05	0.05	0.10	0.24			

- indicates insufficient data to calculate a  $P_n/B$  ratio.

## TABLE VII

										• • • • • • • •	···=····
Season	Station					Samp1	<u>e Siz</u>	е			
beason	JUALION	100	200	300	400	500	600	700	800	900	1,000
•										. *	
Fall	4	1.6	1.6	1.6	1.6	1,6	1.6	1.6	1.6	1.6	1.6
	17	1.1	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
	44	1.2	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4
	97	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Spring	4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	17	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	44	1.3	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
	97	1.8	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
							-				

# MEAN DIVERSITY VALUES OBTAINED BY POOLING SUCCESSIVE SAMPLES FOR THE FALL AND SPRING

· . .

# TABLE VIII

Concer	Station		Sample	Size	
Season	Station	100	200	300	400
· · · · ·					
Fall	4	1.6	1.6	1.6	1.5
	17	1.1	1.2	1.2	1.0
	44	1.2	1.3	1.3	1.4
and a state of the	97	2.2	2.2	2.2	2.2
			·		
				e.	
Spring	4	0.3	0.3	0.3	0.3
	17	0.9	0.9	1.1	1.0
· .	44	1.3	1.3	1.3	1.3
	97	1.8	1.9	1.8	1.8

## MEAN DIVERSITY VALUES OBTAINED FROM INDEPENDENT SAMPLES FOR THE FALL AND SPRING

An analysis of variance was performed to test for significant differences among  $\overline{d}$  values. No significant difference ( $\mathcal{Q} = 0.05$ ) was found among  $\overline{d}$ values of sample size 100 to 400 for any of the stations during the fall or the spring. Therefore, a sample size of 100 was chosen as the optimum number of individuals to count and was used throughout the remainder of the study.

#### Species Diversity

Species diversity,  $\overline{d}$ , tended to increase downstream during all seasons except late summer (Fig. 8). Duncan's New Multiple Range Test was used to determine differences among stations for each season. In fall, minimum diversity was measured at Station 17 and maximum diversity at Station 97. Station 4 and 44 were not significantly different from each other, but were different from the other stations ( $\propto = 0.05$ ).

The lowest diversity values measured in the study were recorded at Station 4 and 17 in winter (Fig. 8). Low values at these stations were due to large concentrations of <u>Euglena viridis</u> which on several sampling dates was the only species collected. Diversity was considerably greater at downstream stations in winter (Fig. 8). The diversity pattern in spring was similar to the winter pattern, except  $\overline{d}$  was greater at Station 4 and 17 in spring. Also, values at Station 17 and 44 were not significantly different from each other in spring (Table IX). Few individuals of <u>Euglena viridis</u> were present at the two upstream stations in the spring.

In summer Station 44 and 97 were not significantly different from each other (Table IX) but were significantly greater than Station 17

× 35



Figure 8. Longitudinal Variation of Species Diversity in Skeleton Creek for the Five Seasons

## TABLE IX

COMPARISON OF d VALUES BY DUNCAN'S NEW MULTIPLE RANGE TEST WITH STATIONS RANKED FROM LOWEST TO HIGHEST DIVERSITY\*

	Fa	11	:	
17	44	4	97	
	· · · · · · · · · · · · · · · · · · ·	<u></u>		
	Win	ter		
4	17	44	97	
	Spr	ing	÷.,	
4	17	44	97	
<del></del>			- -	
,				
	Sum	mer		
17	44	97		
				•
	Late	Summer		
44	97	4	17	
		ter Barthall Angener		
	17 4 4 17 44	Fa 17 44 Win 4 17 Spr 4 17 Sum 17 44 Late 44 97	Fall      17    44    4      Winter      4    17    44      Spring      4    17    44      Summer      17    44    97      17    44    97      44    97    4      97    4    97	17    44    4    97      Winter      4    17    44    97      Spring      4    17    44    97      Gummer      17    44    97      Late Summer      44    97    4    17

\*Stations underscored by the same line are not judged to be significantly different ( $\alpha = 0.05$ ).

(Table IX). An insufficient number of samples were collected at Station 4 to enable statistical treatment of this station.

The late summer period differed from the general pattern of increased diversity downstream. Values of  $\overline{d}$  at the two upstream stations exceeded those at the two downstream stations (Fig. 8 and Table IX). This variation possibly was brought about by high stream flow immediately preceding the exposure period. High stream flow would tend to bring about an uniform distribution of nutrients as well as organisms.

Wilhm and Dorris (1968) have reviewed the application of species diversity of benthic macroinvertebrates in determining degrees of pollution. They found values of  $\overline{d}$  to be less than one in areas of heavy  $\mathbb{Z}$ pollution, between one and three in areas of moderate pollution, and values exceeding three in clean water. Staub et al. (1970) determined d on phytoplankton in various streams receiving domestic and industrial wastes in the vicinity of Memphis and Shelby Counties, Tennessee. They reported d of less than one in areas of severe pollution, from one to two in areas of moderate pollution, from two to three in areas of light pollution, and values above three in clean water zones. A comparison of d values of periphyton obtained from Skeleton Creek with the criteria given by Wilhm and Dorris (1968) and Staub et al. (1970) indicates upstream stations to be in areas of heavy pollution while downstream stations to be in areas of moderate pollution. Although clean water zones in Skeleton Creek were not indicated by  $\overline{d}$  values of periphyton, longitudinal improvement in water quality downstream was suggested by d values.

#### Pigment Diversity

Margalef (1968) discussed a pigment diversity index based on the ratio of the absorbance of 90% acetone extract at 430 nm and 665 nm,  $D_{430}/D_{665}$ . Pigment diversity has been related to nutrients and aging. In the event of a sudden increase in nutrients, chlorophyll <u>a</u> increases at a much greater rate than other pigments and this results in a reduced index. Margalef found the ratio to be lowest in young, growing populations and highest in old, stable populations.

Pigment diversity was calculated only for the winter and summer as these were the only seasons in which a sufficient number of samples were collected. Time had little effect on pigment diversity during the winter (Fig. 9) and summer (Fig. 10). Pigment diversity increased slightly during colonization and then leveled off. Longitudinal variation in pigment diversity was slight during the winter and summer (Fig. 11).

In the present study, pigment diversity ranged from 1.42 to 2.36. Wilhm and Long (1969) found no effect on pigment diversity attributable to nutrients or time in microcosms. They reported values of two to three in their study. Knudson (1970) reported mean phytoplankton pigment diversity values of 2.34 and 2.41 in turbid ponds and values of 2.83 and 3.21 in clear ponds. Kehde (1970) reported values of 2.1 to 3.8 for periphyton in an artificial stream with grazers and values of 1.9 to 3.8 with no grazers.

The lack of an increase in pigment diversity over time or distance downstream in the present study was attributed to a lack of aging in periphyton communities of streams. Patrick (1954) reported that as soon as periphytic diatoms die they no longer adhere to a substrate. Thus as the underlying layers of the periphyton mat die, portions of







the mat float away. Organisms that remain on the substrate are then capable of continued growth and reproduction at a rate determined by available nutrients and are not limited by space. Thus, the community remains young and growing. This is supported by the low pigment diversity (< 2.5) obtained during this study.

#### Autotrophic Index

The autotrophic index proposed by Weber and McFarland (1969) to determine water quality was applied to data from Skeleton Creek. The autotrophic index is the ratio of ash-free weight to chlorophyll <u>a</u> concentration. They stated that periphyton in unpolluted or slightly polluted water are mostly algae and have biomass to chlorophyll ratios similar to those reported for algal cultures. As the level of organic pollution increases, the algae are replaced by filamentous bacteria and other non-chlorophyllous organisms resulting in an increase in the biomass to chlorophyll ratio.

The mean seasonal autotrophic index tended to increase downstream in Skeleton Creek (Table X). However, Duncan's New Multiple Range Test did not indicate a significant difference ( $\propto = 0.05$ ) among stations during the winter or summer. During the late summer, Station 17 was significantly different from Station 97. Other stations were not different.

Autotrophic indices from Skeleton Creek are comparable with those found in unpolluted waters, algal cultures, and artificial streams (Table XI). Highest mean seasonal values in Skeleton Creek are much lower than those reported for a polluted river (Weber and McFarland, 1969).

	Emperance Deviled	Station					
	Exposure reriod		4	17	44	97	
•.	<u></u>		. <u> </u>	<del>* / **********************************</del>			
	Winter		151	103	176	315	
	Summer			116	198	391	
·	Late Summer		117	103	154	292	
		, <sup>s</sup>					

## MEAN SEASONAL VALUE OF THE AUTOTROPHIC INDEX FOR THREE SEASONS IN SKELETON CREEK, OKLAHOMA

TABLE X

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## TABLE XI

## AUTOTROPHIC INDEX CALCULATED FROM DATA IN THE LITERATURE

Investigator	Habitat	Autotrophic Index
	· · · · ·	
Myers, J. and W. A. Kratz, 1955	Algal culture	55
Parsons, T. R., 1961	Algal culture	70
Parsons, T. R. et al., 1961	Algal culture	96
Steemann Nielsen, E., 1961	Algal culture	45
Kehde, P. M., 1970	Artificial stream with grazers	470
Kehde, P. M., 1970	Artificial stream without grazers	720
McIntire, C. D., 1968	Artificial streams (Light intensity- current)	
	(150-0)	267
	(150–14)	123
	(150-35)	127
	(700-0)	32.4
	(700-14) (700-35)	204 212
Weber, C. I. and B. H. McFarland, 1969	Unpolluted river Polluted river	177 1,019

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In the present study, productivity and species diversity indicate an improvement in water quality downstream from the pollution outfalls while no improvement downstream is shown by the autotrophic index. Additional work is needed to determine the effects of pollution on the autotrophic index.

#### CHAPTER V

#### SUMMARY

1. The objectives of the study were to measure spatial and temporal variation in productivity of the periphyton community, to relate species diversity and pigment diversity to levels of productivity and degrees of pollution, and to evaluate the autotrophic index as a method of characterizing water quality. Skeleton Creek, Oklahoma, a stream receiving domestic and oil refinery wastes was studied from October, 1967 to October, 1968.

2. Four stations selected for similarity of light, depth, and current were numbered according to distance downstream from the confluence of Boggy Creek.

3. The study was divided into five exposure periods, each approximately 2 months in duration. Samples were collected at 3 to 7 day\_intervals at each station for each exposure period.

4. Artificial substrate were used to sample the periphyton community. Thirty-six Plexiglas plates each with an exposed surface area of 1  $dm^2$  were attached with bolts and wing nuts to floating redwood rafts. Plates were removed at 3 to 7 day intervals to provide exposure times of increasing duration.

5. Maximum water temperature was 33 C in July. Ice covered all stations in December. The pH was consistently alkaline at all stations.

Little seasonal variation in pH was evident. Dissolved oxygen as determined by daytime grab samples were higher at Station 4 and 97 with lower values at Station 17 and 44. Alkalinity was due primarily to bicarbonate as phenolphthalein alkalinity was detected only on two occasions. Light transmission decreased downstream except in winter. Highest light transmission occurred during the winter when rainfall totaled only 4.1 cm. Conductivity was highest upstream.

6. A discontinuous linear regression model was developed to analyze growth curves of periphyton. Colonization, acceleration, and asymptotic phases of the growth curves were recognized. The slope of the regression line fitted to the acceleration phase of growth was used to estimate productivity.

7. In general, productivity decreased downstream from the pollution outfalls. Higher upstream productivity was due to high nutrient levels resulting from the breakdown of sewage. Reduced productivity downstream indicated an improvement in water quality.

8. Zero slope was maintained during the asymptotic phase of growth as increases in biomass were balanced by sloughing and grazing.

9. The ratio of net productivity to biomass did not indicate spatial or temporal successional stages in the periphyton community.

10. Adequacy of sample size was determined by counting 1,000 individuals from each of four samples from each station for the fall and spring. Graphic evaluation on pooled samples from size 100 to 1,000 did not indicate a difference. No significant difference was found in independent samples of size 100, 200, 300, or 400 for the fall or spring. Therefore, 100 individuals were considered as an adequate sample size.

11. Twenty-two taxa of algae were identified from Skeleton Creek. Diatoms dominated the flora except at Station 4 and 17 during the winter when Euglena viridis was the most abundant.

12. Species diversity increased downstream from the pollution outfalls. This increase indicates a more varied periphyton community and an improvement in water quality.

13. Pigment diversity exhibited little spatial or temporal variation. The lack of variation in pigment diversity was due to the periphyton community remaining young and growing due to losses by sloughing. A continually young and growing community was further suggested by low pigment diversity ( $\leq 2.5$ ).

14. The autotrophic index applied to data from Skeleton Creek did not indicate an improvement in water quality downstream. No significant difference in this index was found during the winter or summer. Differences in the late summer did not indicate an improvement in water quality downstream from the pollution outfalls. Additional work is needed to determine the effects of pollution on the autotrophic index.

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James Mickey Cooper

Candidate for the Degree of

Doctor of Philosophy

Thesis: SPATIAL AND TEMPORAL VARIATION IN PRODUCTIVITY, SPECIES DIVERSITY, AND PIGMENT DIVERSITY OF PERIPHYTON IN A STREAM RECEIVING DOMESTIC AND OIL REFINERY EFFLUENTS

Major Field: Botany

Biographical:

- Personal Data: Born near Centrahoma, Oklahoma, March 29, 1940, son of Sonnie and Edna Sawyers Cooper.
- Education: Graduated from Tupelo High School, Tupelo, Oklahoma, in 1958; received the Bachelor of Science degree from East Central State College, Ada, Oklahoma in August, 1962; received the Master of Science degree in Botany from Oklahoma State University, Stillwater, Oklahoma, in May, 1965; completed requirements for the Doctor of Philosophy degree in May, 1972, at Oklahoma State University.
- Professional Experience: Graduate Teaching Assistant, Oklahoma State University, 1962-1964; Instructor of Biology, Cameron College, Lawton, Oklahoma, 1964-1969; Graduate Teaching Assistant, University of Montana Biological Station, Missoula, Montana, Summer, 1965; National Science Foundation Research Participant, University of Oklahoma Biological Station, Summer, 1966; Assistant Professor of Biology, Cameron College, 1969-1971.
- Member: Phi Sigma Society, Associate Member of Sigma Xi, Southwestern Association of Naturalists, and Oklahoma Academy of Science.