ABSTRACT

AN ECOLOGICAL AND POLLUTION-RELATED STUDY
OF A WARM-WATER STREAM

by Darrell L. King

An extensive ecological study was conducted with special attention to relating changes in production with variation in stream ecology within a 30 mile section of a polluted warm-water stream.

Aufwuchs production was measured from its accrual on plexiglass substrata submerged in the stream. A method was developed for separating autotrophic and heterotrophic aufwuchs production and for separating this production from organic and inorganic sediments. The aufwuchs production in the entire river during the summer of 1961 was 1,246 gm cal. M^{-2} day^{-1}, with the autotrophic organisms contributing 980 gm cal. and the heterotrophic 266 gm cal.

Macrophyte production was measured by the harvest method and during the summer of 1961 macrophyte production amounted to 127 gm cal. M^{-2} day^{-1}.

Total photosynthetic efficiency within the aquatic community was estimated to be 0.045% of the usable solar energy available at the surface of the stream.

Net yield of various aquatic invertebrates was determined by changes in population size from one sampling period to the next. Average net yields for the summer of 1961 were 17 gm-cal. M^{-2} day^{-1} for the herbivorous aquatic insects, 9 gm-cal. M^{-2} day^{-1} for the carnivorous aquatic insects and 13 gm-cal. M^{-2} day^{-1} for the tubificid worms.

Energy budgets were constructed for the entire river and for each of five different ecological zones with the greatest variations being associated with industrial and domestic pollution and the inflow of inorganic sediment from an area of highway construction.

Metal plating wastes eliminate all aquatic macro-fauna except tubificid worms immediately below the point of effluent and substantially reduce macro-fauna populations for a distance of 15 miles downstream. These plating wastes had no measurable affect on the autotrophic organisms.

Untreated domestic wastes and the effluent from a sewage treatment plant increase heterotrophic aufwuchs production and decrease autotrophic aufwuchs production in two areas of the river.

Inorganic sediments from an area of interstate highway construction entered the stream via the tributaries and caused a 68% reduction in net autotrophic aufwuchs production and reduced the heterotrophic members of the community such that their energy requirements were decreased by 58%. Thus, the reduction in the amount of energy required by the heterotrophic community nearly equaled the reduction of energy fixed by the autotrophic community.

In the study stream there is a gradual reduction of autotrophic production as the river flows from upstream to downstream areas which corresponds to the gradual accumulation of the various pollutants.

AN ECOLOGICAL AND POLLUTION-RELATED STUDY OF A WARM-WATER STREAM

By

Darrell L. King

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1964

ACKNOWLEDGMENTS

I extend my heartfelt thanks and personal gratitude to Dr. Robert C. Ball for his helpful supervision of my entire graduate program.

I also wish to thank Dr. Gordon Guyer, Dr. Gerald Prescott, and Dr. Peter Tack for the time they freely gave as members of my guidance committee.

Thanks are also due my fellow graduate students for many stimulating discussions of the ideas presented in this thesis.

This study was financed by a grant from the National Institute of Health (Project WP-00011-06).

****

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF TABLES - Continued

TABLE Page

LIST OF TABLES - Continued

LIST OF FIGURES

LIST OF FIGURES - Continued

INTRODUCTION

Biological studies of aquatic organisms have a long history, but relationships between aquatic productivity and environmental conditions have been investigated only recently. Teal (1957), Odum (1957), and Nelson and Scott (1962) studied community biodynamics in limited areas of streams, but there have been no studies of these relationships in large sections of natural streams. The present study differs from those of Teal, Odum, and Nelson and Scott in that the energy exchange within 30 miles of a polluted warm-water stream was investigated, with special attention to changes in primary and secondary production associated with variation in stream ecology.

Streams are open systems and the type of biotic community present depends largely on the type and amount of material entering from the watershed. Allochthonous material is added to streams from the watershed and both allochthonous and autochthonous materials are transported downstream. Thus, recirculation of nutrients is limited. The biota of most streams depend largely, or at least in part, on allochthonous organic material for the energy necessary for their life processes. However, the addition of organic material exceeding the demands of the biota creates an imbalance within the stream community. The increased oxygen demand caused by the resulting heterotrophic shift often exceeds oxygen production by autotrophic members of the community. The decrease in dissolved oxygen then causes other biotic changes and the stream is said to be polluted.

Other inflowing materials, such as industrial wastes and inorganic sediments, also cause detrimental changes in aquatic biota and reduce water quality. But, these various forms of pollution do not act independently, and the synergistic action of combinations of pollutants represents the greatest danger to our natural waters.

During the period of this investigation, the study stream was receiving industrial wastes, domestic wastes, and large amounts of inorganic sediment. Since these allochthonous substances caused the greatest variations in stream ecology, special attention is given to their influences on community relationships among the stream biota.

THE STUDY AREA

The Red Cedar River, a tributary of the Grand River, is a warm-water stream located in the south-central portion of the lower peninsula of Michigan. The study section consists of 30 miles of the main river, extending upstream from the Michigan State University campus, and drains 355 square miles of rolling farm and suburban land. The width of the river varies from 25 to 80 feet, the average gradient is 2.4 feet per mile (Figure I), and the discharge varies seasonally between the all time low of 3 cfs and the record high of 5510 cfs (Figure II). The Red Cedar arises in an area made up primarily of marsh and wet lands, and much of the upper portion of the river has been dredged to facilitate drainage of the marshes and swamps for agricultural purposes. The area immediately adjacent to the river is predominately woodland, while most of the watershed is utilized for dairy and small grain farming. Edaphic and climatological features of the Red Cedar watershed are described by Meehan (1958) and Vannote (1961) discusses the chemical and hydrological features of the river and its tributaries.

Within the study section, the river touches four urban communities and is locally modified by them. From Fowlerville it receives the effluent of a metal plating plant and domestic wastes from a raw sewage drain. Williamston contributes domestic wastes from a sewage treatment plant and at the time of this study, the Okemos-East Lansing area was contributing domestic wastes from storm drains and septic tank overflow drains.

The study section is divided naturally into five zones (Figure III), the first of which begins at the Farm Lane bridge on the Michigan State University campus and extends upstream to Okemos Road in the village of Okemos. This zone, zone I, is about three and one-half miles in length, is entirely within an urban area, and at the time of this study, received domestic pollutants from a series of storm and septic tank overflow drains.

Figure I. Profile of the Red Cedar River. (Data from U.S.G.S. Topographic maps) showing zones in which experimental work was carried out.

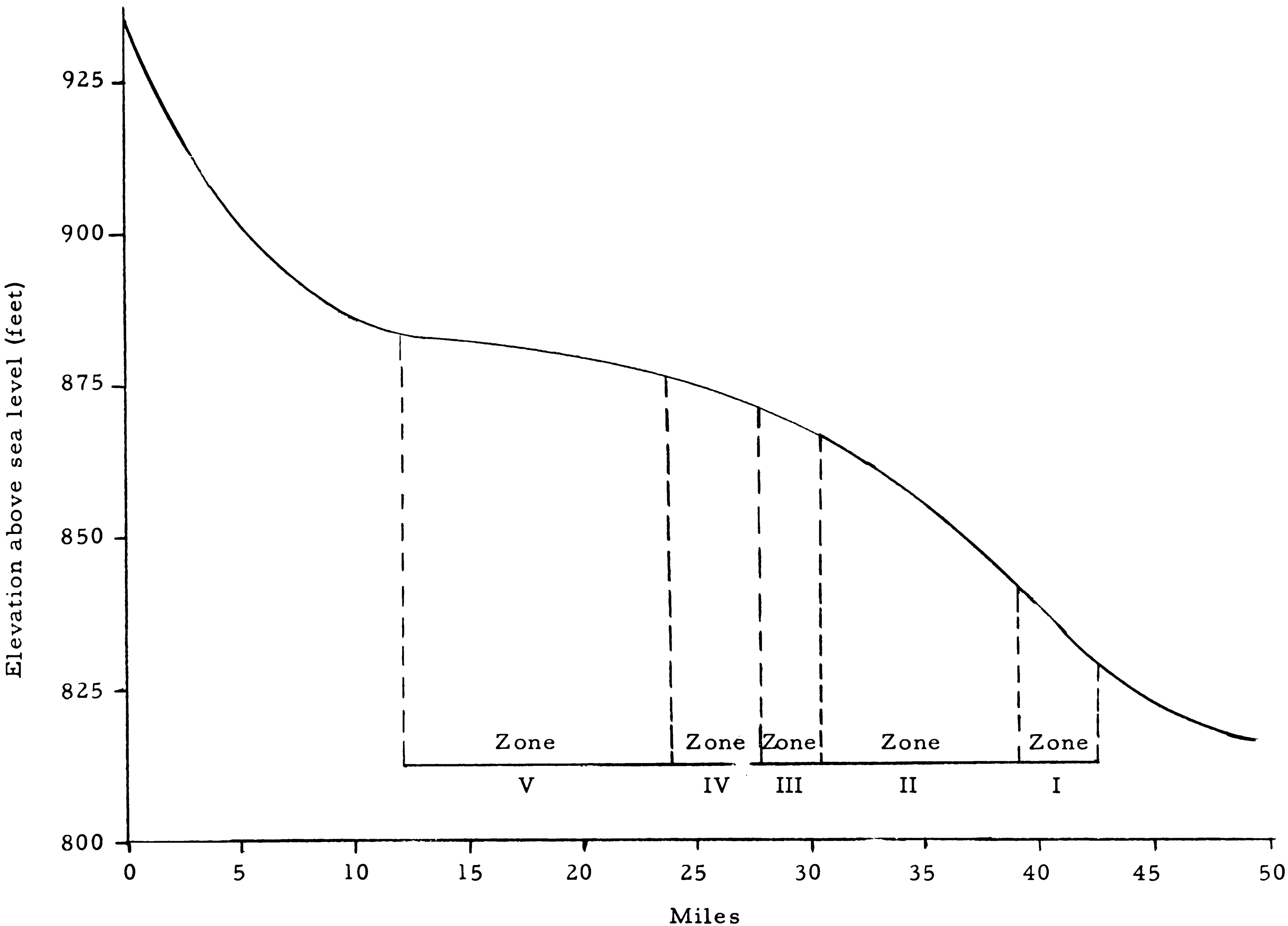

Elevation above sea level (feet)
925
900
875
850
825
800
Zone
V
Zone
IV
Zone
III
Zone
II
Zone
I
0
5
10
15
20
25
30
35
40
45
50
Miles

Figure I

Figure II. Graph of the mean discharge per month in cubic feet per second for the Red Cedar River for 1961 and a ten year average (1946-1956). Data from the U. S. G. S. gauging station at Farm Lane bridge.

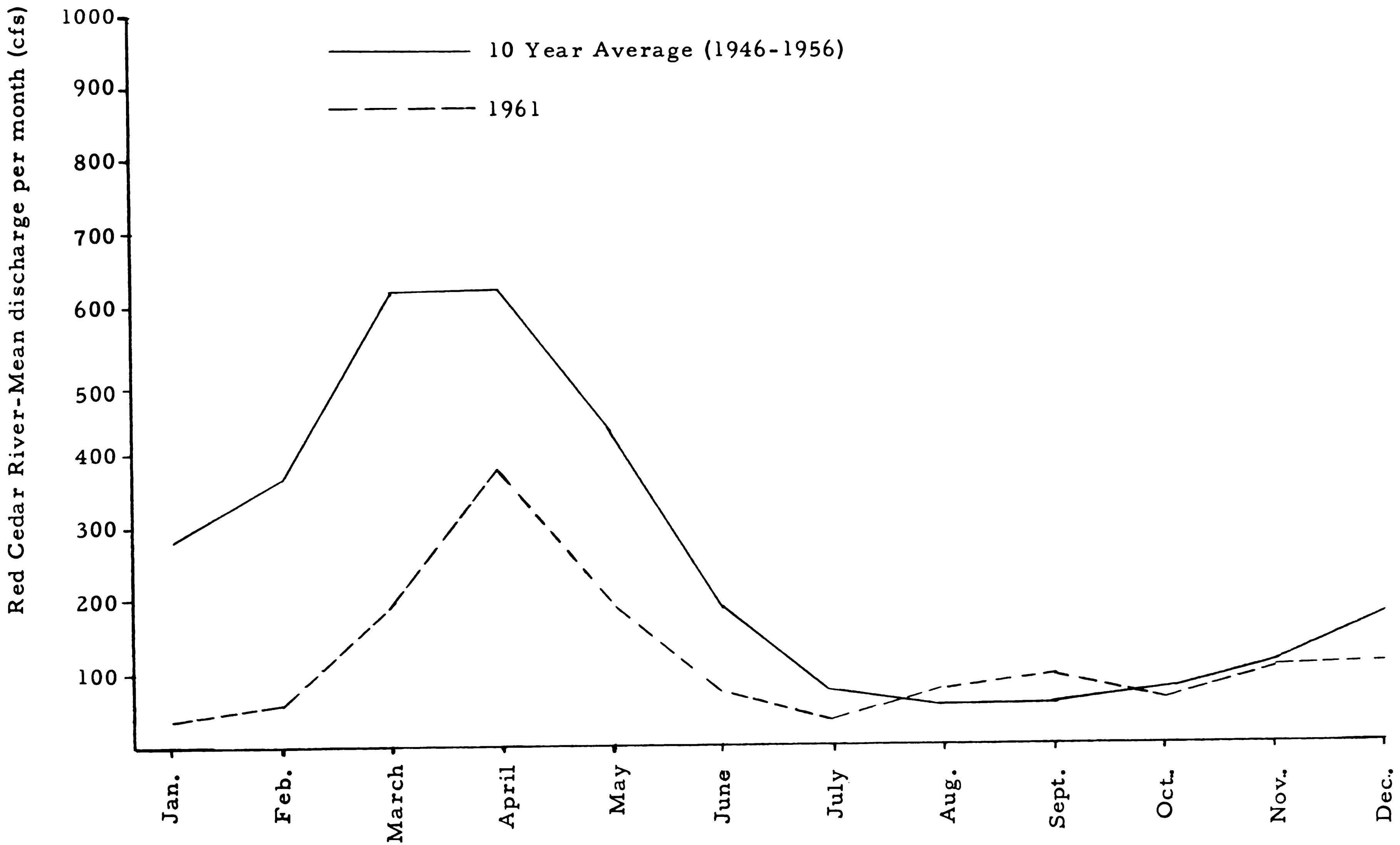
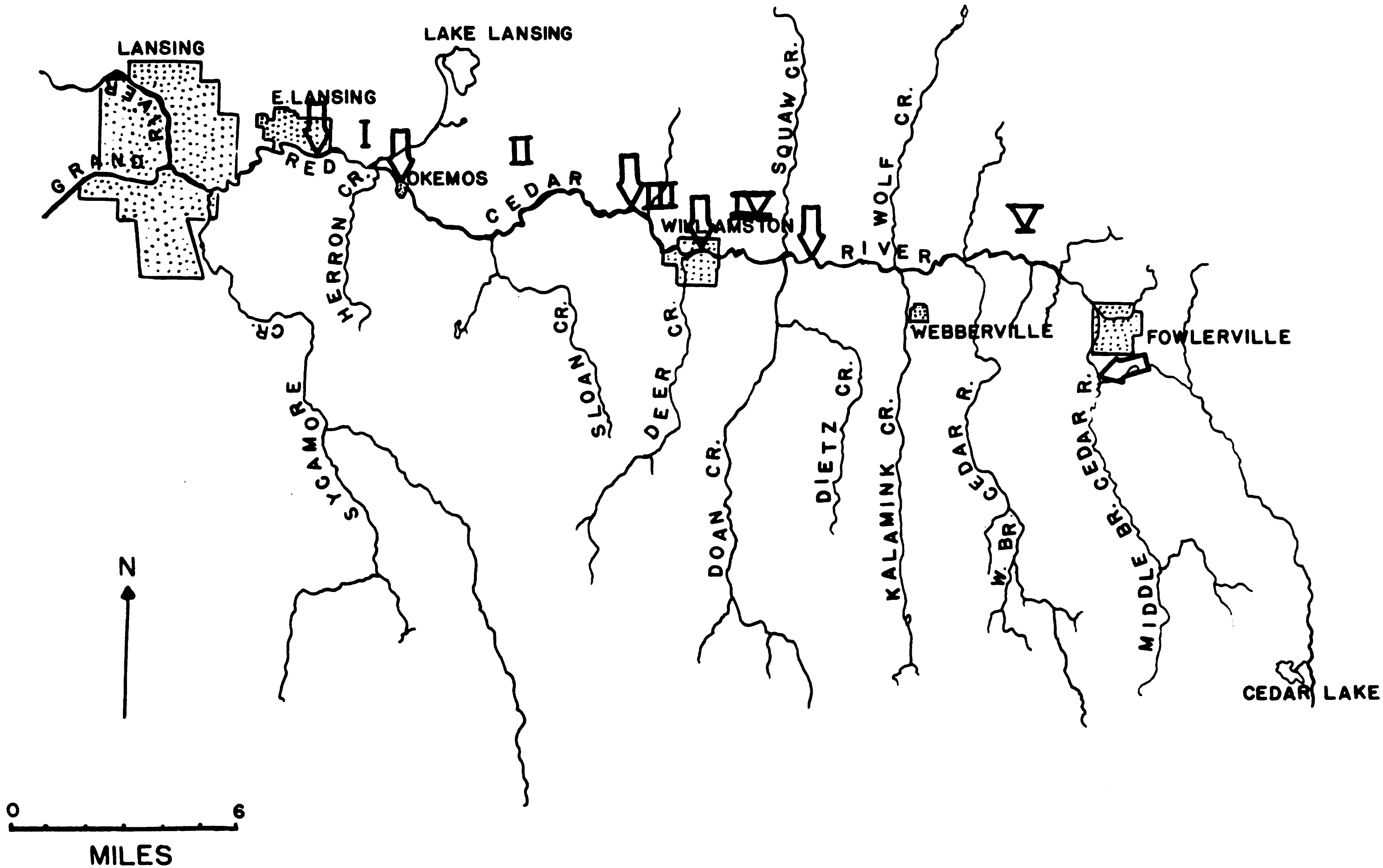

Red Cedar River-Mean discharge per month (cfs)
1000
900
800
700
600
500
400
300
200
100
10 Year Average (1946-1956)
1961
Jan.
Feb.
March
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.

Figure II

Figure III. Map of the watershed of the Red Cedar River showing the five zones of the study section.


LANSING
GRAND RIVER
E. LANSING
LAKE LANSING
OKEMOS
RED CR.
HERRON CR.
SYCAMORE CR.
CEDAR RIVER
SLOAN CR.
DEER CR.
WILLIAMSTON
SQUAW CR.
DOAN CR.
DIETZ CR.
WOLF CR.
KALAMINK CR.
WEBBERVILLE
W. BR. CEDAR R.
FOWLERVILLE
MIDDLE BR. CEDAR R.
CEDAR LAKE
I
II
III
IV
V
N
0
6
MILES

The first of three impoundants on the Red Cedar River is located in zone I on the Michigan State University campus and was constructed primarily for recreational purposes. The bottom type of this zone grades from silt in the impounded area to sand flats in the upper end of the zone. The average depth in the impounded area is less than six feet and the sand flats are covered with about 15 inches of water.

The second zone is eight and one-half miles long, beginning at Okemos Road and extending upstream to Zimmer Road, which is about three miles below Williamston. Woodlands adjoin the river for nearly the full length of this zone. The second impoundment is at a picnic area in Okemos, and the pool behind this stone-ballast dam is used for recreational purposes. The bottom type in this zone ranges from silt and detritus in the pools to sand flats and finally to rubble riffles.

Zone III includes the polluted and recovery areas of the stream below the Williamston sewage treatment plant and is two and one-half miles long. This zone begins at Zimmer Road and ends at the dam in Williamston. The river flows through an urban area for about one mile and then through woodlands for the remainder of zone III. There is a silt and detritus bottom in this zone.

Zone IV includes the section of the river under the influence of the dam at Williamston and is four miles in length, beginning at the dam and extending upstream to Dietz Road. The upper portion of this zone is bordered by woodlands while the lower mile is in the city of Williamston. The bottom material is predominately silt and detritus, although there are occasional areas of hardpan clay or sand. The impoundment at Williamston is truly pond-like while the other two impoundments are better described as slow-flowing river areas.

The fifth zone is twelve miles long and includes the remainder of the study section. This zone is bordered by woodlands and pastures.

Sand and gravel make up the bottom material in the lower portion of this zone, while in the upper dredged portion, silt covers the bottom to a depth of 18 inches. Zone V receives plating wastes and raw sewage at Fowlerville.

METHODOLOGY

The Aufwuchs

The method used in this study to measure the production of aufwuchs was a modification of the method described by Grzenda and Brehmer (1960).

The aufwuchs communities of the Red Cedar River were collected on artificial substrata. The substrata used were cut from flat sheets of $\frac{1}{4}$" plexiglass. Each substrate had a total exposed area, including the sides of 1.4 dm^2 when attached to the supporting racks. These supporting racks were made of a wood crossbar bolted securely to a steel fence post which was driven into the bottom of the stream. Number 125 Acco clamps were attached with screws to this crossbar and the substrata were held securely in these pinch clamps. The clamps were attached directly to the top of the crossbar for the horizontal placement of substrata but were attached to small blocks of wood, which were attached to the crossbar, for the vertical placement of the substrata. Both vertically and horizontally placed substrata were oriented with the $\frac{1}{4}$" edge facing into the current.

The exposure depth of the substrata was maintained at six tenths of the distance from the water surface to the bottom of the river. This made possible the comparison of the accrual rate of aufwuchs in different areas of the river.

Six different exposure periods were allowed for the accrual of aufwuchs; these being 3, 6, 9, 12, 15, and 18 days. All substrata were placed in pairs; that is, there were two substrata placed on the rack for each of the exposure periods. In order to reduce the amount of time spent in collecting the substrata, the following method was used. On day zero the 3, 12, and 18 day substrata were placed on the rack, which was placed at some randomly predetermined point in the river. Since paired substrata were used throughout, there were six separate substrata on the racks when they were placed in the river. After three days, the 3 day substrata were removed and the 9 and 15 day substrata were placed on the rack. Nine days later the 9 and 12 day substrata were removed and the 6 day substrata were placed on the rack. On the eighteenth day the 6, 15, and 18 day substrata were removed and the racks were removed for relocation.

When the substata were removed from the river, they were placed individually in plastic freezer bags and were frozen. Freezing aided in the removal of the accrued material from the substrata and ruptured the plant cells, thereby aiding in the chlorophyll extraction. After freezing, the macrofauna were removed and the accrued material was scraped from the plexiglass substrata with pieces of hard rubber, and rinsed with 95% ethanol. The hard rubber did not remove any of the plexiglass. After the material was removed from the substrata, the phytopigment was extracted by the addition of more 95% ethanol. The bottles containing the accrued material and the ethanol were placed in a refrigerator for a minimum of 48 hours. Grzenda and Brehmer (1960) found that samples can be stored in this manner for at least 30 days without decomposition of the phytopigment.

From this point on, two different methods of treatment of the aufwuchs samples were used. The first of these methods was designed to use the phytopigment absorbancy as a means of estimating organic accumulation. The second method was used to determine the relationships between phytopigment absorbancy and organic weight.

In the first method the samples were removed from the refrigerator, filtered through glass wool, and adjusted to 50 ml, either by evaporation or by the addition of 95% ethanol. The phytopigment absorbancy was determined in a Klett-Summerson colorimeter using a 4 cm solution depth and a red filter (640-700 mμ).

Grzenda and Brehmer (1960) found that the absorption of broad spectrum light (640-700 mμ) is not linearly related to the concentration of ethanol-phytopigment extracts except at very low concentrations. Therefore, due to this deviation from the Lambert-Beer law, the measured absorbancies must be corrected. Since Grzenda and Brehmer based this method on work done in the Red Cedar River, their correction graph was used in this study to correct for this deviation. The corrected absorbancy measurements for the phytopigment-ethanol solutions are referred to as phytopigment units. Thus, 1 adjusted absorbancy unit is equal to 1 phytopigment unit.

Grzenda and Brehmer found that the relationship between phytopigment units and organic weight was such that estimates of the organic weight of an aufwuchs sample could be determined from the phytopigment absorbancy, where it was not greater than 1.3 phytopigment units. Beyond 1.3 phytopigment units the deviation from the Lambert-Beer law is too great to allow correction of the measured absorbancy.

In the second method, the aufwuchs samples were filtered through type HA Millipore Filters with a pore size of 0.45 μ. The absorbancy of the ethanol-soluble phytopigments was determined in the same manner as in the first method. The following procedure was used to determine the organic weight of the material from the substrata. Porcelain evaporating dishes were fired in a muffle furnace until each dish was at constant weight $\pm$ 0.5 mg. After the tare weight of the dish was determined, the Millipore filter was placed in the dish and another weight was determined. A subtraction gave the weight of the Millipore filter.. It was necessary to weigh each Millipore filter because they were found to vary as much as $\pm$ 1 mg.

After the weight of the Millipore filter was determined, the sample was filtered and the absorbancy of the phytopigment extract was determined in the same manner as in the first method. The filter, along with the accompanying residue, was placed back into the dish and the ethanol-phytopigment solution was poured into the dish after the absorbancy determination. The entire sample was then dried at 55°C and the alcohol evaporated, leaving just the sample and the Millipore filter.

When the sample was completely dried, it was weighed again. This value less the tare weight of the dish and the weight of the Millipore filter gave the dried weight of the accrued material. The dish containing the sample was then fired at 550°C in a muffle furnace and upon cooling was weighed again. Since the Millipore filter is ashless, the difference between the dry weight and the ashed weight is the ash free dry weight, or organic weight of the sample.

The Macrophytes

The aquatic macrophytes were sampled at various times throughout the summer at randomly selected sites in each of the five river zones. Each randomly selected site was 100 feet long, and 10 individual samples of one square foot were collected within the 100 foot stratum at randomly selected points. Each square foot sample contained all of the macrophytes, including the roots, attached within the inscribed area.

After collection, the macrophytes were washed to remove all of the silt and sand. This washing did not remove all of the invertebrates or the periphyton growth. These washed samples were drained for a constant period of time and were weighed on a dietetic balance. These wet weight measurements were converted to dry weight by means of the conversion factor derived by Vannote (1963).

After the dry weight of the standing crop was determined, the production rate for each zone was estimated by dividing the average standing crop for a given zone by the number of days in the growth period.

The Invertebrates

All of the invertebrate samples, with the exception of those from the deeper, impounded water of zones I and IV, were taken with a Petersen dredge. The deeper water impoundments necessitated the use of a boat, and an Ekman dredge was used to take the invertebrate samples in these areas. The dredges were chosen as samplers because the current in most areas of the Red Cedar River is not strong enough to sweep the material into the bag on a Surber sampler while the deeper holes preclude the use of a Hess-type sampler.

All sampling sites were randomly selected. The zone to be sampled was first divided into mile units, one of which was selected from a table of random numbers. The chosen mile was then subdivided into portions 1/10 of a mile in length, with one 1/10 of a mile portion being selected from the table of random numbers. The selected 1/10 of a mile portion was then further sub-divided into tenths, one-tenth of which was selected from a table of random numbers. Thus, the area selected for sampling was 0.01 mile, or 52.8 feet long. This is as small an area as it is practical to use for this type of survey, since it is difficult to pinpoint a location on the stream smaller than 0.01 mile.

After the sampling site had been chosen, the sampling points along the transect were chosen by selecting several numbers from the table of random numbers. Ten numbers were selected for all samples during 1961. These numbers were divided into two categories designated even and odd. Every other number was designated even with the intervening numbers being odd. A cable, graduated in feet, was stretched across the river at the randomly selected location. One dredge sample was taken at each of the randomly selected points along this transect. The sample was thus divided into two sub-samples, designated even and odd which were kept separate through all subsequent handling.

After the dredge hauls were taken, the bottom material was screened with a 30-mesh sieve to remove silt and the finer sand. The residue was then placed in buckets and removed to the laboratory. The organisms were picked alive from the bottom material by flotation, utilizing a concentrated sugar solution, the same day they were collected. All final sorting was done by hand, and all organisms were preserved in a 70% ethanol solution containing 5% glycerine.

All organisms were separated into ordinal groups and the aquatic insects were separated into family groups. Prior to weighing, the organisms were removed from the preservative and soaked in tap water for thirty minutes. They were then placed in small 30-mesh screens and spun in a centrifuge for thirty seconds at 1800 r.p.m. to remove the excess moisture. Wet weight was then determined on an analytical balance accurate to 0.001 gm. The weight obtained by this procedure very closely approximates live weight.

Calorimetry

The caloric values of representatives of various trophic levels from the Red Cedar River were determined with a series 1300 plain type Parr Oxygen Bomb Calorimeter according to the methods given in Oxygen Bomb Calorimetry and Combustion Methods, Parr Instrument Company, Manual No. 130, 1960. All samples used for these determinations were dried at 55°C, powdered, and formed into pellets with a Series 2811 Parr pellet press.

RESULTS AND DISCUSSION

PRIMARY PRODUCTION

The Aufwuchs

In aquatic environments there is a great assemblage of organisms which live attached to natural submerged objects. These heterogeneous communities include bacteria, fungi, algae, and often higher forms of plant and animal life. These communities have been described variously, e.g. benthos, periphyton, and aufwuchs. Ruttner's (1953) definition of aufwuchs is used because it includes both attached and free-living organisms and is not as restrictive as the term periphyton. Therefore, the communities which are firmly attached but do not penetrate a natural or artificial substrate are referred to here as aufwuchs.

The technique of collecting the aufwuchs communities from natural waters by means of artificial substrata has been employed for many years. Hentschel (1916) is generally credited with being the first to use this method, but Abdin (1949), Butcher (1932), Castenholz (1960), Ivlev (1933), Grzenda and Brehmer (1960), Kevern (1962), Newcombe (1949, 1950), Patrick et al. (1954), and many others have used artificial substrata to study aufwuchs growth, production, and sucession. Cook (1956) reviewed the history and the general methods of collecting aufwuchs on artificial substrata.

Direct microscopic examination of the exposed substrata has been used by many workers, but Newcombe (1949) did not consider the species composition and was the first to deal only with the production of total organic matter. Grzenda and Brehmer (1960) give a method of estimating aufwuchs production on artificial substrata from phytopigment extracts. They cite Hooper, Ball, and Hayne (ms) as being the first to combine the use of artificial substrata with the phytopigment extract method used by Harvey (1934), Kreps and Verjbinskaya (1930) and Manning and Juday (1941).

Samples of the aufwuchs community in the Red Cedar River were collected during the summers of 1959, 1960, and 1961. The collection dates of these samples are shown in Table 1. Each series consisted of paired samples of accrued material from 3, 6, 9, 12, 15, and 18 day exposure periods from a randomly selected point in each of the five river zones. Horizontal placement of substrata was used in 1959 and 1960, but both horizontal and vertical placement of the substrata were used for the 1961 collections.

The 1959 and 1960 aufwuchs samples were analyzed only for phytopigment absorbancy; i.e., phytopigment units. In 1961, the accrued material from all vertically and horizontally placed substrata from series 1, 3, and 5 was analyzed for both phytopigment absorbancy (phytopigment units) and organic weight. All aufwuchs samples from series 2 and 4 were analyzed only for phytopigment units.

Since more extensive collections of the aufwuchs community are available for 1961 than for the other years, these collections will be discussed first and will be compared later with the 1959 and 1960 collections. The randomly selected aufwuchs sampling locations for the summer of 1961 are given in Table 2.

1961 Aufwuchs Production

Statistical considerations: Organic weight determinations were made gravimetrically for all aufwuchs samples from both vertical and horizontal substrata from series 1, 3, and 5. In order to obtain organic weights for the aufwuchs samples from time series 2 and 4, it was necessary to determine regressions of organic weight on phytopigment units.

Table 1. Aufwuchs sampling periods on the Red Cedar River for the summers of 1959, 1960, and 1961. Horizontal placement of substrata in 1959 and 1960, both vertical and horizontal placement in 1961. Samples were taken from each of the five river zones during each placement.

1959		
	Time Series 1	June 27-July 15, 1959
	Time Series 2	July 17-August 4, 1959
	Time Series 3	August 10-August 28, 1959
	Time Series 4	August 29-September 16, 1959
1960		
	Time Series 1	July 20-August 7, 1960
	Time Series 2	August 18-September 5, 1960
	Time Series 3	September 5-September 23, 1960
1961		
	Time Series 1	June 12-June 30, 1961
	Time Series 2	June 30-July 18, 1961
	Time Series 3	July 18-August 5, 1961
	Time Series 4	August 5-August 23, 1961
	Time Series 5	August 23-September 10, 1961

Table 2. Randomly selected aufwuchs sampling locations for the summer of 1961. River mile zero is at the Farm Lane bridge on the Michigan State University Campus.

Time Series*	River Zone	River Mile
1	I	3.2
1	II	6.9
1	III	13.8
1	IV	17.5
1	V	23.1
2	I	2.5
2	II	9.8
2	III	14.4
2	IV	16.3
2	V	20.9
3	I	0.3
3	II	7.1
3	III	13.5
3	IV	18.2
3	V	19.6
4	I	0.9
4	II	8.7
4	III	13.0
4	IV	16.5
4	V	22.3
5	I	3.0
5	II	10.6
5	III	13.7
5	IV	16.5
5	V	29.0

*Refer to Table 1 for dates.

Regressions of organic weight on phytopigment units were calculated for the vertical and horizontal substrata from series 1, 3, and 5. These six regressions were subjected to an analysis of covariance to determine their common relationships. This analysis indicated that there was a significant difference at the .05 level in the slopes of these regressions (Table 3). Therefore, a common regression, calculated from the six regressions, could not be used as a predictor equation for the organic weight fraction on the substrata from series 2 and 4. Since the substrata from series 5 were in the river during a period of heavy siltation, it was thought that perhaps series 5 could be deleted from these calculations and that a predictor equation could be calculated from the vertical and horizontal substrata from series 1 and 3. When these four regressions were subjected to an analysis of covariance, the slopes were found to be not significantly different at the .05 level. However, there was a significant difference in the intercepts of these four regressions at the .05 level (Table 4).

This negated the possibility of using a common predictor equation for all zones in series 2 and 4 because the high intercept of the common regression introduced appreciable error in those samples containing small amounts of phytopigment and correspondingly small amounts of organic matter.

This variation in the phytopigment to organic weight relationship in the various zones was further pointed out by a three way analysis of variance of the phytopigment x 10^3: organic weight ratio, testing time series, river zones, and vertical and horizontal placement. To obtain this ratio, the phytopigment units of a given sample were multiplied by 10^3 and this value was divided by the number of milligrams of organic weight in that sample. For this test, six of these ratios were selected randomly from each vertical and each horizontal placement in each zone from each of time series 1, 3, and 5. The results of this analysis

Table 3. Summary of the analysis of covariance of the organic weight phytopigment regressions from both vertical and horizontal placement of substrata from series 1, 3, and 5.

Nature of Variation	Symbol	Value	Degrees of Freedom
Group regression coefficients about common coefficient	S_1	11,460.16	5
Scores about regression line for their own group	S_2	411.44	325

$F = \frac{S_1}{S_2} = 27.854$ $F_{95}\ (5,\ 325) = 2.24$

27.854 > 2.24 Therefore, the slopes are significantly different at the .95 level

Table 4. Summary of the analysis of covariance of the organic weight phytopigment regressions from both vertical and horizontal placement of substrata from series 1 and 3.

Nature of Variation	Symbol	Value	Degrees of Freedom
Group regression coefficient about common coefficient	S_1	1,027.91	3
Scores about regression line for their own group	S_2	577.02	211
Scores about regression line with common slope b_w	S_w	583.34	214
Group means about regression line with slope b_w	S_b	6,047.56	3

$F = \frac{S_1}{S_2} = 1.78 \qquad F_{95}\,(3,\ 211) = 2.65$

$1.78 < 2.65$ Therefore, the slopes are not significantly different at the .95 level.

$F = \frac{S_b}{S_w} = 10.367 \qquad F_{95}\,(3,\ 214) = 2.65$

$10.367 > 2.63$ Therefore, the intercepts are significantly different at the .95 level.

indicated a significant difference at the .05 level between series, between zones, and between vertical and horizontal placement of the substrata (Table 5).

Although there was a significant effect indicated for both time series and river zones, the interpretation of these results must be tempered by the fact that a significant interaction existed between those two variables. This interaction negates the use of a multiple comparison test on the overall series and zone means. Since an estimate of the overall effect is not possible, due to that part of the mean attributed to interaction effect not being zero, the particular effect must be studied only with respect to a given condition. Therefore, multiple comparisons were made by Tukey's method on each effect under each condition. The results of these comparisons are given in Table 6.

The significant differences between the three time series, between the five river zones, and between vertical and horizontal placement indicate that a common regression should not be used to determine the organic weight fraction of the substrata from series 2 and 4. Since it was necessary to obtain organic weight estimates for series 2 and 4, and in order to minimize the introduced error in those samples containing small amounts of pigment, separate predictor equations were calculated for the vertical and horizontal substrata in each of the five river zones, utilizing all data from series 1, 3, and 5 from each zone. These predictor equations are given in Table 7. The organic weight which accrued on the substrata of series 2 and 4 was calculated by the use of these predictor equations and the measured phytopigment content of the accrued growth on these substrata.

Average estimates of the organic matter accrual rate on the artificial substrata for all sampline periods during the summer of 1961 are given in Table 8. The 95% confidence limits for the rate of accumulation of organic matter on the horizontal substrate are ± 28.65 mg. organic

Table 5. Summary of a three-way analysis of variance of the phyto-pigment unit x 10^3 : mg. organic weight ratio; testing time series, river zone, and vertical and horizontal placement of the substrata from time series 1, 3, and 5 during the summer of 1961.

Source	Sum of Squares	df	Mean Square	F_{exp}	$F_{.05}$
Series (R)	432.5326	2	216.2663	6.5303*	3.06
Zones (C)	377.5962	4	94.3990	2.8504*	2.43
Placement (L)	156.7626	1	156.7626	4.7335*	3.91
R-C interaction	754.2972	8	94.2871	2.8470*	2.00
C-L interaction	235.3894	4	58.8473	1.7769	2.43
R-C-L interaction	31.2876	8	3.9109	0.1180	2.00
R-L interaction	2.8616	2	1.4308	0.0432	3.06
Within	4,967.5507	150	33.1170		

*Denotes significance at the .05 level.

Table 6. Summary of the multiple comparisons made from the results of the three-way analysis of variance of the phytopigment x 10^3: mg. organic weight ratio of series 1, 3, and 5. The level of significance is .05 and the values given are mean values with units of phytopigment units x 10^3 mg. organic weight^{-1}.

Zone I:

Series 1	Series 3	Series 5
11.6125	5.3016	9.1841

Series 3 significantly different from series 1 and 5

Zone II:

Series 1	Series 3	Series 5
10.5508	14.0583	7.5850

Series 5 significantly different from series 1 and 3 and series 3 is significantly different from series 1.

Zone III:

Series 1	Series 3	Series 5
6.2275	10.6666	1.9050

Series 5 significantly different from series 1 and 3 and series 3 significantly different from series 1.

Zone IV:

Series 1	Series 3	Series 5
10.9816	11.2966	8.7233

No significant difference between series.

Zone V:

Series 1	Series 3	Series 5
10.2208	11.6525	7.7116

Series 5 significantly different from series 3.

Series 1:

Zone I	Zone II	Zone III	Zone IV	Zone V
11.6125	10.5508	6.2275	10.9816	10.2280

Zone III significantly different from all others.

Series 3:

Zone I	Zone II	Zone III	Zone IV	Zone V
5.3016	14.0583	10.6666	11.2966	11.6525

Zone I significantly different from all others and zone II is significantly different from zone III.

Series 5:

Zone I	Zone II	Zone III	Zone IV	Zone V
9.1841	7.5850	1.9050	8.7233	7.7116

Zone III significantly different from all others.

Table 7. **Regressions of phytopigment units vs. organic weight calculated from series 1, 3, and 5 and used to determine the organic weight accrual from series 2 and 4 substrata. The regression formula is $\hat{Y} = a + bX$, where Y refers to organic weight and X to phytopigment units.**

Zone	Horizontal	Vertical
I	$\hat{Y} = 0.667 + 155.799\ X$	$\hat{Y} = 11.648 + 34.741\ X$
II	$\hat{Y} = 5.029 + 86.919\ X$	$\hat{Y} = 7.144 + 78.050\ X$
III	$\hat{Y} = 17.093 + 58.352\ X$	$\hat{Y} = 15.606 + 72.312\ X$
IV	$\hat{Y} = 13.911 + 83.267\ X$	$\hat{Y} = 8.341 + 68.052\ X$
V	$\hat{Y} = 12.692 + 69.482\ X$	$\hat{Y} = 6.178 + 85.694\ X$

Table 8. Average organic matter accumulation on vertical and horizontal substrata in the Red Cedar River during the summer of 1961. Units are mg. organic matter M^{-2} day^{-1}.

Time Series	River Zone	Horizontal placement of substrata	Vertical placement of substrata
1	I	291.1	153.5
1	II	494.8	480.9
1	III	268.5	322.5
1	IV	253.7	262.9
1	V	323.8	210.6
2	I	689.1	305.1
2	II	453.2	349.3
2	III	359.8	349.4
2	IV	664.8	414.7
2	V	416.9	467.6
3	I	156.1	165.8
3	II	286.4	340.4
3	III	324.2	467.9
3	IV	360.0	473.8
3	V	534.2	314.9
4	I	241.7	171.3
4	II	225.4	155.8
4	III	238.2	253.1
4	IV	442.1	294.0
4	V	417.1	546.6
5	I	116.0	61.4
5	II	65.9	46.9
5	III	147.7	151.0
5	IV	301.4	199.0
5	V	213.3	216.0

weight M^{-2} day^{-1}, or ± 8.77% of the mean value of 326.5 mg. organic weight M^{-2} day^{-1}; and for the vertical substrata they are ± 22.42 mg organic weight M^{-2} day^{-1}, or ± 7.90% of the mean value of 283.8 mg. organic weight M^{-2} day^{-1}. The 95% confidence limits for the rate of accrual of organic matter for any sample regardless of placement, time series, or river zone are ± 41.04 mg organic weight M^{-2} day^{-1}, or ± 13.48% of the mean of 305.2 mg. organic weight M^{-2} day^{-1}.

The organic weight fractions of the aufwuchs samples collected in 1961 from all vertically and horizontally placed substrata were subjected to a matched pairs t test to test for differences in organic weight accrual on the two substrate placements. The results of this test ($t = 1.2845$) indicated that there was no significant difference between organic weight accrual on vertical and horizontal substrata at the .05 level and further that there was no significant difference at the .10 level. There was, however, a significant difference indicated at the .20 level, with the horizontal substrata having more organic weight than the vertical.

The next step was to test for differences in the production rate for the six exposure periods. The standing crop of organic weight for each substrate was adjusted to mg. organic weight M^{-2} and this value was divided by the number of days in that particular exposure period. The resulting value, mg. organic weight M^{-2} day^{-1}, was the production rate for that period. Thirty randomly selected production rates from each of the six exposure periods were subjected to a one way analysis of variance. The results (Table 9) indicated no significant difference at the .95 level in the production rate of organic material among the six exposure periods, in fact, the probability of a significant difference was less than 0.01.

There being no significant difference in the production rates for the six exposure periods, the data were subjected to a three way

Table 9. Summary of the one-way analysis of variance testing the organic accrual rates on the 3, 6, 9, 12, 15, and 18 day exposure periods of the artificial substrata.

Source of Variation	Sum of Squares	df	Mean Square	F Ratio
Exposure Periods	35,774.45	5	7,154.89	$F = \frac{7,154.45}{55,519.59} = 0.1288$
Within	9,660,409.24	174	55,519.59	$F_{.95}(5, 174) = 2.26$
Total	9,696,183.69	179		

$F_{exp} = 0.1288$ $F_{.95}(5, 174) = 2.26$ Therefore, there is no significant difference at the .95 level between the accrual rate of organic material on the artificial substrata from the six exposure periods.

analysis of variance, testing time series, river zones, and vertical and horizontal placement of substrata. For this test, six production rates were randomly selected for each vertical and horizontal placement in each series in each zone. Thus, 300 production rates were considered in this analysis.

The results of this test show a significant difference in the accural rates of organic matter at the .05 level between zones and between series but no significant difference was shown between vertical and horizontal placement at the .05 level (Table 10). There was, however, a significant difference indicated in the accrual rate at the .10 level between vertical and horizontal placement.

A significant interaction at the .05 level between series and zones was shown in this test of accrual rates in the same manner as in the test of phytopigment to organic weight ratios discussed previously. For the same reasons cited there a multiple comparison test can not be made on the overall series and zones organic accrual rates. Therefore, as in the other analysis, multiple comparison tests were made by Tukey's method on each effect under each condition. The results of this analysis are shown in Table 11.

Substrate placement and organic loss on removal: Net aufwuchs production was measured in all five study zones from June 12 to September 10, 1961. The algae present in the aufwuchs were almost exclusively diatoms and the most common were *Gomphonema*, *Navicula*, *Fragilaria*, *Cymbella*, *Cyclotella*, and *Synedra*. Peters (1959) found that the plexiglass artificial substrata were not selective but had the same dominant attached organisms as did the natural substrata in the stream. This observation has also been reported by Butcher (1932) and Patrick, Horn, and Wallace (1954) for colonization of algae on glass slides.

Table 10. Summary of the three way analysis of variance of the accrual rate of organic matter (mg. organic weight M^{-2} day^{-1}) testing time series, river zones, and vertical and horizontal placement of the substrata from series 1, 2, 3, 4, and 5 during the summer of 1961.

Source of Variation	Sum of Square	df	Mean Square	F_{exp}	$F_{.95}$
Series (R)	2,394,554.17	4	598,638.54	22.936*	2.41
Zones (C)	1,089,770.12	4	272,442.52	10.438*	2.41
Placement (L)	83,326.67	1	83,326.67	3.192	3.89
R-C interaction	1,700,467.18	16	106,279.19	4.072*	1.69
C-L interaction	234,326.31	4	58,581.57	2.244	2.41
R-L interaction	121,360.33	4	30,340.08	1.162	2.41
R-C-L interaction	593,664.52	16	37,104.03	1.422	1.69
Within	6,525,028.71	250	26,100.11		

*Denotes significance at the .05 level.

Table 11. Summary of the multiple comparisons made from the results of the three way analysis of variance of the accrual rate of organic matter on the substrata during the summer of 1961 in the five series and five zones.

Comparison of Time Series:

Zone I:

series	1	2	3	4	5
1		X	0	0	X
2			X	X	X
3				0	0
4					X

Zone II:

series	1	2	3	4	5
1		X	X	X	X
2			0	X	X
3				X	X
4					X

Zone III:

series	1	2	3	4	5
1		X	X	0	X
2			0	X	X
3				X	X
4					X

Zone IV:

series	1	2	3	4	5
1		X	X	X	0
2			X	X	X
3				0	X
4					X

Zone V:

series	1	2	3	4	5
1		X	X	X	X
2			0	0	X
3				0	X
4					X

Comparison of River Zones:

Series 1:

zones	I	II	III	IV	V
I		X	X	X	X
II			X	X	X
III				0	0
IV					0

Series 2:

zones	I	II	III	IV	V
I		X	X	0	0
II			0	X	X
III				X	X
IV					0

Series 3:

zones	I	II	III	IV	V
I		X	X	X	X
II			X	X	X
III				0	0
IV					0

Series 4:

zones	I	II	III	IV	V
I		0	0	X	X
II			0	X	X
III				X	X
IV					X

Series 5:

zones	I	II	III	IV	V
I		0	0	X	X
II			X	X	X
III				X	X
IV					0

X Denotes a significant difference at the .05 level.
0 Denotes no significant difference at the .05 level.

Prior to the summer of 1961, all aufwuchs collections were made with horizontally arranged substrata. At that time it was felt that the deposition of organic debris was causing an over estimation of the net production of aufwuchs. Therefore, in the summer of 1961 vertical placement of the substrata was used as well as horizontal placement to measure the accrual of organic matter.

Castenholz (1960) in a study of Falls Lake, Washington, found that the organic weight of material collected from vertically and horizontally placed substrata was in a ratio of 1:6.2 during the summer. This value agreed well with Newcombe's (1949) value of 1:6.6 for the ratio of organic weight from vertically and horizontally placed substrata during the summer in a Michigan lake. The average value of this ratio in the Red Cedar River during the summer of 1961 was 1:1.15. Both Castenholz and Newcombe used horizontal placement and indicated that they felt that this placement was better than the vertical. Newcombe further indicated that the loss of organic matter upon removal of the substrata from the water was greater on the vertical than on the horizontal in Sodon Lake. In the Red Cedar River the opposite was found to be true; there being a greater loss upon removal of the substrata from the water from the horizontal than from the vertical.

The Red Cedar is a turbid stream and the algae colonize in and on the silt settling on the horizontal substrata; whereas they grow directly attached to the plexiglass of the vertical substrata. The net result is that upon removel of the substrate from the water, less of the accumulated material is lost from the vertical, where the algae adhere directly to the plexiglass, than from the horizontal, where the algae are not attached directly to the substrate. Because of these differences in attachment, more of the outer, more actively growing portion of the diatom colony is lost from the horizontal than from the vertical substrata.

Organic production of aufwuchs: The organic matter which collects on the artificial substrata in natural waters is made up of three components: 1) organic matter settling out of suspension, 2) autotrophic growth, and 3) heterotrophic growth. More material, both organic and inorganic, settles out of suspension on the horizontally placed substrata than on the vertical. The inorganic material can be separated easily from the organic but the difficulty lies in separating organic sediment from the organic matter produced on site. It is assumed that autotrophic and heterotrophic growth accumulates on the vertical substrata at a rate equal to its accumulation on the horizontal. If the rate of accumulation of both organic and inorganic matter is known for both vertically and horizontally placed substrata, the following method allows separation of organic growth and organic sediment.

Let X = the amount of organic matter settling on the vertically placed substrata.

Y = the amount of organic matter settling on the horizontally placed substrata in excess of that which settles on the vertically placed substrata.

$X+Y$ = the amount of organic matter settling on the horizontally placed substrata.

M = the amount of inorganic matter settling on the vertially placed substrata.

N = the amount of inorganic matter settling on the horizontally placed substrata.

Assume that organic material settles at the same ratio to the inorganic material on the vertical as on the horizontal substrata.

Then: $$\frac{M}{X} = \frac{N}{X+Y}$$

$$MX+MY = NX$$

$$MY = NX - MX \text{ or, } MY = X(N-M)$$

and finally, $$X = \frac{MY}{N-M}$$

Values available for illustrating this method are the following:

283.8 mg. organic weight M^{-2} day^{-1} = the average weight of accrued organic matter on the vertical substrata from the Red Cedar River during the summer of 1961.

326.5 mg. organic weight M^{-2} day^{-1} = the average weight of accrued organic matter on the horizontal substrata from the Red Cedar River during the summer of 1961.

533.5 mg. inorganic weight M^{-2} day^{-1} = the average weight of the accrued inorganic matter from the vertical substrata from the Red Cedar River during the summer of 1961.

925.5 mg. inorganic weight M^{-2} day^{-1} = the average weight of the accrued inorganic matter from the horizontal substrata from the Red Cedar River during the summer of 1961.

Since the algae found on the substrata were almost exclusively diatoms, a correction must be made for the weight of their silica shells. Burlew (1953) found diatoms to be from 35% to 46% inorganic material, while Castenholz (1960) found the diatoms of Washington lakes to range from 42% to 55% inorganic matter. Strickland (1960) suggests that 50% be used for the inorganic percentage of diatoms, and that value will be used here.

Using the above value, the inorganic accrual rate of the artificial substrata due to production of diatom frustules is roughly equal to the organic accrual rate. That portion of the inorganic accrual due to settling of inorganic material from suspension is found as follows:

533.5 - 283.8 = 249.7 mg. inorganic matter M^{-2} day^{-1} on the vertical substrata.

925.5 - 326.5 = 599.0 mg. inorganic weight M^{-2} day^{-1} on the horizontal substrata.

Using these values and the formula $X = \frac{MY}{N-M}$, the net production of organic matter due to autotrophic and heterotrophic growth is found as follows:

Y = the average organic accrual on the horizontal substrata less the average organic accrual on the vertical substrata.

or, $Y = 326.5 - 283.8 = 42.7$ mg. organic weight M^{-2} day^{-1}

$M = 249.7$ mg. inorganic matter M^{-2} day^{-1}

$N = 599.0$ mg. inorganic matter M^{-2} day^{-1}

then; $X = \frac{MY}{N-M} = \frac{(249.7)\ (42.7)}{599.0 - 249.7} = \frac{10,662.19}{349.3} = 30.5$ mg. organic weight M^{-2} day^{-1}

Therefore the amount of organic debris settling on the vertical substrata is 30.5 mg. M^{-2} day^{-1}, and 30.5 + 42.7 or 73.2 mg M^{-2} day^{-1} of organic matter settles from suspension onto the horizontal substrata. The net production rate of organic matter by autotrophic and heterotrophic growth is equal to $283.8 - 30.5 = 326.5 - 73.2 = 253.3$ mg. M^{-2} day^{-1}.

A further indication of the validity of this correction is found in the comparison of the phytopigment to organic weight ratios of the accrued material from the artificial substrata. This ratio is calculated by dividing the phytopigment content of a given sample in phytopigment units x 10^3 by the organic weight of the same sample in milligrams.

For the summer of 1961, the ratio of phytopigment to organic weight for the vertical substrata was 10.06 and for the horizontal substrata it was 8.27. If the organic weight estimate is multiplied by the ratio of phytopigment to organic weight, the result is the phytopigment content of the accrued material in phytopigment units. The accrued material on the vertical substrata had a phytopigment content of 2,855,028 phytopigment units M^{-2} day^{-1}, while the material on the horizontally placed substrata had 2,692,712 phytopigment units M^{-2} day^{-1}.

If these phytopigment-content values are divided by 253.3, the corrected organic production calculated above, the results are 11.27 phytopigment units per mg. organic weight^{-1} for the accrued material from the vertical substrata and 10.63 for the material from the horizontal substrata. The ratio of the initial uncorrected phytopigment to organic weight relationship of accrued material of horizontal to vertical substrata was 8.27:10.06 or, 1:1.22. This ratio after correction was 10.63:11.27 or, 1:1.06.

If autotrophic and heterotrophic production were the same on both the vertically and horizontally placed substrata, this corrected ratio would be 1:1. The experimental corrected ratio is in fact quite close to the theoretical value, indicating that autotrophic and heterotrophic production proceed at about the same rate on the vertically placed substrata as on the horizontally placed substrata.

<u>Colonization rate vs. substrate placement:</u> The accumulation of organic matter on the artificial substrata for the summer of 1961 is given for the six different exposure periods in Table 12 and in Figures IV and V. Figure IV illustrates the accumulation of total organic matter, while Figure V gives accumulation of organic matter corrected for organic sedimentation. This second growth curve of the heterotrophic and autotrophic organisms represents the organic matter actually produced on the plexiglass substrata.

Growth of the aufwuchs (Figure V) occurs at a nearly constant arithmetic rate for exposure periods up to 15 days. At this point the colony stabilizes and the new growth is equal to the organic matter which dies and is sloughed off from the substrata.

It can also be seen from Figure V that the rate of colonization by the aufwuchs is essentially the same for the vertically and horizontally placed substrata. Organic matter, due to on-site growth, from 3 to 15 day exposure periods, accumulated at a rate of 0.2533 grams organic

Table 12. Organic matter accumulation and organic matter accumulation rate, both corrected and uncorrected for organic sedimentation, on the vertical and horizontal substrata from the Red Cedar River during the six different exposure periods in the summer of 1961.

Exposure Period (days)	Horizontal Substrata Accumulation (mg. M^{-2})	Horizontal Substrata Rate (mg. M^{-2} day^{-1})	Vertical Substrata Accumulation (mg. M^{-2})	Vertical Substrata Rate (mg. M^{-2} day^{-1})
Uncorrected for organic sedimentation:				
3	1,208.4	402.8	1,026.3	342.1
6	1,783.8	297.3	1,695.8	282.6
9	2,977.2	330.8	2,552.2	283.6
12	3,782.4	315.2	3,410.5	284.2
15	4,858.5	323.9	4,161.7	277.4
18	5,077.8	282.1	4,195.6	233.1
Corrected for organic sedimentation:				
3	988.8	329.6	934.8	311.6
6	1,344.6	224.1	1,512.8	252.1
9	2,318.4	257.6	2,277.7	253.1
12	2,904.0	242.0	3,044.5	253.7
15	3,760.5	250.7	3,704.2	246.9
18	3,760.2	208.9	3,646.6	202.6

Figure IV. Standing crop of organic matter accrued on the artificial substrata during the summer of 1961 plotted against the six exposure periods.

Figure V. Standing crop of organic matter accrued on the artificial substrata during the summer of 1961, corrected for organic sedimentation plotted against the six exposure periods.

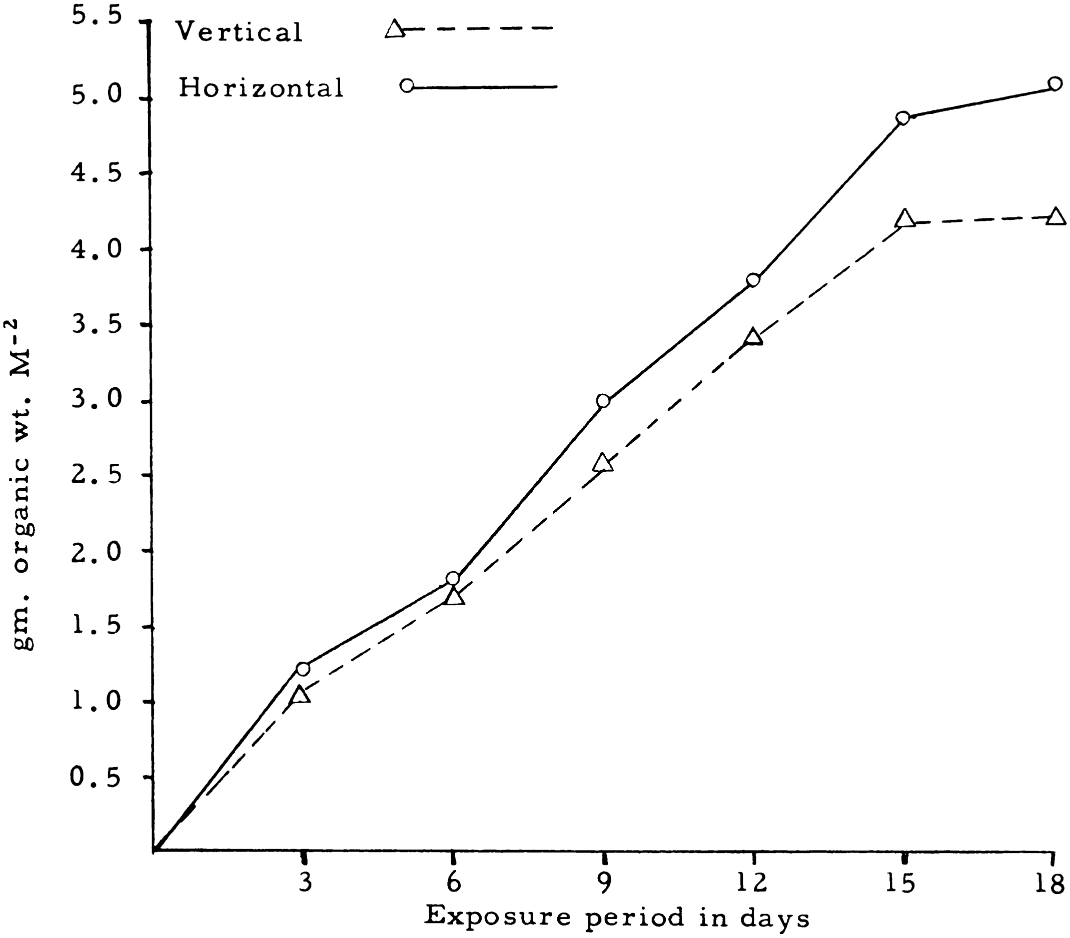


Figure IV

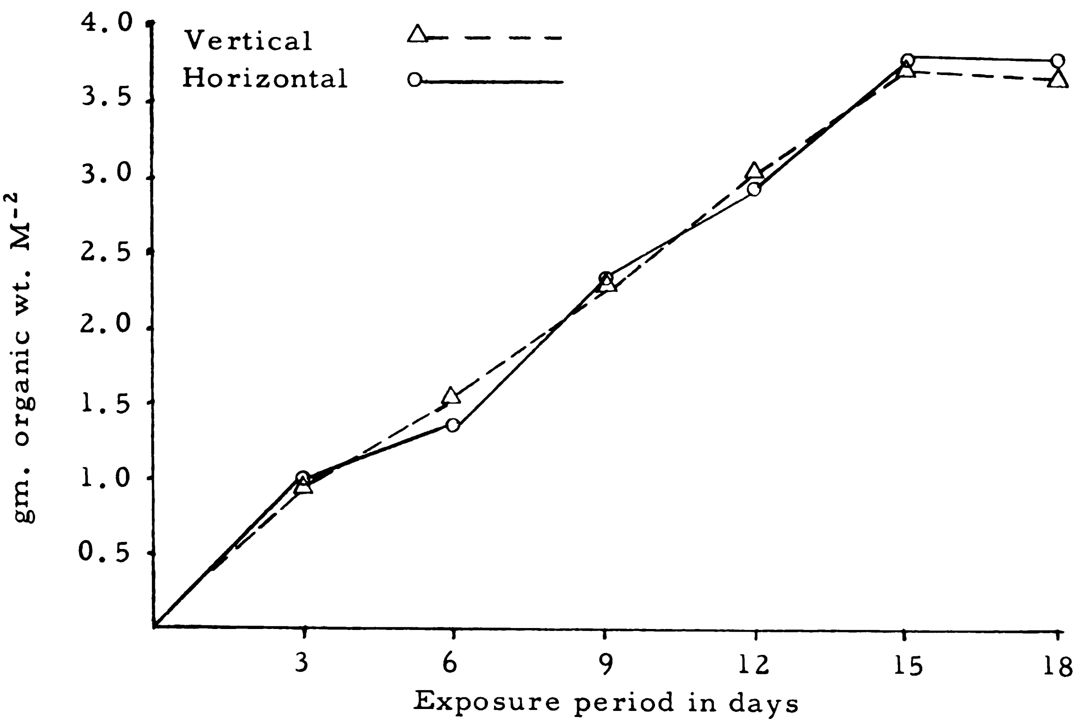


Figure V

matter M^{-2} day^{-1}. Therefore, this value is an estimate of the production rate of the aufwuchs in the Red Cedar River for the summer of 1961.

Inorganic sedimentation: When the total inorganic weight accrual rate on the artificial substrata is corrected for the inorganic weight produced on the substrata by the aufwuchs, the result is the sedimentation rate of inorganic material from the water. Results of this calculation for the five river zones during the summer of 1961 are shown in Table 13 and Figure VI.

The greatest rate of inorganic sedimentation (Figure VI) on the horizontal substrata took place in zone IV, an impoundment. As the river flows into the impoundment the current velocity decreases and the sediment load of the water tends to settle out. The horizontal substrata have a large flat area exposed, and a considerable amount of inorganic sediment settles on this surface.

The greatest rate of inorganic sedimentation on the vertical substrata took place in zone III. This zone receives the effluent from a sewage treatment plant, and most of it lies below the point of effluent. It has been pointed out (Hynes, 1960) that organisms commonly called sewage fungi, such as the filamentous bacteria *Sphaerotilus*, very often are abundant in sewage polluted water. On the vertical substrata these heterotrophic organisms form filamentous colonies which result in a much greater exposed horizontal area than that formed by the more closely applied diatom colonies. Butcher, et al. (1931) found that *Sphaerotilus* traps large quantities of inorganic material as it grows. It will be shown later that zone III, in fact, does have a much greater concentration of heterotrophic growth than any of the other river zones. This heterotrophic growth branches out from the substrata, and the greatest amount of sedimentation on vertical substrata takes place on the ones with the most exposed horizontal surface.

Table 13. Inorganic weight accrual rate on the artifical substrata during the summer of 1961 in the Red Cedar River, and diatom frustule weight correction for the five river zones. All units are mg. M^{-2} day^{-1}.

River Zone	Horizontal Substrata	−	Ditom Shell Weight	=	Inorganic Sediment on Horizontal Substrata	Vertical Substrata	−	Ditom Shell Weight	=	Inorganic Sediment on Vertical Substrata
I	617.6		298.8		318.8	233.6		173.3		60.3
II	760.4		299.3		461.1	507.9		274.7		233.2
III	1,030.2		267.6		762.8	827.7		308.6		519.1
IV	1,328.4		385.8		942.6	673.2		321.1		352.1
V	903.8		379.8		524.0	393.7		341.4		52.3

Figure VI. Weight of the inorganic sediment settling on the vertical and horizontal substrata per day in each of the five river zones during the summer of 1961.

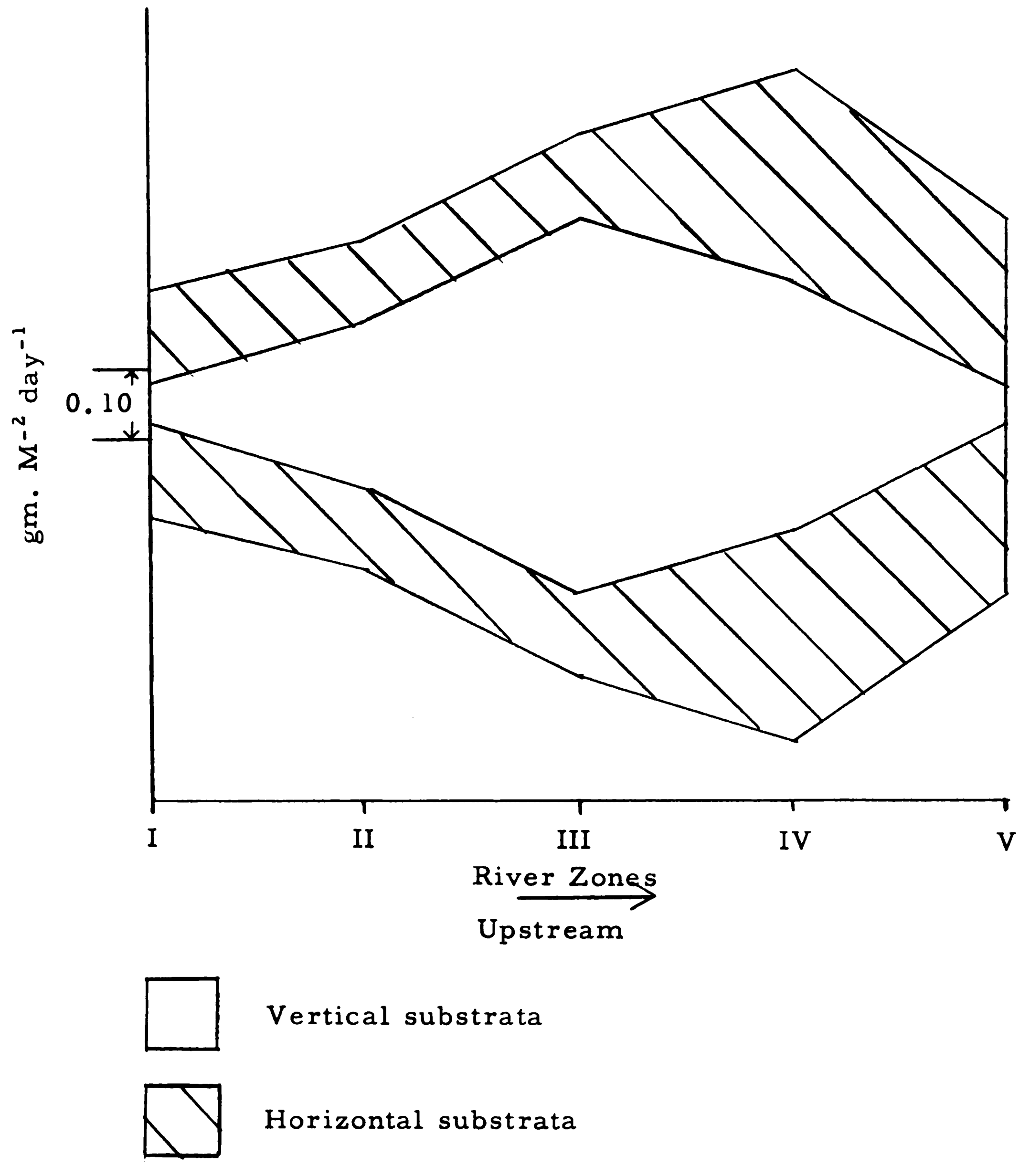

gm. M-2 day-1
0.10
I
II
III
IV
V
River Zones
Upstream
Vertical substrata
Horizontal substrata

Figure VI

Organic sedimentation: When the accumulation rate of total organic material on the artificial substrata is corrected for organic sedimentation, the result is the production rate of organic matter by the aufwuchs. Results of this calculation for the five river zones during the summer of 1961 are shown in Table 14 and Figure VII. Since total organic accrual includes organic sediment and the production of organic matter by aufwuchs, the total organic accrual less organic sediment equals organic production. A positive organic sediment estimate indicates an accrual of organic material in excess of that produced by the aufwuchs, while a negative organic sediment estimate indicates a loss of organic material produced by the aufwuchs.

The negative values for the organic sedimentation rate in zone III (Table 14) indicate that a considerable amount of the aufwuchs production in this zone was lost. Brehmer (1958) working on the river in this same area noted tremendous reductions in the aufwuchs population below Deer Creek following heavy rains. Deer Creek enters the Red Cedar near the upper end of zone III. Another factor, besides the turbidity introduced by Deer Creek, which would tend to scour the aufwuchs from the substrata is the fluctuation in discharge caused by manipulation of the dam at the upper end of zone III.

This dam and a small generator are maintained to provide electrical power for a frozen food plant in Williamston. During the summer when the flow of the river is low, this generator is used intermittently. The impoundment behind the dam is allowed to fill up over a period of several days, and when it is full, is drained rapidly to provide water power to run the generator. This pulse of water often increases river discharge by 2 to 3 times at Farm Lane bridge and the increase in zone III would be much greater. This subject will be discussed more fully later, but is mentioned here as one of the factors causing substantial loss of aufwuchs in zone III.

Table 14. Accrual of organic material on artificial substrata during the summer of 1961 in the five river zones of the Red Cedar given with the organic sediment correction factor. Units are mg. M^{-2} day^{-1}.

River Zone	Horizontal Substrata	− Organic Sediment	= Aufwuchs Production on Horizontal	Vertical Substrata	− Organic Sediment	= Aufwuchs Production on Vertical
I	298.8	154.8	144.0	173.3	29.3	144.0
II	299.3	49.8	249.5	274.7	25.2	249.5
III	267.6	-128.3	395.9	308.6	-87.3	395.9
IV	385.8	103.3	282.5	321.1	38.6	282.5
V	379.8	42.6	337.2	341.4	4.2	337.2

Figure VII. Aufwuchs production rate and accrual rate of organic matter on the vertical and horizontal substrata in the five river zones of the Red Cedar River during the summer of 1961.

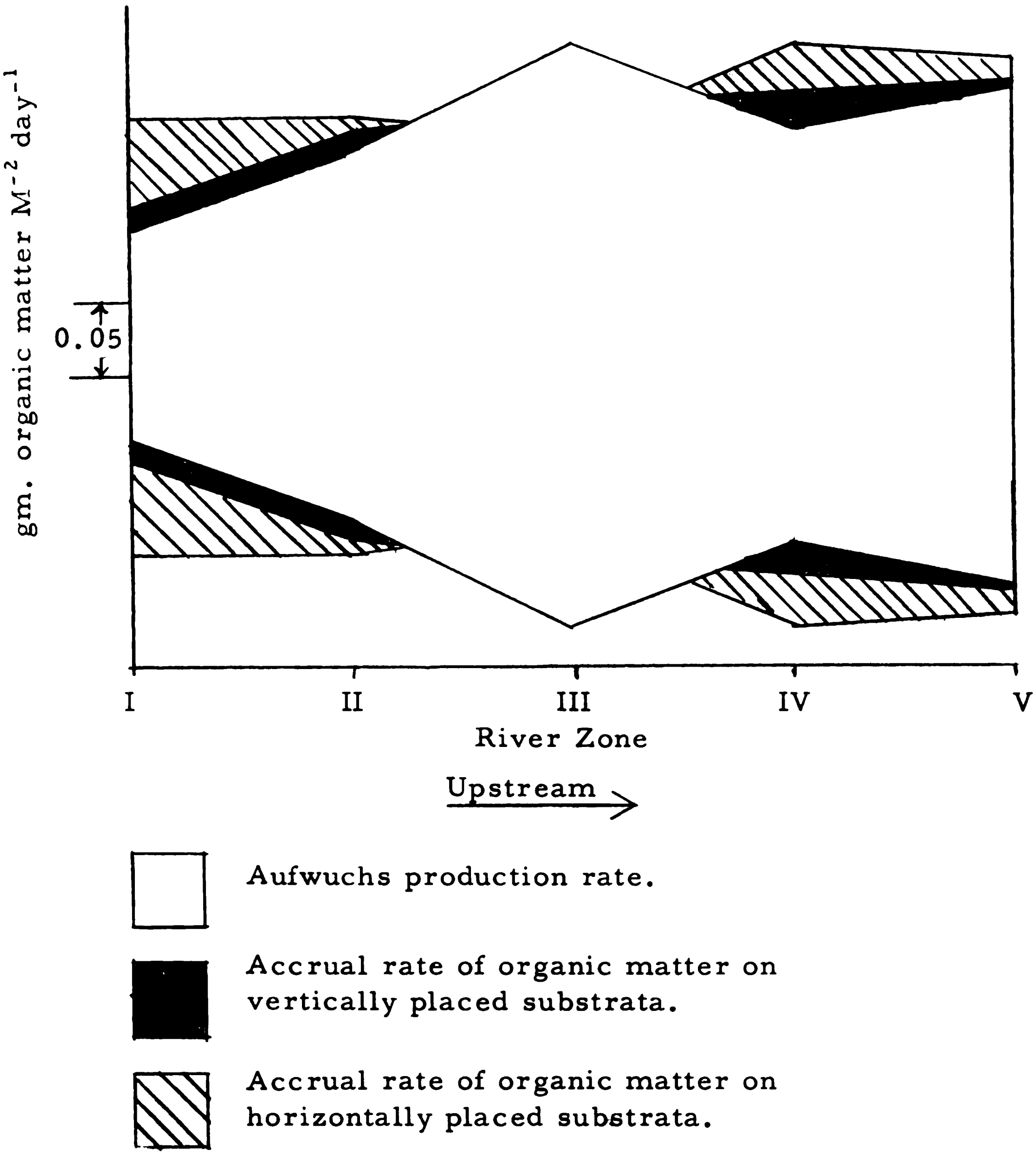

gm. organic matter M^{-2} day^{-1}
0.05
I
II
III
IV
V
River Zone
Upstream
Aufwuchs production rate.
Accrual rate of organic matter on vertically placed substrata.
Accrual rate of organic matter on horizontally placed substrata.

Figure VII

This loss of aufwuchs production in zone III was not considered in the calculation of the average aufwuchs production rate of 253.3 mg. organic weight M^{-2} day^{-1}. Therefore, a better estimate of the average aufwuchs production rate in the Red Cedar River for the summer of 1961 is the average of the aufwuchs production rates in the five river zones. Thus, the average production of aufwuchs in the Red Cedar is estimated at 281.8 mg. organic weight M^{-2} day^{-1}.

Autotrophic and heterotrophic growth: If it is assumed that the organic material settling onto the artificial substrata has no phytopigment, the ratio of phytopigment to organic weight can be corrected to phytopigment to aufwuchs organic weight. This assumption is considered valid since Grzenda (1960), in a study of a section of the river included in the present study, noted that ethanol extractions from suspended materials obtained by filtration of water samples showed a virtual absence of phytopigments. The assumption that the organic sediments contain no phytopigment was used to correct the ratio of phytopigment to organic weight for organic sedimentation in the five river zones (Table 15). This correction was accomplished in the following manner. The total amount of phytopigment produced per M^2 per day was computed by multiplying the ratio of phytopigment to organic weight by the weight of the total organic accumulation. This value was then divided by aufwuchs production (total organic accumulation less organic sediment) in mg. M^{-2} day^{-1} and the result represents the average phytopigment content per mg. of aufwuchs production.

After correction for organic sedimentation has been made, the remaining organic matter is due to heterotrophic and autotrophic production. The autotrophs contain chlorophyll and other phytopigments enabling them to synthesize their own chemical energy source from inorganic material and solar energy. The heterotrophs do not have

Table 15. Ratios of phytopigment unit x 10^3 to mg. organic weight, corrected and uncorrected for organic sedimentation, for the five river zones during the summer of 1961. All units are phytopigment units x 10^3 per mg. organic weight.

River* Zone	Phytopigment: Organic Weight Ratio (uncorrected for organic sedimentation)	Phytopigment: Organic Weight Ratio (corrected for organic sedimentation)	Average corrected phytopigment: organic weight ratio for each river zone
I_H	6.280	13.031	12.894
I_V	10.600	12.757	
II_H	9.824	11.785	11.633
II_V	10.428	11.481	
III_H	6.959	4.680	5.506
III_V	8.124	6.333	
IV_H	8.207	11.208	12.160
IV_V	11.536	13.112	
V_H	10.161	11.444	10.608
V_V	9.651	9.771	

*I_H denotes horizontal placement of substrata in zone I, I_V vertical placement of substrata in zone I etc.

these light sensitive phytopigments and rely on energy-rich organic matter produced by some other level. The autotrophic community in the aufwuchs of the Red Cedar River is made up almost exclusively of diatoms. The heterotrophic community in the aufwuchs is largely bacteria, fungi, and protozoa, since the flow of the river precludes the buildup of large numbers of micro-cructacea, and since all macro-fauna were removed from the substrata before the sample was processed.

Therefore, the higher the phytopigment to organic weight ratio, the greater the amount of phytopigment per unit of organic aufwuchs weight, and the greater the percentage of autotrophic organisms in the aufwuchs.

If it is assumed that the aufwuchs community in the zone with the highest phytopigment content is made up entirely of diatoms, an estimate of the heterotrophic growth can be made for each of the five river zones. This assumption is not strictly true since in natural waters there is always some heterotrophic growth. However, the highest ratio of phytopigment to aufwuchs organic weight encountered was on the vertical substrata in zone IV, and since this zone is further from a known source of organic pollution than any other, the assumption is considered valid.

The ratio of phytopigment to aufwuchs organic weight from the vertical substrata of the reservoir zone is assumed to be that of pure diatom communities. Then, if the average ratio of phytopigment to aufwuchs organic weight in each of the five zones is divided by this ideal ratio, the results represent the percentage of total aufwuchs production due to autotrophic growth in each zone. The following discussion includes an example of this calculation.

For zone I the average corrected phytopigment to organic weight ratio for the summer of 1961 was 12.894 phytopigment units x 10^3 per mg. organic weight.

$$\text{Therefore: } \frac{12.894}{\text{ideal ratio}} = \frac{12.894}{13.112} = 0.983 = 98.3\% = \text{the portion of the aufwuchs production due to autotrophic production.}$$

Therefore: 0.983 x the average aufwuchs production rate = autotrophic production rate.

or, 0.983 x 144.0 mg. M^{-2} day^{-1} = 141.6 mg. autotrophic production per square meter per day.

The heterotrophic production rate is found by subtracting the autotrophic production rate from the total aufwuchs production rate. For zone I the heterotrophic production rate is found as follows:

144.0 - 141.6 = 2.4 mg. heterotrophic organic growth M^{-2} day^{-1}

These values as calculated for each of the five river zones are shown in Table 16. The average aufwuchs production rate for the entire river for the summer of 1961 was 281.8 mg. organic weight M^{-2} day^{-1} of which autogrophic growth produces 212.8 mg. organic weight M^{-2} day^{-1}, and heterotrophic growth produces 69.0 mg. organic weight M^{-2} day^{-1}.

A summary of the rates of accrual of all components contributing to the total accumulation of material on the artificial substrata in the five river zones during the summer of 1961 is given in Table 17.

Estimates of both organic and inorganic weight accrual are available for the artificial substrata from series 1, 3, and 5, but only organic weight accrual estimates are available for series 2 and 4. Table 18 gives the corrected estimates of the rate of accrual of inorganic and organic material on the substrata for series 1, 3, and 5. It can be

Table 16. Calculation of the average autotrophic and heterotrophic production for the five zones of the Red Cedar River during the summer of 1961. All weight units refer to organic weight.

River Zone	Corrected C/W* / Ideal C/W		Aufwuchs Production ($mg.M^{-2}\ day^{-1}$)	Autotrophic Production ($mg.M^{-2}\ day^{-1}$)	Heterotrophic Production ($mg.M^{-2}\ day^{-1}$)
I	$\frac{12.894}{13.112}$	x	144.0 = 0.983 x 144.0 =	141.6	2.4
II	$\frac{11.633}{13.112}$	x	249.5 = 0.887 x 249.5 =	221.3	28.2
III	$\frac{5.506}{13.112}$	x	395.9 = 0.420 x 395.9 =	166.3	229.6
IV	$\frac{12.160}{13.112}$	x	282.5 = 0.927 x 282.5 =	261.9	20.6
V	$\frac{10.608}{13.112}$	x	337.2 = 0.810 x 337.2 =	273.1	64.1
Average for the entire river				212.8	69.0

*C/W = phytopigment units x 10^3 per mg. organic weight.

Table 17. Summary of all components contributing to the total accrued material on the artificial substrata during the summer of 1961. All values are average rates, and the units are mg. M^{-2} day^{-1}.

River* Zone	Inorganic Sediment	Inorganic Material Produced on the Substrata	Organic Sediment	Autotrophic Production (organic)	Heterotrophic Production (organic)
I_H	318.8	298.8	154.8	141.6	2.4
I_V	60.3	173.3	29.3	141.6	2.4
II_H	461.1	299.3	49.8	221.3	28.2
II_V	233.2	274.7	25.2	221.3	28.2
III_H	762.8	267.6	-128.3	166.3	229.6
III_V	519.1	308.6	-87.3	166.3	229.6
IV_H	942.6	385.8	103.3	261.9	20.6
IV_V	352.1	321.1	38.6	261.9	20.6
V_H	524.0	379.8	42.6	273.1	64.1
V_V	52.3	341.4	4.2	273.1	64.1

*I_H refers to the horizontal placement of the artificial substrata in zone I, I_V refers to the vertical placement of the artificial substrata of zone I.

Table 18. Corrected estimates of the accrual rate of inroganic material and the organic production rate of the aufwuchs in the Red Cedar River during time series 1, 3, and 5 in the summer of 1961. All units are mg. M^{-2} day^{-1}.

Time* Series	Inorganic Sediment	Inorganic Material Produced on the Substrata	Organic Sediment	Aufwuchs Production (organic)
1_H**	441.6	329.8	60.4	269.4
1_V	133.9	287.7	18.3	269.4
3_H	537.6	332.2	-80.2	412.4
3_V	400.8	352.6	-59.8	412.4
5_H	948.6	168.8	44.7	124.1
5_V	282.5	137.4	13.3	124.1

*See Table 1 for dates.

**1_H refers to horizontally placed substrata, 1_V refers to vertically placed substrata, etc.

seen from Table 18 that the greatest rate of inorganic sedimentation and the lowest rate of aufwuchs production took place during series 5.

Effect of highway construction: During the summer of 1961, construction of a limited access, interstate highway (I-96) was initiated. This highway parallels the Red Cedar River for the entire length of the study section and crosses most of the major tributaries between two and five miles south of the main river. By the latter part of August, an estimated 1500 acres were bared and many gravel washing sites were in operation in the watershed of the Red Cedar River. This large amount of bare ground coupled with intense rains during late August and early September caused an appreciable increase in the amount of inorganic material entering the Red Cedar River. The highest turbidity measurement recorded for the main river was 387 turbidity units in zone III below Deer Creek on September 4, 1961.

This large influx of inorganic matter into the river had a threefold effect on the aufwuchs. The tremendous increase in turbidity caused a reduction in light penetration, thereby decreasing the amount of solar energy available for photosynthesis. The heavy concentration of inorganic matter in the river water scoured aufwuchs from the substrata, and the inorganic material settling on the substrata smothered the aufwuchs remaining on the substrata.

The net result was that the artificial substrata from the latter part of the summer exhibited the highest rate of inorganic sedimentation and the lowest rate of aufwuchs production.

Effect of the Williamston dam: The effects of increased stream discharge on aufwuchs populations will be discussed in more detail later, but in the Red Cedar the general effect is that as stream discharge increases, aufwuchs production decreases. The discharge of the Red Cedar River for the summer of 1961 as measured at the U. S. G. S.

gauging station at the downstream end of the study section is given in Figure VIII. During most of July and the early part of August there was a great deal of fluctuation in stream discharge. This fluctuation corresponds to the opening and closing of the dam in Williamston. As was described earlier, this dam is maintained to provide electric power, and is operated intermittently during the summer. When the gates of the dam are shut to allow the reservoir to fill up, there is very little water flowing below the dam. When the dam is opened to power the generator, there is a three-fold increase in the stream discharge below the dam during periods of low summer flow.

The organic sediment correction, discussed earlier, gives a measure of the effects of this intermittent flow regulation on the aufwuchs communities in the lower portion of the river. As was shown earlier, a positive organic sediment correction indicates that organic sediments are being added to the organic material produced on the substrata. A negative organic sediment correction indicates a loss of organic material produced on the substrata.

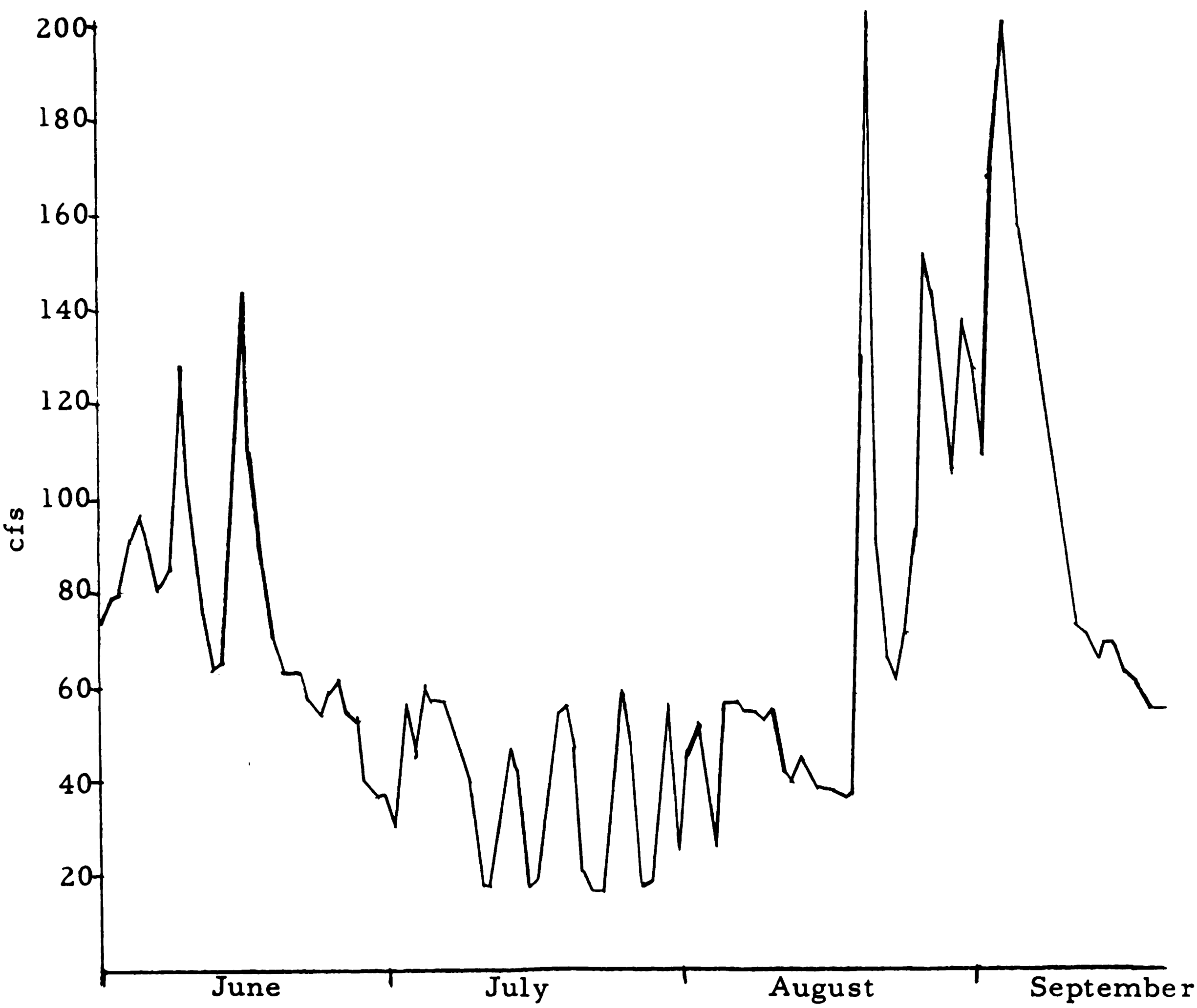
Organic sediment corrections are given in Table 19 for the substrata which were in the river during late July and early August (time series 3). Zone V is above all influence of the dam and thus is not affected by fluctuations of stream discharge caused by manipulation of the dam. This is reflected by the positive organic sediment correction from zone V.

The impounded water behind the dam makes up zone IV. Here the water flows slowly and the aufwuchs colonies on the artificial substrata are more loosely attached than elsewhere in the river. When the dam is opened, the current velocity of the impounded water increases and the water becomes more turbid, resulting in a substantial loss of the loosely attached aufwuchs. This is reflected by the negative organic sediment correction (Table 19).

Table 19. Organic sediment correction factors for the five river zones during time series 3. All units are mg. organic matter M^{-2} day^{-1}.

River zone and substrate placement	Organic sediment correction factor
I_H	-23.4
I_V	-13.7
II_H	-116.4
II_V	-62.4
III_H	-775.3
III_V	-631.6
IV_H	-282.9
IV_V	-169.1
V_H	+282.4
V_V	+63.1

Figure VIII. Discharge of the Red Cedar River as measured at Farm Lane bridge by the United States Geological Survey recording station during the summer of 1961.



	Series 1	Series 2	Series 3	Series 4	Series 5
Maximum cfs	142	60	58	202	199
Minimum cfs	36	17	16	36	60
Average cfs	69	40	36	61	127

Figure VIII

Opening of the dam causes a sudden increase in the volume of water in zone III, immediately below the dam, which has a catastrophic effect on the aufwuchs in this zone. As this pulse of water moves downstream, it spreads out and the increase in volume becomes more gradual. From zone III downstream to the end of the study section there was a decrease in the amount of aufwuchs loss, with the loss in zone I being much less than that in zone II (Table 19).

It is seen, then, that manipulation of the dam at Williamston during periods of low summer discharge has a decided adverse effect on the aufwuchs immediately below the dam, and a lesser adverse effect decreasing with increasing distance below the dam. However, the effect of this fluctuation in discharge was still detectable 14.2 miles below the dam.

Effect of various physical factors: Since no estimates of accrual of inorganic weight are available for series 2 and 4, the production rate of the aufwuchs can not be determined for these time periods. Therefore, in order to correlate organic production on the artificial substrata with various physical parameters over the entire summer, some measure of organic production other than corrected aufwuchs production had to be used. From Figure IX it is seen that the estimates of organic production on the vertical substrata agree much better with the corrected aufwuchs production than do the estimates made from the horizontally placed substrata. Therefore, the estimates of organic production made by vertically placed substrata were used in the following correlations.

Total incident solar energy, as measured by a pyrheliometer, in an open field was found to be positively correlated ($r_{xy} = .50$) with the accrual of total organic material in the Red Cedar River for the summer of 1961. River discharge was found to be negatively correlated ($r_{xy} = -.70$) with the accrual of total organic matter for the same period.

Figure IX. Organic production estimated by horizontal and vertical artificial substrata given with the estimation of aufwuchs organic production corrected for organic sedimentation.

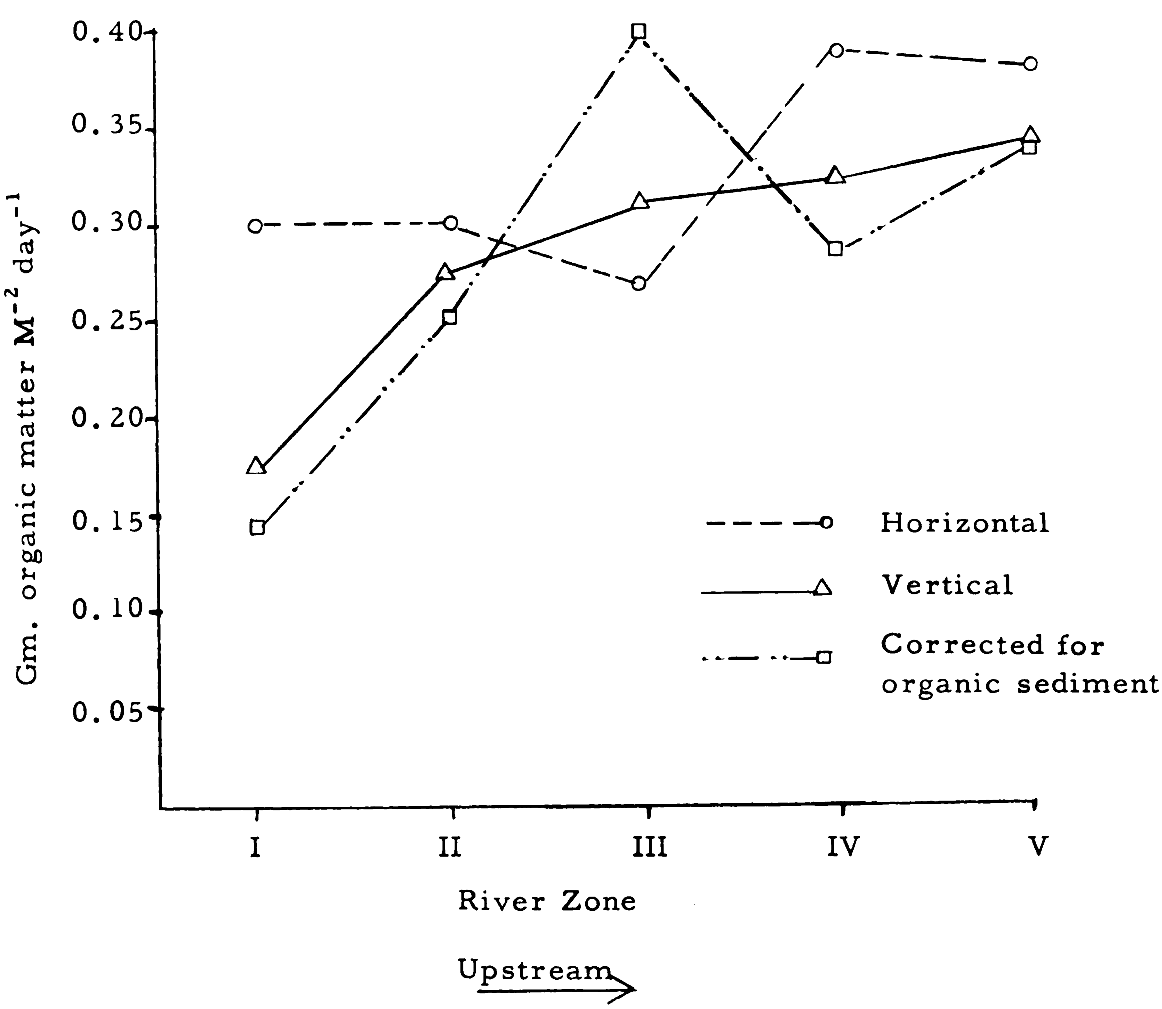

0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
Gm. organic matter M^{-2} day^{-1}
I
II
III
IV
V
River Zone
Upstream
Horizontal
Vertical
Corrected for organic sediment

FIGURE IX

Both of these correlations are significantly different from zero at the .005 level. These correlation coefficients were calculated from the data in Table 20, and the average values of these three parameters for each time series are shown in Figure X.

Since autotrophic growth accounts for 76 percent of the total aufwuchs production in the Red Cedar River, it is not surprising that there is a positive relationship between incident solar energy and organic accrual. These autotrophic organisms utilize electromagnetic energy in the synthesis of their chemical energy source, and hence, the greater the electromagnetic energy, the greater the production of organic matter.

Kevern (1961), in studies of drift material in the Red Cedar River, found that an increase in stream discharge was the greatest single factor causing increases in the concentration and weight of drift material. Vannote (1961) found a direct relationship between phosphorus load and stream discharge in the Red Cedar River. He further noted a distinct phosphorus enrichment gradient from upstream to downstream with the greatest increment being associated with domestic pollution in the lower third of the river.

Therefore, stream discharge is positively related to turbidity and phosphorus, and is negatively related to aufwuchs production. Increased amounts of phosphorus would be expected to increase organic production, or at least would have no adverse effect on it. It is probable that the increase in turbidity with the increase in stream discharge is responsible for the adverse effect on the organic production. The effect of inorganic sedimentation on organic production was discussed earlier and was shown to include three factors; reduction of light at the substrate level, scouring of the aufwuchs from the substrate, and smothering of the remaining aufwuchs.

Table 20. Organic production rate, as measured on the vertical substrata; discharge rate, as measured at Farm Lane bridge; and total incident solar energy, as measured by an open-field pyrheliometer; during the summer of 1961.

Exposure Period (days)	Series 1	Series 2	Series 3	Series 4	Series 5
Organic production rate, organic matter gm. M^{-2} day^{-1}.					
3	0.2209	0.3542	0.6505	0.3576	0.1271
6	0.2875	0.4048	0.2640	0.3102	0.1565
9	0.2806	0.4473	0.3284	0.2869	0.1075
12	0.3374	0.4205	0.2467	0.2888	0.1317
15	0.3317	0.3373	0.3368	0.2305	0.1508
18	0.2635	0.2514	0.2891	0.2214	0.1474
Discharge rate, cfs day^{-1}.					
3	106.3	43.7	40.7	54.7	103.7
6	46.2	34.2	39.8	90.7	117.3
9	68.1	41.8	32.2	42.6	141.8
12	77.7	42.2	34.3	45.6	132.2
15	59.3	38.7	35.3	61.8	132.0
18	67.2	39.6	36.2	60.6	127.3
Total incident solar energy, gm. calories cm^{-2} day^{-1}.					
3	603.9	585.3	489.7	530.5	302.0
6	625.2	517.1	348.4	425.7	422.8
9	561.2	655.1	428.8	509.4	400.1
12	571.9	637.6	447.0	514.7	375.6
15	586.8	599.9	399.1	475.9	409.2
18	589.7	597.5	414.2	485.0	391.3

Figure X. Organic accrual on the vertical substrata, incident solar energy, and river discharge for the five time series in the Red Cedar River during the summer of 1961.

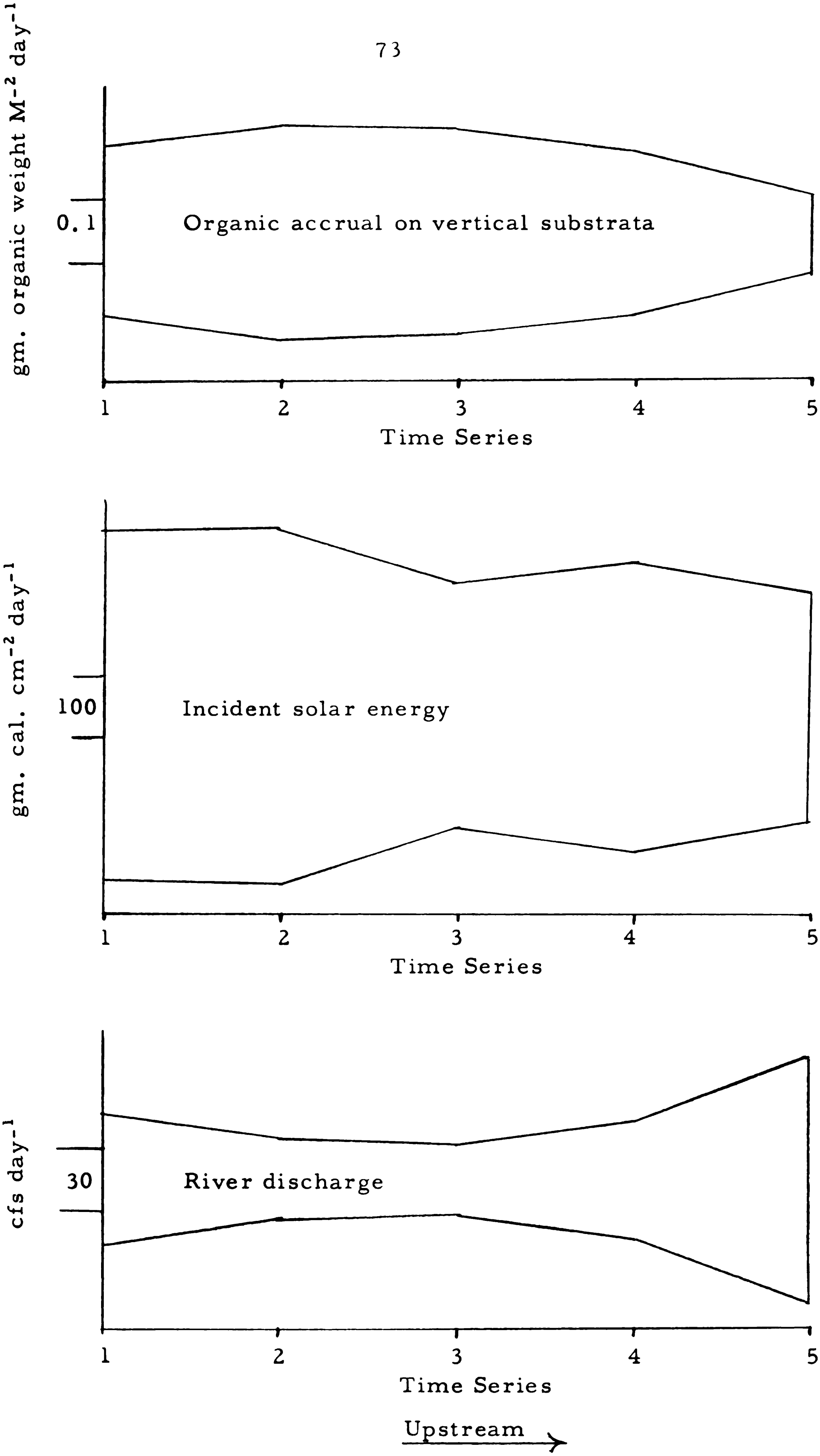

gm. organic weight M-2 day-1
0.1
Organic accrual on vertical substrata
1
2
3
4
5
Time Series
gm. cal. cm-2 day-1
100
Incident solar energy
1
2
3
4
5
Time Series
cfs day-1
30
River discharge
1
2
3
4
5
Time Series
Upstream

Figure X

Steele (1962) indicated that autotrophic production is more dependent on light than on temperature while Moore (1958) stated that heterotrophic growth (bacteria) is temperature dependent. Vannote (1963) postulated that the autotrophic production in the vicinity of the sewage disposal plant in zone III is suppressed at summer temperatures by direct competition of heterotrophic growth. An increase in turbidity with a corresponding reduction in light would also favor heterotrophic organisms over the autotrophic ones.

Vannote, using data collected by Brehmer (1958) and Grzenda (1960), found a linear relationship between stream temperature and the $\log_{10}$ of "periphyton" production during periods of increasing and decreasing photoperiods in a zone of stream enrichment. What he called periphyton is referred to here as total organic accrual on horizontally placed substrata. He then used these relationships to calculate the "periphyton" production in his study area which is included in zone II in the present study. The average total organic accrual rate calculated by Vannote's method yields an estimate of 1.08 gm. M^{-2} day^{-1} which exceeds by 3.5 times the measured accrual rate of organic matter on the horizontal substrata in zone II of 0.305 gm. M^{-2} day^{-1} during the summer of 1961.

This points up the possible error which can arise in using predictor equations based on just one of several interacting physical factors to estimate a biological parameter. Vannote's relationship of temperature to organic accrual was calculated from data collected during 1957 and 1958. Other physical factors affecting organic production may have changed appreciably between 1957 and 1961. For example, runoff from highway construction is known to have increased considerably the sedimentation rate of inorganic material during the latter part of the summer of 1961. Therefore, it is felt that Vannote over-estimated the organic accrual rate on the artificial substrata by a factor of 3.5.

Effect of pollution on the aufwuchs: Butcher (1946, 1947) in studies of English rivers found that organic pollution served to increase the numbers of heterotrophic organisms and to reduce the numbers of autotrophic organisms, with the amount of change being determined by the severity of the pollution. Several authors have noted that the number of diatom species and the number of individuals are reduced by organic pollution (Patrick, 1954). Heavy growths of sewage fungus have been reported below sources of a variety of organic pollutants (Wilson, 1953; Gaufin and Tarzwell, 1952; Rasmussen, 1955; Wiebe, 1927; and Campbell, 1939).

Figure XI gives the average aufwuchs production rate for the five river zones of the Red Cedar River for the summer of 1961. The portions of the total aufwuchs production rate due to autotrophic and heterotrophic growth are also given in this figure.

The Red Cedar River receives untreated sewage from Fowlerville near the upper end of the study section. This source of organic pollution probably accounts for the elevated level of heterotrophic growth found in zone V. From Fowlerville to Williamston, there is no other major source of organic pollution, and there is a corresponding reduction in the level of heterotrophic growth. At Williamston the river receives the effluent from a sewage disposal plant and the heterotrophic production is increased tremendously. There are no major sources of pollution in zone II and the level of heterotrophic growth is again reduced. The estimate of heterotrophic production in zone I is believed to be much lower than the actual value. At the time of this study, zone I was receiving organic pollution from a series of septic tank and storm drains. This low estimate may have been the result of either one or both of two factors. Three of five of the randomly selected sites for the location of the artificial substrata were in the upper portion of this zone, and much of the organic pollution enters

Figure XI. Aufwuchs production rate for the five river zones during the summer of 1961, given with the autotrophic and heterotrophic production rates for the same period.

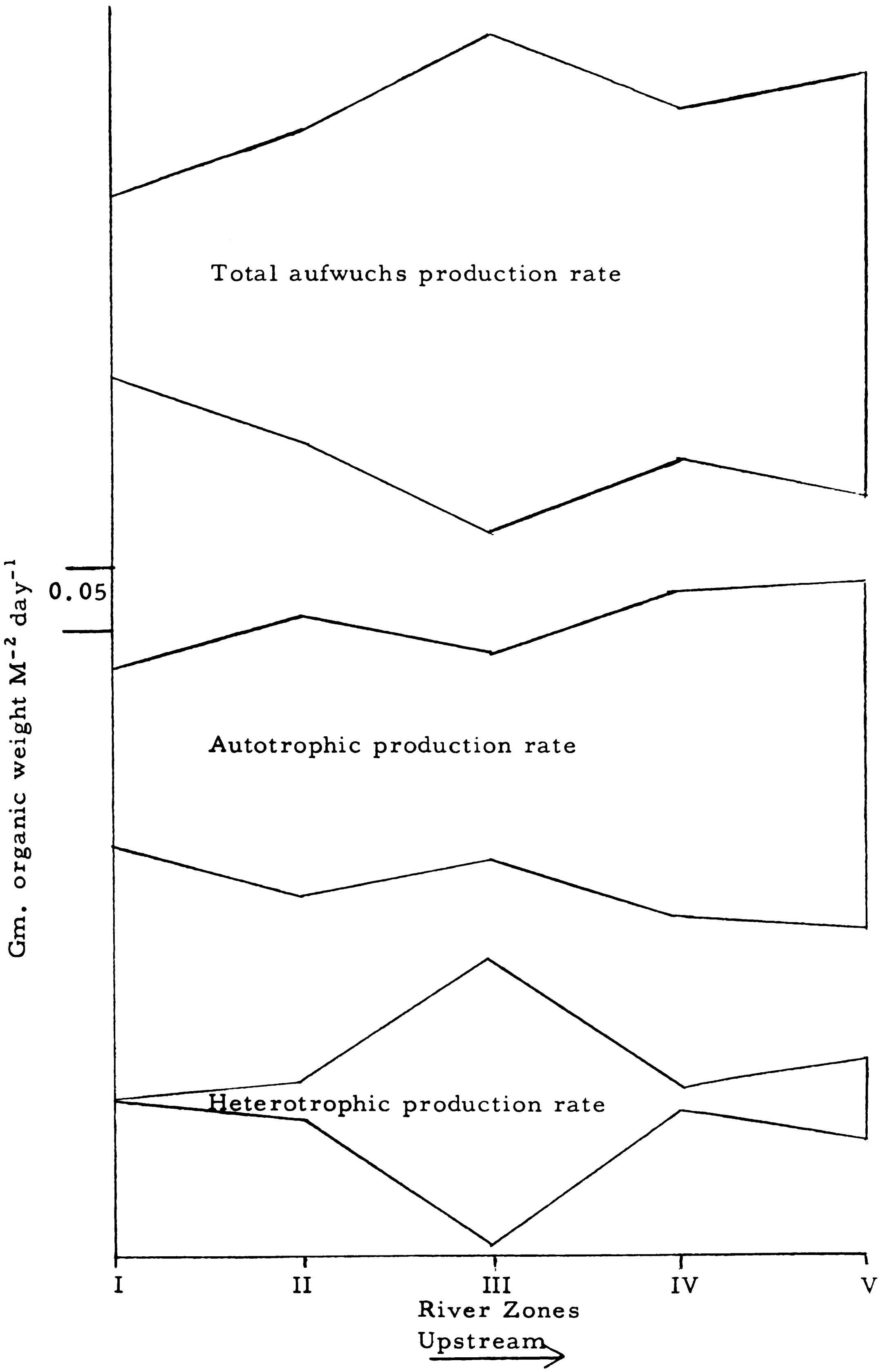

Total aufwuchs production rate
Autotrophic production rate
Heterotrophic production rate
0.05
Gm. organic weight M^{-2} day^{-1}
I
II
III
IV
V
River Zones
Upstream

Figure XI

the river from the middle to the lower end of the zone. The other factor is that during time series 2, the algal production in zone I was much greater than at any other time during the summer. This high algal production for that period tended to overshadow that of all other time periods, and undoubtedly influenced the production rates.

Autotrophic production is reduced from zone V to zone IV and is further reduced in zone III. It is increased in zone II and is reduced again in zone I.

The reduction in autotrophic production in zone III is believed to be due to the adverse effects of the effluent from the Williamston sewage treatment plant. Domestic sewage effluents have been shown to reduce the oxygen content of natural waters and to increase the amount of organic matter in the water. Another factor which would adversely effect algae are toxic wastes such as detergents, laundry bleaches, etc. Reduced oxygen content and increased organic matter serve to increase heterotrophic production at the expense of autotrophic production. Any wastes in the effluent toxic to algae would directly reduce the autotrophic production.

The increased autotrophic production in zone II is believed to be due to an enrichment of the stream by the sewage effluent and to a reduction in competition from heterotrophic growth.

Reduction in autotrophic production from zone V to zone IV could be due to either one or both of two factors. Ruttner (1953) noted that production of benthic algae was greater in flowing water than in standing water. This phenomenon was shown to occur in the Red Cedar River (Rawstron, 1961). Rawstron found that production of aufwuchs was considerably greater in a riffle than in an adjacent pool. The reduction in current velocity in zone IV could account for the reduction in autotrophic production.

Another factor which may account for this reduction is that as the river flows from upstream to downstream areas, there is an increase in the amount of pollutants which enter the river and there is a gradual accumulation of these pollutants. This would explain the gradual reduction in autotrophic production from the upper to the lower end of the study section.

Little is known about the effect of toxic substances on the algae, but considerable work has been done on the effects of toxicants on other organisms. Southgate (1932) and Jones (1939) have shown that many poisons can increase one anothers toxicity by recombination with each other and with natural elements and organic matter in the stream. The physical environment also can affect the toxicity of many substances. Doudoroff and Katz (1953) noted that temperature has a direct influence on toxicity of a variety of poisons to fish. Carpenter (1927) indicated that a rise in water temperature of 10° C reduces by 50% the survival time of fish at constant concentrations of metallic salts. It is obvious that maximum toxicity would result in streams during periods of elevated summer temperatures.

Other physical factors which would affect the toxicity of various poisons are low dissolved oxygen content, variations in dissolved salt content, and variations in pH.

Another more recent source of toxicity to aquatic organisms is the increased use of agricultural pesticides. In many areas of intensive agriculture a pesticide of one kind or another is applied nearly every time the ground is worked. A certain amount of this toxic material will find its way into streams by runoff. These pesticides in all likelihood would add to the accumulating factors detrimental to the natural community of the stream.

All indications are that the nutrient content of the river is such that it can sustain high levels of aufwuchs production in all five river zones, but that other factors serve to depress this production. The increased level of autotrophic production in zone II indicates a response to nutrients introduced by the sewage disposal plant, but this level of production is still less than that of zones IV and V above the sewage treatment plant. Here again the difference in autotrophic production is attributed to the accumulation of a variety of inhibitory pollutional factors.

Comparisons of Aufwuchs Production in 1959, 1960, and 1961

Aufwuchs production was measured in all five river zones of the Red Cedar River from June 27 to September 16, 1959 and from July 20 to September 17, 1960. These measurements were made with horizontally placed substrata and the accrued material was analyzed only for phytopigment content. Therefore, in order to estimate the accrual of organic matter for these periods it is necessary to use the predictor equations calculated from the 1961 horizontal substrata.

The estimates of total organic accrual rate for the summers of 1959, 1960, and 1961 are shown in Table 21 and Figure XII. The accrual rates are plotted at the mid-point of each 18 day time series.

Estimates of the total organic matter accumulation rate in each of the five river zones is shown for the summers of 1959, 1960, and 1961 in Table 22 and Figure XIII.

It is seen from Figure XIII that the pattern of organic accrual is quite similar for the five river zones for the summers of 1960 and 1961, except that the average accrual rate was greater in 1960 than it was in 1961. The estimated accumulation rates in the five river zones for 1959 are different than for either 1960 or 1961.

The river discharge, as measured at Farm Lane bridge, is shown in Figure XIV for the summers of 1959, 1960, and 1961. The discharge rate for 1959 was much greater and much more variable

Table 21. Estimates of total organic matter accrual rate for the entire study section as measured on horizontally placed substrata during the summers of 1959, 1960, and 1961. All units are mg. M^{-2} day^{-1}.

1959		
	June 27-July 15	388.3
	July 17-August 4	290.0
	August 10-August 28	347.2
	August 29-September 16	211.5
1960		
	July 20-August 3	475.1
	August 18-September 5	301.2
	September 5-September 23	519.0
1961		
	June 12-June 30	329.8
	June 30-July 18	510.6
	July 18-August 5	332.2
	August 5-August 23	312.9
	August 23-September 10	168.8

Table 22. Estimates of the total organic matter accrual rate as measured by horizontally placed substrata in each of the five river zones during the summers of 1959, 1960, and 1961. All units are mg. M^{-2} day^{-1}.

River Zone	1959	1960	1961
I	137.5	392.8	298.8
II	332.3	286.0	299.3
III	377.0	345.2	267.6
IV	406.9	475.1	385.8
V	249.3	434.7	379.8

Figure XII. Accrual rate of total organic matter for the entire study section on the horizontal substrata for the summers of 1959, 1960, and 1961.

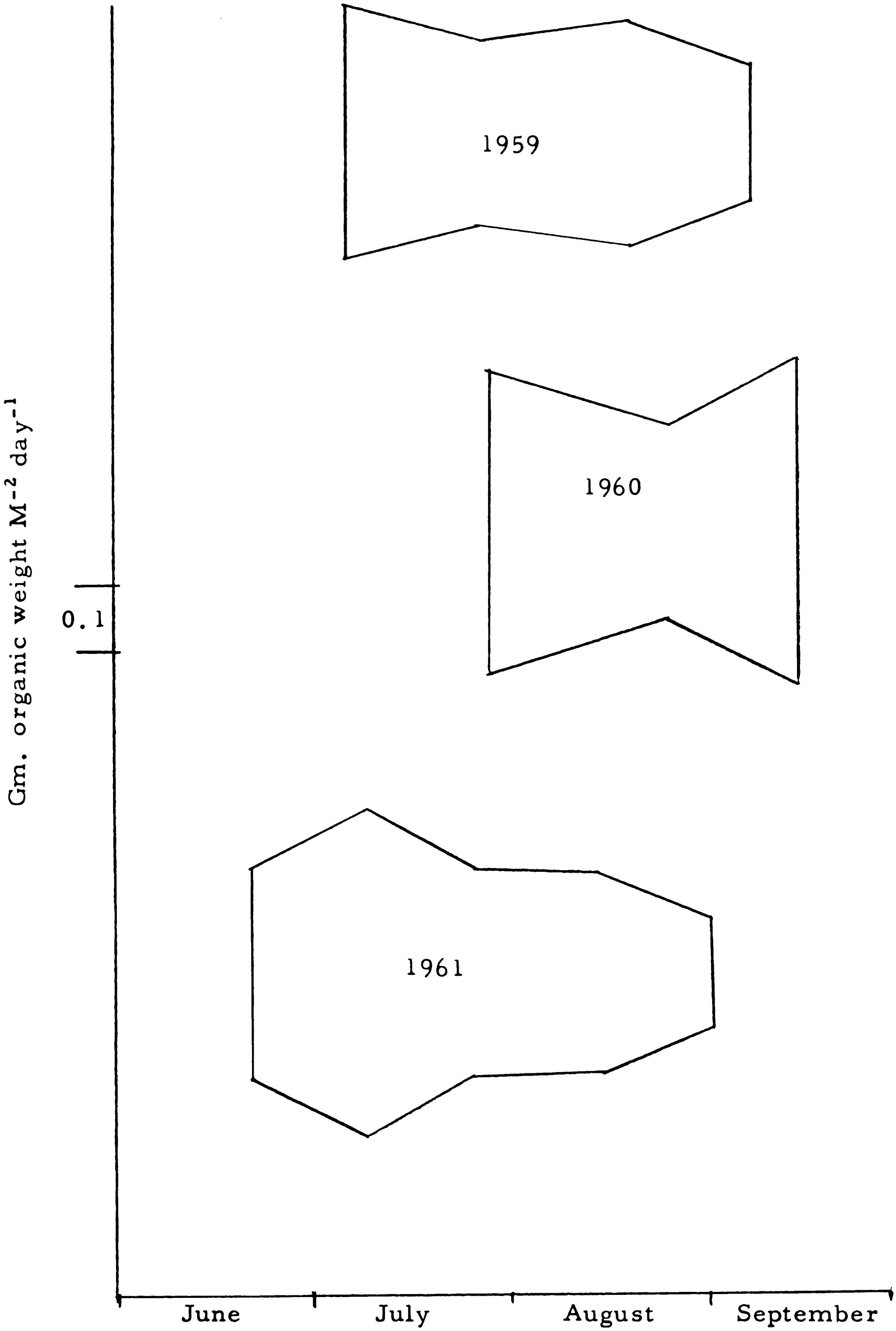

1959
1960
1961
Gm. organic weight M-2 day-1
0.1
June
July
August
September

Figure XII

Figure XIII. Rate of accumulation of organic matter on the horizontal substrata in the five river zones during the summers of 1959, 1960, and 1961.

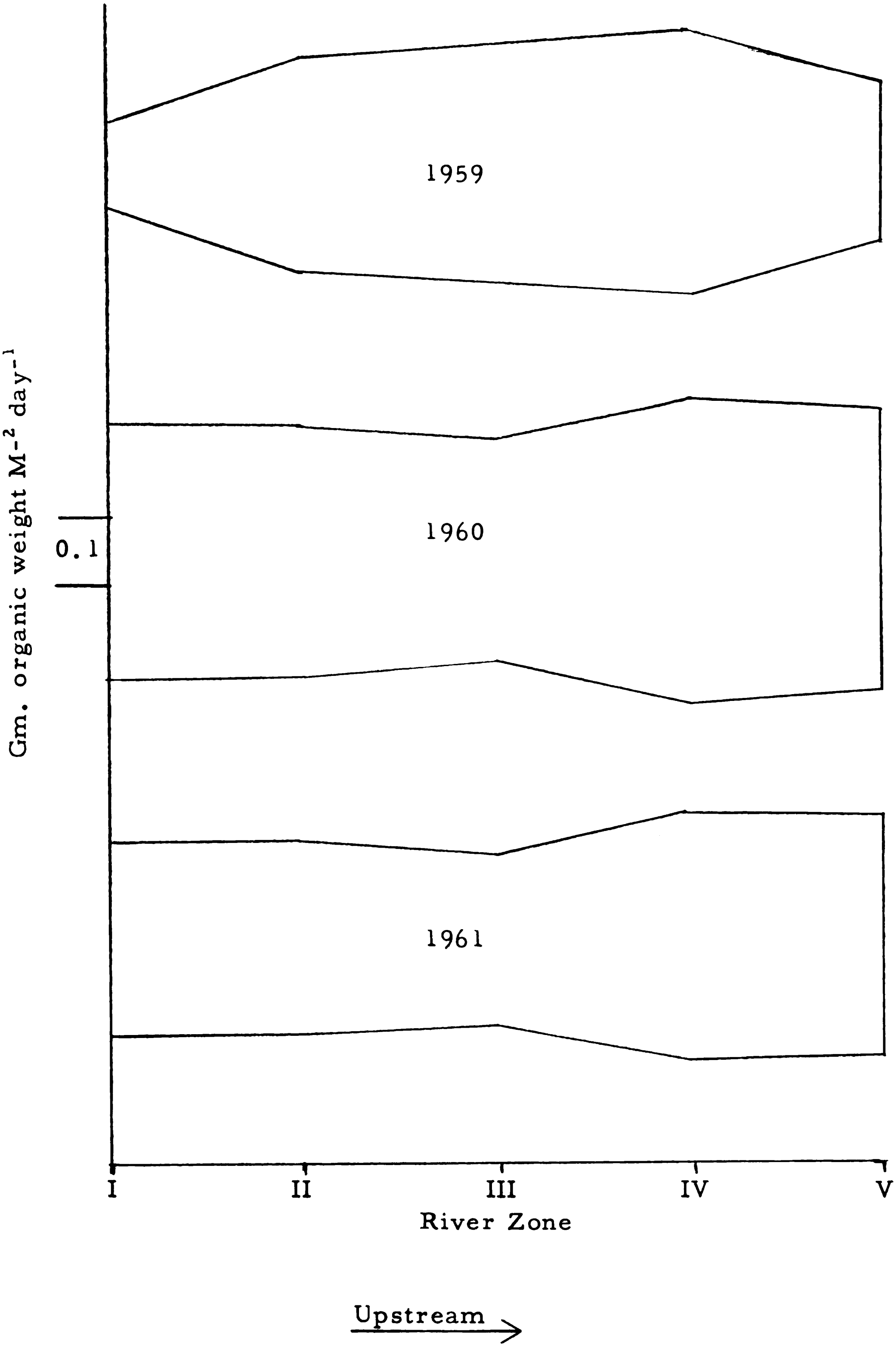

1959
1960
1961
Gm. organic weight M^{-2} day^{-1}
0.1
I
II
III
IV
V
River Zone
Upstream

Figure XIII

Figure XIV. Discharge of the Red Cedar River, as measured at Farm Lane bridge, during the aufwuchs sampling periods during the summers of 1959, 1960, and 1961.

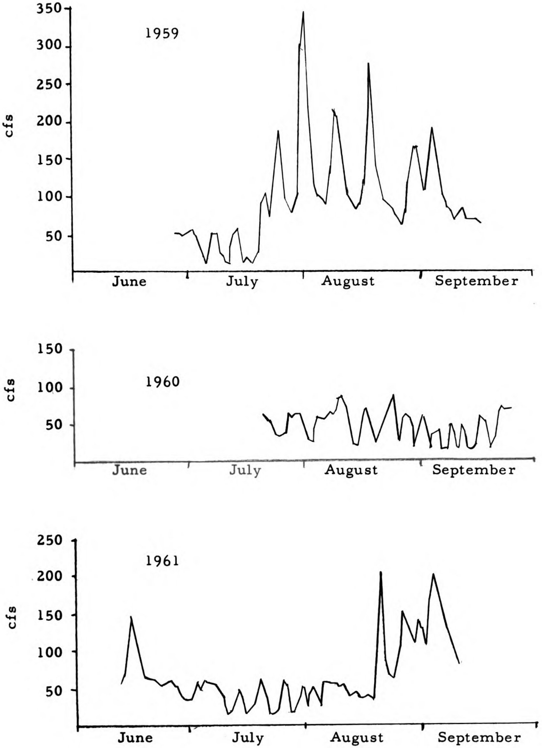

1959
cfs
350
300
250
200
150
100
50
June
July
August
September
1960
cfs
150
100
50
June
July
August
September
1961
cfs
250
200
150
100
50
June
July
August
September

Figure XIV

than for either of the other years. It has already been shown that aufwuchs production is negatively correlated with stream discharge, and it is felt that the unstable discharge during the summer of 1959 was the cause of the low level of organic production in zone I.

The low estimate of organic accrual rate for zone V during the summer of 1959 may be due to toxic wastes from the metal plating plant in Fowlerville. Various copper compounds are recognized as algicides, and any copper in the effluent from this plant would have adverse effects on the aufwuchs. Examination of the effluent indicated that nickel was the dominant metal in the effluent during 1960 and 1961. Indications are that nickel has little effect on aufwuchs production. It is obvious that the effect of the metal plating wastes on the aufwuchs would be dependent on the type of plating operation.

The reduction in the accrual rate from 1960 to 1961 is believed to be due to the increase in inorganic turbidity largely brought about by runoff from the construction site of an interstate highway.

The Macrophytes

The most common aquatic macrophytes in the Red Cedar River are *Vallisneria* and *Sagittaria* but in some areas of the river *Elodea*, *Potamogeton*, *Nuphar*, and *Fontinalis* are locally abundant. During the past several years the distribution of aquatic macrophytes has increased. From 1955-1958 the primary production of the Red Cedar River was due almost entirely to periphyton (Brehmer, 1958 and Grzenda, 1960). Vannote (1963) states that there has been a rapid expansion of the aquatic macrophytes since 1958, and postulated that this expansion may be taking place at an exponential rate.

Samples of the aquatic macrophytes were collected from randomly selected locations in each of the five river zones during the summer of 1962. The sampling method excluded measurements of the plants in the deeper water of the reservoir, so all measurements from zone IV refer to the upper, shallower portion of this zone.

Vannote (1963) states that new macrophyte growth begins in mid May and that the maximum production rate is attained by late June, after which the production rate declines. Vannote estimates a total growing season of macrophytes of 125 days, and states that by late September virtually all of the macrophytes have been detached from the stream bed.

Estimates of the production rate of the macrophytes for the five river zones are given in Table 23. The production rate estimates determined during the early part of September are used as average estimates of the production rate of aquatic macrophytes in the five river zones for the summer. The average production rate of macrophytes for the entire river during the summer was estimated at 0.146 gm dry weight M^{-2} day^{-1}.

Vannote estimated the production rate of the aquatic macrophytes in his 2.2 mile study section of the Red Cedar River to be 1.37 gm. dry weight M^{-2} day^{-1}. His study area is included in zone II of the present study and lies in an area enriched by the sewage treatment plant at Williamston. His study area includes many large, very dense beds of *Vallisneria* and his estimate of the production rate is undoubtedly representative of that area of the stream. In addition to this section of dense macrophyte growth, there are many large barren sand flats included in zone II. Therefore, the estimate of 0.158 gm. dry weight M^{-2} day^{-2} is considered reasonable as an average value for the entire zone II.

Zone V has many areas where the stream bed is completely obscured by the aquatic macrophytes. The dredged portion of this zone has many extensive beds of *Vallisneria* and *Sagittaria* intermixed

Table 23. Estimates of the production rate of aquatic macrophytes in the five river zones of the Red Cedar River during the summer of 1962. All units are gm. dry weight M^{-2} day^{-1}, and include the time period from May 15 to the date indicated.

Sampling			River Zone		
Date	I	II	III	IV	V
June 22					1.110
June 28			0.000	0.056	0.188
July 18	0.218	1.435			
July 19			0.013	0.072	1.084
September 4			0.158		
September 11				0.055	0.225
September 12	0.134	0.158			

with Elodea and Potamogeton and this zone exhibits the greatest production rate of stream macrophytes.

The estimate of macrophyte production rate for zone IV is not representative of the entire zone. The deeper water of the reservoir supports very dense beds of Potamogeton and Nuphar but this area was excluded from the sampling area by the deep water.

Below the dam in Williamston, very dense beds of Sagittaria and Vallisneria extend downstream about a half a mile to the confluence of Deer Creek. From Deer Creek to about a mile below the sewage disposal plant the stream macrophytes are very scarce. This lack of macrophytes is probably due to the adverse effects of the silt entering from Deer Creek and to the pollutants in the sewage effluent. The lower end of this zone supports a lush growth of Sagittaria and Vallisneria.

The upper portion of zone II supports extensive beds of Sagittaria and Vallisneria, but much of the lower half of this zone is made up of extensive sand flats largely devoid of stream macrophytes.

The macrophyte production in zone I is largely due to Sagittaria although there are a few beds of Vallisneria in this area.

Total Primary Production

Total primary production in the Red Cedar River is made up of two components; the algae (chiefly diatoms) and the stream macrophytes. The average production rate of the algae in the Red Cedar River during the summer was estimated at 0.2128 gm. organic weight M^{-2} day^{-1} while for the stream macrophytes the average summer production rate was estimated at 0.146 gm. dry weight M^{-2} day^{-1}.

INVERTEBRATE PRODUCTION

Samples were collected from the invertebrate community of the Red Cedar River during the summers of 1958 and 1959 in an attempt to determine production, standing crop, and the distribution of the aquatic invertebrate fauna (King, 1962). The time interval between sampling periods was 7 days in 1958 and was extended to 14 days in 1959. Distribution and standing crop estimates were calculated from these data, but the increase in biomass between sampling periods was not great enough to allow accurate calculation of production estimates.

In an effort to obtain production estimates in each of the five river zones, the time interval between samples was extended to 28 days and the sampling schedule was expanded considerably during the zummer of 1961. Four complete 28-day series of invertebrate samples were collected from each of the five river zones during the summer of 1961, thereby allowing comparisons of the five zones. The dates of the four time series of invertebrate collections are given in Table 24.

The samples of the invertebrate community collected during the summer of 1961 will be discussed and then will be compared with the collections of invertebrates taken during the summers of 1958 and 1959.

Statistical Considerations: An attempt was made to test the level of significance of the differences in weight between day 0 and day 28 aquatic insect samples. To do this the day 0 and day 28 samples were treated as independent samples and the total weight of each sample (herbivorous insects + carnivorous insects) in pounds live weight Acre^{-1} was the unit used in this analysis.

An analysis of the variances (F test) of the two samples from each location showed a significant difference in the two variances ($F_{19, 20}=0.312$). Since equal variances are a requirement for parametric statistics, it

Table 24. Collection dates of invertebrates from the Red Cedar River during the summer of 1961.

Time Series	Day	Date*
1	0	June 12-June 16
1	28	July 10-July 14
2	0	June 26-June 30
2	28	July 24-July 28
3	0	August 7-August 11
3	28	September 4-September 8
4	0	August 21-August 25
4	28	September 18-September 22

*One zone per day was sampled; zone I, on the first day of a sampling period, zone II on the second day, zone III on the third day etc.

was necessary to use a nonparametric test for this analysis. The test used was the Mann-Whitney U test. It was shown by this test that there was a significant difference at the .1922 level between the day 28 and the day 0 samples using a one-tailed test. This implies that the weight of the day 28 samples was significantly greater than the weight of the day 0 samples at the 20% level.

The .05 level of significance is the generally accepted statistical standard by which data are judged, but this level is often unobtainable in biological investigations and lesser levels of significance may prove useful in comparisons of many inconstant biological populations. I feel that a difference significant at the .20 level for a parameter as subject to wide variation as aquatic invertebrate biomass represents a real difference. An example of just one of the many variables affecting stream invertebrates is stream discharge. Allen (1951) recorded great losses of stream invertebrates during periods of high stream discharge, and Nelson and Scott (1962) noted that the number of invertebrates in a stream was dependent on the stability of water levels. This dependency on stream discharge will be shown also for the invertebrate community of the Red Cedar River.

Aquatic Insects and Tubificid Worms: The invertebrates most commonly encountered in the samples from the Red Cedar River were aquatic insects and tubificid worms. The aquatic insects were identified to family and were then treated as two groups; herbivores and carnivores. Food habits of these organisms were determined from published sources. A list of the herbivorous and carnivorous insects collected from the Red Cedar River is given in Table 25. Since the tubificid worms are detritus feeders, they were considered to be decomposers.

Standing crop: Average standing crop estimates of herbivorous and carnivorous insects and tubificid worms during the summer of 1961 are

Table 25. Herbivorous and carnivorous aquatic insects encountered in the Red Cedar River.

Herbivores	Carnivores
Poduridae	Perlidae
Heptageniidae	Corydalidae
Baetidae	Sialidae
Ephemeridae	Sisyridae
Hydropsychidae	Gomphidae
Psychomyiidae	Cordulegasteridae
Phryganeidae	Libellulidae
Brachycentridae	Coenagrionidae
Limnophilidae	Agrionidae
Hydroptilidae	Gyrinidae
Leptoceridae	Corixidae
Pyralidae	Belastomatidae
Elmidae	Saldidae
Hydrophilidae	Mesoveliidae
Psephenidae	Heleidae
Haliplidae	Tabanidae
Dryopidae	Empididae
Tipulidae	
Culicidae	
Simuliidae	
Tendipedidae	
Ephydridae	

given for all randomly selected sampling sites within the Red Cedar River in Table 26, and are shown in Figure XV. These values are averages of the day 0 and day 28 biomass estimates from each sampling site.

It can be seen from Figure XV that there is a great reduction in standing crop biomass of insects, both herbivores and carnivores, from river mile 28.5 to river mile 27.4. At the same time there is a very large increase in the standing crop of the tubificid worms. Between these two stations the river receives the effluent from a metal plating plant and raw sewage from the City of Fowlerville.

Bartsch (1948) noted that this type of change in the stream biota is common below areas of organic pollution, but it appears that organic pollution is not the dominant factor in this area of the Red Cedar River. The river bottom is rich in organic matter at both sampling sites and the volume of domestic sewage entering the river from Fowlerville does not appear to be large enough to cause this tremendous change in the stream biota.

It is felt that the reduction of the aquatic insects and the corresponding increase in the tubificid worm population is largely due to the effects of the plating wastes. Judging from the size of the tubificid worm population found at river mile 27.4, these organisms apparently are not severely affected by the levels of plating wastes that occur in the river.

By the time the river reached mile 24.0 the biota had begun to recover from the effects of the pollutants. The herbivorous insects exhibited a slight increase in standing crop and the carnivorous insects were at a higher standing crop than at any other area in the river during the summer of 1961.

Fish kills occur below the plating plant at infrequent intervals, usually during periods of high stream discharge. This suggests that the

Table 26. Average standing crop estimates for the randomly selected sampling sites in the Red Cedar River during the summer of 1961. These values are means of the day 0 and the day 28 standing crop estimates and the units are gm. wet weight M^{-2}.

River Zone	River Mile	Time Series	Herbivorous Insects	Carnivorous Insects	Tubificid Worms
I	0.2	4	.1309	.0197	2.1772
I	1.1	3	1.1330	.0074	.2641
I	1.7	2	.7737	.0222	.2298
I	3.4	1	.7964	.2768	.0103
II	4.2	3	.4009	.4191	.0318
II	5.3	4	.5742	.7031	.0633
II	8.6	2	2.3404	.5320	.0915
II	11.2	1	1.3580	.3022	.1845
III	12.2	2	4.0026	.4979	.6951
III	12.4	3	.8629	.3069	.1598
III	13.8	4	.8470	.0024	.1368
III	14.4	1	.9028	.2012	.2498
IV	14.7	4	.4296	.7800	.3060
IV	15.8	3	.6813	.5800	.1457
IV	16.5	2	1.0392	.1037	.1333
IV	17.3	1	.5237	.1837	.6641
V	21.4	3	.4040	.7603	2.6597
V	24.0	4	.1146	1.3767	5.5523
V	27.4	1	.0087	.0000	15.3328
V	28.5	2	1.7537	.3802	.0819

Figure XV. Average standing crop estimates of herbivorous and carnivorous insects and tubificid worms at randomly selected sampling sites in the Red Cedar River in the summer of 1961 plotted against river miles. River mile 0 is at Farm Lane bridge, the lower end of the study section.

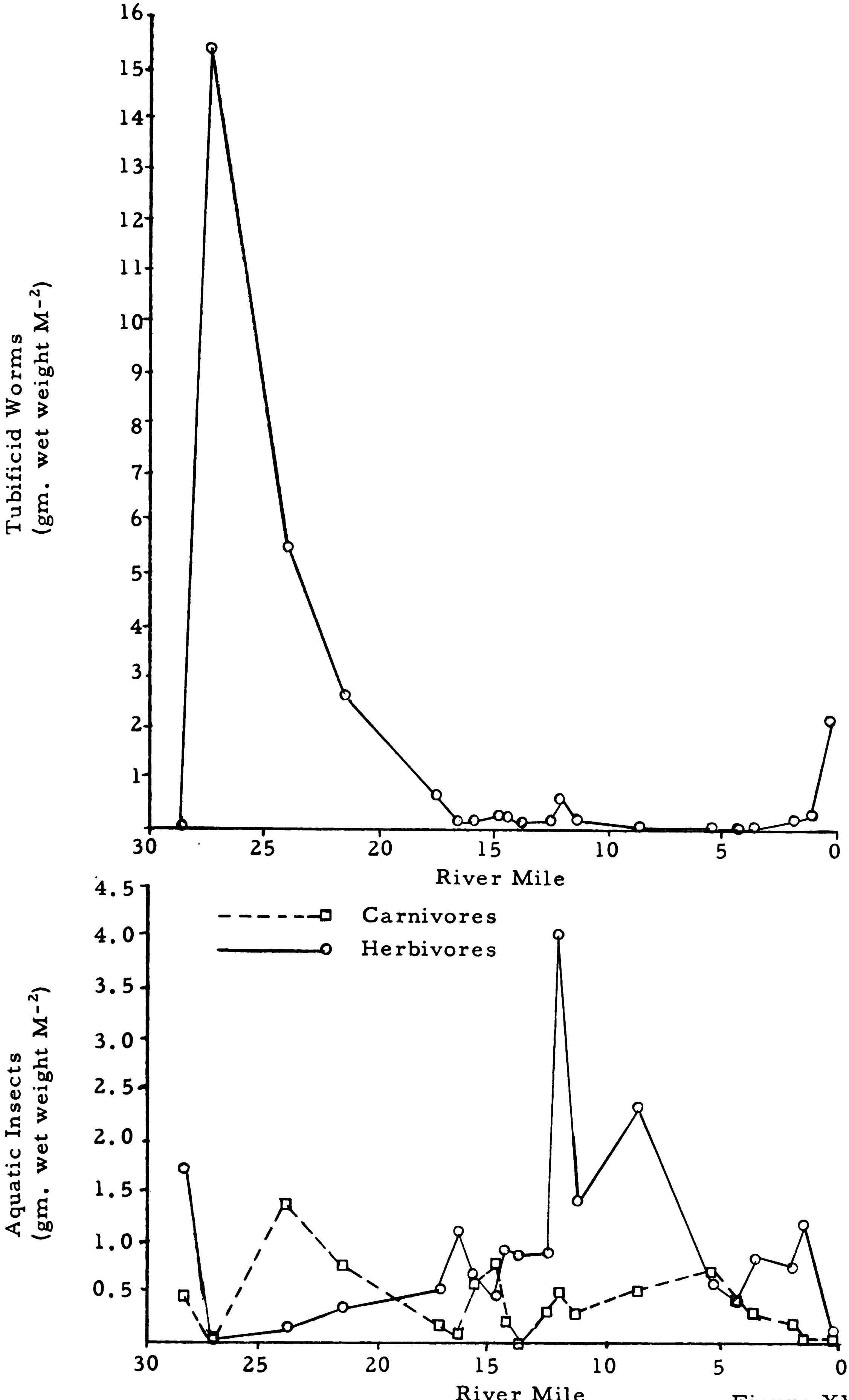

Tubificid Worms
(gm. wet weight M^{-2})
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
30
25
20
15
10
5
0
River Mile
Aquatic Insects
(gm. wet weight M^{-2})
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Carnivores
Herbivores
30
25
20
15
10
5
0
River Mile

Figure XV

settling ponds may be flooded, releasing unsettled wastes into the stream. The last recorded fish kill occurred May 14, 1961, and was estimated to have affected 6 miles of stream (Public Health Service Publication No. 847, 1961). It would seem likely that this sudden release of wastes would affect the carnivorous insects as well as the herbivorous ones. Therefore, judging from the large size of the carnivores (chiefly Odonata) present at river mile 24.0 it appears that they must have migrated into the area from some of the tributary streams. The other possibility is that the Odonata are more tolerant of the plating wastes than are the herbivorous insects, although their absence from the more polluted upstream areas seems to belie this possibility.

From river mile 24.0 downstream to river mile 17.3 there was a reduction in the standing crop of tubificid worms and a corresponding reduction in the standing crop of carnivorous insects (chiefly Odonata). The low population of herbivorous insects at these three stations does not appear to have been capable of supporting the large population of carnivores. Evidently the Odonata must actively feed on the tubificid worms, and the ratio of carnivorous insect standing crop to tubificid worm standing crop remained fairly constant over these 6.7 miles of stream. This ratio for the populations at river mile 24.0 was .248, at river mile 21.4 it was .286, and at river mile 17.3 it was .277.

From river mile 17.3 to river mile 14.7 there is considerable fluctuation in the population levels of all three groups of invertebrates. The populations in this reservoir section appear to be more affected by bottom type than by any residual effects of the pollutants from the Fowlerville area.

Effluent from the Williamston sewage disposal plant enters the river between river mile 14.4 and river mile 13.8. Below this point of effluent there is a reduction in and a suppression of the standing crop of herbivorous insects at river mile 13.8 and 12.4. Below the sewage plant the standing crop of carnivorous insects was considerably reduced and the standing crop of the tubificid worms showed a slight reduction.

In the preceding section, zone III was shown to be physically unstable due to wide variations in turbidity and discharge caused by sudden rains in the Deer Creek watershed and to manipulation of the dam at Williamston. This zone was fairly stable until just before the day 0 samples of time series 4 were collected. After these samples were taken, and before the day 28 samples of time series 3 and 4 were collected, a prolonged period of intense summer rains caused a large increase in the discharge of the river. The runoff from these rains from the construction site of an interstate highway also caused a large increase in the turbidity of the stream. This increase in discharge and turbidity would tend to reduce the insect and tubificid worm populations of the river (Tebo, 1955). The invertebrate population did not suffer any reduction in standing crop during time series 3 at river mile 12.4. Although there was some reduction in standing crop of all three populations at river mile 13.8 during time series 4, the standing crop estimate of the carnivores at day 0 was lower than for any other sampling area in zone III either before or after the intense rains. Therefore, the unstable physical conditions in zone III are reflected by the unstable populations of aquatic insects and tubificid worms.

Variations in standing crop of aquatic insects and tubificid worms from river mile 12.4 to river mile 5.3 are attributed to variation in habitat type, rather than to effects of allochthonous pollutants.

At the time this study was conducted the river was receiving domestic pollutants from a series of storm drains and septic tank overflow drains from river mile 5.3 to the lower end of the study section. The reduction in the standing crop of carnivorous insects and the increase in standing crop of the tubificid worms in this area are attributed to the effects of this pollution. In general, zone I supports smaller populations of herbivorous insects than any of the other zones with the exception of zone V. This also is attributed to the effects of the domestic pollution entering the river from the Okemos-East Lansing urban area.

The average standing crops of these invertebrates for the entire study section during the summer of 1961 were estimated at 0.9539 gm. wet weight M^{-2} for the herbivorous aquatic insects, 0.3728 gm. wet weight M^{-2} for the carnivorous aquatic insects, and 1.4584 gm. wet weight M^{-2} for the tubificid worms.

Production: Estimation of aquatic invertebrate production is generally accomplished by calculation of changes in standing crop from one period to the next. This does not represent the true net production but rather the net yield exclusive of predation, mortality, downstream drift, and emergence. Nelson and Scott (1962) estimated productivity of all trophic levels on a rock outcrop in a Georgia river by considering only the positive changes in standing crop. They assumed that negative changes were due to mortality, emergence, or loss by organisms washing downstream, and that positive changes were due to productivity within the stream. While this method may yield estimates of production which are closer to the true net production on a small area of a stream, it could not be used to estimate invertebrate production over the entire study section of the Red Cedar River under the variable conditions present during the summer of 1961.

The net changes in average standing crop of the herbivorous and carnivorous aquatic insects and the tubificid worms over the entire study section for the four 28 day time series during the summer of 1961 are given in Table 27. From this table it can be seen that there was an increase in standing crop of all three groups during time series 1 and 2 and generally a decrease during time series 3 and 4. The discharge and

Table 27. Net changes in the average invertebrate standing crop in the Red Cedar River during the summer of 1961 based on averages of samples collected from the entire study area during the four 28 day time series. Units are mg. wet weight M^{-2} day^{-1}.

Trophic Group	Series 1	Series 2	Series 3	Series 4
Herbivores	+3.7	+84.1	-8.5	-10.1
Carnivores	+4.2	+18.9	-3.2	+12.8
Tubificid Worms	+88.2	+0.3	-22.9	-30.0

turbidity load of the stream were relatively stable during time series 1 and 2, but both of these parameters increased tremendously during time series 3 and 4. The large increase in turbidity was due to the intense rain storms and the resulting runoff from the previously mentioned area of highway construction.

Scouring and smothering effects of the increased sediment load during late August and early September undoubtedly were the causes of the reduction in invertebrate biomass. An example of the magnitude of this sedimentation was found at river mile 0.2 during time series 4. At the beginning of the collection period the bottom material was a black mud, rich in organic matter, but 28 days later this black material was overlain with .75 inches of finely divided clay and sand.

The adverse effects of the increased discharge and turbidity on the herbivorous and carnivorous insects and the tubificid worms are illustrated for each of the river zones in Figures XVI, XVII, and XVIII. From Figure XVI it can be seen that there was an increase in the net gain of herbivores from series 1 to series 2 in all river zones except zone IV. A net loss in standing crop of herbivores was noted for all river zones during series 4 and for all river zones except zone III during series 3. This reduction in standing crop was due to the increase in discharge and turbidity during late August and early September.

The carnivorous insects showed this same trend during the summer of 1961 (Figure XVII). There was an increase in the net gain of standing crop biomass from series 1 to series 2, followed by a decrease in standing crop during series 3. However, it was noted that the carnivorous insects exhibited an increase in net gain from series 3 to series 4 in river zones II, IV, and V. It appears from this increase that the carnivorous insects may have actively avoided the effects of the heavy silt load of the river by moving to places of cover and then returned to the main stream after the peak of the silt load had passed. The carnivores

Figure XVI. Net change in the standing crop of herbivorous insects during four 28 day periods. These estimates are given for each river zone at each time series.

Time Series 1 -- June 12-July 10
Time Series 2 -- June 26-July 24
Time Series 3 -- August 7 -September 4
Time Series 4 -- August 21-September 18

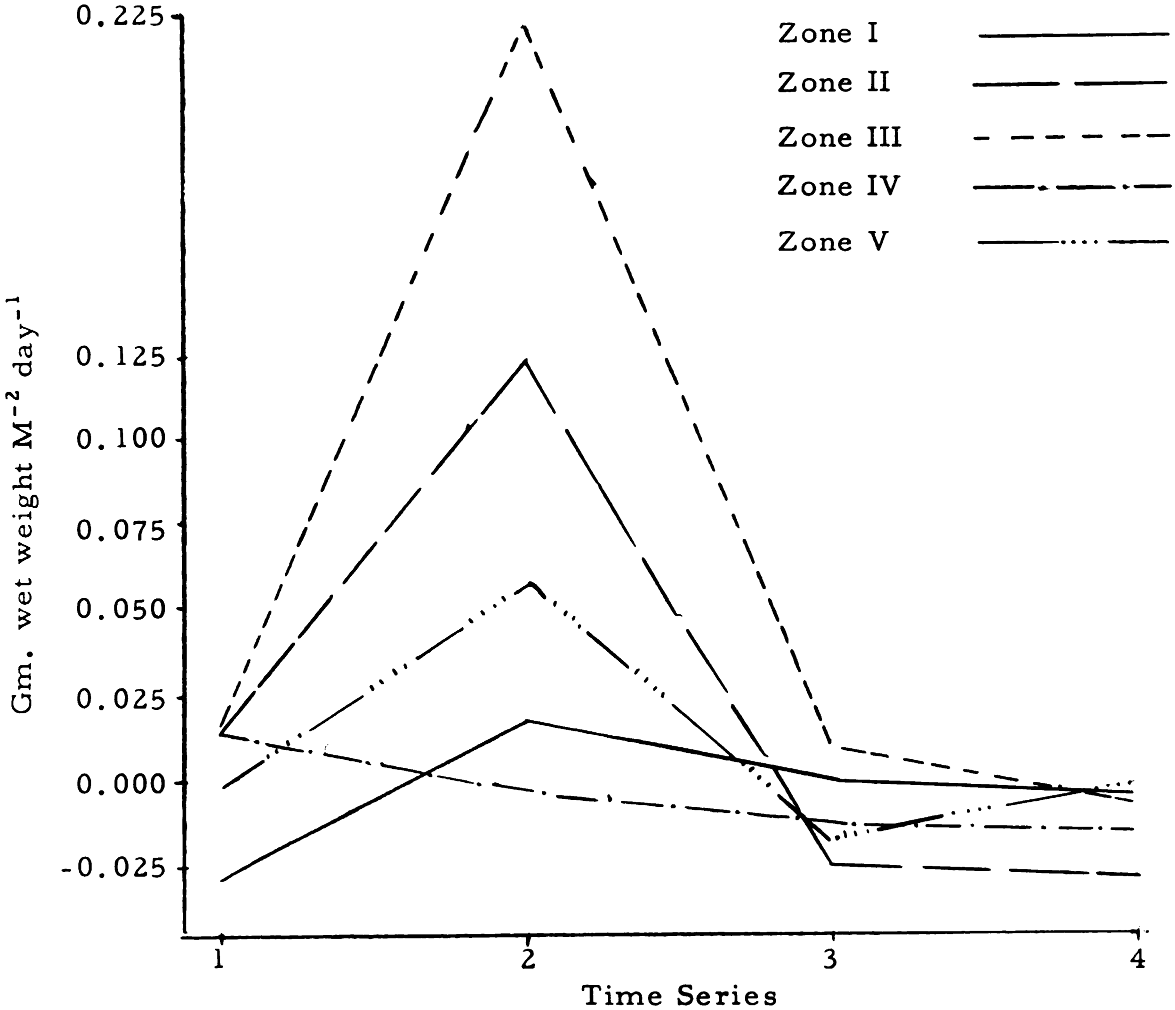

0.225
0.125
0.100
0.075
0.050
0.025
0.000
-0.025
Gm. wet weight M-2 day-1
1
2
3
4
Time Series
Zone I
Zone II
Zone III
Zone IV
Zone V

Figure XVI

Figure XVII. Net change in standing crop of carnivorous insects during four 28 day periods. These estimates are given for each river zone at each time series.

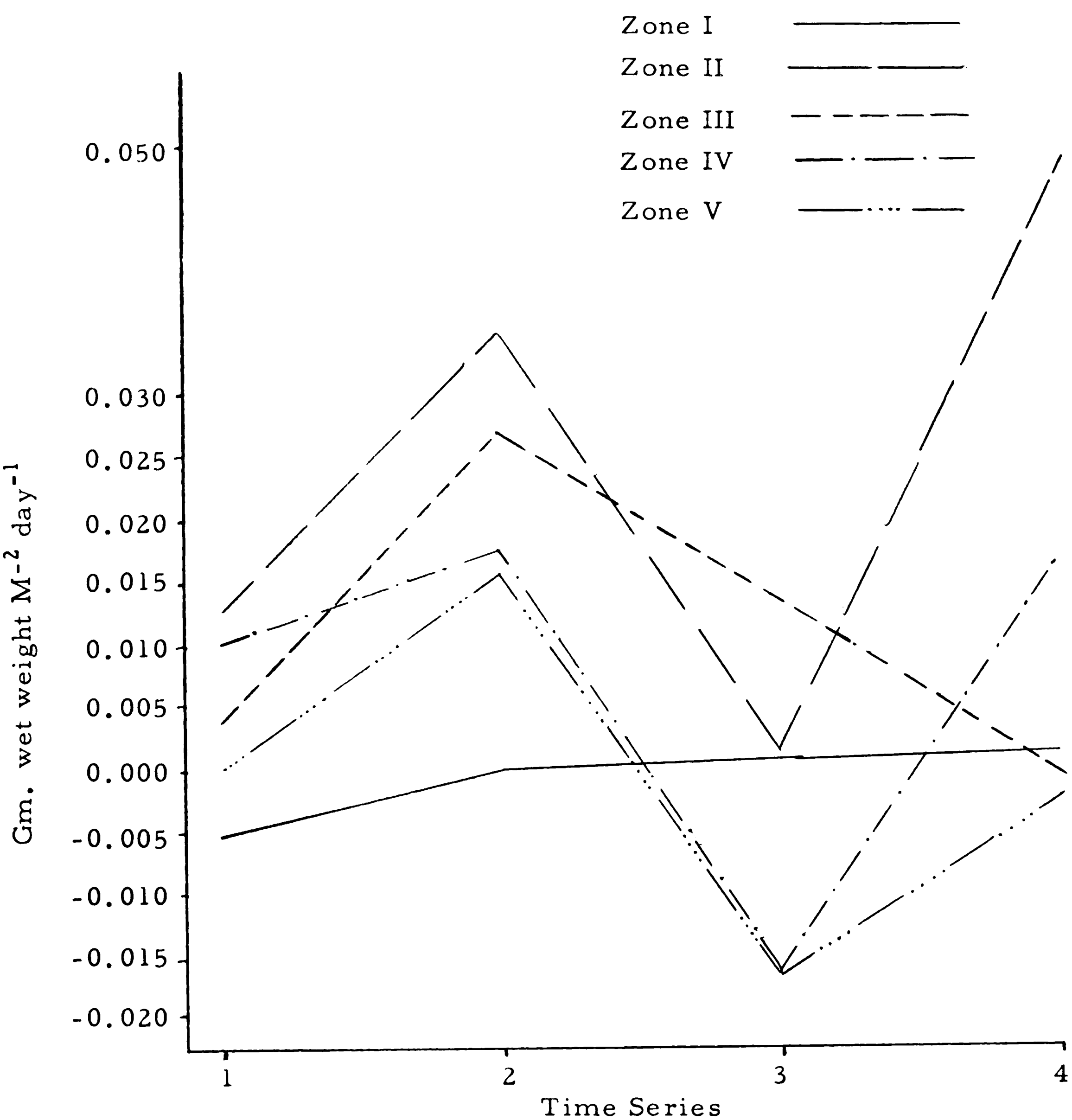

Zone I
Zone II
Zone III
Zone IV
Zone V
0.050
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.020
Gm. wet weight M^{-2} day^{-1}
1
2
3
4
Time Series

Figure XVII

Figure XVIII. Net change in standing crop of tubificid worms during four 28 day periods. These estimates are given for each river zone at each time series.

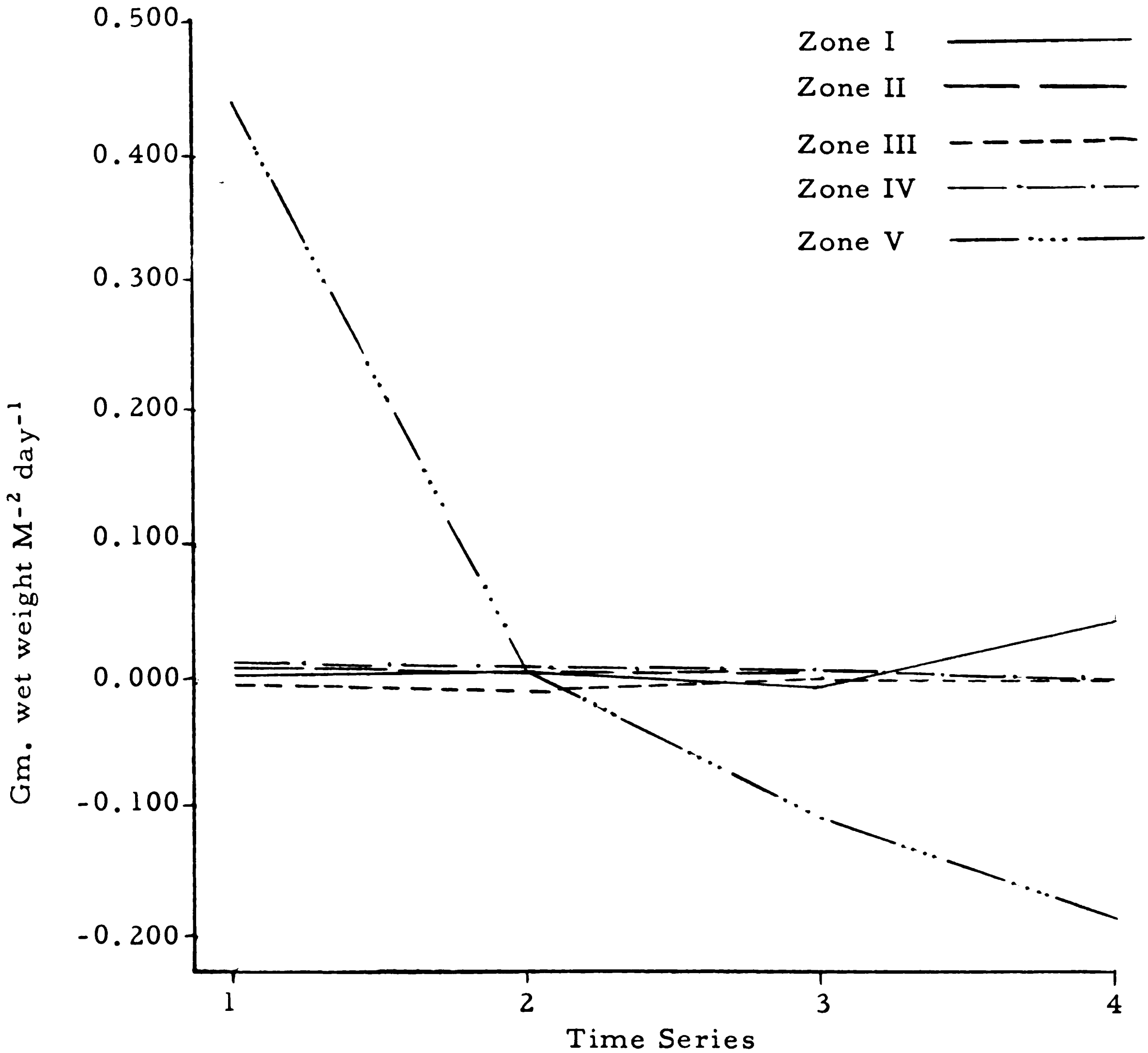


Figure XVIII

are much more active than the herbivores and it is possible that they might have avoided the effects of the increase in sedimentation more successfully than did the herbivores.

Tubificid worm populations remained quite stable in all of the study section except zone V during the summer of 1961. This zone receives the effluent from a metal plating plant at Fowlerville. Above the plating plant the aquatic insects are well represented, both in numbers of families and individuals, and the tubificid worm populations are about equal to those in the lower portion of the study section. Below this plant the aquatic insects are very scarce and the sludge worm populations exceed 137 pounds per acre. The presence of the large populations of tubificid worms indicates that these organisms are not adversely affected by the levels of plating wastes that occur in the river.

Collections were taken from the area above the plating plant during series 2 and the tubificid populations in this area exhibited about the same net gain as in the lower four zones of the study section (Figure XVIII). Collections were taken in the area affected by the plating wastes during series 1, 3, and 4. The large populations of tubificid worms in this area of the stream exhibited a large net gain during series 1 but inorganic sediments from the area of highway construction caused reductions of the tubificids in zone V during the latter two sampling series (Figure XVIII).

Since the large influx of sediment entering the river during late August and early September caused extensive changes in the standing crop biomass of the aquatic insects and the tubificid worms, estimates of average net change were calculated for the portions of the summer before and after the onset of the heavy sedimentation. These estimates for each river zone are given with the average values for the entire summer in Table 28 and the total average estimates are shown in Figure XIX.

Table 28. Net gain of herbivorous and carnivorous aquatic insect and tubificid worm standing crop biomass over the 28 day sampling periods during the summer of 1961. Units are mg. wet weight M^{-2} day^{-1}.

	River Zone				
	Zone I	Zone II	Zone III	Zone IV	Zone V
Herbivores					
Series 1 and 2	-4.5	70.2	118.8	6.2	28.7
Series 3 and 4	-1.6	-25.8	2.2	-12.9	-8.4
Average	-3.0	22.2	60.5	-3.4	10.1
Carnivores					
Series 1 and 2	-2.9	23.8	15.5	13.9	7.8
Series 3 and 4	0.9	25.4	6.7	0.2	-9.4
Average	-1.0	24.5	11.1	7.0	-0.8
Tubificid Worms					
Series 1 and 2	2.2	3.2	-9.6	5.5	220.2
Series 3 and 4	16.5	0.0	-2.4	0.0	-146.4
Average	9.4	1.7	-6.0	2.7	36.8

Figure XIX. Average net gain in standing crop of herbivorous and carnivorous aquatic insects and tubificid worms over the 28 day sampling periods during the summer of 1961 in the Red Cedar River plotted against river zones. Shaded portions of the graphs represent net loss.

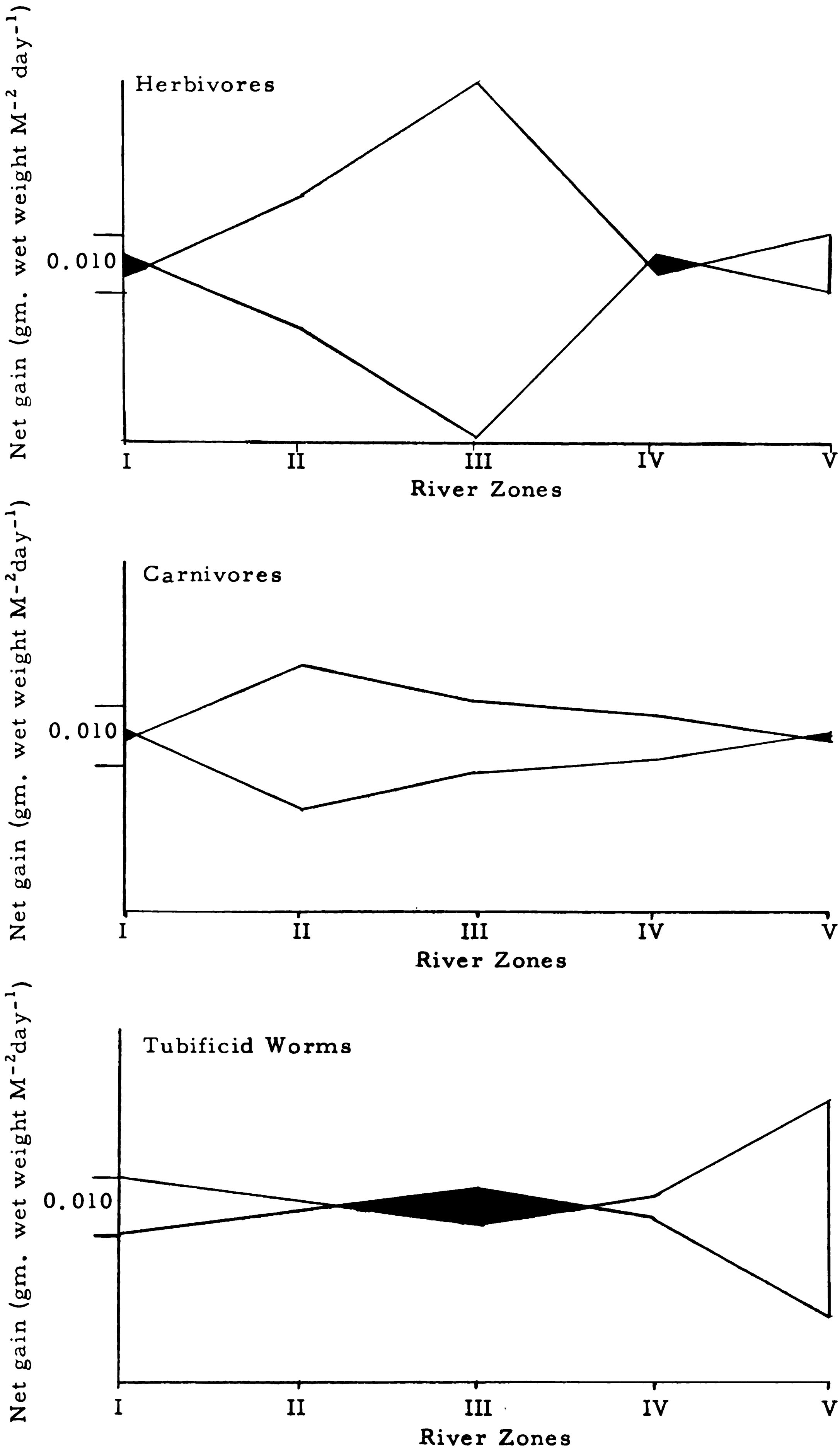

Herbivores
Net gain (gm. wet weight M-2 day-1)
0.010
I
II
III
IV
V
River Zones
Carnivores
Net gain (gm. wet weight M-2day-1)
0.010
I
II
III
IV
V
River Zones
Tubificid Worms
Net gain (gm. wet weight M-2day-1)
0.010
I
II
III
IV
V
River Zones

Figure XIX

The net increase of herbivorous insects was greatest in the zone receiving the effluent from the sewage disposal plant (zone III) and net losses were recorded in the reservoir zone (zone IV) and in the zone furthest downstream (zone I). The net increase of herbivorous insects in zone II was only about a third of that in zone III but was still twice as great as the increase in zone V, which receives the metal plating effluent. The herbivore population in zone III was composed primarily of tendipedid midge larvae and other forms more tolerant of domestic pollution. The reduction of herbivores in zone I is believed to have been due to the combined effects of the increased sedimentation and domestic pollutants entering the stream in this zone. The loss of herbivores in zone IV was largely due to the effects of the increased sedimentation during the latter part of the summer. The net gain in herbivores in zone V was due entirely to the production of the area above the source of pollution at Fowlerville. All sampling sites in zone V below the plating plant showed losses of herbivores. The herbivore standing crop in zone II exhibited a gain during the early part of the summer and a loss during the period of increased sedimentation.

The carnivorous insects exhibited net gains which increased in a downstream direction from zone V to zone II. This downstream increase is believed to have been due to recovery and recolonization of the carnivorous insects from the effects of the metal plating wastes, and appears to reflect modifications of the original stream environment. The loss of carnivores in zone I appears to have been due to the adverse effects of the domestic pollution entering the river in this area. The net loss of carnivores in zone V was due primarily to the changes brought about by the metal plating wastes.

The tubificid worm populations in zone V exhibited the largest increase, due to the large populations present in the polluted area below Fowlerville where they were subjected to little competition and predation. The tubificid populations in zones II and IV exhibited very slight increases, but the standing crop in these areas was much less than that of zone V. The net increase of tubificid worms in zones II and IV was higher during series 1 and 2 than it was during series 3 and 4. Increased sedimentation during the latter two time series was the cause of this reduction. The net loss noted for zone III is thought to reflect the previously discussed instability of this zone.

Other Invertebrates:

Organisms other than the aquatic insects and tubificid worms collected from the Red Cedar River during the summer of 1963 were the following: Decapoda (crayfish), Gastropoda (Snails), Sphaeriidae (fingernail clams), Unionidae (clams), Hirudinea (leeches), Opisthopora (aquatic annelids), Amphipoda (scuds), Hydracarina (mites), Turbellaria (planaria), Ancylidae (limpets), and Isopoda. The mites, planaria, limpets, and isopods were grouped together under a common heading but all of the other organisms were considered separately.

All of the invertebrates except the molluscs were treated in the same manner as the aquatic insects and tubificid worms. Since only total volume was recorded for the molluscs and since the shell weight can not be included as organic weight, factors were determined to allow conversion from total volume to organic weight. Three groups of molluscs were considered in these determinations: the Gastropoda, The Unionidae, and the Sphaeriidae. All snails used to represent the Gastropoda belonged to the genus *Campoloma*, the species not being ascertained. The Sphaeriidae were represented by the genus *Sphaerium*, and again the species was not determined. Four different unionid clams were used in this study, these being *Fusconaia flava*, *Lasmigona costata*, *Strophitus rugosus*, and *Lampsilis ventricosa*. During the past few years there has been a reduction in the number of unionid clams in the

Red Cedar River and it was necessary to include the four genera in order to have a large enough sample to calculate the conversion factors.

Althouth the sample of unionid clams contained four different genera, there was no significant difference in the conversion factors derived for the four groups. The variation of the factors within any group was as large as the variation of the entire sample, so it was considered valid to calculate common conversion factors for the Unionidae from these four genera. The conversion factors calculated for the molluscs from the Red Cedar River are given with the 95% confidence limits in Table 29.

Aquatic invertebrates, other than aquatic insects and tubificid worms were not abundant enough to allow estimation of standing crop. The standing crop estimates are given for these other invertebrates for the individual zones, individual series, and for the entire study section in Table 30.

Crayfish were more common in the samples from the lower three zones of the Red Cedar River and were absent from all but the lower portion of zone V. No crayfish were encountered in zone IV. The estimates of crayfish standing crop are thought to be much too low because they are primarily nocturnal organisms and because they actively avoid the dredge. Vannote (1963) in an extensive study of the crayfish populations in part of the present zone II estimated the average standing crop at 43 gm. M^{-2}.

With the exception of the one sampling location above Fowlerville, molluscs were not found in zone V. This is believed due to the effects of the metal plating wastes. The only molluscs encountered in zone IV were Sphaeriidae. Zone III was found to support very large populations of snails and unionid clams. Zone II exhibited lesser populations of these molluscs while zone I contained fewer yet. The abundance of these molluscs in zone III and the decrease in population size in a downstream

Table 29. Mean values of various conversion factors of molluscs taken from the Red Cedar River given with the 95% confidence limits of the mean.

Conversion Factor	Unionidae	Gastropoda	Sphaeriidae
$\frac{\text{Wet organic wt.}}{\text{Total wet wt.}}$	.480 ± .046	.327 ± .003	.567 ± .005
$\frac{\text{Dry organic wt.}}{\text{Wet organic wt.}}$	.078 ± .010	.134 ± .011	.067 ± .002
$\frac{\text{Dry organic wt.}}{\text{Total wet wt.}}$	.037 ± .005	.044 ± .001	.038 ± .001
$\frac{\text{Dry organic wt.}}{\text{Total volume}}$	.066 ± .008	.048 ± .001	.063 ± .017

Table 30. Standing crop estimates of aquatic invertebrates other than aquatic insects and tubificid worms in the Red Cedar River during the summer of 1961. Units are gm. wet weight M^{-2}.

	Decapoda	Gastropoda	Sphaeriidae	Unionidae	Hirudinae	Opisthopora	Amphipoda	Other
Zone Means:								
I	4.154	1.600	0.468	8.811	0.074	0.239	0.001	0.003
II	2.293	11.968	4.014	49.818	0.026		0.026	0.004
III	6.162	42.680	1.589	100.461	0.093	0.053	0.046	0.024
IV			0.038		0.074		0.254	0.036
V	0.674	1.609		2.170	0.097	1.212	0.011	0.008
Series Means:								
1	3.021	6.852	1.017	57.972	0.076	0.038	0.029	0.022
2	5.160	17.457	0.598	67.622	0.042	0.970	0.025	0.001
3	1.058	21.394	2.512	1.948	0.061	0.181	0.071	0.010
4	1.731	0.582	0.759	1.467	0.112	0.014	0.145	0.028
Mean:	2.656	11.571	1.214	32.252	0.073	0.301	0.067	0.015

direction are attributed to the fertilizing effects of the effluent from the Williamston sewage disposal plant.

Fingernail clams maintained their highest standing crop in zone II. These organisms were more abundant on sand flats, and zone II contains a much larger area of sand than does zone III.

Adverse effects of the excessive siltation during late summer were noted on the unionid clam populations. It is indicated in Table 30 that the average unionid clam population present during time series 3 and 4 was considerably reduced from that present during time series 1 and 2. Many dead clams were noted in the river during the period of heavy siltation and the mortality probably resulted from the abrading and coating action of the silt on the gills of the clams.

Leeches were more common in the polluted zones of the river, and the scuds were most abundant in the reservoir section of zone IV. The larger aquatic annelids and the remaining organisms were encountered in a more random pattern and little can be said about their distribution and abundance.

Comparisons of 1958, 1959, and 1961 Invertebrate Populations:

Estimates of invertebrate populations present in the Red Cedar River during the summers of 1958, 1959, and 1961 are given in Table 31. During the summer of 1958 two samples were taken at a randomly selected location in each of zones I, II, III, and IV. The interval between samples at a given location was 7 days. In 1959 two 10 dredge samples were taken from single randomly selected locations at bi-weekly intervals in each of zones I, II, III, and IV, and two samples were collected from each of three randomly selected sites in zone V.

In 1961 the sampling schedule was expanded considerably and two samples were collected from each of four randomly selected sites in each of the five river zones. The time interval between samples at a given location was 28 days in 1961.

Table 31. Estimates of the invertebrate populations of the Red Cedar River during the summers of 1958, 1959, and 1961. Units are gm. wet organic weight M^{-2}.

Year	River Zone	Insects*	Tubificid Worms	Molluscs	Other Invertebrates	Total
1958						
	I	2.06	0.93	20.52		23.51
	II	2.54	0.38	26.00		28.92
	III	1.81	0.49	17.61		19.91
	IV	2.96	1.48	0.00		4.44
1959						
	I	1.52	0.10	41.20	0.05	42.87
	II	2.24	0.01	44.21	5.30	51.76
	III	0.98	0.24	291.34	3.23	295.79
	IV	1.57	0.86	1.61	0.00	4.04
	V	3.26	7.75	0.10	0.00	11.11
1961						
	I	0.79	0.67	10.88	4.47	16.81
	II	1.66	0.09	65.80	2.35	69.90
	III	1.90	0.31	144.73	6.38	153.32
	IV	1.08	0.31	0.04	0.36	1.79
	V	1.20	5.91	3.78	2.00	12.89

*In 1958 all invertebrates except tubificid worms and molluscs were combined for total volume estimates.

The disparity in the number of sample sites makes difficult any comparison of the invertebrate populations present during each of the three summers. The apparent reduction in insects from 1959 to 1961 is probably due to the reduction brought about by the effects of the heavy sedimentation during the summer of 1961.

The loss of molluscs is more difficult to explain. Silt entering the river during the summer of 1961 undoubtedly caused a reduction in mollusc populations, especially that of the unionid clams. However, siltation is not the sole cause of this mortality. During the past several years, there have been periods during the summer when there was considerable mortality among the unionid clams. This mortality generally occurred during periods of low stream discharge and high water temperature. Under these conditions, low solubility of oxygen and increased heterotrophic respiration combined to reduce night dissolved oxygen concentrations of the water to critical levels. This stress, in addition to those of industrial, domestic, and agricultural pollutants already present in the stream, appears to have exceeded the tolerance of these organisms. Therefore, the mortality of unionid clams in the Red Cedar River during the summer is thought to have been caused by the interaction of several detrimental factors.

The apparent reduction in standing crop of tubificid worms in zone V from 1959 to 1961 is believed to be due to excessive siltation present in 1961.

Larger estimates of standing crop of the remaining invertebrates during the summer of 1961 are due to the increased number of samples causing an increase in the probability of collecting a large crayfish.

CALORIMETRY

In any study of the interrelationships of different types of organisms it is necessary to use a common unit for all measurements. The unit chosen in this study was the gram calorie. Other common units have been used; for example, Gerking (1962) opposed Odum's (1959) use of the calorie and used protein utilization as the comparative factor in his study of bulegill sunfish. He felt that since protein synthesis is essential for the formation of new protoplasm, protein utilization gave more meaningful results than an energy unit such as the calorie. It appears that this conclusion is somewhat misleading since energy is the driving force of all life processes and is certainly basic to the synthesis of protein.

The caloric values of representatives of various trophic levels from the Red Cedar River are given in Table 32. With the exception of the aufwuchs, all caloric values based on dry weight were used directly in converting production estimates from dry weight to the appropriate caloric values. The large quantities of inorganic material which settled on the artificial substrata necessitated a correction of the aufwuchs caloric conversion factor. Therefore, the aufwuchs production estimates were expressed as gram-calories per gram ash-free dry weight rather than on the dry weight basis used for the other levels. The resulting caloric value for total aufwuchs was 4421 gram-calories per gram ash-free dry weight.

Engelmann (1961) found that the mean caloric value of the puff ball was 3856 gram-calories per gram ash-free dry weight and this value was used as the caloric value of the heterotrophic aufwuchs. Since 24.5% of the aufwuchs in the Red Cedar River is composed of heterotrophic organisms, a gram dry organic weight of aufwuchs contains 945 gm-cal. produced by the heterotrophs. The remaining 3476 of the 4421 gm-cal.

Table 32. Caloric values of various organisms collected from the Red Cedar River given with measures of variability. Units are gram-calories per gram dry weight.

Organism	Mean	Number of samples	Standard deviation	±95% confidence limits	95% confidence limits as per-cent of the mean
Tubificid	5261	4	12.42	20	0.38
Crayfish	2901	5	30.10	84	2.88
Aufwuchs*	830	5	22.40	28	3.35
Hydropsychidae	4313	5	331.82	412	9.55
Ephemeridae	4613	2	186.00	1666	36.60
Mixed Insects (carnivores)	5028	2	1025	92	1.83
Unionid Clams	4490	1			

*Uncorrected for sedimentation. The corrected values were 3856 cal/gm ash-free dry weight for the heterotrophic aufwuchs and 4604 cal/gm ash-free dry weight for the autotrophic aufwuchs.

per gram organic weight of total aufwuchs are produced by the autotrophs which account for 75.5% of the sample. The caloric value for the autotrophic aufwuchs, then, is 4604 gm-cal. per gm dry organic weight.

ENERGETICS

Within the stream community the only organisms which can transpose electromagnetic solar energy to chemical energy useful to the heterotrophic members of the community are the autotrophic aufwuchs (chiefly diatoms) and the aquatic macrophytes. These organisms are referred to as primary producers. The energy fixed by the primary producers is used directly by the herbivorous invertebrates and by decomposers such as bacteria, fungi, and tubificid worms. The carnivorous organisms in the stream receive the energy necessary for their life processes indirectly from the primary producers by relying on the energy stored by the herbivorous members of the community.

The transfer of energy within a natural community is a dynamic process and like any other energy transport system, the aquatic community is subject to the second law of thermodynamics. Therefore, not all of the energy fixed by the primary producers can be transported to the next trophic level. A certain amount of this energy is degraded to heat by the metabolic processes which sustain the photosynthetic organisms and additional amounts of energy are metabolically reduced to heat at all levels of the community. Thus, much of the photosynthetically fixed energy is used for maintenance of the community rather than for production.

Odum (1957), Teal (1957), and Nelson and Scott (1962) have attempted to derive the energy budgets of various lotic communities; and Slobodkin (1962) discussed the present knowledge of energy relationships in natural communities.

Odum and Teal confined their measurements to natural springs where environmental conditions approached those of a controlled laboratory system. Nelson and Scott studied a single rock outcrop in a Georgia river. The present study differs from those of Odum, Teal, and Nelson and Scott in that an attempt was made to measure the relationships of various trophic levels within a 30 mile section of a polluted warm-water stream. Included in the study section were zones of industrial and domestic pollution, recovery zones, and relatively unpolluted water.

The size of the study area dictated certain limits for the data but I feel that the estimates of solar insolation, aufwuchs production, and insect and tubificid worm net yield are quite accurate. There is, of course, some speculation in the construction of the energy budgets in that there is a strong reliance on literature values for various parameters.

Energy Budgets

Much can be learned about the dynamics of a community from studies of the energy flow within it. An energy budget is the balance sheet of energy transfer and utilization within an ecosystem, and as such reflects the dependency of the system on external energy sources. In some ecosystems the plants transfer more than enough energy for all of the biotic communities, while in others the organisms depend to varying degrees upon energy from outside sources. In general, natural ecosystems have a tendency to proceed through successive stages until a steady state is reached where production equals total respiration. It is seldom that natural systems, especially streams, progress through these succession stages unmodified. Human influence on streams tends to speed up natural succession to the point where it reaches an unbalanced disclimax. The following energy budgets of the Red Cedar River illustrate the effects of several types of human activity upon natural stream communities.

The production estimates of the autotrophic aufwuchs in the Red Cedar River are believed to very nearly represent the true net production; i.e., total elaboration of chemical energy less the amount of energy needed to maintain these photosynthetic organisms. Therefore, the production estimates of the autotrophic aufwuchs represent the amount of energy they supply to the other trophic levels in the community.

The estimates of macrophyte production were made from collections of these plants taken from the river during the summer of 1962. Vannote (1963) found that the macrophytes were expanding in the river and that the production of macrophytes in 1961 was only 30% of that for 1962. Therefore, the macrophyte production estimates made for the summer of 1962 were corrected by Vannote's factor to correspond with the 1961 estimates of other community levels. Macrophyte production estimates probably approach true net production in that there is little evidence that stream organisms feed directly on the live plants. Vannote (1963) noted that crayfish consume small amounts of Vallisneria and that lesser amounts may be consumed passively by fish while foraging for insects. However, the macrophytes are utilized by the decomposers after they die and become detached.

Production estimates of the heterotrophic aufwuchs probably approach net production, but the degree of predation is unknown. The heterotrophic aufwuchs in the Red Cedar River appear to be roughly the same as the decomposer bacteria described by Odum (1957). Odum estimated that these heterotrophic organisms, living on the surface of the mud, had a tissue growth efficiency (production/assimilation) of 9% and that value will be used in the construction of the following energy budgets.

Estimates of invertebrate production were shown earlier to be estimates of net yield rather than net production in that they did not include predation, mortality, emergence, or downstream drift. Odum found a tissue growth efficiency of 23% for the herbivorous aquatic insects and Teal (1957) estimated the tissue growth efficiency of the carnivorous aquatic insects to be 59%. Teal also noted that the tissue growth efficiency of Limnodrilus, a tubificid worm, was 26%. These values will be used in the construction of the various energy budgets of the Red Cedar River.

Since tissue growth efficiencies are calculated by dividing net production by total assimilation, these efficiencies can not be used to correct net production estimates to true net production. Total assimilation represents all of the energy available to the organism being considered. This includes all of the energy taken in by the organism with the exception of the portion ingested but not digested. Total assimilation, then, is what is often referred to as gross production. Therefore, the accuracy of the estimate of gross production made by this correction depends largely on the accuracy of the net production estimate. The difference between total energy assimilated by an organism and the net energy stored by it is the amount of energy used to metabolically maintain the organism. For these reasons, it appears that Nelson and Scott (1962) were in error when they used tissue growth efficiencies to correct net production estimates for predation and grazing.

During the summer of 1961 some areas of the river sustained net gains in the standing crop of invertebrates while in other areas there was a net loss for the same period. Since these estimates were made by the harvest method, it should be stressed that they refer to average net changes in standing crop; i.e., the changes in standing crop exclusive of predation, mortality, emergence, and downstream drift. These estimates include both gains and losses in standing crop and thus are true averages of the net yield for the river during the summer of 1961. This appears to be a more reasonable approach than that of correcting this value to true net production by the use of several hypothetical correction factors.

Assuming that the tissue growth efficiencies of Odum (1957) and Teal (1957) can be used to estimate the energy assimilation of the biotic community of the Red Cedar River, the amount of energy required to sustain the observed net yield can be determined. This required energy is, of course, a minimum estimate of the energy requirements for the aquatic insects and the tubificid worms since no correction was made for mortality, predation, etc. It was not possible to estimate production of any of the other invertebrates in the stream and thus their energy requirements are not considered in these calculations.

The estimate of total energy available to the community from the primary producers (Table 33) appears to be quite accurate, but the estimate of the amount of energy required to support the remainder of the biotic community is at best a minimum estimate.

The organisms composing the heterotrophic aufwuchs undoubtedly have a very rapid turnover and, if Odum's tissue growth efficiency of 9% can be applied to the Red Cedar River, require an overwhelming majority of the energy necessary to sustain the entire biotic community. From Table 33 it can be seen that the Red Cedar is a heterotrophic stream and that at the very minimum 65% of the energy requirement of the entire biotic community is derived from allochthonous sources. These estimates do not include the energy necessary to sustain the substantial fish populations in the stream. At the present time a study of the fish production of the river is being carried out, but until such time as this study is completed, little can be said about the energy needs of the fish.

The energy budget for each of the five zones of the study section during the summer of 1961 is given in Table 34. In this table it can be

Table 33. Energy budget of the entire study section of the Red Cedar River during the summer of 1961. Units are gm-cal. M^{-2} day^{-1}.

Trophic level	gm-cal. M^{-2} day^{-1}
	Energy Fixed
Autotrophic Aufwuchs	980
Macrophytes	127
Total Primary Production	1,107
Heterotrophic Aufwuchs	266
Herbivorous Insects (net yield)	17
Carnivorous Insects (net yield)	9
Tubificid Worms (net yield)	13
	Energy Required
Heterotrophic Aufwuchs	2,956
Total Insects	139
Tubificid Worms	50
Total Energy Required	3,145
Allochtonous Energy Required	2,038
Percent Allochthonous	64.8%

Table 34. Energy budgets for each of the five river zones during the summer of 1961. Units are gm-cal. M^{-2} day^{-1}.

Trophic level	Zone I	Zone II	Zone III	Zone IV	Zone V
			Energy Fixed		
Autotrophic Aufwuchs	652	1,019	766	1,205	1,257
Macrophytes	116	137	137	48	195
Total primary production	768	1,156	903	1,253	1,452
Heterotrophic Aufwuchs	9*	109	885	79	247
Insects:					
Herbivores (net yield)	-3	22	60	-3	10
Carnivores (net yield)	-1	27	12	8	-1
Tubificid Worms (net yield)	14	2	-9	4	55
			Energy Required		
Heterotrophic Aufwuchs	100	1,211	9,833	878	2,744
Total Insects	-21**	339	348	48	43
Tubificid Worms	54	8	-35**	15	212
Total energy required	154	1,558	10,181	941	2,999
Allochthonous energy needed	0	402	9,278	0	1,547
Percent Allochthonous	0%	26%	91%	0%	52%

*The heterotrophic aufwuchs in zone I were underestimated during the summer of 1961.

**Negative values were not included in the total energy required.

seen that zones I and IV appear to require no allochthonous energy. Heterotrophic aufwuchs production is believed to have been considerably underestimated in zone I during the summer of 1961, due primarily to an algal production which approached bloom proportions during one sampling period. This atypical algal production over-shadowed that of all other periods and influenced the average aufwuchs production in zone I in favor of the autotrophic aufwuchs.

Much of the allochthonous energy required by the biotic community in zone V could be supplied by the effluent from the raw sewage drain in Fowlerville and by leaf fall from the trees which border the stream in this area.

Zone III receives the effluent from the sewage treatment plant in Williamston and the elevated heterotrophic aufwuchs production noted in this zone is undoubtedly due to these domestic wastes. At least 91% of the energy required by the biotic community in zone III is supplied by allochthonous sources.

In a study of the effluents from five different sewage treatment plants with secondary treatment, Bunch et al. (1961) found that from 20 to 30% of the total organic solids in the effluents were filterable, the remainder being present as dissolved solids. The average concentration of the volatile filterable material of the final effluent from the five treatment plants was 89.6 mg. l^{-1}. Assuming that the filterable material composed 30% of the total organic solids, the total organic load would equal 299 mg. l^{-1}, or 1.13 gm. gal.$^{-1}$. Brehmer (1958) estimated that the Williamston sewage treatment plant has a mean base effluent of 150,000 gallons per day. If it is assumed that the effluent from the Williamston sewage treatment plant has roughly the same organic content as that of the plants reported by Bunch et al., an estimate can be made of the organic matter added to the river from this source.

If the effluent from the Williamston sewage treatment plant contains 1.13 gm. gal.$^{-1}$ of dry organic matter, at an effluent output of 150,000 gallons per day the total daily addition of organic matter to the river would be 169,500 grams. This would be equal to 2.09 grams of dry organic material added to each square meter of zone III each day. If this material had a caloric value of 4,705 cal. gm.$^{-1}$, the 2.09 grams M^{-2} day^{-1} would supply all of the estimated required allochthonous energy of 9,833 cal. M^{-2} day^{-1}. This seems to be a reasonable caloric value since this material contains, among other things, a rather large amount of grease (Sawyer, 1960).

The 402 cal. M^{-2} day^{-1} of allochthonous energy required by the biotic community of zone II could be supplied by downstream transport of organic matter from zone III or by the autumnal leaf fall from the trees that line the stream in this area.

During the summer of 1961 there were factors other than domestic and industrial pollution limiting biotic production in the Red Cedar. The greatest single factor limiting the primary producers of the aufwuchs was the silt entering the river from the area of highway construction. Energy budgets for the portions of the summer before and during the period of heavy siltation are given in Table 35.

The silt drastically reduced production of the entire biotic community of the Red Cedar River (Table 35). During the period of siltation there was an apparent reduction of 61% in the primary producers. Since measurements of macrophyte production are not available for each of these two periods, the average summer macrophyte production was used for both portions of the summer. Estimates of autotrophic aufwuchs production are available for both time periods. Since these organisms provide the primary energy source for the herbivorous members of the community, a reduction of autotrophic aufwuchs production would have a greater immediate effect on the community than would a reduction in

Table 35. Energy budgets for the entire study section of the Red Cedar River before and during the periods of heavy siltation in the summer of 1961. Units are gm-cal. M^{-2} day^{-1}.

Trophic Level	Before Siltation	During Siltation
	Energy Fixed	
Autotrophic Aufwuchs	1,140	368
Macrophytes	127	127
Total primary producers	1,267	495
Heterotrophic Aufwuchs	360	170
Insects:		
Herbivores (net yield)	43	-9
Carnivores (net yield)	13	6
Tubificid Worms	65	-39
	Energy Required	
Heterotrophic Aufwuchs	4,000	1,889
Total Insects	283	4
Tubificid Worms	200	-115*
Total energy required	4,483	1,893

*Negative values are not included in the total energy required.

the macrophytes. The heavy siltation and turbidity brought about a 68% reduction in autotrophic aufwuchs production.

The effects of this silt were not confined just to the primary producers. There was also a reduction of 58% in the amount of energy required by the heterotrophic members of the community (Table 35).

It is seen, then, that the silt originating from the area of highway construction caused drastic reductions in all levels of the community, and that the reduction in the amount of energy required approached the reduction in the amount of energy fixed.

Ecological Efficiencies

Recently there has been considerable use made of rather poorly defined ecological efficiencies in the comparison of various ecosystems. These efficiencies, often referred to as trophic level production efficiencies, are the production estimates of a given trophic level divided by the energy of food stuff available to the organisms of that level. Efficiencies have been computed in a variety of ways but now they generally are calculated on an energy basis. However, the use of an energy unit does not mean that efficiencies calculated by different workers are comparable.

Perhaps the most useful ecological efficiency for the comparison of several ecosystems is that of the photosynthetic plants. This efficiency generally refers to the percent of solar insolation fixed as chemical energy by the photosynthetic plants. But, there is considerable difference of opinion in the calculation of these efficiencies; for example, they may be expressed on either a gross production or net production basis. Gross production, in the case of the photosynthetic plants, represents the entire amount of chemical energy fixed by the plants. Net production is equal to gross production less the amount of energy required to metabolically maintain the plants.

Another area of confusion is the type of solar insolation measurement which should be used in these calculations. Rabinowitch (1951) estimated that plants photosynthetically fix about 2 percent of the usable light energy which they absorb. Hand (1946) reported that only 43 percent of the total incident solar energy measured by a pyrheliometer is within the visual spectrum, and Golly (1960) estimated that since infrared radiation is absorbed by clouds, about 50% of total incident solar energy lies within the photosynthetic range. If this is true, only about 1% of total incident solar radiation is photosynthetically fixed by plants. There is a further reduction in solar energy as it passes through water. Riley (1944) noted that the average efficiency of the ocean is 0.18% of the total energy at the surface. Vannote (1963) estimated that the average amount of solar energy available at the substrate level in the Red Cedar River is only 15% of that at the surface.

Ecological efficiencies, then, can be computed in any one of a number of ways. Golly (1960) used 50% of the total incident solar energy as the available energy source for the plants. Nelson and Scott (1962) based their photosynthetic efficiencies on total incident solar insolation, while Odum (1957) attempted to correct total incident radiation for penetration into water and shading by trees.

Vannote (1963) found that the efficiency of primary producers within his study section of the Red Cedar River was 0.23% during the summer of 1962. This estimate is the ratio of net primary production to 50% of total incident solar radiation. Based on light available at the substrate level, this efficiency was 1.5%.

During the summer of 1961 the average efficiency of the primary producers in the entire 30 mile study section was 0.045% based on 50% of total solar energy, and 0.298% when based on available light. Vannote's estimates are roughly five times greater than these, but there are several factors leading to this discrepancy. These estimates are averages of the entire 30 mile study area whereas Vannote's refer to a 2.2 mile section of the river where the aquatic macrophyte beds were much more dense than in other areas of the stream. These efficiencies include only the autotrophic portion of the aufwuchs while Vannote's include the entire organic accrual on the substrata. Another feature contributing to this discrepancy is that Vannote used the 1962 estimate of macrophyte production in his efficiency estimates and I corrected 1962 estimates to the lesser values found during 1961. I feel that the photosynthetic efficiency of 0.045% is representative of the entire river.

Since yield, rather than production estimates, are all that are available for the other trophic levels, no other trophic level efficiencies can be computed.

STREAM POLLUTION

Since the beinning of civilization, man has settled near water. In addition to human agricultural and biological needs, the great industrial expansion leading to modern civilization has been inexorably linked to an abundance of clean water. But, until the middle of the 19th century waste disposal was primarily to prevent the development of a nuisance. In 1855 John Show established a relationship between sewage-polluted water and the spread of infectious diseases. Except for a few specific cases, attempts at waste treatment were confined to the elimination of pathogenic bacteria from that time until well into the 20th century. During the last half-century, expansion of the human population and of modern industry increased the demand for clean water, but at the same time caused a corresponding increase in water pollution. As water quality has decreased there has been a growing awareness that the quantity of water also is limited. But, only within the past 20 years has the maintenance of clean water been intensified.

The problem of water pollution is not static, but continually becomes more complex with the wide variety of new pollutants added to natural waters each year. Domestic and industrial wastes represent only a portion of the total environment of natural waters, and the interaction of pollutants with other environmental factors has been little investigated to date. A pollutant causing a problem in one area may have an entirely different effect in another environment. Thus, the control of water pollution is a many faceted problem and not one where set rules can be applied to all cases.

The Red Cedar River study was originated to determine the energy exchange in a warm-water stream, with special attention to changes in primary and secondary production with variation in stream ecology. Since domestic and industrial pollutants caused the greatest variation in stream ecology, the polluted areas of the stream received considerable attention.

The pollution of the Red Cedar River is probably representative of many mid-western streams. In normal years the Red Cedar receives domestic wastes, industrial wastes, and silt from agricultural areas. During the summers of 1961 and 1962 the river also received a large amount of silt and sand from the construction site of an interstate highway.

Domestic Wastes

When domestic wastes enter a stream there is generally a reduction of the autotrophic organisms (Patrick, 1954) and an increase in the heterotrophic aufwuchs, with the amount of change depending on the severity of the pollution. However, there is an area below many sewage treatment plants in which sewage fungus is the most common organism. In the Red Cedar, the area below the Williamston sewage treatment plant supports large populations of heterotrophic aufwuchs and an autotrophic aufwuchs population smaller than that of other areas of the stream.

Below the area of gross pollution there is usually a section of stream which supports lush populations of autotrophic organisms. This autotrophic growth is supported by the excess nutrients which enter the stream with the sewage effluent. These basic nutrients entering directly from the sewage treatment plant are supplemented by the action of the heterotrophic organisms in the zone of gross pollution. These organisms gain their livlihood by extracting the energy contained in organic matter, and in this reduction of organic sewage large amounts of basic nutrients are released. In the Red Cedar, increased populations of autotrophic organisms below the initial zone of domestic pollution are probably due to two causes; a response to the basic nutrients from the sewage effluent and a reduction in competition from heterotrophic organisms.

In most cases it is obvious that the area of a stream immediately below a domestic sewage outfall is polluted. It is less obvious that the nutrients which are released from sewage and which support large populations of autotrophic organisms below the zone of gross pollution are also a detriment to water quality. These nutrients promote dense beds of aquatic vegetation which filter organic material drifting downstream. This filtering action coupled with the accumulation of dead plants results in large beds of organic matter which are attacked by heterotrophic organisms, thereby lowering the oxygen content of the water and releasing basic nutrients to downstream areas.

It is this biotic stabilization of organic matter that is often referred to as the "self-purification" of a stream. The ability of the biotic community of a stream to stabilize organic matter is limited by such things as the volume, depth, velocity, and temperature of the stream. If the amount of organic material added exceeds the stabilizing ability of the biota, pollution results, with the length of the polluted zone being determined by the amount of organic matter added to the stream.

The enrichment of streams by domestic wastes accelerates natural eutrophication and often results in undesirable changes in the stream biota. Changes take place at all levels of the biotic community. The more desirable game fish are replaced by bullheads and carp and the large numbers of aquatic insect species normally found in unpolluted water are replaced by the tolerant sludge worms.

The area of the Red Cedar below the Williamston sewage treatment plant supports a large population of aquatic insects but it is composed primarily of tendipedid midge larvae and other forms more tolerant of domestic pollution. In the nutrient-rich zone below the initial pollution, dense aquatic vegetation supports a well balanced aquatic invertebrate population. Although this sewage treatment plant causes deterioration of water quality, there is no zone of gross pollution. The nutrients from this sewage effluent are the greatest detriment to the river in that they allow for a continually expanding population of higher aquatic plants.

Industrial Wastes

Toxic industrial wastes may eliminate many members of the stream biota or may be directly harmful only to a few species or life stages. These wastes also can indirectly eliminate organisms such as fish from the polluted area by killing the food organisms which sustain them.

Many industrial effluents receive some form of treatment before they are added to streams. However, just one "spill" of toxic material can eradicate all traces of a recovery of susceptible organisms taking place over a period of years. Thus, if toxic wastes exceed the tolerance of aquatic biota on just one day of the year, the stream becomes unfit for the susceptible organisms.

The Red Cedar receives industrial wastes from a metal plating plant in Fowlerville, and these wastes cause considerable damage to the biota of the stream. Above the plating plant the aquatic insects are well represented, both in numbers of families and individuals. Immediately below this plant the aquatic insects are very scarce and the sludge worms exceed 137 pounds per acre. The presence of the large population of tubificid worms indicates that these organisms are not adversely affected by the levels of plating wastes that occur in the river.

Live molluscs are abundant above the plating plant, and even though empty shells are common below the plant, no live molluscs were found for a distance of 9 miles downstream. This is probably due to the slow dispersal methods of the molluscs. Aquatic insects, with their flying adult stages, could rapidly repopulate the affected area following a spill of toxic wastes. Repopulation of molluscs in the polluted area would take several years, and it appears that large amounts of the wastes are released often enough to preclude re-establishment of the molluscs in this area.

Fish kills occur below the plating plant at infrequent intervals, usually during periods of high water. This suggests that the waste settling ponds may be flooded, releasing large amounts of unsettled toxic wastes to the stream. These wastes remove fish from the area immediately below the plating plant and reduce fish populations for a considerable distance downstream (Linton, 1964).

Inorganic Sediments

Inert inorganic sediments are added to streams from a variety of sources, such as gravel pit washings, mine operations, and highway construction. But, the most common source of inorganic sediment pollution is soil erosion. Whenever land is mis-managed, erosion sets in and soil is lost to nearby streams.

Inorganic materials washed into streams from the watershed affect all levels of aquatic life. Turbidity, caused by inorganic sediment, decreases the amount of solar energy available to the autotrophic plants of the community. Heavy concentrations of inorganic matter in streams scour the attached algae from natural substrata, and the inorganic material settling on the bottom smothers the remaining algae. Since these organisms are the most important members of the community transposing solar energy into chemical energy useful to the heterotrophic organisms, a reduction of the attached algae causes reductions at all subsequent levels.

Bottom type directly affects the productivity of a stream, with rubble and gravel supporting large populations of aquatic organisms while sandy areas are generally quite barren. Areas of shifting sand prevent the establishment of attached algae and higher plants, and the impermanence of this bottom type excludes most of the aquatic invertebrates. When inorganic sediments cover the rubble and gravel areas of a stream, they smother the bottom organisms and bring about a complete change in the entire aquatic community.

Although silt pollution has been shown to harm fish directly by abrasion (Paul, 1952), the greatest detriment of silt to fish is the covering of the spawning sites. Even a very light covering of silt can smother fish eggs; and since the areas most suitable as spawning sites are usually the areas most subject to silt deposition, a small amount of silt can cause large reductions of fish populations. Another adverse factor is the reduction of pool size and depth. Vannote (1963) noted that the smallmouth bass populations of the Red Cedar are closely related to the number and depth of the pools. Since sediments tend to be deposited in the pools, this reduction of habitat will cause material reductions in the bass populations.

During the latter part of the summer of 1961, heavy rains carried large amounts of silt into the Red Cedar from the construction site of a limited access interstate highway. The turbidity and sediment from this source caused a 68% reduction in the production of attached algae, and a reduction of 58% in the amount of energy required to sustain the heterotrophic members of the community. This large reduction of the heterotrophic organisms does not include the harmful effects of the sediment on the substantial fish populations of the stream.

In recent years there has been a growing concern about the effects of highway construction on natural waters. This has been aimed mainly at the changes brought on by stream channel straightening, but other factors have just as far-reaching effects. The highway paralleling the Red Cedar crosses the river in only one place, but it crosses most of the 8 major tributaries between 2 and 5 miles south of the main river. The smothering and abrading effects of the sediments originating from the area of highway construction have caused and will continue to cause changes in the entire biotic community of the river. The reduction of pool size alone may very well make the river unsuitable for the formerly abundant smallmouth bass. There will also be changes in the population structure of the invertebrate community caused by destruction of some species and increases in others.

Thus, the construction of highways can directly affect the biotic community of natural streams, even though there is no modification of the natural stream channel. There was no channel realignment on the Red Cedar, but the silt originating from the highway construction site has caused damage from which the river may never recover.

The Synergistic Action of Pollutants

Since most polluted streams receive more than one type of contamination, a given pollutant seldom acts independently. Poisons can increase one anothers toxicity by recombination with each other and with the natural elements and organic matter in the stream (Southgate, 1932; Jones, 1939). Natural physical factors also affect the toxicity of many substances (Doudoroff and Katz, 1953). When several pollutants enter a stream, they generally enhance each others adverse influence such that the total effect on the stream biota is equal to a sum greater than the sum of the parts.

The Red Cedar receives metal plating wastes and domestic wastes from Fowlerville, domestic wastes from Williamston, and a variety of domestic wastes from the Okemos-East Lansing area. In addition to these wastes from point sources, the stream also is subjected to silt pollution from areas of intensive agriculture. Another more recent source of substances toxic to aquatic organisms is the increased use of agricultural pesticides. As the river flows from upstream to downstream areas, there is a gradual reduction in autotrophic production corresponding to the gradual accumulation of the various pollutants.

During the summer of 1961, the area below Williamston illustrated the synergistic action of several pollutants. The nutrients released from the Williamston sewage effluent promoted large populations of aquatic macrophytes. These aquatic plants, like land plants, can build up soil. They decrease water current velocity and both organic and inorganic materials tend to be deposited in these large beds of vegetation. By late September, the macrophytes die and become detached. Normally these decaying plants are scattered over a large downstream area, but the heavy sediment load from the highway construction covered the plants with a layer of sand. The presence of the decaying vegetation in the sand bars stabilized them and provided rich organic soil building material.

Autumnal leaf fall from the many trees lining the stream contributed additional organic matter to the stream and the net result was that these stabilized sand bars raised the level of the stream bed and filled in many pools. It is expected that this organically-enriched sand will support additional plant growth in the future, and that the vegetation will continue soil building until the end result is the establishment of many well-stabilized mud flats.

In the areas of the stream containing dense beds of aquatic vegetation, the diurnal oxygen pulses ranged from super saturation during peak photosynthetic activity to a low of 2.5 to 3.0 ppm three hours after sunset (Vannote, 1963). These low levels of dissolved oxygen are a function of the total community metabolism and reflect the extent of heterotrophic activity on the excessive amounts of organic material present in the stream. These low dissolved oxygen concentrations border on the lower limit of tolerance of many aquatic organisms, and the addition of other sub-lethal toxicants probably would kill these organisms. For example, the combination of silt pollution and low dissolved oxygen content is thought to be the factor causing large reductions in the unionid clam populations in the river.

During the past few years, there has been a reduction in the number of unionid clams in the Red Cedar, especially in the area below Williamston. These organisms were once common in the river, in fact, van der Schalie (1948) noted that the Grand River and its tributaries were once a productive source of mussels for the pearl button industry. These mussels may be able to tolerate either low dissolved oxygen or the heavy silt, but they can not tolerate low levels of dissolved oxygen when their gills are being abraded and coated by large concentrations of silt and sand.

The most crucial period for the biota of polluted streams is during the summer when stream discharge is low and water temperature is high.

During this period, reduction of organic matter proceeds at an accelerated rate while the addition of domestic wastes proceeds at about the same rate as during the rest of the year. Thus, there is more heterotrophic reduction of more organic matter per unit volume of water during the summer than any other period of the year. This general increase in community metabolism results in very low dissolved oxygen concentrations during summer nights. In streams receiving sub-lethal amounts of toxic substance, it is often the low night-time level of dissolved oxygen that deals the final blow to a biotic community. However, all pollutants in the stream contribute to the removal of the biota and low dissolved oxygen is not the sole lethal agent; for in many cases the biota could withstand the low oxygen content if they were not subjected to other sub-lethal pollutants.

Pollution Detection

When there is an extensive fish kill or other widespread destruction of aquatic biota, the first, and often the only, test used to measure stream conditions is the 5 day BOD. Quite often the stream is found to contain a high BOD, and the fish kill or other biological catastrophy is blamed on the low oxygen content of the water caused by a high biochemical oxygen demand. This is not the complete answer, for the measurement of BOD is not a measure of the cause of the pollution but merely one of many effects.

At the present time there is no one method which can be used to satisfactorily measure and delineate stream pollution, and in order to evaluate the effects of a given pollutant, it is necessary to use a number of methods. Although the determination of the BOD of polluted water is useful, it measures but one of many parameters. Other chemical methods are used in an attempt to quantify stream pollution but studies of stream biota yield more information on the long term effects of pollution than any of the other methods. Aquatic organisms are continually exposed to variations in water quality, and investigations of biotic communities are more apt to show the presence of intermittant pollution or spills of toxic substances than are the various chemical tests. The problems with most biological methods of pollution detection are that they presuppose a high degree of taxonomic training and often require a considerable amount of time. New biological methods for the assessment of water pollution are continually being developed and some show promise as quick, efficient methods requiring little technical training (King and Ball, in press). It is hoped that in the future more attention will be given to the influence of toxic and sub-lethal substances on the biological systems within our waters.

The maintenance of clean water is an expensive and time consuming task, but the rewards of success are great. For, the expansion of our industrial society, and in fact our entire economic growth, is closely tied to the quality and quantity of available water. But, the fact remains that if we want clean water we must pay for it. We now have sufficient knowledge to maintain clean water, but the cost of this operation too often exceeds the desire for a high quality water.

SUMMARY

1. An extensive ecological investigation was conducted on a 30 mile section of a polluted warm-water stream to correlate changes in primary and secondary production with variations in stream ecology.

2. A method was developed for separating heterotrophic and autotrophic aufwuchs production and for separating this production from organic and inorganic sediment.

3. The average aufwuchs production rate for the entire study section during the summer of 1961 was 1,246 gm-cal. M^{-2} day^{-1} of which 980 gm-cal. were due to autotrophic production and 266 gm-cal. were due to heterotrophic production.

4. The average production of macrophytes in the river during the summer of 1961 amounted to 127 gm-cal. M^{-2} day^{-1}.

5. Photosynthetic efficiency was estimated to be 0.045% of the solar energy available at the surface of the stream.

6. Average net yield of aquatic invertebrates was 17 gm-cal. M^{-2} day^{-1} for the herbivorous insects, 9 gm-cal. M^{-2} day^{-1} for the carnivorous insects, and 13 gm-cal. M^{-2} day^{-1} for the tubificid worms.

7. Intermittent flow through a dam was shown to affect aufwuchs production for a distance of 14.2 miles downstream.

8. Inorganic sediments originating from an area of highway construction reduced autotrophic aufwuchs production by 68% and reduced the

heterotrophic members of the community such that their energy requirements were decreased 58%.

9. Metal plating wastes eliminate all aquatic macro-fauna except tubificid worms immediately below the point of effluent and substantially reduce macro-fauna populations for a distance of 15 miles downstream.

10. Domestic wastes from a sewage treatment plant cause a heterotrophic shift in the biotic community in a short section of the stream. Although there is no zone of gross pollution, the nutrients released from this source promote expanding populations of macrophytes downstream.

LITERATURE CITED

Abdin, G. 1949. Benthic algal flora of Aswan Reservoir (Egypt). Hydrobiol., 2: 118-133.

Allen, K. R. 1951. The Horokiwi stream, a study of a trout population. Bull. New Zealand Mar. Dept. Fish., 10: 238 pp.

Bartsch, A. F. 1948. Biological aspects of stream pollution. Sewage Works Jour., 20: 292-302.

Brehmer, M. L. 1958. A study of nutrient accrual, uptake, and regeneration as related to primary production in a warm-water stream. Unpub. Ph. D. thesis, Mich. State Univ., 97 pp.

Bunch, R. L., E. F. Barth, and M. B. Ettinger. 1961. Organic materials in secondary effluents. Jour. Water Pollution Cont. Fed., 33: 122-126.

Burlew, J. S. (ed) 1953. Algal culture: from laboratory to pilot plant. Carnegie Inst. of Wash. Pub. 600, 286 pp.

Butcher, R. W. 1932. Studies on the ecology of rivers, II. The microflora of rivers with special reference to the algae on the river bed. Ann. Bot., 46: 813-861.

_________. 1946. Studies on the ecology of rivers, VI. The algal growth in highly calcareous streams. Jour. Ecol., 33: 268-283.

_________. 1947. Studies on the ecology of rivers, VII. The algae of organically enriched water. Jour. Ecol., 35: 186-191.

Butcher, R. W., F. T. K. Pentelow, and J. W. A. Woodley. 1931. An investigation of the river Lark and the effect of beet-sugar pollution. Fish. Invest. Lond., No. 3, 112 pp.

Campbell, M. S. A. 1939. Biological indicators of intensity of stream pollution. Sewage Works Jour., 11: 123-127.

Carpenter, K. E. 1927. The lethal action of soluble metallic salts on fishes. Jour. Exp. Biol., 4: 378-390.

Castenholz, R. W. 1960. Seasonal changes in the attached algae of freshwater and saline lakes in the lower Grand Coulee, Washington. Limnol. and Oceanogr., 5: 1-28.

Cooke, W. B. 1956. Colonization of artificial bare areas by microorganisms. Bot. Rev., 22: 613-638.

Douderoff, P., and M. Katz. 1953. Critical review of the literature on the toxicity of industrial wastes and their components to fish, II. The metals as salts. Sewage Indust. Wastes, 22: 802-839.

Englemann, M. D. 1961. The role of soil arthropods in the energetics of an old field community. Ecol. Monogr., 31: 221-238.

Gaufin, A. R., and C. M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Publ. Hlth. Rep., 67: 57-64.

Gerking, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecol. Monogr., 32: 31-78.

Golly, F. B. 1960. Energy dynamics of a food chain of an old field community. Ecol. Monogr., 30: 187-206.

Grzenda, A. R. 1960. Primary production, energetics, and nutrient utilization in a warm-water stream. Unpub. Ph. D. thesis, Mich. State Univ., 99 pp.

Grzenda, A. R., and M. L. Brehmer. 1960. A quantitative method for the collection and measurement of stream periphyton. Limnol. and Oceanogr., 5: 190-194.

Hand, I. E. 1946. Pyrheliometers and pyrheliometric measurements. U. S. Dept. Commerce, 55 pp.

Harvey, W. H. 1934. Measurement of phytoplankton populations. Jour. Mar. Biol. Assoc. U. K., 19: 761-773.

Hentschel, E. 1916. Biologische Untersuchungen uber den tierischen und pflanzlichen Bewuchs im Hamburger Hafen. Mitt. Zool. Mus. Hamburg, 33: 1-172.

Hynes, H. B. N. 1960. The biology of polluted waters. Liverpool Univ. Press, Liverpool, England. 202 pp.

Ivlev, V. S. 1933. Ein Versuch zur experimentellen Erforschung der Okologie der Wasserbioconosen. Arch. Hydrobiol., 25: 177-191.

Jones, J. R. E. 1939. The relations between the electrolytic solution pressures of the metals and their toxicity to the stickleback (Gasterosteus aculeatus). Jour. Exp. Biol., 16: 425-437.

Kevern, N. R. 1961. The nutrient composition, dynamics, and ecological significance of drift material in the Red Cedar River. Unpub. M. S. thesis, Mich. State Univ., 94 pp.

________. 1962. Primary productivity and energy relationships in artificial streams. Unpub. Ph. D. thesis, Mich. State Univ., 132 pp.

King, D. L. 1962. Distribution and biomass studies of the aquatic invertebrates of a warm-water stream. Unpub. M. S. thesis, Mich. State Univ., 74 pp.

King, D. L. and R. C. Ball. 1964. A quantitative biological measure of stream pollution. Jour. Water Pollution Cont. Fed., (in press).

Kreps, E. and N. Verjbinskaya. 1930. Seasonal changes in the phosphate and nitrate content and in hydrogen-ion concentration in the Barents Sea. Jour. du Conseil, 5: 329-346.

Learner, M. A. and R. W. Edwards. 1963. The toxicity of some substances to Nais (Oligochaeta). Proc. Soc. Water Treat. and Examin., 12: 161-168.

Manning, W. M. and R. E. Juday. 1941. The chlorophyll content and productivity of some lakes in northeastern Wisconsin. Trans. Wis. Acad. Sci. Arts. Lett., 33: 363-393.

Meehan, W. R. 1958. The distribution of fish in the Red Cedar River drainage in relation to habitat and flow. Unpub. Ph. D. thesis, Mich. State Univ., 136 pp.

Moore, H. B. 1958. Marine Ecology. John Wiley and Sons, New York, 493 pp.

Nelson, D. J. and D. C. Scott. 1962. Role of detritus in the productivity of a rock-outcrop community in a piedmont stream. Limnol. and Oceanogr., 7: 396-413.

Newcombe, C. L. 1949. Attachment materials in relation to water productivity. Trans. Am. Micros. Soc., 68: 355-361.

________. 1950. A quantitative study of attachment materials in Sodon Lake, Michigan. Ecology, 31: 204-215.

Odum, E. P. 1959. Fundamentals of Ecology. 2nd ed. Saunders, Philadelphia, 546 pp.

Odum, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. Ecol. Monogr., 27: 55-112.

Patrick, R. 1954. Diatoms as an indication of river change. Proc. 9th Industr. Waste Conf. Purdue Univ. Eng. Ext. Ser., 87: 325-330.

Patrick, R., M. H. Horn, and J. H. Wallace. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae, No. 259, 12 pp.

Paul, R. M. 1952. Water pollution: a factor modifying fish populations in Pacific Coast streams. Sci. Mon., 74: 14-17.

Peters, J. C. 1959. An evaluation of the use of artificial substrates for determining primary production in flowing waters. Unpub. M. S. thesis, Mich. State Univ., 106 pp.

Rabinowitch, E. I. 1951. Photosynthesis and related processes. Vol. II. Interscience, New York, 1: 603-1208.

Rasmussen, C. J. 1955. On the effect of silage juice in Danish streams. Verh. Int. Ver. Limnol., 12: 819-822.

Rawstron, R. R., Jr. 1962. A study of primary production on artificial substrates in a riffle and pool area of the Red Cedar River. Unpub. M. S. thesis, Mich. State Univ., 73 pp.

Riley, G. 1944. The carbon metabolism and photosynthetic efficiency of the earth. Am. Sci., 32: 132-134.

Ruttner, F. 1953. Fundamentals of Limnology. (transl. by D. G. Frey and F. E. J. Fry). Univ. of Toronto Press, Toronto, Canada, 227 pp.

Sawyer, C. N. 1960. Chemistry for Sanitary Engineers. McGraw-Hill, New York, 365 pp.

Slobodkin, L. B. 1962. Energy in animal ecology, 69-101. In J. B. Cragg, (ed.), Advances in Ecological Research. Vol. I. Academic Press, New York.

Southgate, B. A. 1932. The toxicity of mixtures of poisons. Quart. Jour. Pharm., 5: 639-648.

Steele, J. H. 1962. Environmental control of photosynthesis in the sea. Limnol. and Oceanogr., 7: 137-150.

Strickland, J. D. H. 1960. Measuring the production of marine phytoplankton. Fish. Res. Bd. Canada, Bull. No. 122, 172 pp.

Teal, J. M. 1957. Community metabolism in a temperate cold spring. Ecol. Monogr., 27: 283-302.

Tebo, L. B. 1955. Effects of siltation, resulting from improper logging, on the bottom fauna of a small trout stream in the Southern Appalachians. Prog. Fish. Cult., 17: 64-70.

van der Schalie, H. 1948. The commercially valuable mussels of the Grand River in Michigan. Mich. Dept. Cons. Misc. Pub. No. 4, 42 pp.

Vannote, R. L. 1961. Chemical and hydrological investigations of the Red Cedar River watershed. Unpub. M. S. thesis, Mich. State Univ., 126 pp.

________. 1963. Community productivity and energy flow in an enriched warm-water stream. Unpub. Ph. D. thesis, Mich. State Univ., 156 pp.

Wiebe, A. H. 1927. Biological survey of the upper Mississippi River with special reference to pollution. Bull. U. S. Fish., 43: 137-167.

Wilson, J. N. 1953. Effect of kraft mill wastes on stream bottom fauna. Sewage Industr. Wastes, 25: 1210-1218.