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A QUALITATIVE AND QUANTITATIVE MEASURE OF AUFWUCHS PRODUCTION

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DARRELL L. KING AND ROBERT C. BALL

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A QUALITATIVE AND QUANTITATIVE MEASURE OF AUFWUCHS PRODUCTION¹

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ABSTRACT

Aufwuchs production was measured from its accrual on plexiglass substrates submerged in a stream. A method was developed for separating autotrophic and heterotrophic *aufwuchs* production and for separating this production from organic and inorganic sediments. The average *aufwuchs* production in the entire river during the summer of 1961 was 281.8 mg organic weight M⁻² day⁻¹, with the autotrophic organisms contributing 212.8 mg and the heterotrophic 69.0 mg.

The rate of accumulation of organic material on artificial substrates has been used to estimate production within streams and to estimate the well-being of natural waters. In the past these measurements have included all of the organic matter which accumulated on the substrates and little thought was given to the origin of this material. The method given here allows the separation of this material into the components that are formed on the substrates and those transported from elsewhere and deposited.

The technique of collecting *aufwuchs* communities from natural waters by means of artificial substrates has been employed for many years in a wide variety

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of ecosystems. Hentschel (1916) generally is credited with being the first to use this method; but Butcher (1932), Ivlev (1933), Newcombe (1949, 1950), Patrick, et al. (1954), Castenholz (1960), Grzenda and Brehmer (1960), Kevern (1962), Sladeckova (1962), Wetzel (1963), and many others have used artificial substrates to study *aufwuchs* growth, production, and succession. Cooke (1956) reviewed the history and general methods of collecting *aufwuchs* on artificial substrates.

Direct microscopic examination of the exposed substrates has been used by many workers, but Newcombe (1949) was the first to deal with the production of total organic matter, in contrast to the earlier work that was concerned chiefly with species composition. Grzenda and Brehmer (1960) developed a method of estimating *aufwuchs* production on artificial substrates from phytopigment extracts, and our method employs an expansion and modification of their procedure.

A study was conducted during the summer of 1961 on a 30-mile section of the Red Cedar River, a warm-water stream located in the south central portion of the lower peninsula of Michigan. This 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. The material presented here represents a portion of that study. This section of the river drains 355 square miles of rolling farm and suburban land and for purposes of this investigation was divided into five different ecological zones. The Red Cedar is representative of many midwestern streams in that it receives industrial and domestic wastes and inorganic sediments from agricultural areas.

METHODS

Artificial substrates consisting of flat plexiglass plates, $\frac{1}{4}$ in. thick, having a total exposed area of 1.4 dm² when attached to the supporting racks, were used to measure the rate of growth and accrual of the *aufwuchs* community of the Red Cedar River. The supporting racks were made of a wood crossbar bolted securely to a steel fence post driven into the bottom of the stream. Number 125 Acco clamps were attached with screws to the crossbar, and substrates were held securely in these pinch clamps. Both horizontally and vertically placed plates were oriented with the $\frac{1}{4}$ in. edge facing into the current.

The exposure depth of the substrates was maintained at 0.6 of the distance from the water surface to the bottom of the river to assure representative illumination of all units and make possible the comparison of the *aufwuchs* accrual rate in different areas of the river.

Six different exposure periods were allowed for the accrual of *aufwuchs*; these being three, six, nine, 12, 15, and 18 days. All substrates were placed in pairs; that is, two were placed on the rack for each of the exposure periods.

When the substrates were removed from the river, they were placed individually in plastic freezer bags and frozen. Freezing ruptured the plant cells aiding in the removal of the accumulated material and in the extraction of phytopigment. After freezing, the macrofauna were removed and the attached material was scraped from the substrates with pieces of hard rubber and rinsed with 95% ethanol. The hard rubber did not remove any of the plexiglass. Bottles containing the sample along with 50 ml of 95% ethanol were placed in a refrigerator for at least 48 hrs to permit the extraction of the phytopigments from the periphytic algae. Grzenda & Brehmer (1950) found that samples can be stored in this manner for at least 30 days without decomposition of the phytopigment.

The ethanol-soluble phytopigments were separated from the remainder of

the sample by filtering through a membrane filter with a pore size of 0.45 μ . The absorbancy of the phytopigment was determined in a Klett-Summerson colorimeter using a 4 cm solution depth and a red filter (640–700 m μ).

Grzenda and Brehmer found that the absorption of broad spectrum light is not linearly related to the concentration of ethanol-soluble phytopigment extracts except at low concentrations. Therefore, due to this deviation from the Lambert-Beer law, the measured absorbancies must be corrected. Since Grzenda and Brehmer based their method on work done in the Red Cedar River, their correction graph was used in the present study to correct for this deviation. The corrected absorbancy measurements are designated as phytopigment units.

The ethanol-phytopigment extract was then recombined with the membrane filter and the accompanying residue in a tared evaporating dish. The sample was evaporated to dryness and the dry weight and ash-free dry weight (organic weight) were determined by gravimetric procedures using an analytical balance accurate to the nearest 0.1 mg. Although the filters did not contain a measurable amount of ash, it was necessary to weigh each filter used in the dry weight determination because they were found to vary as much as ± 1 mg.

RESULTS AND DISCUSSION

SUBSTRATE PLACEMENT AND ORGANIC LOSS ON REMOVAL: The algae present in the *aufwuchs* of the Red Cedar River were almost exclusively diatoms and the most common genera were *Gomphonema*, *Navicula*, *Fragilaria*, *Cymbella*, *Cyclotella*, and *Synedra*. Peters (1959) found that the plexiglass substrates were not selective, but supported the same dominant organisms as did the natural substrates. This observation has also been reported by Butcher (1932) and Patrick et al. (1954) for colonization of algae on glass slides.

The organic weight of material collected from vertical and horizontal substrates placed in the Red Cedar River was in a ratio of 1 : 1.15. This ratio for a Washington lake was estimated at 1 : 6.2 (Castenholz, 1960) and agreed well with Newcombe's (1949) estimate of 1 : 6.6 for the ratio of organic weight from vertical and horizontal substrates placed in a Michigan lake. 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 substrates 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 from the horizontal than the vertical substrates when they were removed from the water.

The Red Cedar is a turbid stream and the algae colonize in and on the silt settling on the horizontal substrates; whereas they grow directly attached to the plexiglass of the vertical substrates. The net result is that upon removal 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, actively growing portion of the diatom colony is lost from the horizontal than from the vertical substrates.

The organic weight fractions of the *aufwuchs* collected in 1961 from all vertically and horizontally placed substrates from the Red Cedar River were subjected to a matched pairs "t" test to test for differences in accumulation of organic matter on the two types of placement. The results of this test indicated no significant difference at the 0.05 level in the accrual of organic material between the horizontal and vertical substrates. It was then assumed that the *aufwuchs* production rate was equal on vertical and horizontal substrates, and that the small observed differences were due to the greater rate of organic sedimentation on the horizontal plates.

ORGANIC PRODUCTION OF AUFWUCHS: The material which accumulated on the artificial substrates was composed of inorganic sediments, inorganic material produced on site, organic sediments, and autotrophic and heterotrophic growth. The inorganic matter can be separated from the organic quite easily, but the difficulty lies in separating the three different organic fractions.

The dry weights of the *aufwuchs* samples collected from the vertical substrates were designated as A_v while those from the horizontal substrates were designated A_h . The respective designations for the ash-free dry weight of the samples collected from these two substrate placements were B_v and B_h . Ash-free dry weight approximates organic weight and is equal to the dry weight (A) less the weight of the ash.

The inorganic content of the accrued material is then represented by $A_v - B_v$ for the vertical substrates and by $A_h - B_h$ for those placed horizontally. These values include all inorganic material accumulating on the substrates, both that from inorganic sediment and that produced on site. Because the algae found on the substrates were almost exclusively diatoms, a correction was made for the weight of their silica frustules. 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) suggested that 50% be used for the inorganic percentage of diatoms, and that value was used in this study. Therefore, the inorganic accrual rate due to the production of diatom frustules is roughly equal to the organic accrual rate, so this value (B) subtracted from the total inorganic accumulation rate ($A - B$) yields the rate of inorganic sedimentation. Inorganic sedimentation on the vertical substrates is then equal to $A_v - 2B_v$, and for the horizontal placement it is $A_h - 2B_h$.

Average measurements of the dry weight and ash-free dry weight fractions of the *aufwuchs* collected from the Red Cedar River during the summer of 1961 are given below.

283.8 mg organic weight $M^{-2} \text{ day}^{-1}$ = Average accrual rate of organic material on the vertical substrates (B_v)

326.5 mg organic weight $M^{-2} \text{ day}^{-1}$ = Average accrual rate of organic material on the horizontal substrates (B_h)

817.3 mg dry weight $M^{-2} \text{ day}^{-1}$ = Average accrual rate of all material on the vertical substrates (A_v)

1,252.0 mg dry weight $M^{-2} \text{ day}^{-1}$ = Average accrual rate of all material on the horizontal substrates (A_h).

The total inorganic fraction of the average *aufwuchs* sample from the vertical substrata was calculated to be $533.5 \text{ mg } M^{-2} \text{ day}^{-1}$ ($A_v - B_v$), while for the horizontal placement it was $925.5 \text{ mg } M^{-2} \text{ day}^{-1}$ ($A_h - B_h$). Correcting for inorganic material produced on site gives inorganic sedimentation rates of $249.7 \text{ mg } M^{-2} \text{ day}^{-1}$ ($A_v - 2B_v$) for the vertical placement and $599.0 \text{ mg } M^{-2} \text{ day}^{-1}$ ($A_h - 2B_h$) for the horizontal placement.

It is assumed that organic sediments accumulate at the same ratio to the inorganic sediments on the vertical as on the horizontal substrates; i.e., that the organic and inorganic fractions of the total sediment are independent of substrate placement. This assumption then permits the establishment of the following relationship:

$$1) \quad \frac{A_v - 2B_v}{X} = \frac{A_h - 2B_h}{X + (B_h - B_v)} .$$

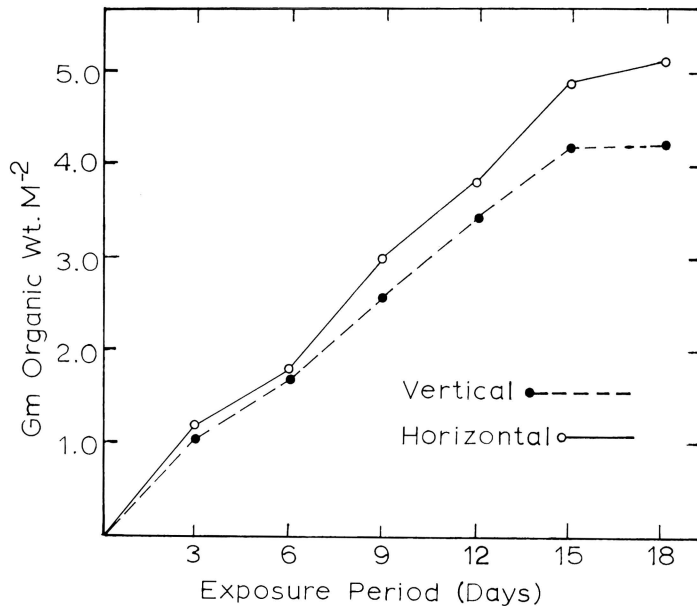


FIG. 1. Standing crop of organic matter accrued on the artificial substrata during the summer of 1961 plotted against the six exposure periods.

Where

X = Organic sedimentation on the vertically placed substrates

$B_h - B_v$ = Organic sedimentation on the horizontally placed substrates in excess of that which settled on the vertically placed substrates. (Assuming organic production is equal on both substrate placements, this excess is entirely sedimentation.)

$X + (B_h - B_v)$ = Organic sedimentation on the horizontally placed substrates

$A_v - 2B_v$ = Inorganic sedimentation on the vertical substrates

$A_h - 2B_h$ = Inorganic sedimentation on the horizontal substrates.

Then, solving for X , equation 1 becomes

$$2) \quad X = \frac{(A_v - 2B_v)(B_h - B_v)}{(A_h - 2B_h) - (A_v - 2B_v)} .$$

Using equation 2 and the above weight measurements determined from *aufwuchs* samples collected from the Red Cedar River, total organic accrual was separated into organic sediment and organic *aufwuchs* production. The amount of organic debris settling on the vertical substrates (X) was calculated to have been $30.5 \text{ mg M}^{-2} \text{ day}^{-1}$, and the organic sedimentation on the horizontal substrates ($X + (B_h - B_v)$) was $73.2 \text{ mg M}^{-2} \text{ day}^{-1}$. The net production of organic matter by autotrophic and heterotrophic growth was then equal to $283.8 - 30.5 (B_v - X)$ or $326.5 - 73.2 (B_h - [X + (B_h - B_v)])$ or $253.3 \text{ mg M}^{-2} \text{ day}^{-1}$.

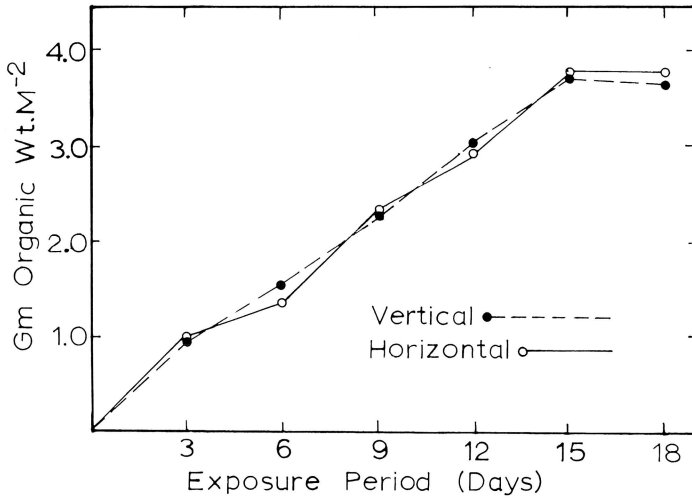


FIG. 2. Standing crop of organic matter accrued on the artificial substrata during the summer of 1961, corrected for organic sedimentation plotted against the six exposure periods.

A further indication of the validity of this correction was shown in the comparison of the ratios of phytopigment to organic weight of the accrued material on the two types of placement. During the summer of 1961, the ratio of phytopigment to total organic weight was 8.27 for the horizontal substrates and 10.06 for the vertical, giving a relationship of 8.27 : 10.06 or 1 : 1.22. After correction for organic sediment, the ratios of phytopigment to organic *aufwuchs* production were 10.63 for the horizontal and 11.27 for the vertical substrates, giving a relationship of 10.63 : 11.27 or 1 : 1.06.

If autotrophic and heterotrophic production were the same on both the vertically and horizontally placed substrates, this corrected ratio would be 1 : 1. The corrected ratio is in fact quite close to the theoretical value, indicating that *aufwuchs* production proceeded at about the same rate on the two types of placement.

COLONIZATION RATE VS. SUBSTRATE PLACEMENT: Colonization by the *aufwuchs* began soon after the substrates were placed in the river (Figs. 1, 2). Following the initial colonization, *aufwuchs* production proceeded at a nearly constant arithmetic rate for exposure periods up to 15 days. At this point the colonies stabilized and the new growth was equal to the organic material sloughed off from the substrates. The accumulation of total organic matter is given in Figure 1, while Figure 2 illustrates the accumulation of organic matter corrected for organic sedimentation. This second growth curve represents the organic matter actually produced on the plexiglass substrates by heterotrophic and autotrophic organisms. The rate of colonization and *aufwuchs* community growth is essentially the same for the vertically and horizontally placed substrates (Fig. 2). Organic matter, due to on-site growth, from three- to 15-day exposure periods accumulated at a rate of 0.2533 grams organic 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.

ORGANIC SEDIMENT: One of the five river zones lies below a dam maintained to power a small generator. During periods of low summer discharge, the gates of the dam are closed until the impoundment fills, and during this time the stream discharge below the dam is very low. When the impoundment is filled,

the gates are opened and the flow below the dam is increased two to three times. This sudden surge of water washed some of the *aufwuchs* colony from both natural and artificial substrates, and the estimate of organic sediment for that period was negative. A negative estimate of organic sediment indicated that some of the organic material produced by the *aufwuchs* had been removed from the substrates.

This loss of *aufwuchs* was not considered in the calculation of the *aufwuchs* production rate of 253.3 mg organic material $M^{-2} \text{ day}^{-1}$. Therefore, a better estimate of the *aufwuchs* production rate over the entire 30-mile study section is the average of the production rates in each of the five zones. Thus, the production rate of *aufwuchs* over the entire study section was estimated at 281.8 mg organic material $M^{-2} \text{ day}^{-1}$.

AUTOTROPHIC AND HETEROTROPHIC GROWTH: It was assumed that the the organic sediment which accumulated on the artificial substrates contained no phytopigment. This implies that all of the phytopigment present in an *aufwuchs* sample was produced by the *aufwuchs*, and that none was added with the organic sediment. Work by Grzenda (1960) substantiates this assumption in that he noted that extractions from suspended materials obtained by filtration of water samples from the Red Cedar River showed a virtual absence of phytopigments. This assumption allowed the ratio of phytopigment to total organic accrual to be corrected for organic sedimentation so that it was then the ratio of phytopigment to organic *aufwuchs* production.

This correction was accomplished in the following manner. The total amount of phytopigment (PU) produced per square meter per day was computed by multiplying the ratio of phytopigment to organic weight (PU/B_v) by the weight of total organic accumulation (B_v). This value was then divided by *aufwuchs* production ($B_v - X$) and the result represents the average amount of phytopigment per mg of *aufwuchs*. The above discussion refers to vertical substrates but the same reasoning is applied to the information obtained from the horizontal substrates.

After the correction for organic sedimentation was made, the remaining organic matter was due to heterotrophic and autotrophic production. The autotrophic members contain chlorophyll and other phytopigments enabling them to synthesize their own chemical energy source from inorganic material and solar energy. The heterotrophic organisms do not have these light sensitive phytopigments and must rely on energy-rich organic matter. The autotrophic community in the *aufwuchs* of the Red Cedar River was made up almost exclusively of diatoms. The flow of the river precluded the buildup of large numbers of micro-crustacea and all macrofauna were removed from the substrata before the sample was processed. Thus, the heterotrophic *aufwuchs* community was composed largely of bacteria, *fungi*, and protozoa.

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

The *aufwuchs* community with the greatest amount of phytopigment was assumed to be composed entirely of diatoms. This assumption is not strictly true since in natural waters there is always some heterotrophic growth.

This assumption is considered valid, however, since the greatest amount of phytopigment per unit organic production was encountered in the zone farthest from a known source of pollution which would encourage heterotrophic growth. The maximum phytopigment content of the *aufwuchs* was 13,112 phytopigment units per mg of organic *aufwuchs*. This value (PU_I) represents the ideal phytopigment content of the autotrophic *aufwuchs*.

TABLE I
Summary of All Components Contributing to the Total Accrued Material on the Artificial Substrata. All Units Are mg M⁻² day⁻¹

River Zone and Placement	Inorganic Sediment	Inorganic Material Produced on Site	Organic Sediment	Autotrophic Production (organic)	Heterotrophic Production (organic)
I _{horiz.}	318.8	298.8	154.8	141.6	2.4
I _{vert.}	60.3	173.3	29.3	141.6	2.4
II _{horiz.}	461.1	299.3	49.8	221.3	28.2
II _{vert.}	233.2	274.7	25.2	221.3	28.2
III _{horiz.}	762.8	267.6	-128.3	166.3	229.6
III _{vert.}	519.1	308.6	-87.3	166.3	229.6
IV _{horiz.}	942.6	385.8	103.3	261.9	20.6
IV _{vert.}	352.1	321.1	38.6	261.9	20.6
V _{horiz.}	524.0	379.8	42.6	273.1	64.1
V _{vert.}	52.3	341.4	4.2	273.1	64.1

The ratio of observed to ideal phytopigment content (PU/PU_I) allowed the separation of *aufwuchs* into autotrophic and heterotrophic fractions. The average observed pigment concentration (PU) of the *aufwuchs* collected from zone I during the summer of 1961 was 12,894 phytopigment units per mg organic weight ($B_v - X$). The ratio of observed to ideal pigment content (PU/PU_I) was 12,894/13,112 or 0.983. Thus, 98.3% of total *aufwuchs* production in zone I was due to autotrophic growth. The average *aufwuchs* production rate in zone I for this same period was 144.0 mg M⁻² day⁻¹ ($B_v - X$). Thus, the autotrophic production rate in zone I was 0.983×144.0 or 141.6 mg M⁻² day⁻¹.

The heterotrophic production rate was equal to the total *aufwuchs* production rate less the autotrophic production rate. During the summer of 1961, the average production rate of heterotrophic *aufwuchs* in zone I was $144.0 - 141.6$ or 2.4 mg M⁻² day⁻¹.

A summary of the rates of accrual of all components contributing to the total accumulation of material on the artificial substrates in the five river zones during the summer of 1961 is given in Table I. The average *aufwuchs* production rate for the entire study section of the Red Cedar River was 281.8 mg organic weight M⁻² day⁻¹ of which autotrophic growth produced 212.8 mg, and heterotrophic growth produced 69.0 mg.

PRECISION OF METHOD: Collection of *aufwuchs* communities on plexiglass substrates is an accurate method of measuring the accumulation of organic material. The 95% confidence limits for the rate of accumulation of organic matter on the horizontal substrates were ± 28.65 mg organic weight M⁻² day⁻¹, or $\pm 8.77\%$ of the mean value of 326.5 mg organic weight M⁻² day⁻¹ and for the vertical substrates they were ± 22.42 mg organic weight M⁻² day⁻¹, or $\pm 7.90\%$ 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 type of placement, time of summer, or area of the river were ± 41.04 mg organic weight M⁻² day⁻¹, or $\pm 13.48\%$ of the mean of 305.2 mg organic matter M⁻² day⁻¹.

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Color Grayscale, 8 bit; Color, 24 bit
File types Tiffs converted to pdf
Notes Greyscale pages cropped and canvassed. Noise removed from background and text darkened.
Color pages cropped.