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Title: Periphyton Standing Crops on Natural and Artificial Substrates in a Flint Hills Stream

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Periphyton standing crops were studied to quantify seasonal levels in a Flint Hills headwater stream in Lyon County, Kansas, from November, 1989, to July, 1990. Samples were scraped from natural rock substrates, and data were compared to that of plexiglass microscope slides and pine dowels. Ash-free-dry weight and chlorophyll_a were measured. Biomass data from rocks displayed a seasonal trend with highest values in late winter (4.184 mg cm^{-2} on February 26) and lowest values in summer (0.3028 mg cm^{-2} on June 25). The significance of a visible trend was supported by ANOVA. Chlorophyll standing crops ranged from .0010 mg cm^{-2} to .0415 mg cm^{-2}. A simple correlation was calculated between chlorophyll and biomass data ($Y = .006479 + .004584\ X$; $r^2 = .4425$, $s_{Y*X} = .0551$ mg cm^{-2}). Predominant algal genera were identified, and quantified in one 499-cell count. Physicochemical conditions were monitored through the study. Nitrate-nitrogen and orthophosphate-phosphorus concentrations were highest through the winter and lowest in spring and summer, and were consistently lower throughout the study than reported levels in area rivers and creeks.

Current flow rates were between 0.20 m^3 min^{-1} and 0.59 m^3 min^{-1}, inclusively. Water temperatures ranged from 0°C to 28°C.

PERIPHYTON STANDING CROPS ON NATURAL AND ARTIFICIAL SUBSTRATES IN A FLINT HILLS STREAM

A Thesis

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by

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[illegible]

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INTRODUCTION

Periphyton

The periphyton community is defined as the community of the attached photosynthetic microorganisms (periphyton) and associated assemblages of bacteria, fungi, protists and other taxa found on submerged rocks, debris and macrophytes (Cole, 1983; United States Geological Survey, 1977). In streams, the standing crops of periphyton may serve as an indicator of water quality because primary production in some streams is chiefly dependent on the autotrophic periphyton. The periphyton serves as an important source of fixed nitrogen, organic phosphorus compounds and essential food materials for stream grazers (Stevenson and Lowe, 1986; Eloranta and Kunnas, 1979).

In streams, production varies with the location of the periphyton relative to current, flow direction and velocity, available photosynthetically active radiation (PAR), and surface texture of the substrate. Differences in these factors affect the colonization and proliferation of the periphyton. Sedimentation of organic particles and detritus also varies with current velocity (Korte and Blinn, 1983; McIntire, 1966) and affects estimates of periphyton production based on biomass. Moderate currents clean photosynthesis-inhibiting organic and particulate matter from the periphyton while replenishing inorganic nutrients. Stronger currents reach a velocity at which scouring of periphyton occurs and surface uptake of nutrients by individual cells is decreased (Horner and Welch, 1981).

Prior to the initial periphyton colonization, newly exposed surfaces experience a process of conditioning which is not reflected on previously exposed surfaces. This conditioning occurs naturally as dissolved, fine and very fine particulate matter, suspended solids, and bacteria attach to the previously bare surface. Korte and Blinn (1983) have shown that this conditioning can be completed in as little as two hours after exposure. Conditioning leaves an organic biofilm to which periphyton can attach (Steinman and Parker, 1990).

Much of the literature on periphyton production is concerned with studies conducted after colonization has begun and during varied phases of community succession. After conditioning, colonization progresses with attachment and growth of diatoms, green algae, cyanobacteria, and additional bacteria and fungi. Variations in production occur because of variations in environmental conditions between the specific locations of substrates within the stream.

Throughout the stream periphyton community, there is variation in standing crops due to specific substrates and the location of substrates within the stream channel. Shading and current velocity varies on a small scale because of the size and shape of substrates and slight shading differences due to presence of trees, larger rocks, etc., affecting photosynthetic activity (McIntire, 1966; Steinman and McIntire, 1986). The attitude of the substrate surface relative to current flow can also affect colonization and proliferation. Shape and surface texture variations of exposed edges and areas of irregular texture, capable of causing turbulent microcurrents, enhance the ability of: a) potential colonizers to contact the substrate surface, and b) nutrients in the water to contact periphytic organisms (Korte and Blinn, 1983; Hamilton and Duthie, 1984; Munteanu and Maly, 1981).

It is uncertain whether artificial substrates offer reliable estimates of periphyton production on natural substrates. This question is evidenced in the literature as authors choose different substrates, exposure times and sample replicate numbers (Cattaneo and Ghittori, 1975; Lamberti and Resh, 1985; Steinman and Parker, 1990). Chlorophyll$_a$ (chl$_a$) and ash-free-dry weight biomass (AFDW) values are considered reliable estimates of periphyton accrual on natural substrates by some researchers (Cattaneo and Ghittori, 1975; Lamberti and Resh, 1985). Weitzel, Sanocki and Holecek (1979) found that species composition, density and diversity of periphyton on artificial substrates resembled that on natural rock substrates, while chlorophyll$_a$ and biomass values are significantly lower than those from rocks.

Objectives

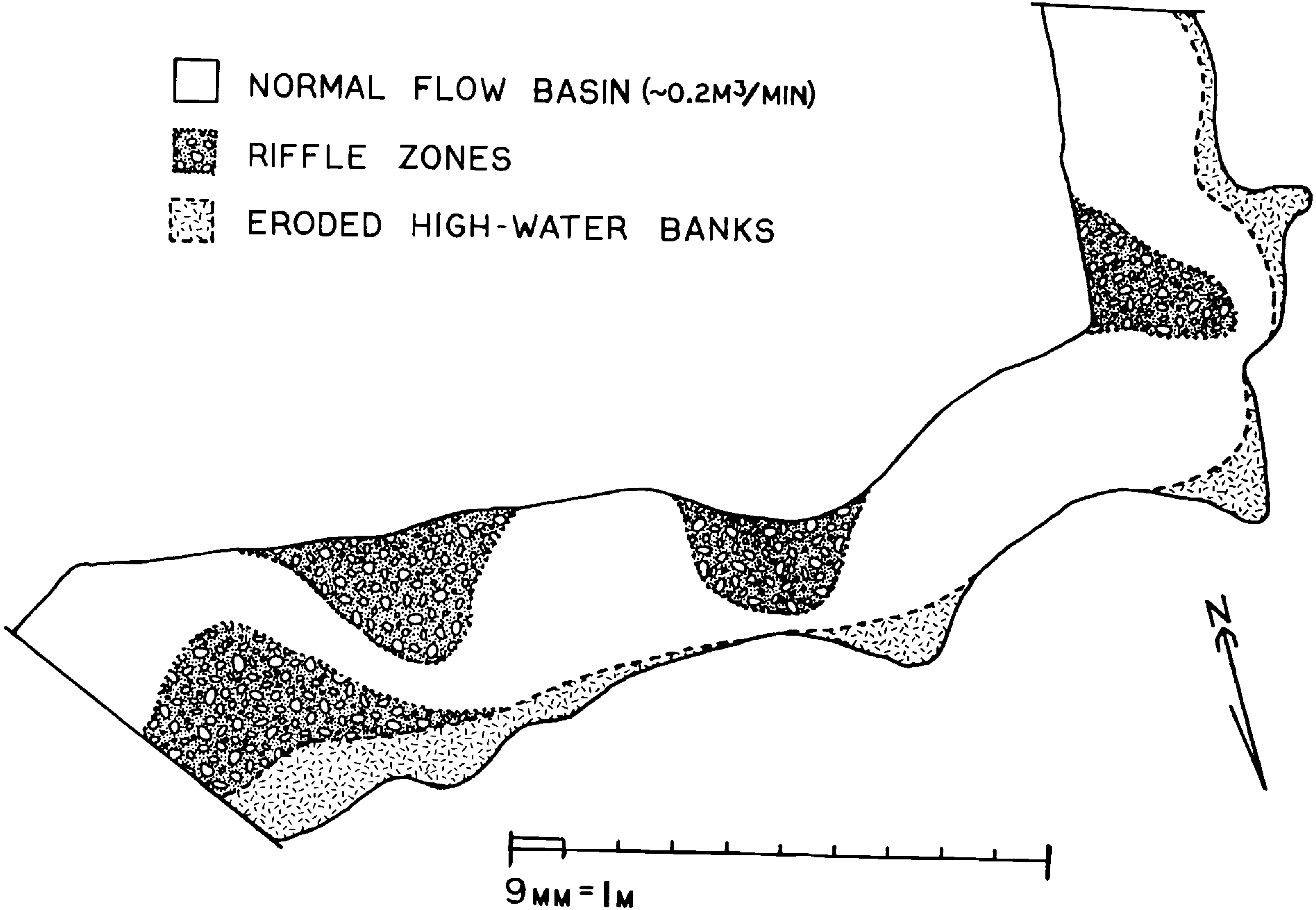
The purpose of this study was to quantify seasonal periphyton standing crops in a Flint Hills headwater stream. Biomass and chlorophyll standing crops on natural rock substrates, microscope slides and dowel rod segments were compared to determine precision of measurements from the artificial substrates. Autotrophic organisms were observed to identify common genera within the periphyton community, and to provide a brief numerical description of the community structure. A secondary objective was to monitor nitrogen and phosphorus concentrations, water temperature and current flow rates, to evaluate their potential effects on the periphyton standing crops.

STUDY AREA

The John P. Coughlen Natural History Area is a 64-acre tract of land located ten miles (16 km) southwest of Emporia, Kansas, in Lyon County (Range 10E, Township 20S; sections 8, 17), and is owned by Emporia State University. It is situated adjacent to the Kansas Turnpike (I-35). The land represents the eastern edge of the Flint Hills upland region of Kansas, and is characterized by moderately sloping (3 - 7%) to moderately steep slopes (5 - 20%) of tall-grass prairie. The soil is classified as part of the Clime-Sogn association. The land on and around Coughlen is underlain by limestone and shale bedrock, with limestone outcrops forming "rocky ledges near ridgetops." Land use in the area is primarily for range on the sloped areas, while crop production is limited to the more shallow slopes or flat areas (0 - 6%) on ridgetops and in valleys (United States Department of Agriculture Soil Conservation Service, 1981).

The Coughlen area is in the Jacob's Creek watershed. Jacob's Creek is a tributary of the Cottonwood River, and drains through parts of Lyon and Chase counties into the Cottonwood River in Lyon County. Three first-order streams flow through the Coughlen area, joining to create a second-order stream before leaving the property. The southernmost of these three streams is considered the major headwater of Jacob's Creek based on flow estimates from observed conditions throughout the drainage. The primary study reach for this research was situated approximately 65 meters downstream from the point where this larger first-order stream enters the property from a drainageway under the highway. Alternating bedrock layers of limestone and shale contribute to a regular pattern of riffles and pools along the study reach (Figure 1).

Figure 1. Primary stretch of the Coughlen Area study stream, showing low flow boundaries, riffle areas and eroded bank areas that extend to the edge of the channel. Outer boundaries of the eroded areas are up to 75 cm higher than low flow water level and are breached by water only during flood events.


NORMAL FLOW BASIN (~0.2M3/MIN)
RIFFLE ZONES
ERODED HIGH-WATER BANKS
N
9MM = 1M

MATERIALS AND METHODS

Physicochemical Survey

All physicochemical values were measured between 11:00 a.m. and 3:00 p.m. on sampling days. Field Measurements of water temperature and current flow were made frequently throughout the study. Relative flow conditions were estimated at a single riffle site, utilizing the formula:

$$R = W\ D\ a\ V. \quad \text{(Robins and Crawford, 1954)}$$

...where R = volume of stream flow, as vol./time
W = width of stream at riffle site
D = average depth at riffle site
a = a bottom surface constant (.8)
V = surface current velocity

Current velocity was estimated at the uppermost riffle in the study reach by timing the passage of a small amount of flourescein dye through two meters of the riffle. Temperature was measured by immersing the thermometer a minimum of 10 minutes in the stream during sampling visits.

For water analysis, six water samples were collected in one-liter polyethylene bottles each sampling day. Two bottles were filled at each of three locations along the study stream. Specific collection points were randomly selected each day from immediately above or below riffles or from pool areas in the study reach. Nitrate-nitrogen concentrations were measured by HACH Nitraver V methods. Orthophosphate-phosphorous concentrations were measured with HACH Stannaver methods. Meaurements of water pH were made with HACH chemistry or an Analytical Measurements Redox pH

Meter, model 707S, on various dates. Spectrophotometry was conducted with a HACH field spectrophotometer.

Periphyton Samples from Rocks

Periphyton was collected by scraping growth from a specific area on natural rock substrates within the primary study channel. The area to be scraped was first marked by pressing the rim of a collection jar lid into the periphyton mat. With the lid in place as a template, the bordering periphyton was removed to mark the sample area. Then the lid was removed, and periphyton was scraped from the marked area with a plastic squeegee. Scraped material was collected as it flowed into a plankton net held in place immediately downstream of the scrape site. Rocks of various sizes were randomly selected. If a rock was so small or irregularly shaped that part of the rim of the lid extended beyond the edges of the rock, the actual area to be scraped was estimated by use of outlining simple geometric shapes with the squeegee upon the available surface. Otherwise, the area of each scrape was approximately 26.42 cm^2.

Because of the heterogeneity of natural rock surfaces, two sampling biases were applied in selection of rocks for periphyton collection. Rocks were selected for having flat surfaces and, in cases where samples were scraped for both biomass and $chlorophyll_a$ analyses, paired scrapes were taken from adjacent areas on larger rocks, or from smaller rocks within 30.5 cm and in similar flow areas.

Artificial Substrates and Placement

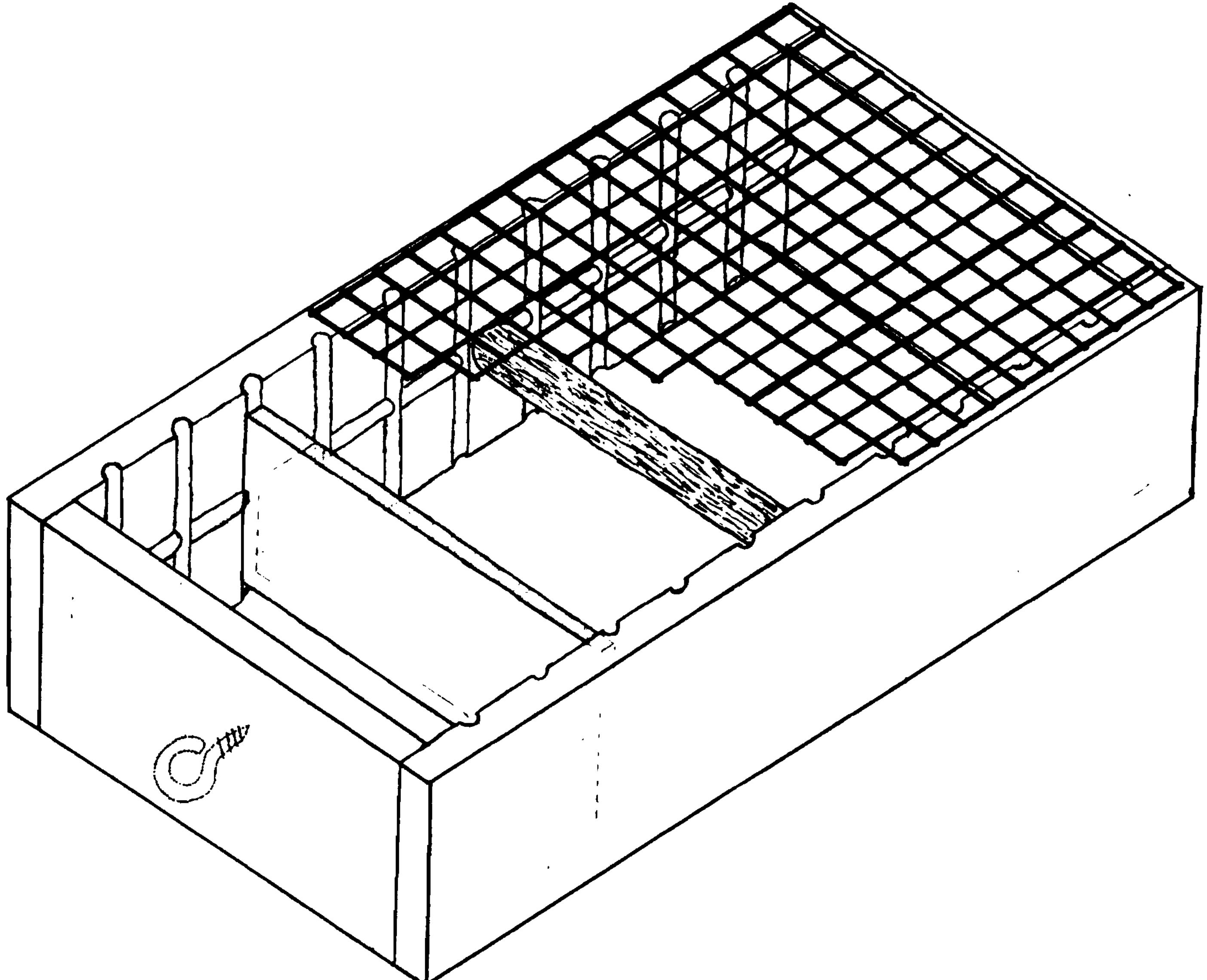
Artificial substrates utilized during this study consisted of Rinzyl Plastic microscope slides (area = 39.01 cm^2) and 3/8-inch pine dowel rod segments (.9525 cm * 7.0 cm; area = 20.78 cm^2). The slides and dowels were deployed in submerged "traps," based on the design of Welch (1948). The traps were constructed of .635 cm clear, colorless plexiglass (Figure 2). The slides were held in traps by vertical grooves, while dowels were secured between two bead rows of clear silicon hot glue. Accidental loss of slides and dowels from a trap was limited by covers of .635 cm mesh hailscreen.

Traps were anchored in the stream by tether lines which were attached to eyelet screws in one end of a trap and tied to tent stakes driven into the stream bed. Styrofoam strips were tied to the sides of a trap to maintain its position just under the surface of the water. The traps were positioned so that the dowels and slides were perpendicular to the direction of stream flow. Traps were placed in flow areas above or below riffles to provide constant tension on tethers to prevent tangling.

Samples from Artificial Substrates

Ten slides or eight dowel segments were placed in each periphyton trap. Traps were then exposed to colonization in the stream for three to 73 days. When collected for sampling, at least eight slides or dowels were carefully

Figure 2. Submersed periphyton trap design, showing a sl
and a dowel substrate in place, hail screen cover (top on
and eyelet for anchoring the unit.



removed from the traps by hand, insuring that the traps did not leave the water. The substrates were then placed, two each in sample collection jars for transport.

In the laboratory, the periphyton that had accumulated on a slide or dowel was removed with a plastic squeegee directly into crucibles for drying and ashing or into a cleaned mortar for grinding prior to chlorophyll$_a$ extraction. Periphyton material remaining adhered to substrate surfaces was washed into the crucibles or mortars with distilled water. Ends of dowel segments and edges of slides were not scraped.

Biomass Procedure

Biomass was measured as ash-free-dry weight. Scraped materials were dried in pretared crucibles for 24 hours at 100 degrees (C), weighed, ashed in a muffle furnace for one hour at 550 degrees (C), and reweighed. Biomass was measured on a METTLER Model B balance and was reported as AFDW per square centimeter.

Chlorophyll$_a$ Procedure

Samples from rock substrates were first placed into centrifuge tubes, centrifuged and decanted prior to grinding of the tissue by mortar and pestel. This removed excess water that had been collected in the plankton net bucket. Artificial substrate samples were scraped directly into the mortar. All samples were ground approximately 75 seconds or exactly 100 strokes with uniform action and interval prior to 24-hour extraction in centrifuge tubes under refrigeration. Extraction was carried out in a 90-percent acetone solution.

Absorbance of extracts was measured with a Bausch and Lomb SPEC 20 spectrophotometer. Readings were taken at 750 nm and 665 nm prior to acidification, and at 750 nm and 665 nm after acidification to measure chlorophyll$_a$ in presence of phaeophytin$_a$ and siliceous turbidity. Chlorophyll$_a$ estimates were made with the following equation:

$$Chl_a \ (\mathrm{mg\ cm^{-2}}) = \frac{26.73\ (665_b - 665_a) * V1}{As * L}$$

...where V1 = volume of extract, (10ml)
As = area of substrate surface,
L = length of light path, (1 cm cuvette)
665_b = absorbance prior to addition of 2 drops of .1 N H_2SO_4 (A750 - A665)
665_a = Absorbance after acidification (A750 - A665)

Taxonomic Survey

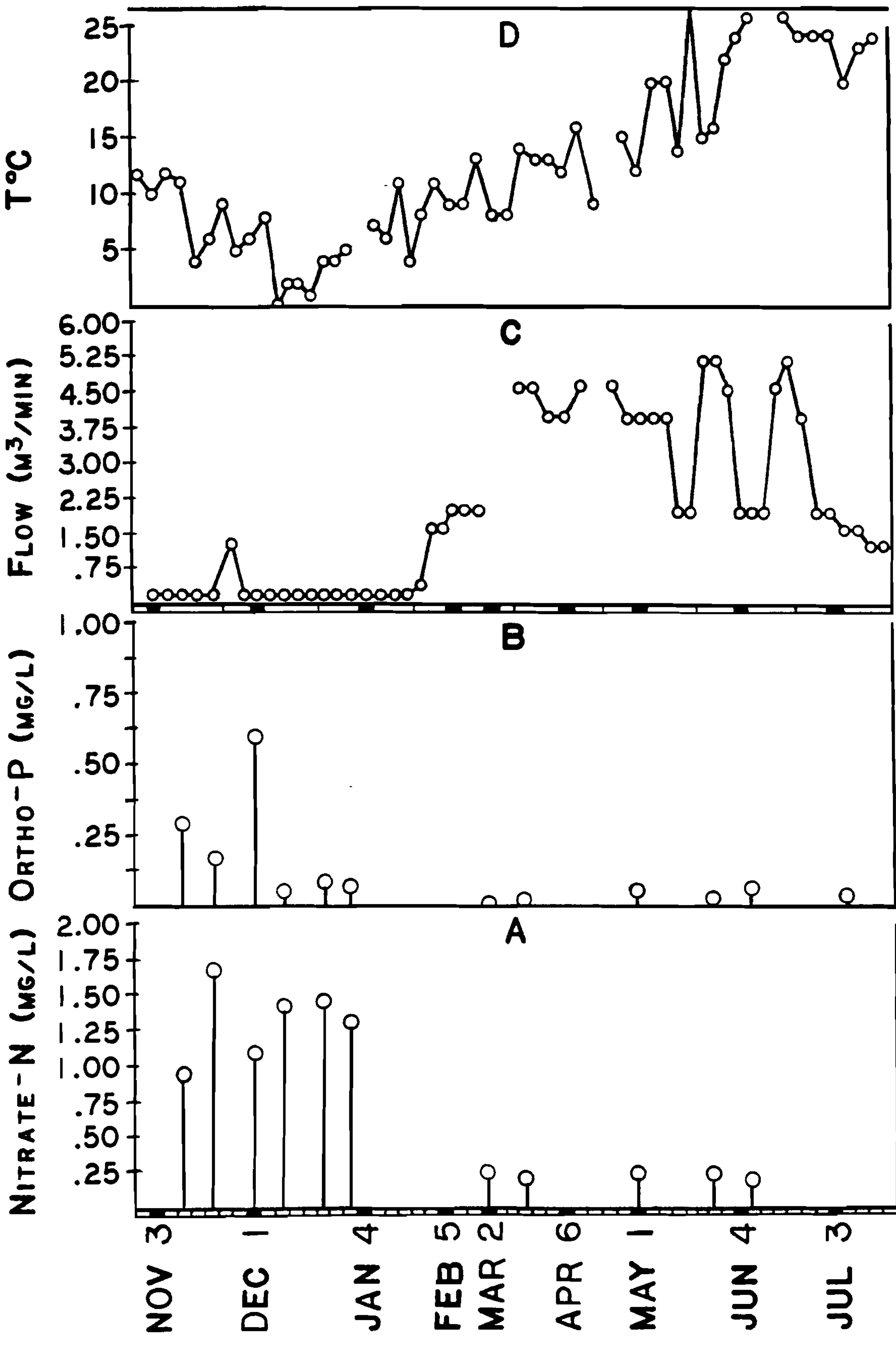
Periphyton samples on microscope slides were observed on several occasions to identify organisms present during various seasons. The slides were exposed in the study stream at least three weeks before collection. Observations were made under 40X by placing the slides directly beneath the objective in a Petri dish with a small amount of stream water. A 499-cell count of periphyton (to genera) was utilized to characterize the community structure and composition (Wietzel, et al., 1979).

RESULTS AND DISCUSSION

Physicochemical Survey

A survey of physicochemical conditions within the study stream was made from October, 1989, through July, 1990. Means of nitrate-nitrogen concentrations, from the six water samples per sampling day, ranged from a high of 1.48 mg l^{-1} in November to a low of 0.20 mg l^{-1} in May (Figure 3a). Means of orthophosphate-phosphorous concentrations, from each sampling day, ranged from 0.592 mg l^{-1} in December, to 0.020 mg l^{-1} in March (Figure 3b). Data were not collected between December 28 and March 2. The mean concentrations of nitrogen and phosphorus were lower than concentrations reported by the U.S. Geological Survey for rivers and creeks within 130 kilometers of the Coughlen area (United States Geological Survey, 1965). Nitrate-nitrogen concentrations averaged 8.92 mg l^{-1} with highest values of 31.0 mg l^{-1} in the Marais des Cygnes in September, 1963, and lowest values of .3 mg l^{-1} in the Walnut River near Eldorado in August,1963. Orthophosphate-phosphorus concentrations averaged 2.62 mg l^{-1} with highest values of 14 mg l^{-1} in the Marais des Cygnes in September, 1963, and lowest values of .1 mg l^{-1} near Marion and Eldorado. Concentrations of nitrate-nitrogen and orthophosphate-phosphorus were measured monthly through the 1963 water year at sites in and near the Flint Hills along the Arkansas, Walnut, and Cottonwood rivers as well as Clear and Mud creeks. Included, for

Figure 3. Physicochemical characteristics of the Coughlen study stream through the study period. Represented are seasonal fluctuations in: a) Nitrate-Nitrogen, b) Orthophosphate-Phosphorus, c) Current Flow Volume, and d) Water Temperature.



D
T°C
25
20
15
10
5
C
Flow (m3/min)
6.00
5.25
4.50
3.75
3.00
2.25
1.50
.75
B
Ortho-P (mg/l)
1.00
.75
.50
.25
A
Nitrate-N (mg/l)
2.00
1.75
1.50
1.25
1.00
.75
.50
.25
NOV 3
DEC 1
JAN 4
FEB 5
MAR 2
APR 6
MAY 1
JUN 4
JUL 3

comparison, were data from the Marais des Cygnes River near Ottawa, Kansas and the Neosho River near Burlington, Kansas. A more recent survey of surface water quality in Chase County, Kansas, (United States Geological Survey, 1988) reported nitrate-nitrogen levels of .070 to 3.87 mgl^{-1} and total phosphorus levels of .290 to .630 mg l^{-1} during the 1987 water year. These values were more similar to data in this study.

Temperature and flow variations showed predictable seasonal trends in response to ambient temperature and precipitation conditions. Total flow volume in the channel varied from a low of 0.22 m^3 min^{-1} through much of the fall and winter to 5.23 m^3 min^{-1} on sampling days following rain events in May and June (Figure 3c). Higher flow rates occurred during and immediately after storm events, but were not measured for safety reasons. Flood levels were noted to reach 61 cm or more above the water level reached at flow conditions of .22 m^3 min^{-1} by observation of flood debris. Flow velocities were estimated between 10 and 20 cm sec^{-1}. Water temperatures varied seasonally from zero to 28 ^oC (Figure 3d).

Taxonomic Survey

A brief taxonomic survey is provided to characterize principle periphytic algae present in the stream along the study reach. Selected and random collections were made from natural and artificial substrates in December, March, May and July. A characteristic description of the periphyton association is provided by the sample of March 19 from a microscope slide that had been exposed since November 3, 1989, and washed downstream several yards from its trap after the trap had opened in moderately heavy flow through January and February. Cell count data are provided below (Table 1). Organisms represented in the sample from March 19 were present in most observations, independent of season. Diatoms were always present. Notable organisms not represented in the sample were characteristic of distinct riffle or pool areas. Cladophora spp. (Chlorophyceae) was present through the study period, and was selectively collected for identification from shallow, rapid riffle areas, where it formed dense, stringy mats clinging to rocks. Spirogyra spp. (Chlorophyceae) was present through the study period and was selectively collected from pool areas where it formed loosely attached, filamentous, suspended mats, remaining planktonic. Spirogyra did occur within the periphyton mat on rocks and both artificial substrates in pool areas, but in limited density. Melosira spp. (Diatomaceae) and Fragillaria spp. (Diatomaceae) were observed in one sample each from slides in July.

Other than Melosira and Fragillaria, all genera were found on all three types of substrates. Notable examples of the affinity of periphyton for various substrates included development of Cladophora and Rhizoclonium spp. on all

Table 1. Taxonomic summary of the periphytic algae community from a slide substrate collected on March 19, 1990. Based upon a 499-cell count.

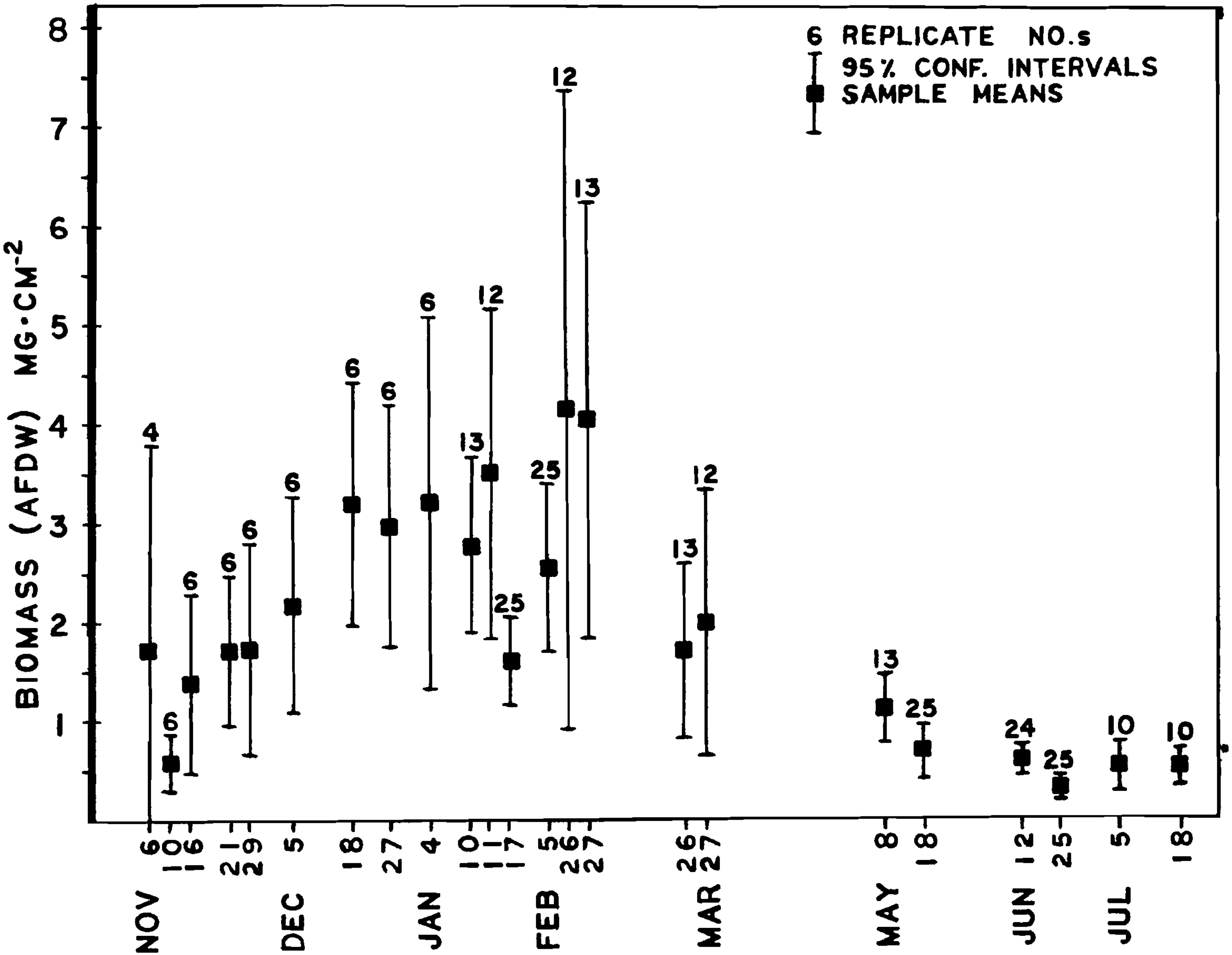
Sub-Phylum	Order	Genus	Ct.	%
Chlorophyceae	Cladophorales	Rhizoclonium	244	49
Diatomaceae	Pennales	Navicula	104	21
Chlorophyceae	Zygnematales	Mougeotia	62	12
Diatomaceae	Pennales	Meridion	42	8
Diatomaceae	Pennales	Asterionella	22	4
Chlorophyceae	Ulothricales	Ulothrix	10	2
Diatomaceae	Pennales	?	8	2
Diatomaceae	Pennales	?	7	1

submersed surfaces, including surfaces of the trap units and trap covers, tether lines and anchoring stakes when traps were exposed in more rapid riffle areas. These two genera are noted to occur together in dense, variable mats in streams (Prescott, 1978). The density of the mats, combined with the shading caused by the filamentous thalli seemed to limit diatomaceous and other periphytic organisms, as displayed in Table 1.

Seasonal Standing Crops

All periphyton biomass samples from natural rocks were collected from the study stream from November 6, 1989, through July 18, 1990. Sample means contributed to the graphically evident trend displayed in Figure 4. Three collection regimes were utilized. Samples from November 6 to January 4 were collected as preliminary data while collection jars were gathered and prior to the calculation of minimum sample size necessary for detecting a true difference by ANOVA (Sokal and Rohlf, 1969). Samples from January 10 through June 25 were collected for use in ANOVA to evaluate the significance of the inclusive portion of the observed seasonal trend. Because a minimum of 24 replicates were required for each data set in ANOVA, smaller samples from consecutive dates on January 10 and 11, February 26 and 27, and March 26 and 27 were pooled after they were tested for significant difference. In each case, the null hypothesis ($\bar{x}_1 = \bar{x}_2$) was accepted ($t_{.05(2),23} = 2.069$; t_{JAN}

Figure 4. Biomass (AFDW) standing crop means and 95% confidence intervals of periphyton scrapes from natural rock substrates in the Coughlen study stream.



6 REPLICATE NO.s
95% CONF. INTERVALS
SAMPLE MEANS
BIOMASS (AFDW) MG·CM^{-2}
NOV
DEC
JAN
FEB
MAR
MAY
JUN
JUL

= -.7961, t_{FEB} = .0458, t_{MAR} = -.3279). The sample from May 8 was not completed because of inclement weather. Samples from July 5 and 18 were collected after ANOVA calculations to insure there was no visible fluctuation in standing crops late in the summer.

Nutrient concentrations were highest in the fall and winter, and the lowest in spring and summer. The biomass of periphyton on natural substrates (Figure 4) was greatest in January and February (4.1484 mg cm^{-2} on Feb. 26). Fall and early winter means showed gradual increase from moderate levels in November, while early spring and summer means were among the lowest recorded in this study (0.3028 mg cm^{-2} on June 25).

Lamberti and Resh (1985) collected periphyton samples from natural substrates in a California stream from mid-May through September, 1980, and found a slight decrease in biomass accrual rates between June and July, and a consistent, gradual increase in biomass standing crop through the summer - fall period. Eloranta and Kunnas (1979) studied the standing crops of periphyton on artificial substrates in a Finland stream from May through October, 1975. They reported a decrease in chlorophyll$_a$ standing crops, and found a similar production decrease between June and July.

If the graphically evident seasonal trend in periphyton standing crop production is significant, one indication would be a significant difference between means as calculated by ANOVA for the inclusive dates of the second sampling regime from January to July, 1990. Results from testing the null hypothesis ($\bar{x}_1 = \bar{x}_2 \ldots = \bar{x}_n$) showed that there was a significant inequality ($P < .005$) between some of the means of the biomass samples (Table 2). Further statistical trend analysis is not suggested for random effects data (Zar, 1984), and was not applied to determine which means were unequal.

Chlorophyll$_a$ Standing Crops

Chlorophyll$_a$ standing crop values from natural rock substrates in the study stream were measured on March 26 and 27, and on July 5 and 18. Individual values ranged from .0010 to .0415 mg cm^{-2} of substrate surface (higher values in March), and sample means ranged from .0068 to .0169 mg cm^{-2}. Autotrophic indices (AI = biomass/Chl$_a$), which express the portion of the periphyton community occupied by photosynthetic organisms, were calculated between adjacent scrapes and ranged from 14.96 to 725.09. This indicates a large heterogeneity in community structure, and variability due to sedimentation of organic particles and debris, which affect total biomass. This extreme range in ratios indicated that a definite dependent relationship between biomass and chlorophyll$_a$ standing crops may not be reflected in the data.

A correlation between biomass and chlorophyll$_a$ standing

Table 2a. Description of 1990 data from individual and pooled samples utilized for ANOVA. b. ANOVA results at $\alpha = .05$ confidence for biomass standing crop (AFDW) measurements from 8 samples collected from the John P. Coughlen Natural History Area.

a.

Sample Date(s)	n	$\bar{x}$	$s_{\bar{x}}$
Jan. 10,11	25	3.134 mg cm-2	*2.271
Jan. 17	25	1.573	0.211
Feb. 05	25	2.548	0.417
Feb. 26,27	25	4.102	*4.690
Mar. 26,27	25	1.860	*1.924
May 18	25	0.684	0.142
Jun. 12	24	0.577	0.069
Jun. 25	25	0.303	0.051

*: Higher $s_{\bar{x}}$ values from pooled samples

b.

	SS	DF	MS
TOTAL	.863072266	198	
GROUPS	.219720484	7	.03138864
ERROR	.643351782	191	$3.368333937 * 10^{-3}$

$F = 9.3187$ $F_{.05(1),7,191} = 2.06$ $P < .005$

crops was calculated to determine if a significant, numerical relation exists between sample data. For the four samples studied, the correlation equation

$$Y = .006479 + .004584\ X \pm .0551$$

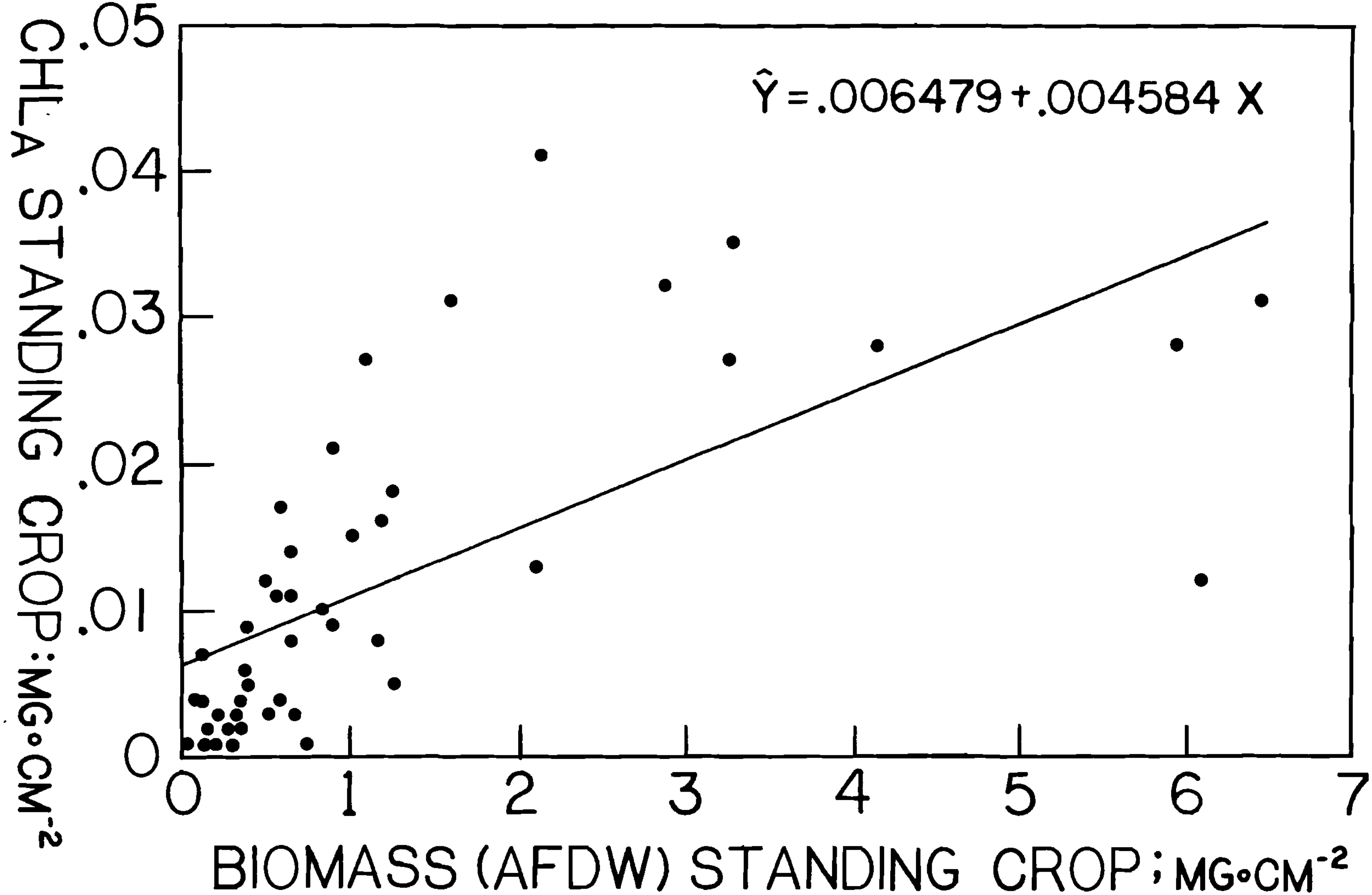
where X = biomass (AFDW) standing crop (mg cm^{-2})
Y = Chorophyll$_a$ standing crop (mg cm^{-2})

was determined by least squares (Zar, 1984). The slope, b, of the equation is not equal to zero, indicating some relation exists between chlorophyll$_a$ and biomass standing crops within the samples, and the implied slope, β, of the existing relationship within the periphyton community is significantly different from zero ($.0005 < P < .001$). Although the correlation reflects a significant relationship, the equation only accounts for 44 percent ($r^2 = .4425$) of the observed data sets (Figure 5). Standard deviation of the equation, $s_{y*x} = .0551$ mg cm^{-2}, was greater than the observed range of chlorophyll$_a$ values.

Chlorophyll$_a$ analysis was performed to provide another estimate of periphyton standing crop production that emphasizes a more narrow evaluation of the accrual of photosynthetic organisms. Photosynthetic organisms observed in the study stream were members of Chlorophyceae and Chrysophyceae (Diatomaceae).

Logically, a cause-effect relation, which can be expressed by a numerical equation, exists between biomass and chlorophyll$_a$ standing crops. Failure of the calculated correlation in this study to explain more than 44 percent of

Figure 5. Chlorophyll$_a$ standing crop data plotted against biomass (AFDW) standing crop data collected from natural rock substrates, with calculated correlation (r^2 = 0.4425, s_{y*x} = 0.0551).



CHL A STANDING CROP; MG•CM-2
.05
.04
.03
.02
.01
0
$\hat{Y}$ = .006479 + .004584 X
0
1
2
3
4
5
6
7
BIOMASS (AFDW) STANDING CROP; MG•CM-2

the variation in the expression of a relation between biomass and chlorophyll$_a$ data in this study can be explained by various sources of error, though biological populations can often display non-linear relations. Prime suspect is the experimental design, which called for collection of separate but adjacent scrapes for determination of biomass and chlorophyll$_a$. Other studies separate individual scrapes into two or more portions for subsequent analyses (Eloranta and Kunnas, 1979; McIntire, 1966; Cushing, et al., 1983). Sedimentation of any organic particulate matter on substrates or among periphytic organisms would likely have dramatic effects on the autotrophic index. Variation of sedimentation can occur on a very small scale due to microcurrent interactions across the substrate material. Another source of error could be the variation of periphyton volume--roughly estimated by biomass--which affects the total extract volume within spectrophotometric cuvettes. All chlorophyll$_a$ calculations were based on a cuvette volume of ten ml, though scrape replicates often had visibly varying (periphyton + sediment)/extract ratios. This apparent source of error was not obvious in samples from artificial substrates.

Production on Artificial Substrates

Biomass (AFDW) and chlorophyll$_a$ standing crop measurements were determined from plastic slide and dowel substrates on random sampling dates. Exposure times of individual substrates varied from three to 70 days. A minimum of eight individual slides or dowels were collected for each sample. Collection of more samples or replicates per sample was limited chiefly by trap space and occasional losses of substrates.

During the preliminary sampling period of November through January, biomass data from slides were acquired from combined samples of five slides each on November 29, and December 5 and 28. These samples were collected to evaluate collection and analysis methods. Later, sample design called for collection of ten slides, but because losses occured, samples of eight replicates each on April 9 and 23, and samples of ten and nine replicates each on June 22 and July 17, respectively, were collected. Sample means ranged from .0487 to .4839 mg cm^{-2}.

Biomass data from dowel substrates were acquired from samples of eight replicates each on April 9 and 23, and from samples of ten and thirteen replicates each on June 22 and July 17, respectively. Sample means ranged from .3730 to 1.3500 mg cm^{-2}.

Biomass data from April 9 and 23, June 22 and July 17, were submitted to analysis by Student's t and Mann-Whitney U tests for each sampling date (Table 3) to determine if periphyton accrual varied between the two substrates. Significant differences in accrual were determined on three of the four dates, with dowel segments producing the highest

Table 3. Student's t and Mann-Whitney U statistical test results between slide and dowel rod substrates; biomass standing crops (AFDW) on 4 sampling dates.

DATE	4-9	4-23	6-22	7-17
SLIDES $\underline{n}_1$	8	8	10	9
SLIDES $\bar{X}_1$ (mg/cm^2)	.3079	.4839	.1864	.3808
DOWELS $\underline{n}_2$	8	8	10	13
DOWELS $\bar{X}_2$ (mg/cm^2)	.4307	.4578	.3730	1.3500
EXPOSURE TIME (days)	21	35	45	70
$t_{(calculated)}$	3.588**	0.199	2.569***	5.626+
$t_{.10(2),v}$	1.761	1.761	1.734	1.725
U	59**	41	10	0
U'	5	23	90*	117+
$U_{.10(2),v1,v2}$	49	49	73	84

+: P < .001
*: P = .002 **: .002 < P < .005 ***: .01 < P < .02

standing crop values. On April 23, periphyton biomass was greater on slides than on dowels, but the difference was not significant.

Difference in periphyton accrual on the two types of artificial substrates is important to note. Differentiation could be due in part to the specific orientation of slides and dowels to a horizontal plane and the plane of current flow as they are deployed in traps. Because slides are perpendicular to current flow and held in traps vertically, they offer less surface than dowel segments for sedimentation of organic solids that could add to measurements of biomass standing crop. Periphyton formed a visibly homogenous mat over the surface of slides, presumably because slide mass did not appreciably decrease useful PAR insolation to either side, but accumulation was visibly limited to top surfaces of dowels--maintained by trap position--which received regular, daily PAR radiation. Substrate surface texture could also be important in supporting colonization and production, and precise sampling efficiency (Grzenda and Brehmer, 1960; Lamberti and Resh, 1985). As previously discussed, substrate shape is also important because of variation in microcurrent characteristics around the substrates.

Chlorophyll$_a$ standing crop data from dowels and slides were collected only on April 9 and 23 because of study limitations caused by loss of adequate numbers of substrates in the stream during exposure periods. Replicate values on April 9 ranged from .0051 to .0141 mg cm^{-2} ($\bar{x}$ = .0100) on dowel rods and .0041 to .0089 mg cm^{-2} ($\bar{x}$ = .0060) on slides. Replicate values on April 23 ranged from .0026 to .0103 mg cm^{-2} ($\bar{x}$ = .0069) on dowels and .0021 to .0075 mg cm^{-2} ($\bar{x}$ = .0051) on slides.

Data from both dates show that, as with biomass standing crops, $chlorophyll_a$ standing crop values are lower on slides than dowels. Autotrophic indices from both dates were variable on both substrates, with ratios less than one in some cases. This indicated a problem with the experimental design, and data were not tested for fit with a correlation equation. $Chlorophyll_a$ standing crop values are believed reliable despite the erratic AI ratios, because, as with natural rocks, separate substrates were utilized for biomass and $chlorophyll_a$ analyses rather than reduction of materials from each substrate unit to separate portions for each analysis. Another source of variation, that could be important in more profuse periphyton mats, is that periphyton tended to be connected between hail screen covers, trap bodies and individual substrates, leading to irregular rates of periphyton removal due to tearing of filamentous algal strands when slides or dowels were removed.

Biomass and $chlorophyll_a$ production values, collected in limited sample numbers, show a slightly different trend from data off natural rock substrates. Means of biomass standing crops show highest cumulative values between the two substrates on July 17, with moderate values in April and lowest values in June. This is not predicted by data from rocks, and may be due to a protective feature of the periphyton traps caused by the trap body, hail screen covers and any periphyton mass attached to the trap that buffer wide fluctuations in flow conditions across the substrate surfaces. This would limit scouring events--noted on rocks--caused by rains.

Natural vs. Artificial Substrates

Estimates of periphyton biomass (AFDW) and chlorophyll$_a$ standing crops from slide and dowel substrates tended not to reflect values expressed by scrapes from natural rocks. Biomass data from the artificial substrates were compared to data from the natural rock surfaces by use of a one-tailed t test. A single mean of 1.7764 mg cm^{-2} was calculated for all periphyton data from natural rocks. Slide and dowel data were evaluated separately against the cumulative rock scrape data mean, except for data of April 23, which showed no significant difference between slides and dowels; an additional evaluation included a cumulative calculation with slide and dowel data as a single sample. All periphyton samples from slides and dowels, as well as the cumulative sample of April 23, were significantly different from the cumulative biomass mean from natural rocks (Table 4).

Table 4. One-tailed t statistical results between periphyton biomass data from individual slide and dowel samples and cumulative data from natural rocks ($\bar{x}$ = 1.7764 mg cm-2). A cumulative sample of both artificial substrates was evaluated for data from April 23, 1990.

Date	t	/t_{calc}/	Significance
4-9-90			
S vs R	$t_{.05(1),7} = 1.895$	3.4569	$.005 < P < .01$
D vs R	"	42.7918	$P < .0005$
4-23-90			
S vs R	"	27.4311	"
D vs R	"	10.5686	"
C vs R	$t_{.05(1),15} = 1.753$	20.6048	"
6-22-90			
S vs R	$t_{.05(1),9} = 1.833$	61.1297	"
D vs R	"	20.5305	"
7-17-90			
S vs R	$t_{.05(1),8} = 1.860$	33.3984	$P < .0005$
D vs R	$t_{.05(1),12} = 1.782$	2.9858	$.005 < P < .01$

S:Slides D:Dowels C:Cumulative R:Rocks

Interesting to note is that $chlorophyll_a$ sample means from artificial substrates tended to overlap those from rocks, though biomass means tended to be comparably lower (Table 5). One explanation for the trend is that sedimentation of organic particulate matter onto artificial surfaces was limited by lack of horizontal surfaces. Sedimentation on rock surfaces may have added variation to biomass estimates, while not appreciably limiting $chlorophyll_a$ production. Grzenda and Brehmer (1960) suggested that artificial substrates be exposed only until periphyton is determined profuse enough that death of periphytic organisms and sloughing of material do not excessively contribute to variation within samples. This would limit the time that sedimentation of organic matter could occur.

Artificial substrates, such as microscope slides and dowel rod segments can be considered for periphyton ecology studies and water quality analyses. More information is needed to evaluate the accuracy of biomass and $chlorophyll_a$ estimates from slides and dowels. Dowels are nearly as easy to handle and deploy as slides, though measured lengths must be carefully cut, and direct microscopic observation of attached organisms is not possible. Glass and plastic microscope slides are popular for this latter utility. Both substrates have uniform surfaces that could serve as control in comparison studies between separate locations or seasons.

Table 5. Ranges of periphyton biomass and chlorophyll$_a$ means of samples from artificial and natural substrates collected from the Coughlen study stream.

Substrate	Biomass (AFDW) Sample Means (mg cm^{-2})	Chl-a Sample Means (mg cm^{-2})
Nat. Rocks	.3028 - 4.1484	.0069-.0170
Mic. Slides	.1864 - .4839	.0037-.0060
Dowel Rods	.3729 - 1.3500	.0069-.0100

Study Dependability

Throughout this study, consideration had to be given to the proximity of the Coughlen area to the Kansas Turnpike. Although there was one incident of human vandalism, the highway presents other potential interference against the reliability of data. Data from the Coughlen channel could conceivably be affected by ongoing repair activities on the highway. Cline, Short and Ward (1982) studied the effects of highway construction on the periphyton community development in Joe Wright Creek, a high mountain stream. They found that reference physicochemical levels, except suspended solids and substrate composition, recovered as soon as two weeks after cessation of construction activities. Periphyton communities recovered less rapidly, but "surprisingly" quickly. That study proposes that periphyton recovery is a function of the flow regime and the seasonal timing of construction.

Rapid recovery in Joe Wright Creek is attributed to the high flow velocity of the stream. Suspended solid levels apparently recovered slowly because new erodable surfaces were exposed in construction. Shifting of the stream bed substrate is consequently a result of varied and generally increased sedimentation activity. These two physical features show similar patterns in the Coughlen channel. After rains, depositional areas for suspended silts and other solids were seen to shift irregularly from heads and tails of riffle areas. Variations in depth of sedimentation included complete removal of silt deposition in some locations and contrasting appearances of new depositions in some locations as flow patterns changed or shifted, moving large rocks along the stream bed.

The ecosystem within the Coughlen channel may not recover from construction activities as rapidly as Joe Wright Creek, because it does not have the flow gradient and comparably high flow velocity. The periphyton community has certainly had time, however, to recover from original highway construction and continues to have months or years to recover between repairs.

Flood events were noted to scour periphytic mats from much of the available rock substrate, often removing layers of the native limestone rock. The scouring effects remained visible for up to two weeks after spate events. Samples collected on March 26 and 27, May 18, June 12 and June 25 were all taken within a week of such spates and failed to show means of "zero" biomass, though reflecting lower standing crop values than previous samples from before heavy rains (Figure 4). Kaufman (1980) found a similar two-week recovery pattern in normally flushed reference laboratory streams and laboratory streams that had been scraped and scoured to be thoroughly depopulated.

SUMMARY

A seasonal production trend was displayed by biomass standing crop data from rocks. The graphically evident trend was supported by ANOVA, and followed nutrient (N,P) concentration maxima. Biomass and chlorophyll$_a$ standing crops from artificial substrates were lower than those from rocks, though chlorophyll$_a$ values were similar. Standing crops from dowel rods were higher than those from slides. Natural rocks are the substrate of choice to accurately determine periphyton standing crops in the ecosystem. Artificial substrates may be useful in taxonomic surveys or for comparison of periphyton production between locations or sample times. Results, showing the seasonal trend, are duplicable within the Flint Hills, based on similarity of the study stream to others in the region, expressed by water temperature and current flow rates, the correlation between nutrient values and standing crops, and the recoverability of the study channel from highway construction.

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PERIPHYTON STANDING CROPS ON NATURAL AND ARTIFICIAL SUBSTRATES IN A FLINT HILLS STREAM

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