THE ROLE OF PERIPHYTON IN THE FEEDING, GROWTH AND PRODUCTION OF *STENONEMA* SPP. (EPHEMEROPTERA: HEPTAGENIIDAE)

By

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ABSTRACT

THE ROLE OF PERIPHYTON IN THE FEEDING, GROWTH, AND PRODUCTION OF STENONEMA SPP. (EPHEMEROPTERA: HEPTAGENIIDAE)

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Field and laboratory studies examined the role of periphyton in the feeding, growth, and productivity of *Stenonema* mayflies. Field studies compared these variables between natural populations living under differing primary production regimes in a 2nd and a 4th order stream. Three species, *S. exiguum*, *S. modestum*, and *S. vicarium*, were found in both streams, and all exhibited univoltine, slow seasonal life history patterns. The proportion of diatoms in gut contents was related to body length, but did not differ greatly between streams, reflecting the similarity of periphyton standing crops. Growth rates did not differ significantly between streams, and were rapid (7.5 %/d) in summer, but much lower in the fall (1.1 %/d). The productivity of each species in each stream was determined largely by the suitability of available substrates, not stream order as hypothesized. Laboratory studies compared the growth of *S. vicarium* on diets of dark-conditioned leaves and natural periphyton. As hypothesized, growth was more rapid on periphyton than on leaves, although seasonal changes in periphyton quality, and possibly endogenous control of growth, may also have influenced growth rates.

To Paula. Thanks for being patient.

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INTRODUCTION AND LITERATURE REVIEW

The concept of trophic structuring of biological communities has been one of the central themes of ecological thought since its introduction by Thienemann (1920). As such, it has served as a conceptual basis for studies of community structure, nutrient cycling, and species interactions, and is closely linked to the niche concept of Elton (1927). Thus, biologists in the early part of this century began to view their subjects not as isolated units but as members of larger communities and ecosystems, complexly interacting with other organisms and the physical environment. The principle of energy flow through biotic communities, first recognized by Lindemann (1942), is one of the most important derivatives of the trophic structure concept.

Determining energy flow through communities involves three basic components: rates of energy exchange between trophic levels, rates of energy loss from and between trophic levels, and rates of energy storage within trophic levels (e.g. Odum 1957). Energy storage within a trophic level is defined as net production and is expressed as units of biomass or energy gained/unit area/unit time. Productivity of lower trophic levels limits the rate of energy transfer to higher trophic levels, and therefore is an important determinant of community structure. Furthermore, production combines in one measurement two population parameters of major ecological significance: individual growth and population survivorship (Benke 1984). Therefore, production is important to the understanding of both population and community level dynamics. Productivity has also been shown to be sensitive to the effects of pollution (e.g. Kimerle and Anderson 1971; Cairns 1977; Uutala 1981).

Historically, much of the early interest in aquatic invertebrate productivity is attributable to fisheries managers who were interested in estimating the amount of "fish food" available in any body of water (e.g. Allen 1949,1951; Boysen-Jensen 1919). Interest in benthic productivity blossomed in the early 1970's largely as a result of the International Biological Programme (IBP). One of the main purposes of the IBP was to study the productivity of terrestrial and aquatic ecosystems to attain a more rational exploitation of the world's biotic resources (Winberg 1971).

Particularly important to the study of benthic productivity was the development by Hynes and Coleman (1968) of the size-frequency method of estimating secondary production from field samples (later corrected and modified by Hamilton 1969, Benke 1979, and Kreuger and Martin 1980). This method estimates the average number of individuals that reach each of a series of size classes over the course of a year, thus creating an apparent survivorship curve by collapsing time (Benke 1984). In so doing, the size frequency method does not require recognition of individual cohorts as do other methods (see reviews by Waters 1977 and Benke 1984 for descriptions of these methods). The average developmental time, or Cohort Production Interval (CPI) of Benke (1979) is the only life history parameter that is required. Therefore, production may be estimated for populations having non-seasonal life history patterns without the need for additional laboratory growth studies (e.g. Cooper 1965). Laboratory growth studies may be necessary for fast-growing species for which CPI is difficult to determine from field samples (e.g. Menzie 1981). With the derivation of a variance estimator for the size frequency method by Kreuger and Martin (1980), specific hypotheses concerning secondary production may be tested.

The relative contribution of allochthonous vs. autochthonous (i.e. exogenous vs. endogenous) energy sources in stream community metabolism has been one of the long standing debates in stream ecology. Many early workers (e.g. Minckley 1963; Minshall 1967; Hynes 1970; Kaushik and Hynes 1971; Cummins 1973) stressed the importance of allochthonous detritus (e.g. leaves) in stream community metabolism. However, Minshall (1978) pointed out a bias in the literature towards low-order, deciduous forest streams where autumnally-shed leaves are the most obvious feature. Thus, some workers (Minckley 1963; Minshall 1967) concluded on the basis of standing crops of detritus and periphyton that autochthonous primary production is of little importance to stream communities. However, such a conclusion erroneously assumes that periphyton and detritus are similar in nutritive value and productivity.

In general, living food resources are nutritionally and calorically superior to detritus (Lamberti and Moore 1984). Since protein is considered to be important in insect nutrition as the principle source of amino acids (Chapman 1982), carbon to nitrogen (C:N) ratios have been used to estimate the protein content of food resources (see review by Cummins and Klug 1979). C:N ratios for periphyton range between 3.7:1 to 7.8:1 (McMahon et al. 1974; McCullough et al. 1979). For comparison, C:N for pure protein averages 3.25:1 (McMahon et al. 1974). C:N ratios for detritus are much higher, with some reported measurements as high as 1340:1 (Cummins and Klug 1979). These high values for detritus reflect its high content of lignin, cellulose and other refractory substances (Lamberti and Moore 1984). Microbes associated with detritus may greatly enhance its nutritional value via biochemical alteration or by supplying metabolites (Lawson et al. 1984). However, reported assimilation efficiencies for algal diets range between 30 to 60% (Trama 1972; McCullough et al. 1979), whereas efficiencies on detrital diets generally range between 10 and 30% (Berrie 1976; Benke and Wallace 1980).

McIntire (1973) demonstrated with a computer simulation that a small (low standing crop) but highly productive periphyton community is capable of supporting a much larger standing crop of consumers. Elwood and Nelson (1972) found that grazing rates of snails in Walker Branch, Tennessee approached net primary productivity, indicating that McIntire's model is probably a realistic simulation. Furthermore, Lamberti and Resh (1983) showed that grazing maintains periphyton communities at a low standing crop but highly productive state. Lamberti and Moore (1984) proposed a graphic model which synthesizes the results of several studies on the effects of grazing on periphyton communities. Their model suggests that periphyton standing crop is inversely related to grazing pressure, but that primary productivity is maximized at intermediate grazing pressures.

Autotrophy may therefore be more important in stream ecosystems than had been previously assumed. By tabulating data on energy sources for a number of stream ecosystems, Minshall (1978) showed that autochthonous inputs either approach or surpass allochthonous inputs in 9 out of 12 reported studies. However, the streams listed comprise an orderly series, ranging from those with negligible autochthonous inputs to those with negligible allochthonous inputs. Minshall (1978) also showed that mean annual gross primary productivity in open-canopied streams generally exeeds input rates of allochthonous detritus, while the opposite is generally true in closed-canopied streams.

Vannote et al. (1980) proposed a model that attempts to explain these patterns. Briefly, this River Continuum Concept states that predictable changes occur in stream communities parallel to longitudinal changes in physical conditions from headwaters to mouth. These community changes should be evidenced by: 1) community Production/Respiration (P/R) ratios less than 1 in low and high order sections and maximum P/R >1 in mid-order sections, due to changes in shading and detrital inputs along a stream's course; and 2) changes in benthic invertebrate community structure which parallel P/R ratio changes. Although the River Contiuum Concept has been criticised as being applicable only to Northern Temperate streams (Winterbourn et al. 1981; Benke et al. 1984), it does appear to hold for streams in that latitude. For example, Naiman and Sedell (1980) showed that predictions concerning community metabolic parameters are accurate for several streams in the Pacific Northwest. In the same set of streams, Hawkins and Sedell (1981) showed that longitudinal changes in benthic invertebrate communities generally follow the predictions of the Concept.

As stated, the River Continuum Concept predicts longitudinal changes in benthic invertebrate community structure in terms of the functional feeding groups of Merritt and Cummins (1984). More fundamentally, the Concept predicts changes in the relative contribution of algal vs. detrital food resources to total benthic secondary production. Since most benthic invertebrates are opportunistic generalists (Chapman and Demory 1963; Cummins 1973), feeding habits of species may vary over life stages, seasons, and stream orders. Functional feeding groups can therefore be taken as only crude approximations of feeding habits. For example, if species "A" is labelled a grazer in a 5th order stream, implying that periphyton is its major food resource, does it ingest less periphyton when it occurs in headwater streams where periphyton is less available? In view of the nutritional superiority of algae over detritus, what are the consequences of such differences in diet for the growth and production of species "A", and furthermore, for the entire benthic community?

One way to approach these questions would be to apply the "trophic basis of production" method of Benke and Wallace (1980) to entire benthic invertebrate communities at two different points along a stream's length. This method combines production, feeding, and bioenergetics to estimate the amount of invertebrate productivity attributable to animal, algal, and detrital food resources. Benke and Wallace (1980) appliedthis method to a guild of filter-feeding caddisflies and showed that although detritus was the predominant food resource, 80% of caddisfly production was attributable to animal food, due to a higher estimated assimilation efficiency for animal food (70% vs. 10% for detritus).

To my knowledge, the "trophic basis of production" method has never been applied to entire benthic communities in the context of the River Continuum Concept. Without such studies, we can only speculate as to the effect of reduced primary productivity in headwater streams on community energy budgets. Obviously, such a study carried out on two entire benthic communities would entail an enormous amount of effort.

This study examined the feeding and production response of one species, *Stenonema vicarium* (Walker) (Ephemeroptera: Heptageniidae) and its congeners, to different primary production regimes in two Michigan streams, Schwartz Creek (2nd order) and the Ford River (4th order). Since aquatic consumers may enhance their growth rates on poor food resources by increasing consumption rates (Cummins and Klug 1979), a laboratory experiment was also performed to determine whether inclusion of periphyton in the diet of *S. vicarium* results in increased growth. The overall objective of this research was to determine the role of algae in the feeding, growth, and production of *Stenonema* spp.

CHAPTER 1

FEEDING, GROWTH, AND PRODUCTION OF *STENONEMA* SPP. IN TWO MICHIGAN STREAMS

INTRODUCTION

The River Continuum Concept (Vannote et al. 1980) presents a holistic view of stream ecosystems in which biological communities are predicted to respond to a continuum of physical conditions from headwaters to mouth. One of the predictions of the Concept is that grazers should be less abundant in headwater streams due to resource limitation. This prediction assumes that grazers are specialized periphyton feeders, and are unable to efficiently utilize alternate food resources. Anderson and Cummins (1979) showed that pupal weights of Glossosoma were positively correlated with community P/R in some Michigan streams, but the effects of P/R were confounded with temperature. Since algae is nutritionally superior to detritus (Lamberti and Moore 1984), reduced productivity of "specialized" grazers in headwaters would be expected. However, most aquatic insects are opportunistic generalists (Chapman and Demory 1963; Cummins 1973), and may increase their ingestion rates to compensate for poor food resources (Cummins and Klug 1979). Differences in resource availability may therefore have little effect on the growth and productivity of generalists.

This study examines the feeding, growth, and productivity of the mayfly Stenonema vicarium (Heptageniidae) and its congeners under different primary production regimes. S. vicarium was chosen because: 1) it is widespread throughout Michigan and occurs in both headwater and mid-order streams (Flowers and Hilsenhoff 1978; Bednarik and McCafferty 1979); and 2) reported feeding habits indicate that it is an opportunistic generalist (Shapas and Hilsenhoff 1976; Edmunds 1984), and therefore is likely to show some response in feeding under different conditions of resource availability.

The overall objective of this study was to determine the importance of periphyton in the feeding, growth, and production of *Stenonema vicarium* and its congeners under differing primary production regimes in 2 Michigan streams: closed canopied Schwartz Creek (2nd order) and the open canopied Ford River (4th order). The specific hypotheses were:

1) *Stenonema* spp. ingest a greater proportion of algae in the Ford River than in Schwartz Creek, due to greater resource availability in the Ford.
2) Growth rates of *Stenonema* are higher in the Ford River than in Schwartz Creek, due to a nutritionally superior diet.
3) Production of *Stenonema* is greater in the Ford River than in Schwartz Creek due to faster growth.

SITE DESCRIPTIONS

Ford River

The Ford River is a low gradient, hard water brook trout stream in Michigan's Upper Peninsula (Lake Michigan drainage). Sampling was conducted in a single riffle and adjacent snags (woody debris) in a 4th order (Strahler 1957) section of the river (Dickinson Co.; T43N R29W sec.14; = site FEX of Burton et al. 1984). The stream channel at the study site was 10-12 m wide and 20-50 cm deep under midsummer baseflow conditions. Riparian vegetation was dominated by Tag Alder (Alnus rugosa), Red-osier Dogwood (Cornus stolonifera), and Balm of Gilead (Populus gileadensis). Discharge during summer normally ranged between 0.5 and 1.0 m^3/s, but reached up to 7 m^3/s during spring and autumn (Burton et. al 1984). Mid-channel current speeds averaged about 20 cm/s. Substrates in the river were predominantly sand, although pebble and cobble (phi -5 and -6, respectively; Hynes 1970) riffles comprised about 30% of the river's bottom in the vicinity of the study site. Snags provided an additional .16 m^2 surface area/m^2 stream bottom for invertebrate habitat. Water temperatures from June to August 1984 ranged between 12.5 and 22.5°C (mean = 18°C; mean daily range = 2.5° C). Other physical and chemical data are presented in Table 1.1.

Table 1.1. Physical and chemical data for Schwartz Creek and the Ford River. All values are mean and (range).

	Schwartz 1983	Ford 1983	Ford 1984
pH	7.9 (7.4 - 8.2)	8.0 (7.7 - 8.3)	7.9 (7.8 - 8.2)
Hardness (mg $CaCO_3$/l)	133 (79 - 217)	160 (124 - 185)	163 (161 - 167)
Alkalinity (mg $CaCO_3$/l)	117 (67 - 143)	144 (114 - 159)	151 (147 - 155)
Conductivity (umhos/cm)	195 (101 - 256)	239 (185 - 270)	261 (204 - 270)
D.O. (mg O_2/l)	8.3 (7.7 - 9.5)	8.9 (8.6 - 9.4)	8.8 (8.5 - 9.8)
Turbidity (NTU)	3.2 (2.0 - 3.5)	1.8 (1.6 - 1.9)	1.3 (1.1 - 1.4)

Data from Burton et al. (1984).

Schwartz Creek

Schwartz Creek is a 2nd order stream in the Escanaba River (Lake Michigan) drainage in northern Dickinson Co., MI. Sampling was conducted in a heavily shaded 50 m section of the stream (T44N R28W sec. 10) approximately 200 m downstream from a marshland. The study site was located in a dense White Cedar (*Thuja occidentalis*) swamp with patches of Tag Alder (*Alnus rugosa*) also occurring along the streambanks. The stream channel was 5-7 m wide and 20-50 cm deep under midsummer baseflow conditions, with a bottom composed entirely of sand. Current speed at the study site was ca. 10-15 cm/s. Large quantities of snags (.68 m^2 surface area/m^2 stream bottom) constituted the only stable substrate for invertebrate activity. Water temperatures from June to August 1984 ranged between 11.5 and 21.5°C, with a mean temperature of 17°C. Data collected during summer, 1983 indicated that Schwartz Creek was physically and chemically similar to the Ford River (Table 1.1).

MATERIALS AND METHODS

Stenonema Populations

Stenonema spp. were sampled on both snag and stone substrates in the Ford River, but only on snags in Schwartz Creek as no other fixed substrates were available.

Ford Riffle - Invertebrate populations in the Ford riffle were sampled as part of the study of Burton et al. (1984). Artificial substrates consisting of semicylindrical plastic baskets (28 cm L x 18 cm W x 10 cm D) lined with 60 µm mesh netting were filled with stream sediments and buried in the stream bed. From June to October, 1983, and May to October, 1984, 5 replicate samples were collected at monthly intervals with replacement of samplers after processing (approx. 30 d colonization periods). Similar methods were used during winter months, but Stenonema did not occur in these samples.

Sample collection proceeded as follows: Each sampler was lifted from the stream and its contents emptied into a bucket of water. Larger rocks in the sample were individually scrubbed and discarded. The remaining sample material was then washed and decanted several times through a 250 µm mesh soil seive. Seive contents were preserved in 70% ethanol until further processing. Aquatic insects were picked from sample debris in the laboratory under a dissecting microscope and separated for later identification and enumeration.

Species of Stenonema were identified using the keys of Bednarik and McCafferty (1979). Nymphs were measured to the nearest 0.1 mm length; those less than 2.5 mm could not be confidently identified to species. Final instar nymphs were identified by their dark wingpads (Burks 1953).

Since substrate surface area limits the availability of periphyton to consumers, surface area of stone substrates in the Ford riffle was estimated to determine effective habitat

(Resh 1979). Twenty 50 cm transects were established by dropping a meter stick into the riffle, with the position and orientation of the meter stick upon retrieval defining the transect, and endpoints taken as the 0 and 50 cm marks. For each transect, the distance between the endpoints was measured directly on the substrate surface using a flexible tape measure, and effective habitat A (m^2 surface area/m^2 quadrat) was estimated as:

$$A = (2d_s)^2$$

where d_s = the distance between points measured directly on the substrate surface. This method is similar to that of Fricke and Thum (1976).

Snags - Three replicate snag samples were collected at biweekly intervals from each stream between June 18 and August 27, 1984, and a final set of replicates was collected on October 19. To minmize sample variance, sampling was limited to snags 5 to 10 cm in diameter and in an advanced state of decay (i.e. no bark and wood soft and pitted; equivalent to class 3 of Dudley and Anderson 1982). Sampling was further limited to snags in current speeds similar to that flowing over the Ford riffle substrate samplers.

Snag sampling proceeded as follows: The sample snag was wrapped with a 250 µm mesh screen to prevent invertebrates from escaping, then cut off approximately 30 cm from the end. The snag piece was transferred to a plastic bag, which was then filled with stream water. The sample was then carbonated (using 100 g baking soda and 250 ml vinegar) to prevent the animals from regurgitating when formaldehyde was added as a preservative (M.P. Oemke, MSU; pers. comm.), thus enabling analysis of feeding habits.

In the laboratory, each snag was placed in a white enamel pan and rinsed under running water. The surface of the snag was examined and any remaining animals were removed. All water used in collecting and processing the sample was washed through a 250 µm mesh soil seive and the seive contents preserved in 70% ethanol. Further processing continued as described above for the Ford riffle samples.

The surface area of each snag piece was estimated by multiplying its length times average circumference. Adjustments were made as needed to account for branches and crevices. Sample sizes ranged between 330 and 840 cm^2.

Length/Weight Relationship

On Oct. 23, 1984, 20 Stenonema vicarium nymphs ranging from 2.9 to 12.2 mm in length were collected from Sloane Creek, Meridian Township, Ingham Co., MI. In the laboratory the nymphs were killed in hot ($70^{\circ}C$) water, then body length (anterior edge of head capsule to posterior end of 10th abdominal tergite) and head capsule width were measured to the nearest 0.1 mm. Nymphs were then blotted dry on tissue paper for 5 s, weighed on a Cahn 27 electrobalance, dried for 24 hr at $50^{\circ}C$, desiccated for 1 hr, and reweighed. The data were $\log_e$ transformed and individual dry weight regressed against length and head capsule width. The length/weight relationship derived for *S. vicarium* in Sloane Creek was assumed to be applicable to the Ford River and Schwartz Creek, since Smock (1980) found that the relationship for *S. annexum* did not differ sigificantly between 2 North Carolina streams.

Characterization of Periphyton Communities

Periphyton communities were characterized in each stream to guage resource availability.

Colonization - Accumulation of periphyton on glass microscope slides was followed in each stream as a rough measure of primary productivity. Colonization data for the Ford River are from Burton et al. (1984). Periphyton samplers consisted of plexiglass slide racks which held 8 standard microscope slides in a vertical position. Racks were tied to bricks and placed in both streams on July 30, 1984 with the slide edges facing into the current. Half of the slides in each stream were removed at 14 and 28 d intervals and frozen until later analysis for biomass (Ash Free Dry Weight), chlorophyll-a, and phaeophytin-a following standard procedures (APHA 1980). Numbers of replicates for chlorophyll and phaeophytin were 20 in the Ford, 4 in Schwartz; for biomass, 10 in the Ford, 4 in Schwartz. Data were analyzed by comparing the slopes of the regression lines of biomass and chlorphyll-a against days elapsed.

Ambient Periphyton Communities - Chlorophyll-a and phaeophytin-a were measured on natural substrates as indicators of periphyton standing crop and physiological condition. Biomass estimates were not possible due to the difficulty in separating periphyton from substrate material. On Aug. 24, 1984, 3 replicate 15 cm^2 periphyton samples were scraped from snag upper surfaces from each stream and extracted in 75 ml acetone for 24 hr at 4^oC. Data on chlorophyll-a and phaeophytin-a on stone substrates in the Ford River were collected by Dr. T.M. Burton (MSU). On Aug. 23, 1984, 5 rocks from the Ford riffle were immersed in separate acetone baths for 24 hr at 4^oC. Prior to extraction, the stones used in these analyses had spent approximately 1.5 hr in clear plexiglass circulating production/ respiration chambers. Analyses of chlorophyll-a and phaeophytin-a proceeded as above. The surface area of each rock was estimated by wrapping it with aluminum foil, and then measuring the area of the foil with an electronic area meter (Licor model LI-3000). Periphyton was assumed to cover half of the total surface area of each stone. Results were analyzed by 1-way ANOVA and designed orthogonal contrasts (Schwartz snags vs. both Ford sources; Ford snags vs. Ford rocks).

Feeding Habits

Midgut contents of Stenonema spp. nymphs from each population were examined using the method of Cummins (1973). After teasing out the midgut in distilled water, the peritrophic membrane and its contents were pipetted into a clean depression slide. Gut contents were emptied into the slide, broken up with forceps, then washed into a clean beaker. After sonicating for 5 s, the contents of the beaker were vacuum filtered (5 psi) through a 0.45 μm Millipore filter. The filter was dried for 10 min at 50°C, cleared in Kleermount mounting medium (Carolina Biological Supply, Burlington, NC), and then slide mounted with a coverslip. Midgut contents of individuals $<$ 5.0 mm length were combined within size classes and species to increase particle density.

Proportions of diatoms and detritus particles on each slide were estimated by a line-transect technique using a phase contrast microscope at 400X. Three 9 mm transects of 30 fields each (300 μm/field) were taken across each slide. For each transect, the proportion of each particle type was estimated as the total number of ocular micrometer units intersected by that particle type, divided by total units for both particle types. The mean of the 3 transects was taken as the overall estimate for the slide.

Individuals less than 1.5 mm length were whole mounted in Hoyer's mounting medium since they were difficult to dissect. Percent diatoms in the gut was determined by estimating the area of diatoms relative to the area of the entire gut.

Growth and Production

Instantaneous growth rates were estimated for the 1984 cohort of each population as the slope of the regression of

$\log_e$ maximum individual dry weight on each sample date against days elapsed. Maximum weights rather than mean weights were used because continuous recruitment occurred in all 3 populations (see results below). Continuous influx of small individuals into a population would lead to low mean weight estimates, thus underestimating growth rate (Waters 1966). Separate estimates were derived for summer (June through August) and autumn (September and October) since other studies have indicated that growth of Stenonema decreases during autumn (Richardson and Tarter 1976; Barton 1980; Kreuger and Cook 1984).

Using the above growth rates, production was estimated for each sample interval by the Instantaneous Growth method (Waters 1977):

$$P = G \times B$$

where P = production in mg dry weight/m^2/days elapsed; G = growth in mg = instantaneous growth rate times days elapsed; B = average biomass in mg dry weight/m^2, estimated as the arithmetic mean of the biomass estimates at the beginning and end of the interval. Total production was the sum of the interval estimates (see example of calculations in Table 1.3).

RESULTS

Stenonema Populations

Ford Riffle - Effective habitat in the Ford riffle (± 95% CI) was estimated to be 1.45 ± .07 m^2 substrate surface/m^2 quadrat. All following references to the riffle population are expressed in terms of effective habitat.

Stenonema vicarium (Walker) [=*fuscum* (Clemens)] predominated in the Ford riffle in both 1983 and 1984. Only a few specimens of *S. modestum* (Banks) [= *rubrum* (McDunnough)] were collected in either year. Nymphs were not found in samples between June and November in either year, although *S. vicarium* had been observed in winter leaf pack samples from the Ford River (pers. obs.). Population density was greater in 1984 than in 1983 (mean density = 473/m^2 and 136/m^2, respectively), but individuals were more evenly distributed over all size classes in 1983 (Figure 1.1). Nymphs larger than 5.0 mm did not occur in the 1984 samples (Figure 1.2). The presence of individuals in the smallest size class from mid-June through mid-October of both years (Figures 1.1, 1.2) indicated that egg hatching was continuous throughout the summer. Mature nymphs were collected in June, 1983, indicating that adult emergence occurred then.

Ford Snags - Numbers of *Stenonema* spp. on Ford snags were extremely low on all sample dates, with most individuals less than 2.0 mm in length (Figure 1.3). As observed in the riffle samples, individuals in the smallest size class

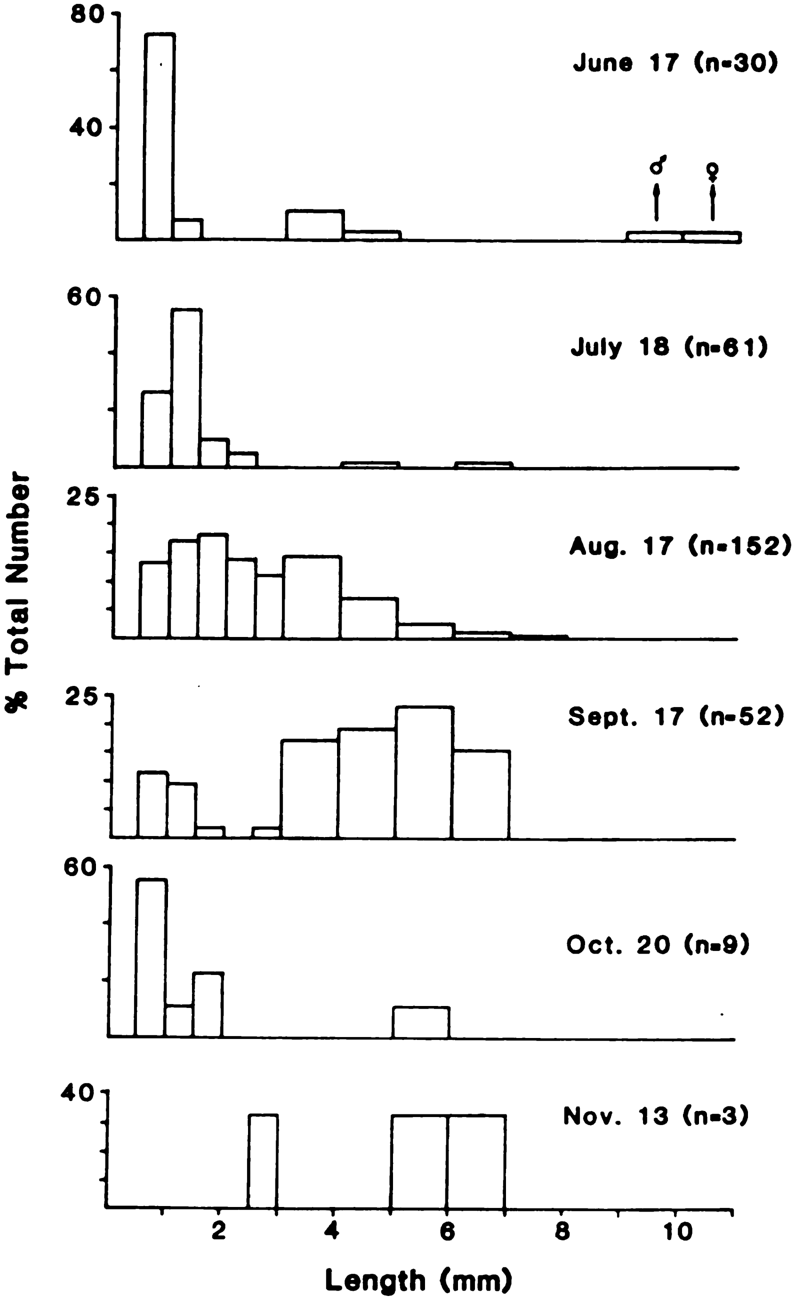

Figure 1.1. Size distribution of nymphs, Ford riffle, 1983.

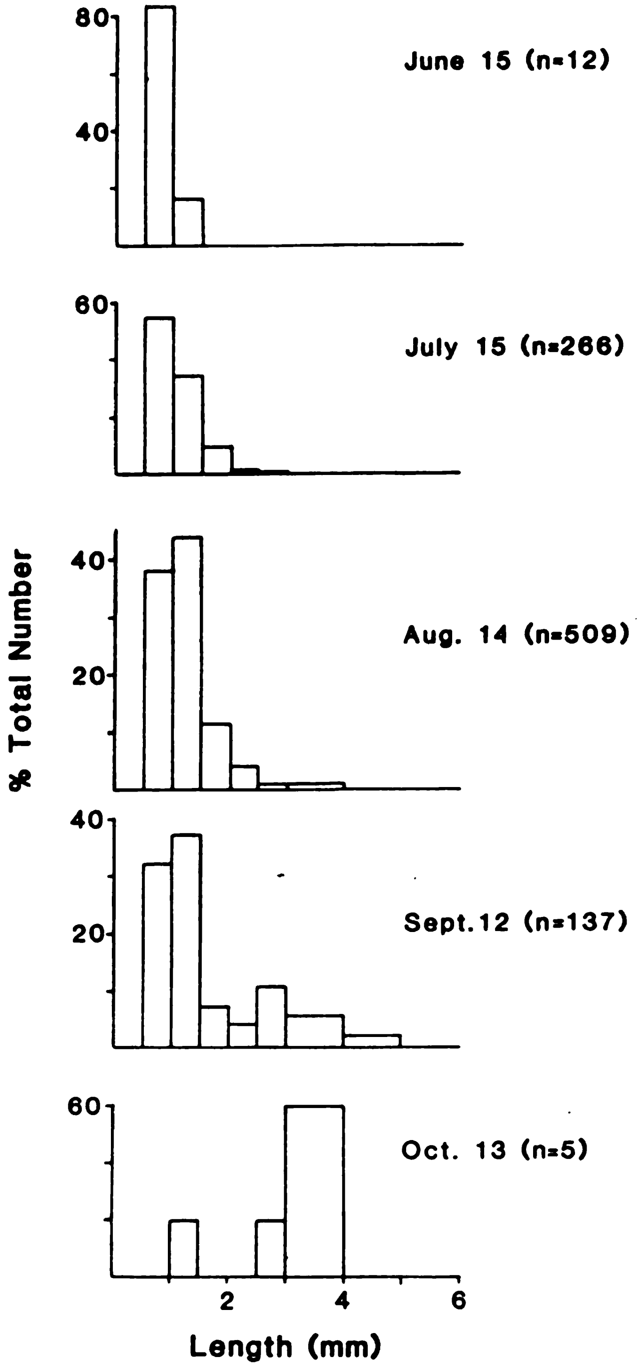

Figure 1.2. Size distribution of nymphs, Ford riffle, 1984.

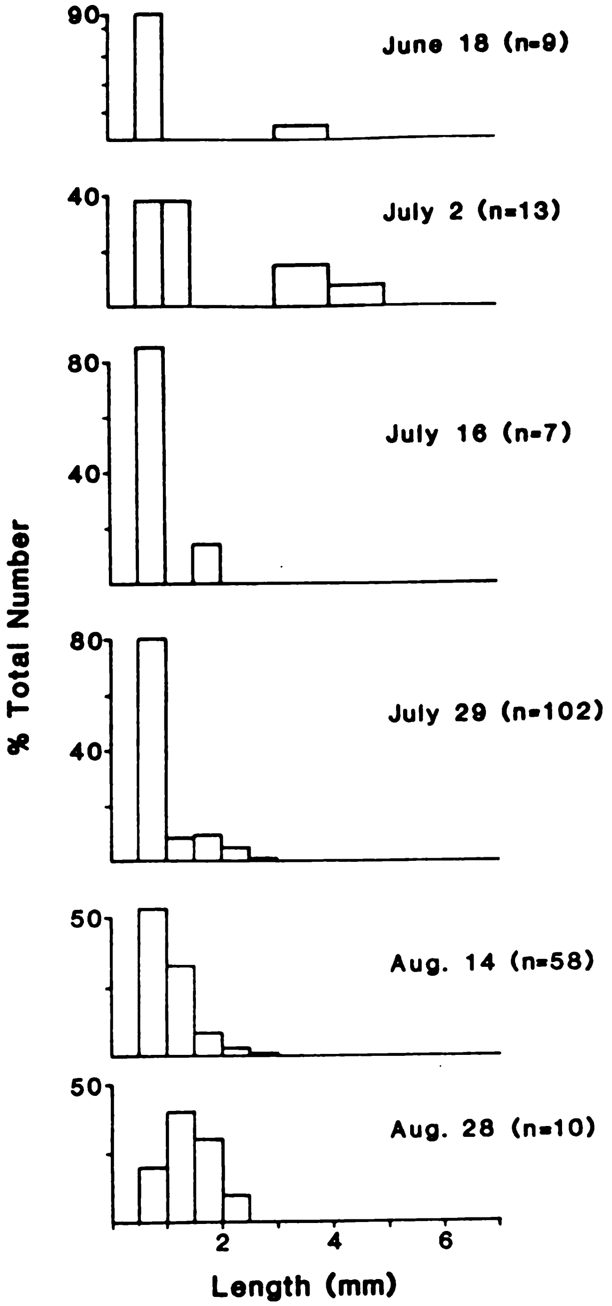

Figure 1.3. Size distribution of nymphs, Ford snags, 1984.

occurred throughout the summer. No *Stenonema* were found in the October samples. All individuals large enough to be identified to species were *Stenonema exiguum* Traver (=*quinquespinum* Lewis), although some individuals less than 2.5 mm length had mouthparts typical of *S. modestum* or *S. vicarium*.

Schwartz Snags - Three species of *Stenonema* were found in Schwartz Creek: *S. exiguum* comprised approximately 53% of the total population, *S. modestum* 35%, and *S. vicarium* 12%. The size frequency distribution of each species taken alone (Figure 1.4) did not differ significantly from the distribution of the combined populations (Figure 1.5; contingency tables; $P > 0.05$ for all species). Therefore, the combined size frequency distribution in Figure 1.5 was taken to be representative of the life cycles of all 3 species, because estimation of growth rates from Figure 1.4 would not have been possible.

The 1983 and 1984 cohorts overlapped considerably, with both egg hatching and adult emergence occurring throughout the sampling period (Figures 1.4, 1.5). Interspecific differences in egg hatching periods were not likely, since members of the 1984 cohort of each species were first observed on the same sample date (Figure 1.4). Adult emergence occurred mainly in June and July (Figure 1.4). Sex ratios were not significantly different from 1:1 (Chi-square tests; $P > 0.50$ for all species). Mean length at emergence for females (9.0 mm) was greater than that of males (7.5 mm), as is typical of mayflies (Burks 1953).

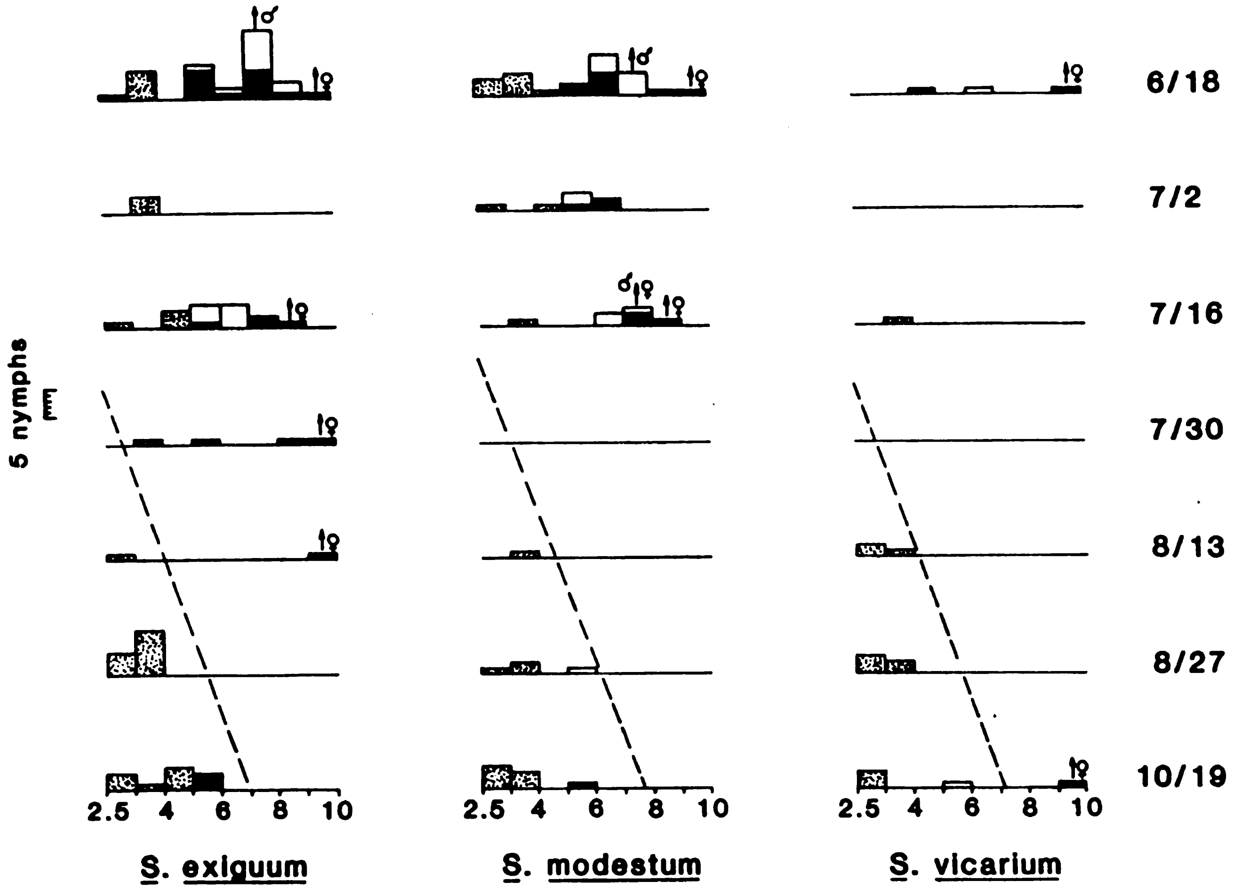

Figure 1.4. Size distribution of Stenonema nymphs in Schwartz Creek, 1984. Solid, open, and stippled bars denote female,male, and unsexed nymphs, respectively. Arrows denote emerging individuals of the indicated sex. Proposed separation of 1983 and 1984 cohorts is indicated with a dashed line.

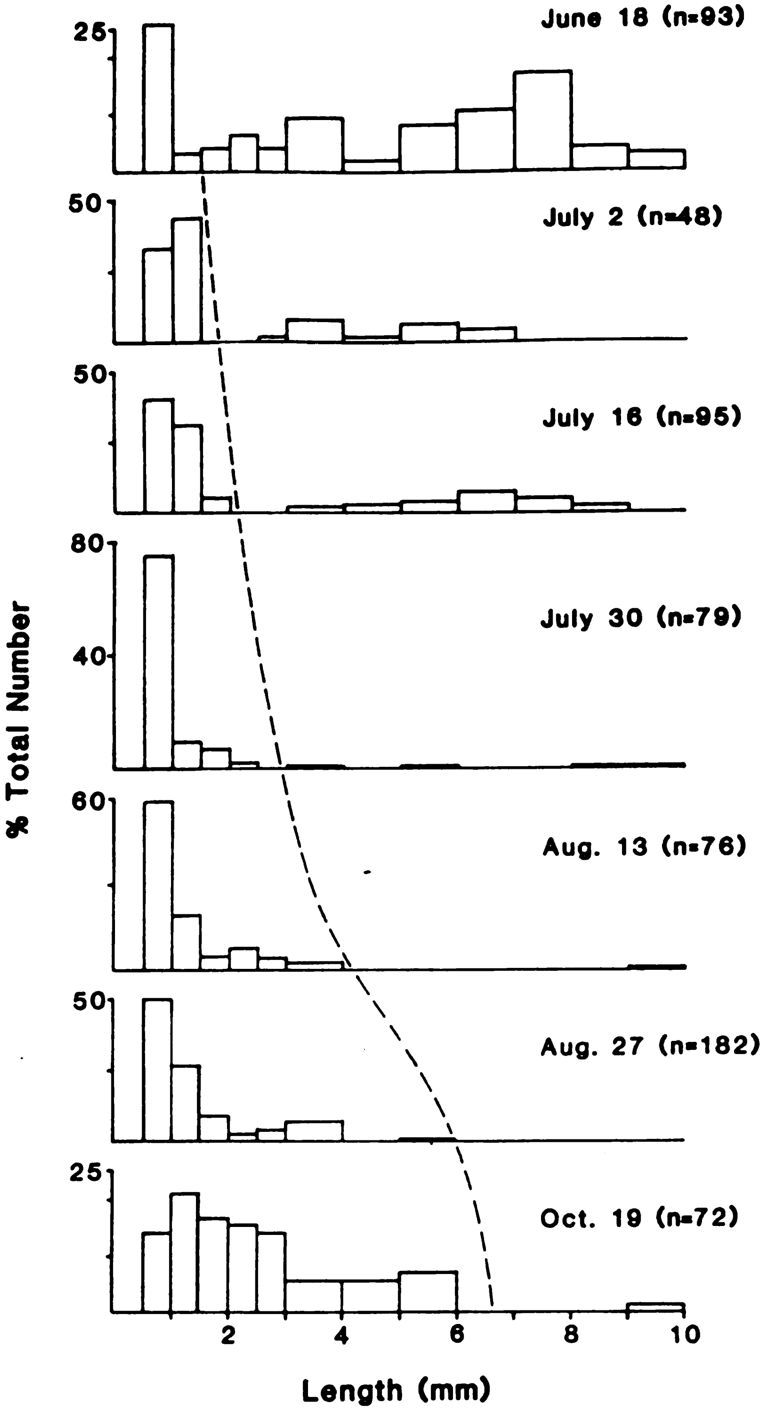

Figure 1.5. Combined distribution of Stenonema spp., Schwartz Creek, 1984. Dashed line indicates separation of 1983 and 1984 cohorts.

Length/Weight Relationship

The relationship between body length and dry weight for *Stenonema vicarium* is presented in Figure 1.6. Length was a better predictor of dry weight ($r^2 = .975$) than was head capsule width ($r^2 = .936$). The relationship in Figure 1.6 was assumed to hold true for all *Stenonema* species found in Ford and Schwartz, since there were no obvious differences in body proportions among these species.

Characterization of Periphyton Communities

As expected, accumulation of periphyton was more rapid in the Ford River than in Schwartz Creek, in terms of both biomass and chlorophyll-a (Figures 1.7-A, B; t-tests; $P < 0.01$ for both). Chlorophyll/phaeophytin ratio (Chl/phaeo) increased linearly in both streams (Figure 1.7-C), and was not significantly different between streams on either sample date (t-tests; $P > 0.05$ in both cases). Autotrophic Index (AI = mean AFDW / mean chlorophyll-a) was greater in Schwartz on both sample dates (Figure 1.7-D).

No difference in chlorophyll-a density on natural substrates was detected between the 2 streams, suggesting that their algal standing crops were similar (Table 1.2). Phaeophytin-a density was significantly lower and chl/phaeo significantly higher on Schwartz snags than on either Ford River substrate (Table 1.2; One-way ANOVA, orthogonal contrasts; $P < 0.01$ for both). Phaeophytin-a and chl/phaeo did not differ among Ford substrates.

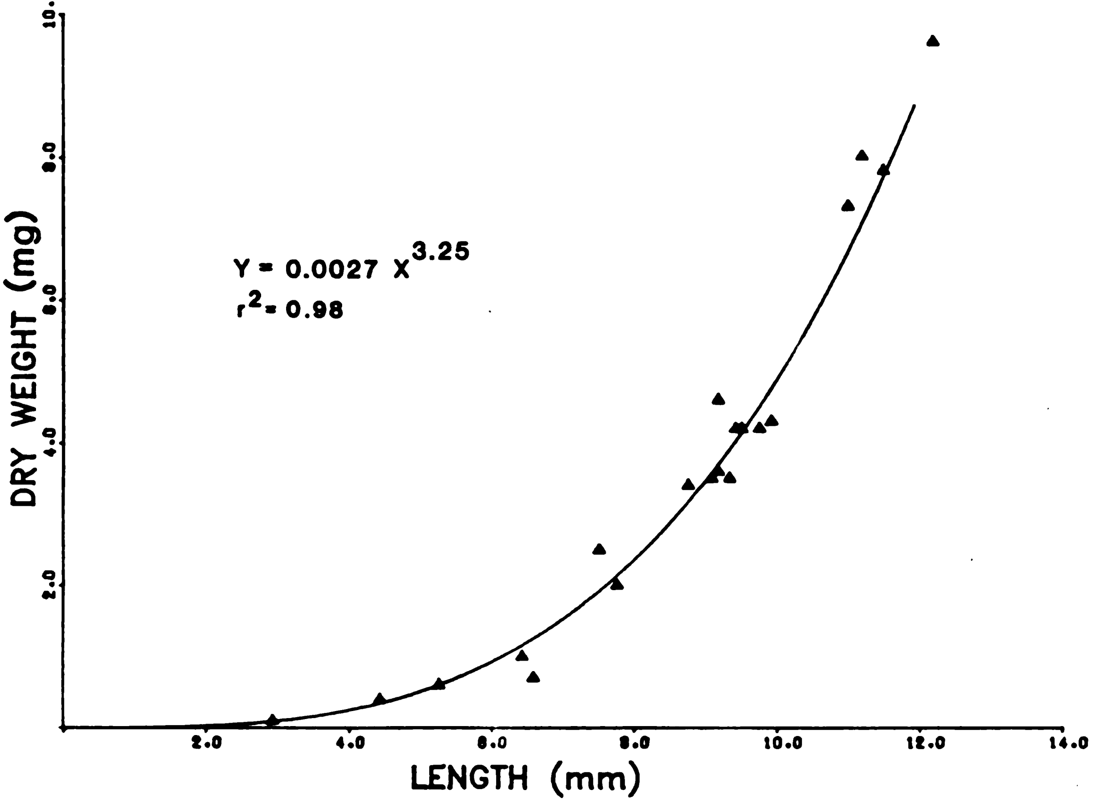

Figure 1.6. Length/weight relationship for S. *vicarium*.

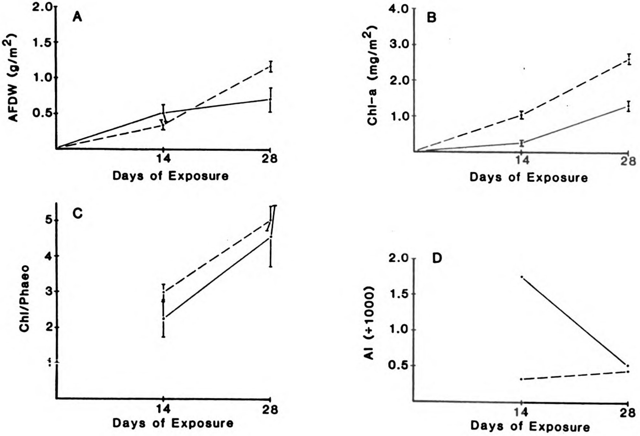

Figure 1.7. Periphyton colonization in Schwartz Creek (---) and the Ford River (——), August, 1984. Vertical bars denote ± SE.

Table 1.2. Characterization of ambient periphyton communities. All values are mean ± SE.

SOURCE	Chlorophyll-a (mg/m^2)	Phaeophytin-a (mg/m^2)	Chl/Phaeo
SCHWARTZ SNAGS (n = 3)	18.15 ± 3.53	3.94 ± 0.96**	4.78 ± 0.39**
FORD SNAGS (n = 3)	18.39 ± 3.53	6.69 ± 0.96	2.62 ± 0.39
FORD ROCKS (n = 5)	19.14 ± 2.74	8.55 ± 0.74	2.26 ± 0.30

** = significantlydifferent from other values in same column; $P < 0.01$, one-way ANOVA, orthogonal contrasts.

Feeding Habits

The proportion of diatoms in guts of Stenonema spp. was positively correlated with body length in all 3 populations (Figure 1.8-A, B). Interspecific differences in feeding were not observed in Schwartz (t-tests; $P > 0.30$ for all combinations), so the length/diet regression in Figure 1.8-B was calculated using the combined data. Length-specific ingestion of diatoms was significantly greater in the Ford riffle than on Schwartz snags (t-test; $P < 0.05$), but Ford snags did not significantly differ from either of these (t-tests; $P > 0.01$ for both).

Growth and Production

Growth rates from June through August, 1984 did not significantly differ among the 3 populations (t-tests; $P > 0.30$ for all combinations). Therefore, the growth rate for all 3 populations during this period was estimated as the slope of the regression line for the combined data sets (Figure 1.9; .0749 mg/mg/d). The autumn growth rate estimated from the combined Schwartz and Ford riffle data was much lower (.0110 mg/mg/d; Figure 1.9), as had been expected. Growth rates estimated using the 5 largest individuals on each sample date were similar (.0730 and .0118 mg/mg/d for suumer and autumn, respectively), so that using only 1 individual per sample date did not bias the the growth rate estimates. Growth rates could not be estimated for the 1983 Ford riffle population because cohorts were not clearly distinguishable (Figure 1.1).

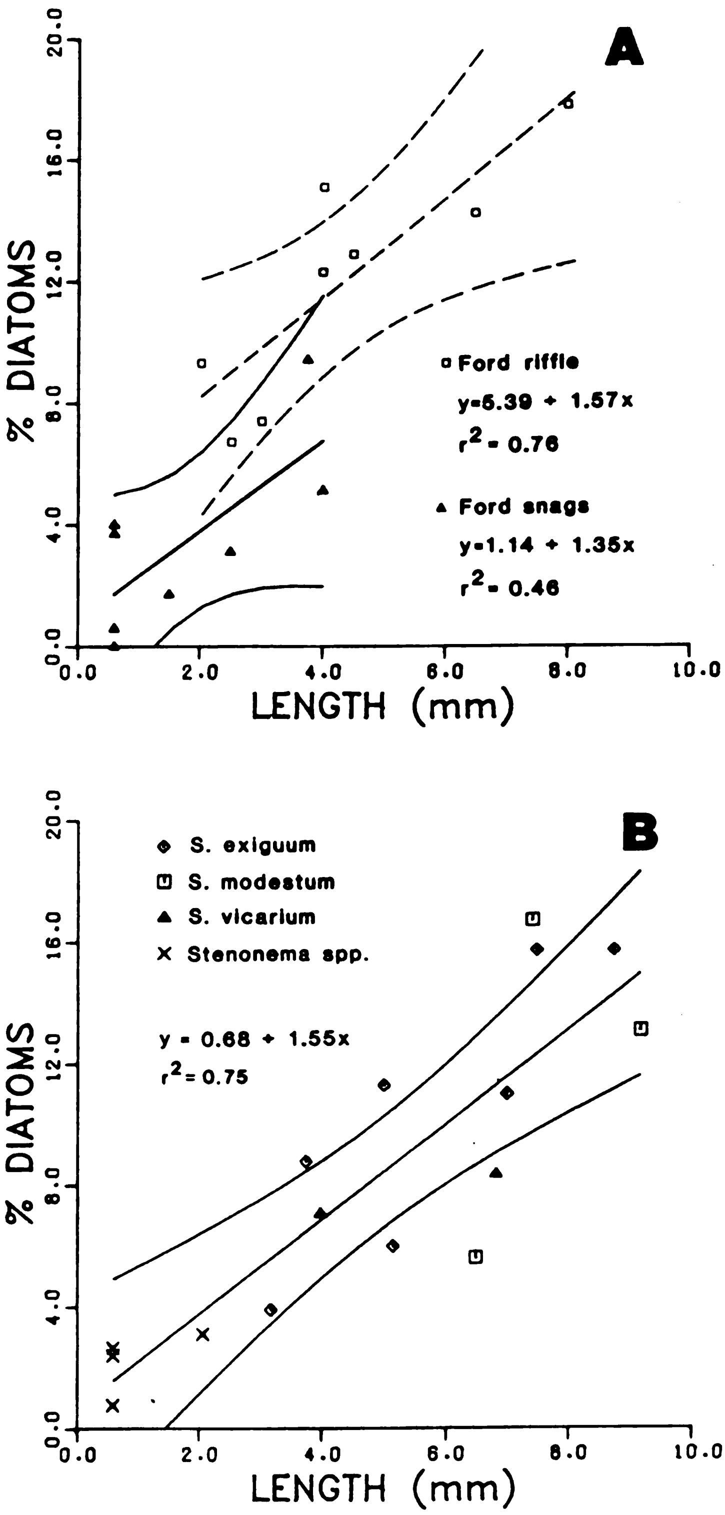

Figure 1.8. Regression of % diatoms in gut contents (± 95% CI for regression line) in: A) Ford River; B) Schwartz Creek.

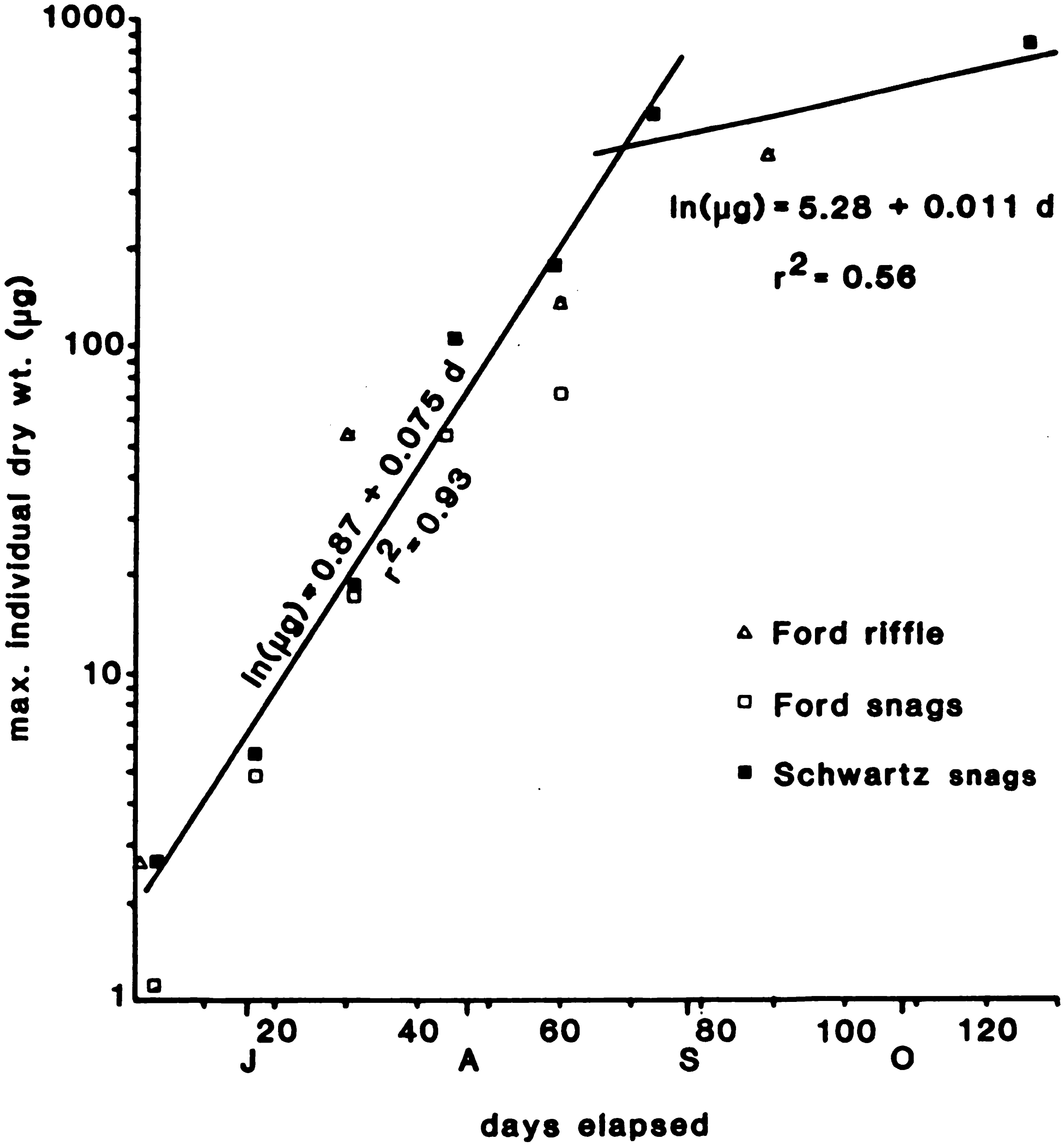

Figure 1.9. Growth regression for the combined populations.

Table 1.3. Calculation of production for the Schwartz 1984 cohort.

Sample Date	Biomass (mg/m^2)	$\bar{B}_i$ Average Biomass	g (mg/mg/d)	$G_i = gd$	$P_i = G_i \cdot \bar{B}_i$
6/18	.1578				
		.3708	.0749	1.0486	.3888
7/2	.5838				
		1.5799	.0749	1.0486	1.6567
7/16	2.5759				
		2.1667	.0749	1.0486	2.2720
7/30	1.7574				
		4.1554	.0749	1.0486	4.3574
8/13	6.5534				
		14.9791	.0749	1.0486	15.7071
8/27	23.4047				
		33.6004	.0110	0.5830	19.5830
10/19	43.7961				
		$\bar{B}$ = 17.1248 mg/m^2			P = 43.9710 mg/m^2/123 d

$P/\bar{B}$ = 43.97 / 17.12 = 2.57

Table 1.4. Summary of production estimates.

POPULATION	COHORT	P*	B*	P/B	DAILY P**
SCHWARTZ	1983 + 1984	538.1	69.0	7.8	4.37
SCHWARTZ	1984				
S. exiguum		21.1	9.0	2.3	.172
S. modestum		11.9	4.1	2.9	.097
S. vicarium		11.0	4.0	2.8	.089
TOTAL		44.0	17.1	2.6	.357
FORD RIFFLE	1984				
S. vicarium (> 95%) *S. modestum* (< 5%)		34.8	6.6	5.3	.290
FORD SNAGS	1984				
S. exiguum other species?		6.2	0.8	7.8	.050

* = mg dry weight / m^2

** = mg dry weight / m^2 / day

Total production of *Stenonema* spp. was greatest in Schwartz Creek, regardless of whether or not the 1983 cohort was considered (Table 1.4). Comparing species individually, *S. exiguum* and *S. modestum* were most productive on Schwartz Creek snags, whereas production of *S. vicarium* was greatest in the Ford riffle. *S. modestum* accounted for less than 5% of the total *Stenonema* production in the Ford riffle. The production of each species in Schwartz was approximately proportional to its relative abundance in the total population. Turnover ratio (P/B) was high (7.8) for the combined Schwartz cohorts and for Ford snags, intermediate (5.3) for the Ford riffle population, and lowest for the Schwartz 1984 cohort (mean = 2.6; Table 1.4).

DISCUSSION

Stenonema Populations

The size freqency distributions of *Stenonema* spp. observed in this study (Figures 1.4, 1.5) are similar to those reported for other northern streams (Coleman and Hynes 1970; Richardson and Tarter 1976; Flowers and Hilsenhoff 1978; Barton 1980; Kreuger and Cook 1984). The apparent similarity of life cycles among *Stenonema* spp. in Schwartz Creek is supported by Flowers and Hilsenhoff (1978), who concluded that life cycles of *Stenonema* spp. in Wisconsin

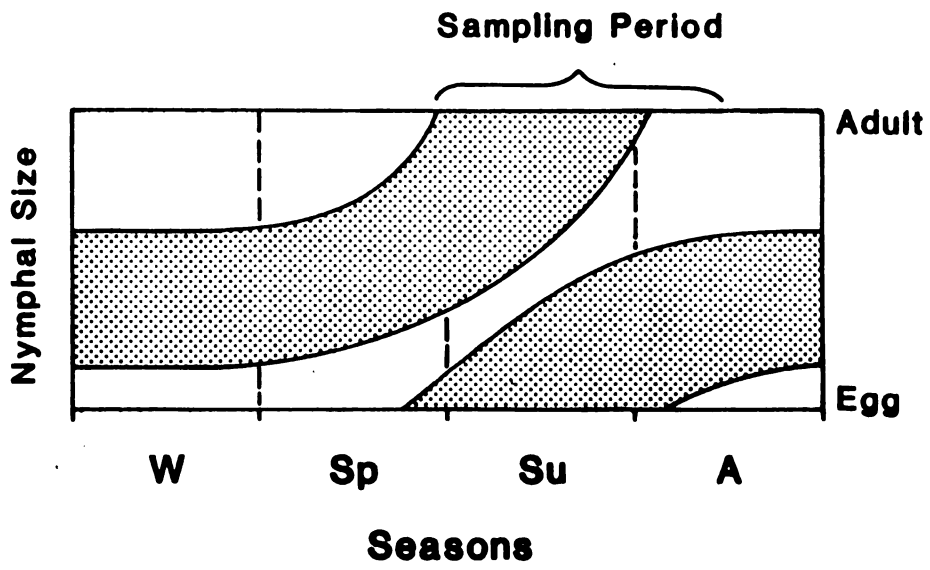

Figure 1.10. Life cycle diagram for Stenonema spp. Shaded area denotes size range of nymphs present as they progress from eggs (bottom line) to emergence (top line). The sampling period for the present study is indicated.

are similar. These life cycles conform to the univoltine "slow seasonal" pattern of Hynes (1970) (Figure 1.10): Eggs hatch after a short incubation period, nymphs grow rapidly until late fall, resume growing in spring when water temperatures rise, and emerge as adults primarily in late spring and early summer. Extended egg hatching as observed in this study has not been reported previously for Stenonema, although the broad size frequency distributions reported in other studies suggest that this phenonmenon may be widespread in the genus (Richardson and Tarter 1976; Flowers and Hilsenhoff 1978; Kreuger and Cook 1984).

Disappearance of Stenonema nymphs from Ford River samples during late autumn was coincidental with rising discharge (Burton et al. 1984). Disappearance of S. vicarium nymphs during winter was also noted by Coleman and Hynes (1970), who suggested that the nymphs may migrate into the hyporheic zone during this period.

Periphyton Communities

The results of the periphyton colonization study suggest that primary productivity was greater in Ford than in Schwartz, since periphyton accumulation was more rapid in the unshaded Ford River (Figure 1.7). Diatom colonization studies carried out in these streams during 1982 and 1983 yielded similar results (M.P. Oemke, MSU; pers. comm.). Periphyton accumulation rates can not be interpreted as net primary productivity, since they represent a composite of colonization and growth (Wetzel 1966). Most important to consumers though, is that the Ford periphyton community regenerates more quickly, and thus may be able to withstand greater grazing pressure.

Autotrophic Indices (AI) and Chlorophyll/phaeophytin ratios (chl/phaeo) are general indicators of the physiological condition of periphyton communities (Weber 1973; APHA 1980). AI roughly corresponds to the proportion of the total periphyton community (including bacteria, fungi, protozoa, and detritus) comprised by algae, and normally ranges between 50 and 200 for "healthy" (predominantly autotrophic) periphyton communities and higher for heterotrophic communities (APHA 1980). Therefore, the greater AI in Schwartz (Figure 1.7) suggests that detritus and heterotrophic organisms comprise a greater portion of its periphyton community, although algal standing crop is similar to that in the Ford (Table 1.2). Chl/phaeo roughly corresponds to the ratio of living (actively photosynthesizing) to senescent algal cells, since phaeophytin-a is the major breakdown product of chlorophyll-a. Thus, the low chl/phaeo on Schwartz Creek snags indicates a periphyton community in better physiological condition to that in the Ford River. This finding is surprising, since physiological condition would be expected to be highly correlated with productivity.

In summary, the results suggest that while algal colonization of substrates is initially more rapid in the Ford River than in Schwartz Creek, both periphyton communities eventually attain a similar standing crop. The high chl/phaeo observed in Schwartz may suggest that this periphyton community was in an actively growing state, whereas low chl/phaeo in the Ford River may indicate self-limitation (i.e. senescence of cells due to competition for light and nutrients; see Lamberti and Resh 1983, 1985).

Feeding Habits

Lack of major differences in the feeding habits of the 3 populations (Figure 1.8) is not surprising, in view of the similarity in periphyton standing crops between the 2 streams (Table 1.2). Since the regression lines of the snag inhabiting populations were almost identical (Figure 1.8), habitat appears to have been a more important factor in the feeding habits of these populations than stream order. Presence of wood particles in the guts of snag-inhabiting nymphs suggests that they ingested substrate material in the process of scraping periphyton. Despite the significant difference in feeding observed between the Ford riffle and Schwartz snags, Hypothesis #1 (algal consumption in the Ford River > Schwartz Creek) is rejected, since it was formulated with the expectation that resource availability would differ between the 2 streams.

Growth and Production

The summer instantaneous growth rate of 7.5 %/d (.0749 mg/mg/d) is higher than most growth rates reported for mayflies living in similar temperature regimes (see Table 6 of McCullough et al. 1979). Higher growth rates have been reported only for species of Tricorythodes: 12.6 %/d for T. minutus (McCullough et al. 1979) and 15.3 %/d for T. atratus (Hall 1975). Tricorythodes spp. are small, multivoltine mayflies with short developmental times. Rapid growth for these species may be an adaptation for producing several generations per year (high "r"). On the other hand, the rapid growth of Stenonema during summer may be necessary to compensate for the extensive period of its life cycle during which there is little growth (Figure 1.10).

Rejection of Hypothesis #2 (Growth in the Ford > Schwartz) could have been caused by several factors: First, Figure 1.8 is only a crude assessment of nymphal feeding habits, particularly with respect to the nutritional quality of the diets. It is possible that part of the "detritus" fraction is actually diatom cell contents. Second, there is no way to determine whether diatoms were alive or dead when ingested. The higher chl/phaeo in the Ford (Table 1.2) suggests that the nymphs ingested proportionally more senescent diatoms than their counterparts in Schwartz Creek, so that ingestion of viable cells could have been comparable in the 2 streams. Third, even if feeding habits were accurately assessed, the diets of the 3 populations probably did not differ enough to produce detectable differences in growth rates. Fourth, nymphs in Schwartz Creek may have increased their ingestion rates to compensate for poor food quality (Cummins and Klug 1979). Temperature probably was not a factor, because the Ford River was only a mean of 1°C warmer than Schwartz Creek. Based on the temperature-growth relationship for the heptageniid *Ecdyonurus dispar* (Humpesch 1981), such a minor difference in temperatures probably would not have had a detectable effect on growth.

The daily production estimates for 1984 cohorts (Table 1.3) suggest acceptance of Hypothesis #3 (Production in the Ford > Schwartz) only with respect to *S. vicarium*, since production of *S. exiguum* and *S. modestum* was greatest in Schwartz Creek. However, the hypothesis can not be accepted because acceptance is dependent on the existence of faster growth in the Ford River. Absence of detectable differences in growth rates indicates that production was determined largely by factors influencing population density and standing stock (e.g. recruitment and mortality).

Among these factors, the substrates available in each stream undoubtedly had major consequences for species composition and population density. *Stenonema exiguum* occurred exclusively on snags, even in the Ford River where alternate substrates were available. The opposite was true of *Stenonema vicarium*, which was dominant in the Ford riffle in both 1983 and 1984, but absent from adjacent snags. *Stenonema vicarium* was also the least abundant of the 3 species on Schwartz snags. *S. modestum* occurred on both stone and snag substrates, but attained its greatest numbers on Schwartz snags. Thus, each species was associated with a specific habitat: *S. exiguum* and *S. modestum* with snags, and *S. vicarium* with stone substrates. Other workers have pointed out similar habitat associations for these species (Leonard and Leonard 1962; Lewis 1974; Flowers and Hilsenhoff 1978; Bednarik and McCafferty 1979), although S. modestum is often associated with stone substrates.

The P/B (turnover) ratios in Table 1.3 can provide valuable insights into the life history characteristics of each population (Benke 1984). Since P = G * B by the Instantaneous Growth method, then cohort P/B should be equal to cohort G. However, this relationship holds true only when both growth and mortality are exponential; inequality of cohort P/B and G therefore indicates deviation from exponential growth and/or mortality (Waters 1966, 1983). It has already been established that growth was not exponential over the entire sampling period of the present study (Figure 1.9). Reduced growth rates in the autumn decreased P/B relative to G, because large, slow-growing individuals increased the average biomass of the cohort (B) without contributing substantially to production (Waters 1983). On the other hand, continuous recruitment and high mortality (or drift) increased P/B relative to G by reducing average biomass (Winterbourne 1974). Calculation of cohort G for these Stenonema populations would be difficult because the sampling period did not cover an entire cohort. However, since all 3 populations were subject to the same conditions of non-exponential growth (Figure 1.9) and continuous recruitment (Figures 1.2 - 1.5), the P/B ratios in Table 1.3 indicate the relative magnitude of the biomass losses from each population. The high P/B ratios observed on snags in both the Ford and Schwartz (combined 1983 and 1984 cohorts) indicate that these populations experienced the greatest biomass losses. Obviously, adult emergence was a major source of losses from Schwartz snags (Figure 1.5). Considering only the 1984 cohorts on snags, biomass losses were much greater from snags in Ford than Schwartz. The disappearance of Stenonema nymphs from the Ford River during autumn undoubtedly accounted for much of the biomass loss from snags, but the magnitude of P/B for Ford snags relative to the riffle indicates that mortality was quite severe. Therefore, both biomass and "survivorship" of Stenonema were greatest on Schwartz snags, suggesting that snags in the 2 streams did not provide equally suitable habitat. One obvious physical difference between them was their degree of lighting; nymphs living on the more brightly-lit snags in the Ford River may have been more susceptible to predation, especially by fish.

General Discussion

The main conclusion that can be drawn from this study is that habitat, not stream order, is the primary factor controlling the productivity of Stenonema spp. in the Ford River and Schwartz Creek. Certainly, the lack of major differences in periphyton availability between streams invalidated the initial hypotheses, since the expected conditions of differing resource avilability were not met. Even if the streams were to differ in periphyton standing crop, it would have been difficult to unequivocally connect

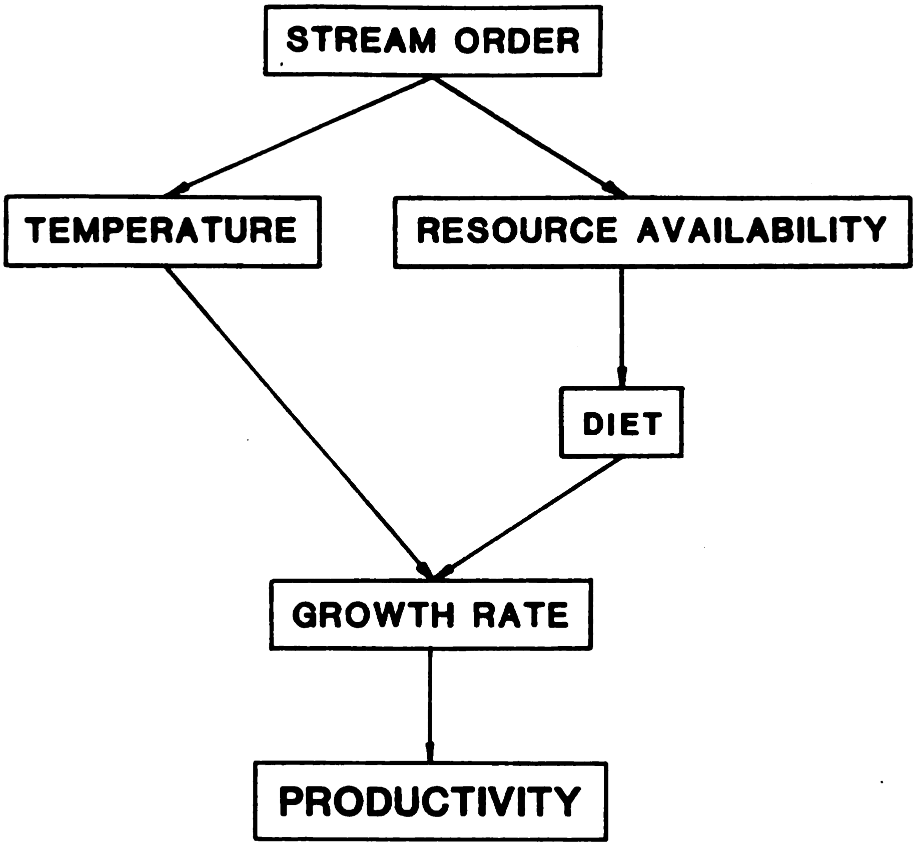

Figure 1.11. Model used in hypothesis formation.

these conditions to the productivity of a single species or group of species. Examination of the model used in hypothesis formulation for this study (Figure 1.11) clarifies this point: stream order was hypothesized to indirectly influence secondary productivity via its effects on resource availability, feeding, and growth. In retrospect, this model is admittedly an extremely simplistic view of stream ecosystems since it fails to account for habitat, competition, predation, etc.; in short, it considers only those factors of interest as if in a controlled laboratory setting.

A more realistic model (Figure 1.12), based on the results of this study and the reviews of Benke (1984) Sweeney (1984), and Minshall (1984) highlights the complexity of factors influencing a species' productivity. Although this model is by no means exhaustive, it is quite clear that comparisons of the productivity of a single species are inappropriate, since the results will be confounded by uncontrollable factors, such as substrate and community structure. Community structure may have played an important role in this study: several potential competitors for Stenonema exist in the Ford River, including 5 other heptageniid species (Burton et al. 1984). In contrast, the invertebrate fauna in Schwartz Creek is much less diverse, with only 2 other heptageniid species occurring in extremely low numbers (pers. obs.). It is possible then that the high productivity of Stenonema spp. in Schwartz Creek is partially caused by the absence of interspecific competition.

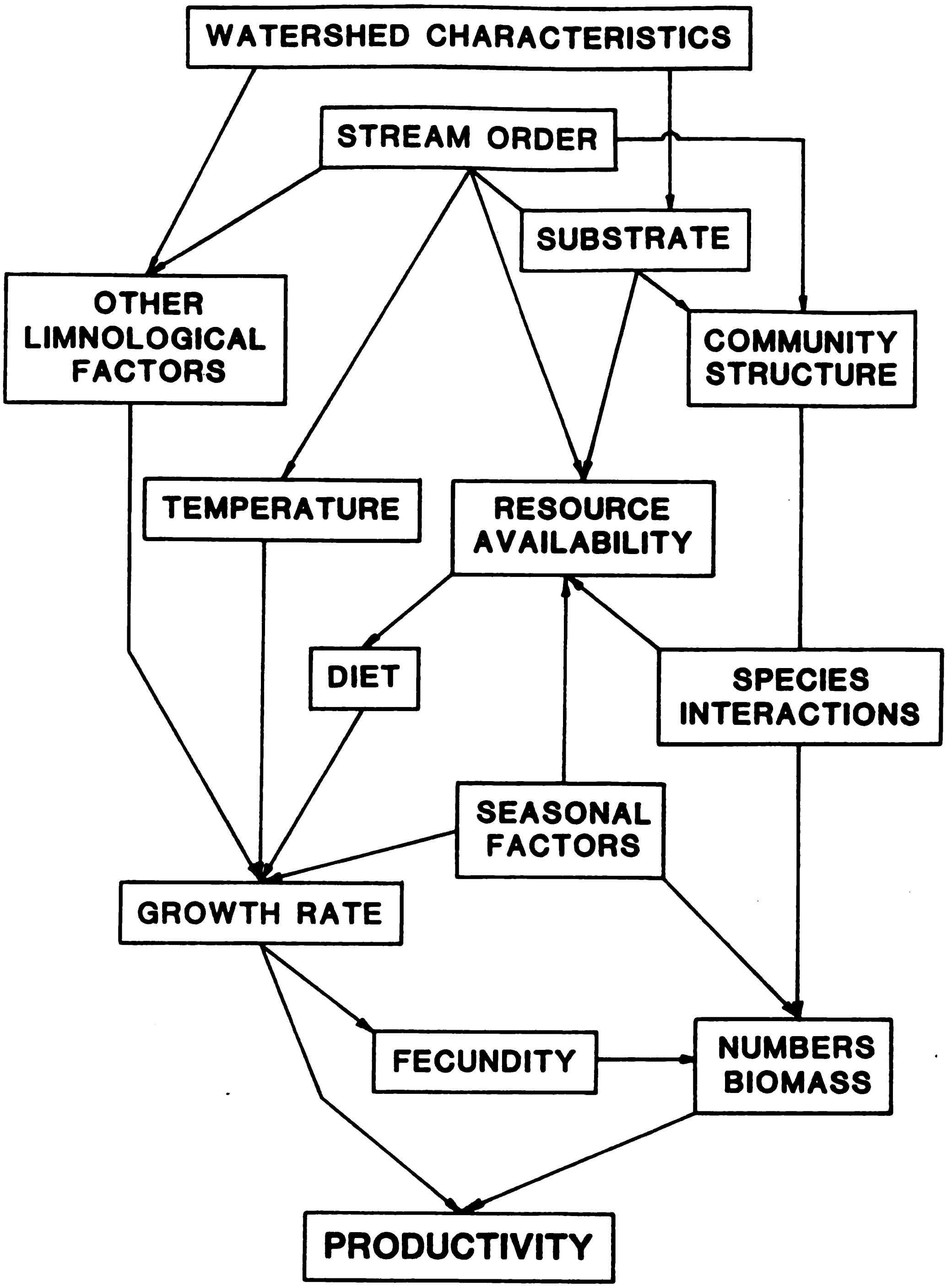

Figure 1.12. Improved model of productivity.

In conclusion, substrate availability had major consequences for the distribution and productivity of *Stenonema* spp. in Schwartz Creek and the Ford River. Since many benthic invertebrates are generally associated with snag habitats (Dudley and Anderson 1982), the distribution and abundance of snags along a stream's course may govern species distributions. Schwartz Creek is typical of headwater streams in the study area, with snags and overhanging vegetation providing the only fixed substrates. Snags are less abundant in downstream reaches as the stream channels widen. Similar snag distributions have been reported in other North American streams (see Wallace and Benke 1984). This pattern of habitat distribution has several implications for the River Continuum Concept (Vannote et al. 1980): First, headwater invertebrate communities may be based on heterotrophy because of ingestion of substrate material, not because of reduced primary productivity as suggested by the Concept. Second, the absence of "specialized" grazers (e.g. *Glossosoma*) in headwaters may only be a result of the unsuitability of snag habitats for their existence (e.g. absence of proper case building materials for *Glossosoma*). Third, utilization of periphyton by species associated with snags may be inefficient, shifting the resource base of the community farther towards heterotrophy than suggested by resource availability. While the River Continuum Concept may be generally accurate, the distribution of habitats along a stream's course must also be considered.

CHAPTER 2

THE INFLUENCE OF DIET ON THE GROWTH OF *STENONEMA VICARIUM* (WALKER)

INTRODUCTION

Food quality is often cited as having pronounced effects on growth and other life history characteristics of aquatic invertebrates in the laboratory (Willoughby and Sutcliffe 1976; Anderson and Cummins 1979; Cianciara 1980; Fuller and Mackay 1981; Sutcliffe et al. 1981; Bird and Kaushik 1984; Sweeney and Vannote 1984). However, the effect of food on growth rates in natural populations is still uncertain, since temperature and other factors are also involved (Sweeney 1984). Separation of the effects of temperature and food is especially important in the context of the River Continuum Concept (Vannote et al. 1980) because longitudinal changes in shading often result in a positive correlation between primary productivity and temperature in many Northern Temperate streams. Thus, the effects of resource availability and temperature may be confounded with respect to stream order (Anderson and Cummins 1979). Unequivocal separation of the effects of these two factors may be possible only in controlled laboratory experiments (Sweeney and Vannote 1984).

Living food resources, such as animal tissue, algae, and fungi, are generally superior to detritus in nutritional quality (Lamberti and Moore 1984). For example, carbon to nitrogen (C:N) ratios for periphyton range between 3.7:1 to 7.8:1 (McMahon et al. 1974; McCullough et al. 1979), indicating a high protein content. In contrast, C:N ratios for detritus may range as high as 1340:1, reflecting a high content of cellulose, lignin, and other refractory substances (Cummins and Klug 1979; Lamberti and Moore 1984). Assimilation efficiencies for algal diets range between 30-60% (Trama 1972; McCullough et al. 1979), whereas efficiencies on detrital diets generally fall between 7-25% (summarized in Berrie 1976). Detritivores may compensate for poor food quality by increasing ingestion rates (Cummins and Klug 1979), ingesting small quantities of animal tissue (Hanson et al. 1983), or by utilizing byproducts of microbial metabolism (Lawson et al. 1984).

Growth rates produced by various food resources have often been used to assess their relative nutritional quality (Willoughby and Sutcliffe 1976; Cianciara 1980; Fuller and Mackay 1981; Sutcliffe et al. 1981; Bird and Kaushik 1984; Sweeney and Vannote 1984). Only 4 studies (McCullough et al. 1979; Cianciara 1980; Bird and Kaushik 1984; Sweeney and Vannote 1984; the latter 2 published since this experiment was conducted) have directly addressed the connection between diet and growth in mayflies. A fourth (Trama 1972) described the bioenergetics of <u>Stenonema pulchellum</u> (Heptageniidae) feeding on diatoms, but did not compare this information with other diets.

This experiment was designed to investigate the role of algae in the growth of <u>Stenonema vicarium</u> (Walker), a common heptageniid mayfly in streams of the Great Lakes region (Flowers and Hilsenhoff 1978; Bednarik and McCafferty 1979). The objective of this study was to determine the relative efficiency of utilization of natural stream periphyton vs. detritus by S. vicarium in terms of its growth rate on each food resource. The specific hypothesis was that growth would be greater on a diet that includes algae than on a diet of leaf detritus.

MATERIALS AND METHODS

The experiment was conducted in 2 thermally controlled artificial stream channels (Frigid Units, Toledo, OH). In each of 3 runs, one channel was manipulated to simulate autotrophic conditions (AUT), while the second was manipulated to simulate heterotrophic conditions (HET). Treatments were randomly assigned to channels for Run 1, switched for Run 2, and randomly assigned for Run 3, so that treatment effects could be separated from channel effects (e.g. residual effects from previous usage of the stream channels). At the beginning of each run, both channels were filled with 160 l of water from the Red Cedar River (RCR), Meridian Township, Ingham Co., MI. Approximately 1/3 of the water in each channel was replaced every 4 d to prevent nutrient limitation. Each channel was provided with 15 plastic cages (16 x 16 x 10 cm, 1 mm mesh screened sides) for individual growth chambers. Both channels were run at 11°C (± 1°C) under a 12:12 hr photoperiod during all runs. Channels were thoroughly drained and cleaned between runs.

Run 1 began on Sept. 29, 1984, Run 2 on Oct. 17, and Run 3 on Nov. 8.

Treatments were conducted as follows: Natural periphyton growing on stones was provided for food in AUT. Stones of about 100 cm^2 upper surface area were collected from Sloane Creek, a tributary of RCR. Stones were replaced every 4 d to prevent food limitation. Residual periphyton on the stone surfaces at each change indicated that food limitation was not a factor in AUT. Care was taken to remove all macroinvertebrates from the stones prior to use in AUT. Lighting over AUT was augmented by flourescent "grow lights", also set at a 12:12 photoperiod, and suspended 40 cm over the water's surface. Dried, autumn scenescent White Ash (*Fraxinus americana*) leaves were provided for food in HET. Leaves were conditioned in the dark for 14 d prior to each run in aerated RCR water at 18° C. To stimulate fungal and bacterial growth on the leaf surfaces, the culture water was supplemented with 25 g KH_2PO_4, 6.5 g NaCl, 18 g $MgSO_4$, 3 g CaCl 2(H_2O), and 37 g KNO_3 (total volume = 37 l; Lawson et al. 1984). Fungi, bacteria, and protozoa were observed on leaf surfaces cultured in this manner; however, algae was never observed. Each growth chamber was provided with approximately 20 entire leaves, and a stone of approximately the same size as those provided in AUT cages. These stones were collected from a gravel pit and washed prior to their use in each run. Lack of extensive skeletonizing of leaf surfaces at the end of each run indicated that food limitation did not occur in HET. All water used in HET was filtered through compressed glass wool to remove algae.

Stenonema vicarium nymphs were collected from Sloane Creek on the day preceding the start of each run, and kept without food overnight in the dark at 10°C. At the start of each run, 30 nymphs were blotted dry on tissue paper for 5 s, then weighed to the nearest 0.1 mg on a Sartorius 1207 MP2 electrobalance. Nymphs were then randomly assigned to cages within each treatment. At the end of 14 days, nymphs were removed from their cages, reweighed, and preserved in 70% ethanol. Exuviae in each cage were also preserved as secondary evidence of growth.

Instantaneous growth rate (% wet weight/d) was calculated for each nymph as per Sutcliffe et al. (1981):

$$G = \lfloor \ln(W_e/W_b)/t \rfloor \times 100\% \tag{1}$$

where W_e = wet weight at the end of the run, W_b = wet weight at the beginning of the run, and t = elapsed time in days.

Growth rate estimates were $\log_{10}$ transformed to correct for heterogeneous variance (Cochran's C; $P = 0.053$), and analysed using the multivariate model:

$$G_{ijkl} = \mu + C_i + D_j + F_k + DF_{jk} + e_{ijkl} \tag{2}$$

where: G_{ijkl} = an individual growth rate
μ = overall mean growth rate
C_i = fixed effect of stream channel i ($i = 1,2$)
D_j = fixed effect of starting date j ($j = 1,2,3$)
F_k = fixed effect of food source k ($k = 1,2$)

DF_{jk} = interaction between starting date and food

e_{ijkl} = random individual error

Since preliminary analysis indicated that channel effects were not significant ($P > .50$), C_i was deleted from model (2) to eliminate confounding of the interaction term (i.e. each channel received only one treatment during each run). Thus, the model was simplified to:

$$G_{jkl} = \mu + D_j + F_k + DF_{jk} + e_{jkl} \qquad (3)$$

where all terms are defined as in (2).

To confirm that the treatments produced the desired nymphal diets, midgut contents of 3 or 4 nymphs in each treatment group were dissected and mounted on microscope slides using the method of Cummins (1973). Approximate proportions of diatoms and detritus were determined by taking a line transect across each slide using a phase contrast microscope at 400X. The proportion of each particle type was estimated as the total number of ocular micrometer units in 30 fields (300 µm each field) intersected by each particle type, divided by the total micrometer units for both particle types.

RESULTS

Mortality was very low or nonexistent in all treatment groups, with only one death each in AUT Runs 1 and 2, and HET Run 1. During Run 3, about 1/2 of the nymphs in each treatment did not molt or grow significantly (Figure 2.1). All other nymphs molted at least once during the experiments. Non-molting individuals were excluded from all statistical analyses.

The overall effect of diet on growth rates was highly significant ($P< 0.01$,Table 2.1),with nymphs in AUT growing an average of 0.22 %/d faster than those in HET (Figure 2.1). However, only Run 1 was significant when treatment means were compared within each run ($P = 0.001$, 0.14, 0.09 for Runs 1, 2, and 3 respectively; orthogonal contrasts). The starting date of each run also had a highly significant effect ($P < 0.01$; Table 2.1), with growth rates generally decreasing over the course of the 3 runs (Figure 2.1). The overall effect of interaction was not significant ($P = 0.31$; Table 2.1), although interaction approached significance ($P = 0.06$) between Runs 1 and 2.

Inspection of midgut contents of nymphs in each treatment group verified that nymphs in HET did not ingest diatoms (Table 2.2). Guts of HET nymphs contained primarily amorphous organic material with fragments of fungal hyphae and mineral particles. Guts of AUT nymphs contained diatoms, mineral particles, and amorphous organic material.

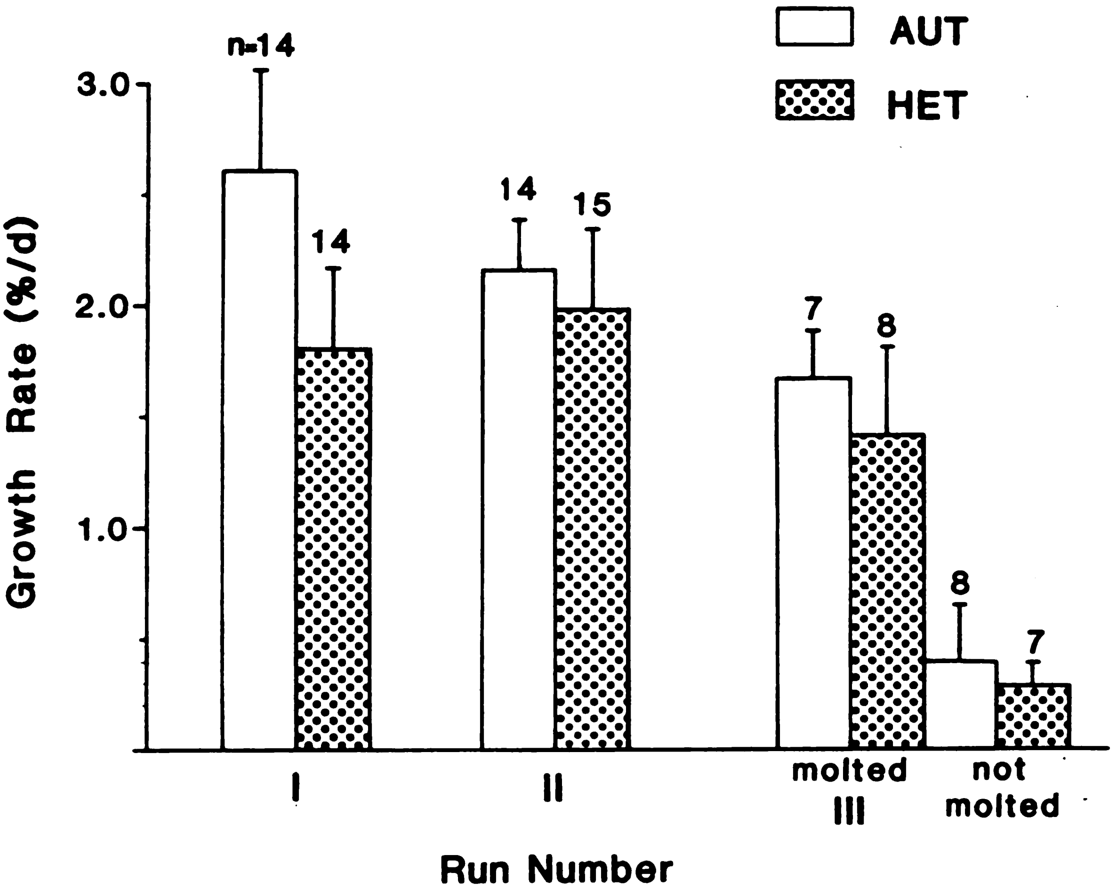

Figure 2.1. Mean growth rate (± 95% CI) of each treatment group.

Table 2.1. MANOVA table for $\log_{10}$-transformed growth rate data.

SOURCE OF VARIATION	SUM SQUARES	df	MEAN SQUARE	F	P
Constant	5.67154	1	5.67154	338.92681	
Date	.21993	2	.10997	6.57156	.002
Food	.18133	1	.18133	10.83605	.002
Interaction	.03993	2	.01997	1.19323	.310
Error	1.10443	66	.01673		
TOTAL	7.21716	72			

The proportion of diatoms in gut contents of AUT Run 3 nymphs was much higher than in previous runs (Table 2.2). Diatoms ingested by AUT nymphs were primarily *Pinnularia*, *Cocconeis*, *Gomphonema*, and *Navicula*.

DISCUSSION

Although the overall effect of diet was highly significant, the results only partially support the initial hypothesis that *S. vicarium* grows better on a diet of periphyton than on leaf detritus. Lack of significant differences in growth rates on the 2 diets during Runs 1 and 2 could have been caused by: 1) contamination by algae in HET Runs 2 and 3, but examination of gut contents (Table 2.2) indicates that this was not the case; 2) changes in the nutritional quality of either of the diets over the course of the experiment; or 3) a greater consumption rate by HET nymphs. Cummins and Klug (1979) suggested that aquatic consumers may increase their consumption rate to compensate for poor food quality, although this parameter was not considered in the present study.

Since periphyton was collected for AUT during a period of rapid seasonal changes in Sloane Creek, it is possible that changes in nutritional quality occurred over the course of the experiment. Qualitative changes in periphyton during autumn, such as an increase in C:N ratio (McMahon et al.

Table 2.2. Feeding habits of S. vicarium nymphs in each treatment group.

			PERCENTAGE IN GUT	
Treatment	Run	n	Diatoms	Detritus
AUT	1	4	33	67
	2	3	28	72
	3	3	76	24
HET	1	4	0	100
	2	4	0	100
	3	3	0	100

Table 2.3. Correlation between individual weight and growth rate in each treatment group.

	Starting weight (mg. wet wt.)				
	Run	x + sd	range	r*	P
AUT	1	15.0 ± 6.0	6.8 - 30.3	-.520	0.06
	2	17.2 ± 8.1	7.9 - 31.9	-.216	0.46
	3	30.2 ± 12.3	10.6 - 51.5	-.282	0.54
HET	1	12.8 ± 5.3	4.1 - 22.7	-.436	0.12
	2	17.9 ± 10.4	4.8 - 37.2	-.596	0.02
	3	26.7 ± 8.2	17.3 - 42.9	-.603	0.11

* Correlation between individual starting weight and growth rate, and significance of r (t-tests).

1974) and decreasing fatty acid content (Moore 1975), could have an adverse effect on consumer growth. In addition, Hornick et al. (1981) showed that an autumnal decline in periphyton productivity in an Appalachian Mountain stream was most highly correlated with changes in lighting, flow, and dissolved organic inputs. In contrast to the susceptability of AUT to seasonal changes, food for HET was prepared in the laboratory under identical conditions, and thus was homogeneous across all 3 runs. Thus, over the course of the 3 runs, the nutritional quality of the periphyton may have decreased relative to that of the leaves.

The greater ingestion of diatoms by Run 3 AUT nymphs may also reflect seasonal changes in the periphyton community in Sloane Creek. Clumps of the filamentous alga *Cladophora* partially covered rocks used in AUT Run 1, but were less prevalent during Run 2 and absent during Run 3. Although *Cladophora* fragments were never observed in gut contents, these clumps may have entrained detritus or hampered the nymphs' ability to scrape diatoms from the rock surfaces.

While the general downward trend in growth rates over the course of the 3 runs could have been caused in part by the increase in starting weight of nymphs used in successive runs, the correlation between individual biomass and growth rate was not significant in most treatment groups (Table 2.3). Since treatment conditions were identical throughout the experiment, factors external to the experimental conditions, i.e. preconditioning of the nymphs, may have had a significant impact on growth rates. This is supported by the fact that many of the Run 3 nymphs in both treatments failed to molt (Figure 2.1). Several workers have concluded that growth of Stenonema spp. slows or stops during winter months (Richardson and Tarter 1976; Barton 1980; Kreuger and Cook 1984; Chapter 1 of the present study). Thus, the seasonal pattern of growth rates observed in the laboratory paralleled that commonly observed in the field. This suggests that growth of S. vicarium is not controlled entirely by the direct effects of temperature, diet, etc. on metabolism, but may also be controlled endogenously using an environmental timing cue (e.g. temperature or photoperiod). Such mechanisms for the timing of life history events have been demonstrated in terrestrial insects (Ricklefs 1973; Chapman 1982), but unfortunately have never been examined in aquatic insects (Sweeney 1984). In view of the low Net Production Efficiency (NPE = Growth/ Assimilation) reported for Stenonema pulchellum (Trama 1972), slower growth during winter may be necessary for survival of the members of this genus. The metabolism of Stenonema may be so low during winter that virtually all assimilated energy must be channelled into maintenance.

Table 2.4 compares the methods and results of this study with those of other studies that compare growth of aquatic insects on diatoms vs. leaf detritus (Fuller and Mackay 1981; Bird and Kaushik 1984; Sweeney and Vannote 1984). In all of these studies, growth rates were greater

Table 2.4. Comparison of methods and results.

Species	T(°C)	Leaf Species	Algal Source	Growth rate (%/d) Leaves	Growth rate (%/d) Algae	% increase*
Chloeon dipterum[1]	25	Hickory	cultured (diatoms)	23.8	27.1	14
(Baetidae)	10			NS	4.7	
Ephemerella subvaria[2] (Ephemerellidae)	15	Maple	natural (periphyton)	1.3	5.6	321
Stenonema vicarium[3] (Heptageniidae)	11	Ash	natural (periphyton)	1.8	2.1	12
Hydropsyche spp.[4]	11	Red Maple	natural (diatom mat)	0.2	1.0	400

1 - Sweeney and Vannote (1984); NS = no survivors.

2 - Bird and Kaushik (1984); second study.

3 - present study; Growth rates are means of all 3 runs.

4 - Fuller and Mackay (1981)

* - % increase = % improvement in growth rate on algae vs. leaves.

on diatoms than on leaves, but the observed improvement in growth differs markedly between studies (Table 2.4; % increase). These discrepancies in results may be explained by several differences in experimental methods. For example, Bird and Kaushik (1984) may have imposed food limitation in their leaf treatment, since only 15 leaf discs (size and species not given) were provided to groups of 10 "early instar" mayfly nymphs every 4 days. Leaf rations were much greater in all other studies. Grafius and Anderson (1980) showed that <u>Lepidostoma unicolor</u> larvae increase their consumption and growth rates as food availability increases. Therefore, in experiments of this type it is prudent to supply all food resources in excess to remove the confounding effects of limitation; i.e. food resources are best compared by the maximum possible growth rates they produce. Food limitation does not appear to have been a problem in any of the other studies, since consumption never exceeded food supply.

The sources of both algal and detrital food resources may also partially explain the differences in results. The ash leaves used in this study were probably of much higher nutritional quality than leaves used in the other studies (Kaushik and Hynes 1971; Peterson and Cummins 1974). McCullough et al. (1979) found that the assimilation efficiency of <u>Tricorythodes minutus</u> is greater on pure diatom cultures (approximated by Fuller and Mackay 1981, and Sweeney and Vannote 1984) than on mixed cultures (as in Bird and Kaushik

1984, and this study).

Unfortunately, these variations in experimental technique obscure interspecific differences in resource utilization efficiency. Assessment of such interspecific differences may lead to clearer definition of the importance of various food resources to stream ecosystems. While measurements of assimilation efficiency or protein content may be more accurate (subject to less variance), the influence of diet on growth (and its consequent effects on other life history features) is ultimately most important to the individual, and consequently to the population and community. However, it is still unclear how minute variations in diet influence growth in natural populations. Growth experiments to date generally use diets that are highly artificial, since food resources rarely occur in isolation in nature (e.g. diatoms colonize leaf surfaces). Further refinement of techniques may produce results that are more comparable to natural situations.

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