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The influence of diet on the growth of *Stenonema vicarium* (Walker) (Ephemeroptera: Heptageniidae)

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Abstract

Laboratory studies compared the growth rate of *Stenonema vicarium* (Walker) nymphs on diets of detritus and natural stream periphyton. In three consecutive runs of the experiment, growth rates were consistently higher on periphyton (mean growth rate = 2.1% wet wt. d^{-1}) than detritus (mean = 1.8% wet wt. d^{-1}). The starting date of each run also significantly influenced growth rates. In each treatment growth rates generally decreased over the course of the 3 runs, and ca. one-half of the nymphs in the last run did not molt or grow. It appeared that growth of *S. vicarium* may be partially controlled by seasonal factors.

Introduction

Food and temperature are among the most influential factors in the life history characteristics of aquatic invertebrates (Sweeney, 1984). Of these, temperature is usually indicated as having the most pronounced effects on growth rates (e.g., Sutcliffe *et al.*, 1981; Merritt *et al.*, 1984; Vannote & Sweeney, 1980, 1986). Food quantity and quality may also play an important role in determining growth rates and other life history parameters (Anderson & Cummins, 1979; Cummins & Klug, 1979; Sweeney & Vannote, 1984). Unfortunately, temperature and food resource availability often covary in natural situations (e.g., changes in riparian vegetation may influence temperature regimes, primary productivity, and detrital inputs). Thus, in the context of the River Continuum Concept (Vannote *et al.*, 1980), it is difficult to determine whether longitudinal differences in growth rates (and productivity) of a given species are primarily attributable to temperature or food (e.g., Hawkins, 1986). Therefore, separation of the effects of these two factors may be possible only under controlled laboratory conditions (Sweeney, 1984; c.f., Sweeney & Vannote, 1986).

This study examined the effect of diet on the growth of nymphs of *Stenonema vicarium* (Walker) (Heptageniidae) under constant temperature conditions in the laboratory. The objective was to determine the relative utilization efficiency of natural stream periphyton vs. leaf detritus by *S. vicarium* nymphs in terms of their growth rate on each food resource. Our specific hypothesis was that growth would be greater on a diet that includes algae than on a diet of leaf detritus because:

1. algae is generally considered to be a superior food resource (Lamberti & Moore, 1984); and/or;
2. members of the Heptageniidae are generally

considered to be grazers or scrapers, and therefore may be "specialized" for feeding on periphyton (Cummins, 1973; Merritt & Cummins, 1984).

Materials and methods

The experiment was conducted in two thermally controlled artificial stream channels (Frigid Units® , Toledo, OH). In each of three runs, *S. vicarium* nymphs in one channel were provided with natural stream periphyton (ALG), while nymphs in the second channel were provided with leaf detritus devoid of algae (DET). 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, 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 × 16 × 10 cm, 1 mm mesh screened sides) for individual growth chambers. Both channels were run at 11 °C (± 1 °C) under a 12:12 h photoperiod during all runs. Channels were thoroughly drained and cleaned between runs. Run 1 began on 29 Sept. 1984, Run 2 on 17 Oct., and Run 3 on 8 Nov.

Treatments were conducted as follows. Natural periphyton growing on stones was provided for food in ALG. Stones of ca. 100 cm^2 upper surface area were collected from Sloane Creek, a tributary of the Red Cedar River. Stones were replaced every 4 d to prevent food limitation. Care was taken to remove all macroinvertebrates from the stones prior to use. Lighting over ALG was augmented by fluorescent "grow light", also set at a 12:12 photoperiod, and suspended 40 cm over the water's surface. Dried, autumn scenescent White Ash (*Fraxinus americana* L.) leaves were provided for food in DET. Leaves were conditioned in the dark for 14 d prior to each run in aerated Red Cedar River 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 during microscopic examination of leaf surface scrapings 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 ALG cages. These stones were collected from a gravel pit and washed prior to their use in each run. Food was provided in excess in both treatments to avoid the effects of food limitation. All water used in DET 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 ranging in size from 3.0–9.0 mm were blotted dry on tissue paper for 5 sec, then weighed to the nearest 0.1 mg on a Sartorius® 1207 MP2 electrobalance. Nymphs were then randomly assigned to cages within each treatment (1 ind./cage). 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^{-1}) was calculated for each nymph as per Sutcliffe *et al.* (1981):

$$G = [\ln(W_e/W_b)/t] \times 100\%$$

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:

$$G_{jkl} = \mu + D_j + F_k + DF_{jk} + e_{jkl}$$

where:

G_{jkl} = an individual growth rate
μ = overall mean growth rate
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_{jkl} = random individual error

Since preliminary analysis indicated that channel effects were not significant (P »0.05), this factor was excluded from the above model.

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). Midgut contents were used because the foregut of *S. vicarium* is not large enough to be sampled by this method. 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

Only three nymphs died during the course of the experiments. During Run 3, ca. 1/2 of the nymphs in each treatment did not molt or grow substantially (Fig. 1). All other nymphs molted at least once during the experiments. Non-molting individuals were treated separately in the statistical analyses.

The overall effect of diet on growth rates was highly significant (P < 0.01, Table 1), with nymphs in ALG growing an average of 0.22% $^{-1}$d faster than those in DET (Fig. 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 1), with growth rates generally decreasing over the course of the 3 runs (Fig. 1). The overall effect of interaction was not significant (P = 0.31; Table 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 DET did not ingest diatoms (Table 2). Guts of DET nymphs contained primarily amorphous organic material with fragments of fungal hyphae and mineral particles. Guts of ALG nymphs contained diatoms, mineral particles, and amorphous organic material. The proportion of diatoms in gut contents of ALG Run 3 nymphs was much higher than in previous

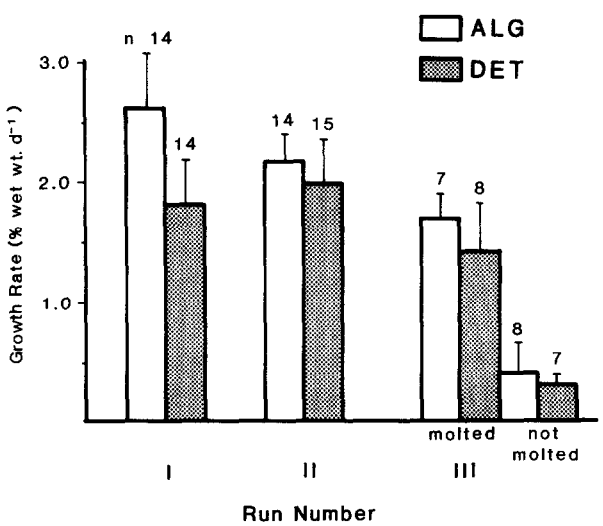

Fig. 1. Mean growth rate (±95% Cl) of each treatment group. Number at the top of each bar is the number of individuals included in each treatment group mean.

Table 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	0.21993	2	0.10997	6.57156	0.002
Food	0.18133	1	0.18133	10.83605	0.002
Interaction	0.03993	2	0.01997	1.19323	0.310
Error	1.10443	66	0.01673		
Total	7.21716	72			

Table 2. Feeding habits of *S. vicarium* nymphs in each treatment group.

Treatment	Run	n	Percentage in gut	
			Diatoms	Detritus
ALG	1	4	33	67
	2	3	28	72
	3	3	76	24
DET	1	4	0	100
	2	4	0	100
	3	3	0	100

runs (Table 2). Diatoms ingested by ALG 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 two diets during Runs 2 and 3 could have been caused by:

1. contamination by algae in DET Runs 2 and 3 however, examination of gut contents (Table 2) indicated that this was not responsible;
2. changes in the nutritional quality of either diet over the course of the experiment; or
3. a greater consumption rate by DET nymphs. Cummins & 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 ALG 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.*, 1974) and decreasing fatty acid content (Moore, 1975), could have had an adverse effect on consumer growth (Hanson *et al.*, 1983; Cargill *et al.*, 1985). 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 susceptibility of ALG to seasonal changes, food for DET was prepared in the laboratory under identical conditions, and therefore 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 ALG nymphs may also reflect seasonal changes in the periphyton community in Sloane Creek, the source of food for ALG nymphs. Clumps of the filamentous alga *Cladophora* partially covered rocks used in ALG 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 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 initial weights of nymphs used in successive runs, the correlation between individual biomass and growth rate was not significant in most treatment groups. 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 (Fig. 1). Several workers have concluded that growth of *Stenomena* spp. slows or stops during winter months (Richardson & Tarter, 1976; Barton, 1980; Kreuger & Cook, 1984; K. M. Webb, unpubl. data). 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 not been as carefully examined in aquatic insects (Sweeney, 1984). In view of the rather low net production efficiency (NPE = Growth/Assimilation) reported for *Stenonema pulchellum* (Trama, 1972), slower growth during winter may be necessary for survival of members of this genus. The metabolism of *Stenonema* may be so low during winter that a significant portion of assimilated energy must be channeled into maintenance.

Table 3 compares the methods and results of this study with those of other published studies comparing growth of aquatic insects on diatoms vs. leaf detritus (Cummins *et al.*, 1973; Fuller & Mackay, 1981; Bird & Kaushik, 1984; Sweeney & Vannote, 1984). In all of these studies, growth rates were greater on diatoms than on leaves, but the observed improvement in growth differs markedly between studies (Table 3; % increase). These discrepancies in results may be explained by differences in experimental methods. For example, Bird & 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 ratios were much greater in all other studies. Grafius & Anderson (1980) showed that *Lepidostoma unicolor* 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. If feeding is selective to any degree, then true food availability can only be measured by the food being selected.

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 & Hynes, 1971; Peterson & Cummins, 1974). McCullough *et al.* (1979) found that the assimilation efficiency of *Tricorythodes minutus* was greater on pure diatom cultures (approximated by Fuller & Mackay, 1981; Sweeney & Vannote, 1984) than on mixed cultures (as in Bird & Kaushik, 1984, and this study).

Unfortunately, these variations in experimental technique obscure interspecific differences in resource utilization efficiency. Assessment of such in-

Table 3. Comparison of methods and results with other published feeding studies.

Species	T (°C)	Leaf species	Algal source	Growth rate (% body wt d^{-1})		% increase*
				leaves	algae	
Chloeon dipterum[1] (Baetidae)	25 10	hickory	cultured (diatoms)	23.8 NS[1]	27.1 4.7	14
Ephemerella subvaria[2] (Ephemerellidae)	15	maple	natural (periphyton)	1.3	5.6	321
Stenonema interpunctatum[3] (& *S. canadense* (Heptageniidae))	5	hickory	cultured (*Ankistrodesmus* sp.)	0.1 – 0.8	not given	-
Stenonema vicarium[4] (Heptageniidae)	11	ash	natural (periphyton)	1.8	2.1	12
Hydropsyche spp.[5] (Hydropsychidae)	11	red maple	natural (diatom mat)	0.2	1.0	400

[1] Sweeney & Vannote (1984); NS = no survivors.
[2] Bird & Kaushik (1984); second study.
[3] Cummins *et al.* (1973).
[4] Present study; Growth rates are means of all 3 runs.
[5] Fuller & Mackay (1981).
* % increase = % improvement in growth rate on algae vs. leaves.

terspecific differences may lead to a clearer definition of the importance of various food resources to stream ecosystems. While measurements of assimilation efficiency, protein or lipid content may be more accurate (subject to less variance), the influence of diet on growth and other life history features is ultimately most important to the individual, and consequently to the population and community. However, one drawback to experiments of this type is that the "acceptability" of a resource influences growth rates as well as digestability (Ward & Cummins, 1979). Furthermore, 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 in techniques may produce results that are more comparable to natural situations.

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