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Biomass Dynamics of *Tipula* (Insecta:Diptera) in Forested Streams of the Interior Highlands, Arkansas

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Abstract.—Abundance patterns of aquatic macroinvertebrates that utilize coarse particulate organic matter as a food source are important indicators of non-point source pollution associated with silviculture activities. This group, referred to as shredders, typically decreases in abundance as its food source—primarily leaves—is removed from the ecosystem. We tested whether larval biomass of the crane fly *Tipula*, a common member of the group, was an effective estimator of shredder abundance. Additionally, we used regression analysis to test whether the length to dry mass relationship of *Tipula* differed among geographic regions, between seasons, and between years. Results did not indicate significant differences in the relationship among these variables. Thus, we concluded that a general length to dry mass relationship was appropriate for *Tipula* in streams of the Interior Highlands. Our results were similar to those reported from North Carolina and Virginia. *Tipula* biomass was positively correlated with the total richness of the macroinvertebrate assemblage, a common measurement of stream quality, but was not correlated with the numerical abundance of the shredder functional feeding group. Thus, we concluded that *Tipula* biomass would not be an effective surrogate for whole assemblage metrics in biological assessments.

Key words:— aquatic macroinvertebrates, non-point source pollution, silviculture, *Tipula,* Interior Highlands, Arkansas.

Introduction

Silviculture is a major land use in Arkansas; over 55% of the state's land cover is commercial forest (AFC 2002). In Arkansas as in other states, biological assessments are performed to examine the impacts of non-point source (NPS) pollution, and one of the potential sources of NPS pollution is silviculture activity. Biological assessments can be effective in testing whether stream impairment is associated with NPS pollution (Rosenberg and Resh 1993). Standard biological assessment requires collecting samples representing the entire invertebrate community of streams in the watershed of interest and comparing characteristics of that community to an unimpaired reference condition. Whole community samples are recommended in order to make this sampling methodology adaptable enough to detect various types of environmental degradation (Barbour et al. 1999). However, these samples require significant investments of money and time to collect and analyze (Davidson and Clem 2002). An assessment procedure that reduces the time and cost spent per sample, but retains the ability to detect environmental impairment, is desirable. For assessments of potential silviculture impacts, focus upon the abundance and biomass dynamics of the shredder functional feeding group may be a solution.

Studies of the effects of forest clearing have frequently noted compositional shifts in aquatic invertebrate communities in streams of the cleared watershed, most often as a reduction in the abundance of the shredder functional feeding group, with corresponding increases in the collector and/or scraper groups (Newbold et al. 1980, Noel et al. 1986, Stone and Wallace 1998). The principal hypothesis of the cause of the shift is that removal of the forest canopy deprives the system of an important energy source—leaf inputs—and results in greater insolation of the stream bottom, stimulating algal growth (Campbell and Doeg 1989).

Important members of the shedder group are *Tipula* spp. (crane flies). This genus has more than 30 species in North America (Byers 1996), and as aquatic larvae, they primarily feed on decomposing plant material and associated microflora (Pritchard 1983). *Tipula* spp. are some of the major shredders, particularly in terms of biomass, in streams of the Interior Highlands (SBM pers. obs.). Most other regional representatives of this functional feeding group are either small taxa (e.g. capniid, leuctrid, and nemourid stoneflies and the chironomid genera *Cricotopus* and *Polypedilum*) or are larger taxa typically found in low numbers (e.g., limnephilid and phryganeid caddisflies; Merritt and Cummins 1996).

Tipula individuals go through four larval instars (Pritchard 1983). Mean length at the end of each instar has been reported for *Tipula sacra* from Alberta, Canada, as follows: first instar—5 mm, second instar—14 mm, third instar—20 mm; fourth instar females reached 50 mm and males reached 30 mm (Pritchard and Hall 1971). Pritchard (1976) reported adult *Tipula* emergence in Alberta through June and July with adults living less than a week. Eggs hatched in a few days, with second instar larvae appearing a few weeks later. They spent about 3 months as a second instar before molting to the third in November and overwintered as third instars. Most individuals molted to the fourth instar in April and May. He found first instar larvae in July through September, and they were the most abundant instar in July. Second instar larvae were present in all months except May and June and were most prominent in August and early September. Third instar larvae were present from August through the following June and were numerically dominant from mid-September to May. Fourth instar larvae were collected in all months and were numerically dominant in June. The typical *Tipula* life cycle is semivoltine with the second year spent as a fourth instar larva (Pritchard 1983). However, Pritchard (1980) hypothesized some cohort splitting may occur in the fall after hatching with most individuals overwintering as third instars, but some others grow rapidly to fourth instar and overwinter in that stage

Researchers addressing ecological questions at various scales recognize invertebrate biomass as an important variable (Benke et al. 1999). However, Rogers et al. (1976) noted that while insect biomass is an important piece of information, direct massing is too time consuming to be practical. Therefore, estimation of biomass is frequently performed using length to mass conversion ratios (Burgherr and Meyer 1997). A common method of performing this conversion is to use regression analysis, typically after log-transformation of raw data, and describe mass as a power function of length (Rogers et al. 1976, Sample et al. 1993, Hodar 1996). Length to mass power functions have been developed for aquatic dipterans (Burgherr and Meyer 1997), for other genera of the family Tipulidae (Meyer 1989), and for *Tipula abdominalis* (Smock 1980, Benke et al. 1999). However, Meyer (1989) questioned whether information from the equations was consistent across different geographical locations. It can be further questioned whether the information is consistent over time, i.e., between seasons and between years.

Tipula spp. are often the major holometabolous shredder in forested, low-order, Interior Highland streams, many of which are temporary streams (Poulton and Stewart 1991, Moulton and Stewart 1996). Holometabolous taxa have been observed to have higher growth ratios than hemimetabolous species (Cole 1980), which may be advantageous for exploiting ephemeral habitats like those prevalent in the Interior Highlands. That is, fast growth may be an adaptive advantage in streams that typically cease flowing for at least a few months every year.

Thus, the purpose of this study was to address the following questions:

1) Do *Tipula* length to dry mass relationships differ among geographically separate streams, between seasons, or between years? Do they differ among larval instars?

2) Do power equations developed for Interior Highland *Tipula* spp. conform to those derived from other sources?

3) Would *Tipula* biomass be useful as a biomonitoring tool for detecting decreased CPOM inputs associated with silviculture?

4) Does seasonal growth for *Tipula* occur faster in temporary streams than in permanent streams?

Methods

Tipula specimens were collected from 5 stream locations in the Ouachita and Ozark highlands in Arkansas from January 2003 through March 2004 using a 23-cm x 46-cm long-handled kick net. Characteristics of the study streams are summarized

Table 1. Characteristics of the 5 study streams. At Caney Creek, 2 sampling stations were on a first order segment of the stream and 1 was on a second order segment. At Thompson Creek, 2 stations were permanently flowing and 1 was on a segment that ceased flowing.

Stream	Ecoregion (subregion)	Order	Flow permanence	Drainage Area (km^2)	Gradient (m/km)
Bailey Creek	Ozark Highlands (Springfield Plateau)	2	Temporary	22.3	10.1
Big Creek	Arkansas Valley --	4	Permanent	89.5	4.3
Caney Creek	Ouachita Highlands (Fourche Mountains)	1/2	Temporary	6.8	16.7
Harris Creek	Ouachita Highlands (Central Ouachitas)	3	Permanent	24.2	12.2
Thompson Creek	Ozark Highlands (Boston Mountains)	2	Permanent, but spring-influenced	10.3	15.8

in Table 1. Sample substrates were predominantly cobble and gravel. At each location, we performed surveys at 3 separate stations, whose lengths of 100 to 200 meters were approximately 20x the average stream width. Collections were made in the early winter and early spring of each year. We collected 3 replicates at each site, for a total of 9 samples (3 replicates x 3 stations) at each stream during each survey period. Samples were preserved in the field with 5 % formaldehyde and transported to the Arkansas State University aquatic biomonitoring laboratory for analysis.

Tipula larvae were identified using Byers (1996) and separated from debris and other invertebrates and transferred to 80% ethanol within one month of collection. Length and mass measurements were taken approximately five months later. It is likely that *Tipula* specimens lost a portion of their dry mass due to leaching (e.g., Howmiller 1972, Landahl and Nagell 1978, Leuven et al. 1985). Even so, this method of preservation, as opposed to live collecting or freezing, is commonly used by entomologists (Rogers et al. 1976, Hodar 1996). Measurements of total length were made with Mecanic Type 6911 calipers, which are accurate to 0.1 mm. Specimens that were obviously contracted from their normal length were infrequently noted (<<1 % of the total individuals measured) and were included in the analysis. Dry mass (DM) was obtained by drying the animals at ~105°C for 16 to 24 hours then massing them on a Mettler-Toledo AB204-S balance accurate to 0.1 mg.

We transformed length and dry mass to $\log_{10}$ values and used linear regression to determine the y-intercept and slope of the line of best fit. We initially performed separate regressions on data from each stream site (n = 5) from each survey season (n = 2) in each year (n = 2). Stations and replicates were pooled within these data sets. We examined the average y-intercepts and slopes and their 90 % confidence intervals, using a Bonferroni adjustment to protect the family of estimates from error inflation. If overlap occurred between confidence intervals, we concluded that the y-intercept and slope coefficients did not differ significantly. Potential experimental outcomes ranged between 20 separate regression lines, if the length to dry mass relationship differed between seasons, years, and between each study stream and 1 line, if the relationship did not differ between season, year, or stream.

Table 2. Mean values (± 1 SE) of y-intercepts and slopes from length vs. mass regressions for samples from all study streams in 2 seasons of 2 years. Regressions used log10-transformed data for total length (mm) and dry mass (mg).

Stream	Year	Season	Y-intercept	Slope	n	r^2
Bailey Creek	2003	Winter	-2.58 ± 0.10	2.57 ± 0.08	172	0.87
		Spring	-2.16 ± 0.11	2.34 ± 0.08	131	0.88
	2004	Winter	-2.76 ± 0.42	2.68 ± 0.55	26	0.50
		Spring	-1.69 ± 0.27	2.04 ± 0.19	38	0.76
Big Creek	2003	Winter	-2.33 ± 0.16	2.45 ± 0.11	62	0.89
		Spring	-2.16 ± 0.32	2.35 ± 0.21	40	0.77
	2004	Winter	-2.43 ± 0.11	2.51 ± 0.08	93	0.91
		Spring	-1.72 ± 0.59	2.18 ± 0.36	31	0.56
Caney Creek	2003	Winter	-2.46 ± 0.38	2.55 ± 0.32	10	0.89
		Spring	-2.02 ± 0.26	2.28 ± 0.17	54	0.78
	2004	Winter	-2.51 ± 0.09	2.53 ± 0.08	342	0.74
		Spring	-1.54 ± 0.21	2.01 ± 0.14	69	0.75
Harris Creek	2003	Winter	-2.36 ± 0.23	2.42 ± 0.17	36	0.85
		Spring	-2.43 ± 0.34	2.51 ± 0.21	24	0.86
	2004	Winter	-2.79 ± 0.17	2.80 ± 0.13	69	0.88
		Spring	-1.93 ± 0.25	2.23 ± 0.16	46	0.81
Thompson Creek	2003	Winter	-2.20 ± 0.26	2.20 ± 0.23	28	0.78
		Spring	-1.56 ± 0.20	1.84 ± 0.15	27	0.86
	2004	Winter	-3.33 ± 0.20	3.23 ± 0.18	99	0.78
		Spring	-1.77 ± 0.22	2.08 ± 0.15	52	0.80

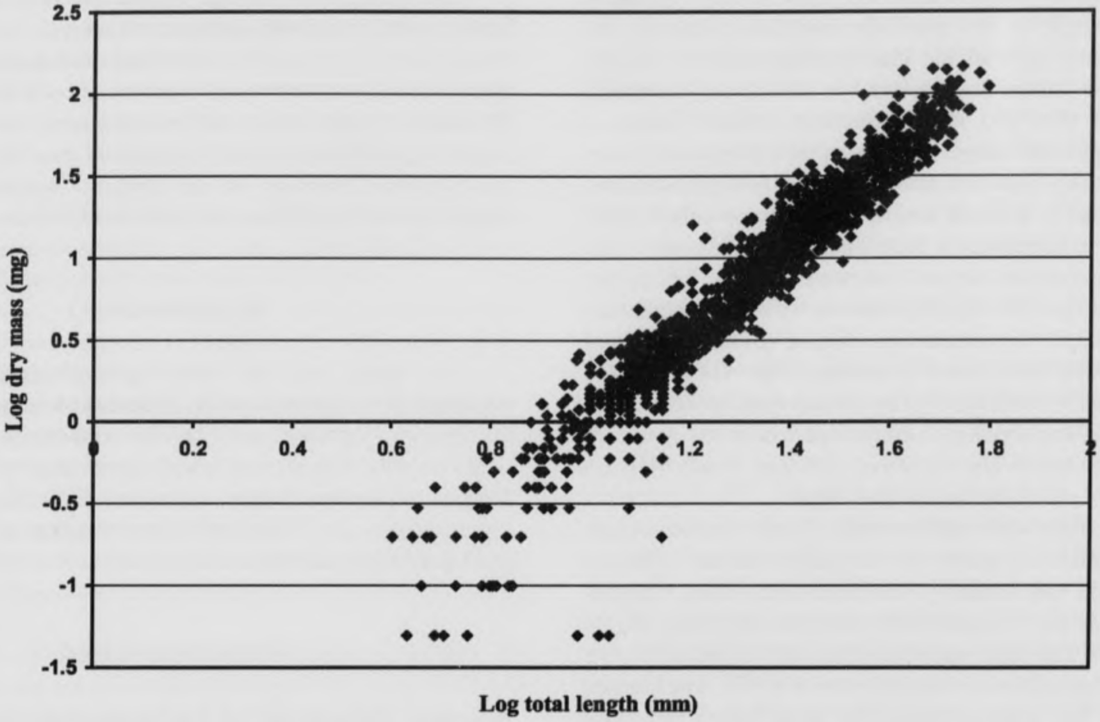

Fig. 1. Log dry mass vs. log length regression line for *Tipula* from 5 Interior Highlands study streams, winter and spring, 2003 – 2004.

Similarly, we used regression analysis to examine whether the length to dry mass relationship differed among the four larval instars. We separated data by total length, but pooled study streams, seasons, and years, using a modification of the instar length ranges reported by Pritchard and Hall (1971). We used 7.5 mm as the boundary between first and second instars and 25 mm as the boundary between third and fourth instars because these were the natural divisions indicated by a length vs. frequency histogram of our data.

If it was determined that annual, seasonal, and geographical differences were not significant, we pooled our data and compared our general length to dry mass relationship to those obtained for aquatic dipterans in other studies by converting the linear regression equation into a power function:

$$DM = aL^{b}$$

where DM is the dry mass of the organism (in mg), L is the total length (mm) and a and b are constants. The exponent b of the power model is the mean slope obtained in the linear regression, and a is the mean antilogarithm of the y-intercept.

We used correlation analysis to examine the relationship between *Tipula* biomass and two invertebrate community characteristics frequently used in biomonitoring studies—total taxa richness and the relative abundance of the shredder functional feeding group (% shredders). Total taxa richness is widely used as a metric in stream quality assessments (e.g., Barbour et al. 1999, OEPA 1987, ADEQ 2002). We chose % shredders as an additional community characteristic since it was expected to be the most sensitive to decreased CPOM inputs as well as being strongly associated with *Tipula* biomass.

We tested whether growth rates differed between *Tipula* collected from temporary streams and those from permanent streams using one-way analysis of variance. Data entered in the analysis were the average lengths for each sample (3 replicates x 3 stations x 2 years) for 4 of the 5 study streams. Bailey Creek and Caney Creek were considered temporary streams, whereas Big Creek and Harris Creek were considered permanent streams using information from 1:24,000 USGS topographic maps. Thompson Creek flowed throughout our study, but it was reported by an adjacent property owner to cease flow in particularly dry years. Since it was difficult to assign Thompson Creek to either treatment group, we excluded it from this analysis.

Results and Discussion

Of the 20 regressions (2 seasons x 2 years x 5 study streams) of *Tipula* total length vs. dry mass, the confidence intervals for the y-intercept and slope of the line overlapped for 17 (Table 2). Two of the 3 exceptions occurred in data from Thompson Creek; although they did not consistently indicate either a steeper or a shallower slope. Since so few regression lines differed significantly between seasons, years, or study streams, we concluded that a general length to dry mass relationship for *Tipula* spp. in the Interior Highlands is appropriate. The relationship for the pooled data is illustrated in Fig. 1. The power equation derived from the log dry mass to log length regression was $DM = 0.002\ L^{2.72}$, which was similar to those reported for *T. abdominalis* from North Carolina ($DM = 0.0015\ L^{2.81}$) and Virginia ($DM = 0.0054\ L^{2.46}$) by Benke et al. (1999). This indicated that the relationship is consistent within the genus, at least in the southeastern United States, and that *Tipula* biomass can be accurately estimated using total length.

Regression lines differed between *Tipula* instars (Table 3), with the shallowest slope for first instar larvae. Second and fourth instars had similar y-intercepts and slopes, whereas third instar larvae had a significantly steeper slope than any of the other instars. The high variability and low r^2 value for first instars likely reflected the low sample size (n = 37). The amount of variation in dry mass explained by total length increased progressively with larger instar larvae; each of the second, third, and fourth instar groups had n > 400.

Tipula biomass was positively correlated with the total richness of the macroinvertebrate assemblage (r = 0.204, *p* = 0.006) but was not correlated to the relative abundance of the shredder functional feeding group (r = 0.021, *p* = 0.779). The latter result suggests that the abundances of other shredder taxa obscure the presence of *Tipula* when the entire assemblage is considered. Also, the significant association with total richness likely reflects the large sample size (n = 180), as the correlation coefficient itself was low. Thus we could not conclude that *Tipula* biomass would be an effective surrogate for whole assemblage metrics in biological assessments. However, there was no impairment gradient in our samples; none indicated even moderate impairment from reference conditions. Therefore, it remains possible that *Tipula* biomass could be an effective indicator of reduced CPOM input into streams.

The increase in average length for *Tipula* did not differ between permanent and temporary streams (F = 0.02, p = 0.886). Mean length increases between winter and spring samples were nearly identical at 16.5 mm for permanent streams and 16.7 mm for temporary streams (n = 54 for each group). Perhaps this is not surprising, as the organisms in this study most likely represented closely related species. In any case, no increased growth rate adaptation in *Tipula* from intermittent streams was evident.

Conclusions

This study indicated that a general length to dry mass relationship is appropriate to estimate biomass for *Tipula* of the Interior Highlands, and that the confidence in the accuracy of the relationship increases with developmental stage. *Tipula* biomass was not strongly associated with whole community characteristics, and therefore does not appear to be an effective indicator of general macroinvertebrate community degradation.

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Table 3. Mean values (± 1 SE) of y-intercepts and slopes from length vs. mass regressions for the four instars of *Tipula* and for pooled data. Regressions used log10-transformed data for total length (mm) and dry mass (mg).

	Y-intercept	Slope	n	r^2
First instar (< 7.5 mm)	-2.08 ± 0.51	1.82 ± 0.66	37	0.17
Second instar (7.6 – 14 mm)	-2.45 ± 0.16	2.47 ± 0.14	413	0.41
Third instar (14.5 – 25 mm)	-3.00 ± 0.12	2.95 ± 0.09	454	0.69
Fourth instar (> 25 mm)	-2.36 ± 0.09	2.48 ± 0.06	545	0.75
All data	-2.72 ± 0.03	2.72 ± 0.02	1449	0.93

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