Variability of Linear Measurements Throughout the Life Cycle of the Mayfly *Leptophlebia cupida* (Say)

(Ephemeroptera : Leptophlebiidae)

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INTRODUCTION

Mayfly nymphs of most species undergo a variable number of molts; instars cannot be determined from head capsule measurements; and, because of environmental factors, increase in size cannot be strictly related to development, larger nymphs sometimes being physiologically younger than smaller nymphs. Pleskot (1962) advocated that mayfly life history data be based on developmental stages instead of or in addition to total length measurements. Developmental stage analysis has been used for mayflies as early as the 19th Century by Vayssiére (1882), utilized successfully in the 1930's by Ide (1935) and Rawlinson (1939), and recently employed by several workers. Still, today, most mayfly life history studies are based on linear measurements. In this respect total length is the most common linear dimension, although occasionally other dimensions are used, e.g. head width (Britt, 1962; Levanidova and Rubanenkova, 1965), head length (Bretschko, 1965), length of wing pads in combination with abdominal tergite width (Ide, 1935) and total length extrapolated from mandible length (Corbet, 1957).

Britt (1953) concluded that, for *Ephemera simulans* Walker nymphs preserved in alcohol, head width was a more reliable index of growth than total length, especially if the specimens had been injured. The variability of linear measurements with age would be a useful criterion for evaluating which measurement gives the best indication of the mayfly's progress through its life cycle. However, one cannot determine absolute age of nymphs from field samples, and without empirical evidence age cannot be strictly correlated with total length. In fact total length should be evaluated along with the other linear measurements. By utilizing developmental stages (each stage representing a different physiological age) and measurements of relative variability, I examined statistically how six linear measurements varied throughout the life cycle of the mayfly *Leptophlebia cupida* (Say). With these procedures it was also possible to evaluate the homogeneity of arbitrarily chosen developmental stages.

THE PAN-PACIFIC ENTOMOLOGIST 46: 98–106. April 1970

METHODS

Specimens of *L. cupida* used for this study were originally collected as part of a continuing limnological program on the Bigoray River, a subarctic brown-water stream of west-central Alberta (Clifford, 1969). The nymphs, initially used for an allometry study (Clifford, 1970), were first grouped into mm size classes (between 10 and 20 nymphs per size class) based on total length excluding the cerci; nymphs 5.0 mm and longer were further separated into males and females. Each nymph was then placed into one of four arbitrarily chosen developmental stages by the appearance and development of the mesothoracic wing pads: Stage I nymphs had no wing pads; Stage II nymphs had small wing pads, their length being shorter than the distance between the two wing pads; Stage III nymphs had a wing pad length greater than the distance separating the two wing pads; Stage IV nymphs had darkened wing pads indicating impending emergence. Stage IV nymphs were in the last nymphal instar; the other stages each represented several instars. Using an ocular eyepiece and dissecting microscope, the following measurements were made to the nearest 0.01 mm on the nymphs of each stage: total length, head length, pronotum width, mesonotum width, mesonotum length, and width of the fourth abdominal tergite. Subimagos and imagos were also measured as above.

The relative variability of each of the six linear measurements throughout *L. cupida*'s life cycle was determined by calculating the coefficient of variation (CV) for each dimension of each developmental stage, where CV is 100 times the standard deviation(s) divided by the mean ($\bar{x}$). The CVs of Stage IV nymphs, subimagos and imagos are comparable to those of other animals; they are homogeneous samples in respect to age and sex. The CV values of the other nymphal stages are only meaningful for comparing the relative variability of the dimensions within a particular stage. However, since each stage represents a progressively older developmental age and collectively all stages represent the entire life span, it is felt this sort of analysis is a valid way to evaluate the variability of the dimensional measurements throughout *L. cupida*'s life cycle.

RESULTS AND CONCLUSIONS

Homogeneity of developmental stages.—Figure 1 shows the seasonal developmental cycle of *L. cupida* when large numbers of nymphs were gathered throughout the year and separated into the four developmental stages. How precise are the arbitrarily chosen stages for inter-

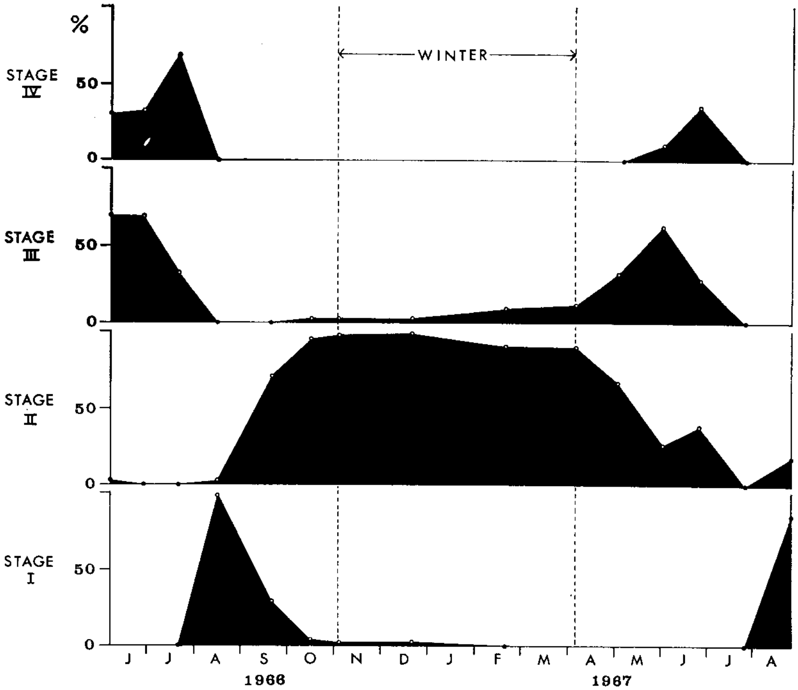

FIG. 1. Seasonal developmental cycle of *L. cupida* nymphs from the Bigoray River; total number of specimens per sampling date (except July 1967) ranged from 94 to 1,302.

preting *L. cupida*'s developmental cycle? The CVs of subimagos and imagos (and Stage IV nymphs except for the small sample size) can be used as a yardstick for judging the homogeneity of the other stages (Table 1). Stages III CVs agree well with those of the winged stages; the CVs of Stage I and especially Stage II are somewhat higher than those of winged stages. For Stage I it was necessary to determine CVs without regard to sex; Stage II included unsexed nymphs (juveniles) as well as nymphs whose sex could be determined, and for this reason the same Stage II juveniles were used to calculate Stage II male CVs as were used to calculate the Stage II female CVs. Even considering the heterogeneity due to the mixing of juveniles with males and females, the high CV values of Stage II, when compared to the other stages, suggest that more accurate information on *L. cupida*'s developmental cycle would be obtained if Stage II nymphs were further subdivided by additional developmental criteria. Although it is obvious from Figure 1 that development is much slower during the long winter than in other seasons, one should, without further subdividing Stage II, use caution in interpreting the precise nature of winter development.

Variability of linear measurements.—Certain nymphal dimensions did have consistently lower CV values (e.g. head length) than other dimensions (e.g. mesonotum length). A clearer picture is obtained by ranking the CV values of each dimension from 1 to 6 within each nymphal stage and each sex. Rank 1 represents the lowest CV value for a given dimension and 6 the highest. Then for a particular body part the rank numbers by sex for the four nymphal stages are added up (including Stage I in summations for both males and females). There was a slightly different sequence for the two sexes (Table 2). For females, both head length and pronotum width had much lower total rank values than the other measurements; for males, abdomen width and head length would be the best linear measurement throughout the entire nymphal life span. For both sexes, mesonotum length was the most variable measurement. Total length was near the middle of each range when the sexes were considered separately.

When the CV of each body part is calculated for each stage without regards to sex (actual tabulated CV values not shown) and then ranked as above, total length has a much higher total rank position, the sequence being head length (8 total rank numbers), pronotum width (12), abdomen width (13), mesonotum width (13), total length (19), and mesonotum length (19).

It would be instructive to know if any of the CVs of Table 1 indicate dimensional measurements statistically more variable than others within a particular stage. Lewontin (1966) shows that the variance of the logarithms (common or natural) of measurements gives a measure of relative variability that is invariant under a multiplicative change, and hence can be used for statistical tests; he also points out for CVs of about 30 or less that the square of the CV (in percentage form) closely approximates the variances of natural logarithms. For *L. cupida* each of the dimensional CVs of Table 1 was squared, and then two null hypotheses were tested. (1) Within a particular stage none of the squared CVs *regardless of sex* is significantly larger (one-sided "F" test, 95% level) than the smallest squared CV of that stage. (2) Within a particular stage none of the squared CVs of a *particular sex* is significantly larger (same test) than the smallest squared CV of that sex.

Certain dimensional measurements of female nymphs and subimagos were significantly more variable than those of males (Hypothesis 1,

TABLE 1. Essential statistics (in mm) and coefficients of variation (CV) of the six dimensional measurements of juvenile (J), male and female nymphs, subimagos and imagos.

	Stage I	Stage II		Stage III		Stage IV		Subimagos		Imagos
	J	J + ♂	J + ♀	♂	♀	♂	♀	♂	♀	♀
Number	17[1]	45	47	19	29	4	4	17	17	19
Total Length										
$\bar{x}$	2.40	5.81	5.80	9.95	10.44	9.71	13.00	10.89	11.79	12.21
s	0.50	1.64	1.58	1.06	1.74	0.50	0.91	1.36	1.32	1.33
CV	20.89	28.17	27.18	10.68	16.67	5.15	6.98	12.52	11.16	10.90
Head length										
$\bar{x}$	0.44	0.97	0.95	1.58	1.58	1.45	1.62	1.03	1.02	1.02
s	0.09	0.24	0.23	0.18	0.17	0.06	0.12	0.09	0.13	0.15
CV	20.45	24.96	23.80	11.35	10.57	3.96	7.12	9.11	12.97	14.96
Pronotum width										
$\bar{x}$	0.65	1.38	1.41	2.24	2.32	1.96	2.35	1.83	1.85	1.82
s	0.13	0.34	0.36	0.26	0.28	0.16	0.17	0.20	0.26	0.27
CV	20.35	24.50	25.31	11.72	11.91	8.34	7.23	11.10	13.78	14.71

[1] Except mesonotum length where there were 13 specimens for Stage I.

TABLE 1. Cont.

	Stage I	Stage II		Stage III		Stage IV		Subimagos		Imagos
	J	J + ♂	J + ♀	♂	♀	♂	♀	♂	♀	♀
Mesonotum width										
$\bar{x}$	0.66	1.38	1.40	2.36	2.41	2.38	2.67	2.11	2.04	2.05
s	0.15	0.32	0.35	0.33	0.32	0.22	0.24	0.24	0.30	0.26
CV	23.38	23.10	24.67	13.91	13.10	9.08	8.92	11.18	14.50	12.49
Mesonotum length										
$\bar{x}$	0.34	0.92	0.93	2.18	2.10	2.60	2.60	2.78	2.79	2.94
s	0.34	0.32	0.33	0.44	0.43	0.14	0.15	0.28	0.46	0.34
CV	99.38	34.46	35.27	20.06	20.42	5.44	5.87	9.89	16.38	11.39
Abdomen width										
$\bar{x}$	0.58	1.41	1.42	2.37	2.62	2.40	2.79	1.97	2.18	2.15
s	0.12	0.36	0.43	0.22	0.32	0.10	0.15	0.18	0.35	0.30
CV	20.26	25.46	29.97	9.43	12.24	4.17	14.03	9.07	16.14	13.73

TABLE 2. Total rank number variabliity of male and female nymphs for the four developmental stages

Body part	Females	Males
Head length	8	10
Pronotum width	11	13
Abdomen width	15	8
Total length	15	14
Mesonotum width	16	17
Mesonotum length	19	22

Table 3). The variability was especially striking for female adbomen width, even in the discrete instars, and possibly reflects egg development in the abdominal region. Considering the sexes separately, mesonotum length (for both males and females) and total length (for females of Stage III) were the only measurements significantly larger than the dimension having the smallest relative variability (Hypothesis 2, Table 4).

In brief, and considering both the rank tabulation and the above statistical tests, one can conclude that no single linear dimension is

TABLE 3. Body parts of a particular stage that had squared CVs significantly larger[1] than the body part[2] with the smallest squared CV of that stage and regardless of sex

Body part	F	D.F.
Stage I (Abdomen width)		
Mesonotum length	4.87	16,16
Stage II (♂ Mesonotum width)		
♀ Mesonotum length	2.33	44,46
♂ Mesonotum length	2.23	44,44
♀ Abdomen width	1.69	44,46
Stage III (♂ Abdomen width)		
♀ Mesonotum length	4.68	18,28
♂ Mesonotum length	4.54	18,18
♀ Total length	3.17	18,28
Stage IV (♂ Head length)		
♀ Abdomen width	12.25	3,3
Subimagos (♂ Abdomen width)		
♀ Mesonotum length	3.28	16,16
♀ Abdomen width	3.16	16,16
♀ Mesonotum width	2.56	16,16

[1] One-sided "F" test, 95% level
[2] In parentheses

TABLE 4. Body parts of a particular stage that had squared CVs significantly larger[1] than the body part[2] with the smallest squared CV of that stage and of the same sex

Body part		F	D.F.
Stage II	Males (Mesonotum width)		
	Mesonotum length	2.23	44,44
	Females (head length)		
	Mesonotum length	2.20	46,46
Stage III	Males (Abdomen width)		
	Mesonotum length	4.54	18,18
	Females (head length)		
	Mesonotum length	3.71	28,28
	Total length	2.52	28,28

[1] One-sided "F" test, 95% level
[2] In parentheses

both least variable for males and least variable for females. Considering the *L. cupida* nymphal population without regards to sex, the usual procedure for interpreting mayfly life history phenomena, head length (or in all likelihood, head width) and pronotum width would be most desirable linear measurements, and mesonotum length would be the least desirable measurement. When the sexes are treated separately, total length is, for most purposes, a seemingly justifiable measurement throughout most of the nymphal life span. But for the larger female nymphs or when the nymphs are treated without regards to sex, several of the other dimensional measurements exhibit much less variability. Finally it is suggested that, because certain females dimensions are significantly more variable than those of males, the most accurate interpretations of size-frequency data would be made when nymphs are separated into males and females, regardless of the linear dimension used.

ACKNOWLEDGMENTS

I am grateful for the technical assistance of Mr. Chi-hsiang Lei. This study was supported by a grant from the National Research Council of Canada.

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