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Why the Simple Frequency and Janetschek Methods are Unreliable for Determining Instars of Mayflies: An Analysis of the Number of Nymphal Instars of *Stenonema modestum* (Banks) (Ephemeroptera: Heptageniidae) in Virginia, USA

by

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ABSTRACT

The Janetschek method is a variation of the simple frequency method and therefore subject to the same limitations. Instars can only be reliably determined by these methods if development within the population is known to be homogeneous. The number of nymphal instars proposed by Kondratieff and Voshell (1980) for an overwintering population of *Stenonema modestum* in the North Anna River of Virginia is not substantiated. These authors estimated a total of 14-15 nymphal instars using the simple frequency and Janetschek methods. A reexamination of their paper indicates that the development of this *S. modestum* population is quite variable and therefore not amenable to successful instar determination using the simple frequency, Janetschek or any other indirect instar determination method. Indirect instar determination methods cannot be considered reliable in determining the number of instars of Ephemeroptera because of the developmental variability characteristic of most, if not all, mayfly species.

INTRODUCTION

Knowing the number of instars for each mayfly species or population could lead to a better understanding of the biology of mayflies. The large number of nymphal molts characteristic of mayflies offers the possibility of closely charting development and thereby correlating development with environmental factors on a fine scale. Unfortunately, mayflies are also characteristically very heterogeneous in growth, morphological development and instar number (e.g. Rawlinson 1939, Hunt 1953, Degrange 1959, Cianciara 1979, Clifford et al. 1979). In heterogeneous development, individuals of different instars overlap widely in size and morphology; thus, discrete instars are not detectable and indirect instar determination methods cannot be relied upon. The simple frequency method, an indirect method, has been shown to be unreliable for populations of other insects whose development is heterogeneous (e.g., Gaines and Campbell 1935, Schmidt et al. 1977). The only reliable mayfly instar determination data have been or will be generated by the direct methods of rearing and the Palmen body (see Fink 1980).

The purpose of this paper is to show why the simple frequency and Janetschek (1967) methods are unreliable for determining instars of mayflies. This is accomplished by analysis of the most current mayfly instar determination effort using these methods. The study chosen for analysis was the life history investigation by Kondratieff and Voshell (1980) of a population of the mayfly *Stenonema modestum* in the North Anna River of Virginia. Only the instar determination and related sections of Kondratieff and Voshell's paper are considered in the present critique.

METHODS

Simple frequency and Janetschek periodic maxima-minima plots were constructed (Fig. 1) for head width frequency data of Kondratieff and Voshell (1980, fig. 4A). Raw data supplied by Kondratieff were actually used to construct Fig. 1 since the data shown in Kondratieff and Voshell's fig. 4A is incomplete (simple frequency values for head width size classes 0.7 and 0.8 mm are 51 and 47 respectively and not 0 and 0 as shown in fig. 4A).

The procedure of the Janetschek method is as follow. Gliding means are calculated from the simple frequency values; the gliding mean of the x_{th} size class, $\bar{Y}_x$, can be calculated as the quantity $((Y_{x-2} + Y_{x-1} + Y_x + Y_{x+1} + Y_{x+2})/5)$ or as the quantity $((Y_x + Y_{x+1} + Y_{x+2} + Y_{x+3} + Y_{x+4})/5)$. The gliding mean values are then subtracted from the respective simple frequency values to yield positive and negative values. Plotting these values results in a graph (periodic maxima-minima) in which each distinctive positive peak may indicate an instar. Imaginary size classes of zero frequency value were used to calculate gliding means for real size classes at the two ends of the simple frequency plot. For example, the gliding mean for the last size class, $\bar{Y}_{xf}$, was calculated as a) $\bar{Y}_{xf} = ((Y_{xf-2} + Y_{xf-1} + Y_{xf} + 0 + 0)/5)$, b) $\bar{Y}_{xf} = ((Y_{xf} + 0 + 0 + 0 + 0)/5)$. In this way all periodic maxima-minima values were preserved in some fashion.

RESULTS AND DISCUSSION

Instar determination by the simple frequency and Janetschek methods involves counting peaks from the plots since a peak may indicate the existence of a discrete instar. About 14-15 peaks should be evident in the simple frequency and Janetschek periodic maxima-minima plots of Kondratieff and Voshell's (1980) head width frequency data for the overwintering brood of *Stenonema modestum* to support their estimate of 14-15 instars. However, only 6-7 prominent peaks plus 2-3 very small peaks are evident in the authors' plots (figs. 4A, 4C) and in my plots of their corrected data (see methods) (Fig. 1). In one sentence of their paper and in personal communication, Kondratieff and Voshell accounted for this discrepancy by citing supplemental rearing and field observations. However, a rearing program and field observations that could accurately account for about 33-50% of

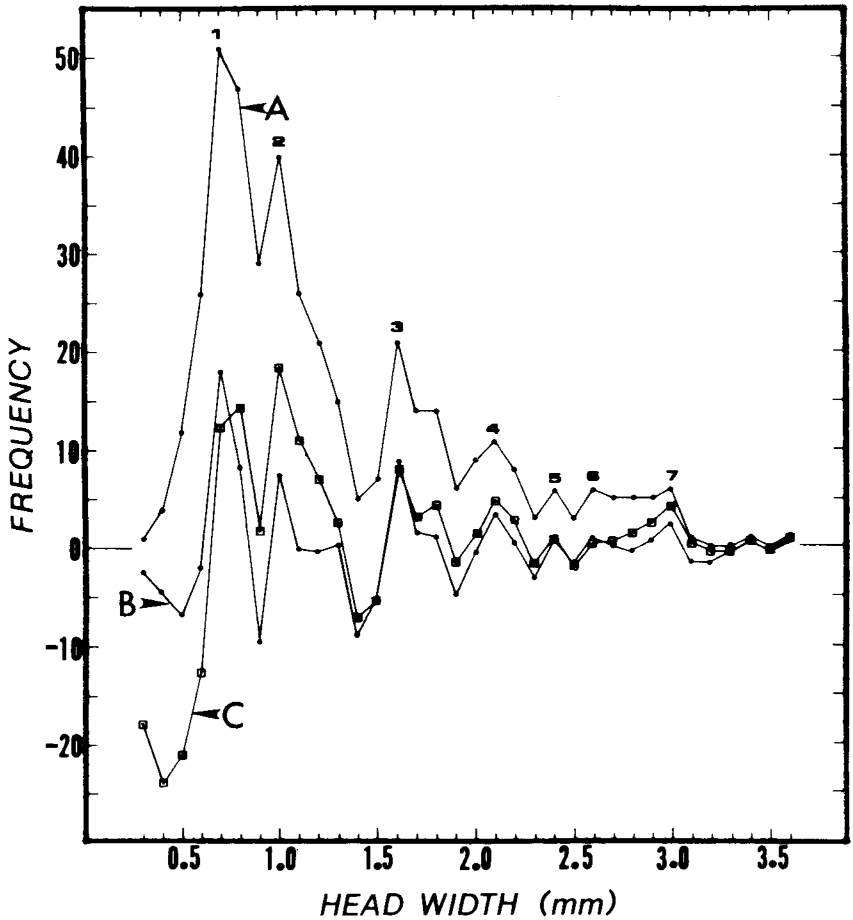

Fig. 1. Instar analysis of male and female *Stenonema modestum* nymphs of the overwintering generation (N = 409, using corrected data, see methods) by the simple frequency and Janetschek methods. A = simple frequency plot; B = Janetschek periodic maxima-minima plot that was calculated using the gliding mean $\overline{Y}_x = ((Y_{x-2} + Y_{x-1} + Y_x + Y_{x+1} + Y_{x+2})/5)$; C = Janetschek periodic maxima-minima plot that was calculated using the gliding mean $\overline{Y}_x = ((Y_x + Y_{x+1} + Y_{x+2} + Y_{x+3} + Y_{x+4})/5)$.

the total number of nymphal instars should have received adequate coverage in their paper.

Although Kondratieff and Voshell (1980) and others (Janetschek 1967, Harper 1973, McClure and Stewart 1976, Oberndorfer and Stewart 1977, Newell and Minshall 1978, Snellen and Stewart 1979) have suggested instar determinations based on the Janetschek method, these estimates are ultimately based on the simple frequency method. The Janetschek method is a variation of the simple frequency method and the gliding mean does not offer any additional insight or information about the data. Subtracting the gliding means from the respective simple frequency values serves only to depress and center around a common horizontal axis (the 0 line) the original simple frequency plot (Fig. 1). This may aid slightly in comparing peaks when compared with the original simple frequency plot but in most cases it is equally as easy to compare peaks in the plots of both methods (Fink, in prep.). The simple frequency and periodic maxima-minima plots are very similar not only in the location of peaks but also in the shape of the plots themselves (Fig. 1). Even calculating the gliding mean in two different ways results in two very similar periodic maxima-minima plots which are in turn very similar to the simple frequency plot (Fig. 1). A further and much more detailed discussion of the Janetschek method is the subject of another paper (Fink, in prep.).

Since the Janetschek method is only a variation of the simple frequency method it is subject to the same limitation, i.e. that simple frequency methods only passively record the distribution of frequency data and do not intrinsically offer any reliable way to determine the significance of that distribution. Peaks indicate instars only if development is homogeneous within the population; otherwise, peaks indicate a relatively random accumulation of specimens of a certain size. The simple frequency plot does offer some clue about the probability that its own peaks and those of the periodic maxima-minima plot might indicate instars. A simple distribution in which peaks are relatively large, distinct and clearly separated might reflect the presence of discrete instars, while a complex distribution indicates the absence of discrete instars and heterogeneous development within the population (Schmidt et al. 1977). The relatively complex simple frequency plot (Fig. 1) and the presence of a wide range of size classes on any sampling date (see Kondratieff and Voshell's fig. 3) indicate that development of the *Stenonema modestum* population studied by Kondratieff and Voshell (1980) is quite heterogeneous and therefore not amenable to instar determination by the simple frequency and Janetschek methods.

Kondratieff and Voshell (1980) also should have assessed the complicating factor of nymphal sexual size dimorphism which probably exists in their *Stenonema modestum* population since female imagines were shown to be considerably larger than male imagines.

In summary, the estimate of the number of nymphal instars proposed by Kondratieff and Voshell (1980) for the overwintering brood of *Stenonema modestum* in the North Anna River of Virginia is not substantiated. The simple frequency method and its variation, the Janetschek method, cannot be relied upon for instar determination in this case. Because of the developmental variability characteristic of most, if not all, mayfly species, indirect instar determination methods cannot be considered reliable in determining the number of instars of Ephemeroptera.

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