

A COMPARISON OF MAYFLY (EPHEMEROPTERA) INSTAR DETERMINATION

METHODS

Thomas J. Fink

Laboratory of Aquatic Entomology
Univ. P.O. Box 111
Florida A&M University
Tallahassee, Florida
U.S.A. 32307

ABSTRACT

Mayfly instar determination methods have been examined to determine their accuracy and usefulness. The Janetschek and Cassie methods are shown not to be an improvement on the simple frequencies from which they are calculated. The Palmen body method suffers from great technical difficulties due to the body's structure and small size. The prospect of associating the number of molts with life history and environmental data is discussed.

INTRODUCTION

Mayflies generally have a large number of postembryonic molts. The number of nymphal instars* (the form of the insect between ecdyses) may range from a high of 40-45 as estimated for *Stenacron canadense* (Walker) (Ide 1935) to a low of 12 estimated for *Baetisca rogersi* Berner (Pescador and Peters 1974).

* The use of the term instar in this paper differs from that of Jones (1978) who defines an instar as the form of the insect between apolyses (i.e. the time the epidermis first separates from the old cuticle prior to the formation of the new cuticle). The term instar as used in this paper is consistent with general use of the term in mayfly literature.

The hardened body parts of mayflies and other arthropods may only increase in size and reveal morphological changes during the short period after a molt and before the new cuticle again becomes hardened. This yields an apparent stepwise growth pattern in which the size gaps or structural differences tend to isolate individuals of different instars. The investigator can determine the number of instars of field collected specimens (collected over the entire life cycle of a generation or cohort) by noting discrete size and morphological groups. This procedure is successful for species whose rate of development and number of instars are fairly homogenous, otherwise problems arise; the gaps become less distinctive as instars overlap in terms of size and morphology.

The rate of development and number of instars are probably controlled by genetic and environmental factors. Nutrition has been considered to be a factor affecting the number of instars in Lepidoptera and other arthropods (Gaines and Campbell 1935; Wigglesworth 1972; Gertsch 1979). Generally poorer nutrition seems to result in a greater number of instars. Cianciara (1979) found that food affected the number of molts of *Cloeon dipterum* (Linnaeus). Temperature, long implicated as a factor influencing size and growth rate in arthropods, may influence the number of molts (Wigglesworth 1972; Roberts *et al.* 1978), and during embryonic life may even affect adult size as discovered for the amphipod *Hyalella asteca* Saussure (de March 1978). Photoperiod has been thought to influence the number of instars in Plecoptera (Khoo 1968).

Genetic control may be implied in the following examples. Degrange (1959) reared, under apparently identical conditions, eggs from one female imago of *Cloeon simile* Eaton and obtained 43 imagoes which had passed through a range of 21 to 30 molts. Hunt (1953) reared *Hexagenia limbata* Serville in aquaria and although eggs were obtained around the same time and individuals in each aquarium were exposed to similar conditions, a large nymphal size range existed in each aquarium. Eason (1969) reared the spider *Pardosa lapidicina* Emerton in the laboratory at $24 \pm 1.7^{\circ}\text{C}$ and found that maturity could be reached in the eighth, ninth or tenth instar.

Mayfly instar determination is particularly difficult due to the generally large number of instars and prolonged immature life cycle. The large number of instars leads to difficulty in resolution of the minute differences, if any, between instars. The prolonged immature life cycle of many mayflies may expose different generations or cohorts and delayed egg hatchlings to widely varying temperature, food and other environmental conditions. These factors may serve to affect the growth rates of the immature insects, the degree of development per instar and the number of instars. Sexual dimorphism is a further obscuring factor and cannot be resolved until late in the life cycle when the sexes can

be identified.

The purpose of this paper is to briefly compare the accuracy and usefulness of those instar determination methods which have been used or may be used on Ephemeroptera. These methods include rearing, morphological characteristics, simple frequency distribution, Cassie, Janetschek, Dyar's law, allometry and Palmen body.

REARING

Rearing involves raising the insect throughout its life cycle and counting all ecdyses. The insects must be reared separately and closely monitored so as to procure all exuviae, the only conclusive proof of molting.

Pescador and Peters (1974) were able to rear *Baetisca rogersi* from eggs to the third nymphal instar. Further specimens were obtained by field-collecting nymphs and rearing them as far as possible. Similarly, Jones (1977), Rawlinson (1939) and Lubbock (1866) obtained most instars of *Neophemera youngi* Berner, *Ecdyonurus venosus* (Fabricius) and *Cloeon cognatum* Stephens (as *dimidiatum* Curtis), respectively. Trost and Berner (1963) reared *Callibaetis floridanus* Banks throughout its life cycle but were unable to find the exuviae of tiny nymphs. Froehlich (1969) was not able to follow the growth of individual *Caenis cuniana* Froehlich nymphs past the fifth instar. Murphy (1922) successfully reared *Baetis posticatus* (Say) from egg to imago and determined 27 nymphal instars; however, results may be viewed with minor suspicion since nymphs were kept in small groups rather than singly. Degrange (1959) successfully reared and counted all ecdyses of 43 *Cloeon simile* individuals. Brittain (1976) determined 17-19 nymphal instars for reared specimens of *Leptophlebia vespertina* (Linnaeus). Illies and Masteller (1977) reared *Baetis vernus* Curtis to the dark wing padded stage which occurred in the 18th instar.

The future of rearing lies in the design and application of equipment expressly for the purpose of rearing such small aquatic insects. Only then will the conflicting needs of ease of observation and simulation of the natural environment be sufficiently balanced to ensure routine success.

The success of rearing means the accurate charting of mayfly development and its possible correlation with environmental conditions under controlled experiments. Even the partial rearing of a large number of nymphs from one clearly defined morphological stage to another equally well defined stage may yield illuminating information on the variability of the number of molts and relative change per molt.

MORPHOLOGICAL CHARACTERISTICS

If mayflies displayed a perfect stepwise growth pattern, instars would be readily separable by the discrete size and structural groupings of nymphs. This is the basis of the morphological characteristics method which entails the detailed description of periodically field-collected nymphs.

Pescador and Peters (1974) determined 12 nymphal instars for *Baetisca rogersi* and Jones (1977) determined 15 nymphal instars for *Neoephemera youngi* using this method in conjunction with rearing. In both studies it was realized that the calculated instars may actually be only developmental stages which are composed of several instars. Ide (1935) reared *Stenacron canadense* and *Ephemera simulans* Walker up to the eighth or ninth instar and obtained other nymphs from periodic field collections. Ide believed that minute changes in morphology were very constant and indicated true instars. The number of segments of the antennae and caudal filaments was believed correlated exactly with the number of molts but difficulty was encountered due to the fusion of segments, the cryptic nature of many sutures, and the ease with which these structures may be damaged. Using morphological criteria Ide estimated between 40-45 and 30 nymphal instars respectively for *S. canadense* and *E. simulans*. Rawlinson (1939) found with *Ecdyonurus venosus* that morphological characteristics were not instar specific, therefore to follow the general course of nymphal development, she designated broad stages based on gill and mesothoracic wing pad development. Similarly, other authors have abandoned instars in favor of broad developmental stages (e.g. Pleskot 1959, Bretschko 1965, Clifford 1970, Lehmkuhl 1970, Hall *et al.* 1975). These stages have been useful in analyzing complex life cycle patterns as demonstrated by McCafferty and Huff (1978) who separated out three overlapping broods found in the life cycle of *Stenacron interpunctatum* (Say).

SIMPLE FREQUENCY DISTRIBUTION

Simple frequency analysis for the determination of instars also relies on the idealized arthropod step-like growth pattern. The method involves frequent periodic field collection of specimens throughout the entire life cycle and the plotting of the number of individuals per size class (all collections combined) for a particular body part measurement. All specimens of a certain instar would be uniform in size and consequently be depicted in the histogram as an isolated peak if there was little or no variation in growth rate and number of instars in the population. Counting the number of peaks would yield the number of instars.

Certain Lepidoptera and other insects do show a homogeneous

rate of development and number of instars and therefore, instars may be adequately detected by the frequency distribution (e.g. Benke 1970, Elliott 1971, Cutler and Harris 1976, Poston *et al.* 1977, Bilapate *et al.* 1978, McNeil 1978, Ross and Craig 1979, Mizell and Nebeker 1979). This is not the case for other species (e.g. Metcalfe 1932, Gaines and Campbell 1935, Kishi 1971, Schmidt and Lauer 1977, Schmidt *et al.* 1977, Ross and Merritt 1978).

In mayflies, the simple frequency data base has been used in an altered form in almost every life history study to provide a meaningful growth trend throughout the life cycle for the purpose of detecting generations or cohorts, and estimating times of growth, egg hatching and emergence. The plot, which may be considered a life history diagram, does not combine collections as above but rather the size class data is plotted for each collection period (e.g. Macan 1957, Thorup 1963, Bretschko 1965, Thibault 1971, Brittain 1972, Pescador and Peters 1974, McClure and Steward 1976, Flowers and Hilsenhoff 1978). In those studies where the simple frequency method has been used in instar determination (McClure and Stewart 1976, Newell and Minshall 1978) only difficult-to-interpret histograms have resulted.

CASSIE METHOD

The Cassie method (Harding 1949, Cassie 1954) is an elaboration of the simple frequency method which attempts to better resolve overlapping distributions. The total distribution is assumed to be the result of a number of normally distributed components, such as the size of particular age classes or instars. The method attempts to uncover the limits of the component distributions.

The Cassie method uses the same data base as the simple frequency analysis except that cumulative size frequency percentages are calculated instead of frequency values. The cumulative frequency percentages are plotted on the probability scale of probability paper versus the size class on the arithmetic scale. A mode in the plot is due to a relatively rapid increase in cumulative frequency due to the accumulation of individuals in a restricted size range, and is assumed to be one component (e.g. age class or instar) distribution of the total distribution. Inflections in the plot are the gaps between the different age class or instar modes.

Harding (1949) and Cassie (1954) used the method with distributions in which the number of components was small and largely known, and both state that the method offers only approximate solutions. Bhattacharya (1967) who developed an additional graphical method for resolving a distribution into its Gaussian (normally distributed) components cautions that the Cassie, his and

other methods adopted by fishery workers, "...are all approximate in the sense that they are applicable only when the components are adequately separated.", and that "The mathematical basis of the probability paper is not very clear."

Recently the Cassie method has been applied to instar determination in mayflies and stoneflies. McClure and Stewart (1976) used it and the Janetschek method for *Choroterpes mexicanus* Allen, while Oberndorfer and Stewart (1977) used the same two methods for the stonefly *Hydroperla crosbyi* (Needham and Claassen).

The Cassie plots of the above two papers were traced on clean white paper (Fink in prep) but no modes or inflections could be determined. This method was also employed on *Baetisca rogersi* and simulated random number data and again no modes and inflections could be determined. Additionally, the Cassie plot of Cassie's (1954) example number 1 was retraced, but only 3 of the 4 inflections stated were present. Even Cassie (1954) admits difficulty in distinguishing the fourth inflection.

The major problem of the Cassie method is the detection of the modes and points of inflection. Although probability paper offers the advantage of estimating the ranges, means and standard deviations of the component distributions, it does so only when they are adequately separated. Probability paper offers a distinct disadvantage in detecting modes and points of inflection over that of the simple frequency method and for this reason the Cassie method cannot be recommended for mayfly instar determination.

JANETSCHEK METHOD

The Janetschek (1967) method is also an elaboration of the simple frequency method which attempts to more clearly resolve significant peaks. Gliding mean values per size class are calculated from the same simple frequency data base resulting in a histogram which supposedly displays a trendline view of growth through the life cycle. The gliding mean values are then subtracted from the simple frequency values to yield positive and negative values. This yields a histogram which is thought to show the periodic maxima of the body part measurement unmasked by the general growth pattern of the population (Janetschek 1967). Significant positive peaks may indicate instars.

The Janetschek method has been used with Collembola (Janetschek 1967), Ephemeroptera (McClure and Stewart 1976, Newell and Minshall 1978) and Plecoptera (Oberndorfer and Stewart 1977, Harper 1973). In these uses of the Janetschek method there has been a somewhat capricious choice of which positive peaks correspond to instar modes. The Janetschek method offers no objective means

of determining significant peaks.

By superimposing the periodic maxima plot on the original simple frequency histogram for each of these studies and for *Baetisca rogersi* and simulated random number data, exact agreement between most peaks of both plots was found (Fink, in prep.).

Thus the Janetschek method is a mathematical exercise which is nothing more than a recapitulation of the simple frequency method, and as such offers no additional information.

DYAR'S LAW

Dyar's (1890) purpose in proposing his empirical generalization was to provide a means to discover overlooked instars of field-collected specimens. His rule states that the discontinuous or step-like increase of sclerotized parts of Lepidoptera larvae usually follow a regular geometric progression. A departure from this progression may then indicate a missing instar or instars. His rule has since been extended to other insects.

Dyar's law can be valid only for those insects whose rate of development and number of instars are homogeneous. Since growth through time of many animals is not constant (Simpson *et al.* 1960), departures from Dyar's law should be commonplace, especially for relatively long lived insects whose life cycles extend over different seasons.

Despite the shortcomings of Dyar's law it remains a useful tool in instar determination. The method is most easily applied by plotting the logarithm of the body measurement on the ordinate versus the suspected instar number on the abscissa. The data points are then connected and a relatively straight line will result if there is some semblance of a regular geometric progression (Forbes 1934). Any large gap between successive data points may indicate a missing instar or instars.

Jones (1977) used this rule for *Neoephemera youngi* and postulated the existence of two additional instars, numbers 3 and 4, between the first two reared instars and the remaining field-collected specimens. Specimens of such a small size (≤ 1 mm in body length) are very difficult to collect in the field. McClure and Stewart (1976) discovered a possible missing second instar for *Choroterpes mexicanus*, which again would be difficult to obtain in field collections. Vaught and Stewart (1974) showed, using Dyar's law semi-logarithmic plot, that nymphal growth of the stonefly *Neoperla clymene* Newman appeared to exhibit 3 distinct phases. Froehlich (1969) appears to have reared the first 5 instars of *Caenis cuniana* and then estimated an additional 10 or 11 instars

using Dyar's law.

ALLOMETRY

The allometric constant α is the ratio of the geometric growth rate of body part y to the geometric growth rate of body part x ($K_{gy}/K_{gx}=\alpha$) (Simpson *et al.* 1960). An allometric plot (logarithm of body measurement y versus logarithm of body measurement x) could be used in the same way as a Dyar's law plot to check for missing instars. The usefulness of this method would depend on the constancy of α over the period of time considered. α is not likely to be constant over the growth period of many organisms as has been shown for *Notonecta undulata* Say (Simpson *et al.* 1960) and *Porcellio laevis* (Latreille) (Nair 1978).

PALMEN BODY

The Palmen body is a ringed structure located in the head of Ephemeroptera at the junction of 4 tracheae. Counting the rings would yield the number of instars if one ring is secreted during the course of each instar. Discovered in 1877 by Palmén, and correlated with the number of molts by Wodsedalek in 1912, it wasn't until 1959 that Degrange tested the Palmen body. Forty-three *Cloeon simile* individuals were separately reared and the Palmen bodies of 1/3 of the specimens successfully counted. The number of instars determined by the Palmen body method and rearing were stated to be identical.

Despite the Palmen body's potential in instar determination only limited use has been made of it. Estimates of the number of molts through at least partial use of the Palmen body have been made by Rawlinson (1939), Taylor and Richards (1963), McLean (1970) and Benech (1972).

The Palmen body method possesses technical difficulties due to the structure's small size and unique morphology. The lower limit of imaginal Palmen body size is probably around 60 μm , as reported by Degrange (1959) for *Cloeon simile*. Consequently, the smallest inner rings are difficult to see, and may be near the limit of resolution in the light microscope.

Thin sectioning is the preferred technique for counting Palmen body rings; however, the problems of orientation of the Palmen to the knife and section thickness make it unlikely that any one section will pass through all rings. Scanning electron microscopy has also been attempted but has failed primarily due to the presence of a persistent coating which does not permit the penetration of electrons to the underlying rings.

The time of Palmen body formation adds an additional problem that should be investigated for each species. Rawlinson (1939) and Degrange (1959) have reported that the tracheal system and Palmen body do not appear until the third or second postembryonic instar respectively for *Eodyonurus venosus* and *Cloeon simile*.

Presently the Palmen body method offers only an estimate of instar number and in many instances the technical difficulties preclude its routine use. It retains, however, the exciting potential of accurate instar determination for every individual and the generation of statistically treatable instar number data.

CONCLUSION

The process of molting is integrally coordinated with mayfly growth and differentiation, and by compartmentalizing development into small units known as instars, may enable the charting of development and the correlation with environmental factors on a fine scale not possible if development were more gradual.

The number of molts or relative change per molt could provide some insight into several important phenomena known or thought to occur in mayflies. One widespread phenomenon is the often large size differences of imagos throughout the emergence period, or of size differences between individuals of different generations or cohorts (e.g. Rawlinson 1939, Macan 1957, Gledhill 1959, Elliott 1967, Minshall 1967, Coleman and Hynes 1970, Bengtsson 1973, Fahy 1973, Sweeney 1978). A related phenomenon has been discussed by Clifford (1970) and Svensson (1977) who have postulated the existence of transitory periods in which cohorts are differentiated or fused. Nymphs having reached a certain physiological stage and minimum size synchronize and increase their development, whereas nymphs not reaching this stage are left behind to complete their development at a later date.

The future of mayfly instar determination should look to the application of new specially designed rearing techniques and the continued testing of the Palmen body. Broad developmental stages and life history diagrams will remain practical means by which mayfly development may be followed, but will result in a more generalized and perhaps schematic history of mayfly development.

ACKNOWLEDGMENTS

This research was supported by a research program (Flax 79009) of SEA/CR U.S.D.A., at Florida A&M University, William L. Peters research leader. I am also grateful for the ideas and assistance of W.L. Peters, M.D. Hubbard and L. Yasui in preparation of this paper.

RESUME

Des études ont été effectuées sur le sujet pour déterminer l'exactitude et l'utilité des différentes méthodes. Les méthodes de Janetschek et Cassie n'ajoutent rien aux fréquences simples à partir desquelles elles ont été mises au point. Celle de Palmen, dite "du corps" se heurte à de sérieuses difficultés techniques en raison de la structure du corps et de sa petite taille. L'ouvrage fait le point sur cette tentative d'association du nombre des mues à la genèse de leur existence et aux données de l'environnement.

ZUSSAMENFASSUNG

Methoden zur Bestimmung von Eintagsfliegen in verschiedenen Stadien zwischen den Häutungen wurden einer Untersuchung unterzogen, um ihre Genauigkeit und Nützlichkeit zu bestimmen. Die Janetschek und Cassie Methoden erweisen sich nicht als Verbesserung gegenüber den einfachen Frequenzen von denen sie errechnet wurden. Die Palmen Körpermethode leidet an großen technischen Schwierigkeiten auf Grund der Struktur des Körpers und der geringen Größe. Vorliegende Arbeit befaßt sich mit der Möglichkeit, die Zahl der Häutungen zu Lebensgeschichte und Umgebungsdaten in Beziehung zu setzen.

REFERENCES

- Benech, V. 1972. Le polyvoltinisme chez *Baetis rhodani* Pictet (Insecta, Ephemeroptera) dans un ruisseau a truites des Pyrénées-Atlantiques, le Lissuraga. *Ann. Hydrobiol.* 3: 141-171.
- Bengtsson, J. 1973. Vækst og livscyklus hos *Baëtis rhodani* (Pict.) (Ephemeroptera). *Flora Fauna* 79: 32-34.
- Benke, A.C. 1970. A method for comparing individual growth rates of aquatic insects with special reference to the Odonata. *Ecology* 51: 328-331.
- Bhattacharya, C.G. 1967. A simple method of resolution of a distribution into its Gaussian components. *Biometrics* 23: 115-135.
- Bilapate, G.G., V.M. Pawar, and A.K. Raodeo. 1978. Biometrical analysis of growth in the larvae of *Heliothis armigera* Hubner. *Proc. Indian Acad. Sci., Sect.* 87: 349-355.
- Bretschko, G. 1965. Zur larvalentwicklung von *Cloeon dipterum*, *Centroptilum luteolum* und *Baetis rhodani*. *Z. wiss. Zool.* 172: 17-36.
- Brittain, J.E. 1972. The life cycles of *Leptophlebia vespertina* (L.) and *L. marginata* (L.) (Ephemeroptera) in Llyn Dinas, North Wales. *Freshw. Biol.* 2: 271-277.

- Brittain, J.E. 1976. Experimental studies on nymphal growth in *Leptophlebia vespertina* (L.) (Ephemeroptera). *Freshw. Biol.* 6: 445-449.
- Cassie, R.M. 1954. Some uses of probability paper in the analysis of size frequency distributions. *Aust. J. Mar. Freshw. Res.* 5: 513-522.
- Cianciara, S. 1979. Some study on the biology and bioenergetics of *Cloeon dipterum* (L.) Ephemeroptera (preliminary data). p. 175-192 In: K. Pasternak and R. Sowa, ed. Proc. Second Int. Conf. Ephem.; August 23-26, 1975: Polish Academy of Sciences Laboratory of Water Biology, Kracow. p. 175-192.
- Clifford, H.F. 1970. Analysis of a northern mayfly (Ephemeroptera) population, with special reference to allometry of size. *Can. J. Zool.* 48: 305-316.
- Coleman, M.J. and H.B.N. Hynes. 1970. The life histories of some Plecoptera and Ephemeroptera in a southern Ontario stream. *Can. J. Zool.* 48: 1333-1339.
- Cutler, B.L., and M.K. Harris. 1976. Head capsule widths of the walnut caterpillar. *J.N.Y. Entomol. Soc.* 84: 156-158.
- Degrange, C. 1959. Nombre de mues et organe de Palmén de *Cloeon simile* Etn. (Ephéméroptères). *C.R. Hebd. Seances Acad. Sci.* 249: 2118-2119.
- de March, B.G.E. 1978. The effects of constant and variable temperatures on the size, growth, and reproduction of the freshwater amphipod *Hyaella azteca* (Saussure). *Can. J. Zool.* 56: 1801-1806.
- Dyar, H.G. 1890. The number of molts in lepidopterous larvae. *Psyche* 5: 420-422.
- Eason, R.R. 1969. Life history and behaviour of *Pardosa lapidicina* Emerton. *J. Kans. Entomol. Soc.* 42: 339-360.
- Elliott, J.M. 1967. The life histories and drifting of the Plecoptera and Ephemeroptera in a Dartmoor stream. *J. Anim. Ecol.* 36: 343-362.
- Elliott, J.M. 1971. The life history and biology of *Apatania muliebris* McLachlan (Trichoptera). *Entomol. Gaz.* 22: 245-251.
- Fahy, E. 1973. Observations on the growth of Ephemeroptera in fluctuating and constant temperature conditions. *Proc. R. Ir. Acad. Sect. B* 73: 133-149.
- Flowers, R.W., and W.L. Hilsenhoff. 1978. Life cycles and habitats of Wisconsin Heptageniidae (Ephemeroptera). *Hydrobiologia* 60: 159-171.
- Forbes, W.T.M. 1934. A note on Dyar's law (Lepidoptera: larvae). *Bull. Brooklyn Entomol. Soc.* 29: 146-149.
- Froehlich, C.G. 1969. *Caenis cuniana* sp. n., a parthenogenetic mayfly. *Beitr. neotrop. Fauna.* 6: 103-108.
- Gaines, J.C., and F.L. Campbell. 1935. Dyar's rule as related to the number of instars of the corn ear worm, *Heliothis obsoleta* (Fab.), collected in the field. *Ann. Entomol. Soc. Am.* 28: 445-461.

- Gertsch, W.J. 1979. American spiders, second ed. Van Nostrand Reinhold, New York. 274 p.
- Gledhill, T. 1959. The life-history of *Ameletus inopinatus* (Siphonuridae, Ephemeroptera). *Hydrobiologia* 14: 85-90.
- Hall, R.J., L. Berner, and E.F. Cook. 1975. Observations on the biology of *Tricorythodes atratus* McDunnough (Ephemeroptera: Tricorythidae). *Proc. Entomol. Soc. Wash.* 77: 34-49.
- Harding, J.P. 1949. The use of probability paper for graphical analysis of polymodal frequency distributions. *J. Mar. Biol. Ass. U.K.* 28: 141-153.
- Harper, P.P. 1973. Life histories of Nemouridae and Leuctridae in southern Ontario (Plecoptera). *Hydrobiologia* 41: 309-356.
- Hunt, B.P. 1953. The life history and economic importance of a burrowing mayfly, *Hexagenia limbata*, in southern Michigan lakes. *Bull. Inst. Fish. Res.* 4: 1-151.
- Ide, F.P. 1935. Post embryological development of Ephemeroptera (mayflies). External characters only. *Can. J. Res.* 12: 433-478.
- Illies, J., and E.C. Masteller. 1977. A possible explanation of emergence patterns of *Baetis vernus* Curtis (Ins.: Ephemeroptera) on the Breitenbach-Schlitz studies on productivity, nr. 22. *Int. Rev. gesamten Hydrobiol.* 62: 315-321.
- Janetschek, H. 1967. Growth and maturity of the springtail, *Gomphiocephalus hodgsoni* Carpenter, from South Victoria Land and Ross Island. p. 295-305 in *Entomology of Antarctica*. Antarct. Res. Ser. 10.
- Jones, J. 1977. The ecology, life cycle, and seasonal distribution of *Neoephemera* (s.s.) *youngi* Berner (Ephemeroptera: Neoephemeridae). M.S. thesis. Florida State University. 152 pp.
- Jones, J.C. 1978. A note on the use of the terms instar and stage. *Ann. Entomol. Soc. Am.* 71: 491-492.
- Khoo, S.G. 1968. Experimental studies on diapause in stoneflies. I. Nymphs of *Capnia bifrons* (Newman). *Proc. R. Entomol. Soc. Lond., Ser. Gen. Entomol.* 43: 40-48.
- Kishi, Y. 1971. Reconsideration of the method to measure the larval instars by use of the frequency distribution of the head capsule widths and lengths. *Can. Entomol.* 103: 1011-1015.
- Lehmkuhl, D.M. 1970. The life cycle of *Rhithrogena morrisoni* (Banks) in western Oregon (Ephemeroptera: Heptageniidae). *Pan-Pac. Entomol.* 46: 124-127.
- Lubbock, J. 1866. On the development of *Chloeon* (*Ephemera*) *dimidiatum*. *Trans. Linn. Soc. Lond.* 24: 61-78, 25: 477-492.
- Macan, T.T. 1957. The life histories and migrations of the Ephemeroptera in a stony stream. *Trans. Soc. Br. Entomol.* 12: 129-156.
- McCafferty, W.P., and B.L. Huff, Jr. 1978. The life cycle of

- the mayfly *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). *Great Lakes Entomol.* 11: 209-216.
- McClure, R.G., and K.W. Stewart. 1976. Life cycle and production of the mayfly *Choroterpes (Neochoroterpes) mexicanus* Allen (Ephemeroptera: Leptophlebiidae). *Ann. Entomol. Soc. Am.* 69: 134-144.
- McLean, J.A. 1970. Studies on the larva of *Oniscigaster wakefieldi* (Ephemeroptera: Siphonuridae) in Waitakere stream, Auckland. *N.Z.J. Mar. Freshw. Res.* 4: 36-45.
- McNeil, J.N. 1978. The number of larval stages of *Thymelicus lineola* (Lepidoptera: Hesperidae) in eastern Canada. *Can. Entomol.* 110: 1293-1295.
- Metcalfe, M.E. 1932. On a suggested method for determining the number of larval instars in *Sitodrepa panicea* L. *Ann. Appl. Biol.* 19: 413-419.
- Minshall, J.N. 1967. Life history and ecology of *Epeorus pleuralis* (Banks) (Ephemeroptera: Heptageniidae). *Am. Midl. Nat.* 78: 369-388.
- Mizell, R.F. III, and T.E. Nebeker. 1979. Number of instars of the southern pine beetle (Coleoptera: Scolytidae) and some comparisons of head capsule widths. *Ann. Entomol. Soc. Am.* 72: 313-316.
- Murphy, H.E. 1922. Notes on the biology of some of our North American species of mayflies. *Bull. Lloyd Libr.* 22, *Entomol. Ser.* 2: 1-46.
- Nair, G.A. 1978. Growth of different instars of *Porcellio laevis* (Latreille) (Isopoda, Porcellionidae). *Comp. Physiol. Ecol.* 3: 120-122.
- Newell, R.L., and G.W. Minshall. 1978. Life history of a multivoltine mayfly, *Tricorythodes minutus*: an example of the effect of temperature on the life cycle. *Ann. Entomol. Soc. Am.* 71: 876-881.
- Oberndorfer, R.Y., and K.W. Stewart. 1977. The life cycle of *Hydroperla crosbyi* (Plecoptera: Perlodidae). *Great Basin Nat.* 37: 260-273.
- Palmén, J.A. 1877. Zur Morphologie des Tracheensystems. Wilhelm Engelmann, Leipzig. 149 p.
- Pescador, M.L. and W.L. Peters. 1974. The life history and ecology of *Baetisca rogersi* Berner (Ephemeroptera: Baetiscidae). *Bull. Fla State Mus.* 17: 151-209.
- Pleskot, G. 1959. Die Periodizität einiger Ephemeropteren der Schwechat. *Wasser Abwasser* 1958: 1-32.
- Poston, F.L., R.B. Hammond, and L.P. Pedigo. 1977. Growth and development of the painted lady on soybeans (Lepidoptera: Nymphalidae). *J. Kans. Entomol. Soc.* 50: 31-36.
- Rawlinson, R. 1939. Studies on the life-history and breeding of *Ecdyonurus venosus* (Ephemeroptera). *Proc. Zool. Soc. Lond. Ser. B* 109: 377-450.
- Roberts, W.P., J.R. Proctor, and J.H.H. Phillips. 1978. Effect of constant temperatures on the number of larval instars of the

- oriental fruit moth, *Grapholitha molesta* (Lepidoptera: Tortricidae). *Can. Entomol.* 110: 623-626.
- Ross, D.H., and D.A. Craig. 1979. The seven larval instars of *Prosimulium mixtum* Syme and Davies and *P. fuscum* Syme and Davies (Diptera: Simuliidae). *Can. J. Zool.* 57: 290-300.
- Ross, D.H., and R.W. Merritt. 1978. The larval instars and population dynamics of five species of black flies (Diptera: Simuliidae) and their responses to selected environmental factors. *Can. J. Zool.* 56: 1633-1642.
- Schmidt, F.H., and W.L. Lauer. 1977. Developmental polymorphism in *Choristoneura* spp. (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.* 70: 112-118.
- Schmidt, F.H., R.K. Campbell, and S.J. Trotter, Jr. 1977. Errors in determining instar numbers through head capsule measurements of a Lepidopteran - a laboratory study and critique. *Ann. Entomol. Soc. Am.* 70: 750-756.
- Simpson, G.G., A. Roe, and R.C. Lewontin. 1960. Quantitative zoology (revised ed.). Harcourt, Brace and Co., Inc., New York. 440 p.
- Svensson, B. 1977. Life cycle, energy fluctuations and sexual differentiation in *Ephemera danica* (Ephemeroptera), a stream-living mayfly. *Oikos* 29: 78-86.
- Sweeney, B.W. 1978. Bioenergetic and developmental response of a mayfly to thermal variation. *Limnol. Oceanogr.* 23: 461-477.
- Taylor, R.L., and A.G. Richards. 1963. The subimaginal cuticle of the mayfly *Callibaetis* sp. (Ephemeroptera). *Ann. Entomol. Soc. Am.* 56: 418-426.
- Thibault, M. 1971. Le développement des Ephéméroptères d'un ruisseau a truites des Pyrénées-Atlantiques, le Lissuraga. *Ann. Limnol.* 7: 53-120.
- Thorup, J. 1963. Growth and life-cycle of invertebrates from Danish springs. *Hydrobiologia* 22: 55-84.
- Trost, L.M.W., and L. Berner. 1963. The biology of *Callibaetis floridanus* Banks (Ephemeroptera: Baetidae). *Fla Entomol.* 46: 285-299.
- Vaught, G.L., and K.W. Stewart. 1974. The life history and ecology of the stonefly *Neoperla clymene* (Newman) (Plecoptera: Perlidae). *Ann. Entomol. Soc. Am.* 67: 167-178.
- Wigglesworth, V.B. 1972. The principles of insect physiology (seventh ed.). Chapman and Hall, London. 827 pp.
- Woodsdalek, J.E. 1912. Palmen's organ and its function in nymphs of the Ephemeridae *Heptagenia interpunctata* (Say) and *Eodyurus maculipennis* (Walsh). *Biol. Bull. (Woods Hole)* 22: 253-273.