NUMBER OF INSTARS IN MAYFLY LARVAE: A PALMEN BODY METHOD APPLIED TO *SIPHLONURUS AESTIVALIS* (SIPHLONURIDAE)

LAURENCE RUFFIEUX, MICHEL SARTORI & GENEVIÈVE L'EPLATTENIER
Musée de Zoologie, Palais de Rumine, Case postale 448, CH-1000 Lausanne 17, Switzerland

To determine exactly how many moults larvae of *Siphlonurus aestivalis* go through in two biotopes, a pond and a river with different temperature regimes, a Palmen Body Method was developed. This method requires little laboratory equipment and gives reliable results with restricted failures.
The number of larval instars for *S. aestivalis* is included between 10-17 for the specimens coming from the pond and 12-17 for the individuals collected in the river. Considering each intralayer space of the Palmen body as the developmental time of each larval instar, the moulting frequency in the two biotopes is compared. A good correlation between mean water temperature and the developmental time of the larval instars is described. Some potential applications of the Palmen body are proposed.

INTRODUCTION

Mayflies spend most of their life in water as larvae. The number of larval instars is the largest within insects (SEHNAL, 1985) and above all, variable within a given species. These reasons have as consequence that mayfly instar determination is generally difficult.
FINK (1980) listed 8 methods used to determine the number of larval instars in mayflies (Table 1). The six first ones rely on morphological measurements and they can only be applied if the rate of development within the population is constant and homogenous. As the variability of the development is characteristic of most mayfly species (FINK, 1982), the only reliable techniques are the rearing and the Palmen body method. As the first one involves rearing the specimens throughout their development, we gave up this method which is too long and we chose the latter. DEGRANGE (1959) was the first to prove that the number of chitin layers making up the Palmen body was correlated with the number of instars. Even though several authors used at least partially the Palmen body to estimate the number of moults (RAWLINSON, 1939; TAYLOR & RICHARDS, 1963; MCLEAN, 1970; BENECH, 1972), no method has been published. FINK (1980) only precised that thin sectioning was the preferred technique for counting Palmen body rings.
Siphlonurus aestivalis larvae inhabit slow-running and standing waters (BRITTAIN, 1978; SARTORI, 1987; STUDEMANN *et al.*, 1992). Two different populations were studied for four seasons (1992-95) in the river Le Veyron and in the nearby pond Les Monneaux (canton de Vaud, Switzerland). The timing and life cycle type of this species agreed well with those described by OTTO & SVENSSON (1981) and SAETTEM & BRITTAIN (1993).
Water temperature was continuously recorded in the two biotopes. Some eggs batches were reared in situ to determine the hatching date of this species. We brought to the fore that the length of the larval life was generally three months shorter in the pond (150 days) than in the river (240 days) (Ruffieux & Sartori, in prep.). On the other hand, nymphs (last instar larvae) coming from the pond were bigger than those of the river. These differences can be explained by the fact that mayfly growth rate is strongly related to temperature (for reviews, see BRITTAIN, 1982; SWEENEY, 1984), the pond presenting a higher mean annual temperature than the river.
So, we tried with this study to answer the following questions:
- Is the number of larval instars related to the length of the larval life and/or to the nymph size?
- What is really the influence of water temperature on the moulting number?

MATERIAL AND METHODS

For this study, *Siphlonurus aestivalis* populations were studied from January to June 1994 with 2 samplings per month. Water temperature was continuously recorded with the help of thermographs. Nymphs were collected between 2 May and 21 June 1994 and immediately conserved in alcohol 70%.
A total of 50 specimens were dissected to obtain the cephalic Palmen body, posterior to the brain, above the oesophagus. They were analysed by using a Palmen body

© MTL, Fribourg - 1997. P. Landolt & M. Sartori (Eds).
Ephemeroptera & Plecoptera: Biology-Ecology-Systematics.

method which is based on the classical techniques used in histology. After being bedded in resin, the Palmen body was cut with an ultramicrotome in sections of 0.75 µm, this thickness being characteristic for *S. aestivalis* (RUFFIEUX *et al.*, 1996). These sections were then placed on a microscopic slide in Canada Balsam. The number of rings and the space between them were respectively counted and measured. In order to compare the moulting frequency in the river and in the pond, we chose ten specimens coming from the two biotopes presenting a twelve-layer Palmen body and we represented the thickness of each ring as a percentage of the trachea length included in the Palmen body (Figs 3a, 3b). Then, we assumed that these intralayer spaces were proportional to the developmental time of each larval instar. So, the total thickness of the rings corresponds to the developmental time of the *S. aestivalis* larvae in the two biotopes, i.e. 240 days in the river and 150 days in the pond.

Table 1. Methods used in mayfly instar determination (summarized by FINK, 1980).

Morphological characteristics
Simple frequency distribution
Cassie method
Janetschek method
Dyar's law
Allometry
Rearing
Palmen body

Moreover we assume that each intralayer space, considered as the developmental time of each instar, could be correlated with an environmental variable such as water temperature. To try to confirm this hypothesis, we calculated the mean temperature of the water during different periods corresponding to the developmental time of each instar (Figs 4a, 4b) and we represented it in function of the thickness of each ring (Figs 5a, 5b).

RESULTS

The diameter of the Palmen body in *S. aestivalis* varies from 200 to 300 µm and the number of layers is included between 10-17 for the specimens coming from the pond and between 12-17 for the individuals collected in the river (Figs 1-2). On 50 analysed larvae, only 4 (8%) had a Palmen body illisible, the rings being not distinct.

The moulting number in all the investigated specimens do not differ significatively between the sexes ($U = 136.5$, $P>0.05$), even if we consider separately the two biotopes ($U = 51.5$, $P>0.05$ for the pond; $U = 13.5$, $P>0.05$ for the river). So nymphs of both sexes are pooled in the results for each biotope. Moreover the number of rings is not significatively different in the pond and in the river ($U = 222.0$, $P>0.05$) and is not correlated with the nymph size ($\tau = -0.127$, $P>0.05$).

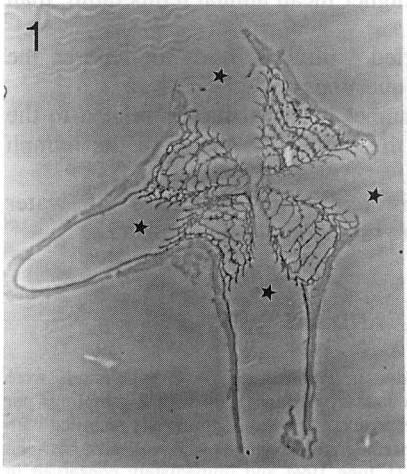

Fig. 1. Central section of a Palmen body: the entry of the four tracheae included in the Palmen body* and the successive chitin layers are well visible. Magnification: 100 x.

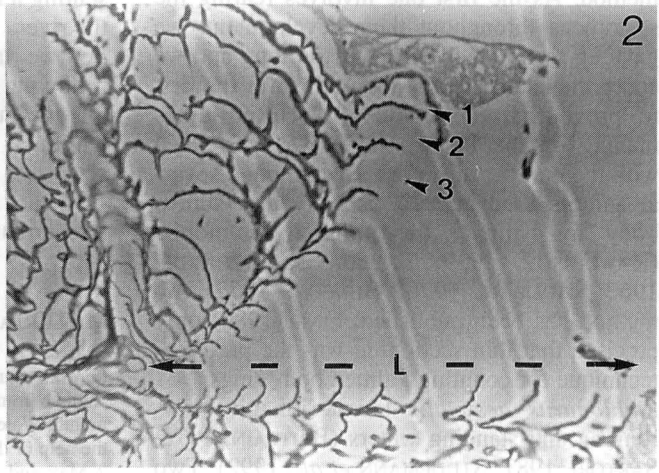

Fig. 2. Magnification of a trachea of a Palmen body with 16 rings. Arrows: successive chitin layers (the more external, No. 1, corresponds to the first larval instar; L: length of the trachea corresponding to the total thickness of the layers. Magnification: 400 x.

The intralayer spaces in the Palmen body are not constant (Figs 3a, 3b). If they were identical each ring thickness would represent 8.33% of the trachea length for a twelve-layer Palmen body. For the specimens coming from the river, the layer thickness varies from about 3.85% to 13.94% (Fig. 3a). This thickness is less variable for the individuals collected in the pond, as it is included between 4.33% and 11.33% (Fig. 3b). For the two biotopes significant differences between each layer of thickness are brought to the fore ($H = 43.224$, $P<0.0001$ for the river; $H = 36.189$, $P<0.0001$ for the pond).

Mean water temperature decreases strongly in the river (Fig. 4a) during the first part of the larval development. At the same time, the moulting frequency is low (important spaces between the points of the curve). In the second part of the graphic, an increase of water temperature and of the moulting frequency can

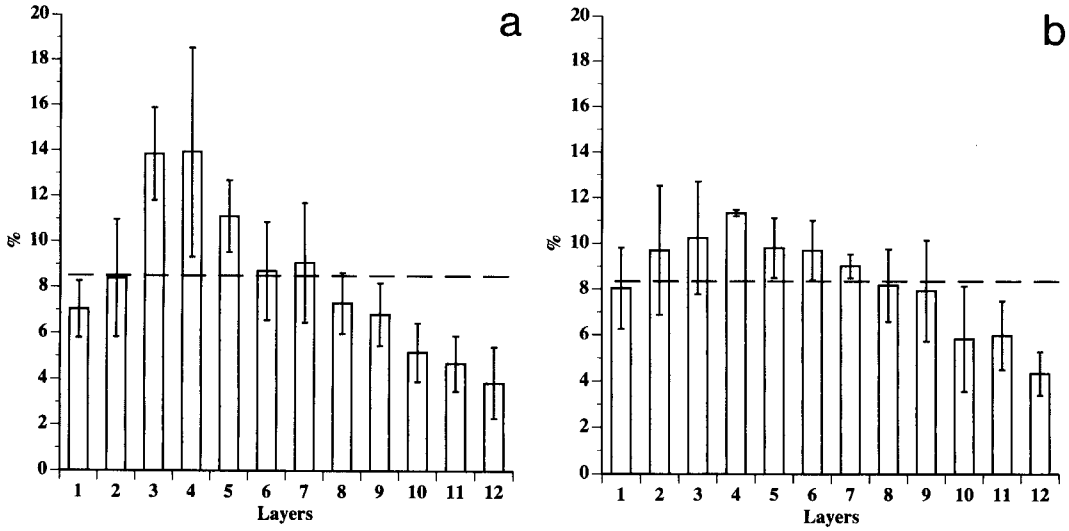

Fig. 3. Thickness of the Palmen body layers (in % of the trachea length, L) in *S. aestivalis* nymphs. a: river Le Veyron; b: pond Les Monneaux. Broken line: mean thickness for a twelve layers Palmen body. Vertical lines show standard deviation.

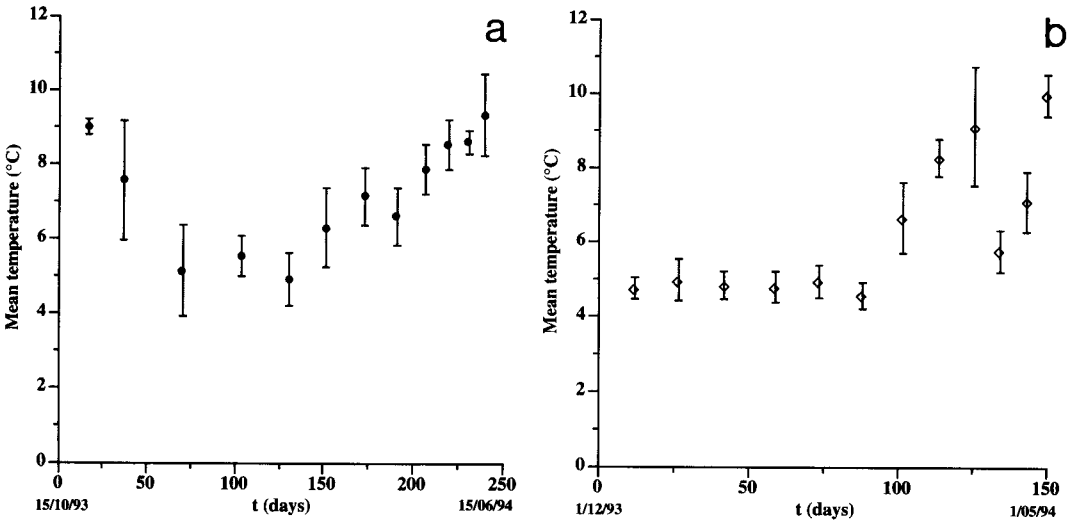

Fig. 4. Mean water temperature calculated during the developmental time of each instar of *S. aestivalis* larvae. a: river Le Veyron, 15/10/93: hatching date, 15/06/94: emerging date; b: pond Les Monneaux, 1/12/93: hatching date, 1/05/94: emerging date. Vertical lines show standard deviation.

be observed. For the individuals collected in the pond (Fig. 4b), the water temperature stays relatively constant during the developmental time of the first larval instars and then increases at the end of the development. So, contrary to the individuals growing in the river, the beginning of the development is more regular in the pond as the duration of each instar is relatively constant and lower, i.e. the moulting frequency is more important. We find a good correlation between the mean temperature of the water and the thickness of each ring, specially for the specimens coming from the river, expressed by the following linear relationship: T = al + b, T = mean water temperature calculated during the developmental time of each instar and l = thickness of the ring as a percent of the trachea length of the Palmen body (a = -0.415, b = 10.658, r^2 = 0.80 for the river; a = -0.630, b = 11.559, r^2 = 0.46 for the pond) (Figs 5a, 5b).

Table 2. Number of chitin layers making up the Palmen body of *S. aestivalis* nymphs collected in the river Le Veyron and in the pond Les Monneaux.

	n (♂)	n (♀)	Mean of layers ± S.E. (♂)	Mean of layers ± S.E. (♀)
Monneaux	14	13	12.643 ± .427	13.000 ± .566
Veyron	5	14	12.600 ± .400	13.357 ± .387

DISCUSSION

The number of rings making up the *S. aestivalis* Palmen body varies between 10-17 for the specimens coming from the pond and 12-17 for the individuals collected in the river. These results agree with those found in literature. In effect, HUMPESCH (1981) considers that the number of instars, including the adult stage, varies from about 10 to 50 for mayflies. And on the basis of several studies (Table 3), it is more frequently situated between 10 and 30.
Contrary to the study of CIANCIARA (1979) on *Cloeon dipterum*, the moulting number does

Table 3. Comparative table presenting the methods used by different authors to determine the number of larval instars in mayflies.

Species	Nb larval instars	Methods
Baetis vagans	27	Rearing (Murphy, 1922)
Cloeon simile	20-29	Rearing and Palmen body (Degrange, 1959)
Callibaetis floridanus	10	Rearing (Trost & Berner, 1963)
Leptophlebia vespertina	17-19	Rearing (Brittain, 1976)
Baetis vernus	18	Rearing (Illies & Masteller, 1977)
Cloeon dipterum	27-38	Rearing (Cianciara, 1979)
Leptophlebia cupida	34	Rearing (Clifford & al., 1979)
Ecdyonurus dispar	24	Rearing (Humpesch, 1981)
Stenacron canadense	40-45	Morphol. characteristics (Ide, 1935)
Ephemera simulans	30	Morphol. characteristics (Ide, 1935)
Baetisca rogersi	12	Morphol. characteristics (Pescador & Peters, 1974)
Neoephemera youngi	15	Morphol. characteristics (Jones, 1977)
Stenonema modestum	14-15	Simple frequency and Janetschek method (Kondratieff & Voshell, 1980)
Baetis rhodani	9-14	Palmen body (Benech, 1972)
Siphlonurus aestivalis	10-17	Palmen body (present study)

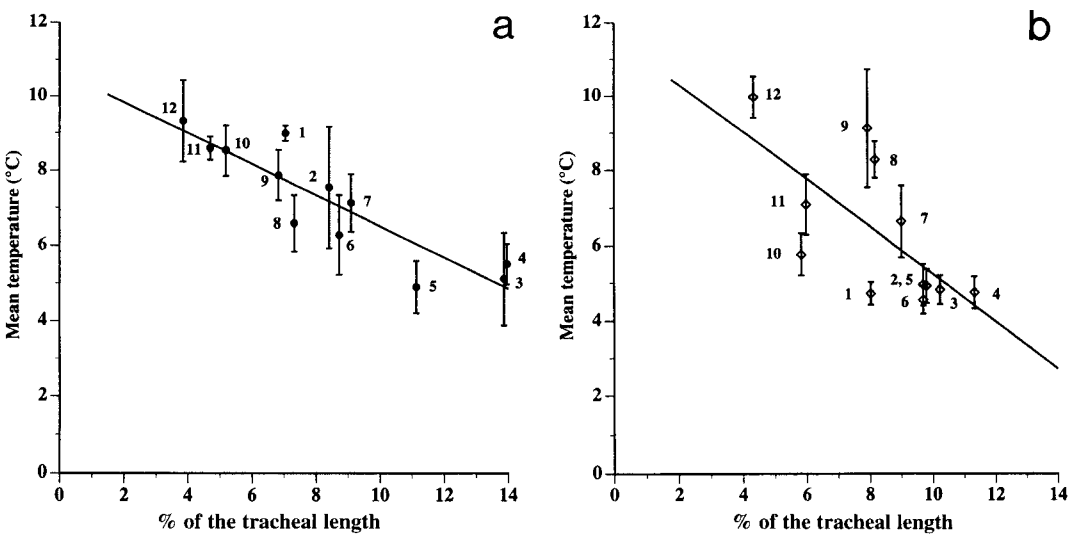

Fig. 5. Regression of mean water temperature calculated during each larval instar and thickness of the Palmen body layers in *S. aestivalis* nymphs (in % of the tracheal length). 1-12: layers numbers corresponding to the successive larval instars. a: river Le Veyron; b: pond Les Monneaux. Vertical lines show standard deviation. For regression coefficients, see text.

not differ between sexes (Table 2), even though female nymphs are bigger than the male ones. As for *Leptophlebia cupida* (CLIFFORD, 1970), the number of larval instars is not correlated with the nymph size.

An increase of mean water temperature involving a faster development (VANNOTE & SWEENEY, 1980; ROSILLON, 1988; GIBERSON & ROSENBERG, 1992), this factor is often described as the more important influencing growth. So, is the number of larval instars correlated with water temperature? The specimens presenting the less larval instars, i.e. 10, were collected in the pond whose mean water temperature is higher than that of the river. So, water temperature seems to play a significant role in the number of moults in *S. aestivalis*. For the specimens coming from the river, the larval growth is more important during the second part of the larval development when the water temperature is increasing (Fig. 4a). This result agrees with that of SAETTEM & BRITTAIN (1993) who noticed that even though *S. aestivalis* stays 8 months in the immature stage, larval growth principally spends during 3 months (April-June). For the individuals collected in the pond (Fig. 4b), the larval growth is relatively stable, the moulting frequency only increasing at the end of the larval development.

Following the hypothesis that there is a correlation between the mean water temperature during a given instar and the thickness of the layer in the Palmen body, we plotted these two variables (Figs 5a, 5b). We find a good correlation, specially for the data of the river. This correlation is expressed by a linear function. So, the higher the mean water temperature is, the thinner the layer thickness is. Or, the higher the water temperature is, the shorter the developmental time is. The results obtained for the pond are globally less decisive. That is certainly due to the numerous overflowings of the river that happen during the larval development. A lot of larvae coming from the river are swept by the current into the pond. So, it is possible that the analysed larvae of the pond have begun their development in the river and in this case, our results are inaccurate.

In conclusion, we see with these results that the thickness of the Palmen body layers is proportional to the developmental time of the larval instars and is correlated with mean water temperature recorded during a given larval instar. In the light of our results, we can advance that the study of the Palmen body does not seem to be limited to the determination of the number of the larval instars. Moreover, in this study, we only focused on individuals possessing the same number of moults, i.e. 12. We are now working on specimens presenting different number of layers in order to see if the patterns observed in this preliminary analysis are comparable.

ACKNOWLEDGMENTS

We thank the Electronic Microscopy Center of Lausanne University and more specially Mrs J. Fakan for technical advice assistance. This study was partly supported by a grant of the «Fondation du 450e anniversaire» of Lausanne University.

REFERENCES

BENECH, V. 1972. Le polyvoltinisme chez *Baetis rhodani* PICTET (Insecta, Ephemeroptera) dans un ruisseau à truites des Pyrénées-Atlantiques, Le Lissuraga. Ann. Hydrobiol. 3: 141-171.

BRITTAIN, J.E. 1976. Experimental studies on nymphal growth in *Leptophlebia vespertina* (L.) (Ephemeroptera). Freshwater Biol. 6: 445-449.

BRITTAIN, J.E. 1978. The Ephemeroptera of Øvre Heimdalsvatn. Holarct. Ecol. 1: 239-254.

BRITTAIN, J.E. 1982. Biology of mayflies. Ann. Rev. Entomol. 27: 119-147.

CIANCIARA, S. 1979. Some study on the biology and bioenergetics of *Cloeon dipterum* (L.) (Ephemeroptera) (preliminary data). *In*: PASTERNAK, K. & SOWA, R. (Eds), Proc. Second Int. Conf. Ephem., pp. 175-192, Polish Academy of Sciences, Kracow.

CLIFFORD, H.F. 1970. Analysis of a northern mayfly (Ephemeroptera) population with special reference to allometry of size. Can. J. Zool. 48: 305-316.

CLIFFORD, H.F., HAMILTON, H. & KILLINS, B.A. 1979. Biology of the mayfly *Leptophlebia cupida* (SAY) (Ephemeroptera: Leptophlebiidae). Can. J. Zool. 57: 1026-1045.

DEGRANGE, C. 1959. Nombre de mues et organe de Palmén de *Cloeon simile* ETN. (Ephéméroptères). C.R. Hebd. Séances Acad. Sci. 249: 2118-2119.

FINK, T.J. 1980. A comparison of mayfly (Ephemeroptera) instar determination methods. *In*: FLANNAGAN, J.F. & MARSHALL, K.E. (Eds), Advances in Ephemeroptera Biology, pp. 367-380. Plenum Press, New York.

FINK, T.J. 1982. 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. Aquat. Insects 4(2): 67-71.

GIBERSON, D.J. & ROSENBERG, D.M. 1992. Effects of temperature, food quantity, and nymphal rearing density on life-history traits of a northern population of *Hexagenia* (Ephemeroptera: Ephemeridae). J. North Am. Benthol. Soc. 11: 181-193.

HUMPESCH, U.H. 1981. Effect of temperature on larval growth of *Ecdyonurus dispar* (Ephemeroptera: Heptageniidae) from two English lakes. Freshwater Biol. 11: 441-457.

IDE, F.P. 1935. Post embryological development of Ephemeroptera (mayflies). External characters only. Can. J. Res. 12: 433-478.

ILLIES, J. & MASTELLER, E.C. 1977. A posssible explanation of emergence patterns of *Baetis vernus* CURTIS (Ins.: Ephemeroptera) on the Breitenbach. Int. Rev. ges. Hydrobiol. 62: 315-321.

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 p.

KONDRATIEFF, B.C. & VOSHELL, J.R. 1980. Life history and ecology of *Stenonema modestum* (BANKS) (Ephemeroptera: Heptageniidae) in Virginia, USA. Aquat. Insects 2(3): 177-189.

MCLEAN, J.A. 1970. Studies on the larva of *Oniscigaster wakefieldi* (Ephemeroptera: Siphlonuridae) in Waitakere stream, Auckland. N.Z.J. Mar. Freshwater Res. 4: 36-45.

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.

OTTO, C. & SVENSSON, S. 1981. A comparison between food, feeding and growth of two mayflies, *Ephemera danica* and *Siphlonurus aestivalis* (Ephemeroptera) in a South Swedish stream. Arch. Hydrobiol. 91: 341-350.

PESCADOR, M.L. & PETERS, W.L. 1974. The life history and ecology of *Baetisca rogersi* BERNER (Ephemeroptera: Baetiscidae). Bull. Fla State Mus. Biol. Sci. 17: 151-209.

RAWLINSON, R. 1939. Studies on the life-history and breeding of *Ecdyonurus venosus* (Ephemeroptera). Proc. Zool. Soc. Lond. B 109: 337-450.

ROSILLON, D. 1988. Food preference and relative influence of temperature and food quality on life history characteristics of a grazing mayfly, *Ephemerella ignita* (PODA). Can. J. Zool. 66: 1474-1481.

RUFFIEUX, L., SARTORI, M. & L'EPLATTENIER, G. 1996. Palmen body: a reliable structure to estimate the number of instars in *Siphlonurus aestivalis* (EATON) (Ephemeroptera: Siphlonuridae). Int. J. Insect Morphol. & Embryol. 25(3): 341-344.

SAETTEM, L.M. & BRITTAIN, J.E. 1993. Life cycle strategies of coexisting Ephemeroptera in an oligotrophic Norwegian lake. Arch. Hydrobiol. 128: 401-408.

SARTORI, M. 1987. Contribution à l'étude systématique et écofaunistique des Ephéméroptères de Suisse (Insecta, Ephemeroptera). Thèse Univ. Lausanne.

SEHNAL, F. 1985. Growth and life cycles. *In*: KERKUT, G.A. & GILBERT, L.I. (Eds), Comprehensive insect, physiology, biochemistry and pharmacology, vol. 2, pp. 2-86, Pergamon Press, Oxford.

STUDEMANN, D., LANDOLT, P., SARTORI, M., HEFTI D. & TOMKA I. 1992. Ephemeroptera. Insecta Helvetica. Fauna. 175 p.

SWEENEY, B.W. 1984. Factors influencing life history patterns of aquatic insects. *In*: RESH, V.H. & ROSENBERG, D.M. (Eds), The ecology of aquatic insects, pp. 56-100, Praeger Publishing, New York.

TAYLOR, R.L. & RICHARDS, A.G. 1963. The subimaginal cuticle of the mayfly *Callibaetis* sp. (Ephemeroptera). Ann. Entomol. Soc. Am. 56: 418-426.

TROST, L.M.W. & BERNER, L. 1963. The biology of *Callibaetis floridanus* BANKS (Ephemeroptera: Baetidae). Fla Entomol. 46: 285-299.

VANNOTE, R.L. & SWEENEY, B.W. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. Am. Nat. 115: 667-695.