THE BIOLOGICAL CYCLE OF *BAETIS PENTAPHLEBODES*, UJHELYI 1966, IN AN OLD MEANDER OF THE RHONE RIVER, FRANCE (EPHEMEROPTERA: BAETIDAE)

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ABSTRACT

Baetis pentaphlebodes described from Hungary by Ujhelyi in 1966, is a recently-discovered species in France. It is abundant in an old meander of the Rhône River now cut off from the main channel and filled by groundwater. Regular sampling demonstrated a bivoltine life cycle of the species and allowed the description of young larvae, so that identification is possible even in the very early stages.

RESUME

Baetis pentaphlebodes, décrit de Hongrie par Ujhelyi en 1966, est une espèce récemment connue de France. Elle est abondante dans un ancien méandre du Rhône, actuellement séparé de l'axe fluvial et alimenté directement par la nappe phréatique environnante. L' étude conduite grâce des prélèvements réguliers effectués sur le terrain, a permis de mettre en évidence le cycle bivoltin de *Baetis pentaphlebodes* dans le milieu prospecté et d' étudier la morphologie des jeunes larves de telle facon que la détermination soit possible, même s'il s'agit de très jeunes stades.

INTRODUCTION

Nymphs of *Baetis pentaphlebodes*, described from Hungary in 1966 by Ujhelyi, were found in the aquatic fauna collected during an interdisciplinary study carried out upstream of Lyon (France) in the Rhône river and its former meanders and channels (Amoros *et al.* 1982, 1987).

In detailed studies of the population of two former meanders, from November 1983 to December 1984, large numbers of nymphs were collected and the life cycle of the species in this region was determined. Because it is necessary to identify the species even in its earliest stages, we studied the variations in the morphology of the nymphs as they developed and were able to demonstrate that the criteria used to identify a species change depending on the maturity of the nymphs.

THE ENVIRONMENT OF *BAETIS PENTAPHLEBODES*

The "Grand-Gravier" and, downstream, "la Chaume", former meanders of the Rhône river are North-East of Lyon on the Jons alluvial plain (Fig. 1). They are oxbow lakes with the latter being the outlet of the former, flowing into the main river after a few kilometers. Previous research has shown that "Grand Gravier" is the drainage system for the aquifer on the North of the plain (Reygrobellet and Dole, 1982). The aquatic

I.C. Campbell (ed.) Mayflies and Stoneflies, 27–34.

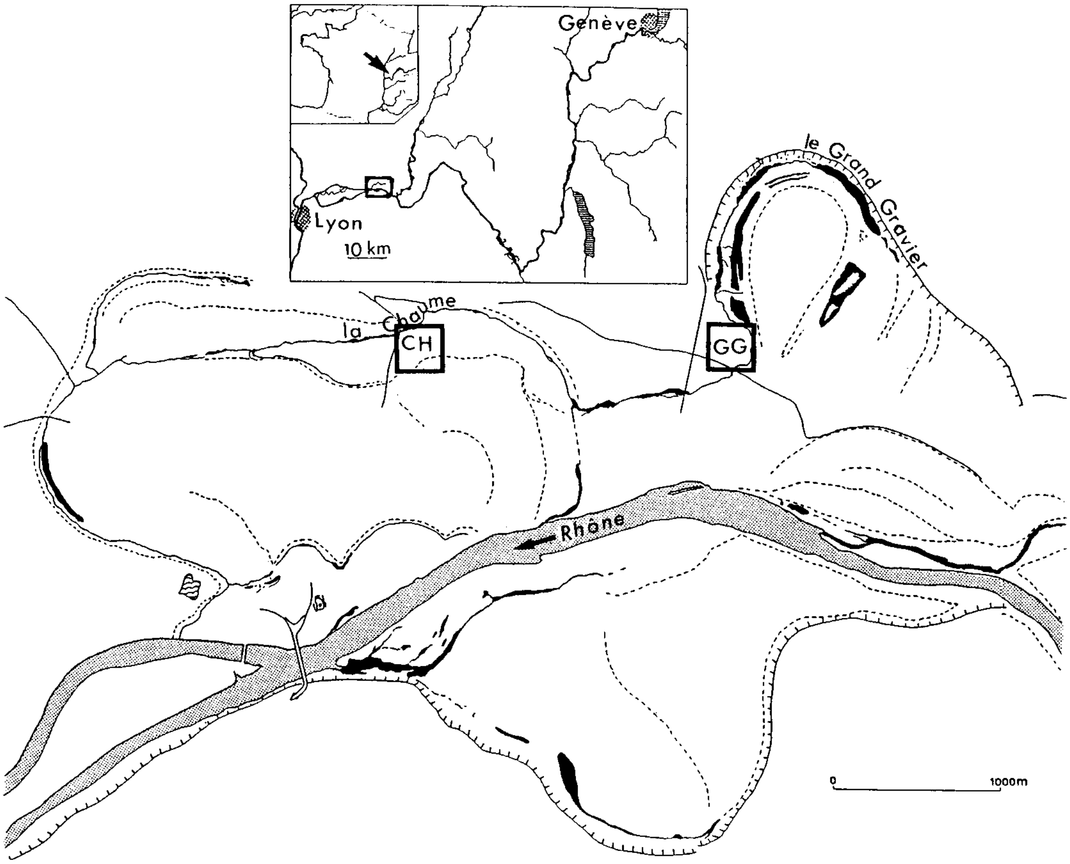


Fig. 1. A map of the sampling sites, CH = La Chaume (IGN map 1/25000 Montluel 1–2, 815,5 × 95,57) and GG = Le Grand Gravier (IGN map 1/25000 Montluel 1–2, 816,67 × 94,82).

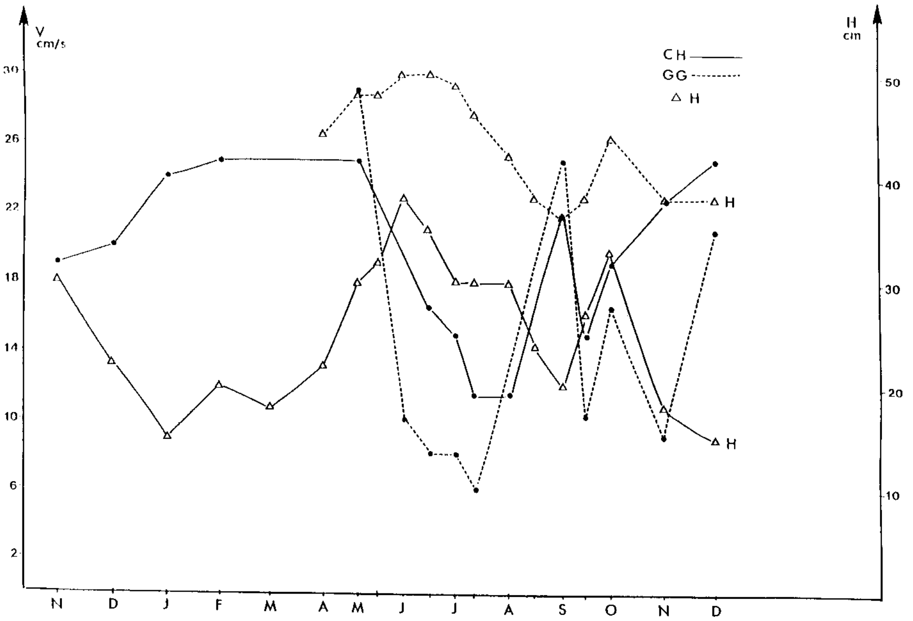


Fig. 2. Current velocity and water level (H) at La Chaume (CH) and Grand Gravier (GG).

vegetation and the fauna are both diverse, and the latter, although not abundant, is highly interesting. Water quality is excellent.

At the first sampling site, "la Chaume" (CH, Fig. 1) early sampling operations revealed an abundant population of *Baetis pentaphlebodes* nymphs, providing a perfect opportunity for quantitative sampling to investigate the life cycle and fluctuations in the population throughout the year. However after January 1984 the numbers of nymphs able to be collected decreased: in February only 12 specimens were found, 1 in March and 0 in April. The Chaume was then prospected for an alternative site, but turned out to be almost completely abiotic, possibly due to pollution from a mosquito control program or fertilizers and pesticides leaching from cultivated lands upstream. It was necessary to find alternative biotopes, and we selected Grand-Gravier oxbow lake 2 km upstream of "la Chaume" (GG, Fig. 1). The study was continued at the second site but regular surveys of CH were continued and, after it was recolonized in August 1984, the two sites were studied in parallel.

Physico-chemical characteristics

Major parameters were measured from November 1983 to January 1985. Current velocity and water level vary in inverse proportion (Fig. 2). Even in the channel where vegetation does not impede flow current velocity is moderate. Water temperature (Fig. 3) is characterised by a steady rise in spring, a maximum in summer and a fall in autumn at both sites. The other major features were: pH about 7.5 at CH and 7.3 at GG, dissolved oxygen 75–110% saturation, conductivity about 400 $\mu S\ cm^{-1}$ (coefficient of variation 0.05), Ca about 90 mg $CaCO_3\ l^{-1}$, TAC about 250 mg $HCO_3\ l^{-1}$, Si about 11 mg $SiO_2\ l^{-1}$.

These former meanders form small brooks with moderate current velocity and oxygen close to, or even above, saturation. Variations in water temperature are relatively gradual and this, together with the considerable mineralisation and high calcium, bicarbonate and silica levels indicates a subterranean origin of the water (Juget *et al.* 1976).

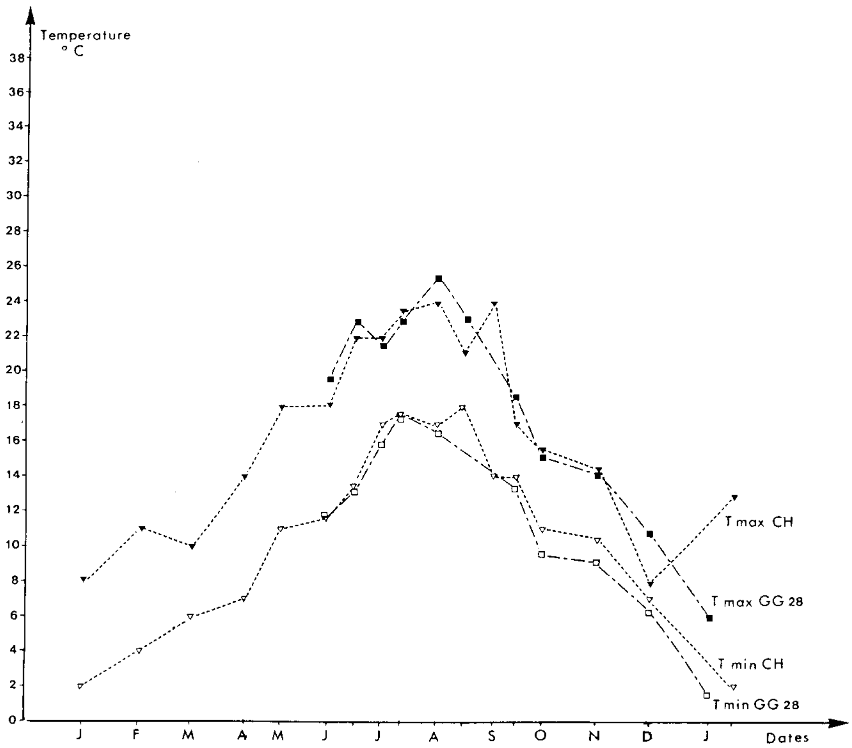


Fig. 3. Water temperatures (minima and maxima) at La Chaume (CH) and Grand Gravier (GG).

The biocenosis

The aquatic vegetation is abundant. Many plant species grow in the open channels, and sometimes, in summer, leave only a narrow space where the water flows freely. Among the plant species providing shelter for *Baetis pentaphlebodes* nymphs were *Nasturtium officinale, Berula erecta* and *Apium nodiflorum* at CH and *Sparganium ramosum, Phragmites australis* and *Hippuris vulgaris* at GG. We collected nymphs clinging to the floating leaves of these plants.

Among the Ephemeroptera, *Baetis pentaphlebodes* is the dominant species at both sites. At CH it accounted for more than 50% of the nymphs collected, together with *Caenis horaria* (21%) and *Cloeon dipterum* (13%). At GG, *Baetis pentaphlebodes* was more numerous than any other ephemeropteran but it only accounted for 21% of the nymphs collected, the other species collected being *Caenis luctuosa* (20%), *Baetis atrebatinus* (18%), *Cloeon dipterum* (17%) and *Caenis horaria* (10%). Nymphs of *B. fuscatus* and *B. vernus* were also collected but represented only about 1% of the mayfly population.

THE LIFE CYCLE OF *BAETIS PENTAPHLEBODES*

Collection of nymphs

Sampling was carried out from November 1983 to December 1984, monthly in autumn and winter and fortnightly in spring and summer, using a 20 × 10 cm Surber sampler with 200 μ mesh. The mesh collected even the smallest nymphs (0.6 mm long), but was coarse enough to prevent rapid clogging. The sampling was quantitative (same zone, same technique) at CH, but at GG the plants were swept until a sufficient number of nymphs had been collected. All nymphs were killed in the field with formalin and measured in the laboratory after being placed in Doetschman solution colored with lignin pink.

Other nymphs collected in the field were reared in the laboratory, at field temperatures, in order to make sure that no generation could occur unobserved between collecting periods.

Measurements

Individuals were measured under a microscope using an ocular micrometer. The parameters used in previous studies may be grouped into 2 categories: biometric characteristics or the progressive appearance of structures. The measurements most widely used are bodylength (excluding caudal filaments) and the width of the head (e.g. Mielewczyk 1981, Olechowska 1981, Rossillon 1984). For structures alar sheaths are most often studied, either in comparison with another structure (El Kallab-Wakim 1978) or to measure the progressive covering of the metathorax, then the first abdominal segments (Humpesch 1979, Cianciara 1979, Jop 1981).

Measurement of size, i.e. of the total length of a nymph, is useful in estimations of the global evolution of population growth, but we consider it too crude to describe the development cycle since the intersegmental membranes are flexible and contract on preservation resulting in shrinking. Also size differences between males and females may lead to incorrect classification, with individuals of different age groups placed in the same category, or individuals of the same physiological age, but separated by a secondary sexual characteristic, placed in different categories.

Measurement of the width of the head is tricky because of the round shape of the head in *Baetis* so we decided to concentrate on the progressive appearance of structures, choosing those which are easily distinguishable and flat to avoid the above-mentioned errors. The sequence of appearance observed on *Baetis pentaphlebodes* was: paracercus, gills and alar sheaths. These organs were previously used by Alba-tercedor (1981 a and b) for *Baetis maurus*.

We subdivided the "gill" phase by measuring the 4th gill, which is large and more often preserved during manipulations than the more posterior gills, using the ratio r where

$$r = \frac{\text{length of the 4th gill}}{\text{medio-dorsal length of the 4th tergite}}$$

For the alar sheaths we used the ratio R where

$$R = \frac{l}{m}$$

and l is the lateral length of the mesothorax from the anterior part to the distal extremity of the anterior alar sheath and m is the medio-dorsal length of the mesothorax.

Lastly we measured the 2328 nymphs collected and distributed them among 8 developmental stages based on the following criteria (Camousseight 1985): Stage 1, paracercus 2 articles or less; stage 2, paracercus > 2 articles and $0.5 \geq r$; stage 3, $1.0 \geq r > 0.5$; stage 4, $r > 1.0$ and $1.10 \geq R$; stage 5, $1.25 \geq R > 1.10$; stage 6, $1.38 \geq R > 1.25$; stage 7, $1.52 \geq R > 1.38$; stage 8, $R > 1.52$.

RESULTS AND DISCUSSIONS

The criteria used avoided all the problems discussed above and allowed each nymph to be fitted into one of the stages without ambiguity.

In La Chaume former meander (CH in Fig. 4) a generation emerged in August-September. Unfortunately the almost total absence of fauna from February until the recolonization in August meant that no conclusions could be drawn concerning the presence of winter generations.

However in the "Grand-Gravier" (GG in Fig. 4) the end of the flight period for the winter generation occurs in late April to early May, while the flight period of a second generation that develops during spring and early summer occurs from late June to early August. Thus, in the Lyon region, as in Czechoslovakia (Soldán 1978), *Baetis pentaphlebodes* behaves like a bivoltine species with two generations per year:

- a generation with slow winter growth over a long period resulting in large nymphs (mean length of mature nymphs, excluding cerci, 9.01 mm);
- a generation growing rapidly in spring and early summer, with small mature nymphs (mean length 7.94 mm). A difference in size between two successive generations of mayflies is well known (Benech 1972, Cianciara 1979).

The period during which last stage nymphs are present is long (Fig. 4), so are these successive cohorts (e.g. Margalef 1974, Begon and Mortimer 1981) or is there another "masked" generation within the long flight period? The behaviour of

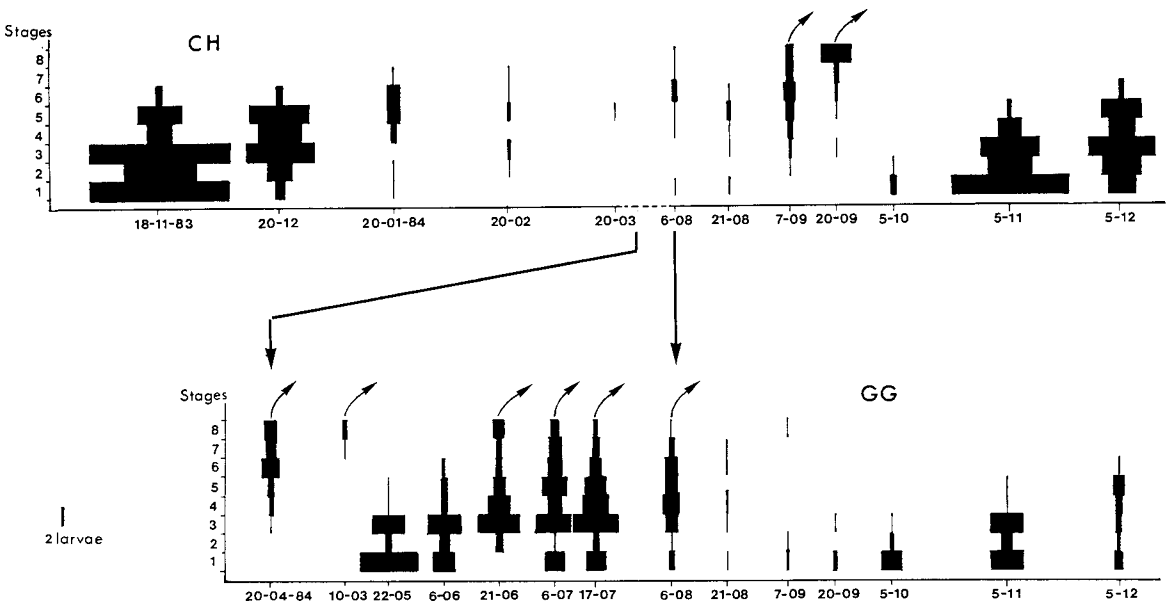
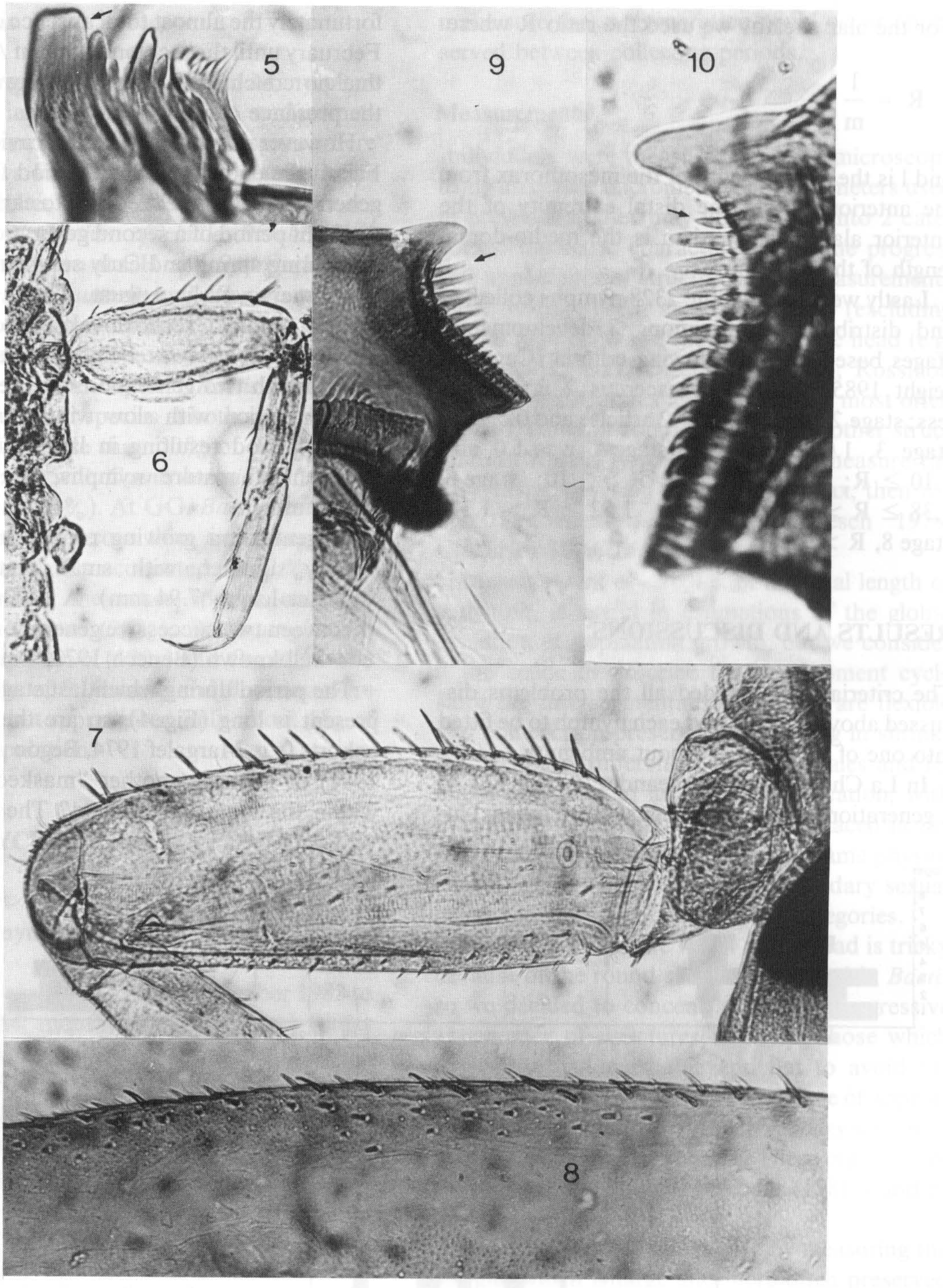


Fig. 4. The life cycle of *Baetis pentaphlebodes* at La Chaume (CH) and Grand Gravier (GG) (the break in the top graph indicates the period when nymphs disappeared).



Figs 5-10. Figs 5–9 *Baetis pentaphlebodes*: 5, canine area of left mandible showing large 1st tooth; 6, femora of a young nymph, stage 1; 7, femora of 4th stage nymph; 8, femora of an old (8th stage) nymph; 9, molar area of left mandible. Fig. 10. *Baetis vernus*, molar area of left mandible.

laboratory reared nymphs tends to negate the latter hypothesis.

There is a time-lag of almost two months between the summer flight period at CH and GG. This is not exceptional since in September 1986 sampling at the two sites gave identical results. Is this second generation time-lag due to local factors such as water temperature (temperature at GG is more variable than at CH) or food (Kondratieff and Voschell 1981, Benech 1972) or could there be three annual generations at CH? The absence of fauna for much of the year prevents any definite conclusion, however a comparison of the CH cycle with the Czechoslovakian population where the summer generation emerges in August-September (Soldán 1978) suggests that the species is bivoltine at both CH and GG but that for the summer generation there is a time-lag between the sites.

CRITERIA FOR RECOGNITION OF *BAETIS PENTAPHLEBODES* NYMPHS

Identification of *Baetis* nymphs has become much easier since the work of Macan (1961) and especially Müller-Liebenau (1969). Hence we had no difficulty distinguishing *large* nymphs of *B. pentaphlebodes, B. atrebatinus, B. vernus* and *B. fuscatus*. Specific criteria used to identify *Baetis* nymphs use organs possessed by medium to large nymphs. Recognition of very small nymphs is much more complex. Several authors who have studied life cycles of Ephemeroptera in their natural habitat have relegated very young nymphs of species belonging to the same genus and sharing a common habitat to a common "undifferentiated" group (e.g. Sowa 1975).

For *Baetis pentaphlebodes* it is difficult to identify the youngest nymphs because of the very small size of the individuals (as small as 0.6 mm) and the absence of specific structures, such as the scale marks on the terga, which appear only later. Some organs which are present in the small nymphs have different shapes and proportions to those in the mature nymphs, a striking example is the femoral spines (Figs 5-8).

We identified the smallest nymphs as *Baetis pentaphlebodes* from the structure of the left mandible (Camousseight 1985). The molar part of this mandible (Fig. 9) shows a row of denticles. For *B. pentaphlebodes* (as for *B. fuscatus*) the more proximal denticle is the longest with the following denticles successively smaller distally. For *B. vernus* and for *B. atrebatinus* the first one or two denticles are shorter than the following one (Fig. 10). Armitage *et al.* (1985) also used characteristics of the left molar to separate damaged nymphs of *B. vernus* and *B. buceratus*. We separated *B. pentaphlebodes* from *B. fuscatus* using the first canine of *B. pentaphlebodes* which is much larger than the others, this is not the case in *B. fuscatus*, and in *B. fuscatus large* scale marks are always present on the tergites while they are absent in the other species. Scale marks in mature *B. pentaphlebodes* nymphs remain small and clearly separated (Camousseight 1985).

CONCLUSION

As in Czechoslovakia, *Baetis pentaphlebodes* in the Lyon region has a bivoltine life history. Identification of young nymphs is essential to establish the correct life cycle, and recognition of the very early stages uses characters which are often different to those used in taxonomic studies.

A comparison of *B. pentaphlebodes* biotopes allows us to define the requirements of the species. Ujhelyi (1966) found it on *Typha* or *Phragmites* leaves in small streams, Soldán (1978) found it in brooks, pond outlets and the lower reaches of rivers where it preferred moderate current and abundant vegetation and Prevot (1984) collected it in a small brook with abundant aquatic plants, pure water a moderate flow and a groundwater supply. Although neither Ujhelyi nor Soldán have mentioned a groundwater supply in *B. pentaphlebodes* habitats, from their descriptions of the habitats this is possible. If this is the case *B. pentaphlebodes* would be an indicator species for streams with moderate current where the subterranean water supply is important. These requirements would explain the discontinuous distribution of the species in Central Europe and France and the rarity of its presence. The establishment of dams on large rivers may be a reason for the disappearance of this species, in the future, from the fauna inhabiting the brooks along the big rivers with a groundwater supply.

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