Internat. Rev. Hydrobiol.	**95**	2010	1	58–71

DOI: 10.1002/iroh.200811197

Manuel Jesús López-Rodríguez, José Manuel Tierno de Figueroa and Javier Alba-Tercedor

Departamento de Biología Animal. Facultad de Ciencias. Universidad de Granada. 18071, Granada, Spain; e-mail: manujlr@ugr.es

Research Paper

Comparative Study of the Nymphal Biology of Two Coexisting Species of Mayflies (Insecta: Ephemeroptera) in a Mediterranean Stream in Southern Europe

key words: mayflies, life cycle, feeding, secondary production, niche overlap

Abstract

We study the life history, nymphal feeding and secondary production of two leptophlebiid mayfly species (*Habrophlebia eldae* and *Paraleptophlebia submarginata*). They cohabit in a Mediterranean stream and present a very high niche overlap in terms of trophic resources. The life cycle was estimated using size-frequency analysis of samples taken throughout a year. Both species have a similar but displaced period of the nymphal development. Secondary production was calculated by means of the size-frequency method. Annual secondary production of *P. submarginata* is much higher than that of *H. eldae* (1.95 g DW m^{-2} $year^{-1}$ *vs.* 0.17 g DW m^{-2} $year^{-1}$), and presents a quite similar annual P/B ratio, but slightly higher in *P. submarginata* (6.97 in *P. submarginata* and 9.21 in *H. eldae*). The study of the gut contents revealed that they are mainly detritivores but, when larger they feed also on CPOM from leaves fallen in the stream. They present an almost total niche overlap in terms of food acquisition. However the previously mentioned shift in trophic resources utilization with size makes it possible that, because no similar size classes of each species are present at the same time, niche segregation exists between the two species. Though further studies are needed to confirm it, this could be the consequence of previous episodes of competition between them.

1. Introduction

Life histories of freshwater invertebrate species, and particularly of mayflies, are conditioned by several factors that can be grouped into two broad classes. These are intrinsic factors (such as morphology, physiology, behaviour, *etc.*), which tend to restrict life history traits within certain genetically or phylogenetically determined ranges, and extrinsic factors (as temperature, photoperiod, nutrition, degree of habitat permanence and presence of other taxa) (Sweeney, 1984; Giller and Malmqvist, 1998). Hence, for a given species, extrinsic factors tend to determine the kinds of strategies associated with different conditions. Life histories of mayflies are extremely variable and depend mainly on the environmental conditions (Brittain, 1982). There are: (1) multivoltine species, with two or more generations within one year, as some populations of *Alainites muticus* (Linnaeus, 1758) or *Baetis alpinus* Pictet, 1843 (*e.g.*, López-Rodríguez *et al*., 2008), (2) univoltine species with the entire cycle

* Corresponding author

lasting one year, as *Serratella ignita* (PODA, 1761) (*e.g.*, LÓPEZ-RODRÍGUEZ *et al*., 2009a), and (3) semivoltine species. Where the life cycle takes two years or more, as in *Ephemera danica* MÜLLER, 1764 (*e.g.*, LÓPEZ-RODRÍGUEZ *et al*., 2009b). The main factors that affect life cycles are temperature, nutrition and photoperiod, although others, such as dissolved oxygen, pH, water current, predation, competition, *etc*., are also of great importance (SWEENEY, 1984; ALLAN and CASTILLO, 2007). Temporal segregation of nymphal development, as a consequence of competition, is one of the most common mechanisms permitting coexistence among closely related species of mayflies (BRITTAIN, 1982). Other factors, such as differences in nutrition, fecundity, predation pressure, and size are also important (BRITTAIN, 1972, 1980). Closely related species that perform a similar trophic function may temporally separate growth and adult emergence within the same stream reach (HAUER and STANFORD, 1982, 1986). The result of this coexistence may also affect secondary production dynamics of the taxa (BENKE, 1984). Others life history attributes, such as natality, abundance, individual growth rate, individual biomass, dispersal, and survivorship, also determine levels of production at the population scale (HURYN and WALLACE, 2000).

In this context we have studied two mayfly Leptophlebiidae, *Habrophlebia eldae* JACOB and SARTORI, 1984 and *Paraleptophlebia submarginata* (STEPHENS, 1835), that coexist in the same site. The former is distributed in the south-western Europe, while the latter is more widely present in Europe, the east-Paleartic region and the Near East (THOMAS and BELFIORE, 2004).

The aims of the present study are: (1) to analyze the life cycle of both species, assess their different life strategies, their nymphal feeding habits and secondary production, discussing the possible reasons for the differences between them, and (2) to estimate the niche breadth of both species in terms of trophic resources utilization and if a niche overlap between them exists.

2. Methods

2.1. Study Area

The study was carried out in Río Fardes (Sierra de Huétor, Granada, Spain; UTM: 30SVG465413, 1200 m a.s.l.), a typical Mediterranean stream with permanent regime. The stream flows over a calcareous substrate and its volume discharge is influenced mainly by rain. The variable flows reflect the highest (usually autumn) and lowest (summer) periods of precipitation. In the sampling site (about 20 m long), the width varied from 1.15 to 3.02 m during the sampling period, and the depth ranged from 0.07 to 0.27 m. No major floods occurred during the sampling period. The substrate was mainly represented by sand (50%), pebbles (35%), and mud (15%). Submerged vegetation was composed of *Nasturtium* sp. and Characeae. The riverside vegetation was abundant and principally represented by Juncaceae or Ciperaceae, and *Salix* sp., and to a lesser extent by Poaceae, *Equisetum* sp., *Mentha* sp. and some *Carduus* sp.

Sampling was carried out monthly from May 2006 to April 2007. A datalogger (HOBO® Water Temp Pro, 0.2 °C accuracy) was placed in the riverbed for registering the temperature hourly, and also for calculating the accumulated degree days between two sampling dates (Fig. 1). Every sampling date we recorded physical parameters *in situ* (oxygen, conductivity and discharge) and transported one litre of water (cold preserved) to the laboratory for analyses of some physicochemical parameters (Table 1). We measured dissolved O_2 with an oximeter (Oxi 320/set Best-Nr. 200 212, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), conductivity with a conductimeter standardized to 25 °C (Ecoscan hand-held series, Euteoh Instrument Technology, Singapore), and discharge with a propeller meter (Global Water Mod. FP101, Global Water Instrumentation, Gold River, CA). The pH was determined by means of a pH meter (CyberScan PH 510, Graintec Scientific Pty Ltd, Toowoomba, QLD), NH_4^+, PO_4^{3-}, NO_3^-, NO_2^- and SO_4^{2+} were determined using a technique of molecular absorption spectrophotometry, Cl^- by means of the Mohr method, alkalinity by means of the potentiometric method, solids in suspension by filtration through a membrane, Ca, Mg and hardness by means of the complexometric

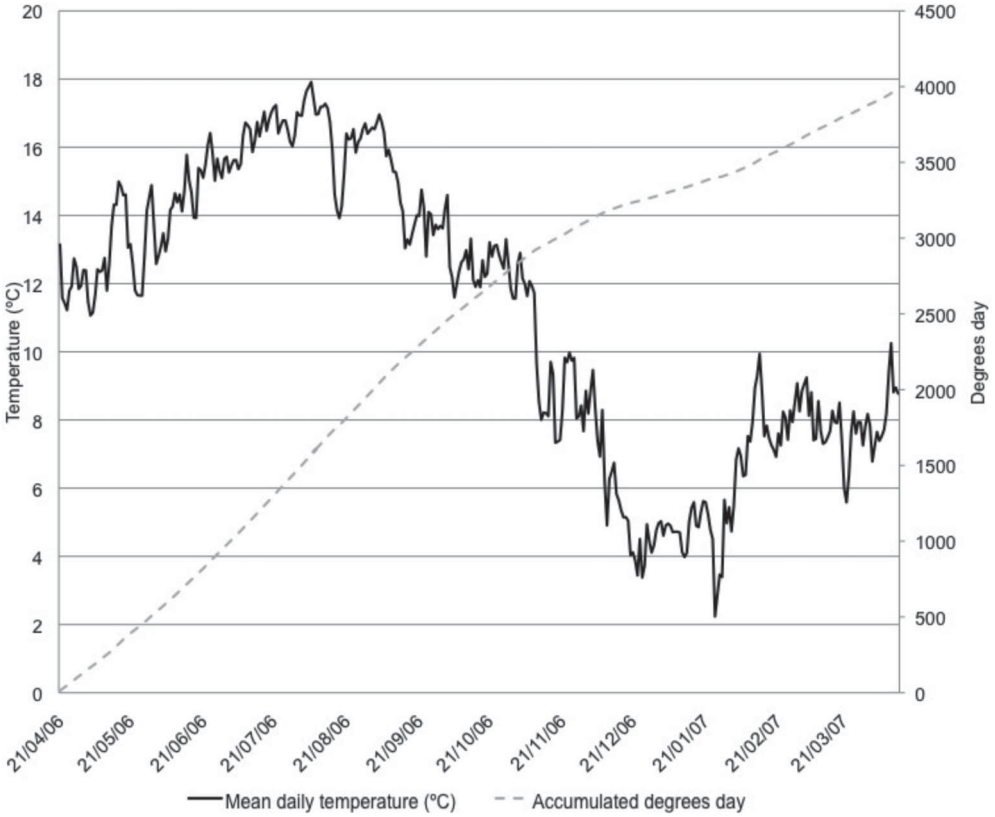

Figure 1. Mean daily temperature and accumulated degrees day at the sampling site during the sampling period.

method, and turbidity using a turbidimeter (Hanna HI 93703-11, Hanna instruments, Eibar, Spain) (Rodier, 1998).

2.2. Collection of Invertebrates

Individuals of both species were collected with a Surber sampler (0.09 m^2 area and 250 μm mesh size). Six replicates were randomly taken for representing the different mesohabitats of each sampling site, both in riffles and pools, and proportionally to their abundances. The organisms were preserved in 4% formalin and transported to the laboratory, and then sieved with a 150 μm mesh size sieve to remove excess formalin and fine detritus. Afterwards, organisms were sorted out and identified to species level.

2.3. Life Histories and Secondary Production Estimates

Each month we measured the total length and pronotum width of 30 individual (when possible) using the micrometer of a binocular microscope (0.01 mm accuracy). Because these two measures were highly correlated (Gamma correlation >0.86 in both species), we used total length for representing the life cycles of the studied species. All the collected individuals were classified into 1 mm intervals. Measures

Table 1. Physicochemical parameters at the sampling site.

	Fardes stream				
	N	Mean	*S.D.*	Minimum	Maximum
pH	12	8.05	0.46	7.03	8.61
Ammonium (mg/l)	12	0.01	0.01	0.00	0.02
Phosphates (mg/l)	12	0.01	0.01	0.00	0.05
Nitrates (mg/l)	12	0.01	0.01	0.00	0.05
Nitrites (mg/l)	12	0.50	1.01	0.03	2.85
Sulfates (mg/l)	12	27.25	20.70	2.43	61.98
Chlorides (mg/l)	12	21.37	9.94	7.10	39.05
Alkalinity (meq/l)	12	51.04	21.73	31.96	114.68
Ss (mg/l)	12	18.78	52.66	1.00	185.80
Ca (mg/l)	12	78.13	49.44	3.90	140.00
Mg (mg/l)	12	43.50	18.12	20.90	82.62
Hardness (mg $CaCO_3$/l)	12	374.11	106.57	95.76	461.66
Turbidity (NTU)	12	2.31	1.74	0.00	6.51
O_2 (% sat)	12	85.08	5.43	76.00	95.00
O_2 (mg/l)	12	8.11	0.74	7.10	9.20
Temperature (°C)	8571	11.13	4.40	0.25	20.39
Conductivity (μS/cm)	12	428.08	102.73	104.00	474.00
Discharge (m^3/s)	12	0.11	0.06	0.05	0.27

were standardized by putting every individual between two slides. Estimation of nymphal biomass was made according to the equation:

$$DW = aX^b$$

or, in natural logarithmic form:

$$\text{Ln DW} = \text{Ln } a + b \text{ Ln X}$$

Where DW = individual dry weight, X = total length, a = constant, and b = slope of the regression. The equations for both species are as follow:

$$\text{Ln DW} = -6.07 + 3.05 \text{ Ln } X, \quad (r^2 = 0.92, F_{1,27} = 312.82, P < 0.05) \text{ for } \textit{H. eldae}$$

$$\text{Ln DW} = -5.17 + 2.71 \text{ Ln } X, \quad (r^2 = 0.94, F_{1,28} = 477.04, P < 0.05) \text{ for } \textit{P. submarginata}$$

For calculating the regression equations, 29 and 30 formalin preserved specimens of *H. eldae* and *P. submarginata* respectively were measured, dried at 60 °C for 24 hours and placed in a desiccator during one hour. After this, they were weighed to the nearest 0.001 mg using a Mettler mod. M3 microbalance.

Mature nymphs (with black wing-pads) were recorded when present.

We used FiSAT II software (Gayanilo *et al*., 2002) for generating the size-frequency graphs representing the life cycles.

Secondary production was calculated by means of the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), because many size classes were present at the same date. Nine size classes were recognized for *H. eldae* and 19 for *P. submarginata.*

Growth was calculated each month as the weighted mean of nymphal total length. Mean was weighted by the number of individuals in each size class.

2.4. Gut Content Analyses and Trophic Basis of Production

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of Ephemeroptera nymphal feeding (*e.g.*, Fenoglio *et al*., 2008; López-Rodríguez *et al*., 2008). We used the same 30 nymphs measured every month for the study of the correlation between pronotum width and total length. Each individual was added to a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. After this, the individuels were mounted on slides for its study under the microscope. We estimated the percentage of the absolute gut content (at 40×). For example, the total was that area occupied by the content in the whole digestive tract, and the relative gut content (at 400×), was that area occupied by each component within the total gut content (using the microscope with an ocular micrometer). Mean, standard deviation, minimum and maximum were calculated. From these data the species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006). We also studied the correlation between size of the nymphs and percentage of the different gut contents, and the contribution of each component of the diet to production. Though assimilation efficiencies and information on dietary enzymatic complexes are not available for these species, we estimated the production derived of each dietary component as described by Benke and Wallace (1980) and assumed a net production efficiency of 40% (Benke and Jacobi, 1994).

2.5. Niche Breadth and Overlap

We used the Levins' index (Levins, 1968) for niche breadth, with the Hurbert's standardization (Hurbert, 1978), to assess if the studied species were more or less generalists. The scale of the standardized index varies between 0 and 1. The higher the value is, the higher the niche breadth, which indicates a more generalist condition. The Levins' index (B) and the Hurbert's standardization (B_A) were calculated as shown below:

$$B = \frac{1}{(\sum p_j^2)}$$

$$B_A = \frac{(B-1)}{(n-1)}$$

being:

p_j = proportion of items in the diet that are of food category j

n = number of possible resource states, evaluated as the whole resources observed in the gut content of all the individuals studied for each species.

We also calculated the niche overlap between the two studied species, in relation with food resources, by using the Simplified Morisita Index proposed by Horn (1966):

$$C_H = \frac{[2 \sum_i^n p_{ij} \cdot p_{ik}]}{[\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2]}$$

where C_H = Simplified Morisita Index of niche overlap between species j and k,

p_{ij} = proportion resource i is of the total resources used by species j,

p_{ik} = proportion resource i is of the total resources used by species k.

The index ranges from 0 (no overlap) to 1 (total niche overlap).

2.6. Statistical Analyses

STATISTICA software (StatSoft, 2005) was used for data analyses. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al*. (2006). Normality of each variable distribution was assessed with the Kolmogorov–Smirnov test. Gamma correlations were used to test for possible associations between total length and protonum width, and size and percentages of diet components. Gamma correlations are thought to be the most appropriate statistic when a high degree of range overlap exists among variables (Guisande González *et al.*, 2006).

3. Results

3.1. Life Histories and Secondary Production Estimates

We captured nymphs of *H. eldae* from November to August. This species presented a univoltine life cycle, with egg hatching starting in November and mature nymphs captured on final June and July. The average nymphal developmental time was approximately nine

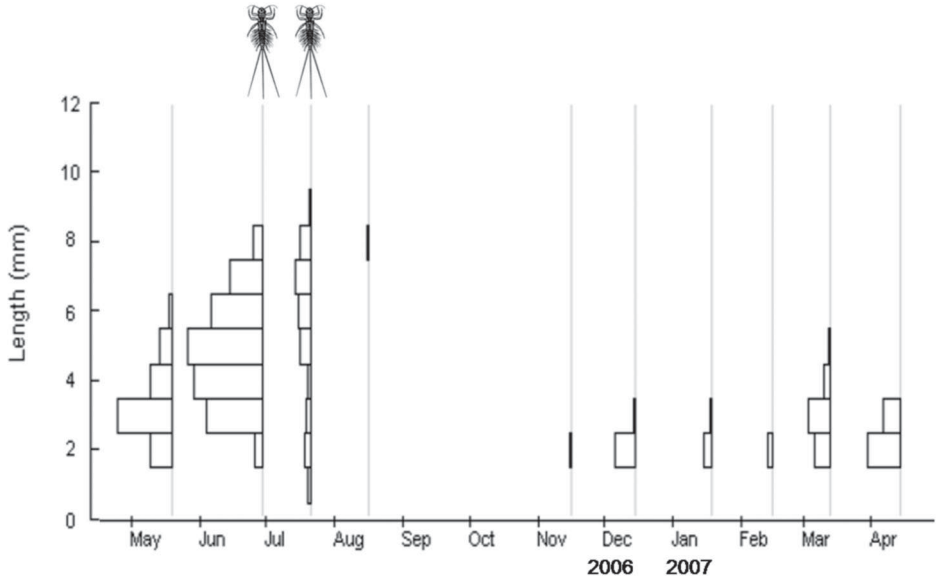

Figure 2. Size-frequency graph representing the life cycle of *Habrophlebia eldae* at the sampling site ($N = 588$). Nymph drawings represent presence of mature nymphs, with black wingpads.

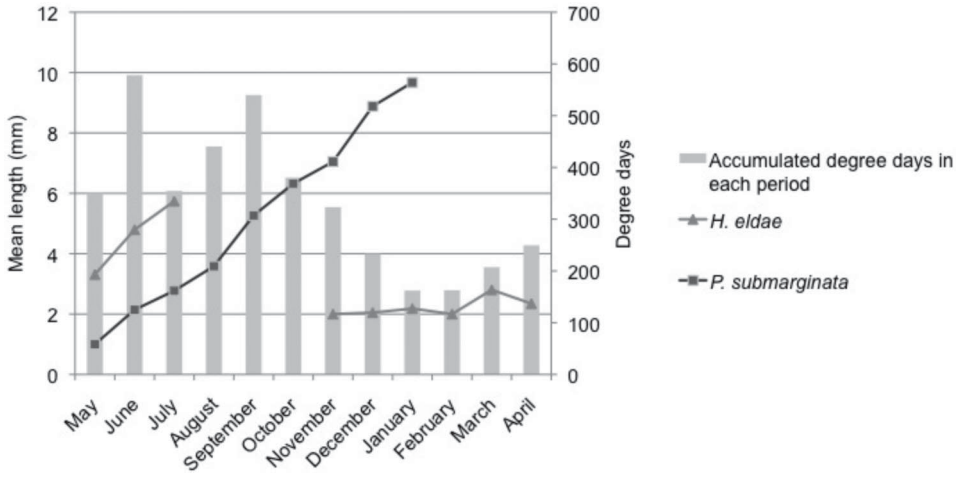

Figure 3. Growth patterns of an average cohort of *Habrophlebia eldae* and *Paraleptophlebia submarginata* and accumulated degrees day between two consecutive sampling dates during the sampling period.

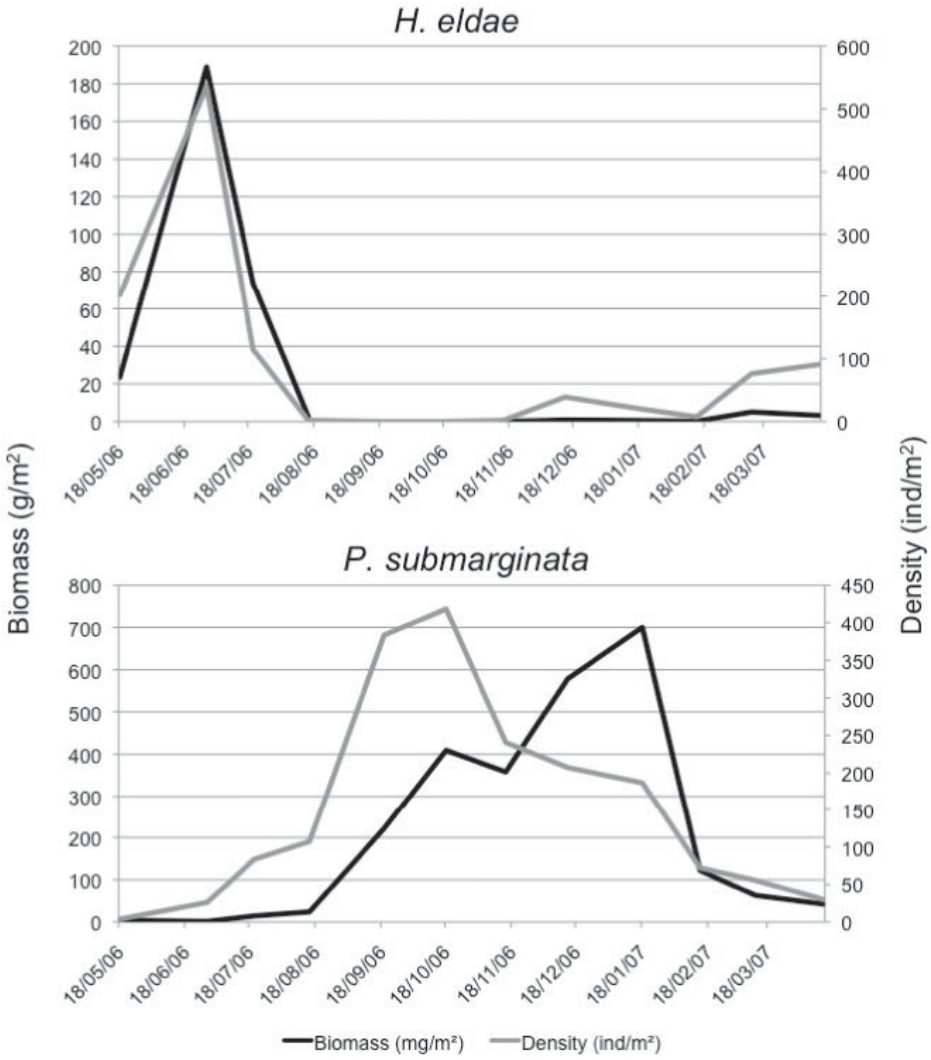

Figure 4. Monthly density and biomass of *Habrophlebia eldae* and *Paraleptophlebia submarginata* during the sampling period.

months (Fig. 2). During this period, the main cohort accumulated 2623 degrees day. Development took place between a mean daily temperature of 2 and 18 °C (Fig. 1). Growth rate was null during the winter months, increased rapidly at the beginning of spring, and was especially noticeful in summer (Fig. 3). Density of individuals and biomass were maximal at the end of the nymphal development (Fig. 4).

The life cycle of *P. submarginata* was also univoltine, with nymphs present in the stream from May to April, probably representing several cohorts. Nymphal development of an average cohort lasted approximately nine months (from May to January). Egg hatching started in May and the maximum nymphal size was reached in January, probably representing

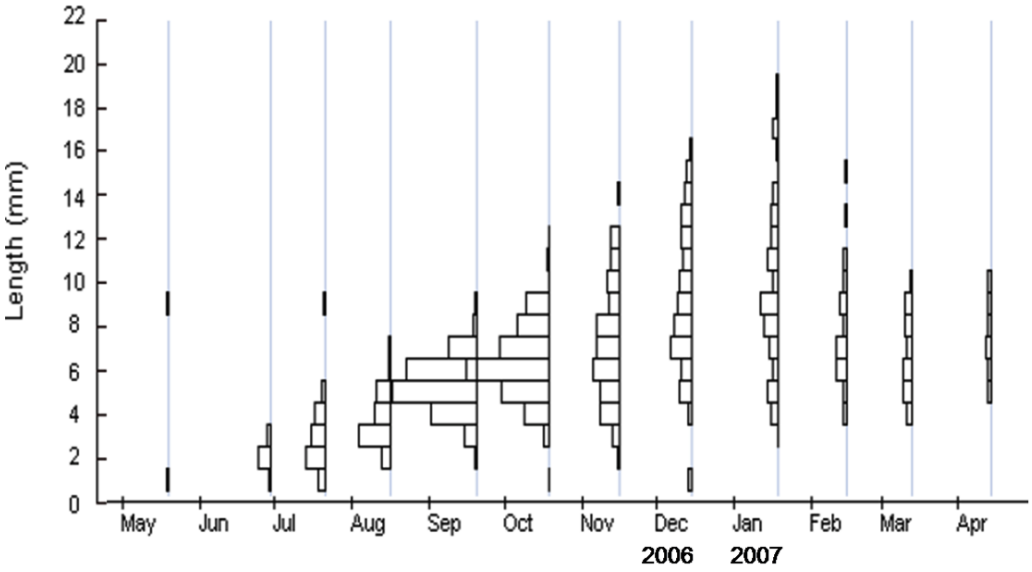

Figure 5. Size-frequency graph representing the life cycle of *Paraleptophlebia submarginata* at the sampling site ($n = 980$).

Table 2. Secondary production parameters of *Habrophlebia eldae* and *Paraleptophlebia submarginata* at the sampling site.

Species	Secondary production (g DW m^{-2})	CPI (months)	Annual secondary production (g DW m^{-2} $year^{-1}$)	Annual P/B ($year^{-1}$)	Cohort P/B
H. eldae	0.13	9	0.17	6.97	5.23
P. submarginata	1.46	9	1.95	9.21	6.91

the beginning of the flight period, although no mature nymphs were recorded in this case (Fig. 5). After this peak, smaller nymphs were still captured from February to April. The total amount of accumulated degrees day by the average cohort from May to January was 3364. Development also occurred between a mean daily temperature of 2 and 18 °C. Growth rate was approximately constant from May to January (Fig. 3). The highest density of individuals took place in October, while the highest biomass was reached in January, coinciding with the beginning of the flight period (Fig. 4).

For the secondary production studies, we estimated the cohort production interval (CPI) of both species in 9 months, representing in each case an average cohort. The annual secondary production of *H. eldae* was equal to 0.17 g DW m^{-2} $year^{-1}$, while the one of *P. submarginata* was 1.95 g DW m^{-2} $year^{-1}$ (Table 2). The cohort production/biomass ratio (P/B) was equal to 5.23 for *H. eldae* and to 6.91 for *P. submarginata*. Both species presented also an approximately similar annual P/B (6.97 and 9.21 for *H. eldae* and *P. submarginata*, respectively).

3.2. Gut Content Analyses and Trophic Basis of Production

Both species fed mainly on detritus and, to a very lesser degree, on coarse particulate organic matter (CPOM). Some other components had sparce representation in the guts of either species (Table 3). When we studied the correlation between percentage of gut con-

Table 3. Nymphal gut contents of *Habrophlebia eldae* and *Paraleptophlebia submarginata* at the sampling site.

	H. eldae				*P. submarginata*			
	N	Mean	*SD*	Min–Max	N	Mean	*SD*	Min–Max
% absolute	196	59.08	27.10	0–100	294	66.62	23.20	0–100
% detritus	176	89.40	14.51	5–100	284	87.62	13.42	0–100
% diatoms	176	1.29	6.96	0–88	284	0.14	1.00	0–10
% hyphae	176	0.45	1.10	0–6	284	0.96	2.83	0–40
% fungi spores	176	0.45	1.11	0–8	284	0.39	1.10	0–10
% CPOM	176	7.43	9.33	0–60	284	9.30	9.99	0–50
% pollen	176	0.43	1.13	0–6	284	1.02	3.23	0–20

Table 4. Gamma correlations between total length and the percentage of the different food items (animal matter not included) in *Habrophlebia eldae* and *Paraleptophlebia submarginata*. Values marked with an asterisk are significant at $P < 0.05$.

	H. eldae total length (mm)	*P. submarginata* total length (mm)
% absolute	–0.23*	0.08
% detritus	–0.47*	–0.47*
% diatoms	0.14	0.26
% hyphae	0.51*	0.48*
% fungi spores	0.37*	0.30*
% CPOM	0.51*	0.41*
% pollen	0.62*	–0.05

Table 5. Estimated contribution of each component of the diet to production in *Habrophlebia eldae* and *Paraleptophlebia submarginata*.

	H. eldae		*P. submarginata*	
	Production attributed to food type (%)	Production attributed to food type (g/m^2 year)	Production attributed to food type (%)	Production attributed to food type (g/m^2 year)
detritus	84.64	0.144	85.56	1.668
diatoms	3.66	0.006	0.41	0.008
hyphae	2.13	0.004	2.81	0.055
fungi spores	2.13	0.004	1.14	0.022
CPOM	7.03	0.012	9.08	0.177
pollen	0.41	0.001	1.00	0.019

tents and size we observed that, in *H. eldae,* detritus was less consumed by larger nymphs, which incorporated a greater quantity of pollen, hyphae, CPOM and fungi spores (Table 4). We noted that larger *P. submarginata* also decreased the intake of detritus when larger and increased the percentage of hyphae, CPOM and fungi spores.

Much of the secondary production (>84%) of both species was due to detritus intake (Table 5). CPOM was the second more important contributor to production, though this resource contributed much more to production in *P. submarginata* (Table 5).

3.3. Niche Breadth and Overlap

Niche breadth values were very low ($B_A = 0.046$ for *H. eldae* and $B_A = 0.055$ for *P. submarginata*). The niche overlap between both species in terms of trophic resources was almost total, with a Simplified Morisita Index (C_H) equal to 0.99.

4. Discussion

4.1. Life Histories and Secondary Production Estimates

The two species studied show a univoltine "slow seasonal" life cycle, following the classification by HYNES (1970). The hatching period of *H. eldae* seems to be relatively prolonged, and this could lead to a wide range of sizes classes present in June and July (Fig. 2). Nymphs of first instars hatching during winter did not grow in this period (Fig. 3), probably due to the low temperatures (Fig. 1). Later, when temperatures started rising, growth rate was high. From the records of mature nymphs we estimate that the flight period was short and concentrated in June and July. The only one nymph captured in August represented an individual with delayed development. In contrast the hatching period of *P. submarginata* started in May and June, although further recruitment seemed to happen throughout the nymphal development (Fig. 5). This was reflected in the presence of mid-size nymphs (after the peak of January, when emergence probably started), representing individuals of several cohorts. Growth of the average cohort was almost constant during the nymphal development (Fig. 3). No clear differences between the period of the nymphal development that took place in spring-summer and the one that took place during autumn-winter were detected. Thus, growth in this species seems to be relatively independent of the temperature.

A similar life cycle for *P. submarginata* was found by ALBA-TERCEDOR (1981) in a close mountain system stream with similar characteristics. This author found nymphs from October to June, with a peak in size in February and March. Nevertheless, this author found no mature nymphs until late April and June, and those were smaller in size than those of the peak. In comparison, the population studied by us had an advanced phenology. In our study, the flight period started in January, but probably extended until April. LANDA (1968) also found a univoltine life cycle in central European populations of this species, but with nymphs reaching maturity in autumn, and extended growing until the spring, when the adults emerged. This latter author classified to *P. submarginata* in the "A1" group (*i.e.*, species whose eggs hatch approximately one month after oviposition and nymphs grow until emergence, which occurs in spring or summer, depending on the altitude). Our population would not completely fit within this or other groups. SOWA (1975) pointed out that nymphs of this species were present in central European streams from August to April–May, when emergence occurred. SOWA (1975) grouped them in the category "B2" (*i.e*., species in which embryonal development of eggs laid in spring, summer or early autumn proceeds without a quiescent period). The young nymphs appear within one to two months, have slow growth

during the season, and even slower growth during winter, though again our population would not fit on this group. In the British Isles this species also displays a univoltine life cycle with overwintering nymphs and adults are present from April to July (Elliott and Humpesch, 1983; Elliott *et al*., 1988). On the other hand, in southern France the flight period extends from March to November (Lavandier and Dumas, 1971). Welton *et al*. (1982), in a experimental stream with temperatures oscillating between 8.1 and 16.8 °C, found a life cycle of seven months, higher growth rates during summer, and an uncertain number of cohorts as in our studied population.

The presence of several size classes of both species at the same time is a common strategy in organisms inhabiting unpredictable aquatic environments, such as those that dry during the aestival period or those that suffer floods during some seasons of the year. Though the stream did not suffer any flood event, during sampling, some years ago the stream flow increased greatly, even modifying its physiognomy (Luzón-Ortega, pers. com.). Thus a wide range of sizes, cohabiting at the same time (more notable in *P. submarginata*), would increase the success of these populations. This asynchronous nymphal development has been found in other studies under different environmental conditions (*e.g.*, Salas and Dudgeon, 2003).

There was a difference in the values of annual secondary production of the studied species, being higher in *P. submarginata*, in spite of having a similar average nymphal development period duration, *i.e*., a similar CPI. This is probably related to its considerably larger size. Nevertheless, if we observe the annual and the cohort P/B we realize that they were also higher, although only slightly, in *P. submarginata*, indicating that this species had a higher biomass turnover than *H. eldae*. The annual secondary production of both species fits within the range of values reported for leptophlebiids (see González *et al*., 2003), but that of *P. submarginata* is particularly high.

4.2. Nymphal Feeding and Trophic Basis of Production

In regard to the feeding behaviour, both species were collector-gatherers, fed mainly on detritus, and had a minor proportion of CPOM in their guts (Table 3). This could be due to a minor shredder role of both species or simply to their capability of ingesting larger particles of detritus when they are also larger. Pleskot (1953) already had pointed out that *P. submarginata* fed collecting detritus, as has been cited for other leptophlebiids (Elliott *et al*., 1988), and concretely for other *Paraleptophlebia* spp. (Shapas and Hilsenhoff, 1976; Mattingli, 1987). We also observed that when the nymphs were larger they fed less on detritus and more on other components, such as CPOM from leaves fallen in the stream and fungi, probably due to their higher shredding power. It is noteworthy that when the percentage of CPOM ingested is higher, the percentage of fungi hyphae and spores is also higher. This probably reflects the biofilm present on the leave's surface. Some of this resource is difficult to assimilate but may contribute to shredder nutrition (Allan and Castillo, 2007). Pollen was mainly consumed by larger nymphs of *H. eldae*, probably because of its coincidence with the flowering season (spring-summer in the study site) of the Angiosperms present in nearby areas.

In spite of having low assimilation efficiencies (Benke and Jacobi, 1994), detritus and CPOM were the two major resources contributing to production in both species (though detritus was much more important in their diets). The net contributions of CPOM seemed particularly high in *P. submarginata*, but were similar in both species in terms of percentage. The remaining components of the diet of these species contributed relatively little to secondary production despite their high assimilation efficiencies. This is due to their little importance on overall diet of both species. Thus growth would be mainly conditioned, from the feeding point of view, by detritus and CPOM intake. Nevertheless more must be known about digestion processes in these species before further conclusions can be presented.

4.3. Niche Breadth and Overlap

We found that both species had very low values of niche breadth in terms of trophic resources, indicating that they fed on few resources (mainly detritus, but also CPOM). Moreover, they showed a very high niche overlap in terms of food resources. So, they fed on the same few resources (mainly detritus and CPOM), creating some competition for food if the resources were limiting. Nevertheless, when they cohabited and were in different developmental stages and sizes, it is probable that the possible resource competition would be lower, because one species would be feeding mainly on detritus (mentioned before for smaller nymphs) whereas the other would be feeding mainly on CPOM.

4.4. Final Remarks

Some aspects of the biology of closely related, and coexisting species at the same site may be modified as consequence of direct competition for food and/or space. The relationship between competition and niche overlap is complex (HOLT, 1987). Trophic niche overlap does not always imply competition, and resources may not always be limiting for populations (ABRAMS, 1980). Nevertheless, niche overlap is a good index of resource sharing between species. In our study two closely related species, cohabiting in the same site, also had low values of niche breadth and a high niche overlap, due to their approximately same use of food resources. As it was previously noted, there was no overlap of size classes between species at the same time, and it seems that the fitness of one of them, *P. submarginata*, was slightly higher than that of the other, taking into account the secondary production analysis results. Thus, it is possible, although not certain, that the displacement of the nymphal development of one species with respect to the other was related to a lower competition for food resources. Considering that the same size classes of both species fed on the same resources, an interspecific competition between them could occur if their nymphal development coincided. The pointed shifts in diet composition among size classes could result in considerable changes in microhabitats, enabling a different habitat choice among the different size classes of both species, as noted by BAEKKEN (1981) for other mayflies. BAEKKEN (1981) thought this could reduce the interaction between nymphal stages within the same species and between two coexisting detritivores species, as seems to be the case in our study. This explanation could be also applied for interpreting the existence of a great range in nymphal size (ARNEKLEIV, 1996), as we found in our study. Our results also support the conclusions drawn by GONZÁLEZ *et al*. (2003). These authors found that two Leptophlebiidae species cohabiting in the same stream showed clear spatial segregation and the authors hypothesized that, even if the different species had coexisted in the same site, temporal segregation in resource use would have reduced interspecific competition.

A clear effect of one species on other is illustrated by BRITTAIN (1982), who also found that two *Leptophlebia* species with essentially similar life cycles throughout Europe, had displaced their life cycles out of step when they occurred together. These two species also presented differences in size at maturity, similar to what we found in our studies. Thus, the requirements of the species studied by us, in terms of developmental temperature needs, food utilization, *etc*., are surely important factors governing life cycle strategies in different study areas. Nonetheless, we cannot ignore the effect that a possible interaction between them could have had on their biology. Although now the possible competition between these two species has been reduced in the way previously discussed, it is possible that these strategies reflect ancient episodes of competition among them. Further studies under controlled conditions should add more light on this topic.

5. Acknowledgements

We want to thank Hydraena S.L.L. personnel for the analysis of the physicochemical samples, Dr. Kenton M. Stewart for his kindness and support, and two anonymous reviewers that greatly improved the original manuscript. This work has been supported by the European research project "Eurolimpacs" (GOCE-CT-2003-505540).

6. References

Abrams, P., 1980: Some comments on measuring niche overlap. – Ecology **61**: 44–49.

Alba-Tercedor, J., 1981: Efemerópteros de Sierra Nevada: ciclos de desarrollo, taxonomía y ecología de las ninfas. – Ph.D. Thesis. University of Granada, Granada. Unpublished.

Allan, J. D. and M. M. Castillo, 2007: Stream Ecology. Structure and function of running waters. Second edition. – Springer, Dordrecht.

Arnekleiv, J. V., 1996: Life cycle strategies and seasonal distribution of mayflies (Ephemeroptera) in a small stream in Central Norway. – Fauna Norv., Ser. B **43**: 19–30.

Baekken, T., 1981: Growth patterns and food habits of *Baetis rhodani*, *Capnia pygmea* and *Diura nanseni* in a West Norwegian river. – Hol. Ecol. **4**: 139–144.

Bello, C. L. and M. I. Cabrera, 1999: Uso de la técnica microhistológica de Cavender y Hansen en la identificación de insectos acuáticos. – Bol. Entomol. Venez. **14**: 77–79.

Benke, A. C., 1979: A modification of the Hynes method for estimating secondary production with particular significant for multivoltine population. – Limnol. Oceanogr. **24**: 168–174.

Benke, A. C. and J. B. Wallace, 1980: Trophic basis of production among net-spinning caddisflies in a southern Appalachian stream. – Ecology **61**: 108–118.

Benke, A. C., 1984: Secondary production of aquatic insects. – *In:* Resh, V. H. and D. M. Rosenberg (eds.): The ecology of aquatic insects, pp. 289–322, Praeger, New York.

Benke, A. C. and D. I. Jacobi, 1994: Production dynamics and resource utilization of snag-dwelling mayflies in a blackwater river. – Ecology **75**: 1219–1232.

Benke, A. C. and A. D. Huryn, 2006: Secondary production of macroinvertebrates. – *In*: Hauer, F. R. and G. A. Lamberti (eds.): Methods in Stream Ecology. Second edition, p. 691–710, Academic Press, New York.

Brittain, J. E., 1972: The life cycles of *Leptophlebia vespertina* (L.) and *L. marginata* (L.) (Ephemeroptera) in Llyn Dinas, North Wales. – Freshwat. Biol. **2**: 271–277.

Brittain, J. E., 1980: Mayfly strategies in a Norwegian subalpine lake. – *In:* Flannagan, J. F. and K. E. Marshall (eds.): Advances in Ephemeroptera Biology, pp. 179–186, Plenum Press, New York.

Brittain, J. E., 1982: Biology of mayflies. – Annu. Rev. Entomol. **27**: 119–147.

Cummins, K. W., 1973: Trophic relations of aquatic insects. – Annu. Rev. Entomol. **18**: 183–206.

Elliott, J. M. and U. H. Humpesch, 1983: A key to the adults of the British Ephemeroptera with notes on their ecology. – Freshwater Biological Association Scientific Publication No. 47, Ambleside, Cumbria.

Elliott, J. M., U. H. Humpesch and T. T. Macan, 1988: Larvae of the British Ephemeroptera: A key with ecological notes. – Freshwater Biological Association Scientific Publication No. 49, Ambleside, Cumbria.

Fenoglio, S., T. Bo, J. M. Tierno de Figueroa and M. Cucco, 2008: Nymphal growth, life cycle, and feeding habits of *Potamanthus luteus* (Linnaeus, 1767) (Insecta: Ephemeroptera) in the Bormida River, Northwestern Italy. – Zool. Stud. **47**: 185–190.

Gayanilo, Jr., F. C., P. Sparre and D. Pauly, 2002: FiSAT II (ver. 1.2.0.). – Food and Agriculture Organization of the United Nations (FAO) (www.fao.org/fi/statist/fisoft/fisat/index.htm).

Giller, P. S. and B. Malmqvist, 1998: The biology of stream and rivers. – Oxford University Press, Oxford.

González, J. M., A. Basaguren and J. Pozo, 2003: Life history, production and coexistence of two leptophlebiid mayflies in three sites along a Northern Spain stream. – Arch. Hydrobiol. **158**: 303–316.

Guisande González, C., A. Barreiro Felpeto, I. Maneiro Estraviz, I. Riveiro Alarcón, A. R. Vergara Castaño and A. Vaamonde Liste, 2006: Tratamiento de datos. – Ediciones Díaz de Santos, Madrid.

HAMILTON, A. L., 1969: On estimating annual production. – Limnol. Oceanogr. **14**: 771–782.
HAUER, F. R. and J. A. STANFORD, 1982: Ecology and life histories of three net-spinning caddisfly species (Hydropsychidae. Hydropsyche) in the Flathead River, Montana. – Freshwat. Invertebr. Biol. **1**: 18–29.
HAUER, F. R. and J. A. STANFORD, 1986: Ecology and coexistence of two species of *Brachycentrus* (Trichoptera) in a Rocky Mountain river. – Can. J. Zool. **64**: 1469–1474.
HOLT, R. D., 1987: On the relation between niche overlap and competition: the effect of incommensurable niche dimensions. – Oikos **48**: 110–114.
HORN, H. S., 1966: Measurement of "overlap" in comparative ecological studies. – Am. Midl. Nat. **100**: 419–424.
HURBERT, S. H., 1978: The measurement of niche overlap and some relatives. – Ecology **59**: 67–77.
HURYN, A. D. and J. B. WALLACE, 2000: Life history and production of stream insects. – Annu. Rev. Entomol. **45**: 83–110.
HYNES, H. B. N. and M. J. COLEMAN, 1968: A simple method of assessing the annual production of stream benthos. – Limnol. Oceanogr. **13**: 569–573.
HYNES, H. B. N., 1970: The ecology of running waters. – University of Toronto Press, Toronto.
LANDA, V., 1968: Developmental cycles of Central European Ephemeroptera and their interrelations. – Acta entomol. Bohemoslov. **65**: 276–284.
LAVANDIER, P. and J. DUMAS, 1971: Cycles de developpement de quelques invertébrés benthiques dan des ruisseaux des Pyrénées Centrales. – Ann. Limnol. **7**: 157–172.
LEVINS, R., 1968: Evolution in changing environments: some theoretical explorations. – Princeton University Press, Princeton, New Jersey.
LÓPEZ-RODRÍGUEZ, M. J., J. M. TIERNO DE FIGUEROA and J. ALBA-TERCEDOR, 2008: Life history and larval feeding of some species of Ephemeroptera and Plecoptera (Insecta) in the Sierra Nevada (Southern Iberian Peninsula). – Hydrobiol. **610**: 277–295.
LÓPEZ-RODRÍGUEZ, M. J., J. M. TIERNO DE FIGUEROA and J. ALBA-TERCEDOR, 2009a: The life history of *Serratella ignita* (PODA, 1761) (Insecta: Ephemeroptera) in a temporary and permanent Mediterranean stream. – Aquat. Sci. **71**: 179–188.
LÓPEZ-RODRÍGUEZ, M. J., J. M. TIERNO DE FIGUEROA and J. ALBA-TERCEDOR, 2009b: Life history of two burrowing aquatic insects in Southern Europe: *Leuctra geniculata* (Insecta: Plecoptera) and *Ephemera danica* (Insecta: Ephemeroptera). – Aquat. Insect. **31**: 99–110.
MATTINGLI, R. L., 1987: Handling of coarse and fine particulate organic matter by the aquatic insects *Paraleptophlebia gregalis* and *P. temporalis* (Ephemeroptera: Leptophlebiidae). – Freshw. Biol. **18**: 255–265.
MERRITT, R. W. and K. W. CUMMINS, 2006: Trophic relations of macroinvertebrates. – *In:* HAUER, F. R. and G. A. LAMBERTI (eds.): Methods in Stream Ecology. Second edition, pp. 585–610, Academic Press, New York.
PLESKOT, G., 1953: Zur Ökologie der Leptophlebiiden (Ephemeroptera). – Öster. Zool. Zeitschrift **4**: 45–107.
RODIER, J., 1998: Análisis de las aguas. – Ediciones Omega, Barcelona.
SALAS, M. and D. DUDGEON, 2003: Life histories, production dynamics and resource utilisation of mayflies (Ephemeroptera) in two tropical Asian forest streams. – Freshw. Biol. **48**: 485–499.
SHAPAS, T. J. and W. L. HILSENHOFF, 1976: Feeding habits of Wisconsin's predominant lotic Plecoptera, Ephemeroptera, and Trichoptera. – Great Lakes Entomol. **9**: 175–188.
SOWA, R., 1975: Ecology and biogeography of mayflies (Ephemeroptera) of running waters in the Polish part of the Carpathians. 2. Life cycles. – Acta hydrobiol. **17**: 319–353.
StatSoft, Inc., 2005: STATISTICA (data analysis software system), version 7.1. (www.statsoft.com).
SWEENEY, B. W., 1984: Factors influencing life-history patterns of aquatic insects. – *In*: RESH, V. H. and D. M. ROSEMBERG (eds.): The ecology of aquatic insects, p. 56–100, Praeger, New York.
THOMAS, A. and C. BELFIORE (coord.), 2004: Fauna Europaea: Ephemeroptera. Fauna Europaea version 1.1. (http://www.faunaeur.org/full_results.php?id=11217)
WELTON, J. S., M. LADLE and J. A. B. BASS, 1982: Growth and production of five species of Ephemeroptera larvae from an experimental recirculating stream. – Freshw. Biol. **12**: 103–122.

Manuscript submitted November 5th, 2008; revised August 25th, 2009; accepted September 21st, 2009

