

Life history of two burrowing aquatic insects in southern Europe: *Leuctra geniculata* (Insecta: Plecoptera) and *Ephemera danica* (Insecta: Ephemeroptera)

Manuel J. López-Rodríguez*, J. Manuel Tierno de Figueroa and Javier Alba-Tercedor

Departamento de Biología Animal, Facultad de Ciencias, Universidad de Granada, Granada, Spain

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Burrowing is a common behavioural adaptation of lotic freshwater invertebrates to avoid the effects of current. This behaviour is accompanied by morphological adaptations. This also applies to the larvae of the stonefly *Leuctra geniculata* (Stephens, 1836) and the mayfly *Ephemera danica* Müller, 1764, both colonising habitats within the substrate and adapted to burrow in it. We have studied their life cycles and their relation with water temperature and day-degrees, feeding and secondary production. The stonefly had a univoltine life cycle with a larval development of 8 months and with an egg incubation period longer than previously reported. The possibility of an egg diapause stage is discussed. The mayfly was semivoltine, completing its larval development in 22 months. Both species mainly fed on detritus, but also ingested a high quantity of CPOM and some other minor components. Annual secondary production in both species was relatively high, being higher in the stonefly.

Keywords: stonefly; *Leuctra geniculata*; mayfly; *Ephemera danica*; life cycle; feeding; secondary production; southern Iberian Peninsula

Introduction

Current is the most significant characteristic of running waters (Hynes 1970; Giller and Malmqvist 1998) exerting evolutive pressure on animals living in lotic environments. Freshwater invertebrates respond to it in several related ways. One of them is by means of morphological adaptations, such as flattened forms, streamlining, the developing of attachment structures or mechanisms such as sucker-like attachment devices, hooks, silk, or sticky secretions, among others. Additionally, several behavioural adaptations exist, such as the reduction or avoidance of flow exposure (Hynes 1970). As a consequence, many aquatic insects spend at least a part of their life deep in the substratum. These burrowing macroinvertebrates perform an important role because their activities help to redistribute particles and fluid in the sediment–water interface, strongly influencing the physical, chemical, and microbiological proprieties of the sedimentary deposits in which they live (Charbonneau and Hare 1998).

In our study we have focused on two burrowing species of aquatic insects, *Leuctra geniculata* (Stephens, 1836) (Plecoptera: Leuctridae) and *Ephemera danica* Müller, 1764 (Ephemeroptera: Ephemeridae). The stonefly *L. geniculata* is distributed in west, south, and central Europe, including the British Islands, and northern Africa (Tierno de

*Corresponding author. Email: manujlr@ugr.es

Figuroa, Sánchez-Ortega, Membiela Iglesias and Luzón Ortega 2003; Fochetti and Tierno de Figuroa 2004). The mayfly *E. danica* is widely distributed in the West Palaearctic region (Thomas and Belfiore 2004). The larvae of both species burrow deeply under stones and/or in the substrate (Hynes 1976; Consiglio 1980; Elliott, Humpesch and Macan 1988). They have certain morphological adaptations that enable them to live there. In *L. geniculata* the main modifications concern the antennae. Lestage (1920) has suggested that the peculiar processes on the antennae, which distinguish the larvae of this species from others of the genus, are an adaptation for burrowing. *E. danica* possesses modified mouthparts and particular processes on the head and prothorax that facilitate burrowing. Numerous hairs help to keep fine particles of the surroundings away from its body, in order to prevent smothering (Hynes 1970). The larvae build U-shaped tunnels and create water current with the aid of their gills (Eastham 1939; Ladle and Radke 1990).

Previous studies on the life cycle of *L. geniculata* have recorded a univoltine life cycle (Hynes 1941; Neveu, Lapchin and Vignes 1979; Elliott 1987b; Ferreras-Romero and Agüero-Pelegrín 1994; Bojková, Špaček and Helešic 2008). For *E. danica*, the life cycle pattern cited by several authors is variable and ranges from being univoltine to semivoltine, or even lasting for three years (e.g. Landa 1968; Sowa 1975; Otto and Svensson, 1981; Elliott et al. 1988; Alba-Tercedor 1990; Aguayo-Corraliza, Ferreras-Romero and Puig-García 1991). The authors who studied the larval feeding of *L. geniculata* reported that this species fed on detritus, leaf fragments, green algae, and diatoms (Hynes 1941; Jones 1949; Azzouz and Sánchez-Ortega 2000). On the other hand, *E. danica* has been shown to feed mainly on detritus, but the way the food is obtained is not clear, and thus it has been placed among different functional feeding groups (Otto and Svensson 1981; Elliott et al. 1988; Ladle and Radke 1990; Merritt and Cummins 2006). In relation to secondary production the studies are much scarcer. To our knowledge, only data for a single population of *E. danica* are available (Tokeshi 1985).

Thus, the aim of this study is to increase the knowledge of some aspects of the larval biology of these two burrowing species in a stream where both coexist, such as their life cycles and the influence of temperature on them, larval feeding and secondary production, and to compare the results with the existing literature.

Materials and methods

The study was carried out in the Río Fardes (Sierra de Huétor, Granada, Spain; UTM: 30SVG465413, 1200 m a.s.l.), a typical Mediterranean stream with permanent regime. In the sampling station, 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. The substrate was mainly represented by a 15% of silt, 35% of pebbles and 50% of sands. Submerged vegetation was composed by *Nasturtium* sp. and Characeae. The riverine vegetation was abundant and principally represented by Juncaceae or Ciperaceae, *Salix* sp., Poaceae, *Equisetum* sp., *Mentha* sp. and some *Carduus* sp.

Samplings were carried out monthly from May 2006 to April 2007. A data logger (HOBO[®] Water Temp Pro, 0.001°C accuracy) was placed in the riverbed for registering the temperature hourly, thereby calculating the accumulated day-degrees between two sampling dates (Figure 1). On every sampling date we recorded physical parameters *in situ* (oxygen, conductivity, discharge) and transported one litre of cold-preserved water to the laboratory to analyse some physicochemical parameters (Table 1).

Specimens of both species were collected with a Surber sampler (0.09 m² area and 250 µm mesh size). The substrate was removed with the aid of a rake reaching an

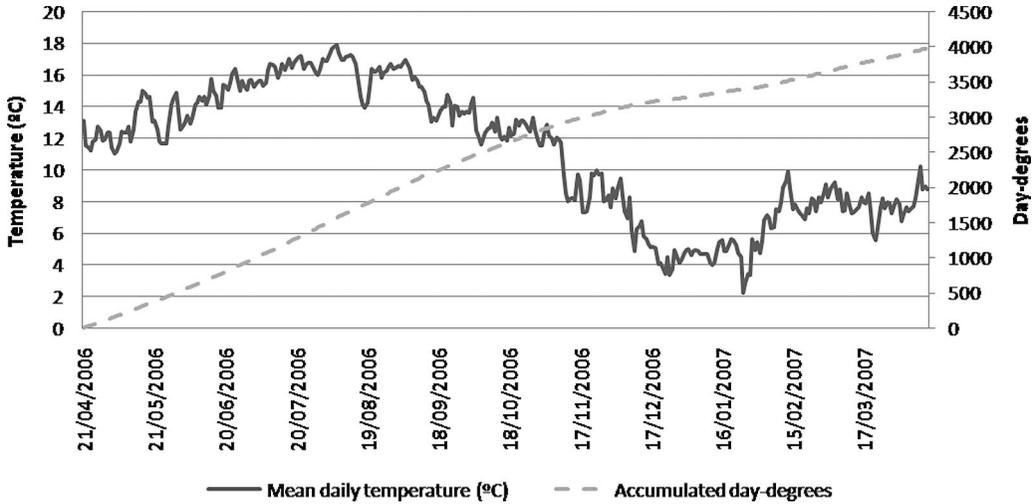


Figure 1. Mean daily temperature and accumulated day-degrees at the sampling site during the sampling period.

Table 1. Physicochemical parameters at the sampling site.

	Fardes stream				
	N	Mean	SD	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

approximate mean depth of 20 cm. Six replicates were taken to represent the different mesohabitats of the sampling site. They were preserved in 4% formalin and carried to the laboratory, where they were sieved with a 150 µm mesh size sieve in order to remove the excess formalin and fine detritus. Afterwards, organisms were sorted out and identified to species level.

Each month we measured the total length and width of pronotum of 30 specimens using the micrometer of a binocular microscope (0.01 mm accuracy). As these two

measurements were highly correlated (with a Gamma correlation of 0.91 for *L. geniculata* and 0.94 for *E. danica*; $p < 0.05$ in both cases), we used the total length to represent the life cycle of the studied species. They were classified into different size classes with 1 mm intervals. Measurements were standardised by placing each specimen between two slides. We used FiSAT II software (Gayaniolo et al. 2002) to generate the size–frequency graphs representing the life cycles.

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

The diet study was performed according to the methodology of Bello and Cabrera (1999) that was also used in other studies dealing with aquatic insect feeding (e.g. Tierno de Figueroa, Vera and López-Rodríguez 2006; Fenoglio, Tierno de Figueroa and Cucco 2008; López-Rodríguez, Tierno de Figueroa and Alba-Tercedor 2008). We used the same 30 individuals per month that had been previously measured to study the correlation between total length and pronotum width. Each individual was introduced into a vial with Hertwig's liquid and heated in an oven at 65°C for approximately 24 hours. Afterwards they were mounted on slides to study them under the microscope. We estimated the percentage of the absolute gut content (at 40 x) as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400 x) as the area occupied for 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, when possible, 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 larval size and percentage of the different gut contents.

Secondary production was calculated by mean of the size–frequency method (Hynes and Coleman 1968; Hamilton 1969; Benke 1979; Benke and Huryn 2006), due to the presence of many size classes at the same date. Estimation of larval 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.

To calculate the regression equation, 30 specimens of each species preserved in formaldehyde were measured, dried at 60°C for 24 hours, and placed in a desiccator for one hour. Then they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance.

For statistical analysis, STATISTICA software (StatSoft 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For election of the proper statistical tests, we followed Guisande González et al. (2006).

Results

Leuctra geniculata

The larval development of *L. geniculata* extended from February to September, taking approximately 8 months for its completion (Figure 2). This clearly points to a univoltine

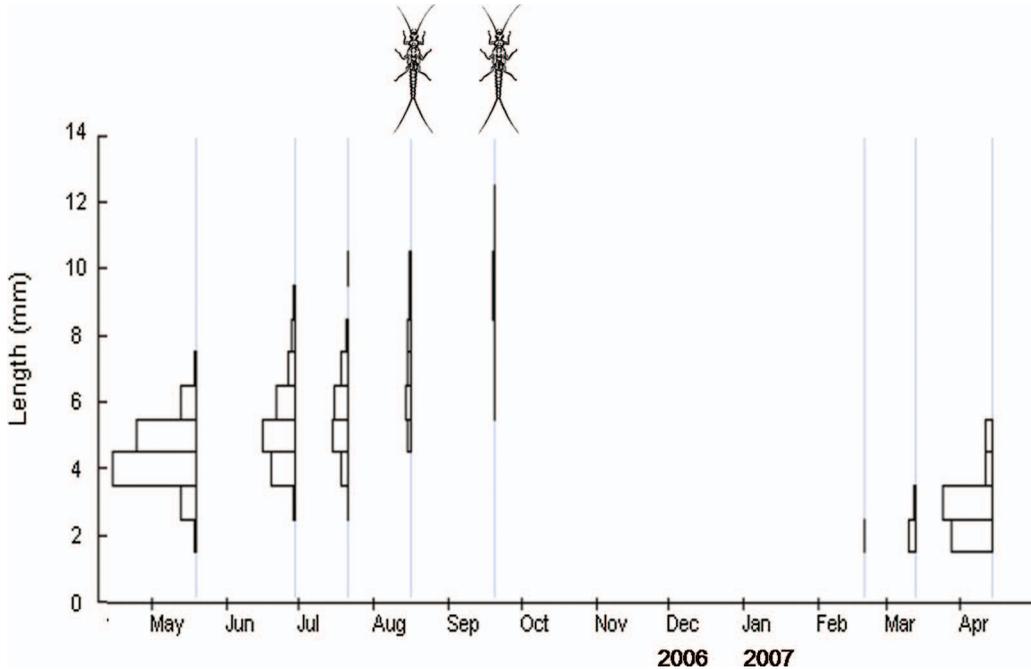


Figure 2. Size–frequency graph representing the life cycle of *Leuctra geniculata* at the sampling site ($N = 1631$). The presence of mature larvae is marked with a respective icon.

pattern. The flight period, estimated from records of mature larvae, took place in August and September. After mating and oviposition, eggs would remain in the hyporheic zone up to 5 months, when hatching would start. Growth was relatively constant, with a slight increase during the last months, coinciding with the emergence (Figure 3). For completing its larval development, *L. geniculata* accumulated a total amount of 2884.30 day-degrees, and growth took place between an approximately mean daily temperature of 5.5 and 18°C.

The larvae of *L. geniculata* mainly fed on detritus, but also ingested a great quantity of coarse particulate organic matter (CPOM) (Table 2). The other components found in their guts were scarcely represented. Less than 2% of the studied individuals contained some animal remains or sand. Some changes in diet composition appeared when the larvae were bigger. Thus, both detritus and diatoms decreased in bigger larvae, and CPOM, pollen and Cyanobacteria increased (Table 3).

Dry weight (DW) was related to body length (X) by the following equation:

$$\text{Ln DW} = -6.39 + 3.10 \text{ LnX}, (r^2 = 0.95, F_{1,28} = 492.87, p < 0.05)$$

Production parameters are summarized in Table 4. The annual secondary production of *L. geniculata* was 7.40 gDWm⁻² year⁻¹, and the cohort production/biomass ratio (P/B) was 6.77. In our calculations we used a cohort production interval (CPI) of 8 months.

Ephemera danica

The mayfly *E. danica* has a semivoltine life cycle, with two generations overlapping at the same time (Figure 4). Larvae hatched approximately in August, after the peak of annual

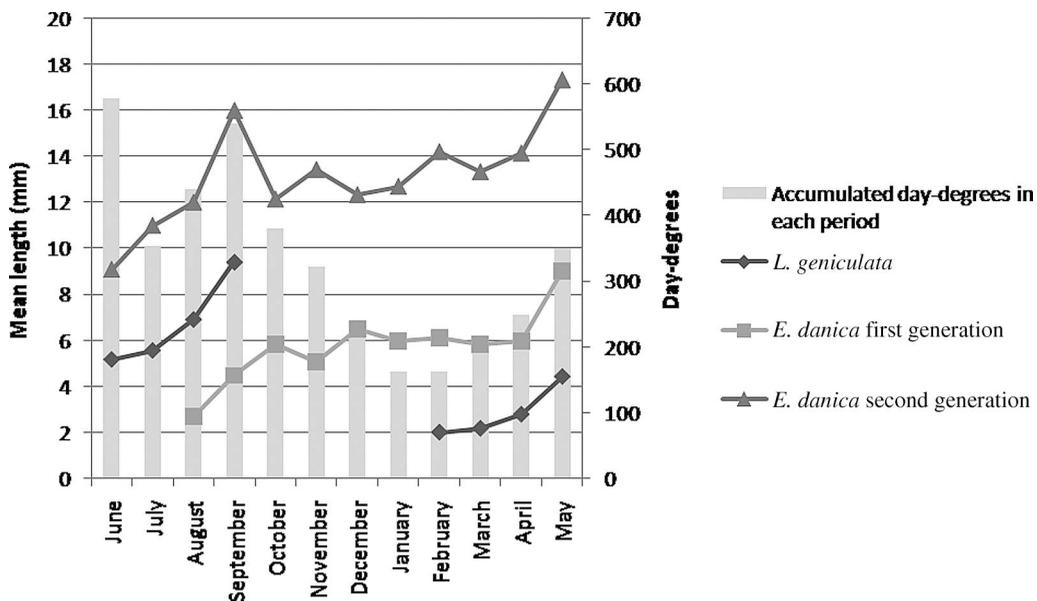


Figure 3. Growth patterns of *Leuctra geniculata* and *Ephemera danica* and accumulated day-degrees between two consecutive sampling dates during the sampling period.

Table 2. Larval gut contents of *Leuctra geniculata* and *Ephemera danica* at the sampling site.

	<i>Leuctra geniculata</i>				<i>Ephemera danica</i>			
	N	Mean	SD	Min–Max	N	Mean	SD	Min–Max
% absolute	206	65.26	29.82	0–100	303	83.40	15.93	0–100
% detritus	182	73.23	26.85	10–100	299	81.20	13.58	8–100
% diatoms	182	1.10	2.79	0–20	299	0.05	0.33	0–4
% hyphae	182	0.89	1.96	0–20	299	0.55	1.21	0–10
% fungi spores	182	0.70	3.25	0–41	299	0.30	1.09	0–10
% CPOM	182	24.27	25.37	0–88	299	17.64	12.75	0–65
% pollen	182	0.20	0.81	0–6	299	0.03	0.22	0–2
% Cyanobacteria	182	0.02	0.18	0–2	299	0.02	0.18	0–2

Table 3. Gamma correlations between total length and the percentage of the different food items (animal matter not included) in *Leuctra geniculata* and *Ephemera danica*. Values marked with an asterisk are significant at $p < 0.05$.

	<i>L. geniculata</i> total length (mm)	<i>E. danica</i> total length (mm)
% absolute	0.06	0.24*
% detritus	-0.16*	-0.47*
% diatoms	-0.40*	-0.14
% hyphae	-0.05	0.07
% fungi spores	0.06	-0.18*
% CPOM	0.21*	0.53*
% pollen	0.28*	0.04
% Cyanobacteria	0.86*	-0.37

Table 4. Secondary production parameters of *Leuctra geniculata* and *Ephemera danica* at the sampling site.

Species	Secondary production (gDWm ⁻²)	CPI (months)	Annual secondary production (gDWm ⁻² year ⁻¹)	Annual P/B (year ⁻¹)	Cohort P/B
<i>Leuctra geniculata</i>	4.93	8	7.40	10.15	6.77
<i>Ephemera danica</i>	10.24	22	5.59	2.80	5.13

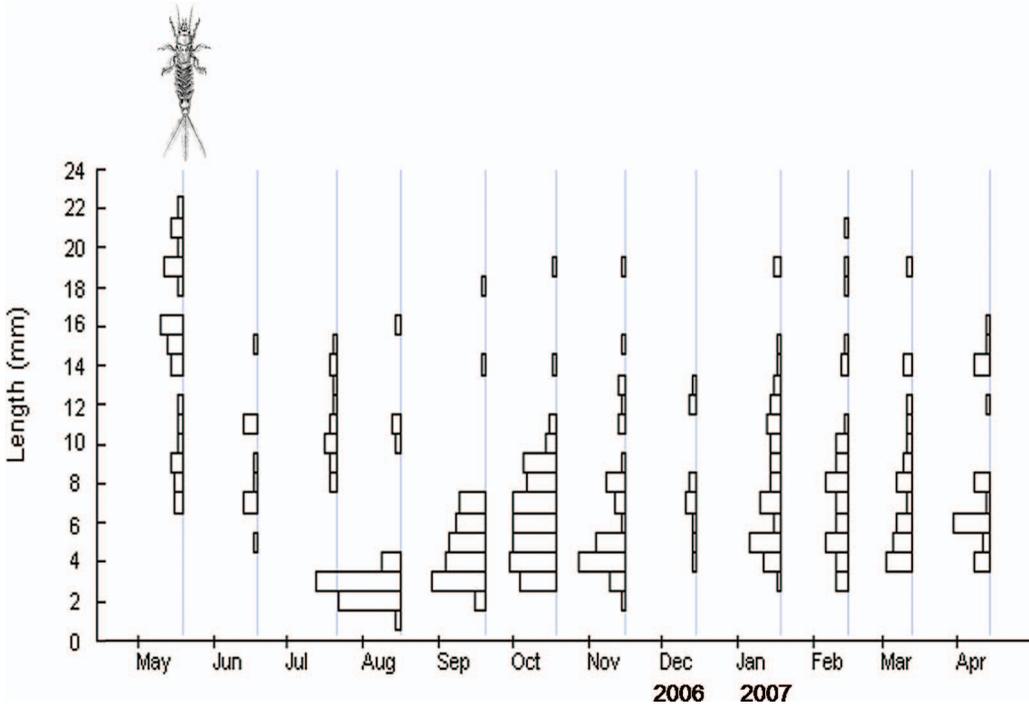


Figure 4. Size–frequency graph representing the life cycle of *Ephemera danica* at the sampling site ($N = 442$). The presence of mature larvae is marked with a respective icon.

temperature, and grew during 22 months to achieve the mature stage in May, almost 2 years later. Eggs laid by individuals emerging in May developed during 2 months for hatching in August. Growth was irregular throughout the larval development (Figure 3). This species accumulated 7033.71 day-degrees during its larval development, that happened between a mean daily temperature of 2 and 18°C.

This species also mainly fed on detritus, also with a high ingestion of CPOM, and other components present to a lesser extent (Table 2). As in the case of the previous species, we found some animal remains and sand in the gut of some larvae, but these were present in less than 2% and 9% of the studied individuals, respectively. Some ontogenetic shifts appeared in bigger larvae, too: a decreasing percentage of detritus and fungi spores and an increase in the ingestion of CPOM by bigger larvae (Table 3).

Dry weight (DW) and body length (X) were related by the following equation:

$$\text{Ln DW} = -6.48 + 3.05 \text{LnX}, (r^2 = 0.95, F_{1,28} = 503.95, p < 0.05)$$

The annual secondary production of *E. danica* was equal to $5.59 \text{ gDWm}^{-2}\text{year}^{-1}$ and the cohort P/B was 5.13. Taking into account the life cycle of this species we used a cohort production interval (CPI) of 22 months.

Discussion

The stonefly *L. geniculata* had a typical univoltine life cycle that could be catalogued as “fast-seasonal” following Hynes’ (1970) classification, although this category requires the presence of a diapause stage during the embryonic development that in our population was not completely confirmed. Eggs started hatching approximately synchronically in February and March, coinciding with the rising of temperature in the stream (Figure 1). The temperature during this period coincided with the one recorded as optimum temperature for egg hatching of this species by Elliott (1987a) in an experimental study (with constant temperatures) in Great Britain, although this author pointed out that eggs would hatch after accumulating 369 day-degrees, and in our case the eggs accumulated 1261.88 day-degrees. This fact could be related to the effect of natural fluctuating temperatures in egg development (Brittain 1990), or, more probably, could be a consequence of the presence of a diapause stage in the studied population, as an adaptation to synchronise larval development with the favourable growing season, and to avoid the low temperatures in winter. In fact, although egg hatching happened in February–March at relatively low temperatures, the larval growth was greater from April–May (Figure 3) when temperatures were higher (Figure 1). Nevertheless, more studies focused on embryonic development are needed in this population to interpret these data. Larval development was fairly synchronic, with little spread among size classes, and so an approximately clear cohort could be identified. Mature larvae were collected just in two months (August and September), indicating a short emergence period and a flight period inside the limits of this species in the Iberian Peninsula (Tierno de Figueroa et al. 2003). In fact, previous studies on adult stoneflies in the same mountain system showed an early autumnal flight period for this species (Luzón-Ortega et al. 1998; López-Rodríguez et al. 2004). A similar, univoltine life cycle was found by Ferreras-Romero and Agüero-Pelegri (1994) in the Sierra Morena, a mountain system in South-Western Iberian Peninsula, although they hypothesised that some of the larvae could remain in the stream a second winter, probably in quiescence, and emerge the following spring. Elliott (1987b) also found a univoltine life cycle, but with a longer larval development lasting from October to August–September, with the smallest larvae approximately coinciding in size with those found at the end of winter in our study. These data could support the possible existence of an embryonic diapause in our study area that would explain the longer egg period found. Several authors have studied the life cycle of this species in several parts of its distribution (Hynes 1941; Neveu et al. 1979; Pañil et al. 2008), always confirming the univoltinism. This pattern of univoltinism in our study area is also supported by the existence of a regular flight period in different years (Luzón-Ortega, Tierno de Figueroa and Sánchez-Ortega 1998; López-Rodríguez, Luzón-Ortega, Palomino-Morales and Tierno de Figueroa 2004; present data for 2006).

The life cycle of *E. danica* in the studied site was clearly semivoltine, “non-seasonal” following the classification of Hynes (1970). It fitted in the “A1” category of Sowás (1975)

classification, where species are placed with semivoltine life cycles, short hatching periods (1–2 months) and long larval development (more than 20 months). Within Landa's (1968) classification it can be catalogued in the "C1" group that unites all the mayfly species with a two year cycle. Eggs started hatching in August, just 2 months after oviposition by adults. Larvae grew during 22 months before reaching the mature stage. Growth was less evident during the winter period, and greater during spring and summer, when temperatures were higher (Figures 1 and 3). Egg hatching was approximately synchronic, but spread in larval size increased with time, mainly due to sexual dimorphism, which is more evident in bigger larvae. Alba-Tecedor (1990) found a semivoltine pattern in which part of a population from southern Iberian Peninsula developed in one year, while the other part lasted 2 years. The life cycle of the two year cohort was similar, but slightly delayed to the one found in our study, probably due to the different thermal regime of the stream. A univoltine life cycle has been also pointed out by Aguayo-Corraliza et al. (1991) in a south-western Iberian Peninsula stream. Several other studies have focused on the life cycle of *E. danica* throughout Europe (see Alba-Tecedor 1990 for a review; Otto and Svensson 1981; Tokeshi 1985; Elliott et al. 1988), indicating variations in its duration, from 1 to 3 years, and even variations among the same population, with different cohorts of the same generation lasting different time to complete the entire development (1–2 years or 2–3 years).

Regarding feeding, both species are mainly detritivorous, but to a considerable degree also phytophagous (Table 2). The ingestion of detritus by *L. geniculata* has already been noted by Jones (1949), who pointed out that this species also fed on leaf fragments and green algae, although the amount of these components was very small. A similar result was found in a population of North Africa, where larvae ingested mainly detritus but also vegetal matter (diatoms and algae), which supposedly made up to 30% of the diet (Azzouz and Sánchez-Ortega 2000). Hynes (1941) indicated that this species showed a higher preference for algae than other species belonging to its genus, but this is not supported by our results. Thus, we can classify *L. geniculata* as gatherer-collector, also with an important role as shredder.

In relation to *E. danica*, several studies on feeding agree that this species ingests mainly detritus (see Elliott et al. 1988 for a review). The way of acquisition of this detritus is a more discussed matter. Otto and Svensson (1981) and Ladle and Radke (1990) pointed out that this species uses its forelegs to filter a water current produced by the respiratory movements of the gills, through its burrow. Thus, the species would be filterer-collector, as pointed out by Wallace and Merritt (1980) for some ephemeroptera. Other authors classify this species as gatherer-collector (Merritt and Cummins 2006), so its assignment to a main FFG remains unclear. Apart from this, in our study the species also showed an important role as shredder of leaves that probably are obtained from the substrate outside of its tunnel.

Animal remains were found in the gut of few larvae of both species, but their low proportion indicates accidental ingestion, probably when collecting detritus. This was also found by Hynes (1941) for *L. geniculata*. The same reason can be argued for the sand found in their guts.

In relation to the secondary production of *L. geniculata*, no previous data are available as far as we know. In this stream we recorded a relatively high annual secondary production if we compare this with other macroinvertebrates (Huryn and Wallace 2000) or within gatherer-collectors that also have a high annual P/B (Benke 1993).

Annual secondary production of *E. danica* was extremely similar to that obtained by Tokeshi (1985) in a British population ($5.58 \text{ gDWm}^{-2}\text{year}^{-1}$ of this author's study versus

5.59 gDWm⁻²year⁻¹ in our study), although the annual P/B ratio was slightly higher in our population. If we compare our results with those obtained by Lee, Hwang and Bae (2008), we observe that the annual secondary production in our study is higher than that of the majority of multivoltine and univoltine species, including some species of the genus *Ephemera*. Poepperl (2000) also estimated the secondary production of two ephemerid species, finding lower values than ours. In relation to the FFG of this species in our study, both if we consider it as filterer-collector or gatherer-collector, the annual secondary production is relatively high, although the annual P/B would be among the more common values (Benke 1993).

If we compare one species with the other, taking into account that the diet is approximately the same, we observe that both have similar values of annual secondary production despite having very different duration of life cycle. Also, the cohort P/B is similar and around 5, the most typical value for freshwater invertebrates (Benke 1993). Nevertheless, we observe that the annual production and the cohort P/B are slightly higher in *L. geniculata*, probably conditioned by the life cycle duration, due to species with a two year life cycle exhibiting lower P/B ratios than those with a one year or shorter life cycle (Waters 1977).

In conclusion, we can see that these two species, belonging to different taxonomic groups, make an optimum utilisation of the same habitat. They greatly differ in life cycle duration, but share approximately the same diet and population dynamics (in terms of biomass production), maybe as a consequence of a convergence in exploiting the available resources.

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