

# Life history of two aquatic insects in the Cacín stream (SE Spain): *Leuctra geniculata* (Plecoptera) and *Serratella ignita* (Ephemeroptera)

## Estrategias vitales de dos insectos acuáticos en el río Cacín (SE Spain): *Leuctra geniculata* (Plecoptera) and *Serratella ignita* (Ephemeroptera)

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Recibido el 9 de octubre de 2012. Aceptado el 22 de octubre de 2012.

ISSN: 1130-4251 (2012), vol. 23, 57-68

**Key words:** stonefly, mayfly, life cycle, feeding, secondary production.

**Palabras clave:** plecóptero, efemeróptero, ciclo de vida, alimentación, producción secundaria.

### ABSTRACT

The life history, nymphal feeding and secondary production of the mayfly *Serratella ignita* (Poda 1761) and the stonefly *Leuctra geniculata* (Stephens, 1836) are studied. Both species are univoltine and complete their nymphal development in the same time (seven months), but *L. geniculata* growth is constant, while nymphs of first stages of *S. ignita* do not almost grow during the first months after hatching. Nymphs of both species feed mainly on detritus, but also incorporate other trophic resources to their diet, as diatoms, fungi and coarse particulate organic matter. Some ontogenetic shifts are detected in both species. The annual secondary production is very low for *L. geniculata*, and it has also low values of cohort production/biomass ratio. In the case of *S. ignita*, due to the low number of individuals collected, it was not possible to evaluate it.

### RESUMEN

Se estudian las estrategias de vida, la alimentación ninfal y la producción secundaria del efemeróptero *Serratella ignita* (Poda 1761) y el plecóptero *Leuctra*

*geniculata* (Stephens, 1836). Ambas especies son univoltinas y completan su desarrollo ninfal en el mismo tiempo (siete meses), pero el crecimiento de *L. geniculata* es constante, mientras que las ninfas de primer estadio de *S. ignita* casi no crecen durante los primeros meses después de la eclosión. Las ninfas de ambas especies se alimentan principalmente de detritus, pero también incorporan otros recursos tróficos a su dieta, como diatomeas, hongos y materia orgánica particulada gruesa. Se detectan algunos cambios ontogenéticos en ambas especies. La producción secundaria anual es muy baja para *L. geniculata*, y tiene también valores bajos del ratio producción/biomasa de cohorte. En el caso de *S. ignita*, debido al bajo número de individuos colectados no fue posible evaluarla.

## INTRODUCTION

Aquatic insects are the most diversified and abundant group of invertebrates in freshwaters, being the Plecoptera and Ephemeroptera some of the most important animal components in stream ecosystems (Fochetti & Tierno de Figueroa, 2008; Brittain & Sartori, 2009). They also represent a very large proportion of the biomass of aquatic ecosystems. Therefore, and not surprisingly, they have an important role in the cycle of matter and the flow of energy through the freshwater food-webs. Their importance in this flow may be seen in the magnitude of their production (Huryn & Wallace, 2000). Both Plecoptera and Ephemeroptera are essential links in the food-webs in this kind of ecosystems. They may have different functional roles using many different resources and being part of the diet of many predators, including fishes, amphibians, birds and other invertebrates (Tierno de Figueroa *et al.*, 2003; Brittain & Sartori, 2009).

In running waters, these organisms have evolved several strategies, such as the reduction or avoidance of flow exposure, although many other factors are also important selective pressures for animals living in lotic environments (Hynes, 1970; Giller & Malmqvist, 1998). Among all variables, temperature (together with nutrition) is one of the most important factors governing their life histories (Lillehammer, 1975; Brittain & Sartori, 2009). Temperature affects both the egg and the nymphal stages, being their duration inversely proportional to the temperature in most cases (e. g. Humpesch, 1984).

The aim of this paper is to analyze the nymphal biology (life cycle, growth, feeding behaviour and secondary production) of the stonefly *Leuctra geniculata* (Stephens, 1836) and the mayfly *Serratella ignita* (Poda, 1761) in a permanent Mediterranean stream, the Cacín stream (SE Iberian Peninsula). These species are widely distributed and diverse aspects of their nymphal biology have been widely studied and compared in a great

part of their distribution areas (e.g. Elliott, 1987; Pařil *et al.*, 2008 for *L. geniculata*; see López-Rodríguez *et al.*, 2008 for a wide discussion on *S. ignita*'s biology). This allows us to compare the results of this study with those obtained particularly in previous works in other permanent streams from southern Iberian Peninsula with different thermal regimes (López-Rodríguez *et al.*, 2008, 2009a, 2009b).

## MATERIAL AND METHODS

The study was carried out in the Cacín stream (N 36° 56' 33.7" W 3° 51' 47.8", 842 m a.s.l., Sierra de Almirajara, Granada province, Spain). This permanent stream has a hydrologic cycle characterized by a maximum flow during spring and a reduction in summer, due to low rainfall and high temperatures. Some anthropogenic alterations of the stream occur a few tens of meters upstream of the sampling site, mainly in spring and summer, as there is a recreational area.

Macroinvertebrates were collected with a Surber sampler (0.1 m<sup>2</sup> area and 250 µm mesh size) from Nov 18<sup>th</sup> 2010 to Oct 17<sup>th</sup> 2011, approximately every 30 days. Six replicates were taken to represent the different mesohabitats in the study area. With the intention of obtaining a better representation of individuals for statistical analysis, *kick* sampling was performed with ten replicates to represent the mesohabitats as with the Surber sampler. The streambed was composed mainly by gravels, pebbles and sand and, in a lower concern, rocks. Its average width during the sampling period was 7.81±0.70 m and its average depth of 0.25±0.08 m. The study area was surrounded by well-developed riparian vegetation, constituted mainly by *Populus alba*, *Salix* sp., Juncaceae and Poaceae. Physic-chemical parameters (conductivity, pH, % O<sub>2</sub> and O<sub>2</sub> concentration) were recorded in each sampling date (Table I). Moreover, a temperature datalogger was placed in the streambed during the whole study period, which recorded temperature hourly (Fig. 1).

All individuals collected were preserved in bottles with 4% formalin and brought to laboratory, where the samples were washed and sieved to remove excess formalin and detritus. Afterwards, all nymphs of mayflies and stoneflies were sorted and *L. geniculata* and *S. ignita* were identified using an optical stereomicroscope. To generate the life cycle graphs, both pronotum width and total body length were measured. Data were obtained with the micrometer of a binocular microscope at 4x for the pronotum measures and 2x for the total length. Given the fact that the correlation between pronotum width and total length was statistically significant (Gamma correlation = 0.91, p < 0.05), only total body length was used to describe

Table I.—Main physic-chemical parameters recorded during the sampling period in the Cacín stream.

Tabla I.—Principales parámetros físico-químicos registrados durante el periodo de muestreo en el río Cacín.

	Mean	SD	Min-Max
Temperature (° C)	13.20	3.21	6.89-18.71
Dissolved Oxygen (mg/l)	8.77	0.65	7.73-9.58
Dissolved Oxygen (%)	92.23	5.56	82.80-101.00
Conductivity (µS/cm)	335.35	32.92	294.20-392.00
pH	8.45	0.08	8.31-8.54
Discharge (m <sup>3</sup> /s)	0.86	0.31	0.34-1.16

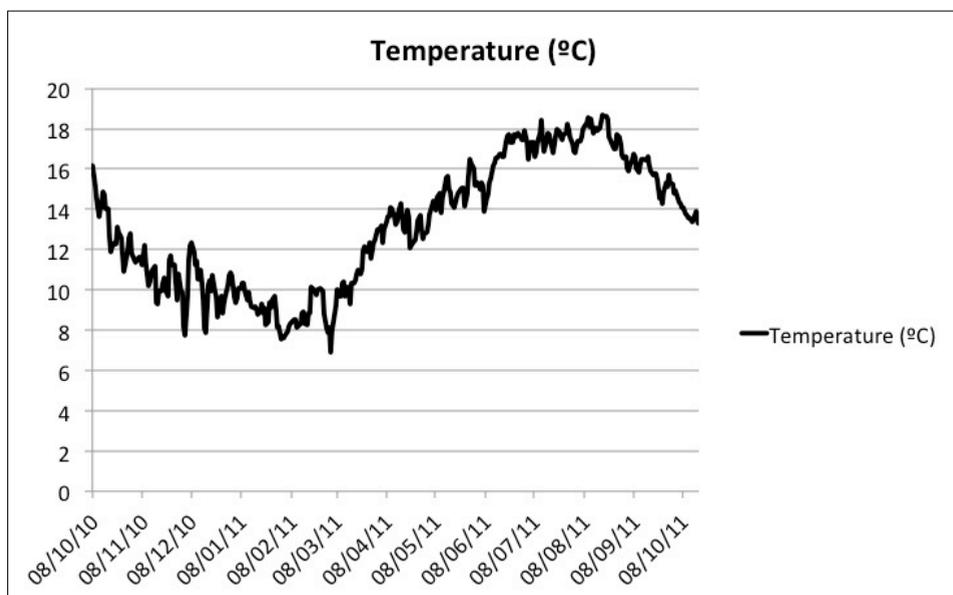


Fig. 1.—Graph showing mean daily water temperature oscillations during the sampling period. Dates expressed as day/month/year.

Fig. 1.—Gráfica mostrando las oscilaciones diarias de la temperatura del agua durante el periodo de muestreo. Fechas expresadas como día/mes/año.

the life cycle. Nymphs were classified into 0.5 mm intervals. To realise the size-frequency graphs and represent the life cycles, the FiSAT II software was used (Gayanilo *et al.* 2002).

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of aquatic insects feeding (e.g. Tierno de Figueroa *et al.*, 2009; Gallo *et al.*, 2010). The individuals were introduced in vials with Hertwig's liquid (a variation of Hoyer's liquid) for 20-24 hours and put them into an oven at 65° C. After this, the specimens already clarified, were put on a slide glass with a cover glass on, and brought to a microscope, where the absolute percentage of gut content (measured as percentage of occupied area) at 40x, and the relative percentage of each component present in the gut at 400x were estimated. In order to detect the variation of the nymphal diet along their development we correlated percentage of each food item with the nymphal size in both species.

Finally, the secondary production was calculated by means of the size-frequency method (Hynes & Coleman, 1968; Benke & Huryn, 2006) due to the broad variety of sizes of individuals in each date. For this reason, the nymphal stages cannot be clearly defined and this non-cohort method is appropriate for this kind of populations, where nymphal recruitment is continuous and size spread is high, as in our case (Benke & Huryn, 2006). When the nymphal development period is less than a year is necessary to apply a correction factor, known as cohort production interval (CPI). CPI is the mean development period from hatching to final size (Benke, 1979) and represents the number of months that the studied species were in the nymphal stage. Annual production was calculated by multiplying the total production obtained by 12/CPI. Nymphal biomass was obtained using the equation parameters in López-Rodríguez *et al.* (2009a, 2009b). For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. The variables studied were not normally distributed, so to study correlations between variables, a non-parametric Gamma test was used.

## RESULTS

### Life histories

A total of 286 *L. geniculata* nymphs were collected and measured from February to August. The nymphs of the first sampling did not have the compound eyes developed yet, but they had ocelli spots instead. Thus we can assure that these individuals were newly hatched. The development period lasted for seven months (Fig. 2). Growth was relatively constant with a slight increase since mid-April. After August no nymphs were found in the stream, which indicated that the flight period occurred in the late summer, coinciding with the peak of the warmest season (Fig. 1). After mating and

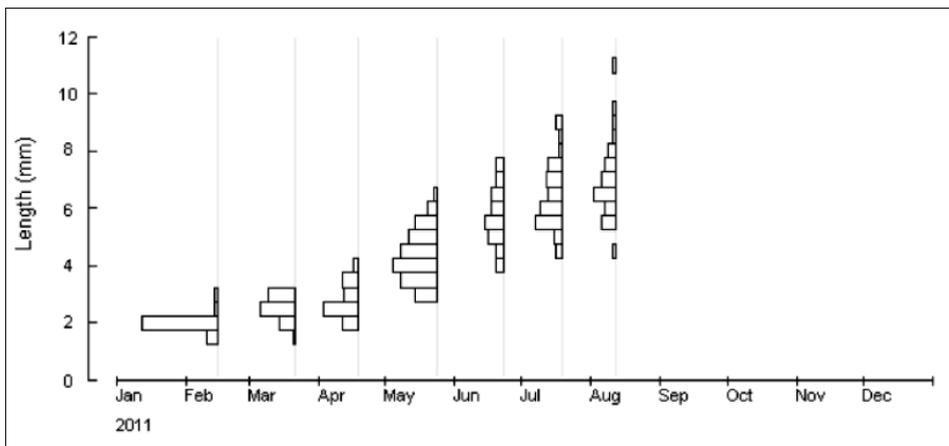


Fig. 2.—Size-frequency graph representing the life cycle of *Leuctra geniculata* at the sampling site.

Fig. 2.—Gráfica de frecuencia de tamaños que representa el ciclo de vida de *Leuctra geniculata* en el lugar de muestreo.

oviposition, the eggs probably remained in the hyporheic zone for 5 months before hatching. Nymphs needed a total of about 2533.29 day-degrees to complete their development.

For *S. ignita* a total of 79 nymphs were collected and measured from December to June, but in January no one was collected. Individuals sampled in February showed ocelli instead of compound eyes, so they were individuals from the first nymphal stage. The main development period lasted for seven months (Fig. 3). Development and growth was quite fast. Moreover, this development was not constant, having a growth spurt in late April and early May, coinciding with the first adult emergences, as saw from the development of the wing pads of several nymphs. After June no more nymphs were found. The flight period occurred in early summer coinciding with the beginning of a lower flow period. Nymphs needed around 2206.81 day-degrees to complete their development.

## Feeding

Individuals of both species fed mainly on detritus followed by a lower percentage of other resources (Tables II and III). *Leuctra geniculata* ingested a greater proportion of coarse particulate organic matter (CPOM) than *S. ignita*, but the latter showed a much higher consumption of fungi. Comparing

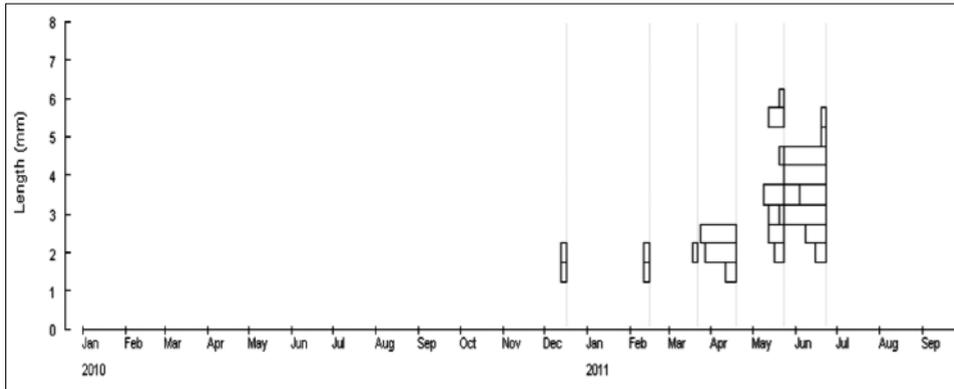


Fig. 3.—Size-frequency graph representing the life cycle of *Serratella ignita* at the sampling site.  
 Fig. 3.—Gráfica de frecuencia de tamaños que representa el ciclo de vida de *Serratella ignita* en el lugar de muestreo.

Table II.—Nymphal gut contents of *Leuctra geniculata*.

Tabla II.—Contenido digestivo de las ninfas de *Leuctra geniculata*.

	Valid N	Mean	S.D.	Min-Max
% absolute	208	39.0	31.7	0-100
% detritus	160	68.9	17.9	0-100
% diatoms	160	12.5	17.0	0-100
% CPOM	160	6.4	7.0	0-25
% pollen	160	4.3	6.0	0-40
% fungi	160	6.5	5.8	0-30

Table III.—Nymphal gut contents of *Serratella ignita*.

Tabla III.—Contenido digestivo de las ninfas de *Serratella ignita*.

	Valid N	Mean	S.D.	Min-Max
% absolute	68	47.8	27.5	0-100
% detritus	63	61.3	21.3	0-100
% diatoms	63	12.5	17.8	0-70
% CPOM	63	1.9	2.8	0-10
% pollen	63	3.3	3.0	0-10
% fungi	63	21.0	22.1	0-100

the variation of the total length of nymphs along the cycle with the food resources used, some ontogenetic shifts were detected (Table IV). On the one side, in *L. geniculata*, the percentage of CPOM increased with the nymph size, while on the other, *S. ignita* showed a significant increase in

Table IV.—Gamma correlations between total length and the percentage of the different food items in *Leuctra geniculata* and *Serratella ignita*. Values marked with an asterisk are significant at  $p < 0.05$ .

Tabla IV.—Correlación Gamma entre la longitud total y el porcentaje de los diferentes tipos de alimento en *Leuctra geniculata* y *Serratella ignita*. Los valores marcados con un asterisco son significativos para  $p < 0.05$ .

	<i>Leuctra geniculata</i>	<i>Serratella ignita</i>
% detritus	0.098	0.034
% diatoms	-0.450*	-0.524*
% CPOM	0.681*	-0.070
% pollen	0.119	0.075
% fungi	0.122	0.381*

the use of fungi (packs of hyphae) when larger. Finally, in both species the percentage of diatoms decreased significantly when larger and the presence of pollen in the gut contents was no significantly correlated with nymphal size ( $p > 0.05$  in both species).

## Secondary production

The secondary production of *S. ignita* could not be calculated due to the low number of nymphs captured during the study. In *L. geniculata*, a secondary production equal to  $88.65 \text{ mgDWm}^{-2}$  was obtained, but when corrected for the CPI of seven months, the annual secondary production was equal to  $151.18 \text{ mgDWm}^{-2}\text{year}^{-1}$ . The cohort production/biomass ratio (P/B) was 4.38 and the biomass turnover (annual P/B rate) was  $7.51 \text{ year}^{-1}$ .

## DISCUSSION

### Life histories

Following the classification of Hynes (1970), *L. geniculata* has a typical “fast-seasonal”, univoltine life cycle. As pointed by this author, this cycle requires a diapause stage in the embryonic development, but this has not been confirmed by our results. In a previous study on the same species in the Fardes stream (Sierra de Huétor, SE Spain, 1200 m a.s.l.), López-Rodríguez *et al.* (2009a) found that eggs start hatching in February and March, coinciding with the rising of temperature in the stream. In the

present study eggs start hatching in January, one month before, but under similar temperatures (approximately 8° C). Nevertheless, the accumulated day-degrees in the Cacín stream are lower than in the Fardes stream (2533.29 day-degrees vs. 2884.30 day-degrees). Our data support what Brittain (1990) pointed out for Plecoptera in general, i.e. that the development of nymphs is faster in warmer streams.

The population of *S. ignita* in the Cacín stream shows also a “fast-seasonal”, univoltine life cycle (Hynes, 1970; Clifford, 1982). The nymphal growth is very slow at the beginning of the nymphal development, under low temperatures, and increases in spring (Fig. 2). The life cycle of *S. ignita* is slightly displaced in relation with the cycle found in other nearby streams of the same regions (López-Rodríguez *et al.*, 2008; López-Rodríguez *et al.*, 2009b). In Sierra Nevada, López-Rodríguez *et al.* (2008) found that, at higher altitude, the nymphal development is very fast (three-four months), and that nymphal development occurs from May-June to August, while in the Cacín population this development period last for seven months from December to June. Thus, the nymphal development would be slightly advanced in the latter, probably due to higher temperatures. In the Fardes stream, the nymphal development last four months but is slightly delayed in comparison with the Cacín population (López-Rodríguez *et al.*, 2009b). In the Cacín stream, nymphs accumulated a higher amount of day-degrees than in Sierra Nevada or Sierra de Huétor (Fardes stream) populations. Nevertheless, this great difference could be due to for the calculation of the day-degrees in the Cacín population the whole nymphal development period is considered, even when there is no or little growth. In addition, the temperature at the time of hatching in Válor, Poqueira (Sierra Nevada, SE Spain, 1840 m a.s.l. and 1540 m a.s.l. respectively) and Fardes streams is always higher than 8°C, as in the Cacín stream. It seems that contrary to what would be expected, these populations have shorter cycles and less accumulated day-degrees at higher altitudes.

## Feeding

The feeding habits of both species are very similar. *Leuctra geniculata* and *S. ignita* are mainly detritivorous, but they use other resources. Thus, they can be catalogued as mainly collector-gatherers, but in a lower concern, as scrapers (*S. ignita* and *L. geniculata*) or even shredders (*L. geniculata*).

The population of *L. geniculata* from the Cacín stream, like the population from the Fardes stream studied by López-Rodríguez *et al.* (2009a), feed mainly on detritus. Nevertheless, the proportion of other components differs between both populations: CPOM is more abundant in the gut of

the nymphs from the Fardes stream, while diatoms and fungi are almost absent. When observing the correlation between size and percentages of the different diet resources, a significant increase in CPOM consumption is detected, as previously noted in the Fardes population (López-Rodríguez *et al.*, 2009a) and in other species of Plecoptera with similar trophic behaviour (López-Rodríguez *et al.*, 2010, 2012). This is probably related to the higher chewing capacity of larger nymphs that have more powerful mouthparts.

In the case of *S. ignita*, in addition to detritus, fungi and diatoms are relatively important components of its diet in our study area. The high percentage of diatoms found in our study has been also detected in populations of this species of other permanent streams from southern Iberian Peninsula (López-Rodríguez *et al.*, 2008, 2009b), without reaching the percentage found in a studied population from the temporary stream Despeñaperros (Sierra de Despeñaperros, SE Spain, 560 m a.s.l.; López-Rodríguez *et al.*, 2009b). Moreover, the Cacín stream population ingests a great quantity of fungi hyphae, becoming the 100% of the gut content in some nymphs at the end of their development period (a significant increase in fungi consumption is detected in relation with size, as well as a decrease in diatoms). Any of the other populations of *S. ignita* studied in southern Spain showed elevated percentages of fungi in their guts (López-Rodríguez *et al.*, 2008, 2009b). Finally, unlike of the Sierra Nevada populations (López-Rodríguez *et al.*, 2008, 2009b), mosses (bryophytes) were not found in the nymphal gut in the Cacín stream.

As shown by this study and its comparison with previous ones, general patterns can be observed for some species, but outstanding peculiarities appear in different populations. This justifies the interest of studying different populations of the same species in order to achieve a better knowledge of their trophic role.

## Secondary production

*Leuctra geniculata* shows low values of annual secondary production in relation with other studied populations of the same species (López-Rodríguez *et al.*, 2009a). The annual secondary production of *L. geniculata* in the Cacín stream is only the 2% of the Fardes stream population (151.18 mgDWm<sup>-2</sup>year<sup>-1</sup> vs. 7400 mgDWm<sup>-2</sup>year<sup>-1</sup>). On the other hand, its cohort P/B ratio is within the range pointed out by Benke (1993) and Benke & Huryn (2006) for freshwater invertebrates (between three and eight, being the most typical around five). Finally, it also has an annual P/B rate value within the range pointed by Waters (1977) for benthic invertebrates (from one to ten).

The extremely different values of annual secondary production between this population and some others are probably related with the lower density of *L. geniculata* in the Cacín stream. These low values also indicate that the fitness of the species could be lower than in other parts of the same region, and this could reflect the anthropogenic disturbances that occur some tens of meters upstream of the sampling site. Thus, secondary production would highlight the effect of a possible stressor on this population. Nevertheless, more studies are needed to confirm this possibility.

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