

Manuel Jesús López-Rodríguez¹, José Manuel Tierno de Figueroa², Tiziano Bo³,
Alessandro Moggi³, Stefano Fenoglio³

¹Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, Campus
Fuentenueva s/n, 18071, Granada, Spain. E-mail: manujlr@ugr.es

²Departamento de Biología Animal, Facultad de Ciencias, Universidad de Granada,
Campus Fuentenueva s/n, 18071, Granada, Spain.

³Dipartimento di Scienze dell'Ambiente e della Vita, University of Piemonte Orientale
Via T. Michel 11, 1512, Alessandria, Italy.

**Living apart together: on the biology of two sympatric *Leuctra* species (Plecoptera,
Leuctridae) in an Apenninic stream (Italy)**

Keywords: Stoneflies, *Leuctra fusca*, *Leuctra hippopus*, life cycle, feeding habits.

Abstract

Plecoptera are a good subject of study in many fields of biological sciences. Leuctridae is the richest stonefly family on the European continent, with many species of *Leuctra* often coexisting in sympatry in the same lotic environment. In this work we studied the life cycle and feeding habits of two species (*Leuctra fusca* and *L. hippopus*) living in a small Apennine stream. Particularly interesting is the fact that the two species life cycles are absolutely non-overlapping, with periods of higher nymphal growth and adult emergence not concurrent. Both species feed mainly on detritus, though they also ingest important quantities of coarse particulate organic matter (CPOM) and diatoms. Nymphs

of both species ingested more CPOM and fewer diatoms when larger, among the main components of their diet. This reflects a similar trophic behaviour of both species, which change their main trophic resource as they grow. Hence, they may act as collector-gatherers in an early stage of development and behave either as collector-gatherer or as shredder in a posterior stage. Both hypotheses are discussed in this article.

1. Introduction

Aquatic insects represent the most diversified and abundant group of invertebrates in freshwaters, and in particular Plecoptera is one of the most important and often dominant orders in stream ecosystems (Fochetti and Tierno de Figueroa, 2008a).

Leuctridae Klapálek 1905 represents the richest family of the European stonefly fauna, with the genus *Leuctra* including fifty-nine species currently described for Italy (Fochetti and Tierno de Figueroa, 2008a, Vinçon and Graf, 2011). *Leuctra* are small sized stoneflies, with long, slender and light coloured nymphs and blackish adults, characterised by wings held wrapped around the abdomen. *Leuctra* nymphs are generally detritivores, feeding mainly on coarse particulate organic matter, although their diet may also include algae, fungi and bacteria (Feminella and Stewart, 1986). The emergence usually occurs at night. The flight period may have a different duration, but is generally quite long and adults can feed on pollen, cyanolichens, cyanoprocariota and fungal hyphae (Tierno de Figueroa and Sánchez-Ortega, 2000).

Many studies underlined that different species of *Leuctra* can coexist in the same stream reach. For example, Ravizza and Ravizza Dematteis (1976) reported that four *Leuctra* species can live in sympatry in an Alpine creek while, more recently, Bo *et al.* (2009) reported that six species cohabit in the Curone creek, an Apenninic lotic system. Because of the presence of several species often coexisting in the same environment, this genus represents an excellent subject for ecological-evolutionary studies. The aim of this research was to study the biology of two populations of *Leuctra*, one of *Leuctra fusca* (Linnaeus, 1758) and one of *L. hippopus* (Kempny, 1899) that inhabit a small creek, analysing their occurrence, diet, life-cycle and adult phenology, and discussing the results on the light of an evolutionary and ecological approach.

2. Methods

The study was conducted in the Albedosa creek (4°41'14'' N, 8°43'19'' E; 190 m a.s.l.), an Apenninic small tributary of the Orba river, north-western Italy. The climate is temperate-Mediterranean, with high autumnal rainfalls. The stream flows through a small valley in a secondary deciduous forest. The riparian vegetation is dominated by *Robinia pseudoacacia* (L., 1758), *Alnus glutinosa* Gaertn, 1790, *Corylus avellana* L., 1753 and scattered *Castanea sativa* (Miller) and *Quercus* spp. In the sampling station, the channel width was about 2.5-3.5 m, with a mean depth of 25-30 cm. The stream substrate consisted mainly of sands (40 %), gravels (30 %), cobbles (20 %), and masses (10 %). Samples were collected randomly in the stream bed, in order to represent every possible microhabitat, by using a Surber sampler (area: 0.0625 m²; mesh size: 250 µm). All macroinvertebrates were collected and preserved in 85% ethanol. In the laboratory, *Leuctra fusca* and *L. hippopus* nymphs were separated, counted and measured.

We measured total body length and pronotum width of each nymph with the micrometer of a binocular microscope. A high correlation existed between both measures in both species (Gamma correlation= 0.85, $p < 0.05$ for *L. fusca* and Gamma correlation= 0.71, $p < 0.05$ for *L. hippopus*), so we used only total length for all the analyses. Nymphs were classified in 1 mm length size intervals. The life cycle was represented by means of size-frequency graphs obtained with FiSAT II software (Gayanilo *et al.*, 2002).

To study the diet of the nymphs, we used the method proposed by Bello and Cabrera (1999), as in other papers on Plecoptera nymphal feeding (e.g. López-Rodríguez and Tierno de Figueroa, 2006; Fenoglio *et al.*, 2009; Kozáčková *et al.* 2009). Once each nymph was measured (to the nearest 0.01 mm), each individual was

placed in a vial completely cover with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. Afterwards individuals were mounted on slides to study their gut content under the microscope. We use a microscope equipped with an ocular micrometer to estimate the percentage absolute gut content at 40x (as percentage of total area occupied by the contents in the whole digestive tract) and the relative abundances of food items in the gut content at 400x (as percentage area occupied by each component of the total gut contents). We calculated the mean, standard deviation, minimum and maximum of occupation of each component. We also studied the correlation between nymphal size and gut contents using a Gamma correlation.

All the statistical analyses were performed under STATISTICA v.7.1 software (StatSoft, 2005). As the analysed variables were non-normal distributed, nonparametric statistics were used.

3. Results and discussion

Both species have a univoltine life cycle in the studied site, but nymphal development does not coincide in time (Fig. 1). The average nymphal development period of *L. fusca* is 3 months, from July to September, though some medium-size nymphs are also present in October. These nymphs, albeit do not have a large size, are mature. The flight period of this species begins in September and ends in November.

On the other hand, *L. hippopus* has a wider nymphal development period, ranging from September to February-March, though even in February some small nymphs are captured. The flight period is shorter than the one of *L. fusca*, and adults were only captured in March.

Quantitatively, more nymphs of *L. fusca* were captured than of *L. hippopus*. The nymphal feeding study shows that both species feed mainly on detritus, but also ingest

important quantities of coarse particulate organic matter (CPOM) and diatoms (Table 1). In the gut of some nymphs of both species there is mineral matter too. Positive significant correlations between size and percentage of occupancy in the gut are found for fungi hyphae and spores, and CPOM, in *L. fusca*, and for fungi hyphae, CPOM and mineral matter in *L. hippopus* (Table 2). Negative significant correlations were found for diatoms and mineral matter in *L. fusca*, and for diatoms in *L. hippopus*.

The univoltine life cycle of the Albedosa population of *L. fusca* is similar to the one found in other parts of Europe, such as Great Britain, Sweden, France and Norway (Hynes, 1941; Brinck, 1949; Lavandier and Dumas, 1971; Lillehammer, 1988). Nymphal growth is also fast in this species, as first nymphs reaching maturity do it in just three months, and takes place in summer. A maximum nymphal growth in summer was recorded by several authors (e.g. Brinck, 1949; Lillehammer, 1988) in different populations, but never so rapid. For instance, Lavandier and Dumas, 1971 pointed out that nymphs of *L. fusca* are more abundant in spring and summer, and that they require eight to nine months for nymphal development. This authors captured nymphs from January to November, a wider period than the one in the Albedosa creek. Some mature nymphs were also captured in October, but with a relatively smaller size than those of September. They would be either males (generally of smaller size) or some nymphs that did not achieve a higher size before the arrival of the emergence period. The flight period in the Albedosa creek is mainly autumnal, as recorded in several studies (In Tierno de Figueroa *et al.*, 2003; Fochetti and Tierno de Figueroa, 2008b), though in some parts of Europe it can be extended to winter (e.g. Luzón-Ortega *et al.*, 1998). Adults are present also after one month of the capture of the last nymph, probably indicating that they can survive several weeks in this stage.

Leuctra hippopus has also a univoltine pattern that coincides with the one pointed out in other European studies (e.g. Lavandier and Dumas, 1971; Lillehammer 1988). The growth of this species is relatively slower than the growth of the previous one, lasting from seven to eight months, and approximately constant. This period is similar to the one found in a population of the French Pyrenees, where first nymphs in reaching maturity did it in approximately eight months (Lavandier and Dumas, 1971). Nevertheless, in that study the authors found nymphs the 12 months of the year, and we only captured them in eight months. This may be due to, as pointed out by Lillehammer (1988), *L. hippopus* seems capable of given rise to local populations with different types of life history, due to egg hatching and nymphal development are greatly temperature-dependent. In relation with this, some degree of asynchronous hatching have been detected in the study site, represented in some small nymphs that were captured in February, probably representing individuals that hatched late and did not achieve to complete their nymphal development before the warmer period. In the Albedosa creek, adults were captured only in one month, March. In other parts of Europe, the flight period is also spring (Fochetti and Tierno de Figueroa, 2008b), spring-summer (Lillehammer, 1988) or even winter-spring, the latter in the French Pyrenees (in Tierno de Figueroa *et al.*, 2003). Nevertheless, there is a great difference with the results obtained by authors such as Lavandier and Dumas (1971) who collected adults from February to June, a wider period than that of the Albedosa population.

Regarding to the feeding habits, both species behave mainly as collector-gatherers. Albeit the relatively high content of CPOM and diatoms could suggest they played an important role as shredders and scrapers too, the presence of mineral matter in the gut may indicate that they swallowed these resources when collecting detritus. Nevertheless, the first hypothesis cannot be rejected, as CPOM is more ingested by

larger nymphs, as well as hyphae (and spores in *L. fusca*). This would support the fact that larger nymphs, with more robust mouthparts, could fragment CPOM and ingest, together with it, the fungi that colonize it and condition it. Feeding on detritus and CPOM by *L. fusca* and *L. hippopus* had been already reported by several authors (Brinck, 1949; Lillehammer, 1988), as well as ingestion of diatoms and mineral matter in *L. fusca* (Brinck, 1949). In this study the two *Leuctra* species seem to have different thermal requirements for nymphal development. Apparently it seems that nymphs of *L. fusca* need warmer temperatures than those of *L. hippopus*. In fact, Fochetti and Tierno de Figueroa (2008b) classified *L. fusca* as mesotherm reophil and *L. hippopus* as reophil and crenophil in Italy.

4. Conclusions

The fundamental question of how congeneric and similar species manage to coexist with one another in the same environment is an intriguing subject of study. The coexistence of two or more congeneric species in the same environment is possible because adaptive mechanisms have evolved; in fact, related species are more likely to interact and/or compete because the great part of behavioural and ecological activities is strictly linked with phylogenetic and morphological characters (Losos, 1990)

In this context, the well-documented tendency of sympatry in *Leuctra* is a fascinating subject. Probably the most outstanding result of this study is the complete absence of overlapping in the development of two congeneric and morphologically similar species: *L. fusca* and *L. hippopus*. We can hypothesize that this offset in the life cycles could be due to: i) a strategy to minimize possible competition for trophic resources if they were limiting in the past. Because both species show detritivorous feeding habits, the temporal segregation of the period of intense nymphal growth would

be fundamental in minimizing trophic resource interaction, as pointed out in previous studies on other aquatic insects (Brittain, 1982; López-Rodríguez *et al.*, 2010). ii) The no coincidence between the two flight periods. Adults of these two sympatric species never meet. We could suppose that this fact could be of great importance in avoiding erroneous mating [as previously pointed out by Tierno de Figueroa *et al.* (2001), for some *Leuctra* species coexisting in the same streams].

Obviously, both hypotheses [decreasing the ecological competition in accordance with the principle of Gause (Gause, 1935) and strengthening reproductive isolation, according to the Wallace effect (Sawyer and Hartl, 1981)] may help to explain this asymmetry in the life cycles of these congeneric species. Nevertheless, according to Krebs (2009), Gause's principle should not be used as a general ecological model for competition, since even the smallest differences can allow coexistence of closely related species. Moreover, given the fact that in other parts of their distributional range, even when they do not coexist, they maintain these life history patterns (Graf *et al.*, 2009), this displacement in the life cycles of both species would be due to an evolutionary constrain of each species more than due to the "ghost of competition past" (Connell, 1980) in this stream.

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6. References

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Tables and figures

Table 1. Nymphal gut contents of the studied species in the Albedosa creek.

	<i>Leuctra fusca</i>					<i>Leuctra hippopus</i>				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
% detritus	61	57.61	24.44	0.00	95.00	33	61.76	23.83	0.00	100.00
% hyphae	61	2.34	3.39	0.00	15.00	33	4.03	6.21	0.00	30.00
% spores	61	0.85	1.80	0.00	10.00	33	2.85	9.20	0.00	50.00
% CPOM	61	16.46	22.35	0.00	100.00	33	12.76	18.12	0.00	95.00
% pollen	61	0.61	1.45	0.00	8.00	33	0.21	0.74	0.00	4.00
% diatoms	61	15.82	17.79	0.00	65.00	33	13.42	18.81	0.00	90.00
% mineral matter	61	6.64	4.36	0.00	15.00	33	5.24	6.62	0.00	35.00

Table 2. Gamma correlations between size and gut contents of the studied species in the Albedosa creek. *= significant at $p < 0.05$.

	<i>L. fusca</i> total length (mm)	<i>L. hippopus</i> total length (mm)
% absolute	-0.05	0.11
% detritus	0.05	-0.20
% hyphae	0.36*	0.29*
% spores	0.35*	0.14
% CPOM	0.19*	0.53*
% pollen	0.05	0.00
% diatoms	-0.29*	-0.27*
% mineral matter	-0.26*	0.40*

Figure 1. Nymphal development of *L. fusca* (dashed line) and *L. hippopus* (points line).
* = mature nymphs; arrow = adults.

