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Imaginal biology of the stonefly *Hemimelaena flaviventris* (Pictet, 1841) (Plecoptera: Perlodidae)

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We studied various aspects of the imaginal biology of the stonefly *Hemimelaena flaviventris* in the southern Iberian Peninsula. This species was found to have a spring flight period (May and June). A great variety of components were recorded in its diet (e.g., pollen grains, especially of *Pinus*; cyanoficeas; cyanolichens; coarse particulate matter), indicating little dietary selectivity. We also analysed male drumming (three beats with intervals of 122.39 and 82.25 ms) as well as female drumming (1–3 beats, mean = 1.41; intervals between beats = 97.10 and 120 ms), suggesting a simple, monophasic model of intersexual vibration call. Mating duration ranged from 39 to 106 min (mean = 63 min/copulation, SD = 20.6). Both the males and the females proved able to copulate on more than one occasion. Finally, we described the morphology and size of the eggs of this species, indicating some parameters pertaining to the clutch, such as maximum fecundity encountered (357 eggs per female), the maximum number of clutches per female (*n* = 4), and the number of eggs per clutch (mean = 87.7, range = 22–195).

1. Introduction

The stonefly, *Hemimelaena flaviventris* (Pictet, 1841), is a thermophilous Betic-Rif species (Aubert 1963a). It was first described in Portugal (Pictet 1841), and it is now known throughout a large part of the Iberian Peninsula and in the northern part of Africa (Sánchez-Ortega & Alba-Tercedor 1987, Sánchez-Ortega & Tierno 1996).

Up to now, no aspect of its imaginal biology has been described. Previous works report no more than observations of this species in several localities (Aubert 1963a, Sánchez-Ortega & Tierno 1996), and the oldest works provide an external description of the insect (Pictet 1841, Aubert 1963b).

The aim of this work was to conduct a detailed study of different aspects of the biology of this species (temporal distribution, feeding, drumming, reproduction, etc.). The results obtained have increased the knowledge of the imaginal biology of the stonefly, an aspect poorly studied in this insect group.

2. Material and methods

The specimens were collected in three seasonal streams from the Sierra de Huétor (Granada, Spain):

— Barranco del Majalijar, Huétor-Santillán, 1 320 m a.s.l., U.T.M: 30SVG586287.
— Afluente del Arroyo de las Perdices, Huétor-Santillán, 1 380 m.a.s.l., U.T.M: 30SVG574277.
— Arroyo de las Perdices, Huétor-Santillán, 1 350 m a.s.l., U.T.M: 30SVG582278.

The captures were made during a sampling survey aimed at describing the fauna and phenology of the stoneflies in this mountain system (Luzón-Ortega *et al.* 1998). The sampling was carried out monthly from September 1996 to August 1997, but *Hemimelaena flaviventris* specimens were collected only in May and June.

In each station, two samples were taken simultaneously but independently by two people for 30 min, across a fixed transect by the river banks. For this, we swept the riparian vegetation. In addition, some specimens were captured directly from the vegetation with entomological tweezers. Some of the collected specimens were preserved in 70% alcohol in plastic dram bottles. In the laboratory, they were identified, labelled and preserved in glass vials. Other specimens were placed in empty dram bottles, which were kept in an ice chest, to maintain the insects alive for laboratory studies.

The gut contents were studied by a transparency method for slides (C. L. Bello, pers. comm.). These insects were kept in vials containing Hertwig's liquid (a variation of Hoyer's liquid), and placed in an oven, where they were kept at 65°C for 24 hours. Next, the specimens were mounted on a slide with Hoyer's liquid and then placed in the oven for one or two days. The slides were sealed with enamel.

For the quantitative analysis of the gut contents, the percentage of different types of food was estimated using an ocular micrometer. The different components of the digestive contents were identified and quantified using an Oxford Trade microscope (60×, 150×, 600×).

Nymph and imaginal mouth pieces were also studied in slides mounted with Hoyer's liquid, to examine the degree of their reduction from the nymph to the adult stages.

To study stonefly communication, we used adults collected in the Arroyo de las Perdices (a tributary of the Arroyo de las Perdices) on 5 May 1997. These specimens were transported to the laboratory in dram bottles kept in an ice chest. The drumming signals were recorded over the following two days between 9.00 and 13.00 at a temperature between 23.3 and 24.3°C, under light that oscillated between 54.6 and 1380 lux and with a relative humidity at 44 to 44.3%.

Male calls were recorded by placing individuals in a 2.5 × 2.5 × 2.5 cm bottomless plastic box resting directly on a 7.5 cm in diameter, 8 ohm, 0.8 W speaker connected to a Philips AZ8052 model cassette-recorder. By finger tapping directly on the speaker membrane, we occasionally stimulated males to drum. Sometimes a female answered, whereupon we placed her close to the males in a separate bottomless plastic box similar to the one containing the males, permitting one insect to receive the call of the other.

Recorded calls were displayed and measured using the IBM-compatible computer program Creative Wave Studio for Windows 1993. Fig. 1 was drawn using the SoundEdit™ Macrecorder®Sound system 2.0.5.

To study the mating behaviour of this species, we kept different stonefly males and females in 7 × 5 × 5 cm plastic boxes and observed the behavioural cues. We quantified the mating duration, noting the number of copulations for males and females, and any copulations occurring between ovipositions.

The eggs were obtained from ovipositions in the laboratory. To encourage oviposition, females with eggs on the end of the abdomen were enclosed over a Petri dish filled with water (*see* Khoo 1964) or, in the case of dead females, the clutch on the end of the abdomen was removed. In some cases, the clutch was deposited by the female directly onto a solid substrate.

To study the maximum number of eggs that a female could produce, we dissected five females which had the most swollen abdomens. Nevertheless, as these were specimens taken from the field, it was possible that they had previously laid some eggs; therefore, the observed clutch could be smaller than the maximum capacity of that individual. Maximum fecundity was expressed as the sum of the eggs laid by the same female in all the clutches laid in the laboratory, plus the eggs which remained inside the insect.

In all cases, the eggs were counted with a Zeiss binocular microscope model (magnification 10× to 40×) and preserved in 70% alcohol in crystal vials.

To visualize the eggs, we used optical microscopy as well as Scanning Electron Microscopy (SEM).

The data were analyzed with the STATISTICA package for Windows 4.0 (1993).

3. Results and discussion

3.1. Temporal distribution

From the data reported by several authors in different localities where *H. flaviventris* was captured (Sánchez-Ortega & Alba-Tercedor 1987), we found the spring flight period in this species to take place between April and June; however, adults of this species were also found in March in Estremadura, Portugal, by Whytton da Terra (1979), and in July in León, northern Spain, by Vidal (1992) and Vidal and Membiela (1993, 1994).

In the study area, the observed flight period was similar to that mentioned above (May–June), without outstanding peak captures, coinciding with the complete drying of the streams in the stonefly habitat.

3.2. Feeding

We studied the gut contents from ten males and eight females. A relative reduction of the size of mouth pieces in relation to the size of nymphs was noted in both sexes, similar to that found in other Perlodidae such as the genus *Isoperla* (Brinck 1949, J. M. Tierno de Figueroa & A. Sánchez-Ortega, unpubl.).

Only the guts of two males (20% of the total) contained food. The first contained a great quantity of food, consisting mainly of cyanolichens (98%), and partly of Pinaceae pollen (2%) and other pollens (quantities of less than 1%). The second contained little digestive content: cyanolichens (55%), Pinaceae pollen (40%), *Fraxinus* pollen (2%), and other unidentified pollens (3%).

Some food — highly variable in composition and, in all cases, very scant — was found in the guts of four females (50% of the studied females). In the first specimen, the gut contained only cyanophicea; in the second, ascospores (with a low percentage of pollen, less than 1%); in the third, ascospores (50%), Pinaceae pollen (25%) and other not identified pollens (25%); and in the fourth, coarse particulate matter (vegetable remains, 50%), Pinaceae pollen (17%), cyanolichens (15%), and other unidentified pollens (13%).

The results showed no clear food selection but rather considerable variation in this regard. The exine of the pollen grains remained intact because the content was extracted by diffusion, which is also common in many pollen feeding insects.

The percentages of specimens with gut content in both sexes appear to indicate that the ingesta can be more important to females than to males, which is perhaps related to egg production during their imaginal stage. In fact, dissections of mature nymphs of *H. flaviventris* did not show developed eggs.

Despite that the quantity of ingested food was not excessive, the results confirm that the feeding process forms part of the adult life in this species, as in other Perlodidae species (J. M. Tierno de Figueroa & A. Sánchez-Ortega, unpubl.).

3.3. Reproductive biology

3.3.1. Drumming

Sixty-nine male drumming-calls (from five different individuals) and twenty-nine female drumming-calls (from two different individuals) were analysed.

The male call is composed of three consecutive beats with intervals of 122.39 ms between the first and the second beat, and 82.25 ms between the second and the third beat (Table 1; Fig. 1).

The female call comprised one to three beats (mean = 1.41; mode = 1) with average intervals of 97.10 ms between the first and the second beat, and 120.00 ms between the second and the third one (Table 1). The same female can answer with one, two or three beats.

The average time between the last male-call beat and the first female-call beat was 210.00 ms (Table 1). This time was significantly longer when the female responded with a single beat rather than more than one (Lilliefors $p < 0.01$; U-test Mann-Whitney $Z = -2.86770$, $n_1 = 19$; $n_2 = 10$; $p < 0.01$) (Siegel & Castellan 1988).

This species male-call pattern resembles that of other Perlodidae species, such as *Susulus venus-*

Table 1. Drumming call characteristics of *H. flaviventris.* — I1°–2°♂: interval between the first and the second beat of the male. — I2°–3°♂: interval between the second and the third beat of the male. — I♂–♀: interval between the third beat of the male and the first beat of the female. — I1°–2°♀: interval between the first and the second beat of the female. — I2°–3°♀: interval between the second and the third beat of the female.

	N beats/signal		I1°–2°♂	I2°–3°♂	I♂–♀	I1°–2°♀	I2°–3°♀
	♂ *n* = 69	♀ *n* = 29	*n* = 69	*n* = 69	*n* = 29	*n* = 10	*n* = 2
mean ms	3	1.41	122.39	82.25	210.00	97.10	120.00
SD	0	0.63	7.47	7.21	34.00	46.87	49.50
SE	0	0.12	0.90	0.87	6.31	14.82	35.00
Range	–	1–3	107–142	69–119	168–355	68–227	85–155

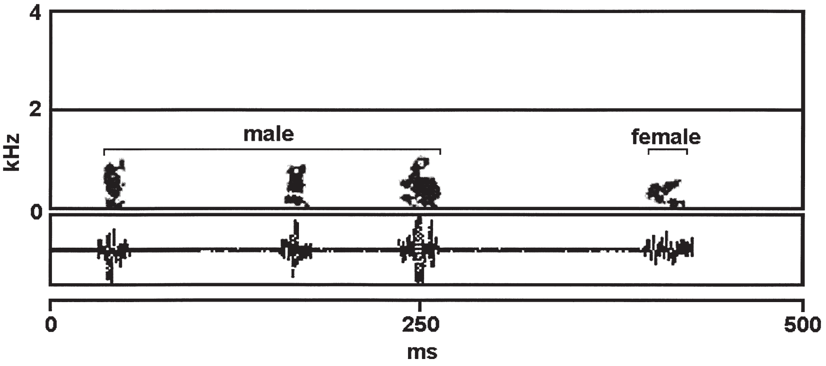

Fig. 1. *H. flaviventris* male and female drumming call. Upper box: sonogram of *H. falviventris* drumming signal. Lower box: oscillogram of *H. falviventris* drumming signal.

tus Jewett, 1965 (Bottorff *et al.* 1989) or *Cosumnoperla hypocrena* Szczytko and Bottorff, 1987 (Stewart *et al.* 1995), or species of different stonefly families (Stewart & Maketon 1991) having the three-beat patterns; the time interval between beats varies among the different species.

The simplicity of the *H. flaviventris* call — monophasic and with a low number of beats — gives it a primitive and ancestral character (Zeigler & Stewart 1987). Being a monospecific genus, it probably has not had the necessity of call diversification as a reproductive isolating mechanism. This situation appears to have developed in some species of the genus *Isoperla* (Bottorff *et al.* 1990, Tierno de Figueroa & Sánchez-Ortega 1998).

In addition, it has been suggested that a simple call with three beats could be an evolved model of simplification. This type of a call implies a simpler model that provides sufficient information. In this way, even among phylogenetically closely related species inhabiting the same area, different time intervals between beats could act as an isolating mechanism (Stewart *et al.* 1995).

3.3.2. Mating behaviour

Once the duet of the male and female calls is established, the male rapidly approaches the female and directly mounts her wings, seizing the female's thorax, and mating ensues. During mating, the male arches its abdomen underneath the abdomen of the female (in the characteristic S-form), assumes a position parallel to the female, and penetrates it with his penis, transmitting his sperm, in a way similar to that of other Perlodidae species (Brinck 1956, Berthélemy 1979). During the mating process, the male touches the female with its antennae, which is perhaps a tactile stimulation (Stewart *et al.* 1969). The couple remains immobile during the entire copulation.

The use of drumming in nature apparently facilitates the encounter between the two sexes, as in other Plecoptera (Abbott & Stewart 1993, Alexander & Stewart 1996). Nevertheless, drumming does not appear to be an obligatory courtship pattern or a requisite for mating, since on some occasions, copulation takes place directly after a male finds a female (without calls) which has also been found in other species (Berthélemy 1979, Bottorff *et al.* 1990).

In the laboratory, we studied ten matings of six different females. The number of copulations observed per female ranged from one to three (average = 1.7 copulations/female; SD = 0.8), though this number could be greater, since we did not know whether the female had mated previously in nature before being captured.

In all the observed cases, the female rejected the male's second mating attempt, and did not become receptive again until after she had deposited her clutch of eggs. The female could oviposit several times without the need for intermediate matings.

The duration of the mating varied between 39 and 106 minutes (average = 63 minutes/copulation; SD = 20.6), and in no case was the mating seen to be interrupted by foreign elements (presence of other males, movements in the mating box, etc.). No reduction or increase was noted in copulation time between successive matings of the same female.

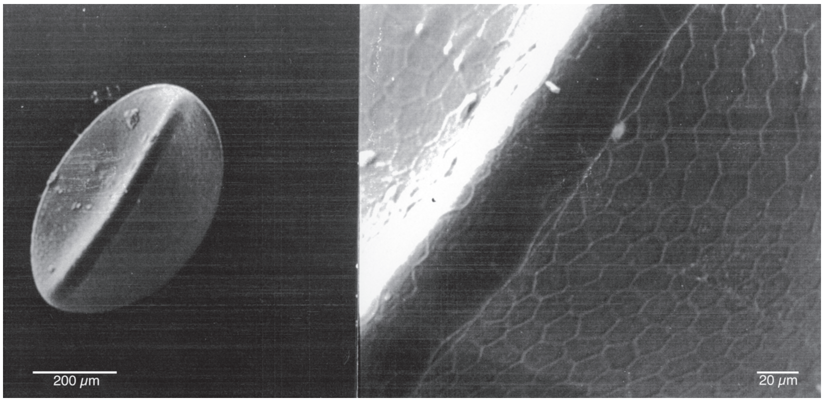

Fig. 2. SEM photographs of *H. flaviventris* egg. Left: egg morphology. Right: chorionic surface detail showing the follicle cell impression.

3.4. Clutches and eggs

The average number of eggs per clutch was 87.7 (SD = 42.8, SE = 7.3, CV = 0.49) in 34 clutches obtained in the laboratory. The number of eggs per clutch decreased in general for the same female between successive matings (A: 105-73-64-53; B: 122-33; C: 109-56-56-22; D: 103-66; E: 170-101-35-28; F: 153-66-35; G: 116-112; H: 120-54; I: 115-106; J: 195-61-42). Only in one case did the number of eggs per clutch increase in successive clutches: 95 and 100 eggs, respectively. In the rest of the cases, only one clutch was observed per female.

A maximum of four clutches per female were observed (in three of the 15 females which oviposited), although this number could be greater, since we did not know whether the females had oviposited in nature before being captured. The clutch size was highly variable, ranging from 22 to 195 eggs.

The maximum fecundity found was 357 eggs per dissected female, and 386 eggs after adding the number of eggs laid per female plus those which were found by dissection.

To calculate the egg size, we measured the long axis and the short axis in 15 eggs. The results were: 0.545 mm on average for the long axis (SD = 0.012), and 0.374 mm. on average for the short axis (SD = 0.010). Compared to data reported for other eggs of Perlodidae (Brinck 1949), *H. flaviventris* eggs are large.

The eggs were yellow-brown, and had an oval form with a transverse section in the form of a three-pointed star (Fig. 2). This peculiar egg morphology could be a mechanism of saving space within the oviduct, as suggested by Khoo (1964) to explain the deformations which often appear in some eggs of *I. grammatica*. The eggs of *H. flaviventris* do not have the collar or the anchor typical to eggs of other Perloidea (Brinck 1949). Berthélemy (1973) associated this lack of the anchoring structure in *H. flaviventris* eggs (although according to the same author, the citation might correspond to *Afroperla* sp. and not to *H. flaviventris*), and eggs of other species of warm water with the need to enter interstitial environments to avoid exposure to the air when the streams become dry.

The clutch, spherical in shape, was transported to the end of the abdomen by the female, which, during oviposition, shook the apex of the abdomen in the water to free the egg mass. This movement may be an adaptation of these insects to shallow streams with almost no current, where a female this way disperse the eggs.

Contrary to reports on many Perloidea (Brinck 1949, Britain 1990), we found no mature eggs in the nymphs of this species during their last developmental stage.

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