

Biometric relationships among female size, fecundity, and flight period in *Isoperla nevada* Aubert 1952 (Plecoptera: Perlodidae)

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Abstract. Relationships among female size, fecundity (measured as number of eggs and/or egg size) and flight period in a population of *Isoperla nevada* from Southern Iberian Peninsula have been studied. Bigger females produce a higher number of eggs, although not necessarily bigger eggs. It has been also observed a reduction of the fecundity along the flight period associated to the fact that females emerging later were smaller. These data indicate that female size is a good indicator of its reproductive potential, and that an early emergence would have a good adaptive value for females.

Résumé. Relation biométriques entre la taille des femelles, la fécondité et la période de vol chez *Isoperla nevada* Aubert 1952 (Plecoptera: Perlodidae). Les rapports entre la taille de la femelle, la fécondité (mesurée en tant que nombre d'œufs et/ou taille de l'œuf) et la période de vol ont été étudiés dans une population d'*Isoperla nevada* dans le sud-est de la péninsule ibérique. Les femelles les plus grandes produisent un plus grand nombre d'œufs, mais pas nécessairement des œufs plus grands. Il a été aussi observé une diminution de la fécondité le long de la période de vol associée à la diminution de la taille de la femelle. Ces données montrent que la taille de la femelle est un bon indicateur de son potentiel reproductif, et que l'émergence au début de la période de vol aurait une valeur adaptative pour les femelles.

The relationship existing between female size and clutch size has been studied in several insect groups (Berrigan 1991; Stewart *et al.* 1991; Honěk 1993). In Plecoptera, some publications mention the existence of a positive relationship between both (Khoo 1964; Elliott 1988; Brittain 1990; Peckarsky & Cowan 1991).

It is commonly accepted in Plecoptera that the number of eggs a female may produce is directly proportional to its size (Khoo 1964; Brittain 1990; Peckarsky & Cowan 1991). In our research we have analyzed the relationship existing not only between the female size and the number of eggs, but also between its size and the egg size. Particularly we have focused on *Isoperla nevada* Aubert 1952. This species belongs to the superfamily Perloidea, where the egg is little affected by differences in the hydration of the surrounding membrane (which avoids mistakes in egg measurements). Furthermore, eggs are completely mature in nymphs of

last instars in this species (Tierno de Figueroa & Sánchez-Ortega 1999), so the female will not produce more eggs along its adult life.

The flight period of *I. nevada* and its relationship with imaginal size are well known in the study area (Tierno de Figueroa *et al.* 2001; Tierno de Figueroa & Sánchez-Ortega 2004). Here we also want to point out the relationship existing between female fecundity and the moment of the species flight period in which each female emerges.

Material and methods

Individuals were collected in Sierra Nevada (SE of Iberian Peninsula), in a study area sited in the Barranco de los Tejos, ED50 UTM 30SVG920871, 1660m. Samplings were made from June to September of 1992 (Tierno de Figueroa *et al.* 2001).

Adults were caught as described in Tierno de Figueroa & Sánchez-Ortega (2004), i.e., with the aid of an entomological net along the riparian vegetation. They were preserved in 70% ethanol.

Table 1. Descriptive statistics for wing length, femur length, egg number and egg volume.

| | Valid N | Mean | Median | Minimum | Maximum | Std.Dev. |
|--------------------------------|---------|-----------|-----------|-----------|------------|----------|
| Wing length (cm) | 49 | 0.934 | 0.945 | 0.710 | 1.070 | 0.067 |
| Femur length (mm) | 49 | 8.122 | 8.100 | 7.050 | 9.450 | 0.527 |
| Egg number | 49 | 218.347 | 201.000 | 100.000 | 457.000 | 83.987 |
| Egg volume (μm^3) | 49 | 8 574.794 | 8 629.200 | 7 301.800 | 10 560.600 | 599.673 |

For the biometric study, females with both wings and femurs well-preserved were considered, and with aspect of having all the eggs (swollen abdomen) ($N = 49$). Two measures were taken: wing length, from base to distal end, and hind femur length on the outer side. Data were obtained with the micrometer of an Olympus binocular microscope ($10\times$ for wing length and $40\times$ for femur length). These variables were chosen because they are clearly adaptative, as shown in Tierno de Figueroa & Sánchez-Ortega (2004).

Egg number of each female was count, obtained by dissection. They were measured with the micrometer of an Olympus microscope ($100\times$). When calculating egg volume, ellipsoid formula was used: $V = 4/3 \pi R r^2$. Twenty eggs were taken from each female. In order to know which number of eggs was significant to measure we used the running mean method (Chalmers & Parker 1986), obtaining that the value of the mean did not vary starting from the 10th measured egg (Tierno de Figueroa & Sánchez-Ortega 1999).

For wing and femur lengths, and for egg measures, measurement-error percentage (% ME) and repeatability (R) were calculated (Møller & Zamora-Muñoz 1997):

$$R = [100 - \% \text{ME}] / 100.$$

The % ME was calculated according to Yezerinac *et al.* (1992):

$$\% \text{ME} = S^2 \text{ within} / [S^2 \text{ within} + S^2 \text{ among}] \times 100,$$

$$\text{where: } S^2 \text{ among} = [\text{MS among} - \text{MS within}] / m$$

$m = \text{number of repetitions.}$

MS within (mean squared deviations of scores within each individual measures) = S^2 within (variance within each individual measures)

MS among = mean squared deviations of scores among different individual measures

Results (wing length: $R = 0.990$; $F_{9,10} = 191.778$; $P < 0.01$; % ME = 1.037; femur length: $R = 0.963$; $F_{9,10} = 53.545$; $P < 0.01$; % ME = 3.667; egg volume: $R = 0.972$; $F_{9,10} = 70.407$; $P < 0.01$; %ME = 2.800)

indicated that the % ME was low and that there was a high R .

Variations observed in the number of eggs could be due to the female oviposition before collected. Nevertheless the probability of having oviposited a number of times is the same for every female independently of its size, so this should not influence the results.

Previously to the data analysis, a normality test (Kolmogorov-Smirnov test) was applied to all the variables. In order to see if there were significant differences in the egg size between specimens, an ANOVA Kruskal-Wallis test was run. For seeing if there was a relationship between the animal size (measured as the femur or wing length) and its reproductive efficiency (measured as number of eggs and mean egg size), a Pearson correlation coefficient was used. All the statistics analysis were carried out with Statistica for Windows version 4.0 (StatSoft Inc 1993).

RESULTS AND DISCUSSION

A basic statistical analysis of the data is shown in table 1. Significant differences on egg size among individuals were found [Kolmogorov-Smirnov, $d = 0.07$; $p < 0.01$; $H(9, N = 200) = 81.53$; $p < 0.01$], showing the existence of variations in the mean egg volume among individuals.

The results of application of the Kolmogorov-Smirnov test to the following variables pointed out a normal distribution: wing (Kolmogorov-Smirnov, $d = 0.10$; $p > 0.20$), femur (Kolmogorov-Smirnov, $d = 0.06$; $p > 0.20$), egg number (Kolmogorov-Smirnov, $d = 0.11$; $p > 0.20$), and mean egg volume (Kolmogorov-Smirnov, $d = 0.12$; $p > 0.20$).

When applying the correlation coefficient mentioned before (see material and methods) we obtained the results shown in table 2. As we can see there is a high correlation between wing and femur length (Pearson $r = 0.75$; $p < 0.01$), and between both and the number of eggs that females lay down (Pearson $r = 0.61$ and Pearson $r = 0.62$, respectively; $p < 0.01$), which shows that biggest

Table 2.

Correlation coefficient between the different parameters measured (significant values, $p < 0.01$, in bold italics).

| | Wing length | Femur length | Egg number | Egg volume |
|--------------|-------------|--------------|-------------|-------------|
| Wing length | 1.00 | 0.75 | 0.61 | 0.02 |
| Femur length | | 1.00 | 0.62 | 0.05 |
| Egg number | | | 1.00 | -0.12 |
| Egg volume | | | | 1.00 |

females have highest number of eggs in clutches. These results confirm that obtained for other insect species, in which the reproductive capacity, measured as number of eggs, increases proportionally to the female size (Stewart *et al.* 1991; Honěk 1993).

Eggs from bigger females are not significantly bigger than those from smaller ones (Pearson $r = 0.02$ y Pearson $r = 0.05$, respectively to the wing and femur length; $p > 0.01$). Thus, egg size in this species seems to be independent of female size.

Clutch size (as number of eggs) is not negatively correlated in a significant manner with the mean egg size per clutch (Pearson $r = -0.12$; $p > 0.01$). If this correlation had been significant, it would imply a balance of the reproductive effort between the production of higher number of eggs or the production of bigger eggs, both also limited by the necessity of maintaining a non-excessive abdomen size that would difficult flight. This is especially important in good flyer insects (Berrigan 1991), although this is not the case.

When we studied the variation of female fecundity along the flight period we found that, when advancing in this period, there is a decrease in wing length (Pearson $r = -0.61$; $p < 0.01$), in femur length (Pearson $r = -0.62$; $p < 0.01$) [coinciding with that pointed by Tierno de Figueroa & Sánchez-Ortega (2004)], in the number of eggs per female (Pearson $r = -0.42$; $p < 0.01$), and in the mean egg volume (Pearson $r = -0.29$; $p < 0.01$).

In the light of these data we may think that biggest females will have more eggs. This is probably because they had a longer nymphal period for developing or a higher proportion of developing in that period due to environmental conditions (Sweeney 1984). Nevertheless, this may be influenced by individual differences with genetic base. In fact, as pointed out by Honěk (1993), both size and fecundity are influenced by genetic factors and development conditions.

The lower size of the females in the late flight period could be explained because females which emerge before are those which have already reached the optimum size, whereas those which emerge at the end of the flight period may have been forced to delay the emergency trying to reach an optimum degree of development. This would make the number of eggs they are able to produce to be lower and their size smaller. Some authors have demonstrated in stoneflies how insufficient quantities of nutrients, intraspecific competition or non-optimum thermal regimes during nymphal stage have negative effects on adult size and fecundity, as a delay in the emergency (Perry *et al.* 1987; Peckarsky & Cowan 1991) or a functional reduction, due to physiological fertility is determined during the nymphal stage (Peckarsky & Cowan 1991; Honěk 1993). This is especially evident in many Perloidea, which emerge with all or almost every egg already formed.

A factor to take into account when we analyse the results obtained in the study of the variation of fecundity along the flight period is that the probability of finding a female that has oviposited once or more times is higher at the end of it, because they can be older. This could also explain the smaller mean size of the eggs in the late flight period, due to the percentage of eggs that could not rise the optimum size may be higher. In order to avoid this problem in our study we worked with females full of eggs that very probably had not oviposited (see material and methods).

In fact, in some species of insects it has been found that the mean egg size varies with female age and with temperature (Honěk 1993). Nevertheless, intraspecific variations in egg quality (size or another indicators, such as weight, lipid proportions, etc.) have poorly being studied in aquatic insects, although it is though that environmental factors would affect more to the quantity than to the quality of eggs (Sweeney 1984).

It is for all the aspects mentioned above that results in the variation of fecundity along the flight period for a given species must be taken into account with the limitations shown before.

For *I. nevada*, female size is probably a good indicator of its reproductive potential, and emergence at the beginning of the flight period may have a good adaptive value for females, which would have the capacity of leaving a higher number of descendants.

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