*International Journal of Odonatology*
2024, Vol. 27, pp. 77–84
doi:10.48156/1388.2024.1917271

# Geographical variation in size of the three final stadia of *Cordulegaster boltonii* (Donovan, 1807) larvae in the Iberian Peninsula (Odonata: Cordulegastridae)

M. Ángeles Hernández[a,*], Patricia Casanueva[b], Javier Oscoz[a], Luis Fernando Sánchez-Sastre[c], Manuel Ferreras-Romero[d] & Francisco Campos[e]

[a] Departamento de Biología Ambiental, Facultad de Ciencias, Universidad de Navarra, 31080 Pamplona, Spain

[b] Departamento de Ciencias Experimentales, Universidad Europea Miguel de Cervantes, 47012 Valladolid, Spain

[c] Área de Ingeniería Cartográfica, Geodésica y Fotogrametría, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, 34004 Palencia, Spain

[d] Calle Gerona 9, 41003 Seville, Spain

[e] Paseo de Zorrilla 100, 47006 Valladolid, Spain

*Corresponding author: Email: mahermin@unav.es

**Research Article**



**Published:** 13 March 2024
**Received:** 5 December 2023
**Accepted:** 1 March 2024

**Citation:**
Hernández, Casanueva, Oscoz, Sánchez-Sastre, Ferreras-Romero & Campos (2024): Geographical variation in size of the three final stadia of *Cordulegaster boltonii* (Donovan, 1807) larvae in the Iberian Peninsula (Odonata: Cordulegastridae). *International Journal of Odonatology, 27,* 77–84 doi:10.48156/1388.2024.1917271

**Data Availability Statement:**
All relevant data are within the paper.

**Abstract.** A two-variable analysis of male and female *Cordulegaster boltonii* larvae (head width and hindwing sheath length) in specimens from five Iberian populations was carried out with the objective of ascertaining whether these traits differ between populations. The results indicate that the southernmost population (Los Alcornocales, Andalusia) have larger sizes for both variables and a lower growth rate between the penultimate and final stadia. Winter water temperatures, which may cause a longer arrest of larval growth in northern populations, and possible differences in the number of larval stadia could be the origin of the size divergences.

**Key words.** Anisoptera, dragonflies, growth rate, larval stadia, southern Europe

## Introduction

*Cordulegaster boltonii* (Donovan, 1807) is a species widely distributed throughout the western Palaearctic. It ranges from North Europe (southern Scandinavia) to Northwest Africa (Morocco) (Boudot & Holuša, 2015; Dijkstra et al., 2020). Cordulegastrid dragonflies typically occur and breed in lotic waters, generally small streams and brooks, where larvae of the genus *Cordulegaster* Leach, 1815 live as shallow burrowers in the sediment. This species is widespread throughout all the mountainous areas of the Iberian Peninsula (Portugal and Spain) and is often locally abundant.

Information on the life cycle of *C. boltonii* throughout its distribution range is sparse (Brettfeld, 1989; Corbet et al., 1960; Lloyd & Ormerod, 1992; Ormerod et al., 1990; Schütte, 1997). In the Sierra Morena Mountains (southern Spain) *C. boltonii* presents itself mainly as a partivoltine 'spring species' *sensu* Corbet (1954, 1964), with its larvae spending the winter before emergence in the last two stadia (mostly in F-0), but a low proportion of the larvae require only two years to complete their development (semivoltinism). Emergence takes place early in the year and in a relatively synchronized manner, and the species skips an embryonic diapause (Ferreras-Romero & Corbet, 1999). Current knowledge of the life cycles of Odonata is biased towards more northern temperate areas and to some extent those with larvae that live in ponds (Norling, 2021). The Mediterranean area is thus interesting in bridging the gap to the subtropics.

Little is known about how geographical location of a population may affect the biometric peculiarities of *C. boltonii*. Recently, differences in the wing venation between northern and southern Iberian Peninsula populations of *C. boltonii* have been found, which also differ with regard to the venation patterns found in European populations inhabiting regions outside that peninsula (Nunes et al., 2023). Likewise, Iberian populations differ regarding head width and prementum size in final-stage exuviae (Casanueva et al., 2020; Hernández et al., 2022). Larval growth rates of this species have not been analyzed until now on a broader geographical scale such as the Iberian Peninsula. Using anatomical characteristics of larvae, the aim of this study is to demonstrate the existing differentiation in both larval size and growth rate between *C. boltonii* specimens from five Iberian areas located from near the Strait of Gibraltar (warmer) to the Pyrenees (colder).

## Material and methods

In the Iberian Peninsula, five mountainous were selected and specified as "Pyrenees", "Sierra La Culebra", "Central System", "Sierra Morena", and "Los Alcornocales", respectively. The first three of these are situated in the northern half, and the other two in the southern half (Fig. 1). The first belongs to the Iberian Euro-Siberian region, and the other four to different bioclimatic zones of the Mediterranean region. Their basic characteristics of altitude (m a.s.l.), mean annual temperature (°C), and annual precipitation (mm) are summarized in Table 1. In each area, 1–5 streams were sampled. For the calculations, the data from all the streams were pooled per area.

Larvae were collected using hand nets and fixed in 70% ethanol, except those from the Sierra La Culebra and Central System, which were measured *in situ* and returned to the water. The preserved larvae are now deposited in the Science Museum of the University of Navarra (Pyrenees) and the personal collection of Manuel Ferreras-Romero (Sierra Morena and Los Alcornocales).

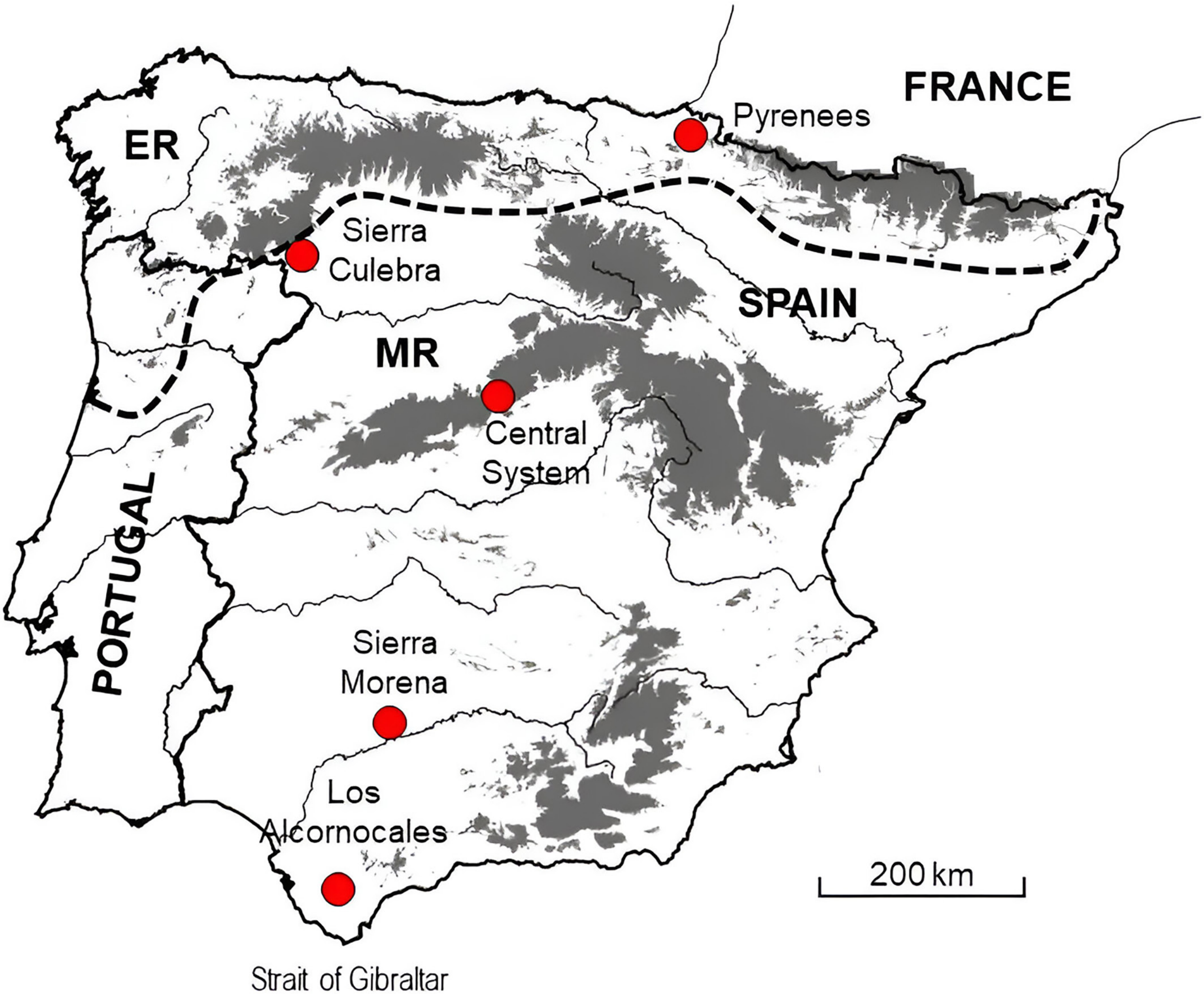


**Figure 1.** Distribution of the five areas sampled (red dots) on the Iberian Peninsula: Pyrenees, Sierra La Culebra, Central System, Sierra Morena and Los Alcornocales. Shaded area: altitude ≥ 1000 m a.s.l.. The dashed line roughly separates the Euro-Siberian Region (ER) from the Mediterranean Region (MR).

**Table 1.** Some characteristics of the five sampling areas in Spain: latitude and longitude as decimal coordinates, altitude in m above sea level, Tm (mean annual temperature) in °C, and Pa (annual precipitation) in mm. Tm and Pa were obtained from AEMET (2023). * location of the natural park's administration offices.

| | Pyrenees | Sierra La Culebra | Central System | Sierra Morena | Los Alcornocales |
|---|---|---|---|---|---|
| Province | Navarra & Huesca | Zamora | Segovia & Ávila | Córdoba | Cádiz |
| Municipality | Bértiz | Ferreras de Arriba | San Ildefonso | Sta. María de Trassierra | Alcalá de los Gazules* |
| Latitude | 43.1611 | 41.8967 | 40.8488 | 37.9366 | 36.4721 |
| Longitude | -1.6306 | -6.1971 | -4.0179 | -4.8488 | -5.7243 |
| Altitude (m a.s.l.) | 280 | 899 | 1200 | 400 | 130 - 620 |
| Tm (°C) | 12.7 | 9.9 | 10.1 | 16.5 | 17.6 |
| Pa (mm) | 1716 | 782 | 1075 | 769 | 824 |
| Sampling period | 2001-2016 | 2023 | 2017-2018 | 1990-1992 | 1999-2005 |

Two characteristics were analyzed for each specimen: head width (HW), i.e., the maximum distance between the lateral margins of the compound eyes, and the length of the right metathoracic (hind) wing sheath (WSL), each measured to the nearest 0.1 mm. On the basis of HW and WSL, each larva was either assigned to one of the last three instars or considered a 'smaller larva' (Ferreras-Romero & Corbet, 1999). Here we follow common practice, designating the final, penultimate, and antepenultimate stadia as F-0, F-1, and F-2, respectively. The sex of each larva was identified according to the presence (female) or absence (male) of gonapophyses on the ventral surface of the eighth and ninth abdominal segments. Growth rates (GR) for HW and WSL were calculated by dividing the mean value for a stadium by the mean value for the preceding stadium (Tennessen, 2017).

Differences between sexes for each studied were subjected to an ANOVA in STATA 12.1 software, after verifying that the criteria for normality and homogeneity of variance were met. If this was not the case, a non-parametric Kruskal-Wallis analysis was performed. Pairwise comparisons of the two variables were also made with STATA 12.1 software for both sex and instar for from the different areas of the Iberian Peninsula.

Using Past 3.15 software (Hammer et al., 2001), a clustered multivariate analysis was conducted to group the variables in a quest to achieve maximum homogeneity in each group and show up the greatest difference between the groups. In this analysis, the unweighted pair-group average (UPGMA) was used as an algorithm, and the Euclidean and constrained distances as the similarity indices. Clusters are joined based on the average distance between all members in the two groups. With a bootstrapping of N = 10,000, the percentage of support is given in each of the nodes.

## Results

A total of 1256 larvae of *C. boltonii* (602 males, 654 females), that were in one of the last three stadia of development, were analyzed. Of these, 35 (16 males, 19 females) were from the Pyrenees, 166 (81, 85) from the Sierra La Culebra, 388 (185, 203) from the Central System, 432 (205, 227) from the Sierra Morena—only the Bejarano stream—, and 235 (115, 120) were from Los Alcornocales.

### *Differences between sexes*

In all the areas analyzed, the values in both HW and WSL were higher in females than in males in the larvae of the three instars studied (Table 2). These differences were always statistically significant, except for HW (F-1 and F-2 stadia) and WSL (F-2 stadium) from the Pyrenees (Table 3). Therefore, these two variables had to be analyzed separately for males and females.

### *Differences within sexes*

Average values of HW differed significantly between male larvae (F-0, F-1, and F-2) from Los Alcornocales and those examined from the other areas (Table 4). However, for WSL this was only true in F-1 and F-2 stadia since the F-0 larvae from Los Alcornocales and the Central System had similar average values. With regard to the females, the average values of both HW and WSL for the three stadia analyzed were again significantly higher in larvae from Los Alcornocales than in those from the remaining four areas (Table 5).

### *Geographical variation*

The clustered multivariate analyses of the two variables revealed quite similar grouping patterns in both males and females, although with different distances between groups and support percentages (Fig. 2). The F-0 larvae from northern areas (Pyrenees, Central System, and Sierra La Culebra) appear grouped, while those from southern locales (Sierra Morena and Los Alcornocales) are separated from each other and from the aforesaid group. A fairly similar grouping pattern emerged for F-1 female larvae. The groupings of the F-2 larvae of both sexes were different from the previous ones, but again positioned those from Los Alcornocales clearly separated from our samples from the four remaining areas (Fig. 2).

**Table 2.** Mean values ± SD of wing sheath length (WSL) and head width (HW) (both in mm) in larvae of males and females of *Cordulegaster boltonii* from five Iberian sampling areas (sample size in brackets).

| | | Instar | Pyrenees | Sierra La Culebra | Central System | Sierra Morena | Los Alcornocales |
|---|---|---|---|---|---|---|---|
| Males | WSL | F-2 | 2.33 ± 0.10 (6) | 2.37 ± 0.20 (34) | 2.19 ± 0.26 (56) | 2.13 ± 0.20 (51) | 2.60 ± 0.25 (43) |
| | | F-1 | 4.19 ± 0.87 (4) | 4.17 ± 0.50 (19) | 4.37 ± 0.26 (15) | 4.05 ± 0.23 (44) | 4.63 ± 2.21 (25) |
| | | F-0 | 8.85 ± 0.51 (6) | 8.70 ± 0.44 (28) | 9.06 ± 0.34 (114) | 8.24 ± 0.24 (110) | 9.06 ± 0.30 (47) |
| | HW | F-2 | 4.93 ± 0.27 (6) | 4.98 ± 0.17 (34) | 4.74 ± 0.23 (56) | 4.75 ± 0.19 (51) | 5.23 ± 0.24 (43) |
| | | F-1 | 5.93 ± 0.30 (4) | 6.26 ± 0.21 (19) | 6.17 ± 0.23 (15) | 6.00 ± 0.20 (44) | 6.55 ± 0.21 (25) |
| | | F-0 | 7.97 ± 0.24 (6) | 7.96 ± 0.13 (28) | 7.94 ± 0.21 (114) | 7.51 ± 0.18 (110) | 8.13 ± 0.18 (47) |
| Females | WSL | F-2 | 2.37 ± 0.42 (6) | 2.49 ± 0.24 (31) | 2.52 ± 0.18 (54) | 2.32 ± 0.20 (53) | 2.79 ± 0.23 (42) |
| | | F-1 | 4.68 ± 0.31 (9) | 4.69 ± 0.41 (17) | 4.72 ± 0.32 (22) | 4.44 ± 0.19 (52) | 4.94 ± 0.29 (24) |
| | | F-0 | 9.70 ± 0.61 (4) | 9.41 ± 0.32 (37) | 9.73 ± 0.28 (127) | 8.90 ± 0.35 (122) | 9.88 ± 0.31 (54) |
| | HW | F-2 | 5.27 ± 0.32 (6) | 5.24 ± 0.23 (31) | 5.04 ± 0.23 (54) | 5.10 ± 0.21 (53) | 5.56 ± 0.17 (42) |
| | | F-1 | 6.64 ± 0.12 (9) | 6.71 ± 0.25 (17) | 6.61 ± 0.25 (22) | 6.41 ± 0.18 (52) | 7.01 ± 0.21 (24) |
| | | F-0 | 8.68 ± 0.17 (4) | 8.56 ± 0.19 (37) | 8.48 ± 0.20 (127) | 8.12 ± 0.19 (122) | 8.76 ± 0.27 (54) |

**Table 3.** Statistical analysis between the sexes of *Cordulegaster boltonii* larvae in wing sheath length (WSL) and head width (HW) of the stadia F-0, F-1, and F-2. F: ANOVA, M: Kruskal-Wallis test, *p*: probability. Significant values are in boldface.

| Instar | Variable | Pyrenees | Sierra La Culebra | Central System | Sierra Morena | Los Alcornocales |
|---|---|---|---|---|---|---|
| F-0 | WSL | F = 25.3<br>***p* = 0.001** | M = 35.224<br>***p* = 0.0001** | F = 617.89<br>***p* = 0.0001** | M = 165.29<br>***p* = 0.0001** | M = 68.31<br>***p* = 0.0001** |
| | HW | F = 5.74<br>***p* = 0.043** | M = 46.276<br>***p* = 0.0001** | M = 146.21<br>***p* = 0.0001** | M = 142.53<br>***p* = 0.0001** | F = 179.11<br>***p* = 0.0001** |
| F-1 | WSL | M = 7.714<br>***p* = 0.0055** | M = 17.1<br>***p* = 0.0001** | F = 29.19<br>***p* = 0.0001** | F = 108.48<br>***p* = 0.0001** | F = 58.33<br>***p* = 0.0001** |
| | HW | M = 0.595<br>*p* = 0.440 | M = 18.572<br>***p* = 0.0001** | F = 12.54<br>***p* = 0.0011** | M = 48.941<br>***p* = 0.0001** | F = 18.95<br>***p* = 0.0001** |
| F-2 | WSL | F = 4.04<br>*p* = 0.072 | F = 4.37<br>***p* = 0.0406** | F = 51.26<br>***p* = 0.0001** | M = 48.137<br>***p* = 0.0001** | M = 32.945<br>***p* = 0.0001** |
| | HW | M = 0.028<br>*p* = 0.8728 | F = 27.12<br>***p* = 0.0001** | M = 48.771<br>***p* = 0.0001** | F = 24.31<br>***p* = 0.0001** | F = 12.97<br>***p* = 0.0005** |

### *Growth rate*

In the five areas here compared, the GR for head width for the moult from F-1 to F-0 varied from 1.24 to 1.34, whereas the GR for hindwing sheath length varied from 1.95 to 2.11. The range of variation for both values in females fall within those seen in males (Table 6). Likewise, the GR for head width for the moult from F-2 to F-1 varied from 1.20 to 1.31, whereas the GR for hindwing sheaths varied from 1.76 to 1.99.

In males, the highest growth rates between F-2 and F-1, for both HW and WSL, were found in larvae from the Central System (Table 6). For their part, the F-1 and F-0 instars of the larvae from the Pyrenees exhibited higher growth rates than those recorded from larvae from the remaining sampling areas. In females, the highest HW growth rates were again noted in our samples from the Central System (step F-2/F-1) and the Pyrenees (step F-1/F-0). However, the highest growth rates for WSL, both moults, were recorded in larvae from the Pyrenees (Table 6). The lowest growth rates in the final larval moult (F-1 to F-0), in both sexes and for both HW and WSL, were seen in the larvae from the southernmost population (Los Alcornocales), followed by the one from the Sierra Morena.

## Discussion

Previous works have demonstrated that males and females of Iberian *C. boltonii* differ in larval biometry (Casanueva et al., 2020; Ferreras-Romero & Corbet, 1999). Females are larger in size than of males in both larvae and F-0 larva exuviae (Hernández et al., 2022). Our results indicate that this is also true for larvae of the last three stadia of development at the five areas here investigated and probably throughout the Iberian Peninsula.

Growth rate (F-0/F-1) value ranges obtained for HW in this work (1.24-1.34) are within those observed by Tennessen (2017) who compared nine species of American odonates (1.15–1.44). Likewise, our range is

**Table 4.** Pairwise comparisons of wing sheath length (WSL) and head width (HW) in male larvae from the five areas analyzed. 1: Pyrenees, 2: Sierra La Culebra, 3: Central System, 4: Sierra Morena, 5: Los Alcornocales. For each variable, the diagonal separates the values of pairwise comparisons of means (lower zone) from the probability value (upper zone). Statistically significant values are in boldface.

| Instar | Variable | Area | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| F-0 | WSL | 1 | | 0.832 | 0.508 | 0.0001 | 0.517 |
| | | 2 | -1.05 | | **0.0001** | **0.0001** | **0.0001** |
| | | 3 | 1.58 | -5.39 | | 0.0001 | 1.000 |
| | | 4 | -4.65 | 6.98 | -19.55 | | 0.0001 |
| | | 5 | 1.57 | -4.83 | 0.1 | 15.1 | |
| | HW | 1 | | 0.818 | 0.372 | 0.0001 | 0.921 |
| | | 2 | -1.08 | | 0.334 | 0.0001 | 0.0001 |
| | | 3 | -1.81 | 1.87 | | 0.0001 | 0.0001 |
| | | 4 | -5.21 | 11.09 | -13.66 | | 0.0001 |
| | | 5 | 0.83 | -4.68 | 8.3 | 18.84 | |
| F-1 | WSL | 1 | | 1.000 | 0.861 | 0.935 | 0.104 |
| | | 2 | -0.08 | | 0.423 | 0.668 | 0.0001 |
| | | 3 | 0.99 | -1.73 | | 0.014 | 0.128 |
| | | 4 | -0.78 | 1.34 | -3.22 | | 0.0001 |
| | | 5 | 2.48 | -4.52 | 2.38 | 6.96 | |
| | HW | 1 | | 0.043 | 0.260 | 0.962 | 0.0001 |
| | | 2 | 2.83 | | 0.745 | **0.0001** | 0.0001 |
| | | 3 | 2.03 | 1.21 | | 0.070 | 0.0001 |
| | | 4 | -5.21 | 4.4 | -2.64 | | 0.0001 |
| | | 5 | 5.42 | -4.47 | 5.44 | 10.25 | |
| F-2 | WSL | 1 | | 0.998 | 0.612 | 0.222 | 0.054 |
| | | 2 | 0.31 | | 0.006 | 0.0001 | 0.0001 |
| | | 3 | -1.43 | 3.45 | | 0.543 | 0.0001 |
| | | 4 | -2.11 | -4.72 | -1.53 | | 0.0001 |
| | | 5 | 2.72 | -4.57 | 8.87 | 10.12 | |
| | HW | 1 | | 0.984 | 0.228 | 0.284 | **0.009** |
| | | 2 | 0.53 | | 0.0001 | 0.0001 | **0.0001** |
| | | 3 | -2.09 | 5.22 | | 0.999 | 0.0001 |
| | | 4 | -1.97 | 4.91 | 0.25 | | 0.0001 |
| | | 5 | 3.34 | -5.31 | 11.6 | 11.13 | |

quite similar to the range given by Schütte (1997) for the first nine stadia of *C. boltonii* in southern France (1.23–1.38). On the other hand, throughout the five areas here compared, the GR for hind WSL was constantly higher for the moult from F-1 to F-0 than that for the previous moult (Table 6). Thus it can be stated that Iberian *C. boltonii* larvae exhibit WSL growth rates that are similar in variation between stadia to those recorded from other Odonata species (Tennessen, 2017). However, this progressive increase of GR in the last two developmental stages did not always occur for HW in both males and females (Table 6). Probably, each population has adapted its growth rates to the environmental conditions under which it develops.

Within the Iberian Peninsula, the sizes of some *C. boltonii* structures vary with the geographical situation of the individual populations. Indeed, the prementum sizes of F-0 exuviae are greater in southern populations than in northern ones, in both males and females (Hernández et al., 2022). El Haissoufi et al. (2018) and Casanueva et al. (2020) analyzed head width in exuviae of F-0 larvae (only females) from Iberia and North Africa and showed that those from the Rif Mountains (Morocco) are clearly larger than those inhabiting the Iberian Peninsula. These authors did neither study specimens from the Pyrenees (the northern limits of Iberia) nor Los Alcornocales (the southern tip of Iberia), but the Rif Mountains are located only about 150 km south of Los Alcornocales.

In the present study, the data obtained from larvae of the last three developmental stadia once again showed biometric differences across the Iberian Peninsula. The highest average values were found in the southernmost population (Los Alcornocales); the climate in the Iberian south is similar to that of North Africa. Our results demonstrate that the *C. boltonii* larvae from our sample sites Pyrenees, Sierra La Culebra, and Central System, i.e., the three northern areas

**Table 5.** Pairwise comparisons of wing sheath length (WSL) and head width (HW) in female larvae from the five areas analyzed. 1: Pyrenees, 2: Sierra La Culebra, 3: Central System, 4: Sierra Morena, 5: Los Alcornocales. For each variable the diagonal separates the values of pairwise comparisons of means (lower zone) from the probability value (upper zone). Statistically significant values are in boldface.

| Instar | Variable | Area | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| F-0 | WSL | 1 | | 0.435 | 1 | **0.0001** | 0.818 |
| | | 2 | -1.7 | | 0.0001 | 0.0001 | 0.0001 |
| | | 3 | 0.16 | -5.24 | | 0.0001 | 0.0001 |
| | | 4 | -4.94 | 8.61 | -20.47 | | 0.0001 |
| | | 5 | 1.08 | -6.81 | 2.93 | 18.78 | |
| | HW | 1 | | 0.818 | 0.372 | **0.0001** | 0.921 |
| | | 2 | -1.08 | | 0.334 | **0.0001** | **0.0001** |
| | | 3 | -1.81 | 1.87 | | 0.0001 | 0.0001 |
| | | 4 | -5.21 | 11.09 | 13.66 | | 0.0001 |
| | | 5 | 0.83 | -4.68 | 8.3 | 18.84 | |
| F-1 | WSL | 1 | | 1 | 0.996 | 0.133 | 0.121 |
| | | 2 | 0.12 | | 0.998 | **0.014** | **0.044** |
| | | 3 | 0.38 | -0.32 | | **0.001** | 0.064 |
| | | 4 | -2.36 | 3.22 | -3.94 | | 0.0001 |
| | | 5 | 2.41 | -2.82 | 2.68 | 7.27 | |
| | HW | 1 | | 0.928 | 0.998 | **0.025** | **0.0001** |
| | | 2 | 0.81 | | 0.626 | **0.0001** | **0.044** |
| | | 3 | 0.3 | 1.41 | | 0.002 | 0.0001 |
| | | 4 | -3.03 | 5.1 | -3.82 | | 0.0001 |
| | | 5 | 4.56 | -4.57 | 6.44 | 11.65 | |
| F-2 | WSL | 1 | | 0.726 | 0.447 | 0.986 | **0.0001** |
| | | 2 | 1.24 | | 0.943 | 0.007 | 0.0001 |
| | | 3 | 1.68 | -0.75 | | 0.0001 | 0.0001 |
| | | 4 | -0.51 | 3.43 | -4.89 | | 0.0001 |
| | | 5 | 4.47 | -5.9 | -5.96 | 10.51 | |
| | HW | 1 | | 0.998 | 0.115 | 0.369 | **0.014** |
| | | 2 | -0.29 | | 0.001 | 0.036 | 0.0001 |
| | | 3 | -2.42 | 4.04 | | 0.665 | **0.0001** |
| | | 4 | -1.81 | 2.87 | 1.34 | | 0.0001 |
| | | 5 | 3.2 | -6.44 | 11.84 | 10.53 | |

studied in this work, appear grouped in our analysis and are separated from those from the two southern areas.

However, the sizes of the larvae from the Pyrenees, Sierra La Culebra, and the Central System are larger than those of the larvae from the Sierra Morena population, located at a lower latitude. Consequently, our results only partially agree with what was suggested by Hernández et al. (2022) on the adjustment of the species *C. boltonii* in the Iberian Peninsula as per Bergmann's inverse rule (Bergmann, 1847; Mousseau, 1997), because specimens are not always smaller at higher latitudes. Many scientists have posited that ectotherms may display a "converse-Bergmann" cline, in which organisms are smaller in colder climates and larger in warmer ones, a theory inspired by the heat-dependent growth rates and metabolic rates of ectotherms (Makarieva et al., 2005; Winterhalter & Mousseau, 2008). However, Shelomi (2012) concluded that Bergmann's rule, in any variation or definition, can never be readily assumed to apply to insects.

The variation in size recorded in the present work could be partially explained by the higher environmental temperature average in the southern parts of the Iberian Peninsula (IGN, 2019 and Table 1), which as a consequence promotes higher water temperatures. Altitude and temperature are two factors related to each other (Montgomery, 2006), which would also explain why the larvae from Los Alcornocales, located mainly in watercourses at below 400 m a.s.l., are the largest. According to Suhling et al. (2015), growth rate in odonates increases with temperature, although there is an optimum temperature for growth for each species, but this value is as yet unknown for *C. boltonii*.

However, the F-0 larvae from Los Alcornocales, even though they were the largest throughout the areas analyzed, were the ones with the lowest growth rates. This fact is not readily understood. It suggests that the larvae

**Table 6.** Growth rates during the three final stadia of larval development in *Cordulegaster boltonii* males and females of the five sampling areas.

| | | Instar | Pyrenees | Sierra La Culebra | Central System | Sierra Morena | Los Alcornocales |
|---|---|---|---|---|---|---|---|
| Males | WSL | F-2 / F-1 | 1.795 | 1.765 | 1.993 | 1.907 | 1.777 |
| | | F-1 / F-0 | 2.113 | 2.085 | 2.073 | 2.033 | 1.958 |
| | HW | F-2 / F-1 | 1.203 | 1.258 | 1.303 | 1.264 | 1.251 |
| | | F-1 / F-0 | 1.345 | 1.272 | 1.287 | 1.252 | 1.242 |
| Females | WSL | F-2 / F-1 | 1.977 | 1.886 | 1.870 | 1.914 | 1.771 |
| | | F-1 / F-0 | 2.074 | 2.007 | 2.061 | 2.005 | 1.999 |
| | HW | F-2 / F-1 | 1.261 | 1.281 | 1.311 | 1.257 | 1.260 |
| | | F-1 / F-0 | 1.307 | 1.275 | 1.283 | 1.267 | 1.250 |

from this population are larger by consequence of other factors driving growth rate that were not analyzed in this work. Improved availability of food (Corbet, 2004) and significantly more moderate winter water temperatures could facilitate larval growth with hardly any season-induced interruptions (Molina Rodríguez et al., 2022). On the other hand, the number of times Odonata moult between hatching and metamorphosis to the adult stage varies from 9 to 17 (Corbet, 2004). The total number of stadia appears to vary also within species, and an egg mass from a single female can produce nymphs that undergo different numbers of moults despite their being subjected to identical environmental conditions and feeding rates (e.g., Tennesen, 2017). It is probable that the larvae of the southernmost population pass through a higher number of instars to reach the final one.

On the other hand, in *Boyeria irene* (Odonata, Aeshnidae) larvae, another Iberian rheophilic species, latitudinal variation in size has been recorded between populations from the southern half of the peninsula, with larvae of the F-1 and F-0 stadia being larger in populations from the southernmost locales than in those at more northern latitudes (Molina Rodríguez et al., 2022). Our results for *C. boltonii* in the southern half of the Iberian Peninsula confirm that they follow a similar pattern of latitudinal size variation .

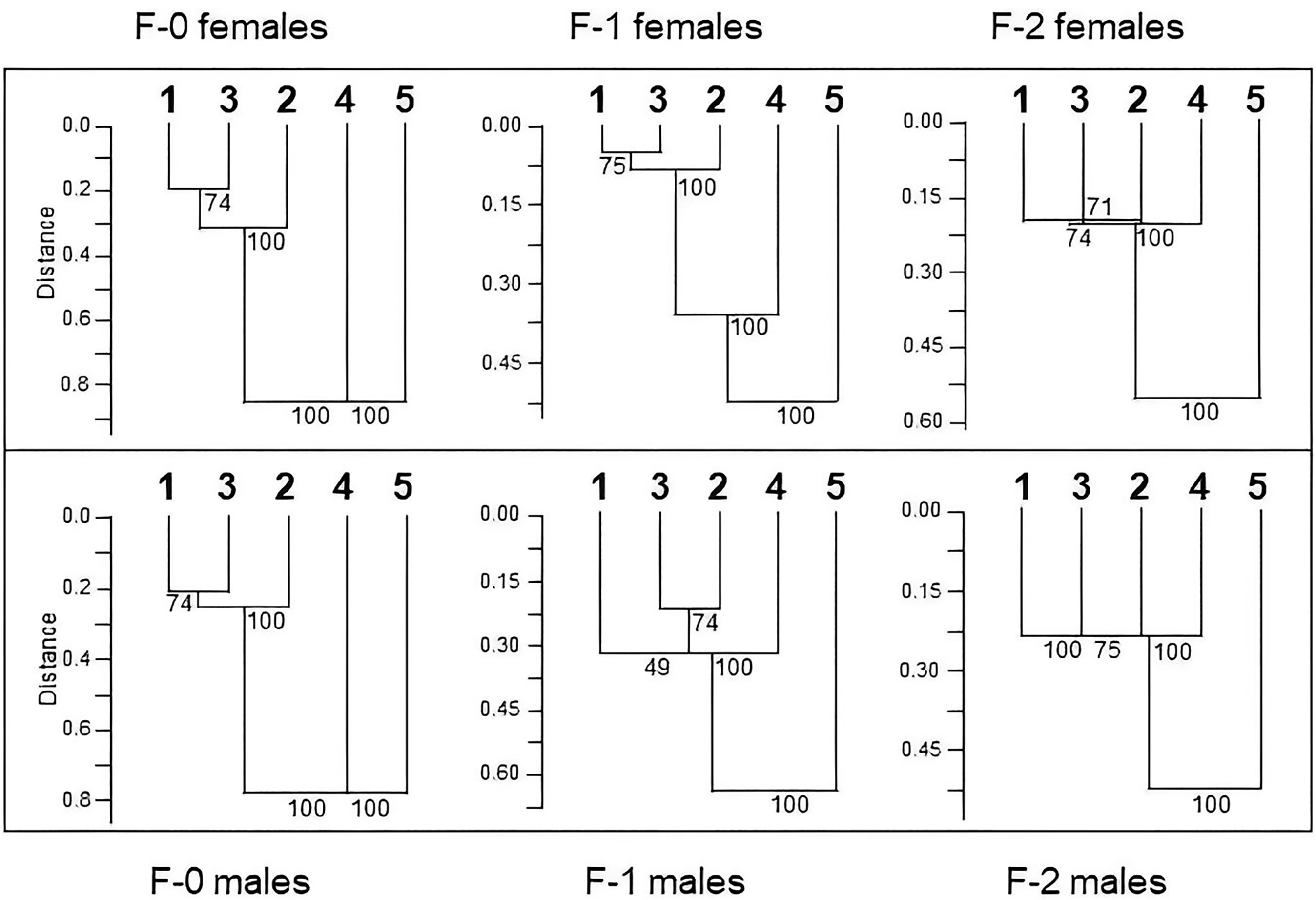


**Figure 2.** Dendrograms grouping the five areas analyzed based on head width and wing sheath length of the three final larval stadia of *Cordulegaster boltonii*. Numbers at nodes indicate bootstrap support for that particular branch.

Finally, the increase in environmental temperature recorded in the Iberian Peninsula (AEMET, 2023) has undoubtedly brought about higher water temperatures in the streams where *C. boltonii* develops. If the temperature increases, the duration of the larval period can be expected to be shortened, as is suggested by the fact that this species spends 2–3 years as a larva in the Sierra Morena (Ferreras-Romero & Corbet, 1999), but more years in northern Europe where temperatures are lower (Donath, 1987; Schütte, 1997). Whether this factor also causes larvae to be larger or smaller is something that needs to be investigated in the future.

**Acknowledgements**

Our thanks go to Ana Amezcua, Ángel Chaves, María Imas, and Enrique Baquero (Science Museum of the University of Navarra) who facilitated the examination of specimens from the Pyrenees contained in the collection under their care. The Regional Government of Castilla and León granted permits to handle certain specimens.

**References**

AEMET, Spanish State Meteorological Agency (2023). *Informe sobre el estado del clima de España 2022*. Madrid: Ministerio para la Transición Ecológica y el Reto Demográfico.

Bergmann, C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, *3*, 595–708.

Boudot, J.-P. & Holuša, O. (2015). *Cordulegaster boltonii* (Donovan, 1807). In J.-P. Boudot & V. J. Kalkman (Eds.), *Atlas of the European dragonflies and damselflies*. pp. 213–215. The Netherlands: KNNV Publishing.

Brettfeld, R. (1989). Die Zweigestreifte Quelljungfer (*Cordulegaster boltoni* Donovan) aus der Gruppe der Fliessgewässerlibellen (Insecta, Odonata). *Veröffentlichungen des Naturhistorischen Museums Schleusingen*, *4*, 2–12.

Casanueva, P., Santamaría, T., Hernández, M. A., Sánchez-Sastre, L. F., Teixeira, A., Bennas, N., El Haissoufi, M., Ferreras-Romero, M. & Campos, F. (2020). Biometric differences in Iberian and Maghrebi populations of *Cordulegaster boltonii* (Odonata: Cordulegastridae). *European Journal of Entomology*, *117*, 260–264. doi:10.14411/eje.2020.028

Corbet, P. S. (1954). Seasonal regulation in British dragonflies. *Nature*, *174*, 655. doi:10.1038/174655a0

Corbet, P. S. (1964). Temporal patterns of emergence in aquatic insects. *The Canadian Entomologist*, *96*, 264–279. doi:10.4039/Ent96264-1

Corbet, P. S. (2004) (revised edition). *Dragonflies: behaviour and ecology of Odonata*. Colchester: Harley Books.

Corbet, P. S., Longfield, C. & Moore, N. W. (1960). *Dragonflies*. London: Collins.

Dijkstra, K-D. B., Schröter, A. & Lewington, R. (2020). *Field Guide of the Dragonflies of Britain and Europe*. Second edition. London: Bloomsbury Publishing.

Donath, H. (1987). Untersuchungen in einer Larvenkolonie von *Cordulegaster boltoni* (Donovan) in der Niederlausitz. *Libellula*, *6*, 105–116.

El Haissoufi, M., Bennnas, N., Hernández, M. A., Casanueva, P. & Campos, F. (2018). Biometrics of exuviae of *Cordulegaster boltonii algirica* Morton, 1916 (Odonata: Cordulegastridae). *Aquatic Insects*, *39*, 407–413. doi:10.1080/01650424.2018.1476720

Ferreras-Romero, M. & Corbet, P. S. (1999). The life cycle of *Cordulegaster boltonii* (Donovan, 1807) (Odonata: Cordulegastridae) in the Sierra Morena Mountains (southern Spain). *Hydrobiologia*, *405*, 39–48. doi:10.1023/A:1003763819991

Hammer, O., Harper, D. A. T. & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, *4*, 9 pp.

Hernández, M. A., Casanueva, P., Nunes, L., Santamaría, T., Sánchez-Sastre, L. F., Ferreras-Romero, M. & Campos, F. (2022). Geographical variation of prementum size in Iberian *Cordulegaster boltonii* (Odonata: Cordulegastridae) populations. *International Journal of Odonatology*, *25*, 56–61. doi:10.48156/1388.2022.1917169

IGN, Instituto Geográfico Nacional. (2019). *España en mapas. Una síntesis geográfica*. Available at www.ign.es

Lloyd, E. C. & Ormerod, S. J. (1992). Further studies on the larvae of the Golden-ringed Dragonfly, *Cordulegaster boltoni* (Donovan) (Odonata: Cordulegastridae), in upland streams. *Entomologist's Gazette*, *43*, 275–281.

Makarieva, A. M., Gorshkov, V. G. & Li, B.-L. (2005). Temperature-associated upper limits to body size in terrestrial poikilotherms. *Oikos*, *111*, 425–436. doi:10.1111/j.1600-0706.2005.14095.x

Molina Rodríguez, J., Hernández, M. A. & Ferreras-Romero, M. (2022). Biometric differences across three populations of *Boyeria irene* from the southern Iberian Peninsula (Odonata: Aeshnidae). *Odonatologica*, *51*, 247–262. doi:10.60024/odon.v51i3-4.a3

Montgomery, K. (2006). Variation in temperature with altitude and latitude. *Journal of Geography*, *105*, 133–135. doi:10.1080/00221340608978675

Mousseau, T. A. (1997). Ectotherms follow the converse to Bergmann's rule. *Evolution*, *51*, 630–632. doi:10.1111/j.1558-5646.1997.tb02453.x, doi:10.2307/2411138

Norling, U. (2021). Growth, winter preparations and timing of emergence in temperate zone Odonata: Control by a succession of larval response patterns. *International Journal of Odonatology*, *24*, 1–36. doi:10.23797/2159-6719_24_1

Nunes, L. F., Santamaría, T., Casanueva, P., Sánchez-Sastre, L. F., Ferreras-Romero, M., Romero, A., Campos, F. & Hernández, M. A. (2023). Patterns of variation in wing venation of Iberian *Cordulegaster boltonii* (Donovan, 1807) (Odonata: Cordulegastridae). *International Journal of Odonatology*, *26*, 164–171. doi:10.48156/1388.2023.1917226

Ormerod, S. J., Weatherly, N. S. & Merrett, W. J. (1990). The influence of conifer plantations on the distribution of the Golden Ringed Dragonfly *Cordulegaster boltoni* (Odonata) in upland Wales. *Biological Conservation*, *53*, 241–251. doi:10.1016/0006-3207(90)90095-7

Schütte, C. (1997). Egg development and early instars in *Cordulegaster boltonii immaculifrons* Selys: a field study (Anisoptera: Cordulegastridae). *Odonatologica*, *26*, 83–87.

Shelomi, M. (2012). Where are we now? Bergmann's rule sensu lato in insects. *The American Naturalist*, *180*, 511–519. doi:10.1086/667595

Suhling, F., Suhling, I. & Richter, O. (2015). Temperature response of growth of larval dragonflies—an overview. *International Journal of Odonatology*, *18*, 15–30. doi:10.1080/13887890.2015.1009392

Tennessen, K. (2017). A method for determining stadium number of late stage dragonfly nymphs (Odonata: Anisoptera). *Entomological News*, *126*, 299–306. doi:10.3157/021.126.0407

Winterhalter, W. E. & Mousseau, T. A. (2008). The strength of temperature-mediated selection on body size in a wild insect population. *Journal of Orthoptera Research*, *17*, 347–351. doi:10.1665/1082-6467-17.2.347