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Diurnal and Sex Ratio Flight Activity of Rare Cavity-Dweller *Eucnemis capucina* Ahrens, 1812 (Coleoptera: Eucnemidae) in Lowland Deciduous Forest: Case Study from Czech Republic

Oto Nakládal, Jiří Synek and Václav Zumr *

Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha 6, Suchdol, Czech Republic; nakladal@fld.czu.cz (O.N.); synekj@fld.czu.cz (J.S.)
* Correspondence: zumr@fld.czu.cz

Abstract: The cavity-dwelling saproxylic beetles are very poorly studied because of their hidden way of life, and they are threatened by forest management. *Eucnemis capucina* is a rare and sporadic species in all of its distribution area. This species is associated with old hollow trees, which may be why it is on the red lists of many European countries; however, this association could be an indicator of diverse forest structure. We monitored diurnal and seasonal flight activity with intercept flight traps installed on massive ash trees during three seasons. The observed peak of seasonal activity was in May and June. Contrasting most eucnemids, *E. capucina* is clearly a daytime species, with flight activity between 8:00 and 20:00 and peaking at 14:20. The peak of flight activity is the same for both sexes, but males are more active at the beginning and end of the flight period compared to females. An unequal sex ratio 1.91:1 (F:M) of captured individuals was recorded. The increasing body size of females affected the number of eggs in the body of adult females. The average potential fecundity of a female was 54 eggs, 10 eggs per 1 mm of female body length. The eggs themselves were oval in shape and only the length of the eggs correlated to the body size of the females; the width did not change with body size and did not correlate with egg length, either. We also confirmed that even a single hollow tree can host high numbers or whole populations of rare species and, thus, have a high conservation value. Our study may help better understand the biology of cavity-dwelling beetles and their active life.

Keywords: saproxylic beetle; dead wood; Coleoptera; hollow trees; beetle fecundity; egg size

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1. Introduction

Saproxylic beetles are the most studied group of invertebrates in forests [1]. These beetles depend on dead wood substrate [2] and create the most beetle-species-rich forests in their origin state [3]. One of the most valuable habitats for saproxylic beetles are tree cavities and large pieces of dead wood [4–6]. However, this attribute of forests is lacking in today's managed forest landscape [7,8]. This leads to a steady decline in saproxylic beetle richness [9,10], which is supported by the fact that the volume of dead wood is deeply below the optimal threshold [11]. Furthermore, in the Czech Republic, 40% of this group of beetles is included on the red list [12], and across Europe, the number of threatened species has increased during the last decade from 436 [13] to 693 [9]. Much of the established scientific work deals with the wider community, diversity of saproxylic beetles in terms of forest management effects, dead wood volumes, microhabitats, or canopy in general and their overall interactions, e.g., [14–17]. Few recent studies deal directly with single saproxylic species [18–21]. However, a majority of these detailed studies focus on popular "umbrella" dead-wood-dwelling species. These studies have also examined habitat parameters outside of flight activity, which can be important for understanding beetle species, e.g., *Cerambyx cerdo* (Linnaeus, 1758) [22] and *Rhysodes sulcatus* (Fabricius, 1787) [23], and cavity-dwelling species *Osmoderma eremita* (Scopoli, 1763) [24], *Limoniscus violaceus* (P.W.J. Müller, 1821) [25], and *Elater ferrugineus* (Linnaeus, 1758) [26]. Smaller beetles could also have equal or higher importance to biodiversity and insect conservation, such as the family Eucnemidae (the false click beetles), where the whole family is typically saproxylic and often associated only with hollow trees [27]. Eucnemidae (Melasidae), our family of focus, is a relatively small group of beetles with approximately 1500 species worldwide, belonging to 190 genera [28]. They are typically found in the tropics and subtropical regions; their occurrence in the temperate zone is quite low [29]. Eucnemidae consists of 31 species in Europe, and about half of them are endemic [13]. In Europe, 23% of these species are classified with a conservation status of Endangered (EN), Vulnerable (VU), or Near Threatened (NT). In individual countries, a majority of the Eucnemidae species are threatened, e.g., 70% of species in Italy [30] and 95% in the Czech Republic [31]. For these reasons, the false click beetles (Eucnemidae) are great indicators of a diverse forest structure [32]. However, their biology is very poorly known because they are rare, have short seasonal activity, are nocturnal, and have a hidden way of life [29]. The larvae of almost all species develop in decaying wood, which is principally colonized by white-rot fungi (Ascomycota and Basidiomycota), and feed on the hyphae using extraoral digestion [33]. Specimens collected by sweeping and beating vegetation are unusually rare and sporadic [27]. It is more effective to find them by signs of their presence, such as the presence of emergence holes on the stems of trees with removed bark, emerged larval galleries in big holes cut out by woodpeckers, and hollow trees with decaying wood [29]. Development time is about 2 years, but some larvae from the same population can pupate after 3 or 4 years. In Central Europe, most of species overwinter in the larval stage, rarely as pupa (Eucnemis) or adult (Melasis). The pupal stage usually takes 2 weeks [29].

The study species *Eucnemis capucina* (Figure 1) is distributed in Europe and west Siberia [28]. However, this species is rare and sporadic in all distribution areas [29]; hence, in Europe, it has an LC (Least Concern) status; [9] however, specific countries denote it varying statuses. For example, the Czech Republic lists this species with a higher conservation status, as Endangered (EN) [31], Germany lists it as Vulnerable (VU) [34], and Italy lists as Near Threatened (NT) [30]. The larvae of *E. capucina* feed on rotting wood [28], and according to Muona [33], on moist, very soft white rotten wood. It can inhabit various deciduous trees [27]. The knowledge about the diurnal flight of *E. capucina* is somewhat inconsistent. Freude et al. [35] noted activity during the warm summer days; however, Burakowski [29] considers Eucnemidae as a group with night activity in general. Other authors have observed the activity of some individuals from the afternoon to early evening [27]. From these perspectives—its understudied life activity and potential fecundity—we constructed the following. The goals of this study were to observe the diurnal flight activity of *E. capucina* (1), test differences in flight activity between both sexes (2), compare the sex ratio of captured individuals (3), and determine the dependence of female size on the number and size (length, width, content, volume) of the eggs in the abdomen (4).

Figure 1. *Eucnemis capucina* (foto Leica).

2. Materials and Methods

2.1. Study Site

The study was performed in the Czech Republic in the Litovelské Pomoraví Protected Landscape Area at the National Natural Reserve Vrapač. Vrapač (NNR) is situated in the southern part of north Moravia, approximately 0.5 km northeast of the village of Mladeč (Litovel env.). Geographical coordinates (GPS) are as follows: 49°42′31″ N; 17°02′23″ E (centroid). The studied territory comprises 80.69 ha, at altitudes of 236–237 m a.s.l. The majority of the floodplain area is covered by hardwood forests (association *Querco-Ulmetum*). There are few smaller deciduous trees, mostly lining the banks of the Morava river. These habitats are represented by the existence of *Salicetum-albae* or *Salicetum-triandrae*. Only a small part of the studied area consists of open habitats, inhabited by *Caricetum-buekii* and *Rorippo-Phalaridetum arundinaceae*, or gravel habitats without any vegetation. Two rivers are present, the Morava river and Malá Voda river, and the channels of both are undisturbed along the entire length of the NNR.

2.2. Data Collection

Seasonal flight activity was monitored in the 2011, 2012, and 2013 seasons. During the first two seasons, 12 hollow ash trees (*Fraxinus excelsior* L.) with different parameters were monitored to describe seasonal flight activity and to determine the most important parameters for the model species. Only four of the trees were selected as the most favorable, and traps were installed on these four during 2013 to monitor diurnal activity. Data were collected using cruciform window flight intercept traps (unbaited) (WTs), which were installed in front of the entrance to the tree cavity (Figure 2). Traps were 35 × 50 cm (width × height), covered by a roof on the top and with a funnel on the bottom, and joined to the collecting container with saturated salt water as an inert fixative liquid. The collecting containers of all the WTs were checked every 2 weeks in 2011 and 2012 from March to November. Diurnal flight activity was monitored only in the 2013 season. In 2013, the collecting containers of all the WTs were checked daily from the 1st of March 2013 (date of installing the WTs) to the 21st of May (when first *E. capucina* was captured). Consequently, the daily checking interval was changed to an hourly interval from 21st May to 23rd of June, and then was changed back to a weekly period. Captured beetles were stored in a freezer. For each individual captured in 2013, body size, sex, and, in the case of females, the number of eggs and their size were measured by dissection. Dissection was conducted by a Leica S9E stereomicroscope (Leica Camera AG, Wetzlar, Germany), and measurements were taken by a ProgRes CT1 1,3 Mpix camera with a measuring table (Jenoptik AG, Jena, Germany) and Nikon NIS-Elements software (Version 4.1, Nikon Instruments Inc., Melville, NY, USA).

Figure 2. Trap in entrance to an ash tree cavity.

2.3. Data Analysis

Diurnal flight activity was tested by Rayleigh Test in the Oriana software (Version 4.02, Kovach Computing Services, Pentraeth, Wales, UK) for circular data, tested separately for males, females, and *E. capucina* as a species. The real sex ratio was tested by Pearson's chi-squared test in Microsoft Excel 2003 (Version Standard S.P. 11.0.8173.0, Microsoft Corporation, Redmond, WA, USA). The difference between male and female diurnal flight activity was tested by Pearson's chi-squared test (Yates correction), taking into account the real sex ratio. The correlation between body size and number and size of eggs was evaluated by a linear regression model in Statistica 13 (Version 13.0, StatSoft, Inc., Tulsa, OK, USA) [36].

3. Results

The total captured individuals during three years of the study was 254. Divided by years, we captured 79 individuals in 2011, 111 individuals in 2012, and 64 individuals in 2013. Fifty-four individuals were captured by hourly sampling. We found an unequal sex ratio of 1.91:1 (F:M) (n(F) = 42, n(M) = 22, $\chi^2 = 6.250$, $p < 0.012$) for the 2013 season, and for unfavorable trees during the first two seasons, the ratio was 5.2:1 (F:M) (n(F) = 26, n(M) = 5, $\chi^2 = 14.226$, $p < 0.000$).

E. capucina is clearly a species with daytime activity, which starts at 8 a.m. and ends at 8 p.m. The period exactly in the middle of the recorded activity (13:00–14:00) was also the hour with the most beetles recorded. Only 16 beetles were trapped during the first five hours, and 28 after 14:00. This means that the real peak of the diurnal flight activity occurred after 14:00, exactly at 14:20 based on statistical analysis (Table 1). Males and females showed almost the same diurnal flight activity, with peaks at 14:31 for males and 14:16 for females (Figure 3). However, based on Figure 4, the flight activity in females appears to start 1 h later than in males. Furthermore, female flight activity ends about 1 h earlier. Unfortunately, the low number of trapped beetles did not allow us to statistically test this phenomenon. However, beetles trapped during the morning (first 3 h of recorded flight activity, 8:00–11:00) showed a statistically higher male proportion than the real sex ratio (n(F) = 2, n(M) = 4, χ^2Yates = 4.393, $p < 0.036$). Additionally, beetles trapped during the evening (last 3 h of recorded flight activity, 17:00–20:00) showed a higher male proportion than the real sex ratio, but with very low statistical significance (n(F) = 3, n(M) = 5, χ^2Yates = 4.194, $p < 0.041$). Lastly, the middle 6 h period (11:00-17:00) showed a slightly higher proportion of females (n(F) = 31, n(M) = 9, $\chi^2 = 2.496$, $p < 0.114$). The mean number of eggs in a female body was 54. The number of eggs linearly increased with the body size of the female adults ($R^2 = 0.59$, $F_{1,27} = 39.0$, $p < 0.000$). The egg length increased with body size ($R^2 = 0.48$, $F_{1,26} = 24.3$, $p < 0.000$), and egg width was not dependent on body size ($R^2 = 0.07$, $F_{1,26} = 1.9$, $p <$ n.s.). There was no linear correlation between egg length and width ($R^2 = 0.01$, $F_{1,27} = 0.3$, $p <$ n.s.), indicating that they are always longer than they are wider. The content of the eggs is depends on the body size increasing ($R^2 = 0.39$, $F_{1,26} = 16.5$, $p < 0.000$), and also with the volume of the eggs ($R^2 = 0.26$, $F_{1,26} = 9.1$, $p < 0.006$) (Figure 5). The body length of adults did not indicate any differences between the sexes: female mean size was 5.2 mm (min 3.6, max 6.4) and male mean size was 5.2 (min 4.2, max 6.2). The end of May and beginning of June is the obvious peak of the seasonal flight activity, based on all 254 trapped beetles. The last recorded beetle was from a period between 8 and 23 August (Figure 6).

Table 1. Results of diurnal flight activity for the species, and separately for males and females (2013).

Statistical Characteristics	Both Sexes	Males	Females
Top of flight activity (time)	14:20	14:31	14:16
Circular Standard Deviation	2:48	3:53	2:11
N	54	18	36
Circular Variance	2.478	0.405	0.152
Length of Mean Vector (r)	0.763	0.595	0.848
Rayleigh Test (Z)	31.462	6.366	25.893
Rayleigh Test (*p*-value)	$<10^{-12}$	0.001	2.41×10^{-11}

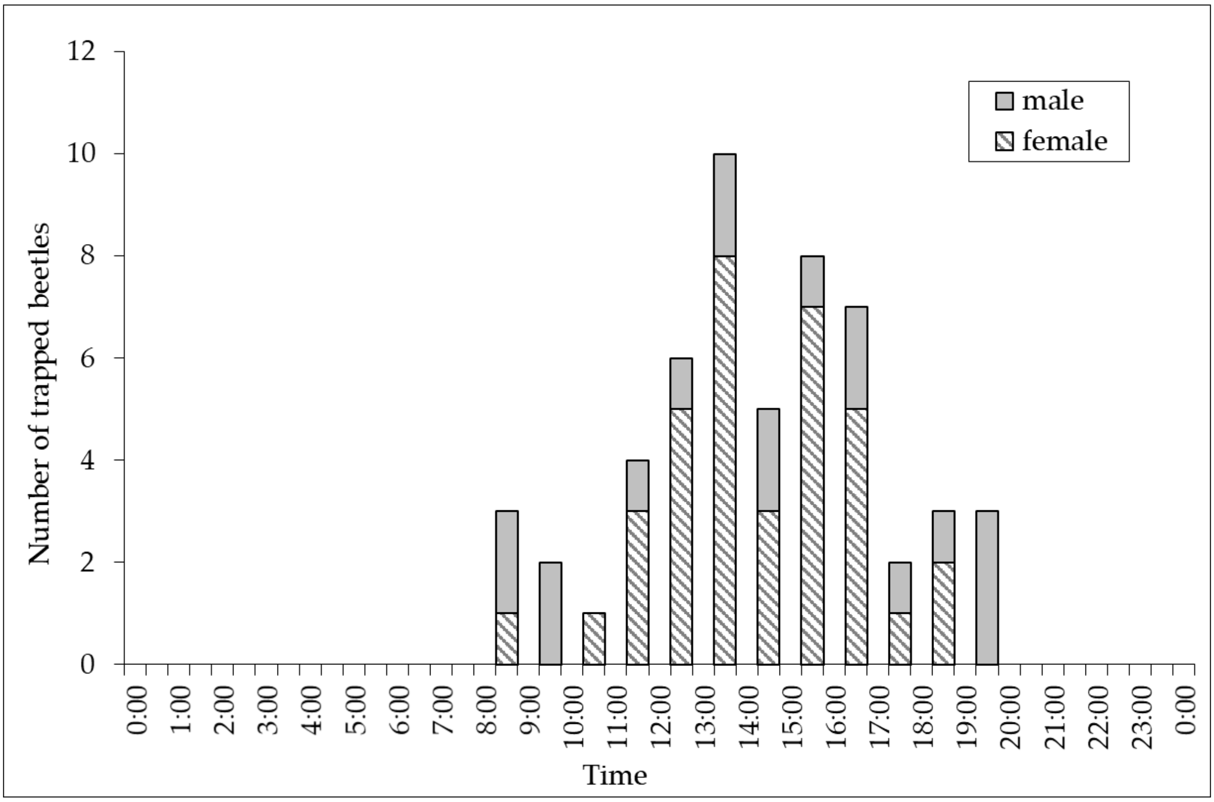

Figure 3. Diurnal flight activity recorded by window traps in period 21–23 May–June 2013.

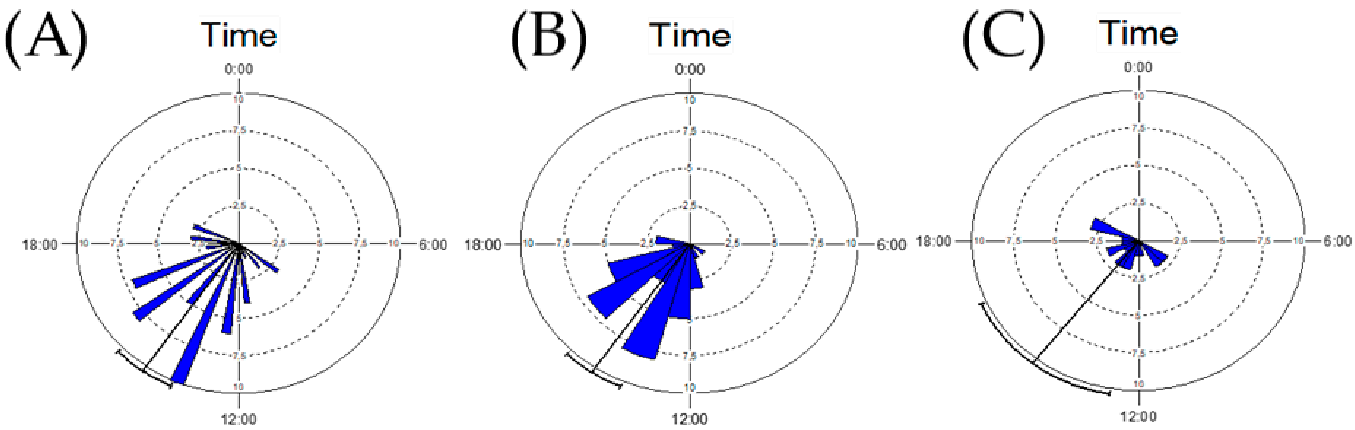

Figure 4. The flight activity by sex: both sexes (**A**), females (**B**), and males (**C**). Peaks of flight activity are indicated with a confidence interval of 95%.

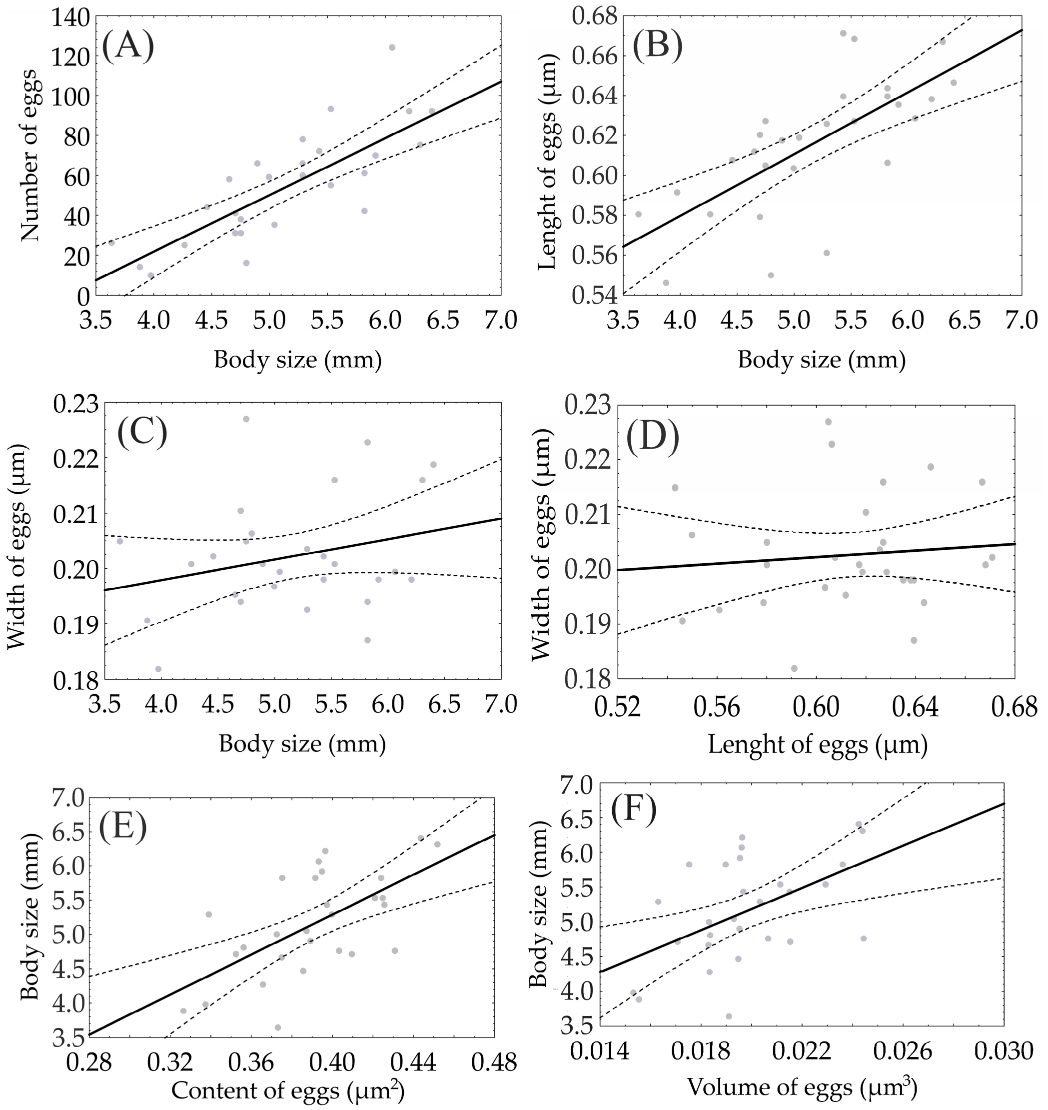

Figure 5. Linear regression with 95% confidence interval (dashed lines) of the female individuals and their relationship to number of eggs and body size (**A**), body size–length of eggs (**B**), width (**C**), length and width (**D**), content (**E**), and volume of eggs (**F**).

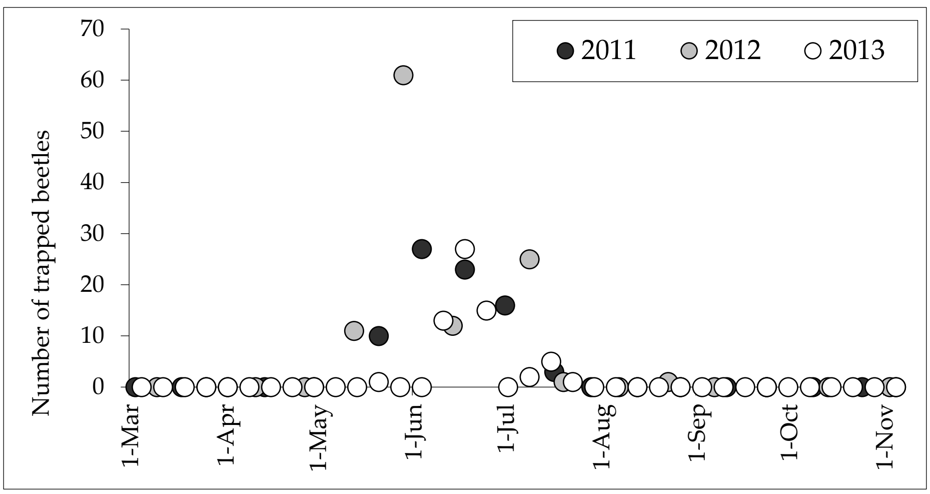

Figure 6. Seasonal flight activity of *E. capucina* based on trapped individuals in the period March–November during all study years.

4. Discussion

This study provides the first insight into the diurnal activity of the rare cavity-dwelling species *E. capucina*. This species is sporadically represented throughout Europe [32]. We found 254 individuals within three years with a relatively low number of traps in a lowland alluvial forest. The high occurrence could be supported by the fact that thermophytic alluvial forest habitats are very biologically rich [37], and by the fact that *E. capucina* is a poorly abundant species only residing in specific habitats. Müller et al. [38] captured a total of 94 individuals of the model species in beech forests with 1500 traps. At the same time, Mertlik [27] presented only 266 well-dated records from the whole Czech and the Slovak Republics from the period of 1895–2008. However, we studied hollow trees with a high potential for their occurrence, hence our finding may be an overestimation, as rare species have low mobility, especially cavity-dwelling ones [39,40]. On the other hand, this supports the opinion that cavities are important habitats for rare saproxylic beetles [41], and that hollow trees in forest stands are bioindicators with high conservation values. We found the species to be strictly diurnal and to move around their cavity extensively, in contrast to another cavity-dwelling umbrella species, *Osmoderma eremita*, which only leaves the cavity in a fraction of the cavity population [42]. Occasionally, we found a beetle sitting on the bark of a tree near the original cavity at night, but the beetle had been there for several hours in the same place and the same position. This lead us to the conclusion that the *E. capucina* do not fly during the night and, furthermore, they are completely inactive at this time.

We determined that May and especially June are the peaks of their seasonal activity. Seasonal activity strongly decreases in July, and individuals caught later are very rare. There are several records from August and even from September and October. While the beetles caught in the WTs were always caught alive, we do not have information on the status of the beetle specimens found in the case of the faunistic data published by Mertlik [27] and, based on this fact, we can consider that these specimens were dead upon capture. Since all these late beetles were found in a relatively low altitude, we can exclude longer development with the combination of late emergence due to the overall colder developmental environment. Furthermore, *Eucnemis capucina* larval development takes at least two years, depending on the habitat it overwinters in as larvae, prepupae, or pupae [32]. Therefore, findings of imagoes in wood before winter can be also excluded. On the other hand, the seasonal flight activity seems to be strongly dependent on weather. We trapped the first specimen of *E. capucina* on 21 May after several warm and sunny days. After that followed sixteen cold and cloudy days, when no specimen were captured. Nine beetles were trapped on 7th June, again after two warm and sunny days. The literature shows seasonal activity (recorded beetles) during March and April. March activity is restricted only to its second half, according to the data published by Mertlik [27]. We trapped the first 11 beetles in the period between 27 April and 13 May 2012 in the WTs. This is almost 2 months later than the first records published by Mertlik [27].

The observed sex ratio could be sensitive to the trapping method used and, in some cases, the real sex ratio can be extremely different from the sex ratio recorded by the trapping methods. The females of saproxylic beetles could be more attracted to the suitable habitats. Graf et al. [43] found that saproxylic beetles are attracted by potentially suitable wood substrates. However, within interspecific variability, there is considerable variation in flight ability comparing the sexes [44]. Many insects have females that are unable to fly because of their wing brachyptery, e.g., many Blattodea, Lepidoptera, Coleoptera, and Hemiptera, e.g., [45,46]. In these cases, trapping by WT could strongly skew the proportion of males, while the real sex ratio could be the opposite. In some cases, females have normal wings, but they still do not fly, as in the European population of *Lymantria dispar* (L., 1758) (Lepidoptera:Lymatriidae) [47], or the males may be flight active like *Ips grandicollis* (Coleoptera:Curculionidae) [48]. This situation has the same impact on estimating the real sex ratio. Generally, we can calculate the real sex proportion with the use of WTs only in species with the same flight mobility and natality in both sexes. In our study, with the use of WTs, we found both sexes in high numbers, and this indicates the same flying ability. Many insects have a tendency to reduce the proportion of males in favor of females, with a goal to increase the population growth of the species, e.g., aphids [45,49]. Generally, a higher proportion of males than usual suggests the population development is decreasing, such as in some forest pests, e.g., the bark beetle [50]. In this study, the captured sex ratio could be standard for *E. capucina* populations developing in good environments, taking into account the large size of the original trees and also the large size of the hollows. We found that the average number of eggs per female was 54, with a ratio of 10 eggs/mm (number of eggs/female mean body size, mm). This is a relatively small number compared to that of bark beetles, *Ips typoghraphus* and *Pytiogenes chalcogprahus* (both Coleoptera: Curculionidae), with body size length 2–5 times smaller than our model species and a fecundity of about 100 and 40 eggs per female [50]. These species have a ratio of about 55 eggs/mm and 50 eggs/mm of female body length, respectively. In contrast, a big saproxylic species, *Cerambyx cerdo* (Coleoptera:Cerambycidae), has a mean fecundity of 144 eggs and a mean body length to create 3 eggs/mm [51]. Our findings corroborate the findings of [51], that a female's size affects the size of their eggs. This may create an evolutionary disadvantage in the environment when trying to increase the population of our model species and other endangered saproxylic species. This synergistically increases the beetles' threatened potential, along with their unique habitat requirements.

5. Conclusions

Our study provided us insight into the life of a rare saproxylic species. We also confirmed that even just a few hollow trees are very important sanctuaries for saproxylic beetles and can host whole populations. Some of the cavities were multiple times richer than others, and we agree with the view of the high heterogeneity of the cavity environment posed by Cuff et al. [52]. In general, there is very little information about the Eucnemidae family and their active life [27]. Based on our results, we monitored and described the active life of *E. capucina* as one of the Eucnemidae species. *E. capucina* is clearly a diurnal species with flight activity period between 8:00 and 20:00, peaking at 14:20. The peak of flight activity is almost the same for both sexes, but males show more activity at the beginning and end of the flight period compared to females. On the other hand, both sexes had a relatively similar flight activity during the peak of their diurnal and seasonal flight activity. This is opposed to many other rare species, where females are less mobile [44]. Our study may help better understand the biology and active life of the Eucnemidae family and cavity-dwelling beetles in general.

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Data Availability Statement: Data upon request in authors.

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