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ASSESSMENT OF RIVERINE DRAGONFLIES (ODONATA: GOMPHIDAE) AND THE EMERGENCE BEHAVIOUR OF THEIR LARVAE BASED ON EXUVIAE DATA ON THE REACH OF THE RIVER TISZA IN SZEGED

G. Horváth

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Abstract. Abundance, phenology, sex ratio, emergence pattern, mortality and larval emergence behaviour of riverine dragonflies (Odonata: Gomphidae) were studied at the Lower-Tisza reach at Szeged (168–173 rkm) during the emergence period in 2011. Three 20 meter long sampling sites were chosen and searched systematically for exuviae, dead specimens and dragonfly wings, which were left behind by bird predators. At the studied reach of the river two species form stable populations: *G. flavipes* and *G. vulgatissimus. G. flavipes* was much more abundant than *G. vulgatissimus*. Exuviae indicated the excess of females in the *G. vulgatissimus* population (altough there were no significant difference between sexes), while in the case of *G. flavipes* the number of individuals in both sexes were almost the same. *G. vulgatissimus* started to emerge first as a 'spring species', while *G. flavipes* started to emerge about a month later showing the characteristics of a 'summer species'. The rate of mortality in the *G. flavipes* population during emergence was slight and quite normal compared to the abundance of the species. Selection of emergence support of *G. flavipes* showed that the significant majority of the larvae chose soil, but this could have been caused by the notable minority of other types of substrates at the sampling sites. The distance crawled by the larvae from the water-front to the emergence site differed significantly between the two species, *G. vulgatissumus* crawled further, and in the case of *G. flavipes* the effect of the measured background variables to the distance had not been proven.

Key words: Gomphus flavipes, G. vulgatissimus, collections of exuviae, abundance, emergence pattern, sex ratio.

G. Horváth, Department of Ecology, University of Szeged, H-6726 Szeged, Közép fasor 52., Hungary

Introduction

If we want to examine Odonata (in this particular case Gomphidae) populations, there are three different methods to carry out the work:

The most difficult way is the imago based examination because of the excellent manoeuvring skills and hiding behaviour of the adults. The collection of larvae is also not easy, sampling can be problematic in large watercourses. In the case of these two methods there is another disadvantage in a conservational point of view, as imagines and larvae may die during the collection.

Knowing all those, the most reliable and the simplest method to define the population size and emergence specificity of riverine dragonflies is the regular quantitative collection of their exuviae. The best time to estimate the accurate size of the Gomphidae population is during the emergence period (Suhling and Müller – cit. Farkas *et al.* 2012a). Beside species composition and abundance, exuviae provide information about phenology, pattern of emergence, sex ratio, mortality during the emergence, we could make statement about morphological features or even about the moult-strategy of the larvae (Berzi-Nagy 2011, Farkas *et al.*

2009, 2011, 2012a,b; Jakab 2006). The application of the method is highly recommended, because it does not require the collection of living animals so it is not objectionable in a conservational point of view either. Furthermore, exuviae of Anisopterans remain intact for a long time, even under unsuitable weather conditions (Jakab 2006) so there is no need for daily collection.

During the past few years, many studies were published about the riverine dragonfly populations of the Upper- and Middle-Tisza regions (Bánkuti *et al.* 1997; Mátyus 2006 – cit. Berzi-Nagy 2011; Berzi-Nagy 2011; Farkas *et al.* 2009; Farkas *et al.* 2012a; Jakab 2006). Nevertheless, this present study is the first to discuss the Gomphid assemblages of the Lower-Tisza region.

According to literature two Gomphidae species, the River Clubtail (*Gomphus flavipes*) and the Common Clubtail (*Gomphus vulgatissimus*) were expected to occur with abundance big enough to form stable populations along this region. In the light of the current Gomphidae based works my goals were to reveal the sex-ratio and emergence characteristics (phenology, emergence pattern) of the riverine dragonflies that inhabit the reach of the river Tisza near Szeged. I also examined the emergence behaviour (the distance larvae crawled from the waterfront and correlation with the variables, and substrate preference) and mortality during the emergence.

Materials and Methods

Study sites and sampling

Sampling was carried out at the bank of the river Tisza within the administrative territory of Szeged (between 168-173 river kilometers).

I chose 3 different sampling sites, one on the left bank [L.I. (46°12'46.71"N, 20° 7'42.43"E)], and two on the right bank of the river [R.II. (46°14'25.53"N, 20°8'57.53"E); R.III. (46°13'31.74"N, 20°8'23.20"E)], each site was 20 m long.

Each sampling site differed from the others in their characteristics. L.I. site was sunny, cover of vegetation was low, and the inclination angle of the riverbank was little. The R.II. site was shaded the whole day, cover of vegetation was low, and the inclination angle of the riverbank was high. The R.III. site had sunny and always shaded parts too, cover of vegetation was relatively high, the inclination angle of the riverbank was medium compared to the other two sites.

Sampling was performed between 6 May and 18 August in 2011 twice a week, usually in the third and the fifth day.

I checked the bank of the river carefully twice (the soil and the vegetation) in a 4-5 m zone from the water-front. I recorded the emergence support and the distance crawled from the water-front to the emergence support, then I collected the exuviae with tweezers and stored them in boxes in dry conditions.

To study the substrate-preference I determined 8 different support-types (artificial objects in the watercourse, dead fallen leaves, exuviae, green leaves, objects washed up by the river, roots, soil and thin branches).

To study the mortality during the emergence, I recorded data of individuals that were captured by predators (wings near the exuviae indicate bird predation), or died during the emergence due to other reasons (e.g. abnormal moulting or abnormal wing decompression). To determine total mortality, I also paid attention to young adult dragonflies that were damaged. These individuals would not live enough to mate, they usually die shortly after emergence. I did not count these imagines in the total number of individuals, because in most cases I found their exuviae next to them.

Processing of the exuviae took place at the laboratory of the Department of Ecology of the University of Szeged. Identification of the specimen to species and gender level had been carried out with a stereomicroscope. I used the keys and descriptions of Askew (1988), Gerken and Sternberg (1999) and Raab *et al.* (2006). To separate the sexes I used the work of Berzi-Nagy (2011).

The water level and water and air temperature data came from the on-line database www.vizadat.hu, data of the measure station in Szeged (173,6 river kilometer) were used as background variables.

Statistical analysis

PAST (Hammer *et al.* 2001) and R (R Development Team 2009) softwares were used to the statistical analysis of the dataset.

To the comparison of the sex ratio of *G. vulgatissimus* and *G. flavipes*, $\chi 2$ test was used.

To compare the distance crawled from the waterfront to the place of emergence by *G. vulgatissimus* and *G. flavipes* larve, Kruskal-Wallis test was used.

The number of *G. vulgatissimus* larvae was so low that if the data of this species were used by ANOVA and linear regression the results would be quite questionable, so during the following statistical methods I used only the data of *G. flavipes* exuviae.

Linear regression was used to examine the relationship between the amount of emerged *G. flavipes* specimens and that of captured by birds.

The analysis of the substrate-preference of *G. flavipes* was carried out with one-way ANOVA, and Tukey-test was used to the pairwise comparisons.

Multiple linear regression was used to reveal the connection between the distance crawled by *G. flavipes* larvae and the background variables (water level, water temperature and air temperature) and between the number of the exuviae and the background variables. The best model had been chosen with Stepwise models election based on Akaike information criterion (AIC).

Results

Abundance of species

During the examination period, 1217 exuviae were found. Thirty two (2,6%) of them were *G. vulgatissimus*, 1183 (97,2%) *G. flavipes* and 2 (0,2%) were Green Snaketail (*Ophiogomphus cecilia*). The 1217 exuviae come from 3 study sites, so the average number on a 20 meter long study site is 406. In the case of *G. vulgatissimus* exuviae this number is 11 and the *G. flavipes* is 394. In the case of *O. cecilia*, there is no point talking about population density, because of their low number.

In the case of *G. vulgatissimus*, there was no big difference between the number of the individuals at the three study sites. However, in the case of *G. flavipes*, the number of the exuviae in the L.I. site exceeded the combined number of the individuals of the R.II. and R.III. (Fig. 1.).

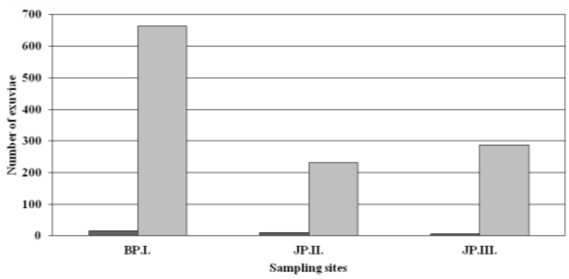

Figure 1. Distribution of *G. vulgatissimus* (dark grey columns) and *G. flavipes* (light grey columns) exuviae between the sampling sites.

Sex ratio

From the 32 *G. vulgatissimus* exuviae there were 19 (59,4%) female and 13 (40,6%) male specimens. In the case of the 1183 *G. flavipes* exuviae, there were 590 (49,8%) female and 591 (49,9%) male, while the sex of 2 individuals were uncertain. There was no significant difference between the ratio of sexes either in case of *G. vulgatissimus* ($\chi 2=0{,}0008$; df=1; p=0,98) or *G. flavipes* ($\chi 2=1{,}125$; df=1; p=0,29).

Pattern of the emergence

G. vulgatissimus started to emerge on the 6th May. The emergence of *G. flavipes* started on the 25th May.

The pattern of the emergence is fundametally different between the two Gomphid species (Fig. 2.). In the case of *G. vulgatissimus* the whole population emerged within a month (19 days), the EM50 value (the time needed for the 50% of the population to emerge) is 4 days, the curve of the emergence is steep – the species act as a 'spring species'. In the case of *G. flavipes*, however, the emerging of the total population took more than two months (72 days), the EM50 value is 17 days – the species act as a 'summer species'.

If we examine the pattern of the emergence on the basis how many exuviae had been found each day, two peaks can be seen in the case of *G. flavipes* (Fig. 3.).

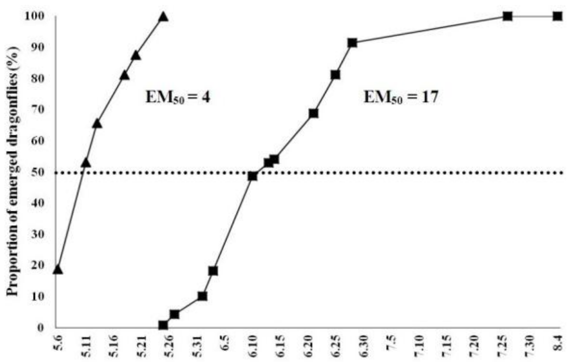

Figure 2. The emergence curve of *G. vulgatissimus* and *G. flavipes* at the investigated reach of the river Tisza at Szeged in 2011 (EM50 :the time needed for the 50% of the population to emerge; ▲–*G. vulgatissimus* ■–*G. flavipes*).

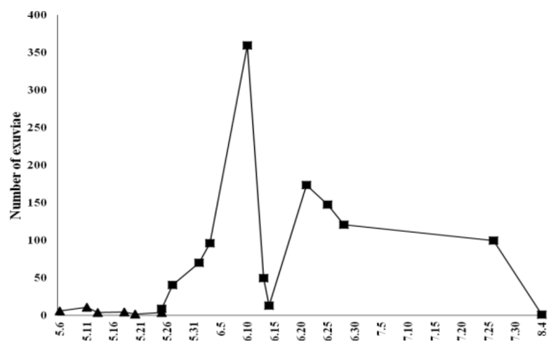

Figure 3. The emergence pattern of *G. vulgatissimus* and *G. flavipes* at the investigated reach of the river Tisza at Szeged in 2011 (▲–G.vulgatissimus ■–G.flavipes).

Mortality of G. flavipes during the emergence

Total mortality of *G. flavipes* during the emergence period was 5,58%. According to the literature (Farkas *et al.* 2011, 2012b) this is a normal value by this abundance of the species. Larvae consumed by predators (4,9%) make up the largest proportion of the total value, and within predation birds are liable for the most consumed larvae (4,48%). These specimens can be easily distinguished from those that were consumed by other, unknown predators (0,42%) in most cases. When birds eat the emerging dragonflies they leave the uneatable wings of the insects behind, so if the wings are found nearby the exuviae that refers to bird predators. Linear regression shows that there is a significant and positive correlation between the amount of emerged dragonflies and mortality caused by birds (β=0,52; F=15,4; df=1 and 12; p=0,002, n=12).

The remaining proportion of mortality was caused by abnormal moulting (0,34%) and the abnormal decompression of the wings (0,34%).

Substrate pereference of G. flavipes

I used data of 763 specimen of *G. flavipes* exuviae to the examination of substrate preference, because the original support could be identified without doubts by that many exuviae (in the other 420 case the exuviae were found lying on their back, or due other reasons I could not identify the original support). According to one-way ANOVA there is a significant difference between the support choice (F=7,832; df=7,16; p<0,0003), the majority of the larvae chose soil as an emergence support (Table 1.). Tukey's pairwise comparison shows (Table A.1.) that soil is significantly differ from any other supports and between other support types there are no significant difference in terms of preference.

Table 1. Substrate types chosen by *G. flavipes* larvae at the reach of the river Tisza in Szeged.

support type	number of individuals	percentage of individuals
fallen leaves	14	1,83
exuviae	5	0,66
roots	6	0,79
artificial object	10	1,31
washed up objects	7	0,92
soil	687	90,04
branches	12	1,57
green leaves	22	2,88
Total	763	100

Distance crawled from waterfront, number of exuviae and connection with background variables

According to the distance data crawled by larvae, there is a significant difference between *G. vulgatissimus* and *G. flavipes* populations (Kruskal-Wallis-test: H=13,35; Hc=13,35; p<0,0005). *G. vulgatissimus* crawl greater distance (horizontal+vertical) from the waterfront than the other species, although there was no vertical movement observed of *G. vulgatissimus* specimens. (Figure 4. and Table 2.).

Water level, water temperature and air temperature (investigated their effects individually) have no significant effect on the distance crawled by *G. flavipes* larvae (Linear regression: β=-0,24; F=0,35; df=7,3; p=0,79; n=12), and they have no significant effect if we assume interaction between them either (Linear regression: β= 0,07; F=1,11; df= 7,3; p=0,51; n=12). None of the models were supported by the AIC.

Nevertheless, marginally significant positive connection was found between water temperature and the number of exuviae (Linear regression: β=0,52; F=14,1; df= 1,11; p=0,003; n=13).

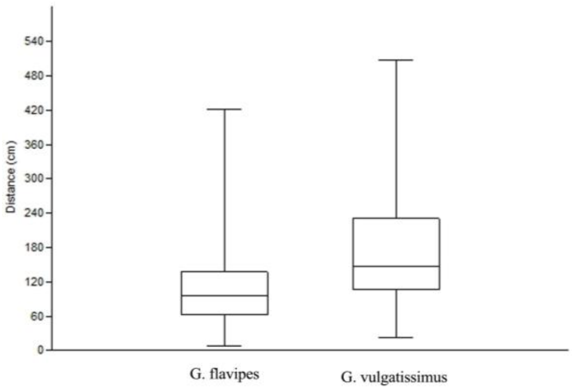

Figure 4. Distance crawled by the larvae of *G. flavipes* and *G. vulgatissimus* from the waterfront to the emergence substrate at the reach of the river Tisza in Szeged.

Discussion

At the investigated reach of the river Tisza *G. vulgatissimus* and *G. flavipes* seem to form stable populations. Although there is a huge difference between the abundance of the two species this phenomenon seems to be normal along the river Tisza and every other places where the two species occur together. (Jakab and Dévai 2008). In 2011 at Szeged the abundance of *G. flavipes* was 36 times bigger than *G. vulgatissimus*, many authors inform about a similar result, nevertheless, the differences in

Table 2. The mean, standard deviation and maximum values of distances (horizontal, vertical and total) crawled by the larvae of *G. flavipes* and *G. vulgatissimus*.

Species	Horizontal distance		Vertical distance		Total distance		N
	Mean±SD	Max.	Mean±SD	Max.	Mean±SD	Max.	
G.flavipes	104 ± 63	420	1 ± 6	72	105 ± 62	420	763
G.vulgatissimus	174 ± 114	506	0	0	174 ± 114	506	26

abundance quoted by these papers are greatly variable. According to Jakab (2006) at the reach between Tiszafüred and Tiszacsege in 2001 the abundance of *G. flavipes* was 8 times greater, during the following years the abundance of *G. flavipes* was much more greater than the abundance of *G. vulgatissimus*: 11 times greater in 2002; 23 times greater in 2003 and 26 times greater in 2012 (Farkas *et al.* 2012a). At Vásárosnamény in 2008 the abundance of *G. flavipes* was 2 times greater than the abundance of *G. vulgatissimus* (Farkas *et al.* 2008). As we can also see the differences increase toward the south greatly, it could be possible that the southern regions of the river Tisza can provide better conditions for the populations of *G. flavipes*, as this species, in his paper Berzi-Nagy (2011) made the same conclusions.

In the case of *O. cecilia* it is quite sure that the species has no stable population at the investigated reach of Tisza. This species, as well as the Small Pincertail (*Onychogomphus forcipatus*), the fourth occurring Gomphid in Hungary, prefers small rivers and streams with high oxygen level and moderate flow (Raab *et al.* 2006). The two specimens might have drifted from the river Maros, where they form populations (Jakab and Dévai 2008).

The result of the investigation shows that in 2011 at Szeged there was no significant difference between the ratio of sexes either in case of *G. vulgatissimus* or *G. flavipes*. In the case of *G. flavipes* the number of individuals in both sexes are almost the same. Although, for the subgenus Anisoptera it is general that the number of females is higher than the number of males (Berzi-Nagy 2011; Farkas *et al.* 2009; Jakab 2006) similar result may occur (Jakab 2006). It is also an example that the sex ratio of a certain species differ at the same reach of a river between years (Corbet 1999 – cit. Farkas *et al.* 2009), so it is possible that next years the sex ratio of the *G. flavipes* also will change.

In 2011 at Szeged the *G. vulgatissimus* acted as a 'spring species' (the species emerged strongly synchronized within a short time) the *G. flavipes* as a 'summer species'. (the emergence was less synchronized and stretched in time) This phenomenon can be observed at other regions of the river Tisza during the last decade as well. According to Berzi-Nagy (2011) the emergence pattern of *G. flavipes* showed the trait of a 'spring species' at the Middle-Tisza region near Szolnok. Jakab (2006) reported the same phenomenon from that region, but during his three year long investigation the pattern of the emergence of *G. flavipes* varied between years, too, and the differences were significant. Similarly to sex ratio, the pattern of emergence can vary between years, and also between different reaches of one certain river. Variability could be caused by water temperature: lower water temperature in winter and higher one during summer caused more synchronized emergence (Suhling 1995 – cit. Jakab 2006).

The emergence pattern of *G. flavipes* shows two peaks, but this cannot be explained with weather conditions, because these peaks do not coincide with the highest air temperatures. So in this case, cohort splitting seems to be the best explanation to the emergence pattern as cohort splitting and unsuitable weather conditions can cause a long-drawn emergence period too. The reason of cohort splitting is that females lay eggs during the entire emergence period and some larvae winter in the final (F0) larval, while some in the penultimate (F-1) larval stage. Those that winter in F-1 stage will emerge a few days or weeks later and they cause the second peak in the emergence pattern.

The fact that more than 90% of the larvae chose soil as emergence support does not necessarily mean that there is a specific attachment to the soil as substrate. 92% of the 763 *G. flavipes* larvae emerged within 2 meters from the waterfront and 78% of them within 1,5 metres. There is a possibility that the over-representation of the soil has caused this phenomenon, as during most of the emergence period there were no – or was in very low proportion of – other substrates in the first 1,5-2 meter zone from the waterfront. Former studies (Farkas *et al.* 2009, 2011) claim that in the case of *G. flavipes* larvae there is no substrate-specific attachment, but they choose supports that are available within a certain distance from the waterfront. This idea is supported by the observation that larvae that chose green leaves (second most frequently chosen emergence support) did not crawl further than the mean distance but most of them emerged in late June and July, when the vegetation had grown in this zone too (68% of them emerged within 2 metres; 68 % emerged in July and 54% of them emerged within 2 metres in July).

During the emergence period in 2011 there were 66 *G. flavipes* exuviae, larvae or young imagines found consumed by predators or wounded mortally at the investigated reach of the river. This proportion at this density is quite normal (Farkas *et al.* 2011, 2012a,b). Due to the emergence strategy of the species [larvae emerge close to the waterfront and the entire process takes 15-59 minutes, which is very short compared to other Anisoptera taxa (Farkas et al 2012b)] the major factor for mortality is predators, especially birds as common blackbird (*Turdus merula*) and white wagtail (*Motacilla alba*) (personal observation and Farkas *et al.* 2012a,b).

Results of the present study shows that *G. vulgatissimus* larvae crawl greater distances from the waterfront than *G. flavipes* larvae, as there is a strong significant difference between the crawled distance (from the waterfront to the emergence support) of the two species. This seems to be general along the river Tisza (Farkas *et al.* 2009, 2011, 2012a,b), and the explanation is that *G. vulgatissimus* starts emerging in late April or early May when greater fluctuations of the water-level is possible, while in late May or early June, when the *G. flavipes* starts the emergence, there is a less chance of the fluctuation of the water level (Farkas *et al.* 2012b).

The background variables could have a strong effect to the emergence of the Gomphidae species: Berzi-Nagy (2011) claims that the level and temperature of water could influence the rate of synchronization and the timing of emergence. Moreover, according to former studies (Farkas *et al.* 2009) water level has a positive and water temperature has a negative effect on the crawled distance. In the case of this present study, the fact that none of the background factors showed to effect the distance, might be due to the low sample size.

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Appendix

Table A.1. Tukey's pairwise comparisons for the substrate types (* marks the significant differences).

	fallen leaves	exuviae	roots	artifical ob.	washed ob.	soil	branches	green leaves
fallen leaves	–	1	1	1	1	0,001*	1	1
exuviae	0,1053	–	1	1	1	0,0009*	1	1
roots	0,0936	0,0117	–	1	1	0,0009*	1	1
artifical ob.	0,0468	0,0585	0,0468	–	1	0,0009*	1	1
washed ob.	0,0819	0,0234	0,0117	0,0351	–	0,0009*	1	1
soil	7,877	7,982	7,97	7,923	7,959	–	0,001*	0,0011*
branches	0,0234	0,0819	0,0702	0,0234	0,0585	7,9	–	1
green leaves	0,0936	0,199	0,1873	0,1404	0,1756	7,783	0,117	–