NEW DATA REGARDING THE PRESENCE OF TWO INSECT LARVAE SPECIES – *GOMPHUS* (*STYLURUS*) *FLAVIPES* (ODONATA) AND *PALINGENIA LONGICAUDA* (EPHEMEROPTERA) – IN THE LOWER SECTOR OF THE DANUBE RIVER

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Abstract. The paper presents data regarding two larvae insect populations – *Gomphus (Stylurus) flavipes* (Charpentier, 1825) and *Palingenia longicauda* (Olivier, 1791), inhabiting in the communities existing at the water/sediment interface along the Danube River. The dragonfly *Gomphus (Stylurus) flavipes* (Charpentier, 1825) is one of the most important indicator species, listed in Annex IV of the EU Habitats Directive (EU Directive 92/43/EEC) and included in the IUCN Red List of Threatened Species, 2014. This species of dragonfly has become an endangered species in most Western European countries due to water pollution and river regulation. The other important larvae species, mayfly *Palingenia longicauda* (Olivier, 1791), listed in Annex II of the Bern Convention, is considered critically endangered in Europe. Both require a specific habitat conditions in order to complete their life cycle. During the spring campaigns conducted during 2012-2015, the presence of the two species was reported in 26 profiles from the Lower Danube Sector, *Gomphus (Stylurus) flavipes* in 18 profiles, and *Palingenia longicauda* in 10 profiles. The most abundant occurrences were recorded in 2015, at Km 4 – Măcin Arm (59.2 ind/m^2) for *Gomphus (Stylurus) flavipes*, respectively, in 2012, at Km 8 – Sf. Gheorghe) (125.8 ind/m^2) for *Palingenia longicauda*. The presence or absence of larvae in samples, as well as their abundance, are strictly dependent on ecological conditions, the type of substrate representing the decisive factor in the microdistribution of the two larvae.

Key words: Danube River, aquatic ecosystems, insect larvae, IUCN Red List, Bern Convention, EU Habitats Directive

1. INTRODUCTION

Within an interdisciplinary approach, the benthic fauna is relevant as bioindicator of a rigorous characterization of the specific environments in the Danube river systems. Any change on particular species (appearance, disappearance, replacement, morphological anomalies, population dynamics, etc.), could be useful for highlighting a stressful environment linked with natural or anthropogenic impacts.

The Danube River is one of the most important natural water "highways" for Europe and, certainly, the most important in Eastern Europe countries. Both terrestrial and aquatic flora and fauna from the Eastern Danube – Lower Danube Sector have a leading role in assessing the pollution degree status in this area.

The investigations present data regarding two endangered species: *Gomphus (Stylurus) flavipes* (Charpentier, 1825) and *Palingenia longicauda* (Olivier, 1791), from the lower Danube sector. One of the most important clean water indicator species is the dragonfly *Gomphus (Stylurus) flavipes*, listed in Annex IV of the EU Habitats Directive (EU Directive 92/43/EEC) and the IUCN Red List of Threatened Species, 2014. Because of water pollution and river regulation, *Gomphus*

(Stylurus) flavipes has become an endangered species in most Western European countries.

The other target species, the mayfly *Palingenia longicauda*, listed in Annex II of the Bern Convention, is considered critically endangered in Europe. It has been included in the Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention, 1998), in the Carpathian List of Endangered Species (Czech Republic, Romania, Poland, Slovakia, Ukraine and Hungary, 2003) and The Red book of Ukraine (2009). *Palingenia longicauda* disappeared totally in the 1930s from many European rivers. Soldán *et al.* (2009) identified *Palingenia longicauda* swarms, on the Sf. Gheorghe Branch, near Murighiol, in 2007 and 2008. Also, Bulánková *et al.* (2013) found *Palingenia longicauda* near Tulcea (Western Danube Delta).

Both, *Gomphus (Stylurus) flavipes* and *Palingenia longicauda*, require specific habitat conditions in order to complete their life cycle.

2. MATERIALS AND METHODS

During the spring periods (March-April) of the years 2012 – 2015, 70 profiles with 197 sampling stations have been investigated within the Lower Danube River Sector, the larvae of the species mentioned above being reported in 26 profiles. Several environmental samples were collected, in order to assess the conservation status of the habitats, as follows: 70 water samples, 197 sediment samples and 70 benthic fauna quantitative samples (Fig.1).

The water quality assessment was made according to Order no. 161 from 16 February 2006, for the approval of the Normative on the Classification of Surface Water Quality in order to establish the ecological status of the water bodies.

In all research expeditions carried out over several years, the sediment samples and the zoobenthos quantitative samples were collected using a Van Veen grab. The results were expressed as number of individuals per unit area (1 m^2), using a multiplication factor of 7.4 (SR EN ISO 10870:2012).

The samples were washed aboard the R/V Istros through a 0.250 mm sieve, in order to remove the excess sediment particles and keep the fauna. A mixed solution of Rose Bengal and 4% buffered formaldehyde was used for fixation, staining and further preservation until subsequent analysis of benthic organisms. In laboratory, the samples were sorted, and the organisms were identified at the lowest taxonomical level possible using a Carl Zeiss SteREO Discovery V8 microscope and an Axiostar microscope. The taxonomic identification was done according to Godeanu (2002). All organisms within a sample have been counted. The sample processing and analysis were carried out according to the SR EN ISO 5661-1:2008. But, as mentioned earlier, the main objective of this paper is to focus on the two larvae of insects.

3. RESULTS AND DISCUSSION

The two species were reported from 2012 to 2015 in 44 sampling stations, located on 26 transversal profiles distributed along the Danube River and the main branches of the Danube Delta: Km 8 Sf. Gheorghe Arm, Km 1 Sf. Gheorghe Arm, Km 108+500 Sf. Gheorghe Arm, Km 20 Vâlcov-Periprava (Chilia Arm), Km 40 Downstream Chilia Veche (Chilia Arm), Hm 72 Sulina Channel, Mile (M) 2.8 Sulina Arm, M 43.5 Ceatal Ismail, M 42 Tulcea Arm, Km 4 Măcin Arm, M 54 Isaccea, Km 167 Downstream Brăila, Km 174 Upstream Brăila, M 78 Downstream Galaţi, Km 159 Upstream Siret River mouth, Km 2 (246) Borcea Arm, Km 253 Upstream Hârşova, Km 294 Seimeni, Km 301 Upstream Cernavodă Bridge, Km 375 Chiciu-Silistra, Km 481 Downstream Giurgiu, Km 551 Downstream Zimnicea, Km 557 Upstream Zimnicea, Km 604+400 Olt River mouth, Km 687 Kozlodui, Km 789 Downstream Calafat (Table 1).

Gomphus (Stylurus) flavipes was identified in 18 profiles (30 sampling stations) and *Palingenia longicauda* in 10 profiles (16 sampling stations); only 2 samples contain both species (Table 1).

Palingenia longicauda (Fig. 2) is probably the oldest known mayfly Ephemeroptera, the first published data on this species being those of Clutius, 1634 (in Mol, 1984) and Swammerdam, 1675 (in Cobb, 2000).

According to Russev (1987), this species was reported in the past from the Netherlands to Ukraine, being known as a Central European species. Around the 1950s the most important populations of this species met in the Danube and some of its tributaries. In 1936, Prof. C. Motaş describes in the scientific journal „V. Adamachi" the flight of „swarms of hundreds of individuals per square meter".

A review about the distribution and ecology of *Palingenia longicauda* has been published by Soldán *et al.* (2009).This species was originally distributed in almost all great and numerous middle-sized European rivers in Western, Central and SE Europe. *Palingenia longicauda* has disappeared since the end of the 19th century from France, Belgium and Germany. The last records from the Netherlands, Poland, Czech Republic and SW Slovakia are from early 20th century. In SW Europe it probably vanished since mid-20th century. During the 20th century, industrialization led to river degradation and water pollution, causing the disappearance of *P. longicauda* in most of its former distribution area in Europe (Russev, 1987, Soldán *et al.*, 2009).

Approximately 98% of *P. longicauda* populations have disappeared in the last century (Bálint *et al.*, 2012). Between 1946 and 1973, *Palingenia longicauda* was considered a common species, being found regularly (Yaroshenko, 1957 and Mushchinskij, 1972, in Munjiu, 2017). Prior to 1960, on the parts of the river characterized by clay sediments, *Palingenia longicauda* contributed significantly to the total zoobenthos density and biomass (Byzgu *et al.,* 1964, in Munjiu, 2017).

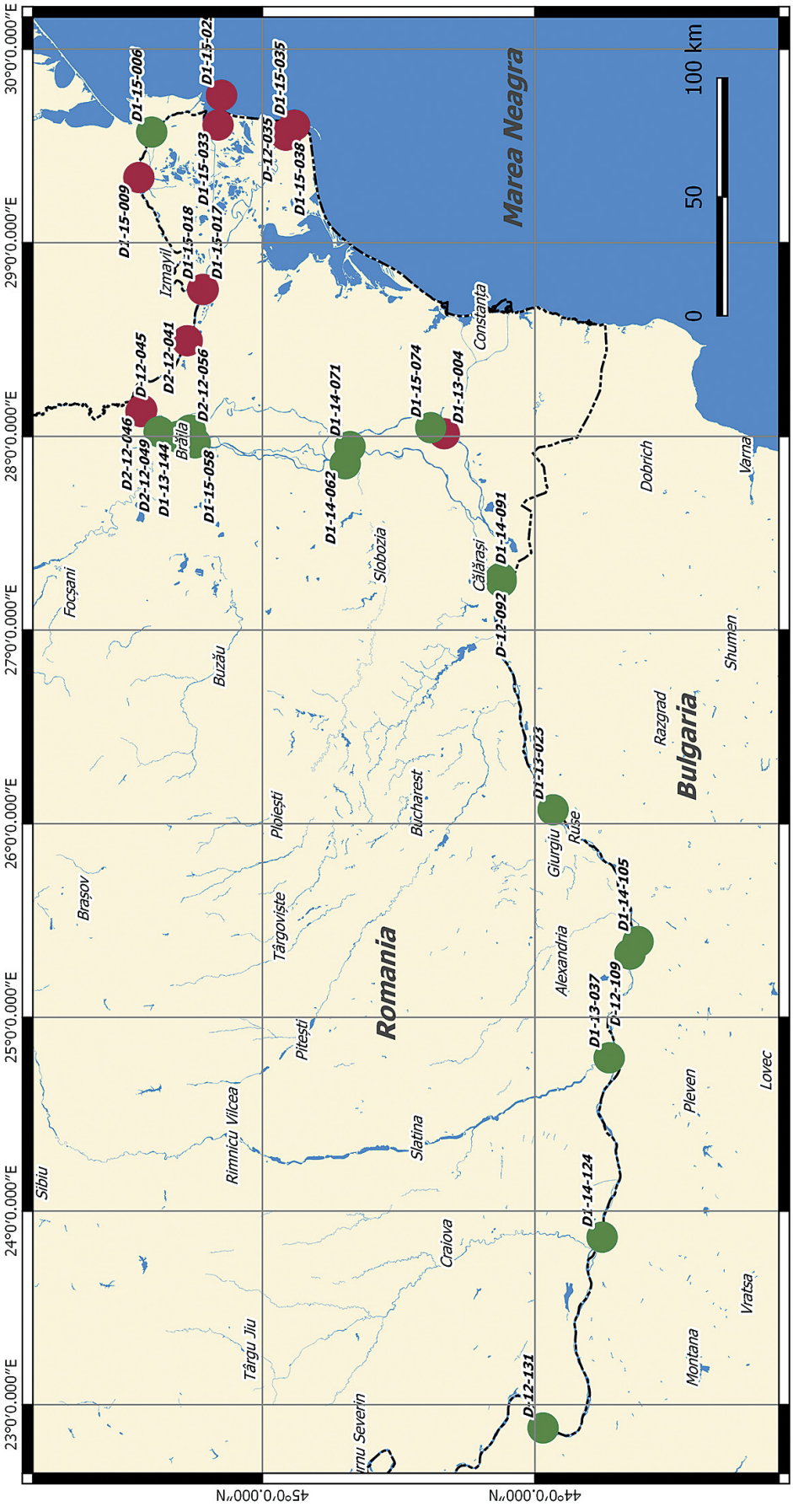

Fig. 1. Position of the sampling points from the Lower Danube River Sector (red bullets – presence of *Palingenia longicauda*, green bullets – presence of *Gomphus* (*Stylurus*) *flavipes*).

Table 1. The distribution of the two types of larvae on the Lower Danube River

Nr. crt.	Station	Date	*Palingenia longicauda*	*Gomphus (Stylurus) flavipes*	Distance from shore (m)		Water Depth	Abundance	Lat. (α)	Long. (λ)
			Presence/ Absence		Left	Right	m	m²		
1	D-12-035 Km 8 Sf. Gheorghe Arm	22.03.2012	p	a		58	7.40	59.2	44⁰ 54′52.3″ N	29⁰ 33′25.1″ E
2	D-12-045 M 78 Downstream Galați	24.03.2012	p	a	45		16.72	29.6	45⁰ 26′23.6″ N	28⁰07′44.8″E
3	D-12-052 Km 167 Downstream Brăila	25.03.2012	a	p		110	4.97	22.2	45⁰ 18′12.2″ N	28⁰00′04.1″E
4	D-12-053 Km 167 Downstream Brăila	25.03.2012	a	p	69		8.94	14.8	45⁰ 18′13.1″ N	27⁰59′43.2″E
5	D-12-056 Km 4 Măcin Arm	25.03.2012	a	p	31		5.40	14.8	45⁰ 15′53.8″ N	28⁰01′27.6″E
6	D-12-072 Km 253 Hârșova	26.03.2012	a	p	19		2.40	22.2	44⁰ 40′38.0″N	27⁰57′03.5″E
7	D-12-092 Km. 375 Chiciu-Silistra	27.03.2012	a	p		25	11.60	14.8	44⁰ 07′29.6″N	27⁰15′01.2″E
8	D-12-109 Km. 557 Upstream Zimnicea	31.03.2012	a	p	34		5.81	22.2	43⁰38′47.6″N	25⁰19′35.2 ″E
9	D-12-131 Km. 789 Downstream Calafat	02.04.2012	a	p	58		6.37	29.6	43⁰58′09.7″N	22⁰52′44.8″E
10	D2-12-036 Km 8 Sf. Gheorghe Arm	21.08.2012	p	a	46		7.50	65.8	44⁰ 54′53.1″ N	29⁰ 33′38.3″ E
11	D2-12-041 M 54 Isaccea	22.08.2012	p	a		96	13.10	29.6	45⁰ 16′21.7″ N	28⁰29′41.7 ″E
12	D2-12-046 M 78 Downstream Galați	22.08.2012	p	a	93		14.80	29.6	45⁰ 26′14.7″ N	28⁰08′11.9″E
13	D2-12-049 Km 159 Upstream Siret	23.08.2012	a	p	122		4.10	29.6	45⁰ 22′27.3″ N	28⁰01′28.9″E
14	D2-12-055 Km 4 Măcin Arm	23.08.2012	a	p		72	3.80	14.8	45⁰ 15′59.9″ N	28⁰01′26.5″E
15	D2-12-056 Km 4 Măcin Arm	23.08.2012	a	p	48		4.10	14.8	45⁰ 15′56.3″ N	28⁰01′23.8″E
16	D1-13-176 Km 8 Sf. Gheorghe Arm	06.04.2013	p	a	70		8.40	14.8	44⁰ 54′54.3″ N	29⁰ 33′36.1″ E
17	D1-13-004 Km. 301 Upstream Bridge Cernavodă	12.04.2013	a	p	185	192	10.70	14.8	44⁰ 20′02.8″N	28⁰00′52.0″E
18	D1-13-023 Km. 481 Downstream Giurgiu	15.04.2013	a	p	45		7.00	22.2	43⁰55′55.9″N	26⁰04′19.7″E
19	D1-13-037 Km. 604+400 Olt River	17.04.2013	a	p	265	132	4.20	14.8	43⁰43′26.0 ″N	24⁰47′27.5 ″E

Table 1 ***(continued)***

Nr. crt.	Station	Date	*Palingenia longicauda*	*Gomphus (Stylurus) flavipes*	Distance from shore (m)		Water Depth	Abundance	Lat. (α)	Long. (λ)
			Presence/ Absence		Left	Right	m	m²		
20	D1-13-144 Km. 167 Downstream Brăila	04.04.2013	a	p		324	8.80	22.2	45⁰ 18′14.2″N	28⁰ 00′04.2″E
21	D1-14-055 Km. 4 Măcin Arm	19.03.2014	a	p		56	6.83	22.2	45⁰ 15′48.0″ N	28⁰01′57.5″E
22	D1-14-056 Km. 4 Măcin Arm	19.03.2014	a	p	50		5.24	14.8	45⁰ 15′41.4″ N	28⁰01′57.7″E
23	D1-14-059 Km. 174 Upstream Brăila	19.03.2014	a	p		70	5.76	22.2	45⁰ 14′38.7″ N	27⁰58′04.6″E
24	D1-14-062 Km. 2 (246) Borcea Arm	20.03.2014	a	p	66		9.70	14.8	44⁰ 41′51.7″ N	27⁰51′33.6″E
25	D1-14-071 Km. 253 Hârșova	20.03.2014	a	p		31	7.53	14.8	44⁰ 40′48.9″N	27⁰56′52.9″E
26	D1-14-091 Km. 375 Chiciu-Silistra	22.03.2014	a	p		37	7.44	22.2	44⁰07′30.3″N	27⁰16′05.3″E
27	D1-14-105 Km. 551 Downstream Zimnicea	25.03.2014	a	p		87	4.68	14.8	43⁰36′59.5″N	25⁰23′29.1″E
28	D1-14-124 Km. 687 Kozlodui	26.03.2014	a	p		62	3.25	14.8	43⁰45′00.9″N	23⁰51′52.5″E
29	D1-15-006 Km. 20 Vâlcov-Periprava	11.03.2015	a	p	30		5.24	7.4	45⁰ 24′04.0 ″N	29⁰ 34′20.5″E
30	D1-15-009 Km. 40 Aval Chilia	11.03.2015	p	p	15		28.50	7.4	45⁰ 26′51.3″N	29⁰ 20′16.5″E
31	D1-15-015 M 43,5 Ceatal Ismail	12.03.2015	p	a	23		5.12	81.4	45⁰ 13′40.0″N	28⁰ 43′38.7″E
32	D1-15-017 M42 Tulcea Arm	12.03.2015	p	a		28	22.21	7.4	45⁰12′53.6″ N	28⁰ 45′35.1″E
33	D1-15-018 M 42 Tulcea Arm	12.03.2015	p	a	19		2.00	7.4	45⁰ 13′00.5″N	28⁰ 45′34.9″E
34	D1-15-026 Km. 108+500 Sf. Gheorghe Arm	12.03.2015	p	a		31	5.46	14.8	45⁰ 10′53.1″N	28⁰ 53′38.0 ″E
35	D1-15-029 Hm 72 Sulina Channel	13.03.2015	p	p	34		12.25	14.8	45⁰ 08′54.2″ N	29⁰ 45′45.4″ E
36	D1-15-032 M 2,8 Sulina Arm	13.03.2015	p	a		26	11.25	7.4	45⁰ 09′35.9″ N	29⁰ 36′30.0″ E
37	D1-15-033 M 2,8 Sulina Arm	13.03.2015	p	a	17		13.50	7.4	45⁰ 09′39.2″ N	29⁰ 36′32.9″ E
38	D1-15-035 Km. 1 Sf. Gheorghe Arm	13.03.2015	p	a		104	7.09	7.4	44⁰ 52′58.4″ N	29⁰ 36′29.3″ E

Table 1 ***(continued)***

Nr. crt.	Station	Date	*Palingenia longicauda*	*Gomphus (Stylurus) flavipes*	Distance from shore (m)		Water Depth	Abundance	Lat. (α)	Long. (λ)
			Presence/ Absence		Left	Right	m	m^2		
39	D1-15-038 Km. 8 Sf. Gheorghe Arm	14.03.2015	p	a		42	7.20	7.4	44⁰ 54'52.6" N	29⁰ 33'23.8" E
40	D1-15-055 Km. 4 Măcin Arm	18.03.2015	a	p		71	8.70	22.2	45⁰ 15'50.9" N	28⁰01'49.5"E
41	D1-15-056 Km. 4 Măcin Arm	18.03.2015	a	p	35		6.85	14.8	45⁰ 15'45.0" N	28⁰01'47.3"E
42	D1-15-057 Km. 4 Măcin Arm	18.03.2015	a	p	70	210	5.56	22.2	45⁰ 15'40.9" N	28⁰02'02.6"E
43	D1-15-058 Km. 174 Upstream Brăila	18.03.2015	a	p		95	15.96	22.2	45⁰ 14'38.4" N	27⁰58'03.7"E
44	D1-15-074 Km. 294 Seimeni	19.03.2015	a	p		75	9.44	14.8	44⁰ 23'02.1"N	28⁰02'44.9"E

In 1961, Dediu & Val'kovskaja (in Munjiu, 2017) recorded *Palingenia longicauda* as a common species for the Prut River.

At present, the species is extinct or at least missing at most of its original areas, except for relatively small refugia in the Tisza basin in Hungary, Serbia and, probably, also, Slovakia and Ukraine (Soldán *et al.*, 2009; Bálint *et al.*, 2012). Soldán *et al.* (2009) identifies *P. longicauda* swarms on the Sf. Gheorghe arm, near Murighiol in 2007 and 2008, and Bulánková *et al.,* (2013), near Tulcea (Channel Mila 35).

These insects usually appear in an explosive manner in May, hence the popular English name "mayfly". In Romania, the popular term is "rusalie" (plural "rusalii"), associated with *"Rusalii"*, the Romanian name of *Pentecost*, a Christian celebration that takes place on the 50th day after Easter, so in May or June. They live in the larval stage for 1-3 years on the lush bottom of the river, at present, being a very rare species in the big rivers of Europe. After 39-45 days, depending mainly on water temperature (at an average temperature of 21.4 °C it lasts 26 days), the larvae hatch from the eggs (Landolt *et al.,* 1997; Tittizer *et al.,*2008). Those are living in mud tunnels at a density of 400 tunnels per square metre, building U-shaped tubes of clay, in which water current is created by gill movement (Csoknya & Halasy, 1974; Russev, 1987).

At the time of shedding, gas is formed between the pulp shell and the new coating, in order to raise it to the surface of the water. Here the shell is detached, and the insect becomes winged being able to breed.

Grouped in compact swarms, they perform the nuptial flight that takes place at sundown. They no longer eat in the adult stage, since their oral device is no longer functional, and the digestive tract contains nothing but air. Their entire adult life takes a few hours, so after mating ends, their life cycle also ends in 15 minutes. With the end of the mating act, the insects die, leaving eggs on the bottom of the water. It is a unique phenomenon all over the world of insects, all their existence being directed to one purpose: reproduction (Godeanu, 2002).

Eggs of some females (up to 50%, according to Landolt *et al.,* 1997) develop parthenogenetically, this being probably relevant for small populations.

In the past, the mayflies were encountered in large numbers throughout the Danubian area, but at present these have become an endangered species, due to water pollution, which largely affected the Danube course (Robinson, 2005). Larvae of *Palingenia longicauda* are highly sensitive to changes in abiotic factors and disappear rapidly from regulated rivers or from sections with organic pollution (Landolt *et al.,* 1997). Their presence varies from one location to another, depending on the different ecological conditions: hydromorphological, hydrochemical level of anthropogenic loading and type of substrate; sediment granulometry appears to be the most important factor that may affect the distribution of *Palingenia* larvae (Table 2).

Regarding the granulometry, *Palingenia* larvae distribution is influenced by the type of sediment, the most appropriate

Fig. 2. *Palingenia longicauda* (Olivier, 1791)

Table 2. The granulometry of the sediments in the profiles where the two larvae were identified.

Nr. crt.	Sample	Composition				Shepard classif.
		Gravel %	Sand %	Silt %	Clay %	
1	D-12-035 Km 8 Sf. Gheorghe Arm	0.00	8.94	62.53	28.55	Silty clay
2	D-12-045 M 78 Downstream Galați	0.00	5.25	65.94	28.81	Silty clay
3	D-12-052 Km 167 Downstream Brăila	0.00	11.78	67.74	20.49	Silty clay
4	D-12-053 Km 167 Downstream Brăila	0.00	6.23	70.85	22.93	Silty clay
5	D-12-056 Km 4 Măcin Arm	0.00	87.36	10.93	1.72	Sand
6	D-12-072 Km 253 Hârșova	0.00	22.53	63.15	14.32	Silty sand
7	D-12-092 Km. 375 Chiciu-Silistra	0.00	21.64	61.78	16.58	Silty sand
8	D-12-109 Km. 557 Upstream Zimnicea	0.00	14.37	66.01	19.63	Silty clay
9	D-12-131 Km. 789 Downstream Calafat	0	41	47	12	Silty sand
10	D2-12-036 Km 8 Sf. Gheorghe Arm	0	11.12	64	24.88	Silty clay
11	D2-12-041 M 54 Isaccea	0	10	65.7	24.3	Silty clay
12	D2-12-046 M 78 Downstream Galați	7.3	92.7	0	0	Gravel sand
13	D2-12-049 Km 159 Upstream Siret	0	43	47	10	Silty sand
14	D2-12-055 Km 4 Măcin Arm	0	42.5	48.34	9.16	Silty sand
15	D2-12-056 Km 4 Măcin Arm	0	41.25	48.45	10.3	Silty sand
16	D1-13-004 Km. 301 Upstream Bridge Cernavodă	0	94	5.34	0.66	Sand
17	D1-13-176 Km 8 Sf. Gheorghe Arm	0	13.76	62.8	23.44	Silty clay
18	D1-13-023 Km. 481 Downstream Giurgiu	0	87.37	10.43	2.2	Sand
19	D1-13-037 Km. 604+400 Olt River	17.09	82.91	0	0	Gravel and Sand
20	D1-13-144 Km. 167 Downstream Brăila	0	2.14	71.45	26.41	Silty clay
21	D1-14-055 Km. 4 Măcin Arm	0	84.55	12.77	2.68	Sand
22	D1-14-056 Km. 4 Măcin Arm	0	40.74	46.69	12.57	Silty sand
23	D1-14-059 Km. 174 Upstream Brăila	0	91.43	7.33	1.24	Sand
24	D1-14-062 Km. 2 (246) Borcea Arm	0	40.65	46.83	12.52	Silty sand
25	D1-14-071 Km. 253 Hârșova	0	93.9	5.5	0.6	Sand
26	D1-14-091 Km. 375 Chiciu-Silistra	0	41.5	47.3	11.2	Silty sand
27	D1-14-105 Km. 551 Downstream Zimnicea	6.98	93.02	0	0	Gravel and Sand

Table 2 ***(continued)***

Nr. crt.	Sample	Composition				Shepard classif.
		Gravel %	Sand %	Silt %	Clay %	
28	D1-14-124 Km. 687 Kozlodui	0	96.07	3.22	0.71	Sand
29	D1-15-006 Km. 20 Vâlcov-Periprava	0	92.906	6.152	0.942	Sand
30	D1-15-009 Km. 40 Downstream Chilia	0	97.816	1.91	0.274	Sand
31	D1-15-015 M43,5 Ceatal Ismail	0	90.623	7.84	1.537	Sand
32	D1-15-017 M 42 Tulcea Arm	0	8.271	72.193	19.536	Silty clay
33	D1-15-018 M 42 Tulcea Arm	0	8.187	71.393	20.42	Silty clay
34	D1-15-026 Km. 108+500 St. Gheorghe Arm	0	1.13	63.966	34.904	Silty clay
35	D1-15-029 Hm 72 Sulina Channel	0	12.376	68.102	19.522	Silty clay
36	D1-15-032 Mila 2,8 Sulina Arm	0	93.572	5.973	0.455	Sand
37	D1-15-033 Mila 2,8 Sulina Arm	0	80.115	14.89	4.995	Sand
38	D1-15-035 Km. 1 St. Gheorghe Arm	0	21.734	59.841	18.425	Silty sand
39	D1-15-038 Km. 8 St. Gheorghe Arm	0	25.237	59.315	15.448	Silty sand
40	D1-15-055 Km. 4 Măcin Arm	0	75.879	20.224	3.897	Sand
41	D1-15-056 Km. 4 Măcin Arm	0	17.939	57.135	24.926	Silty clay
42	D1-15-057 Km. 4 Măcin Arm	0	8.354	69.603	22.043	Silty clay
43	D1-15-058 Km. 174 Upstream Brăila	0	6.867	71.755	21.378	Silty clay
44	D1-15-074 Km. 294 Seimeni	0	93.009	6.52	0.471	Sand

consisting mainly of clay and silt. (Russev 1968; Csoknya & Ferencz, 1972, 1975; Csoknya & Halasy, 1974). Our results show that, in most cases, sediments colonized by *Palingenia* larvae consist of more than 30% clay. A proportion of more than 20%of sedimentary particles, especially coarse sand, is incompatible with colonization of *Palingenia* larvae.

Gomphus (Stylurus) flavipes (Fig. 3) belongs to Odonata order and is a species widespread in the past in Europe's western rivers (Loire, Rhine), being common in large rivers in Eastern Europe, with preference for slow flowing sections and sandy bed. It was believed to have been extinct in the West and Central Europe but has experienced a strong recovery since the 1990s (Dijkstra & Lewington, 2006).

The larvae are reophile-potamophilous and predatory; they are buried in the sand or marsh of the flowing waters at quite large great depths, reaching even 8-18 cm (Godeanu, 2002). The life cycle of *Gomphus (Stylurus) flavipes* is characterized by an incomplete metamorphosis, including the following stages: egg, larvae and adult, lacking a nymph stage.

The laying of the eggs takes place directly in the water, or in vegetal tissues, generally under water. When deposited, the eggs have a clear, whitish colour, which later changes and becomes brown. Hatching of eggs occurs usually after four weeks. Exceptions are eggs that, in unfavourable weather conditions, enter the diapause, a stage in which they stagnate over winter. Larvae hatch from eggs with a pulsatile organ called the cephalothoracic heart, an organ that has an existence span from a few seconds to a few minutes. The hatching takes place following a stimulus: an example of a stimulus is the exposure to water of the eggs laid on water (usually in the spring, with the rise of waters) or the increase of the water temperature (Cîrdei & Bulimar, 1965). Both larvae and adults are carnivorous, feeding on almost any small animal, sometimes even bigger than them. The larva, during its development, passes through a number of 10-15 shedding episodes (usually 12-13), that occur faster in warmer months and when food is abundant (Corbet, 2004).

The last larval stage undergoes some changes (before the last „transformation"), so its colour becomes darker, the colour of the eyes becomes more intense, the mask starts to regress, and the feeding is stopped. The larvae are found in places with shallow water, they climbs on the shore or on vegetation, the respiratory activity intensifies, the larvae partially emerging from the water for a surplus of oxygen, necessary for the following metamorphosis. All these changes are controlled by hormones (Wigglesworth, 1984; Corbet, 2004). The development from egg to adult generally lasts three years, so that habitat conditions do not undergo much change during such a period (Trpiš, 1957).


Foto: Ana-Bianca Pavel

Fig. 3. *Gomphus flavipes* (Charpentier, 1825)

The preferred habitat of *Gomphus (Stylurus) flavipes* is the shallow, low-flow portion of the river characterized by a sandy substrate. As with mayflies, it is an endangered species, due to the pollution of the waters, in the past, dragonflies being seen in large numbers throughout the Danube. The larvae live in the sediment, preferring places with higher temperatures. The exuviae can be found near the water, indicating restored wetlands (Bulánková *et al.*, 2013). David (2005) states that adults should reach 10 copies/100 m of coastline to be in line with the favourable conservation status of European importance species.

In the 1950s, large populations of *Gomphus (Stylurus) flavipes* and *Palingenia longicauda* were reported on the lower course of the Danube (Soldán *et al.*, 2009; Bulánková *et al.*, 2013). Due to anthropogenic activities, such as hydrotechnical constructions and organic water pollution, the populations of these two species have diminished considerably, even leading to their disappearance from certain areas of the sector (Soldán *et al.*, 2009; Málnás *et al.*, 2011). These huge construction works and many other small dams have led to morphological changes and degradation of the riverbed and have adversely affected the environmental conditions for these sensitive species. In addition, water pollution caused by industrial activities and the excessive use of pesticides in agriculture could have resulted in the disappearance of their larvae on the Lower Danube. In spite of this, more recent data indicate stable populations of these two larvae for this area (Bulánková *et. al.*, 2013; Petrović *et. al.*, 2017; Munjiu, 2017).

Soldán *et al.* (2009) and Málnás *et al.* (2011) highlight the fact that larvae of the two species prefer large, unpolluted rivers with low to moderate current speed, with steep shores of clay (for *Palingenia* larvae) or silty sand (for *Gomphus* larvae). Our research carried out during 2012-2015 is confirming them by noticing the same ecological preferences for *Gomphus* and *Palingenia* larvae.

Studies performed in this period show an uneven distribution of *Gomphus (Stylurus) flavipes* in the lower course of the Danube. Larvae of this species were reported in almost all the stations analysed throughout all the years of study, with relatively low population numbers. In a single location (Km 4 – Măcin Arm), the species were found in 2012, 2014 and 2015, having relatively large abundances (37-59.2 ind/m^2), indicating a stable population in that area (Fig. 4). The large number of individuals can be attributed to the dependence of this species on a certain type of substrate, in this area the larvae being encountered in silty sediments. In spite of this, the sediment type is not the only limiting factor, the microdistribution of this larva being in direct correlation with other physico-chemical parameters (water temperature, oxygen and nutrient concentration).

The frequency of occurrence of *Palingenia longicauda* larvae in samples, in the years of the study was rather low, they being sporadically encountered in relatively low population numbers. Larvae of this species are very sensitive to changes in abiotic factors, the type of substrate playing an important role in their distribution. Thus, in 2012, the highest abundance was encountered in Km 8 Sf. Gheorghe profile (125.8 ind/m^2), *Palingenia longicauda* having a high affinity for the clay sediments found in this area (Fig.5). However, from 2012 to 2015, the populations of *Palingenia*, was decreasing in this location, fact that can be attributed to the disturbing anthropogenic factor. Although research conducted by Russev (1987) show that *Palingenia* larvae were not reported in habitats with sandy substrates, in 2015, our studies show a rather high abundance of larva in the M 43.5 Ceatal Ismail area, under the conditions of a sandy bottom sediment, uncharacteristic to the species. Large abundance

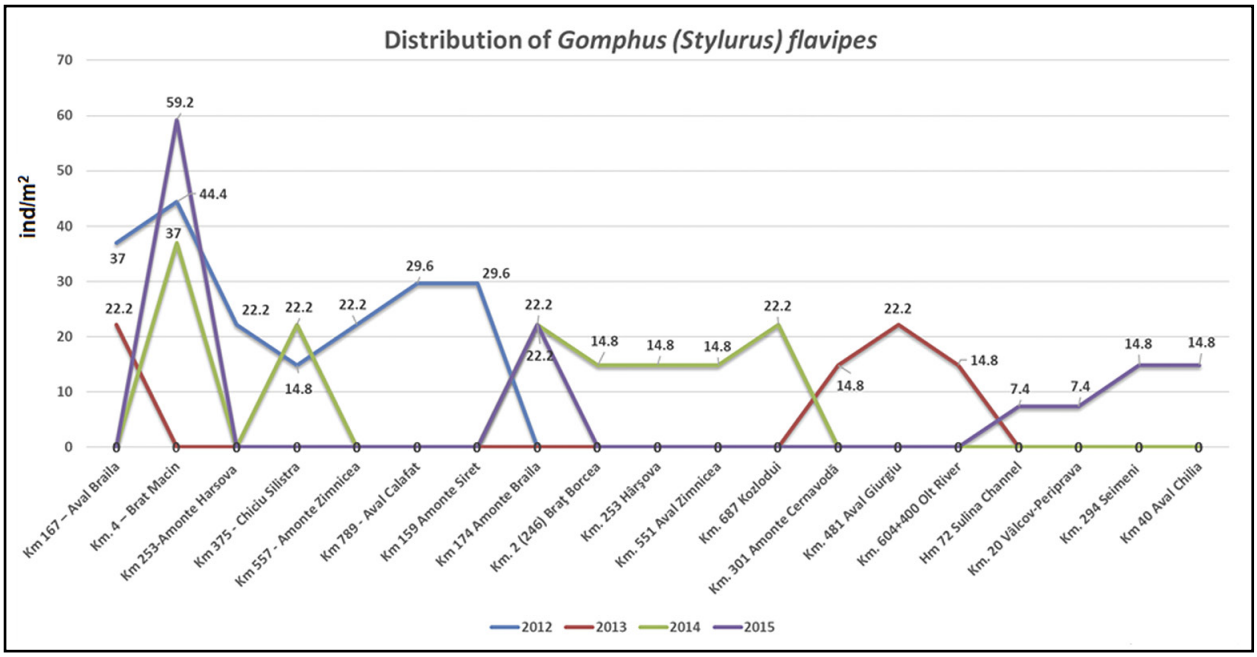

Fig. 4. Distribution of the *Gomphus* (*Stylurus*) *flavipes* during the 2012-2015 period.

could be explained by the optimal abiotic conditions for species development (high oxygen concentrations: 11.39 mg/l and low organic pollution).

The distribution of the two larvae populations can also be influenced by the riverbank morphology (undergoing erosion, or characterized by deposition), it being more abundant in areas with active sediment deposition. So, on the profiles Km 557 Upstream Zimnicea and Km 789 Downstream Calafat, the larvae were found on the left shore, instead, in the others two profiles, Km 551 Downstream Zimnicea and Km 687 Kozlodui, the larvae were found on the right shore. All cases represent accretionary bank deposits (Fig. 6).

By comparing the data, the prognosis is favourable for both species. The reappearance of the Palingenia and Gomphus larvae was probably linked to the improvement of water quality since 2000 compared to the previous period. Following the adoption of the Water Framework Directive (WFD), Romania has taken action in line with the requirements of the Directive, which have led to a significant improvement in the Lower Danube River situation (Theodosiu *et al.*, 2009).

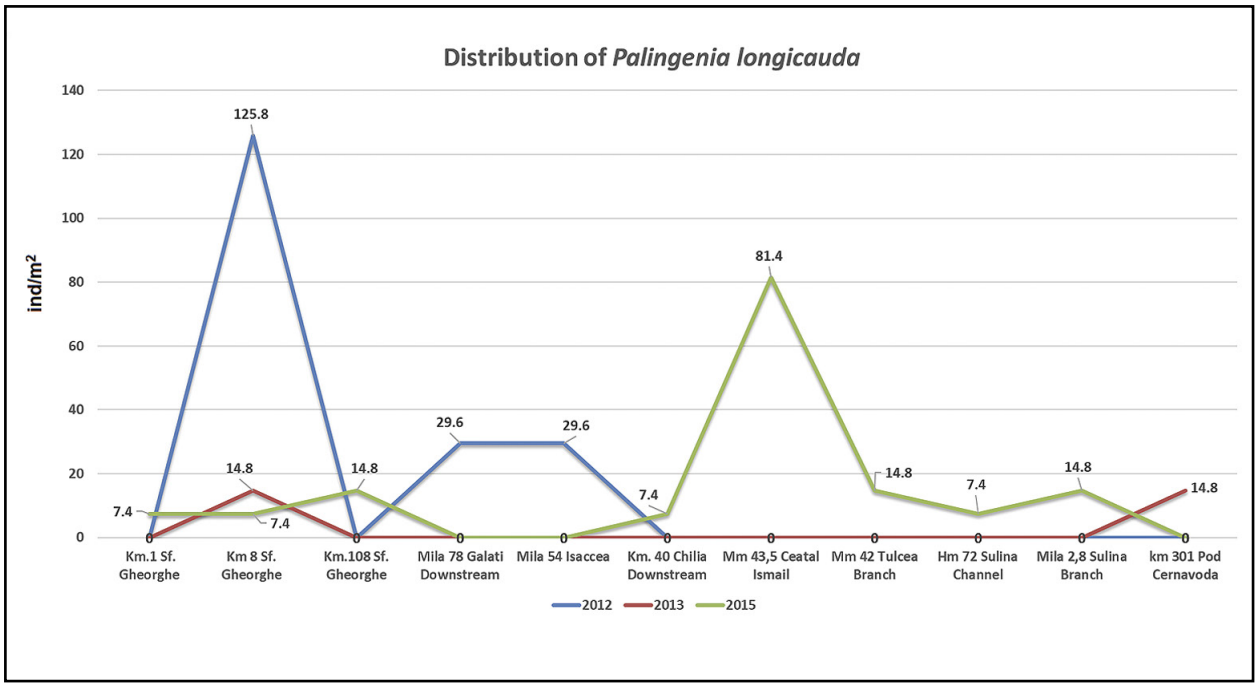

Fig. 5. Distribution of the *Palingenia longicauda* during the 2012-2015 period.

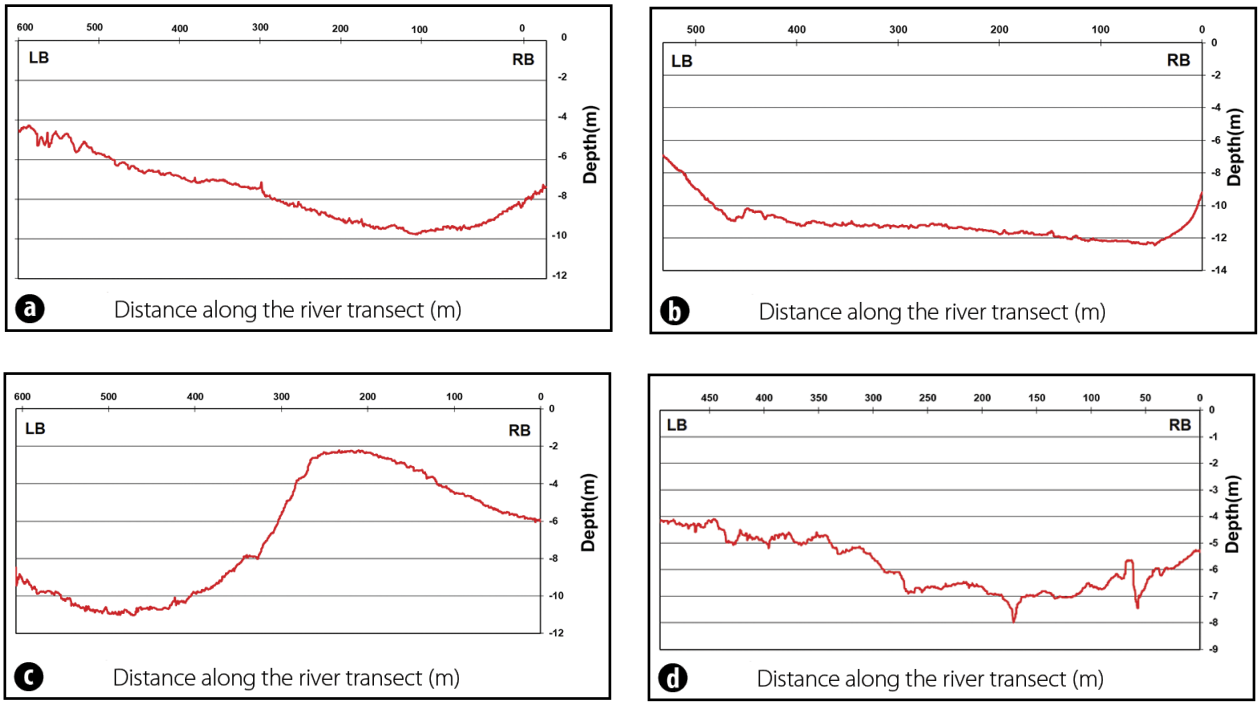

Fig. 6. Cross sections on the Danube River (LB – Left bank; RB – Right bank). (**a**) Km 557 Upstream Zimnicea; (**b**) Km 789 Downstream Calafat; (**c**) Km 551 Downstream Zimnicea; (**d**) Km 687 Kozlodui

4. CONCLUSION

The occurrence of large populations of the two endangered potamal species, *Gomphus (Stylurus) flavipes* and *Palingenia longicauda,* is reported in the Romanian Danube River (from km 1 Sf. Gheorghe to Km 301 Upstream Cernavodă Bridge for Palingenia and from Km 20 Vâlcov-Periprava to Km 789 Calafat for *Gomphus*). The results confirm the Danube river and Danube Delta high importance for aquatic biodiversity conservation.

More of *Gomphus* and *Palingenia* individuals than it has been expected were found along the Lower Danube Sector. The occurrence of rare and vulnerable species may be attributed to the improvement of water quality status of the river (Liška *et al.,* 2008).

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