BABEȘ-BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL IN INTEGRATIVE BIOLOGY

Summary of PhD thesis

Taxonomic revision of *Habroleptoides carpatica/H. confusa* stat. nov. (Insecta: Ephemeroptera) and the effect of global warming on the species

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Ephemeroptera, *Habroleptoides confusa*, *H. carpatica*, synonym, morphological characters, rearing, global warming, temperature, advanced emergence

1 General introduction

1.1 Characterization of mayflies (Insecta: Ephemeroptera)

1.1.1 Biology of mayflies

Ephemeroptera are hemimetabolous insects with complex life cycles involving both aquatic (egg, larva) and aerial stages (subimago, imago) (Bauernfeind & Soldán, 2012) (Fig. 1).

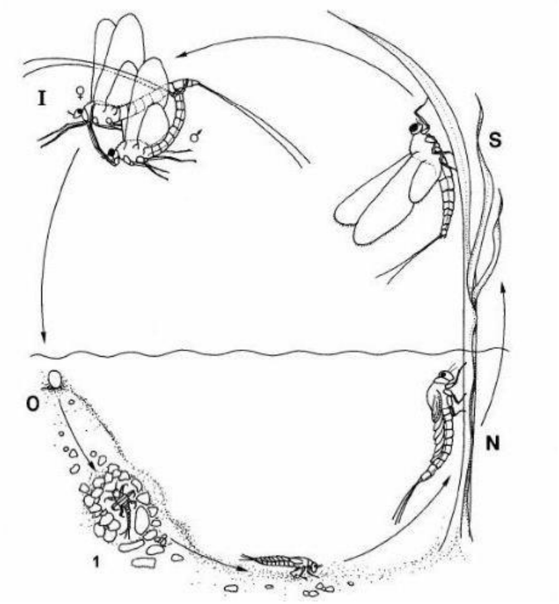

Fig. 1. Life cycle of mayflies (Studemann et al., 1992)

Mayflies are unique among the insects due to the existence of two winged stages separated by moult, the subimago and imago (Edmunds & McCafferty, 1987). As long as the duration of the subimaginal stage ranges between a few minutes and even 4 days, the individual life span of imagines usually lasts no more than 24 hours, with extremes between only a few minutes and several days (Bauernfeind & Soldán, 2012). The role of imagines resumes to the propagation of species (Bauernfeind & Soldán, 2012).

Mayflies spend most of their life in aquatic environment either as eggs or in larval stage (Brittain & Sartori, 2003). Embriogenesis ranges from a couple of weeks to nearly a year (Bauernfeind & Soldán, 2012). Depending on species and environmental factors, the larval stage lasts from four weeks (Funk et al., 2010; Harker, 1997) to three years (Kriska et al., 2007; Soldán et al., 2009). During this time mayflies pass through 15-25 postembryonic moults

(Brittain & Sartori, 2003). Larval growth, consequently the frequency of moulting depends mostly on environmental factors such as temperature, food quality and food availability, body size and age, light conditions as it increases the temperature and facilitates algal growth (Bauernfeind & Soldán, 2012; Giberson & Rosenberg, 1992).

Although pupal stage is missing, larvae morphologically differ from adult insects by the existence of fully developed and functional mouthparts and gills but also by the lack of wings and functional reproductive organs (Bauernfeind & Soldán, 2012).

Most mayfly larvae are adapted to consume detritus and peryphyton (Brittain, 1982; Brittain & Sartori, 2003). Based on the morphology of their mouthparts, mayfly larvae can be divided in a few functional feeding groups: grazers-scrapers, schredders, gatherers-collectors, active and passive filterers, predators (Bauernfeind & Soldán, 2012). Despite of the mentioned categories, feeding type was shown to be highly flexible even within species and depends on larval age, seasonal dynamics of algae and availability of food types (Elpers & Tomka, 1994; López-Rodríguez, Tierno de Figueroa & Alba-Tercedor, 2008).

Oxygen uptake of mayfly larvae is realized mainly through abdominal gills, but the occurrence of cutaneous respiration was also documented (Morgan & Grierson, 1932; Bauernfeind & Soldán, 2012). Additionally several lineages of the extant Ephemeroptera (Baetidae, Colubriscidae, Isonychiidae, Nesameletidae, Oligoneuriidae, Rallidentidae, Siphluriscidae) present accessory gills on different body parts like maxillae, labium, coxae, or ventral thoracic surfaces, and even if not proved experimentally, they most likely support the function of gills (Staniczek, 2010).

The transition from aquatic to aerial habitat usually lasts about 5-10 minutes (Bauernfeind & Soldán, 2012) and represents a critical period for mayflies. During this time they become highly vulnerable to both aquatic and aerial predators (Brittain, 1982). Metamorphosis is determined mainly by temperature and light intensity (Bauernfeind & Soldán, 2012), most of the species exhibiting clear diurnal emergence patterns (Brittain, 1982).

1.1.2 Life cycle

Depending on their life cycles, mayflies can be grouped in three main categories: univoltine species with one generation per year, multivoltine species with two or more generations per year or semivoltine species whose development requires more than a year (Brittain, 1982).

Though the interspecific variation of voltinism is implicit, the life cycle pattern of the same species might be characterized by high flexibility allowing such species to inhabit a wide range of climates and habitats (Sand & Brittain, 2009). Amongst others, this is the case of *Baetis rhodani*, which is bivoltine in West Norway (Baekken, 1981), polivoltine in Italian prealpine streams with three generations per year (Erba et al., 2003), but the most conspicuous life cycle shift is documented for central Southern Norway, where the species turns from one generation a year at 1090 m a.s.l. to semivoltinism above 1100 m a.s.l. in the same catchment area, larvae reaching the imago stage in two years (Sand & Brittain, 2009).

1.2 *Habroleptoides* within Ephemeroptera

The genus *Habroleptoides* encompasses 17 species including one species with uncertain taxonomic position (*H. carpatica*), all of them inhabiting watercourses in Western Palaearctic (Bauernfeind & Soldán, 2012; Kazanci & Türkmen, 2011). No representatives have been identified from Fennoscandia, the British Isles and Central Lowlands (Bauernfeind & Soldán, 2012). As long as larval and female imaginal stages of most of the species are not described, males can be identified based on the genitalia (shape of basal projection of first segment of forceps) and venation of wings.

H. confusa represents the most studied species of the genus owing to detailed description for all developmental stages (Biancheri, 1956; Sartori & Jacob, 1986; Bauernfeind & Soldán, 2012; Schönemund, 1929; Sartori, 1986) and the large areal located between Portugal and South Caucasus (Sartori & Jacob, 1986), with records in Turkey as well (Tanatmiş, 2004). It is considered an expansive holomediterranean species (Haybach & Jacob, 2010).

1.3 Global climate change: recent trends, causes and projections

In the last 140 years (between 1880-2012) air temperature rose with 0.85 °C (0.65-1.06 ºC) (IPCC, 2013) therefore the 20th century became the warmest ever since 1500 AD (Jylhä et al., 2010) and 2015 the warmest year since instrumental recordings are dated (Hansen et al., 2016). Temperature growth led to alterations in the hydrological cycle, dramatic decline of arctic sea ice extent, mass loss of glaciers, global mean sea level increases and warming of the water, changes in ocean biochemistry (IPCC, 2013).

As long as in earlier times global warming was considered mainly a CO_2 mediated climatic event (Ramanathan & Feng, 2009), recent studies list increased concentrations of other greenhouse gases (GHG) like CH_4, N_2O and CFCs, land cover changes (mainly due to deforestations and urbanization), air pollution among the drivers of recent global climate change (IPCC, 2013)

According to the projections, temperature increses will range between 1 °C-4 °C, while other weather events would continue the observed trends over the last half century (IPCC, 2013).

2 On the identitiy of *Habroleptoides carpatica* Bogoescu & Crăsnaru, 1930 and *H. confusa* Sartori & Jacob, 1986 (Insecta: Ephemeroptera: Leptophlebiidae: Habrophlebiinae)

2.1 Introduction[1]

Even if major revision of the genus *Habroleptoides* was undertaken by Sartori (1986), Sartori & Jacob (1986), Sartori & Thomas (1986) and new species are described (Kluge, 1994; Kazanci & Türkmen, 2011), the status of *Habroleptoides carpatica* Bogoescu & Crăsnaru, 1930 is still not clear. The species was described at imaginal and larval stages from Valea Căşăriei brook close the Zoological Research Station of Sinaia (Romanian Carpathians) (Bogoescu & Crăsnaru, 1930). Since then, *H. carpatica* was reported mainly from Romanian watercourses (Bogoescu, 1932a, Bogoescu, 1958, Miron, 1959, Szállassy, 1999), but also from the Serbian Carpathians (Filipovic, 1979) and Serbian and Eastern Bosnian-Herzegovinan Dinaric Alps (Filipovic 1975, Tanasijevic, 1970, 1973).

The species was described by the two segmented maxillary palps and labial palps, venation of the hind wings, the shape of the last abdominal sternite of female imagines (Bogoescu & Crăsnaru, 1930). Later on Bogoescu (1958) completed the description with further characteristics of the male genitalia. When Sartori & Jacob (1986) established the new name *Habroleptoides confusa* for the widespread but misidentified taxon *H. modesta* sensu Schönemund et auct. sequ. (nec *Potamanthus modestus* Hagen, 1864) they supposed that the discriminating characters observed by Bogoescu & Crăsnaru (1930) in imagines might fall into the natural variation of *H. confusa*. However, in absence of the type material (or topotypic material) of *H. carpatica* the authors refrained from any decision about the status of *H. carpatica*.

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[1] Váncsa et al., 2013

The aim of the study is to assess the variation of morphological characters of *H. confusa* sampled from a wide range of habitats and to ascertain the taxonomic position of *H. carpatica* based on reared material from the type locality (Valea Căşăriei brook, Sinaia, Baiului Mountains, Romania).

2.2 Materials and methods[1]

As the type material of *H. carpatica* was declared lost by Romanian researchers and staff of museums and research stations on the 17th of April 2009 a total of 5 *Habroleptoides* larvae was collected from the type locality of *H. carpatica*, Valea Căşăriei brook (45.366944 N 25.556389 E, 928 m alt.) (Fig. 2) and reared. Two nymphs died during rearing process and three specimens developed to imagines. All the material, including the nymphal skins, was preserved in 70% ethanol.

Fig. 2. Type locality of *H. carpatica:* Valea Căşăriei, Sinaia

Additionally *H. confusa* specimens were sampled from different locations. Last instar larvae were also reared. Slides from museum collections were examined.

Specimens were analyzed under a Hund Wetzlar stereomicroscope and Olympus microscope. Slides were realized: mouthparts, legs, genitalia were fixed with Liquid de Faure (Adam & Czihak, 1964), gills, terga, sterna, eggs and the entire exuvia were mounted in PVA (Heinze, 1952). Photographs were taken and measurements were realized with the help of the Cell^A software. The chorionic structure of the eggs of *H. carpatica* was investigated with a Jeol JSM-7401F scanning electron microscope at 4kV.

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[1] Váncsa et al., 2013

The analysis of the diagnostic characters mentioned by Bogoescu & Crăsnaru (1930) and Bogoescu (1958) for *H. carpatica* formed the basis of our investigastion. Other characters such as groups of bristles were identified on the mouthparts, legs and pronotum of the larvae or nymphal exuviae and were counted. Number of teeth on the claws was also registered. In imaginal stage the study was completed with measurements of the distance between eyes, length of segments of fore legs, length of wings.

Examined material:

Habroleptoides carpatica Bogoescu & Crăsnaru, 1930: 2 nymphs, 1♂ and 1♀ (reared) with their nymphal exuviae: Valea Căşăriei brook.

Habroleptoides confusa Sartori & Jacob, 1986: 31 larvae (2 ind. Switzerland, 5 ind. from Austria, 10 ind. from Eastern Carpathians, 1 ind. from Southern Carpathians , 5 ind. from Western Carpathians, 2 ind. from Czech Republik) and 23 imagines (1♂ Switzerland: the holotype, 7 ♂ & 2 ♀ from Romania, 3 ♂ & 2 ♀ from Bulgaria, 2 ♂ from Germany, 3 ♂ from Czech Republik, 5 ♂ from Spain).

2.3 Results

The specimens collected in Valea Căşăriei brook resemble *H. confusa* both in larval and imaginal stage in all respects (Fig. 3-6).

Fig. 3. A, B: Larvae of *Habroleptoides carpatica*

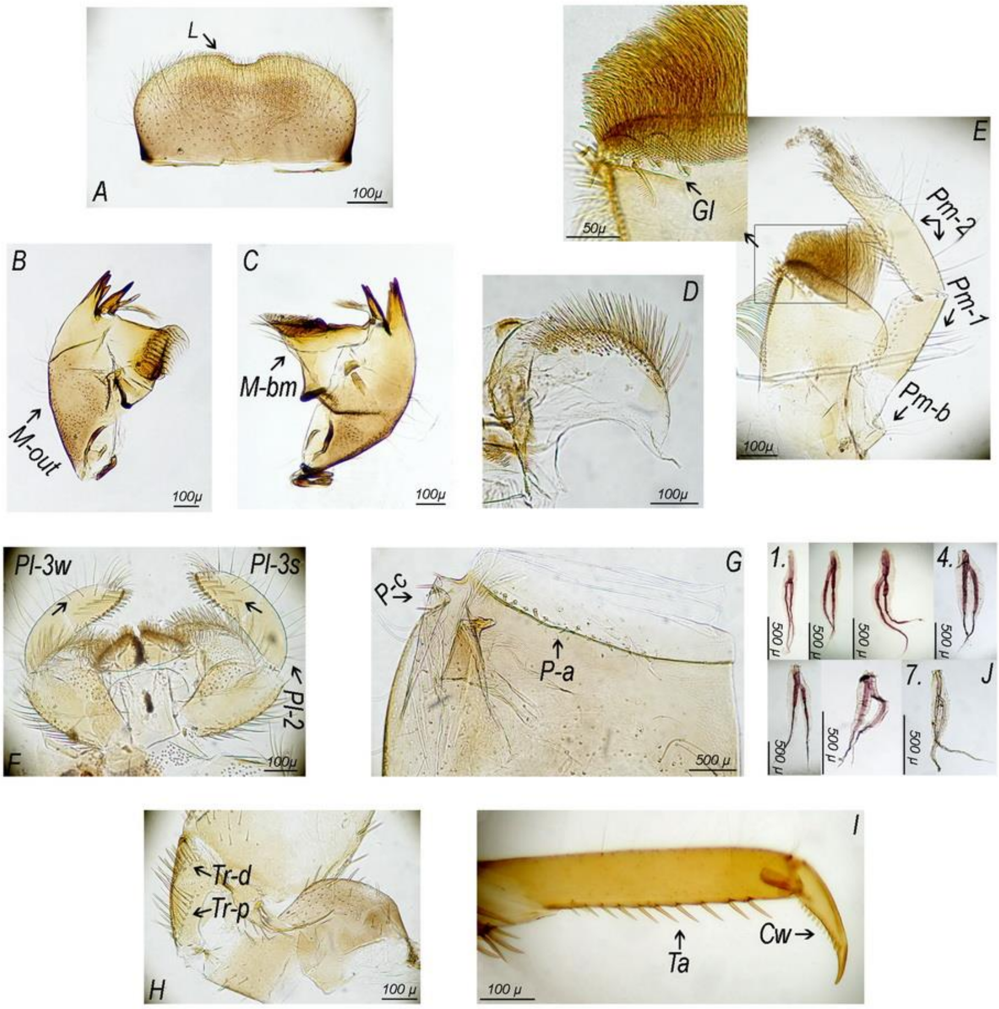

Fig. 4. *H. carpatica*: A: labrum, L - row of bristles along the anterior margin of the labrum, B: left mandible, M-out – row of bristles along the outer margin of the mandible, C: right mandible, M-bm – row of bristles on the right mandible, below the mola, D: hypopharingeal superlingua, E: maxilla, Gl – row of comb-shaped bristles on lacinia, Pm-b – group of bristles at the base of the maxillary palp, Pm-1 – row of bristles along the 1st segment of the maxillary palp, Pm-2 – row of bristles along the 2nd segment of the maxillary palp, F: labium, Pl-2 – row of bristles along the margin of the 2nd segment of the labial palp, Pl-3w – row of thick bristles on the 3rd segment of labial palps, Pl-3s - row of thin bristles on the 3rd segment of labial palps, G: pronotum, P-a – row of bristles along the anterior margin of the pronotum, P-c – group of bristles in the antero-lateral part of the pronotum, H: coxa and trochanter (1. leg), Tr-d – row of bristles on the distal part of the trochanter, Tr-p – row of bristles on the proximal part of the trochanter, I: tarsus (3. leg), Ta – bristles along the inner margin of the tarsus, Cw – teeth on the claw, J: gills

Fig. 5. *H. carpatica*: ♂ imago

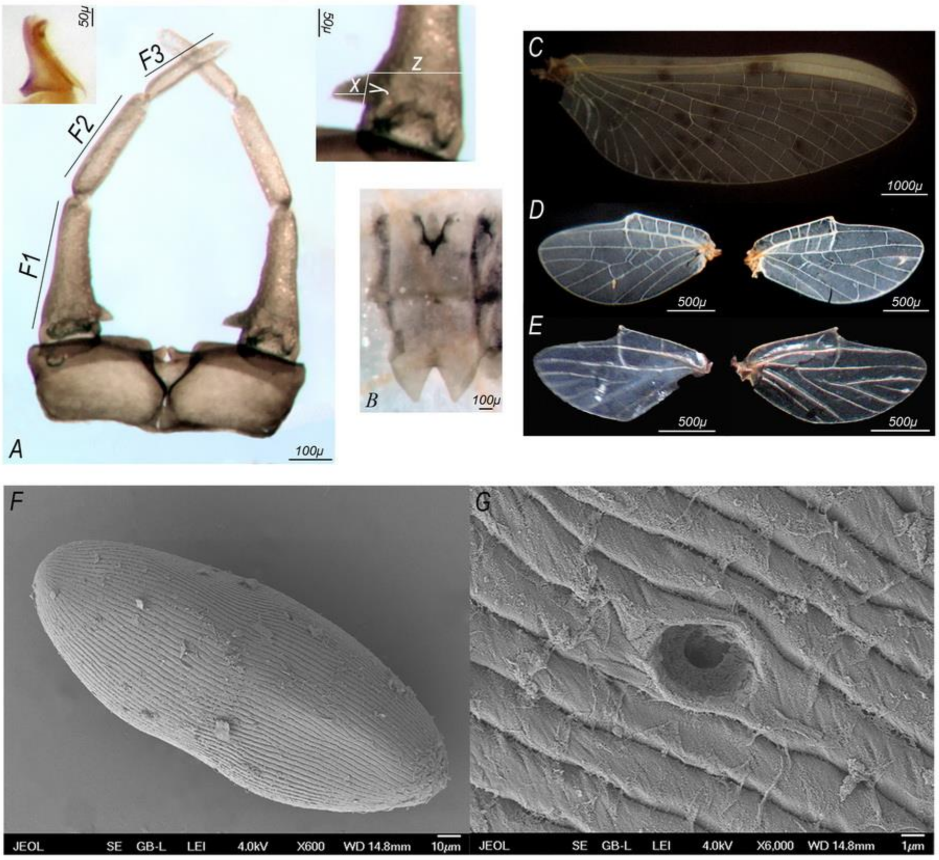

Fig. 6. *H. carpatica*: A: ♂ genitalia, F1– length of the first segment of the forceps, F2 - length of the second segment of the forceps, F3 - length of the third segment of the forceps, x – length of the internal process, y – width of the internal process, z- width of the basal segment of the forceps, B: ♀ last sternum, C: ♂ fore wing, D: ♂ hind wings, E: ♀ hind wings, F: egg, G: micropyle

The most relevant larval characteristics of *H. carpatica* are the three-segmented maxillary and labial palps (Fig. 4. E, F), the relatively similar length of the first pair of gills with the following ones (Fig. 3, Fig. 64. J), variation of the hind wing venation, observed even in a single individual (right and left wings) (Table 2, Fig. 6 D, E), shorter 3rd segment of the forceps relative to the 2nd segment, well developed, relatively slender, slightly bent and pointed internal projection of the basal segment of the forceps, and the constitution of the egg chorion from ridges arranged in parallel, longitudinal rows are emphasised (Fig. 6 F, G). Further characteristics of *H. carpatica* larvae and imago in comparison with *H. confusa* can be seen in Table 1 and Table 2[1]

Analyzed characters		*H. carpatica*		*H. confusa*	
		variation	N ind.	variation	N ind.
1 leg	Cw	9-13	4	11-15	28
	Tr-p	9-14	4	8-20	25
	Tr-d	14-19	4	15-30	20
2 leg	Cw	9-13	4	10-15	28
	Ta	10-11	4	8-14	27
	Tr-p	2-4	3	0-9	22
	Tr-d	4-8	4	3-13	23
3 leg	Cw	12-13	3	10-16	24
	Ta	10-11	3	8-14	24
	Tr-p	1	3	1-3	18
	Tr-d	4	3	1-9	20
Mouthparts	L (half)	10-12	4	8-16	27
	L (entire)	21-23	4	17-31	27
	M(left)-bm	8-11	4	7-14	21
	M(left)-out	11-14	3	8-28	20
	M(right)-out	12-14	3	6-28	19
	Pm-b	4-5	3	4-6	26
	Pm-1	6-8	4	3-11	27
	Pm-2	3-5	3	2-5	27
	Gl	4-5	4	4-6	26
	Pl-2	8-11	3	7-13	27
	Pl-3s	4-6	3	4-8 (12)	26
	Pl-3w	4	3	2-7	26
Pronotum	P(left part)-a	12-14	4	7-17	22
	P(right part)-a	11-13	4	9-23	22
	P(left part)-c	6-11	4	4-15	23
	P(right part)-c	4-11	4	4-13	23

Table 1. Numerical characters of *H. carpatica* and *H. confusa* larvae (for abbreviations of the analyzed characters see Fig. 4)

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[1] Váncsa et al., 2013

Analyzed characters		*H. carpatica*		*H. confusa*	
		variation	N ind. gen	variation	N. ind. gen
Distance eyes (µm)	compound	175	1 ♂	154-224	12 ♂
	ocelli	222	1 ♂	222-280	12♂
1. leg – length (µm)	Femora	1980 / 1593	1 ♂ /1 ♀	1769-2329 / 1463	10♂ / 1 ♀
	Tibia	2898 / 1896	1 ♂ / 1♀	2482-3016 / 1949	12♂ / 1 ♀
	Tars 1	1063 / 205	1 ♂ / 1♀	919-1140	12♂
	Tars2	990 / 158	1 ♂ / 1♀	871-1200	12♂
	Tars3	772 / 131	1 ♂ / 1♀	678-908	12♂
	Tars4	263 / 253	1 ♂ / 1♀	119-360	12♂
Wing length (µm)	fore wing	7803	1 ♂	7334-8657 / 8482	14 ♂ / 1♀
	hind wing	1852 / 1519	1 ♂ / 1♀	1759-2212 / 1754-2187	9♂, 2♀
Fore wing – crossveins (N)	pterostigma	14	1♂	10-14	7♂, 2♀
Fore wing – intercalary veins (N)	cubital field	5	1♂, 1♀	4-5	15 ♂, 2♀
Hind wing – transversal veins (N)	R-M	0-3	1♂, 1♀	1-4	17 ♂, 2♀
	Cu-M	0-1	1♂, 1♀	0-1	17 ♂, 2♀
Genitalia (µm)	x	63	1 ♂	53-83	18♂
	y	52	1 ♂	42-67	18♂
	z	101	1 ♂	91-142	18♂
	F1	325	1 ♂	302-392	18♂
	F2	280	1 ♂	261-327	15♂
	F3	233	1 ♂	206-272	15♂

Table 2. Numerical characters of *H. carpatica* and *H. confusa* imagines (for abbreviations of the analyzed characters see Fig. 6)

2.4 Discussion

The present study confirms that the *Habroleptoides* specimens collected at the type locality of *H. carpatica* cannot be separated from *H. confusa* neither in the larval nor in the imaginal stages. The morphological traits suggested by the original description of *H. carpatica* are either unreliable or non-existent. Thus the examination of the mouthparts of *Habroleptoides* larvae and larval exuviae originating from Sinaia clearly indicated that the maxillary and labial palps are composed of three segments each, instead of the two described, which is obviously based on a misinterpretation by Bogoescu & Crăsnaru (1930). The measurements made on the male genitalia also disagree with the findings of Bogoescu (1958). Even though the third segment of the forceps turned to be shorter than the middle one in all the analyzed specimens, including *H. confusa*, the difference between the segments of the two species was not as pronounced as it was suggested. Likewise the internal projection of the forceps was about half the width of the basal segment right above the process in all measured males, instead of being about the same width in *H. carpatica* and 3 times thinner in *H. confusa*. Slight deflections from the vertical position of the process during the measurements could explain the differences recorded by Bogoescu (1958). Both species are also supposed to differ by the number of intercalary veins present in the hind wings, but our results clearly deny the reliability of this diagnostic character (Fig. 6 D, E). The variation of hind wing venation in *H. confusa* had been previously documented by Biancheri (1956 [sub *H. modesta*]) and Sartori & Jacob (1986). The egg chorionic structures have been studied by Gaino et al. (1993), and both species present the same chorionic structure. The present analysis, including additional larval and imaginal structures as well (Tables 1, 2) did not reveal the existence of any species-specific morphological trait[1].

In conclusion the analyzed morphological and numerical characteristics clearly revealed that the diagnostic characters suggested by Bogoescu & Crăsnaru (1930) and Bogoescu (1958) for *H. carpatica* fit into the natural variation of *H. confusa*. Therefore both taxa should be considered as synonyms[2].

Following the principle of priority article 23.1 (ICZN, 1999), the valid name of the species should be *H. carpatica*.[3] Taking in consideration that the requirements of article 23.9.1.1 are violated, as the senior synonym has been used as a valid name, though only on a few occasions, and conditions requested for article 23.9.1.2 are met, the same species appears under the name *H. confusa* in more than 150 publications by more than 60 authors (Appendix 3), including the European legislation and projects like AQEM (www.aqem.de), STAR (www. eu-star.at) and others[4], therefore the usage of the name *H. carpatica* would create confusions, Váncsa & Sartori (2013) proposed the maintenance of prevailing usage. In December 2015 the International Comission of Zoological Nomenclature accepted the proposal and precedence was given over *H. carpatica* (ICZN, 2015).

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[1] Váncsa et al., 2013

[2] Váncsa et al., 2013; Váncsa & Sartori, 2013

[3] Váncsa et al., 2013; Váncsa & Sartori, 2013

[4] Váncsa & Sartori, 2013

3 Adaptation of a simple technique for rearing lotic mayfly (Insecta: Ephemeroptera) larvae: the reversed-funnel technique

3.1 Introduction[1]

Though aquatic insects represent only 5.75% of the total number of animal species recognized globally (Balian et al., 2008), they are well represented in the scientific literature and in many of them field surveys are completed or replaced by laboratory investigations. From the wide range of applications realized with the aid of a vast assortment of rearing facilities varying from simple eqippments (Rosillon, 1988; Finlay, 2001) till model streams (Cobo, 2005) it is highlighted the importance of reared material in the description of new species (Haybach, 2005; Sroka & Godunko, 2012), and realization of more complete faunistic inventories (Bonada et al., 2004) or the role of ex situ outdoor or indoor surveys in understanding important aspects in the biology, ethology and ecology of a high number of aquatic taxa (Elpers & Tomka, 1995; Lieske & Zwick, 2008). Along with modeling studies (Domisch et al., 2013; Sauer et al., 2011), the results of rearing experiments (McKee & Atkinson, 2000; Rosillon, 1988) combined with long term field observations (Durance & Ormerod, 2007) offer a realiable overview on the impact of projected global climate change upon model species and aquatic ecosystems.

The purpose of the study is to introduce and adapt an old water filtration and aeration technique used by aquarists (Horn & Zsilinszky, 2005; Józsa, 1958) into the macrozoobenthos-related research and to test the efficiency of this method on lotic mayflies.

3.2 Materials and methods

3.2.1 Description of the rearing facility

The rearing chambers (Fig. 12) were constructed from 5 l plastic soft drink recipients with plain bottom, cut at the level where the sides start to converge to the lid. A plastic funnel with a small hole on the conical surface and 4 - 5 triangular cuttings located at the edge of the mouth-like part was placed into each rearing chamber with the wider side down and fixed with washed, coarse sand. The aquarium tubing with 0.4 mm interior diameter was introduced into the funnel throughout the hole located at the conical surface of it. The other side of the tubing was

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[1] Váncsa et al., 2016

connected to an aquarium air pump. Stream water was filled into the rearing chambers till it totally covered the funnel. Each chamber was equipped with a dried *Rubus idaeus* stick and 1 or 2 small and thin pieces of Styrofoam to facilitate emergence of specimens. In order to prevent the escape of the freshly emerged subimagines, chambers were covered with a silky white material.

By aeration of this facility recirculation and mechanical filtration of water is also realized. Thus the bubbles entering the funnel from the airline pump water through the funnel up and this current pulls water from the rearing chamber down through the sand, which filters it. Water enters the funnel via the triangular cuttings while the impurities from it are trapped in the sand.

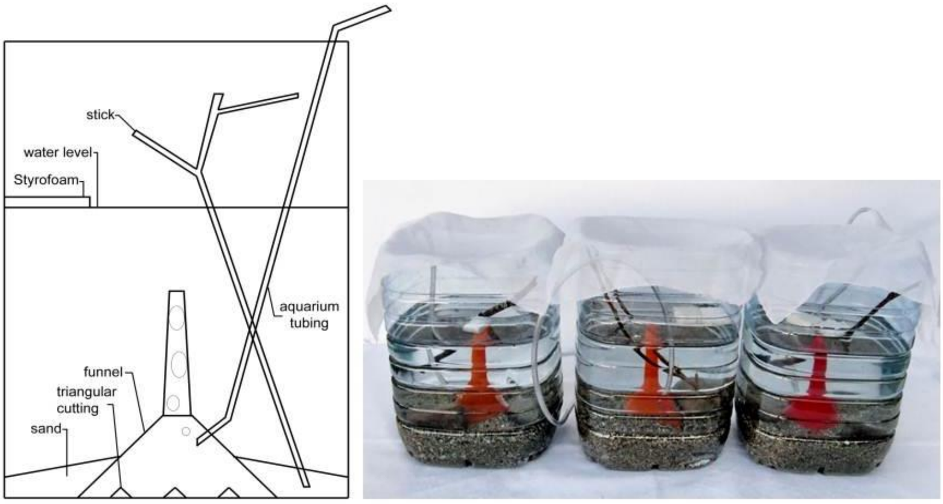

Fig. 7. The rearing facility

3.2.2 Evaluation of the rearing facility[1]

The efficiency of the rearing facility was tested on lotic mayfly nymphs in 2008 and 2009. In 2009 the investigation was completed with young instars of *Ecdyonurus* sp. Specimens were collected from 3 creeks of the Eastern Carpathians: Aita (45.974581 N 25.626739 E, 530 m a.s.l.), Cormoș (46.175967 N 25.627689 E, 554 m a.s.l.) and Ozunca (46.093814 N 25.722458 E, 533 m a.s.l.). Biofilm covered stones were also collected as the algae from it constituted the main food source of the larvae. Chamberes were filled with stream water.

A total of 14 chambers were connected to a Boyu S-2000 air pump.

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[1] Váncsa et al., 2016

A maximum of five last instar mayfly individual or just one young *Ecdyonurus* larva was introduced into each rearing chamber. The evaluation was realized with 341 undamaged nymphs and 45 intact inmature *Ecdyonurus* larvae. In case of young larvae water was not replaced, but completed due to the occurring evaporation. Food consisting of biofilm covering the stones was replaced at monthly intervals.

All chambers were checked daily: dead specimens, exuviae were removed; subimagines were transferred into small plastic salt cellars equipped with shoots of wandering jew (*Tradescantia fluminensis*) for ensuring the appropriate humidity for the final moult. Dead specimens, larval and subimaginal exuviae and the imagines were introduced into Eppendorf tubes filled with 70% ethanol.

Slides were realized according to the methods described in the previous chapter. Specimens (nymphal exuviae and imagines) were identified with the aid of the specific literature (Bauernfeind & Humpesch, 2001; Haybach, 1999; Bauernfeind & Soldán, 2012).

Emergence success (% of subimagines (SI) emerging from reared specimens), rearing success (% of imagines (I) in relation to the reared specimens) and mortality in the larval and subimaginal stage were calculated.

3.3 Rezults

3.3.1 Rearing of mayfly nymphs

Though the last instar mayfly nymphs spent up to 14 days in aquatic environment, the majority of them emerged within 4-5 days after the introduction into the rearing devices. The reared individuals were distributed into 24 taxonomic units including 19 species (Table 3). 78% of the mayfly nymphs emerged and due to the low subimaginal mortality, the rearing success also attained high levels (68.57%). Species mortality in the subimaginal stage was considerably lower than the nymphal mortality. The rearing success of several species reached the maximum level (Fig. 8).

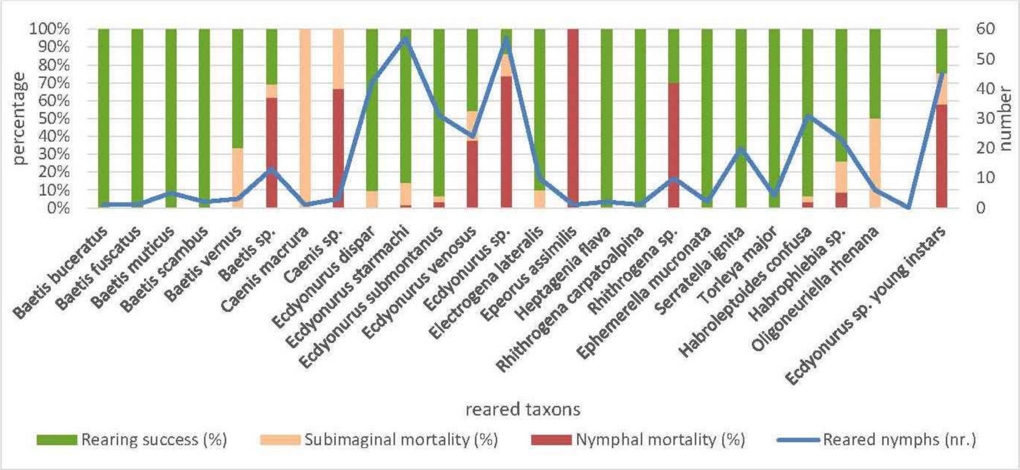

Fig. 8. Rearing success, larval and subimaginal mortality of the reared young and last instar larvae

3.3.2 Rearing of young instars of *Ecdyonurus*

Young instar *Ecdyonurus* larvae survived up to 130 days in the designed chambers and including the nymphal moult, cast their skins 7 times the most. The high mortality in the aquatic and first alar stage implied a modest rearing success (Fig. 8).

3.4 Discussion

Laboratory rearing of aquatic insects allows researchers to regulate environmental variables that are rather difficult to control in the field. The adaptation of an old water filtration technique used by aquarists (Horn & Zsilinszky, 2005; Józsa, 1958) to the requirements of aquatic insects has furter advantages: the construction of it is easy, cheap and time effective, requires modest maintenance actions, is available both for scientists and enthusiast amateurs without requiring well-equipped laboratories. The reversed-funnel technique yields promising results especially for rearing nymphs. In their case both the emergence and rearing success fluctuated around 70% highly exceeding the one experienced by Finlay (2001). Though the nymphs were collected from just 3 creeks, the emergence and rearing success varied between the species. Several species were characterized by maximal rearing success, but, with the exception of *Serratella ignita*, an eurytopic species colonizing the rhithron of almost all types of running water (Bauernfeind & Soldán, 2012), the results are not reliable due to the low number of specimens introduced into the experiment. The reason of the recorded variation in emergence succes, subimaginal mortality and rearing success of the mayfly species implied in our experiment lies behind the discrepancies in the biology and ecology of the species.[1] This is sustained by a recent study (Rowsey et al., 2015). Thus the good results obtained in case of *Serratella ignita* or *Habroleptoides confusa* are due to their euritop distribution, while *Oligoneuriella rhenana*, which colonizes the rhithron and epipotamon of fast flowing, well oxigenated rivers (Bauernfeind & Soldán, 2012; Jansen et al., 2000) transformed to subimagines in a proportion of just 50 %. Even so, this result is highly surprising in a condition when the majority of nymphs died during the nearly one hour transportation time.

Contrary to mayfly nymphs, the rearing of young instars of *Ecdyonurus* species showed modest results. Their mortality in the aquatic stage exceeded more than 2 times the mortality of last instar *Ecdyonurus* nymphs'. As in similar systems used in aquaculture, wastes and accumulated organic matter were reported to be trapped in the sediment during water recirculation (Horn & Zsilinszky, 2005), where a microbial community was established (Józsa, 1958), we presumed that sediment filtration and biodegradation occurring in the substrate can substitute water replacement, in consequence waste removal and can ensure the necessary amount of nutrients for periphyton growth. Previous studies also sustain indirectly the necessity of food supply by confirming that *Ecdyonurus* species, like other members of the feeding guild reduce periphyton biomass and change community composition by sweeping with their brush-like mouthparts the more accessible algal physiognomies (Wellnitz & Ward, 1998; Wellnitz & Ward, 2000).[2].

4 Evaluation of the impact of global warming on a model species, *Habroleptoides confusa*

4.1 Introduction

There is growing evidence that freshwaters are highly sensitive to global climate change (Carpenter, Stanley & Vander Zanden, 2011; Sala et al., 2000). Among them, streams and rivers seem to be the most threatened systems (Domisch et al., 2013) due to the combined effects of climate change (warming, increased frequency of droughts and floods) and multiple, on-going anthropogenic stressors (habitat loss, habitat fragmentation and degradation, modification of flow, overexploitation, pollution, introduction of non-native species) (Revenga et al., 2000; Allan et al., 2005). This is confirmed by recent studies, which highlight that over

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[1] Váncsa et al., 2016

[2] Váncsa et al., 2016

the past decades freshwater biodiversity declined far greater than either terrestrial or marine one (Carpenter et al., 2011; WWF, 2012) and is likely to keep the leading position in the 21st century as well, with a biodiversity loss falling within the range of estimates for tropical rainforest communities (Ricciardi & Rasmussen, 1999).

Nevertheless, similarly to terrestrial species, both the observed and projected reactions of freshwater populations and species can be grouped in four major categories: adaptation, demographic change, emigration/immigration and local extinction of taxa (Brittain, 2008). The severity of responses depends not only from the intensity of climate change related events, frequency, duration of future extreme events, but mostly on the ecology of species. Thus the predicted temperature increase of up to 5°C by the end of the 21st century (IPCC, 2007) will particulary influence cold- or warm-adapted species with limited distribution (Domisch et al., 2013; Conti et al., 2014), (Domisch et al., 2013; Conti et al., 2014; Brown et al., 2007), endemic species or highly specialized taxa (Hering et al., 2009), which colonize alpine or montane habitats (Muhlfeld et al., 2011) or are restricted to spring-zones of the watercourses (Hering et al., 2009), have short embryogenesis, synchronous egg hatching and larval development, large larval size and fixed, univoltine life cycle (Brittain, 2008).

The aim of the study is to investigate the effects of rising temperature on a model species, *Habroleptoides confusa* (Ephemeroptera) with the aid of long-term laboratory experiment and to analyze the size and fecundity of field-collected *H. confusa* specimens.

4.2 Materials and methods

4.2.1 Laboratory rearing experiment

The impact of global climate change upon aquatic macroinvertebrates was investigated at the Department of Limnology and Conservation (Gelnhausen) of the Senckenberg Research Institute. The mayfly *Habroleptoides confusa* was chosen as model organism.

The species has a univoltine winter life cycle (Clifford, 1982; Pleskot, 1953; Petrovici, 2009; Hefti & Tomka, 1990). The collector-gatherer detritivor larvae (Pleskot, 1953), colonize the crenon, rhithron of streams and rivers situated at 200-1200 m a.s.l. with warmer oligo- β-mezosaprob (Bauernfeind & Soldán, 2012) waters, with moderate flow (Bogoescu, 1932b) with stony gravel substrat (Pleskot, 1953).

The model species was reared on 3 temperature regimes, the A2 and B2 storyline, projecting a 4°C, respectively 2°C global warming by the end of this century (IPCC, 2007) and the control temperature. The reversed funnel facility (Váncsa et al., 2016) was used for laboratory rearing of organisms. Mayflies were reared in 1 l glass jars with the interior diameter of 9 cm equipped with polypropylene funnels connected to a MEDO 1.6 (28 l/min) and MEDO 2.7 (45 l/min) air pump through aquarium tubing.

Each temperature regime consisted of 40 replicates (chambers), which were introduced in boxes filled with water (Fig. 9). The water from the boxes of each treatment was circulated with HQB-4900 submercible pump (4500 l/h) through a Hailea HC-500A chiller (experimental temperatures) and HC-1000A chiller (control treatment) and ensured the desired temperature for the main treatments. Water temperature from each shelf was recorded every two hours by loggers.

Fig. 9 Rearing facility

In 2010 September young instars of *H. confusa* were sampled from Elmbach and transported to the laboratory in a refrigerator bag, in plastic bottles filled with water. Additionally stream conditioned alder leaves (*Alnus sp.*) were collected, as the detritus from its surface constituted the main food source for the larvae. Stream water was also taken to the laboratory to ensure the appropriate environment for the larvae. Each chamber received 2 larvae, an alder leaf, a *Rubus idaeus* stick and 2 pieces of Styrofoam to facilitate the transformation of nymphs to subimagines. Each chamber was covered with white dense mesh (net) for prevention of escape of the subimagines.

After the acclimatization period, temperatures of the experimental treatments were raised 1°C each day till it reached the desired value. During the experimet, temperatures were adjusted to the rearing protocol in order to follow the temperature changes observed in the field. Chambers received natural daylight, therefore the light-dark cycle was similar to field conditions.

Food consisting of one entire stream conditioned alder leaf was replaced every second week. Evaporation of water requested completion of it. Total replacement of water occurred 2 times during the experiment and it was followed by a partial water replacement.

Chambers were verified daily and the shed exuviae were removed and preserved in Eppendorf tubes filled with 70% ethanol. Deaths were also recorded and dead specimens preserved. The emerged subimagines were removed and placed in plastic salt cellars where the final moult occurred. Humidity from the cellars were ensured by shoots of wandering jaw. Imagines and subimaginal exuvia were also preserved in 70% ethanol.

4.2.2 Field observation

Between the 25th and last day of the rearing experiment *H. confusa* larvae were collected from Elmbach on 11 occasions and preserved in 70% ethanol. Nymphs were also reared to imagines. Size and fecundity of field preserved and reared specimens represented the investigated parameters.

Aditionally fecundity and wing length of a total of 93 *H. confusa* individuals from the collection of the Department of Limnology and Conservation (Gelnhausen) of the Senckenberg Research Institute was identified. The analyzed material was collected in Groβe Steinau (Harz) (Schindehütte, 2000).

4.2.3 Data preparation

Following the principle applied in the evaluation of the reversed funnel technique, emergence success, rearing success, larval and subimaginal mortality of *H. confusa* reared under different temperature regimes were calculated.

Size of the specimens was determined to the nearest 0.01 µ with the aid of the Cell^A Software. In case of exuviae and larvae collected from Elmbach body length, mesonotum & wing pad length, last sternite width were measured. For imagines body length and first wing length were taken into account. Additionaly, in case of female imagines, with the exception of specimens collected in last larval instar and reared in the laboratory, the sternite width was also registered. In order to determine the fecundity of specimens reared on different treatments or developed in field (Elmbach and Groβe Steinau), eggs were removed from the abdomen of the female imagines or subimagines and counted.

Larval developmental time, moulting number and moulting frequency were determined for each individual.

4.2.4 Statistical analysis

The statistical analysis was performed with R version 3.2.2 (R Development Core Team, 2008) using the next packages: matrixStats (Bengtsson, 2015), dunn.test (Dinno, 2016), ggplot2 (Wickham, 2009), mgcv (Wood, 2011), multcomp (Hothorn et al., 2008).

For the determination of control temperature regime the database of the Senckenberg Reasearch Institute was analyzed and temperature data were pooled off for 23 sites of 20 watercourses, where the species *Habroleptoides confusa* occurred. Based on this recordings, a naturally fluctuating theoretical control temperature regime was predicted with the aid of the LOESS regression model with 0.15 span. The predicted theoretical control temperature and realized control, middle and high temperature recordings were plotted with the aid of a linear model with quadratic term. Emergence success, moulting number and moulting frequency on different treatments was compared with Kruskall-Wallis and Dunn’s test with bonferroni correction. As larval sheds might have remained undetected, the outliers were removed from the dataset regarding moulting frequency of specimens.

Growth dynamics of the larvae was analyzed. Based on the measurements and recorded moulting date, the size of every single reared specimen was determined for each day till emergence. From these data mean daily body length, mean daily mesonotum & wing pad length and mean daily last sternite width was calculated for each treatment and plotted. Larvae with just one measurement were omitted from the analysis.

Size of the specimens reared on different treatments were compared on two different developmental stages: nymphal and imaginal stage. Multiple linear regression models were designed to investigate whether treatment, aquatic developmental time, gender and the interaction of them define nymphal body length, mesonotum & wing pad length, last sternite width and imaginal body and wing length. Model selection followed the principle of parsimony and was done by backward elimination of non-significant covariates, covariate-interactions. Best fit models were validated by graphical residual analysis. Significant treatment effects were further investigated with the glht function of the multcomp package. Sternite widths of female imagines and fecundity data were compared with two-way ANOVA using treatment, developmental time and the interaction of them as independent variables. Significant main effects were further investigated with Tukey-test. In order to identify the impact of developmental time on size or fecundity of the specimens, general additive models (GAM) were developed using smooth terms represented by penalized cubic regression splines. The relationship between fecundity and body size was also analysed with the aid of GAM models.

Size and fecundity of the specimens reared under control temperature regime was compared with the size of the specimen sampled in Elmbach in larval stage or reared to imagines from nymphs by analysis of variance. The dataset resulting from the analysis of specimens developed in Groβe Steinau was also included in the statistical analysis.

4.3 Results

4.3.1 Rearing temperature

The analysis of the temperature data recorded by loggers clearly revealed a difference between the theoretical (predicted) and control temperature regime especially during winter, but also highlights that the experimental temperatures were successfully adjusted to it (Fig. 10). Thus mean water temperature in control treatment reached 8.61 ºC instead of 7.5 ºC. Middle mean temperatures was with only 1.37 ºC higher, while at the highest scenario average temperatures were with 3.43 ºC above the control one.

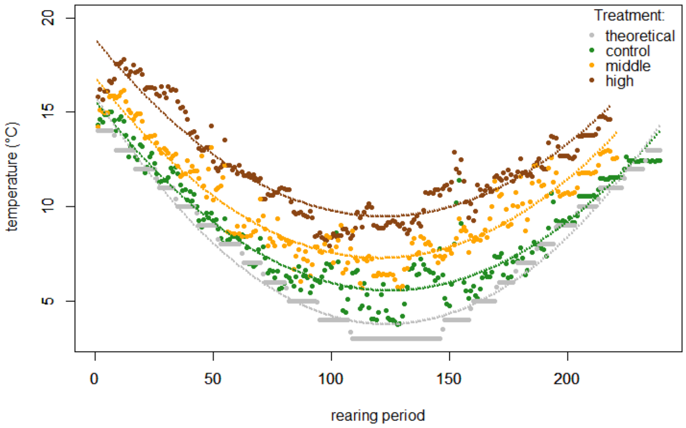

Fig. 10. Theoretical control and realized mean daily temperature on treatments

4.3.2 Emergence and rearing success

From the 240 young instar *H. confusa* larvae distributed evenly among the three treatments a total of 42 deaths were recorded during the aquatic developmental stage. Greatest larval mortality was recorded at the middle temperature treatment (21.25 %). Both the emergence and rearing success attained high values on all scenarios (Table 3). According to the statistical analysis, the difference between the emergence success of mayflies reared on different treatments is not significant (Kruskal-Wallis: chi-sq> 1.28, p=0.53)

Treatment	Nr.	%			
	Reared larvae	Larval mortality	SI mortality	Emergence success	Rearing success
Control	80	15.00	10.29	85.00	76.25
Middle	80	21.25	12.70	78.75	68.75
High	80	16.25	11.94	83.75	73.75
Total	240	17.50	11.62	82.50	72.92

Table 3. Emergence and rearing success of *H. confusa* larvae

4.3.2.1 *Larval developmental time (=emergence day)*

Depending on treatment, mayfly larvae spent up to 239 days in laboratory conditions, excluding the accommodation period. According to the statistical analysis of our data (TukeyHSD post-hoc test run on ANOVA), rearing temperature significantly shortened the aquatic stage of *Habroleptoides* larvae (Fig. 11). The difference was significant between all treatments (p<0.001). Relative to the control treatment, the 1.37ºC and 3.43ºC mean temperature increase shortened the aquatic life stage on average with 5 (middle treatment) and 22.1 days (high).

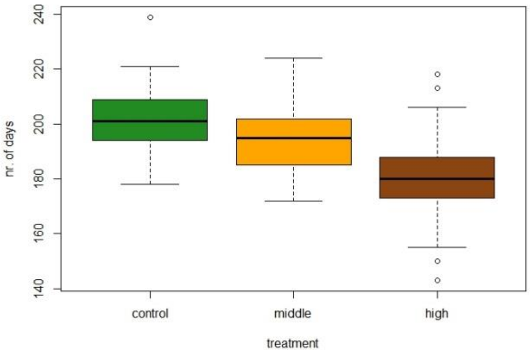

Fig. 11. Duration of aquatic stage of *H. confusa* on treatments

4.3.2.2 *Moulting number and moulting frequency*

According to the Kruskall-Wallis test rearing temperature had a significant impact both on moulting number and moulting frequency (Fig. 12, Fig. 13). Thus at high treatment scenario, specimens moulted more times and more frequent than those reared at control temperature (Dunn-test: $p<0.001$) or middle treatment (Dunn–test: $p<0.001$). The difference between moulting number and moulting frequency of specimens developing under control and middle treatments was not significant. Thus 3.43 °C above optimal increased maximal molting number with 2 in more than 21 % of the specimens emerging after the 8th or 9th exuvial shed, whereas at control and middle treatment emergence occurred after the 7th shed, the most. Relative to control treatment, mean moulting frequency shortened with 4.98 days in middle treatment and more than 11 days in case of specimens reared 3.43 °C above control temperatures

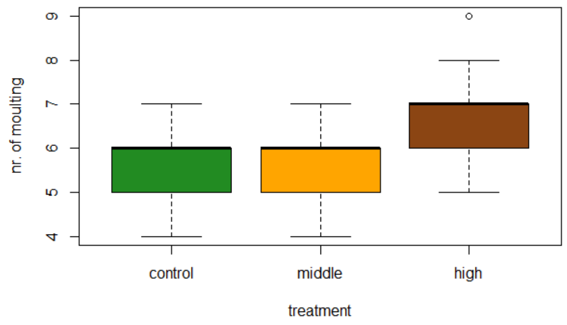

Fig. 12. Moulting number of *H. confusa* on treatments

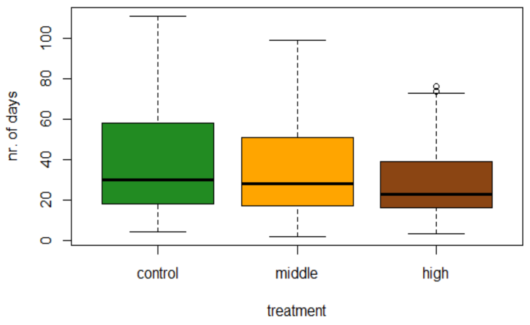

Fig. 13. Moulting frequency of *H. confusa* on treatments

4.3.2.3 *Growth dynamics of larvae*

The calculation and analysis of mean daily body sizes clearly revealed, that growth dynamics varied not only according to the measured body parameter, but it was also influenced by the temperature regime the larvae were reared on. As long as the elongation of mesonotum & wing pad lengths during the aquatic developmental stage was continuous regardless of the seasonally fluctuating temperature (Fig. 15), the increase of body length (Fig. 16) and last sternite width (Fig. 17) was characterized by discontinuity and considerable differences between treatments. Nevertheless both mean body lengths and mean sternite widths strongly and in some cases gradually increased during autumn and moderate during spring, but growth was ceased for different periods. For instance mean body length remained unchanged from the middle of December till the end of January at middle treatment, in January at control scenario, and only last week of December at high treatment (Fig. 15).

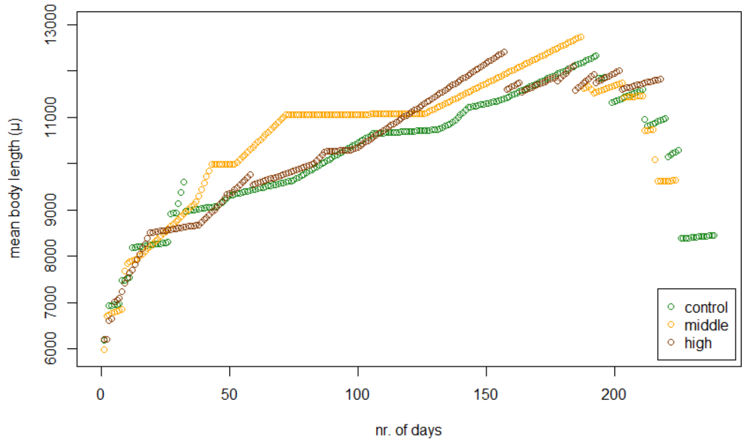

Fig. 14. Growth dynamics of mean body length of *H. confusa* larvae on treatments

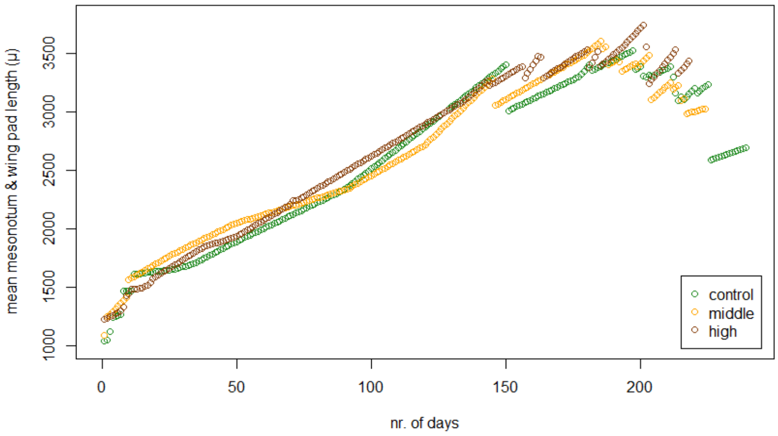

Fig. 15. Growth dynamics of mean mesonotum & wing pad length of *H. confusa* larvae on treatments

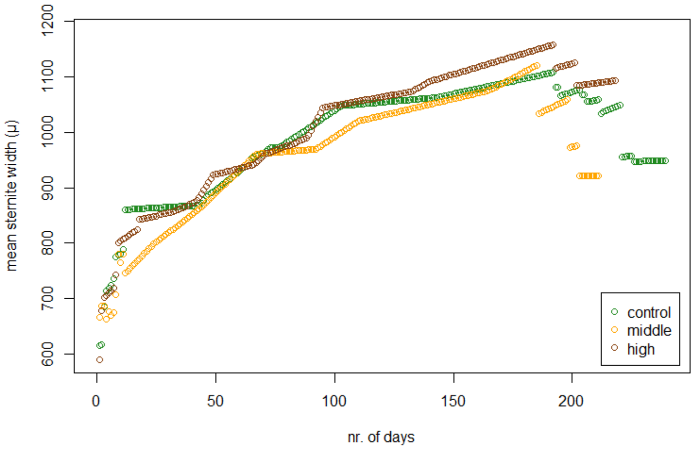

Fig. 16. Growth dynamics of mean last sternite width of *H. confusa* larvae on treatments

4.3.2.4 *Size of nymphal exuviae and imagines*

According to the multifactorial linear models nymphal body size is influenced by a series of factors: temperature, gender, length of aquatic developmental stage and the interaction of these variables. High treatment specimens had significantly longer bodies and mesonotum & wing pads, wider last sternites relative to control and middle treatment specimens. The difference between body size of specimens developing under control and middle temperatures were not significant (Fig. 17 -19).

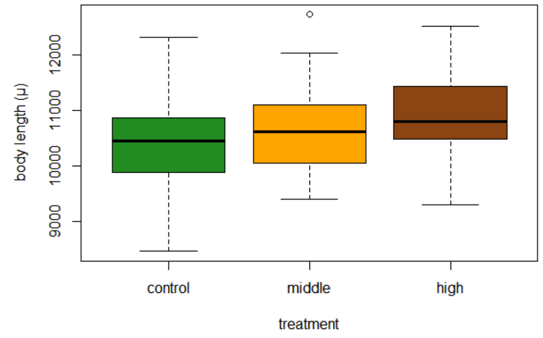

Fig. 17. Body length (R2=0.46, p<0.001; h-c: t= -3.36, p=0.003; h-m: t= 3.34, p=0.003, c-m: t= 3.34, p=0.003)

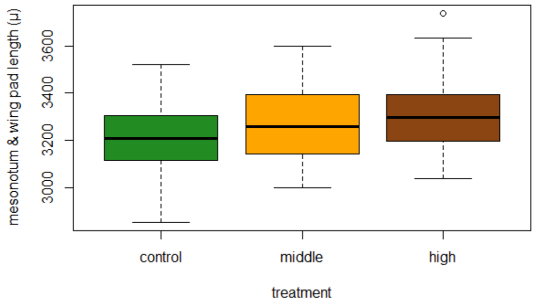

Fig. 18. Mesonotum & wing pad length (R2=0.305, p<0.001; h-c: t= -4.09, p<0.001; h-m: t=0.77, p<0.001; m-c: t=0.77, p=0.72)

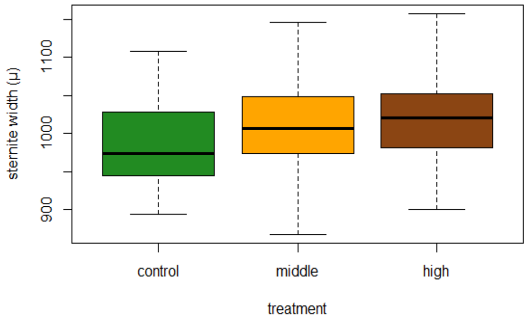

Fig. 19. Last sternite width (R2=0.406, p<0.001; h-c: t=-4.63, p<0.001; h-m: t=4.89, p<0.001; m-c: t=0.18, p=098)

The size of imagines was not influenced by the rearing temperature.

4.3.3 Correlation between body size and duration of aquatic stage

According to the GAM models there is positive correlation between body size of specimens reared at high treatment and developmental time and negative correlation in case of middle and control treatments (Fig. 20 – 22).

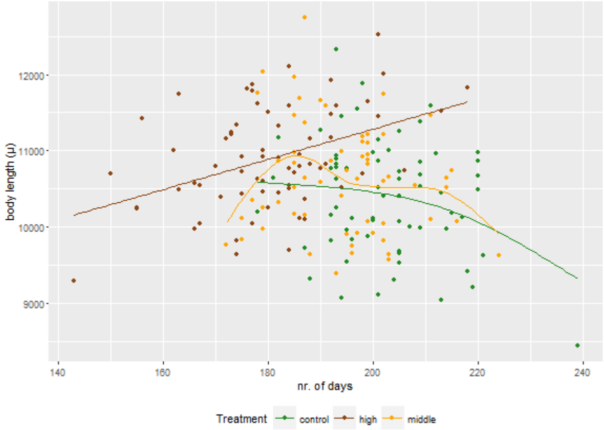

Fig. 20. Change of body length of nymphs according to the developmental time (control: F=2.22, p=0.095; middle: F=1.57, p=0.203; high: 17.6%, F=13.84, p<0.001)

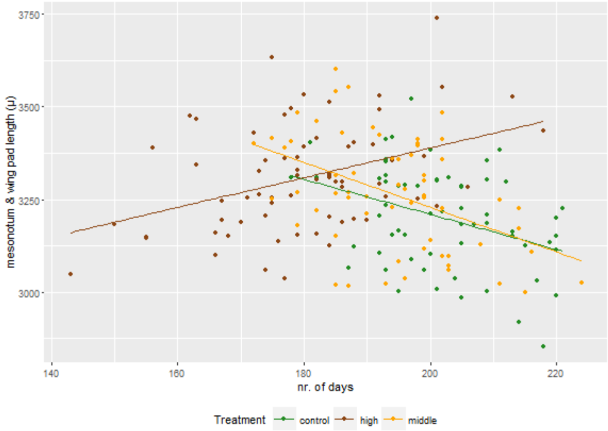

Fig. 21. Change of mesonotum & wing pad length of female and male nymphs according to the developmental time (control: F=9.07, p=0.003; middle: F=14.87, p<0.001; high: F=11.73, p=0.001)

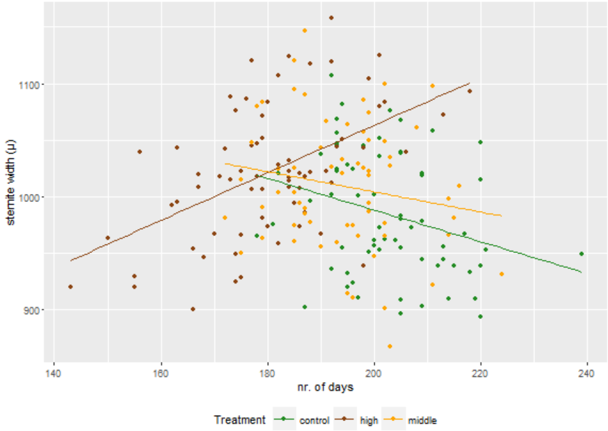

Fig. 22. Change of sternite width of nymphs according to the developmental time (control: F=5.89, p=0.018; middle: F=1.92, p=0.17; high: F=23.06, p<0.001)

The correlation between body size of imagines and developmental time is not so unequivocal in case of imagines (Fig. 23 – 25).

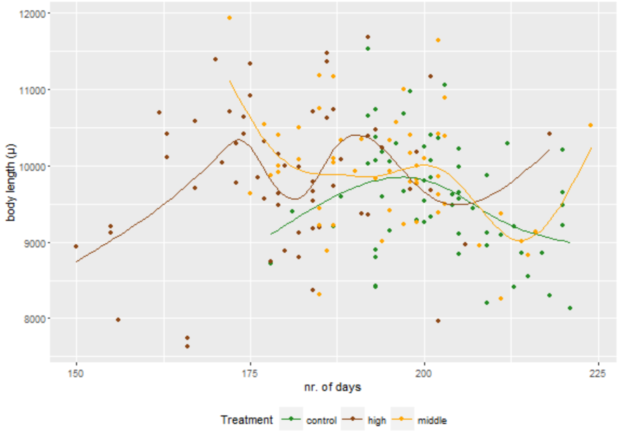

Fig. 23. Change of imaginal body length according to the developmental time (control: F=3.3, p=0.018; middle: F=2.66, p=0.02; high: F=1.63, p=0.12)

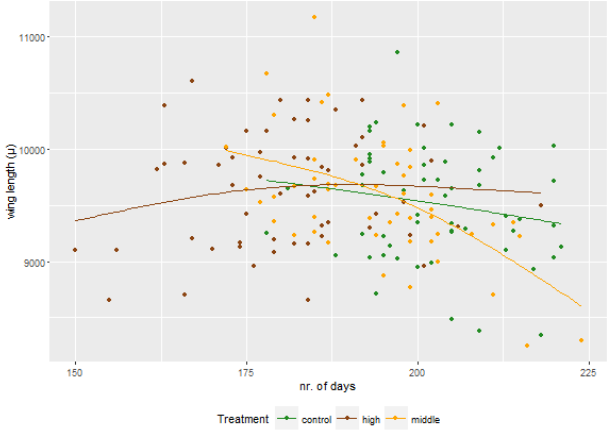

Fig. 24. Change of imaginal wing length according to the developmental time (control: F=1.48, p=0.2; middle: F=9.58, p<0.001; high: F=0.82, p=0.44)

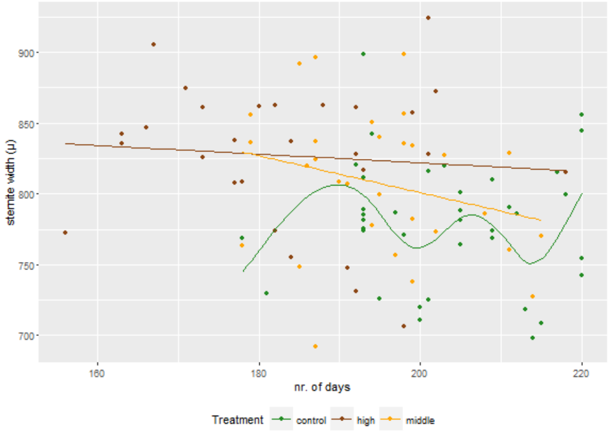

Fig. 25. Change of sternite width of female imagines according to the developmental time (control: F=1.33, p=0.27; middle: F=2.058, p=0.16; high: F=0.2, p=0.66)

4.3.4 Fecundity

The number of eggs varied between broad scales, the less fertile specimen having less than 358 eggs (high treatment) while the highest record was of 3386 (middle treatment). The two-way ANOVA revealed that fertility of specimens significantly depends on aquatic developmental time (F=20.72, p<0.001) and not on treatment (F=0.6, p=0.55) or interaction of treatment and emergence (F=0.79, p=0.46). Nevertheless an increasing tendency in fecundity was detected along with the increase of rearing temperature. Thus relative to control treatment, the fecundity of specimens reared at middle scenario increased with 106, while those reared at high treatment with 137 (Fig. 26).

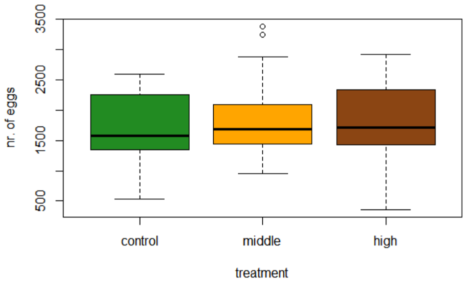

Fig. 26. Fecundity of *H. confusa* on treatments

The GAM model revealed a significant negative correlation on all treatments between fecundity and developmental time (Fig. 27) and positive correlation between imaginal wing length and fecundity (Fig. 28).

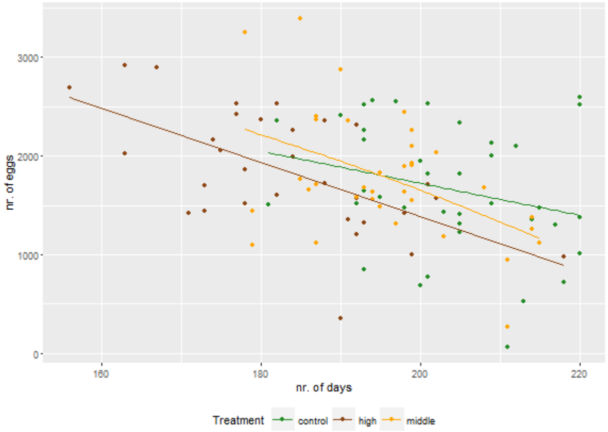

Fig. 27. Change of fecundity according to the developmental time (control: F=2.09, p=0.16; middle: F=6.55, p=0.015; high: F=16.83, p<0.001)

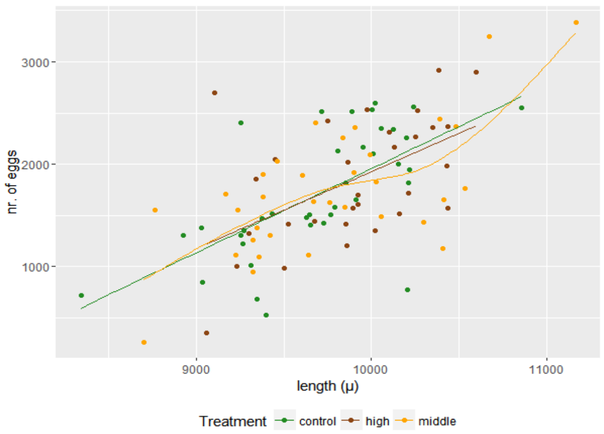

Fig. 28. Change of fecundity according to the imaginal wing length (control F=7.54, p<0.001; middle: F=24.32, p<0.001; high: F=2.65, p=0.03)

4.3.5 Emergence temperature

The average temperature on the day when the first specimen emerged was 7 ºC on control treatment, 8 ºC on middle treatment and 10 ºC on high scenario.

4.3.6 Variation of field collected specimens relative to specimens developing in control treatment

According to the statistical analysis field collected specimens were significantly smaller than those reared in laboratory on control temperatures (p<0.001). As long as the difference between the fecundity of specimens reared to imagines from last instar nymphs sampled in Elmbach and control treatment specimens was not significant (ANOVA: p=0.45), the individuals collected in Große Steinau were significantly less fecund (ANOVA: p<0.001) (Fig. 29).

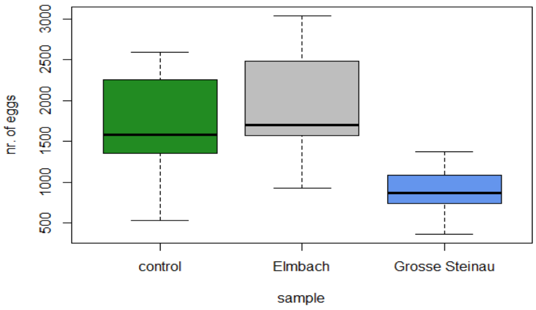

Fig. 29. Fecundity of mayflies sampled in Elmbach and Große Steinau and in control treatment

4.4 Discussion

The rearing experiment aimed to identify and characterize the effect of a 2 ºC (B2 scenario) and 4 ºC (A2 scenario) temperature increase projected by the end of this century (IPCC, 2007) on a model species, the mayfly *H. confusa*. The analysis of the data recorded by loggers clearly revealed, that the fluctuating control temperature regime predicted by the LOESS regression model (mean 7.5 ºC) could not be sustained, but the middle and high scenarios were successfully adjusted. Thus mean middle and high temperature regimes were just 1.37 ºC and 3.43 ºC higher than the control one (mean 8.61ºC) and in the last, Fifth Assessment Report (IPCC, 2013) correspond to the projections of RCP 4.5 scenario by year 2060, respectively RCP 8.5 by 2100 The increase of control rearing temperatures (consequently the middle and high temperatures) can be explained by the lack of a climate control unit from the laboratory, the technical characteristics of the coolers, and the large surface of the shelves, which permitted temperature exchange.

Species exhibit diverse strategies when adjusting to water temperature (Li et al., 2011). According to our experiment adaptation of *H. confusa* to warmer habitat conditions was realized mostly by phenological shifts, but morphological changes were also detected. Earlier differentiation of males, increased moulting frequency, advanced emergence, changes in growth dynamics and increased body size in nymphal stage are the main observed responses. Elevated rearing temperatures had hardly any impact on survival in aquatic stage, imaginal body size and fecundity of mayflies.

A high number of studies specify temperature as the main driver of larval growth (Huryn, 1996; Knispel, Sartori & Brittain, 2006), which is possible due to the moulting of the chitinized exoscheleton after a critical size is attained (Merzendorfer & Zimoch, 2003; Soluk, 1990). In consequence moulting frequency and moulting number is a good indicator of the impact of temperature on growth of specimens. In our experiment both variables increased at high treatment scenario. Moulting is stresful and energetically extremely costly (Camp, Funk & Buchwalter, 2014; Sweeney & Vannote, 1981), when along with the exoskeleton, the linings of the tracheae, foregut and midgut are also discarded and replaced (Speight et al., 2008). during this period respiration is ceased (Malmqvist & Sjöström, 1980; Camp et al., 2014) and become highly vulnerable against predators (Soluk, 1990). On the basis of the above mentioned data, it is highly possible, that due to the increased frequency of moulting number and moulting frequency, elevated rearing temperatures will result in higher mortality of *H. confusa* larvae and the population size will decrease. Nevertheless the incresed fecundity comepansate the negative effect of increased moulting number and moulting frequency.

According to our observations, regardless of the treatment *H. confusa* larvae moulted more often during autumn and spring and their growth continued even during winter as it was documented by previous studies as well (González et al., 2003; Kukula, 1995; Sowa, 1975), though at lower rates as proved by scarce exuvial sheddings. But growth of the measured body parts was not proportionate and with the exception of mesonotum & wing pad length, was highly affected by treatment. Especially winter temperatures caused stepwise increase of mean body length and mean sternite width of mayflies reared on different scenarios for time intervals varying both in length and in moment of onset. Taking in consideration the recorded growth patterns we are highly confident that growth regulation is a complex process and is a result of the impact of environmental factors upon endogeneous mechanisms. Thus growth of *H. confusa* specimens was ceased after probably attaining a temperature dependent critical size. The growth dynamics of the measured variables clearly revealed that the reared species is capable of rather continuous growth even during winter provided optimal temperatures are ensured, but higher temperatures modify the growth pattern due to faster development of larvae proved by increased frequency of moultings.

Body size and fecundity of specimens is influenced by a series of factors, such as temperature and developmental time, gender and the interaction of them. Thus it was observed an increase of all the measured nymphal parameters if temperature increase was higher than 2 °C. These results contradict Bergman's rule, according which body size shoul decrease if temperatures are higher than optimal (Atkinson, 1995; Atkinson, 1994). Our observations are sustained by a high number of exceptions (Atkinson, 1995; Watanabe & Kuroda, 1985).

As mayflies and other aquatic insects have both aquatic and aerial life stages, the latter being required for reproduction, their existence depends on environmental conditions of both of their habitats and the timing of emergence is crucial for the survival of the species (Nebeker, 1971). In our experiment higher temperatures significantly reduced the developmental time of *H. confusa* larvae as it was previously observed by Pleskot (1953).

Previous studies confirm that emergence is initiated by a minimum water temperature (Brittain, 1979; Humpesch, 1971). Taking in consideration that at experimental treatments the emergence temperature increased, most surely the onset of emergence is influenced by other factors as well like light conditions. This is sustained by the mayfly *Baetis alpinus*, which has not moulted to subimago when reared under constant dark conditions even if the optimal emergence temperature was ensured (Humpesch, 1971).

The above mentioned results completed with literature data confirm, that though in laboratory conditions we try to investigate the impact of temperature as single and independent variable and drive conclusions, under field conditions the phenological and morphological traits of the species are influenced by a series of additional habitat characteristics including both biotic and abiotic functions, species ecology and the interaction of them as it is sustained by the comparison of size and fecundity of control treatment specimens with field collected ones. Field collected specimens were smaller in all the cases. Moreover fecundity of specimens collected in Groβe Steinau reduced with 50 % and was due to the smaller size determined by the high incidence of nematodes in the abdomen of the specimens.

Conclusions

- The reversed-funnel technique adapted to the requirements of aquatic macroinvertebrates yields promising results both in short term and long term rearing of lotic mayflies and will likely provide a wider access to a vast range of experiments upon mayflies and other lotic taxonomic units
- *Habroleptoides carpatica* represents the senior synonim of *H. confusa* and ICZN accepted prevailing usage
- As a result of the phenological and morphological plasticity, *H. confusa* will likely adapt to the increased temperatures projected by the end of year 2100 due to the:
    1. Reduction of aquatic developmental time, but adapted to the external conditions
    2. More pronounced development, but adapted to the external factors
    3. Increase of nymphal size
    4. Increase of fecundity

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List of publications

Publications directly related to the topic of the PhD thesis

Váncsa, É., Csata, Z. & Rákosy, L. (2016): Adaptation of a simple technique for rearing lotic mayfly (Insecta : Ephemeroptera) nymphs. Entomologica Romanica, 19(2014/15): 5–12.

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