# 4. Principal conclusions of the thesis

European necrophagous species are overlooked, but they deserve much more attention, because they play major role in nutrient cycling and information about their ecology and evolution can have great impact on applied fields of science like forensic entomology and nature protection (Lomolino *et al.*, 1995; Lomolino & Creighton, 1996; Ratcliffe, 1996; Begon *et al.*, 2006; Looney *et al.*, 2009; Crawford & Hoagland, 2010; Midgley *et al.*, 2010; Jurzenski *et al.*, 2014; Houston *et al.*, 2015).

During the field work on article **A** and **C** we captured and identified 15 species of Middle European carrion beetles. Three of which are currently considered as rare and are on the Czech Red List of Invertebrates as Endangered (*N. antennatus* and *N. germanicus*) or Nearly Endangered (*N. sepultor*) (Růžička, 2005). The last ecological studies of these species in Europe were done almost 50 years ago when they were probably much more common (Novák, 1966; Petruška, 1968).

The most frequently observed species was *T. sinuatus*, whose dominance was overshadowed by *N. vespillo* and *T. rugosus*, but only in autumn. All these species seem to be very common in open landscape habitats and our findings confirm the earlier observations of (Novák, 1962, 1965, 1966; Petruška, 1964). Both species of genus *Thanatophilus* are therefore fairly common in the Czech Republic and they visit regularly carcasses of large animals.

*T. sinuatus* and *T. rugosus* are considered to be co-occurring species without spatially or temporally differentiated niches (Novák, 1966). The higher abundance of *T. rugosus* in autumn samples could indicate a temporal niche differentiation.

All these traits could be used in forensic entomology, but larval identification, instar determination and thermal summation models are not established for them jet. In contrast, successful case report of estimation of PMI based on closely related African species of genus *Thanatophilus* (*T. mutilatus* and *T. micans*) was published last year (Ridgeway *et al.*, 2014). These species were only recently studied and proposed thermal summation models made them available for use in forensic entomology (Midgley & Villet, 2009; Ridgeway *et al.*, 2014). *T. sinuatus* and *T. rugosus* are showing similar qualities and options for future research looks very promising.

We were able to show in the article **A** that the abundance of seven of the carrion beetles (*N. antennatus, N. germanicus, N. humator, N. interruptus, N. sepultor, Silpha obscura obscura* and *T. sinuatus*) differed significantly in areas with chernozem and fluvisol soils, and therefore soil type is an important factor in determining the occurrence of these carrion beetles. Our findings are supported by the results of the CCA analysis and Wilcoxon rank-sum tests (article **A**).

CCA analysis in article **A** also revealed that the factors that are significantly associated with the species composition are both soil types (chernozem and fluvisol) and also three crops (*Zea mays*, *Hordeum vulgare* and *Heliantus annus*). The association between the abundance of the carrion beetles and these crops is very interesting and we did not expect such a relationship. We think that it could be due to the microclimatic conditions in fields with these crops. Based on our experience, *Z. mays* provides a much more humid and cooler environment than *H. vulgare* and *H. annus*. Further study is needed to reveal causality.

Association between abundance of Nicrophorinae and soil characteristics was assumed by many authors (Pukowski, 1933; Paulian, 1946; Theodorides & Heerdt, 1952; Novák, 1961, 1962). Heretofore, this phenomenon was empirically proven only for North American species (Muths, 1991; Bishop *et al.*, 2002; Looney *et al.*, 2009), where the association of beetles of the subfamily Silphinae with a particular soil was previously reported by (Bishop *et al.*, 2002).

Although burying beetles are good fliers and can cover long distances they mostly choose to stay close to their original locations (e.g. *Nicrophorus americanus*, which is a relatively large and robust beetle, is capable of flying as far as 7.41 km in a single night, but more typically travels less than 1.6 km/night) (Jurzenski *et al.*, 2011). Limited mobility coupled with adaptation to local conditions could cause the observed spatial structure rather than individual habitat choice (preferential colonization). This is also in line with general local adaptation hypotheses (Alstad, 1998). It is also possible that the abundances of those species are different throughout their distribution area, as pointed out by (Scott, 1998).

Among other species, which were more abundant on cernozem, we also identified three endangered species (*N. antennatus*, *N. germanicus* and *N. sepultor*). Our finding can help in actions towards the conservation of these species, which is currently not a topical issue in the Czech Republic and Europe generally, but these beetles are charismatic and could become a widely accepted flagship species for stakeholders and policymakers, as indicated by the public interest in and the vast number of studies on the American burying beetle (*N. americanus*) (Anderson, 1982; Lomolino *et al.*, 1995, 1999; Lomolino & Creighton, 1996; Bedick *et al.*, 1999, 2004; Szalanski *et al.*, 2000; Sikes & Raithel, 2002; Bishop *et al.*, 2002; Walker & Hoback, 2007; Crawford & Hoagland, 2010; Jurzenski *et al.*, 2011, 2014; Black, 2012; Hall *et al.*, 2015; Houston *et al.*, 2015).

The article **B** provides parameter estimates of thermal summation model of *Sciodrepoides watsoni* together with new character for instar determination. *S. watsoni* is very common necrophagous beetle (Růžička, 1994, 2000; GBIF, 2015), which occurs across Holarctic region (Peck & Cook, 2002; Perreau, 2004). This species was offered as with some utility for forensic entomology by (Schilthuizen *et al.*, 2011). This view was recently challenged by (Kilian & Mądra, 2015), but they unfortunately did not show any evidence to support their claim that this species is irrelevant for forensic entomology.

The mean development time decreased with increasing temperature (article **B**, Fig. 6), except for L2 and L3 instars in the 25°C treatment. This might indicate that between 21°C and 25°C should be an optimal temperature for the development of these two stages. Optimal temperatures for lower stages are probably even higher. This agrees with findings of (Engler, 1981), who reported *S. watsoni* as warm season species in contrast to some species of Choleva and Catops that prefers to breed during the winter season and their optimal temperatures for development were below 16°C.

During the breeding we observed very high levels of mortality in later stages of development (third larval instar and pupae). It is more likely that separation from other larvae and adults was the reason for this phenomenon. (Peck, 1975) mentioned that *Ptomaphagus hirtus* (Tellkampf, 1844) (Leiodidae: Cholevinae: Ptomaphagini) needed soil from the cave of its origin to successfully complete the development. Soil bacteria probably played some part in this process, because specimens did not develop on autoclaved soil (Peck, 1975). It is possible that adults feeding along with larvae could provide such bacteria in our case. Another explanation could be that feeding of multiple individuals is much more effective or improves the quality of the food source.

During the observation of freely breeding specimens we did not see any signs of cannibalism between individuals as reported by (Kilian & Mądra, 2015), but it is possible that we missed it, because estimated number of individuals in the box was close to one hundred.

Methodology of measuring the size of the instars of *S. watsoni* was based on continual observation of individuals from egg until pupation. This approached differs from other studies with similar goals (Velásquez & Viloria, 2010; Fratczak & Matuszewski, 2014), where authors tried to estimate the stage of development based on the size of selected characters without prior knowledge of the true stage of the specimen. This approach is from my point of view a little bit problematic, because those measured characters are correlated, therefore bigger larvae could be misidentified as higher instar than they really are. This bias would probably not affect the obtained mean values, but it would give distorted picture about variation.

As you can see on Fig. 8 and Table 3, all instars have some overlap in the head widths. This is especially true for the first and second instar. It would not do us any good to measure more characters, because they are correlated, but we offer different solution. First instar larva has only primary setae on its body, but after molt to the second instar a secondary set of setae will emerge and they are present unchanged also on the third instar larvae. Thus chaetotaxy can be used for the discrimination of the first and second instar larvae. For additional differential diagnosis of those morphological characters, see (Kilian & Mądra, 2015).

We established developmental parameters for *Sciodrepoides watsoni* together with the new and reliable character for instar determination. This species is so far the smallest necrophagous beetle with known thermal summation model. The developmental characteristics provided in this study will help to estimate the PMImin in cases where it was not possible before. The instar determination is the integral part of the PMImin estimation, because without accurate determination it we could not reach the right conclusion. We strongly encourage other authors to adopt our methodology for establishing the size-based instar characteristics, because it provides correct picture about its variability.

Together these tree articles aim to contribute to growth of our knowledge about ecological and evolutional strategies of necrophagous beetles and I hope that I bring some of them to attention of professional public.