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Thermal summation model and instar determination of all developmental stages of necrophagous beetle, *Sciodrepoides watsoni* (Spence) (Coleoptera: Leiodidae: Cholevinae)

Pavel Jakubec

Necrophagous beetles are underrepresented in forensic entomology studies despite their undeniable utility for the field. In our article we would like to address this problem and provide information regarding developmental biology and instar determination of *Sciodrepoides watsoni* (Spence, 1813), which is a very common species occurring across the Holarctic region. We collected adult specimens from several localities across the Czech Republic to establish a laboratory culture with constant temperature regime and long day photoperiod. These adults were divided between five treatments that differed only in temperature (15, 18, 21, 25 and 28°C). Emerging larvae were separated and their individual development was photographically documented every day until adulthood. Parameters of thermal summation models and their standard errors were calculated for each developmental stage. We also propose head width as a new character for larval instar determination together with a new methodology for future studies of size based characters.
Thermal summation model and instar determination of all developmental stages of necrophagous beetle, Sciodrepoides watsoni (Spence) (Coleoptera: Leiodidae: Cholevinae)

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Abstract

Necrophagous beetles are underrepresented in forensic entomology studies despite their undeniable utility for the field. In our article we would like to address this problem and provide information regarding developmental biology and instar determination of Sciodrepoides watsoni (Spence, 1813), which is very common species occurring across the Holarctic region. We collected adult specimens from several localities across the Czech Republic to establish a laboratory culture with constant temperature regime and long day photoperiod. These adults were divided between five treatments that differed only in temperature (15, 18, 21, 25 and 28°C). Emerging larvae were separated and their individual development was photographically documented every day until adulthood. Parameters of thermal summation models and their standard errors were calculated for each developmental stage. We also propose head width as a new character for larval instar determination together with a new methodology for future studies of size based characters.

Introduction

Forensic entomology is a rapidly developing new field of science (Midgley et al., 2010). New methods and models for estimation of minimum post mortem interval (PMImin) are developing at a very rapid pace (e.g., pre-appearance interval, gene expression during larval development, quantile mixed effects models, generalized additive modeling or generalized additive mixed modeling) (Matuszewski, 2011; Tarone & Foran, 2011; Baqué et al., 2015a, 2015b), but even the well-established models lack actual data for their further use and application. A good example is the commonly used thermal summation model (Richards & Villet, 2008). This model, which is based on the assumption that development of immature stages is linear, has been known for several decades (Higley et al., 1986), but it is still not established for the majority of forensically important species of invertebrates, which would be a great contribution to legal investigations.

Currently these models are known for a number of fly species (Diptera) (Nabity et al., 2006; Villet et al., 2006; Richards et al., 2009; Voss et al., 2010a, 2010b, 2014; Tarone et al., 2011; Nassu et al., 2014; Zuha & Omar, 2014), but because the utility of beetles in forensic entomology
was overlooked for a long time (Midgley et al., 2010), there are only a three species of beetles with known thermal summation models (Midgley & Villet, 2009a; Velásquez & Viloria, 2009; Ridgeway et al., 2014).

However, using beetles for PMImin estimation has several benefits compared to flies. Beetles tend to have longer development therefore they can be found on and around the carrion for a longer period of time (Villet, 2011). They also do not form a maggot ball like flies and they can be reared individually so they are easier to handle in laboratory conditions (Midgley et al., 2010). However, we think that the biggest advantage is the possibility of cross validating PMImin estimates between species and groups, such as flies and mites. This is important mainly in cases when one of these groups or species could have been affected by external factors (restricted access to body, temperature too high or low, etc.) and give biased estimate (Šuláková 2014, pers. comm.).

As mentioned above, statistically robust thermal summation models are only known for three species of necrophagous beetles, all of them belonging to the family Silphidae. These are Thanatophilus micans (Fabricius, 1794) (Ridgeway et al., 2014), T. mutilatus (Castelnau, 1840) (Ridgeway et al., 2014) and Oxelytrum discicolle (Brullé, 1840) (Velásquez & Viloria, 2009). T. micans occurs mainly in Africa and extends to Yemen on the Arabian Peninsula (Schawaller, 1981; Růžička & Schneider, 2004), T. mutilatus has a geographical distribution restricted to South Africa region (Schawaller, 1981, 1987) and O. discicolle inhabits Central and South America (Peck & Anderson, 1985). This leaves North America, Europe and most of Asia without a single beetle species with a known thermal summation model.

Models alone are not sufficient to make a species available for use in legal investigation. There are other criteria to be fulfilled. Any forensic entomologist has to be able to identify those species in every stage of development and discriminate between larval instars. Without reliable instar determination it is not possible to expect reliable PMImin estimates. But this is sometimes complicated, because beetle larvae often lack any morphological characters, which would allow such identification. Therefore size based models were developed instead (Midgley & Villet, 2009b; Velásquez & Viloria, 2010; Fratczak & Matuszewski, 2014), but larval instars of only two European species can be identified in this way, namely Necrodes littoralis (Linnaeus, 1758) (Silphidae) and Creophilus maxillosus (Linnaeus, 1758) (Staphylinidae) (Fratczak & Matuszewski, 2014).

Sciodrepoides watsoni (Spence, 1813) is one of the most widespread and abundant species of necrophagous beetles in the Holarctic region (Peck & Cook, 2002; Perreau, 2004). Robust occurrence data are available especially for Europe (see Fig. 1). This saprophagous beetle belongs to subfamily Cholevinae (Leiodidae) and is rather inconspicuous, because the whole body is brown and about 3 millimeters long (Szymczakowski, 1961; Perreau, 2004) (see Fig. 2). Adults can be fairly easily distinguished from the other European species of genus Sciodrepoides by the shape of the antennal segments (Szymczakowski, 1961). The main peak of activity is during the warmer parts of the year (late spring and summer) (Růžička, 1994). All stages can be
found on decaying corpses of vertebrates in various types of habitats where they feed and develop 
(Růžička, 1994; Peck & Cook, 2002; Topp, 2003).

Egg, all larval instars and pupae of this beetle were properly described recently by (Kilian & 
Mądra, 2015) and also DNA barcode for possible validation is available (Schilthuizen et al., 
2011). Therefore identification of this species in every stage of development is not an issue.

Instar determination of S. watsoni larvae is also partially possible thanks to (Kilian & Mądra, 
2015), but they found morphological differences only between the first and second instar, which 
is not enough for future application for PMImin estimation.

We would like to improve the utility of S. watsoni for PMImin estimation by finding the 
parameters of its thermal summation model and also offering a new method for identifying larval 
stages based on combination of morphological features mentioned by (Kilian & Mądra, 2015) 
and the size based characters.

**Material and Methods**

A laboratory colony was started with adults of S. watsoni, which were collected in spring of 2012 
and/or 2013 from five localities in the Czech Republic (Prague – Suchdol (15 May – 12 April 
2012, 15 May – 12 April 2013), Běstvina (7 – 11 April 2012, 6 – 10 April 2013), Domažlice (28 
May – 12 April 2013) and Klato 

Beetles were collected using 10 baited pitfall traps, placed at each locality. The traps composed of 
1,080 ml plastic buckets (opening of 103 mm and 117 mm deep). These buckets were embedded 
in substrate up to the rim to eliminate any obstructions which could deter beetles from entering. 
As protection against rain we put metal roofs (150x150 mm) over the traps. The roof was 
supported by four 100 mm nails, one in each corner, and placed approximately two centimeters 
above the surface. The bait, ripened cheese (Romadur) and fish meat (Scomber scombrus 
Linnaeus, 1758), was placed directly inside the bucket on a shallow layer of moist soil. This 
created good conditions for survival of the trapped beetles between servicing, which was usually 
done once a week.

After transport to our laboratory we confirmed identification and sexed the beetles under 
binocular microscope (Olympus SZX7). Most of the beetles were than randomly assigned to form 
breeding groups of at least four individuals (2 males and 2 females). Specimens from the same 
locality were kept together regardless of capture date to eliminate cross-breeding of different 
populations. These groups were formed to produce new progeny, which we than observed 
throughout of their development (breeding experiment).

These groups were kept in Petri dishes with the layer of soil and small piece (approx. 5x5 mm) of 
fish meat (Scomber scombrus) as a food source. The content of the dish was lightly sprayed with 
tap water every day and food was provided ad libitum and changed if we spotted any sign of 
fungal growth.
The dishes were randomly placed in one of six climatic chambers (custom made by CIRIS s.r.o.). The chambers were set up at constant temperature (15, 18, 21, 25 or 28°C) and 16 hours of light and 8 hours of dark photoperiod regime, maintained by fluorescent light (Osram L 8W/640). We tried to have a similar number of breeding groups from the same locality in each chamber. We accomplished that in case of beetles from Praha and Běstvina, but it was not possible for beetles from Domážlice and Klatovy, because of a low number of adults obtained. Therefore we kept them together in one treatment (18°C).

We also started an observation study of their natural behavior. The study was conducted in a small plastic box (15x6x2 centimeters) with 12 adult individuals (7 females and 5 males) from Prague population. In this colony we did not separate larvae from adults or each other, but we allowed them to interact freely and without our intervention. The box itself was placed in 18°C treatment and its inhabitants were attended in the same way as the specimens in the breeding experiment (regular water spray and meat replaced if we saw a sign of fungal growth).

In the breeding experiment we slightly changed our method of handling eggs and first instar between the years to improve accuracy of our observations. During the first year of experiment (2012) we searched the dishes for eggs and then we transferred them individually to separate dish. But due to the fact that eggs of *S. watsoni* are very small and adults tended to hide them in the substrate, we struggled to find them right after laying. Due to that our estimation of egg and L1 development for the first year were inconsistent and we did not use them for models.

To minimize this error we chose different approach for the second year (2013). We instead transferred the whole breeding group to a new Petri dish every day. The old dishes were marked and kept in the same climatic chamber as the parents. We checked them every day for emergence of the first instar larvae that were further separated into their own dishes. The time when the eggs were laid, was estimated as a half-time between the transfers of the breeding group.

Every larva from the second year (2013) breeding experiment was photographed every day, starting with their occurrence as the first instar larvae and we continued until pupation. In this way we documented morphological changes during their development. The whole process of finding the larva and taking a picture did not usually take more than 1 minute in total. Key developmental stages of each larva with the accurate date and time could be distinguished based on those photographs simply by keeping track of the change in the width of their head capsule, because its size expand after each molt.

It happened sometimes that we were unable to find some larva in the Petri dish. In that case we treated the dish as full and put it back into its treatment and tried another day. If the larvae changed instars before we found it, we counted both instars as NAs and we tried to keep track of it all the time in the next stage.

We also used obtained photographs for the instar determination. Because, the dorsal side of all the larvae was photographed daily, we had plenty of characters to choose from. However, the thorax and abdomen of the *S. watsoni* larvae are not strongly sclerotized (see Fig. 3), so we omitted these parts, and also the body length, as good characters for instar determination.
Measuring of some smaller parts such as urogomphi or antennae was impractical, because our camera had low resolution and those parts would be very challenging to measure accurately.

The most stable and reliable feature for the instar determination of *S. watsoni* larvae appears to be the head capsule. This part of the body is strongly sclerotized, therefore it is not affected by water or food content, but it changes its size after each molt so it is tightly linked with individual growth. Also the head does not change its size in different fixation media or even after desiccated, thus the instar can be identified even for very poorly handled and long dead specimens. Ultimately, we chose the head width over its length for a practical reason. Head width of living larvae do not change on the pictures captured from above, but length varies a lot.

For estimating the mean and standard deviation of the head capsule width we used all photographs where the head was clearly visible and was sharp enough to make a precise measurement. All measurements were done with graphical program EidosMicro calibrated by precise ruler.

Parameters of thermal summation model (lower developmental threshold (t) and sum of effective temperatures (k)) were estimated for each developmental stage using the major axis regression method ([DT] = k + tD) where D is duration of development, T is environmental temperature (°C). This formula was developed by (Ikemoto & Takai, 2000) and is commonly used for estimation of thermal summation parameters and their standard errors in forensic entomology (e.g., (Midgley & Villet, 2009a; Ridgeway et al., 2014)). (Ikemoto & Takai, 2000) method is based on standard linearized formula (1/D = — (t/k) + (1/k)T), but it weights out the data points in lower and upper part of the temperature range to obtain more reliable estimates of the parameters.

Normality of all the data was confirmed by evaluation of the qqplots and histograms. The significance level was set at 5%. Data management and all analysis were carried out using R statistical program (R Core Team, 2015). Graphical outputs were handled by ggplot2 and ggmap R packages (Wickham, 2009; Kahle & Wickham, 2013).

**Results**

In total, we were able to catch 81 adult specimens of *S. watsoni* and they produced 399 first instar larvae (Prague – 174, Běstvina - 178, Klatovy - 19, Domažlice - 28) for the breeding experiment. Because we obtained only twelve adults from Klatovy and six from Domažlice, it was impossible to split them between all our treatments. Therefore we decided to keep them all at 18°C.

In the breeding experiment we observed, directly or indirectly, and recorded duration of the development of all *S. watsoni* stages, namely egg, three larval instars (L1, L2 and L3) and pupae. These observations were made on 399 specimens in total starting with the first instar larvae.

Higher temperatures (25 and 28°C) were probably limiting to breeding activity of our beetles in the experiment. Ultimately we did not obtain any larvae from the 28°C treatment. Mortality in the other treatments was also quite high especially for the third instar and pupae (see Fig. 4) and only 23 individuals developed until adulthood.
The development times differed between stages (Fig. 5) and the mean development time decreased with increasing temperature (Fig. 6), except for L2 and L3 instars in 25°C treatment. The sum of effective temperatures (k) and lower developmental threshold (t) values were calculated for all developmental stages of *S. watsoni* with their expected errors (see Table 1 and Fig. 7).

Mortality of the specimens in the observation study could not be measured, but the colony itself prospered very well and number of adults increased steadily, which is in contrast with what we observed in the breeding experiment. The observed females tended to hide their eggs in small holes or crevices in the substrate. Newly hatched larvae could be found mostly around the food source. The third instar larvae after few days of feeding dug underground and created small chamber where they pupate. No cannibalism or hostility of any kind between individuals was recorded.

For the instar determination measurements we made 2,104 photographs, but only 1,731 were good enough to allow precise measurements of the head width. Those pictures covered all three larval instars (L1 = 591, L2 = 500 and L3 = 640 pictures). The bias in number of pictures between different stages was caused by difference in the duration of development of these instars (lower stages of development are shorter in duration) and it was also much more challenging to take a usable picture of the first or second instar larvae.

The mean width of the head appears to be a good character for the instar determination (see Table 2 and Fig. 8). Standard deviations are well separated and there is only a small overlap between 75th and 25th quintiles across all instars. We recorded some extreme values on the both sides of the spectrum, but these were very rare.

**Discussion**

We did not obtain any larvae from the 28°C treatment probably because adults did not oviposit in this temperature or egg mortality was too high. The second claim is little bit more likely from our point of view, because we did not find any eggs. But as we mentioned in the methodology section, eggs of *S. watsoni* are tiny and we could simply overlook them during our controls in the Petri dish's substrate even under the binocular microscope.

Mortality of our specimens in the breeding experiment was very high over the all treatments especially in the later stages (L3 and pupae). This was in a sharp contrast with what we saw in the observation study. The whole colony in the observation study prospered and even increased in the number of adult over time. Only difference between these two was that we did not separate individuals and we also did not have to handle the larvae for photo documentation.

We did not observe any hostility between specimens in the observation study or signs of cannibalism between individuals as reported by (Kilian & Mądra, 2015), but it is possible that we missed it, because the estimated number of individuals in the box was close to one hundred.
We think that photographing process was not so intrusive to be responsible for such high mortality rates thus it is more likely that separation from other larvae and adults was the reason for that. (Peck, 1975) mentioned that *Pptomaphagus hirtus* (Tellkampf, 1844) (Leiodidae: Cholevinae: Ptomaphagini) needed soil from its cave of origin to successfully complete the development. Soil bacteria probably play some part in this process, because specimens did not develop on autoclaved soil. It is possible that adults feeding along with larvae could have provided 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.

We had to change our methodology of egg extraction for the second year due to the fact that eggs could be easily overlooked in the substrate and beetles refused to lay their eggs in offered damp cotton wool balls or small pieces of paper. To prevent bias in recorded time we introduced dish rotation methodology and adults stayed in the same dish only one day and then were moved to another. Those used dishes were then regularly searched for emerging larvae. The main issue with this approach (dish rotation) is that we could not measure egg mortality, because we could not count the original number of eggs.

The mean development time decreased with increasing temperature (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.

As you can see in Table 1, we had low number repeats for L3 and pupae. This was caused by high mortality rates of both instars. Measuring development time for pupae was even more challenging and we had difficulties measuring it precisely due to the fact that they did not pupate close to the wall of Petri dish. Therefore we had to search for them. This was sometimes unsuccessful and some specimens surprised us after time when they appeared as adults, because they had been missing and presumed dead.

Our methodology of measuring the size of the instars 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 our 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 a distorted picture of variation.

As can be seen 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 help to measure more characters, because they are correlated, but we offer a different solution. A first instar larva has only primary setae on its body, but after molting to the second instar a secondary set of setae will emerge and
they are also present unchanged 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 a 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 of instar we could not reach the right conclusion. We strongly encourage other authors to adopt our methodology for establishing size based instar characteristics, because it provides an accurate picture of its variability.

**Acknowledgements**

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**References**


Table 1: Summary of development constants for *S. watsoni* for five developmental stages. Sum of effective temperatures (k) and lower developmental threshold (t) shown as means with the standard errors.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature range</th>
<th>$R^2$</th>
<th>Df</th>
<th>$p$ value</th>
<th>k ± SE</th>
<th>t ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>15-25</td>
<td>0.8134</td>
<td>22</td>
<td>2.20E-16</td>
<td>929.354 ±49.111</td>
<td>11.400 ±0.368</td>
</tr>
<tr>
<td>L1</td>
<td>15-25</td>
<td>0.9375</td>
<td>17</td>
<td>1.10E-16</td>
<td>233.683 ±27.031</td>
<td>15.437 ±0.305</td>
</tr>
<tr>
<td>L2</td>
<td>15-25</td>
<td>0.8768</td>
<td>20</td>
<td>1.20E-16</td>
<td>243.945 ±45.301</td>
<td>15.689 ±0.410</td>
</tr>
<tr>
<td>L3</td>
<td>15-25</td>
<td>0.8199</td>
<td>27</td>
<td>1.49E-11</td>
<td>2602.996 ±297.464</td>
<td>9.375 ±0.846</td>
</tr>
<tr>
<td>Pupae</td>
<td>15-21</td>
<td>0.8563</td>
<td>10</td>
<td>1.61E-05</td>
<td>1207.431 ±489.288</td>
<td>12.535 ±1.624</td>
</tr>
</tbody>
</table>

Table 2: The head widths (in millimeters) of all three larval instars of *S. watsoni*.

<table>
<thead>
<tr>
<th>Instar</th>
<th>max.</th>
<th>min.</th>
<th>mean</th>
<th>stand. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.392</td>
<td>0.270</td>
<td>0.329</td>
<td>0.017</td>
</tr>
<tr>
<td>L2</td>
<td>0.479</td>
<td>0.350</td>
<td>0.421</td>
<td>0.021</td>
</tr>
<tr>
<td>L3</td>
<td>0.582</td>
<td>0.451</td>
<td>0.522</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Fig. 1: Occurrence of *S. watsoni* in Europe based on our own observations and records from the GBIF database (GBIF, 2015). Underlying map generated by package ggmap (Kahle & Wickham, 2013).
Fig. 2: Habitus of the *S. watsoni* male from dorsal view.
Fig. 3: Dorsal (A), lateral (B) and ventral (C) side of the third larval instar of *S. watsoni*. Point where the head width was measured is shown (a).
Fig. 4: Mortality rates between developmental stages of *S. watsoni*. The 28°C treatment is not shown, because breeding did not occur.
Fig. 5: Observed range of development times of *S. watsoni* over four experimental treatments (15, 18, 21, 25 °C) for each developmental stage (2012 data were excluded for egg and L1). The horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the 75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile ranges. Small, black dots are outliers. Small red dots are the mean values of development time.
Fig. 6: Bar plot of mean development time (in hours) of all observed stages (2012 data were excluded for egg and L1) of *S. watsoni* over the whole range of experimental temperature except the 28°C, where beetles did not breed successfully.
Fig. 7: Major axis regression for all stages of development in *S. watsoni*. Black line shows median and grey area around is standard error. DT is the time in days to reach the stage multiplied by the constant rearing temperature. 2012 data were excluded for egg and L1.
Fig. 8: Box plot graph of lengths of all three instars (L1, L2 and L3) of the *S. watsoni* larvae. The horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the 75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile ranges. Small, black dots are outliers.