

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 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.

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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

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

32 Currently these models are known for a number of fly species (Diptera) (Nabity *et al.*, 2006;
33 Villet *et al.*, 2006; Richards *et al.*, 2009; Voss *et al.*, 2010a, 2010b, 2014; Tarone *et al.*, 2011;
34 Nassu *et al.*, 2014; Zuha & Omar, 2014), but because the utility of beetles in forensic entomology

35 was overlooked for a long time (Midgley *et al.*, 2010), there are only a three species of beetles
36 with known thermal summation models (Midgley & Villet, 2009a; Velásquez & Viloria, 2009;
37 Ridgeway *et al.*, 2014).

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

47 As mentioned above, statistically robust thermal summation models are only known for three
48 species of necrophagous beetles, all of them belonging to the family Silphidae. These are
49 *Thanatophilus micans* (Fabricius, 1794) (Ridgeway *et al.*, 2014), *T. mutilatus* (Castelnau, 1840)
50 (Ridgeway *et al.*, 2014) and *Oxelytrum discicolle* (Brullé, 1840) (Velásquez & Viloria, 2009). *T.*
51 *micans* occurs mainly in Africa and extends to Yemen on the Arabian Peninsula (Schawaller,
52 1981; Růžička & Schneider, 2004), *T. mutilatus* has a geographical distribution restricted to
53 South Africa region (Schawaller, 1981, 1987) and *O. discicolle* inhabits Central and South
54 America (Peck & Anderson, 1985). This leaves North America, Europe and most of Asia without
55 a single beetle species with a known thermal summation model.

56 Models alone are not sufficient to make a species available for use in legal investigation. There
57 are other criteria to be fulfilled. Any forensic entomologist has to be able to identify those species
58 in every stage of development and discriminate between larval instars. Without reliable instar
59 determination it is not possible to expect reliable PMI_{min} estimates. But this is sometimes
60 complicated, because beetle larvae often lack any morphological characters, which would allow
61 such identification. Therefore size based models were developed instead (Midgley & Villet,
62 2009b; Velásquez & Viloria, 2010; Fratzczak & Matuszewski, 2014), but larval instars of only two
63 European species can be identified in this way, namely *Necrodes littoralis* (Linnaeus, 1758)
64 (Silphidae) and *Creophilus maxillosus* (Linnaeus, 1758) (Staphylinidae) (Fratczak &
65 Matuszewski, 2014).

66 *Sciodrepoides watsoni* (Spence, 1813) is one of the most widespread and abundant species of
67 necrophagous beetles in the Holarctic region (Peck & Cook, 2002; Perreau, 2004). Robust
68 occurrence data are available especially for Europe (see Fig. 1). This saprophagous beetle
69 belongs to subfamily Cholevinae (Leiodidae) and is rather inconspicuous, because the whole
70 body is brown and about 3 millimeters long (Szymczakowski, 1961; Perreau, 2004) (see Fig. 2).
71 Adults can be fairly easily distinguished from the other European species of genus *Sciodrepoides*
72 by the shape of the antennal segments (Szymczakowski, 1961). The main peak of activity is
73 during the warmer parts of the year (late spring and summer) (Růžička, 1994). All stages can be

74 found on decaying corpses of vertebrates in various types of habitats where they feed and develop
75 (Růžička, 1994; Peck & Cook, 2002; Topp, 2003).

76 Egg, all larval instars and pupae of this beetle were properly described recently by (Kilian &
77 Mądra, 2015) and also DNA barcode for possible validation is available (Schilthuizen *et al.*,
78 2011). Therefore identification of this species in every stage of development is not an issue.

79 Instar determination of *S. watsoni* larvae is also partially possible thanks to (Kilian & Mądra,
80 2015), but they found morphological differences only between the first and second instar, which
81 is not enough for future application for PMImin estimation.

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

86 Material and Methods

87 A laboratory colony was started with adults of *S. watsoni*, which were collected in spring of 2012
88 and/or 2013 from five localities in the Czech Republic (Prague – Suchdol (15 May – 12 April
89 2012, 15 May – 12 April 2013), Běstvina (7 – 11 April 2012, 6 – 10 April 2013), Domažlice (28
90 May – 12 April 2013) and Klatovy (14 – 28 May 2013)).

91 Beetles were collected using 10 baited pitfall traps, placed at each locality. The traps composed of
92 1,080 ml plastic buckets (opening of 103 mm and 117 mm deep). These buckets were embedded
93 in substrate up to the rim to eliminate any obstructions which could deter beetles from entering.
94 As protection against rain we put metal roofs (150x150 mm) over the traps. The roof was
95 supported by four 100 mm nails, one in each corner, and placed approximately two centimeters
96 above the surface. The bait, ripened cheese (Romadur) and fish meat (*Scomber scombrus*
97 Linnaeus, 1758), was placed directly inside the bucket on a shallow layer of moist soil. This
98 created good conditions for survival of the trapped beetles between servicing, which was usually
99 done once a week.

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

106 These groups were kept in Petri dishes with the layer of soil and small piece (approx. 5x5 mm) of
107 fish meat (*Scomber scombrus*) as a food source. The content of the dish was lightly sprayed with
108 tap water every day and food was provided *ad libitum* and changed if we spotted any sign of
109 fungal growth.

110 The dishes were randomly placed in one of six climatic chambers (custom made by CIRIS s.r.o.).
111 The chambers were set up at constant temperature (15, 18, 21, 25 or 28°C) and 16 hours of light
112 and 8 hours of dark photoperiod regime, maintained by fluorescent light (Osram L 8W/640). We
113 tried to have a similar number of breeding groups from the same locality in each chamber. We
114 accomplished that in case of beetles from Praha and Běstvina, but it was not possible for beetles
115 from Domažlice and Klatovy, because of a low number of adults obtained. Therefore we kept
116 them together in one treatment (18°C).

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

123 In the breeding experiment we slightly changed our method of handling eggs and first instar
124 between the years to improve accuracy of our observations. During the first year of experiment
125 (2012) we searched the dishes for eggs and then we transferred them individually to separate
126 dish. But due to the fact that eggs of *S. watsoni* are very small and adults tended to hide them in
127 the substrate, we struggled to find them right after laying. Due to that our estimation of egg and
128 L1 development for the first year were inconsistent and we did not use them for models.

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

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

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

145 We also used obtained photographs for the instar determination. Because, the dorsal side of all
146 the larvae was photographed daily, we had plenty of characters to choose from. However, the
147 thorax and abdomen of the *S. watsoni* larvae are not strongly sclerotized (see Fig. 3), so we
148 omitted these parts, and also the body length, as good characters for instar determination.

149 Measuring of some smaller parts such as urogomphi or antennae was impractical, because our
150 camera had low resolution and those parts would be very challenging to measure accurately.

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

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

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

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

174 Results

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

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

182 Higher temperatures (25 and 28°C) were probably limiting to breeding activity of our beetles in
183 the experiment. Ultimately we did not obtain any larvae from the 28°C treatment. Mortality in the
184 other treatments was also quite high especially for the third instar and pupae (see Fig. 4) and only
185 23 individuals developed until adulthood.

186 The development times differed between stages (Fig. 5) and the mean development time
187 decreased with increasing temperature (Fig. 6), except for L2 and L3 instars in 25°C treatment.
188 The sum of effective temperatures (k) and lower developmental threshold (t) values were
189 calculated for all developmental stages of *S. watsoni* with their expected errors (see Table 1 and
190 Fig. 7).

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

198 For the instar determination measurements we made 2,104 photographs, but only 1,731 were
199 good enough to allow precise measurements of the head width. Those pictures covered all three
200 larval instars ($L_1 = 591$, $L_2 = 500$ and $L_3 = 640$ pictures). The bias in number of pictures
201 between different stages was caused by difference in the duration of development of these instars
202 (lower stages of development are shorter in duration) and it was also much more challenging to
203 take a usable picture of the first or second instar larvae.

204 The mean width of the head appears to be a good character for the instar determination (see Table
205 2 and Fig. 8). Standard deviations are well separated and there is only a small overlap between
206 75th and 25th quintiles across all instars. We recorded some extreme values on the both sides of the
207 spectrum, but these were very rare.

208 Discussion

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

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

219 We did not observe any hostility between specimens in the observation study or signs of
220 cannibalism between individuals as reported by (Kilian & Mdra, 2015), but it is possible that we
221 missed it, because the estimated number of individuals in the box was close to one hundred.

222 We think that photographing process was not so intrusive to be responsible for such high
223 mortality rates thus it is more likely that separation from other larvae and adults was the reason
224 for that. (Peck, 1975) mentioned that *Ptomaphagus hirtus* (Tellkampf, 1844) (Leiodidae:
225 Cholevinae: Ptomaphagini) needed soil from its cave of origin to successfully complete the
226 development. Soil bacteria probably play some part in this process, because specimens did not
227 develop on autoclaved soil. It is possible that adults feeding along with larvae could have
228 provided such bacteria in our case. Another explanation could be that feeding of multiple
229 individuals is much more effective or improves the quality of the food source.

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

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

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

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

257 As can be seen on Fig. 8 and Table 3, all instars have some overlap in the head widths. This is
258 especially true for the first and second instar. It would not help to measure more characters,
259 because they are correlated, but we offer a different solution. A first instar larva has only primary
260 setae on its body, but after molting to the second instar a secondary set of setae will emerge and

261 they are also present unchanged on the third instar larvae. Thus chaetotaxy can be used for the
262 discrimination of the first and second instar larvae. For additional differential diagnosis of those
263 morphological characters, see (Kilian & Mądra, 2015).

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

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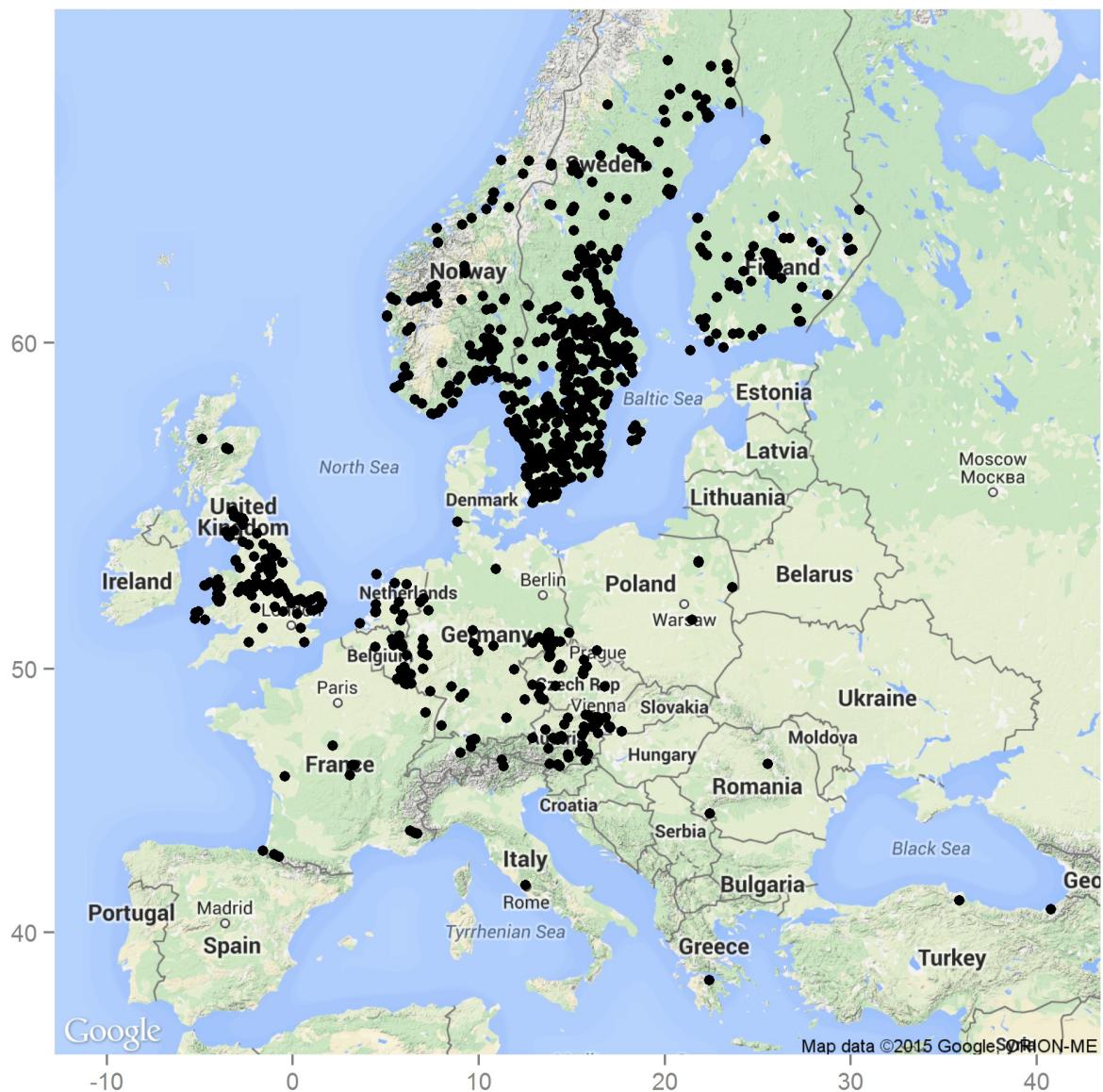
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392 Table 1: Summary of development constants for *S. watsoni* for five developmental stages. Sum of
 393 effective temperatures (k) and lower developmental threshold (t) shown as means with the
 394 standard errors.

Stage	Temperature		R ²	Df	p value	k	t
	range						
Egg	15-25		0.8134	22	2.20E-16	929.354 ±49.111	11.400 ±0.368
L1	15-25		0.9375	0	2.20E-16	233.683 ±27.031	15.437 ±0.305
L2	15-25		0.8768	17	2.20E-16	243.945 ±45.301	15.689 ±0.410
L3	15-25		0.8199	20	1.49E-11	2602.996 ±297.464	9.375 ±0.846
Pupae	15-21		0.8563	6	1.61E-05	1207.431 ±489.288	12.535 ±1.624

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

Instar	max.	min.	mean	stand. dev.
L1	0.392	0.270	0.329	0.017
L2	0.479	0.350	0.421	0.021
L3	0.582	0.451	0.522	0.021



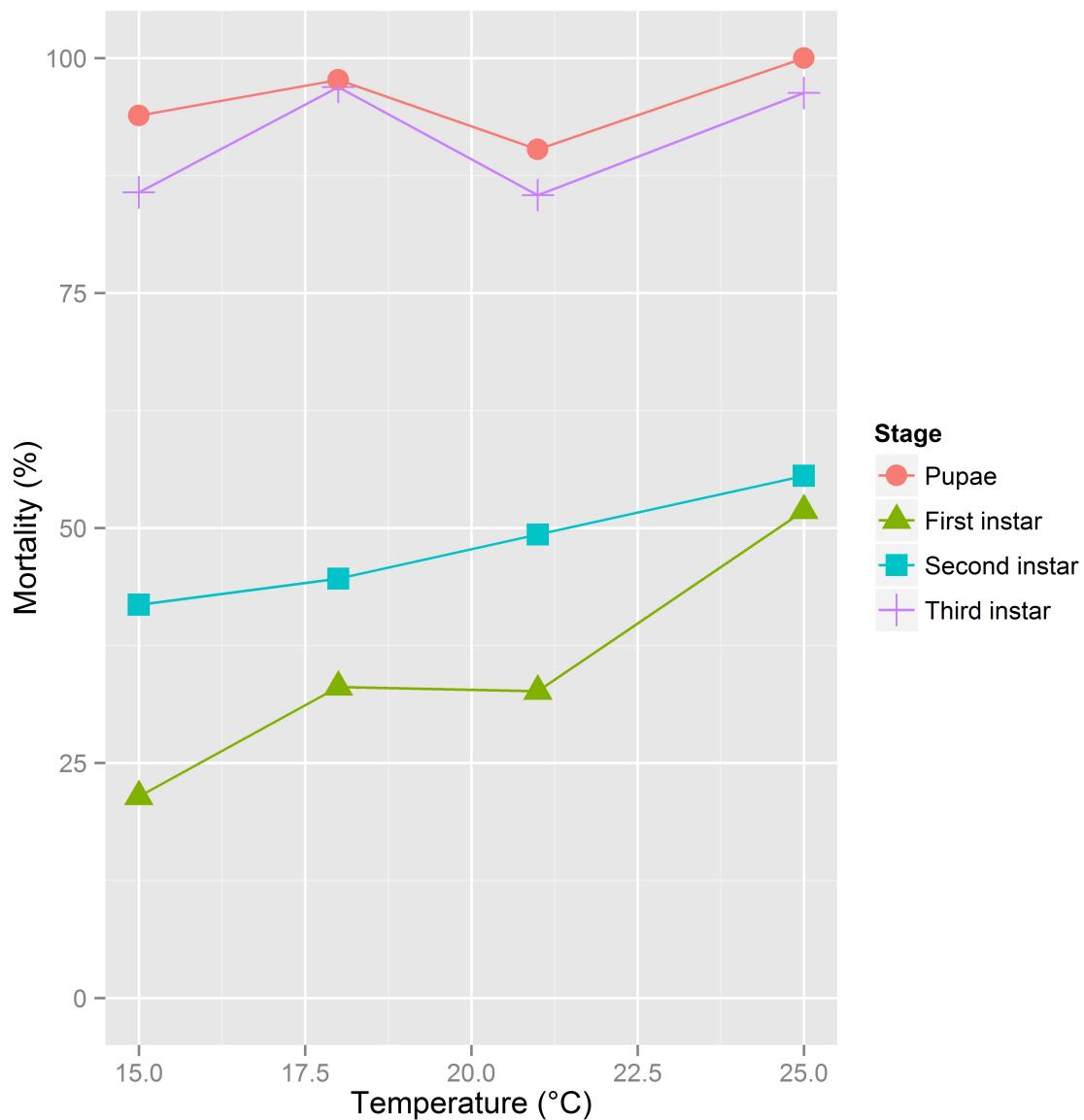
396 Fig. 1: Occurrence of *S. watsoni* in Europe based on our own observations and records from the
397 GBIF database (GBIF, 2015). Underlying map generated by package ggmap (Kahle & Wickham,
398 2013).



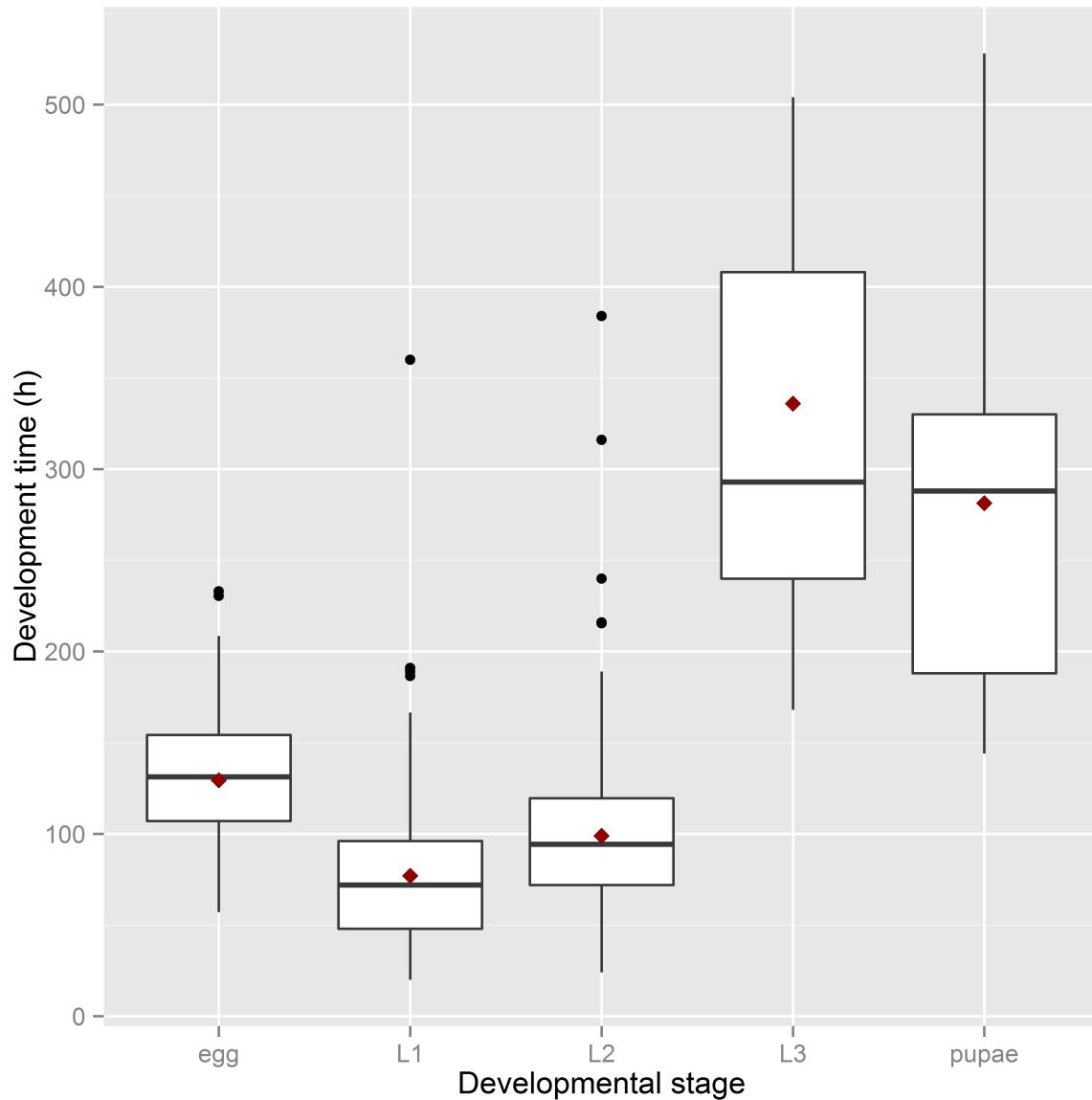
399 Fig. 2: Habitus of the *S. watsoni* male from dorsal view.



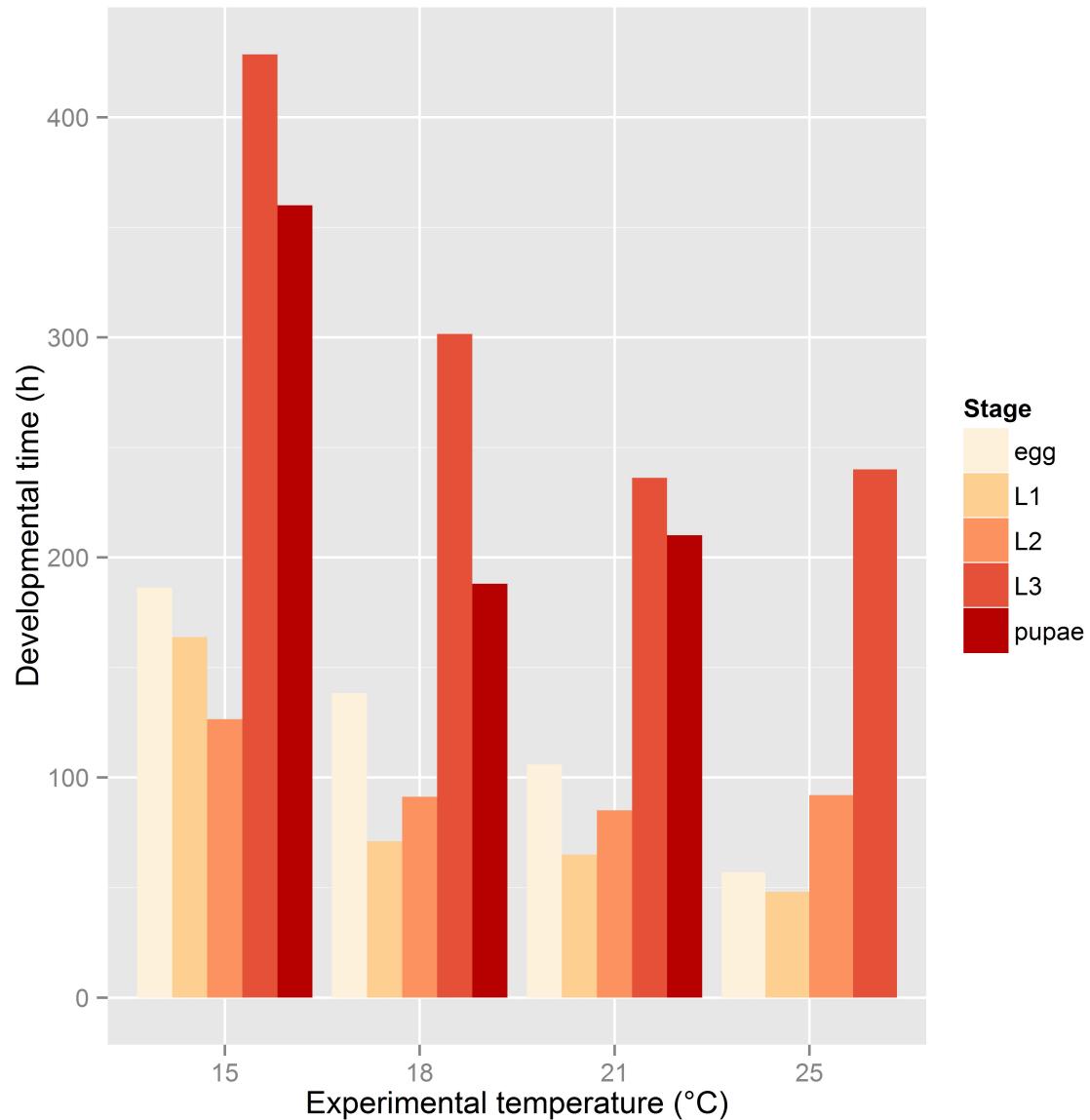
400 Fig.3: Dorsal (A) lateral (B) and ventral (C) side of the third larval instar of *S. watsoni*. Point
401 where the head width was measured is shown (a).



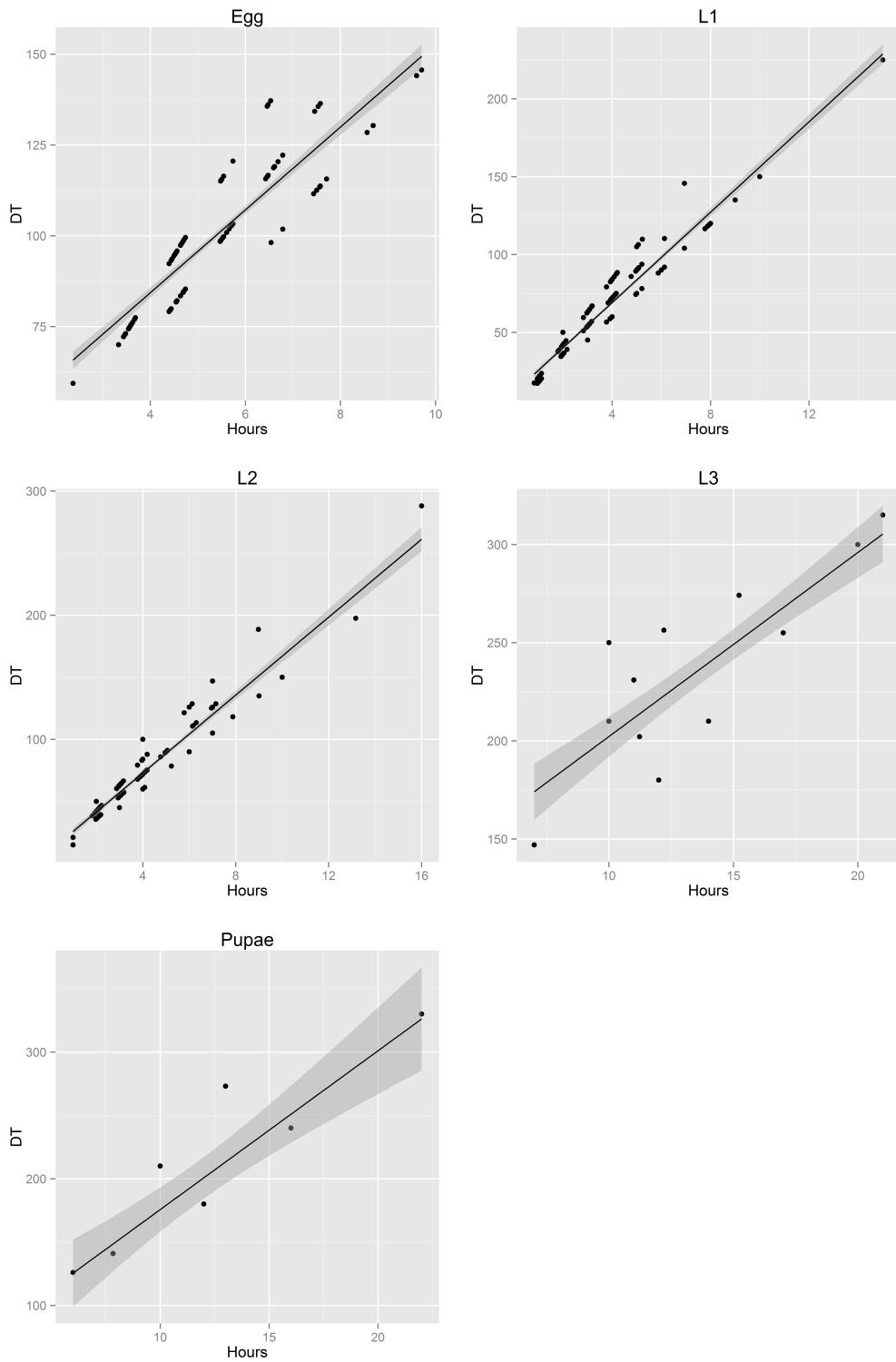
402 Fig. 4: Mortality rates between developmental stages of *S. watsoni*. The 28°C treatment is not
403 shown, because breeding did not occur.



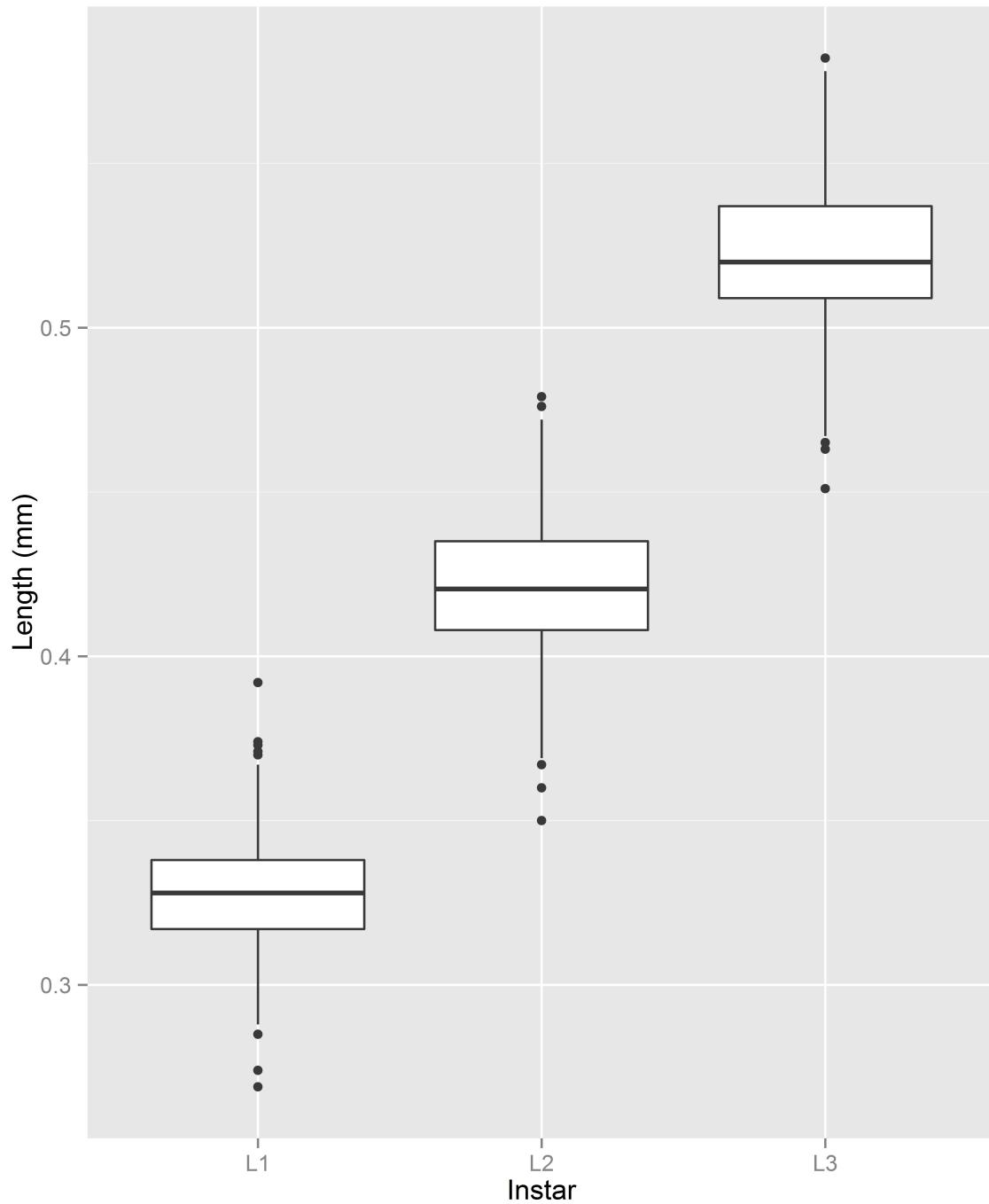
404 Fig. 5: Observed range of development times of *S. watsoni* over four experimental treatments (15,
405 18, 21, 25 °C) for each developmental stage (2012 data were excluded for egg and L1). The
406 horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the
407 75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile
408 ranges. Small, black dots are outliers. Small red dots are the mean values of development time.



409 Fig. 6: Bar plot of mean development time (in hours) of all observed stages (2012 data were
410 excluded for egg and L1) of *S. watsoni* over the whole range of experimental temperature except
411 the 28°C, where beetles did not breed successfully.



412 Fig. 7: Major axis regression for all stages of development in *S. watsoni*. Black line shows
 413 median and grey area around is standard error. DT is the time in days to reach the stage
 414 multiplied by the constant rearing temperature. 2012 data were excluded for egg and L1.



415 Fig. 8: Box plot graph of lengths of all three instars (L1, L2 and L3) of the *S. watsoni* larvae. The
416 horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the
417 75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile
418 ranges. Small, black dots are outliers.