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 the present article, information is presented regarding the developmental biology and instar determination of *Sciodrepoides watsoni* (Spence, 1813), a very common species occurring across the Holarctic region. Wild collected beetles were kept in climate chambers at constant temperature (12, 15, 18, 21 and 28°C) and their development was documented regularly. Parameters of thermal summation models and standard errors were calculated for each developmental stage. These models may be used for an estimation of post mortem interval in legal investigations after further validation on local populations of *S. watsoni*. An additional methodology is introduced for future studies of size-based characteristics, addressing instar identification bias. The methodology provided estimations (mean, standard error and standard deviation) of *S. watsoni* larval head capsule width for preliminary larval instar determination. The methodology may be used with other morphological features to improve instar determination accuracy.

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Abstract

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10 undeniable utility for the field. In the present article, information is presented regarding the
11 developmental biology and instar determination of *Sciodrepoides watsoni* (Spence, 1813), a very
12 common species occurring across the Holarctic region. Wild collected beetles were kept in
13 climate chambers at constant temperature (12, 15, 18, 21 and 28°C) and their development was
14 documented regularly. Parameters of thermal summation models and standard errors were
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16 mortem interval in legal investigations after further validation on local populations of *S. watsoni*.
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Introduction

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

33 Currently these models are known for a number of fly species (Diptera) (Nabity *et al.*, 2006;
34 Villet *et al.*, 2006; Richards *et al.*, 2009; Voss *et al.*, 2010a, 2010b, 2014; Tarone *et al.*, 2011;
35 Nassu *et al.*, 2014; Zuha & Omar, 2014), but only for a few necrobiont beetles.

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

44 Statistically robust thermal summation models are available only for three species of
45 necrophagous beetles, all belonging to the family Silphidae. These models are for *Thanatophilus*
46 *micans* (Fabricius, 1794) (Ridgeway *et al.*, 2014), *T. mutilatus* (Castelnau, 1840) (Ridgeway *et*
47 *al.*, 2014) and *Oxelytrum discicolle* (Brullé, 1840) (Velásquez & Viloria, 2009). *T. micans* occurs
48 mainly in Africa and extends to Yemen on the Arabian Peninsula (Schawaller, 1981; Růžička &
49 Schneider, 2004), *T. mutilatus* has a geographical distribution restricted to the South Africa region
50 (Schawaller, 1981, 1987) and *O. discicolle* inhabits Central and South America (Peck &
51 Anderson, 1985). Therefore, North America, Europe and most of Asia lack a single beetle species
52 with a thermal summation model.

53 Models alone are not sufficient for species to be useful in legal investigations. There are other
54 criteria to be fulfilled. Any forensic entomologist has to be able to identify those species in every
55 stage of development and discriminate between larval instars. Without reliable instar
56 determination, it is not possible to expect reliable PMImin estimates. However, this is sometimes
57 complicated, because beetle larvae often lack morphological characteristics specific to particular
58 instars, which would allow such identification (Velásquez & Viloria, 2009). In such cases the
59 body size frequency distributions are commonly used (see Logan et al., 1998).

60 Not all size-based characteristics are equally appropriate for instar determination. Dyar (1890)
61 observed that head width is a very advantageous characteristic for larval instar determination. He
62 supported his claim by showing that head width stays the same across intermoult intervals and
63 also that for some lepidopteran larvae it follows a geometrical progression. These properties
64 enable estimation of number of instars even from incomplete developmental information, when
65 some instars were unobserved and are widely used for instar determination (e.g. Delbac,
66 Lecharpentier & Thiery, 2010; Wu, Wang & Wu, 2012a,b; Gomez et al., 2015).

67 Head width or mean head width can be found for many species in their published descriptions
68 together with other morphological and size based characteristics. Unfortunately these
69 measurements are often based on a small number of animals, because they are not intended as
70 statistically robust models, thusthe true mean value could be very different from the reported one.
71 This bias is a serious problem for instar determination in applied disciplines like forensic

72   entomology, especially when we are dealing with animals from spatially or temporally distant
73   populations (Stillwell & Fox, 2009). For several species of necrobiont beetles statistically robust
74   models were developed, based on measuring not only head width, but also other size based
75   characteristics (Midgley & Villet, 2009b, Velásquez & Viloria, 2010 and Fratczak &
76   Matuszewski, 2014). However, these models are available only for two European species, namely
77   *Necrodes littoralis* (Linnaeus, 1758) (Silphidae) and *Creophilus maxillosus* (Linnaeus, 1758)
78   (Staphylinidae) (Fratczak & Matuszewski, 2014) and even those models should be used with
79   great care as I will discuss later in the text.

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

90   Egg, all larval instars and pupae of *S. watsoni* were described recently by Kilian & Mądra (2015),
91   and also a DNA barcode for validation is available (Schilthuizen *et al.*, 2011). Therefore
92   identification of this species in every stage of development is not an issue. Instar determination of
93   *S. watsoni* larvae is also possible (Kilian & Mądra, 2015), but its natural variability was not
94   covered in the case of size based characteristics.

95   This study attempts to improve the utility of *S. watsoni* for PMImin estimation by calculating the
96   parameters of thermal summation models for each stage (egg, three larval instars and pupae) and
97   developing an additional characteristic for instar determination, based on photographic
98   documentation and measurement of larval head capsule width. The latter methodology may be
99   developed to cover natural variability and can be easily observed, measured, and evaluated.
100   Combined with a morphological feature unique to specific instars, these data provide accurate
101   identification of larval instar and may be integrated into PMImin estimation models.

Material and Methods

103   A laboratory colony was started with adults of *S. watsoni*, which were collected in spring of 2012
104   and/or 2013 from five localities in the Czech Republic (Prague – Suchdol (15 May – 12 April
105   2012, 15 May – 12 April 2013), Běstvina (7 – 11 April 2012, 6 – 10 April 2013), Domažlice (28
106   May – 12 April 2013) and Klatovy (14 – 28 May 2013)).

107   Beetles were collected using 10 baited pitfall traps, placed at each locality. The traps were
108   composed of 1,080 ml plastic buckets (opening of 103 mm and 117 mm deep). These buckets
109   were embedded in substrate up to the rim to eliminate any obstructions which could deter beetles
110   from entering. As protection against rain metal roofs (150x150 mm) were put over the traps. The

111   roof was supported by four 100 mm nails, one in each corner, and placed approximately two
112   centimeters above the surface. The bait, ripened cheese (Romadur®) and fish meat (*Scomber*
113   *scombrus* Linnaeus, 1758), was placed directly inside the bucket on a shallow layer of moist soil.
114   This created good conditions for survival of the trapped beetles between servicing, which was
115   usually done once a week.

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

122   Groups were kept in Petri dishes with a layer of soil and small piece (approx. 5x5 mm) of fish
123   meat (*Scomber scombrus*) as a food source. The content of the dish was lightly sprayed with tap
124   water every day and food was provided *ad libitum* and changed to prevent fungal growth.

125   Dishes were randomly placed in one of six climate chambers (custom made by CIRIS s.r.o.). The
126   chambers were set up at constant temperature (12, 15, 18, 21, 25 or 28°C) and 16 hours of light
127   and 8 hours of dark photoperiod regime, maintained by fluorescent light (Osram L 8W/640). A
128   similar number of breeding groups from the same locality were placed in each chamber for
129   beetles from Praha and Běstvina. However, because few adults were obtained from Domažlice
130   and Klatovy, this was not possible, and they were kept together in one treatment (18°C).

131   An observation study of their natural behavior was also conducted in a small plastic box (15x6x2
132   centimeters) with 12 adult individuals (7 females and 5 males) from the Prague population. In
133   this colony, larvae were not separated from adults or each other, but were allowed to interact
134   freely and without intervention (measuring, photographing or other manipulations). The box itself
135   was placed in an 18°C treatment, and its inhabitants were attended in the same way as the
136   specimens in the breeding experiment (regular water spray and meat replaced to prevent fungal
137   growth).

138   In the breeding experiment, the method of handling eggs and first instar was changed slightly
139   between the years to improve the accuracy of observations. During the first year of experiment
140   (2012), dishes were searched for eggs which were transferred individually to a separate dish.
141   However, it was difficult to locate eggs of *S. watsoni* as they were very small and adults tended to
142   hide them in the substrate. Therefore, the estimation of egg and L1 development for the first year
143   was inconsistent and was not used in the models. In the second year (2013), the entire breeding
144   group was transferred to a new Petri dish every day. The old dishes were marked and kept in the
145   same climate chamber as the parents. Dishes were checked every day for emergence of the first
146   instar larvae that were further separated into their own dishes so that individual development
147   could be observed. The time when the eggs were laid was estimated as a half-time between the
148   transfers of the breeding group.

149 Larvae from the second year (2013) breeding experiment were photographed every day, starting
150 with their occurrence as the first instar larvae until pupation. In this way, morphological changes
151 were continuously documented during their development. To do this, the Petri dish was removed
152 from the climate chamber and was placed under the stereoscopic microscope to locate the larva,
153 which tended to stay near the food source. The larva was transferred with a fine brush to a white
154 sheet of paper and photographed (microscope Nikon SMZ-2T with attached camera Sony with
155 resolution of 768 x 576 pixels). Once a usable picture was obtained, the larva was returned to
156 Petri dish and back to the corresponding climate chamber. The whole process of finding the larva
157 and taking a picture did not usually take more than 1 min. Key developmental stages of each
158 larva, with the accurate date and time, could be distinguished based on photographs simply by
159 keeping track of the change in the width of their head capsule, because the width expanded after
160 each molt. Increase in the width over 0.1 mm was considered as a clear sign of the molt. This
161 strategy was very useful for data collection, because exuvia were not needed for confirmation of
162 molt to the next instar.

163 Because the dorsal side of larvae was photographed daily, many characteristics were monitored.
164 However, the thorax and abdomen of the *S. watsoni* larvae are not strongly sclerotized (Fig. 3)
165 and were thus omitted for instar determination, as well as the body length, which has too much
166 variation. Measuring of some smaller parts, such as urogomphi or antennae, proved impractical,
167 because it was very challenging to measure them accurately on a living and moving animal.
168 Chilling or CO_2 immobilization could not be used, because it would stress the specimens even
169 more and it could potentially affect the length of development.

170 The most stable and reliable feature for the instar determination of *S. watsoni* larvae was the head
171 capsule. This part of the body was strongly sclerotized, therefore it was not affected by water or
172 food content, but it changes in size after each molt so it is tightly linked with individual growth.
173 Also, the head does not change its size in different fixation media, or even after desiccation, thus
174 the instar can be identified even for very poorly handled and long dead specimens. Ultimately, the
175 head capsule width was chosen over its length for a practical reason. Head width of living larvae
176 did not change on the pictures captured from above, but length varied considerably.

177 For estimating the mean and standard deviation of the head capsule width (measured in the
178 widest point, see Fig. 3), I used all photographs where the head was clearly visible and was sharp
179 enough to make a precise measurement. All measurements were with graphical program
180 EidosMicro, calibrated by a precise ruler.

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

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

Results

194 In total, 81 adult specimens of S. *watsoni* were collected (Prague – 174, Běstvina - 178, Klatovy -
195 19, Domažlice - 28), and they produced 399 first instar larvae for the breeding experiment.
196 Because only twelve adults were obtained from Klatovy and six from Domažlice, it was
197 impossible to split them between temperature treatments, and they all were reared at 18°C.

198 In the breeding experiment, the duration of development of all *S. watsoni* stages was recorded,
199 namely egg, three larval instars (L1, L2 and L3) and pupae. These observations were made on
200 399 specimens in total, starting with the first instar larvae.

201 Higher temperatures (25 and 28°C) were probably limiting to breeding activity of the beetles in
202 the experiment. Ultimately I did not obtain any larvae from the 28°C treatment. Mortality in the
203 other treatments was also quite high, especially for the third instar and pupae (see Fig. 4) and
204 only 23 individuals developed until adulthood. Low temperature also prevented breeding, as I did
205 not observe any larvae in the 12°C treatment.

206 The development times differed between stages (Fig. 5) and the mean development time
207 decreased with increasing temperature (Fig. 6), except for L2 and L3 instars in the 25°C
208 treatment. The sum of effective temperatures (k) and lower developmental threshold (t) values
209 were calculated for all developmental stages of *S. watsoni* with expected errors (Table 1 and Fig.
210 7).

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

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

223 The mean width of the head capsule was a good additional characteristic for the instar
224 determination (see Table 2 and Fig. 8). Standard deviations were well separated, and there was
225 only a small overlap between 75th and 25th quintiles across all instars. I recorded some extreme

226 values on the both sides of the spectrum, but those were very rare. If head capsule measurement
227 is used along with morphological characters like chaetotaxy and brown spot on the head, as
228 described by Kilian & Mądra (2015), the accuracy and precision of larval instar determination of
229 *S. watsoni* may be improved.

Discussion

231 No larvae were obtained from the 28 and 12°C treatment, probably because adults did not
232 oviposit at this temperature or egg mortality was too high. The second claim is supported by the
233 fact that no eggs were found. However, as mentioned in the methodology section, eggs of *S.*
234 *watsoni* are tiny and may be overlooked, especially if there were only a few.

235 Mortality of the specimens in the breeding experiment was very high over all treatments
236 especially in the third instar. High mortality in this stage is not uncommon, but in this case it was
237 in sharp contrast with what was found in the observation study. The entire colony in the
238 observation study prospered and even increased in the number of adults over time. The only
239 difference between these two studies was that individuals were not separated and photographed in
240 the observation study.

241 Despite the increased mortality of some stages in the breeding experiment, the total length of
242 development (from egg until adulthood) did not differ significantly from values in the
243 observation study (ca. 28 days at 18°C) and also those reported by Kilian & Mądra (2015) (ca. 20
244 days at 20°C). Therefore larvae in the breeding experiment likely did not prolong development
245 due to unfavorable conditions. Larvae also did not increase or decrease number of their instars,
246 and they had to undergo three larval instars before maturation, which was also reported by Kilian
247 & Mądra (2015). Aggression or hostility was not observed between specimens, nor was there
248 cannibalism as has previously been reported for this species (Kilian & Mądra, 2015). However, it
249 was possible that it was missed due to the large number of larvae and adults in a box, close to one
250 hundred. The photographing process was not so intrusive to be responsible for high mortality
251 rates, and thus it is more likely that separation from other larvae and adults was probably the
252 reason for the increased mortality. Peck (1975) mentioned that the cave adapted beetle
253 *Ptomaphagus hirtus* (Tellkampf, 1844) (Leiodidae: Cholevinae: Ptomaphagini) needed soil from
254 its cave of origin to successfully complete development. Soil bacteria probably played some part
255 in this process, because specimens did not develop on autoclaved soil. In the experiments, it is
256 possible that adults feeding along with larvae could have provided such bacteria. Another
257 explanation could be that feeding of multiple individuals is much more effective or improves the
258 quality of the food source. As a support for this hypothesis, I observed a very rapid growth of
259 some fungi in Petri dishes with a single larva, but almost none in the observational study
260 containers where a large number of individuals were feeding.

261 The methodology of egg extraction was changed in the second year because eggs were easily
262 overlooked in the substrate, and beetles refused to lay their eggs in offered damp cotton wool
263 balls or small pieces of paper. To prevent bias in recorded time, a dish rotation methodology was

264 introduced, and adults stayed in the same dish only one day and then were moved to another.
265 Those used dishes were then regularly searched for emerging larvae. The main issue with this
266 approach (dish rotation) is that egg mortality could not be determined.

267 The mean development time decreased with increasing temperature (Fig. 6), except for L2 and
268 L3 instars in the 25°C treatment. Therefore, the optimal temperature of *S. watsoni* L2 and L3
269 instars may be between 21°C and 25°C (e.g., temperature with highest developmental rate where
270 the specimen is still able to reach maturity). Optimal temperatures for lower stages may be
271 higher, which agrees with the findings of Engler (1981), who reported *S. watsoni* as a warm
272 season species in contrast to some species of *Choleva* and *Catops* that prefer to breed during the
273 winter season, and their optimal temperatures for development were below 16°C (e.g., *Catops*
274 *nigricans*, C*holeva agilis* and *Ch. elongata*). This hypothesis also agrees with the findings of
275 Ridgeway et al. (2014) who reported that the optimal temperature for Afrotropic *Thanatophilus*
276 *mutilatus* is between 14 - 25 °C.

277 Measuring development time for pupae was even more challenging due to the fact that they did
278 not pupate close to the wall of the Petri dish. Searching for them was sometimes unsuccessful,
279 and some specimens surprised us when they reappeared as adults, because they had been
280 recorded as missing and presumed dead.

281 The methodology of measuring the size of the instars was based on continual observation of
282 separated individuals, from egg until pupation, so stage-specific information was available
283 regardless of their size. This approach differs from other studies with similar goals (Velásquez &
284 Viloria, 2010; Fratczak & Matuszewski, 2014), where authors tried to estimate the stage of
285 development based on the size of selected characteristics without prior knowledge of the true
286 stage of the specimen. The latter approach can be problematic, because measured characteristics
287 are correlated, and therefore larger larvae could be misidentified as a later instar. This bias would
288 probably not affect the obtained mean values, but it would give a distorted picture of variation
289 and ultimately give false confidence in determinations. This approach could be corrected if
290 information about the risk of erroneous determination would be calculated beforehand (see
291 Merville et al., 2014).

292 The data demonstrated that instars have some overlap in the head widths, especially true for the
293 first and second instar. In these regions the uncertainty of determination based solely on the head
294 width is very high, so the use of some additional morphological characteristics is in place. A first
295 instar larva has only primary setae on its body and the head is without any colored spots, but after
296 molting to the second instar, a secondary set of setae will emerge and a brown spot will appear on
297 the head (light brown and not fully defined) (Kilian & Mądra, 2015). Setae are also present
298 without any change on the third instar larvae, but the brown spot is much darker with sharp and
299 well defined edge (Kilian & Mądra, 2015). Thus chaetotaxy and pigmentation of the head can be
300 used for the discrimination of all instars. The data provide developmental parameters for *S.*
301 *watsoni* together with a new and reliable characteristic for instar determination. This species is so
302 far the smallest necrophagous beetle with a known thermal summation model. The developmental
303 characteristics provided in this study will help to more accurately estimate the PMImin.

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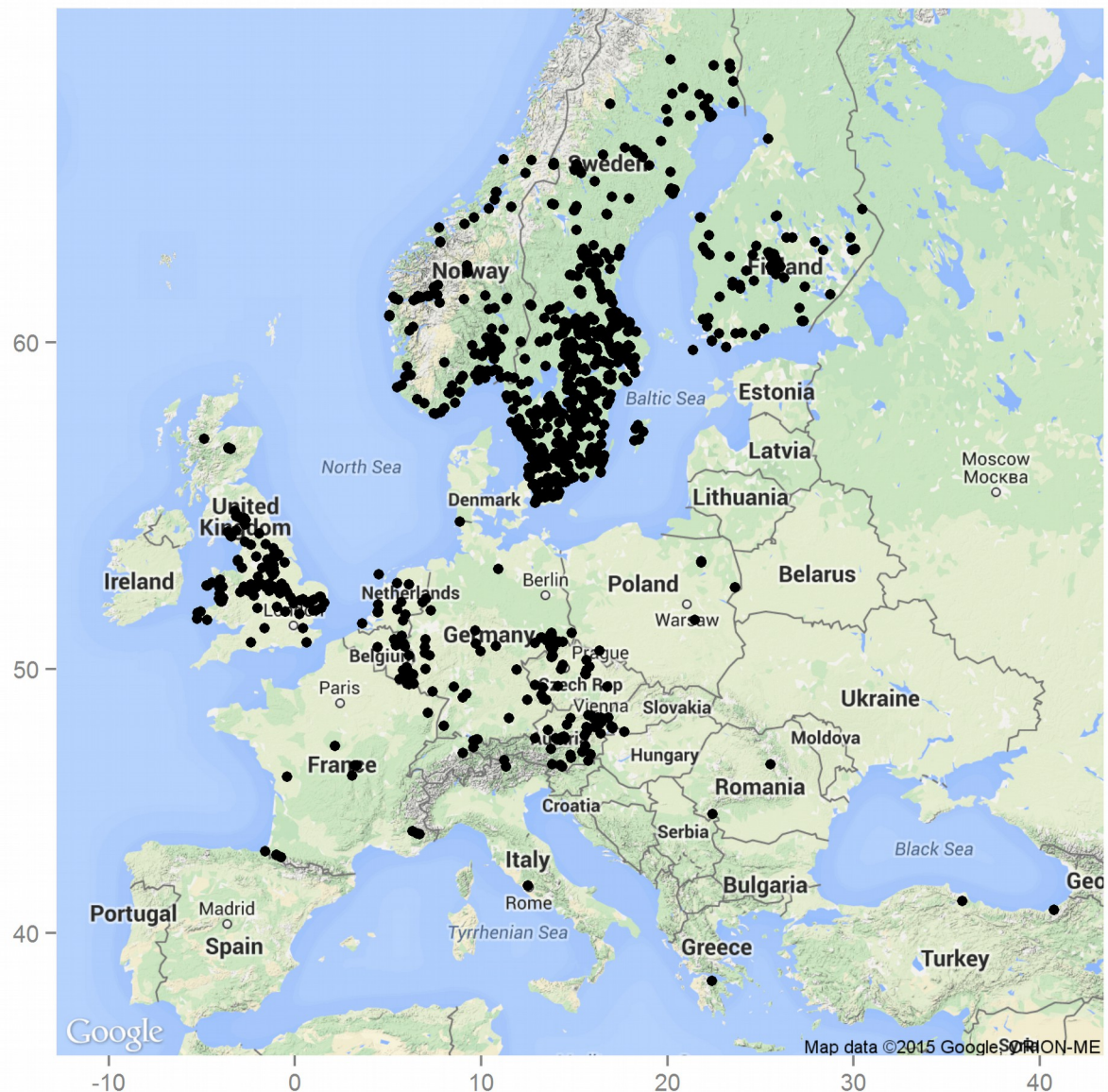
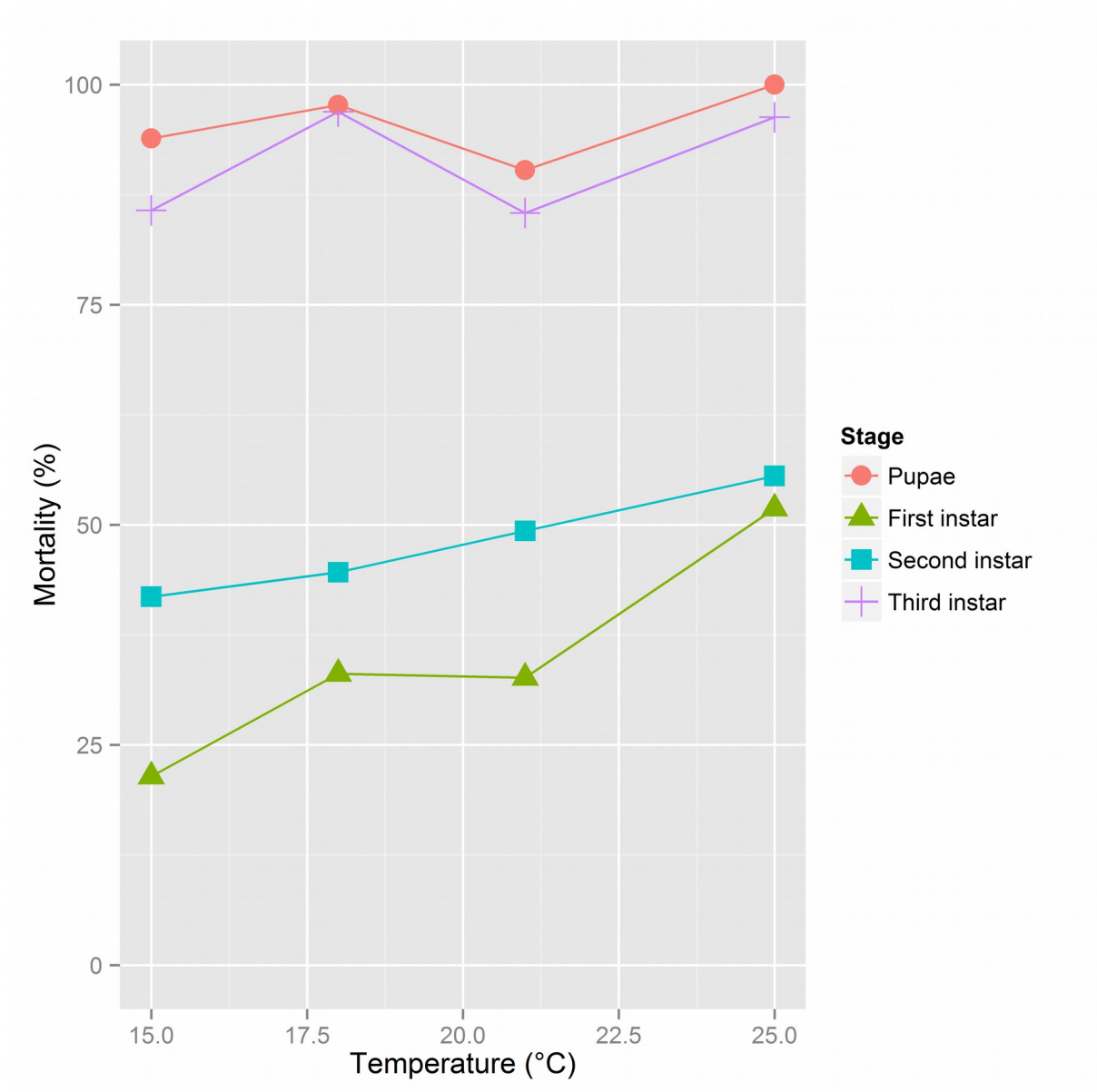
444 Table 1: Summary of development constants for *S. watsoni* for five developmental stages. Sum of
445 effective temperatures (k) and lower developmental threshold (t) shown as means with standard
446 errors (coefficient of determination (R2) and degrees of freedom (Df) and p values are provided).

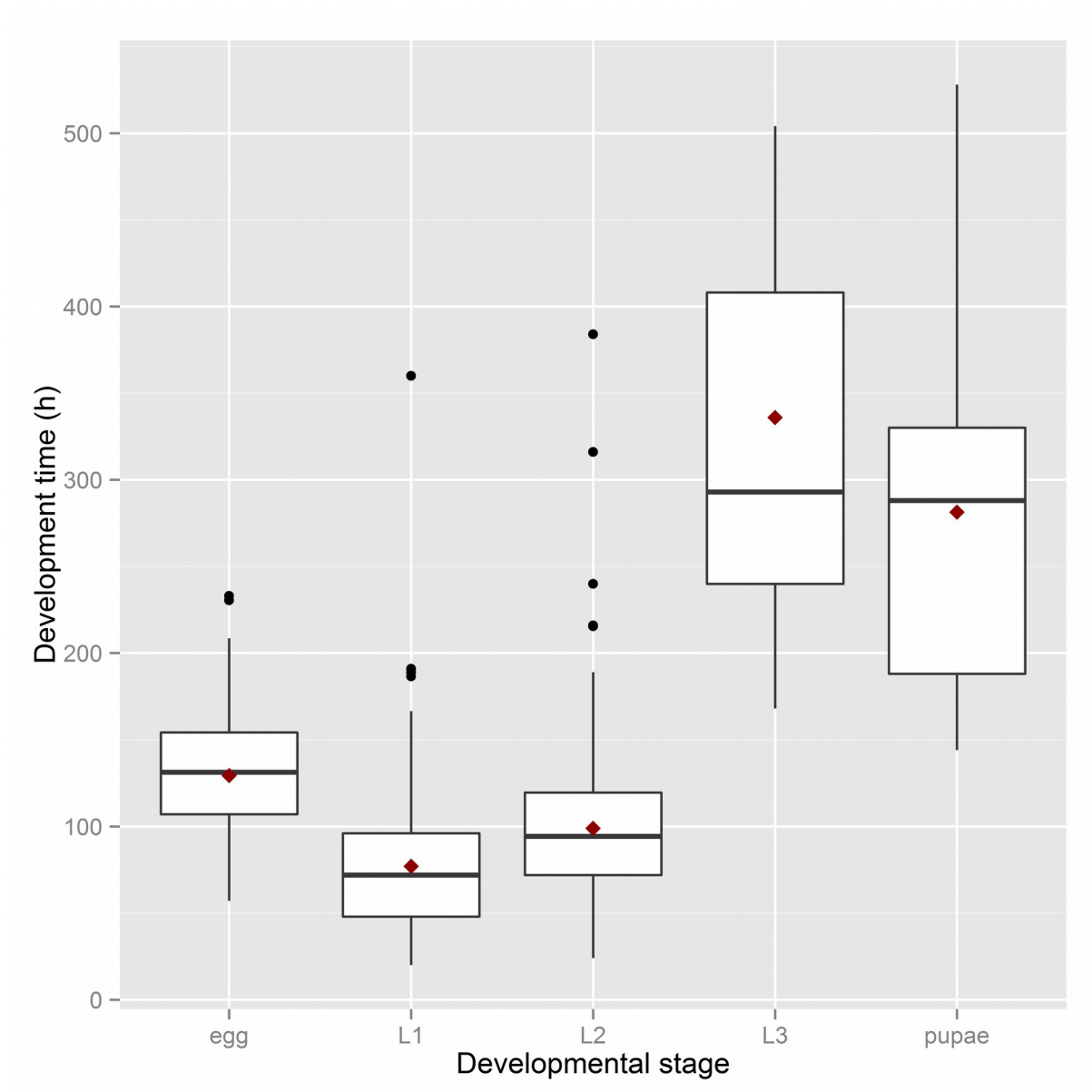
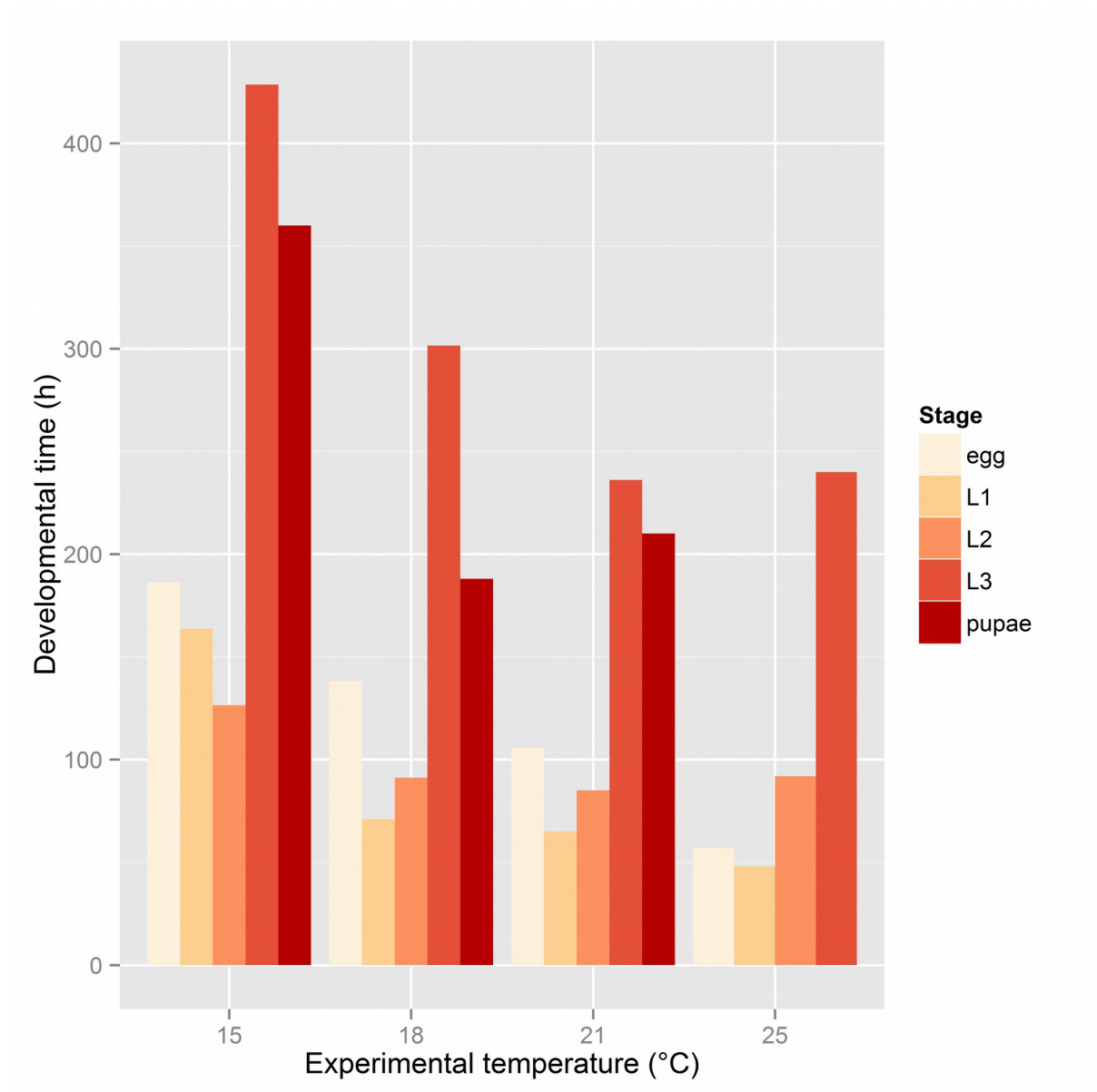
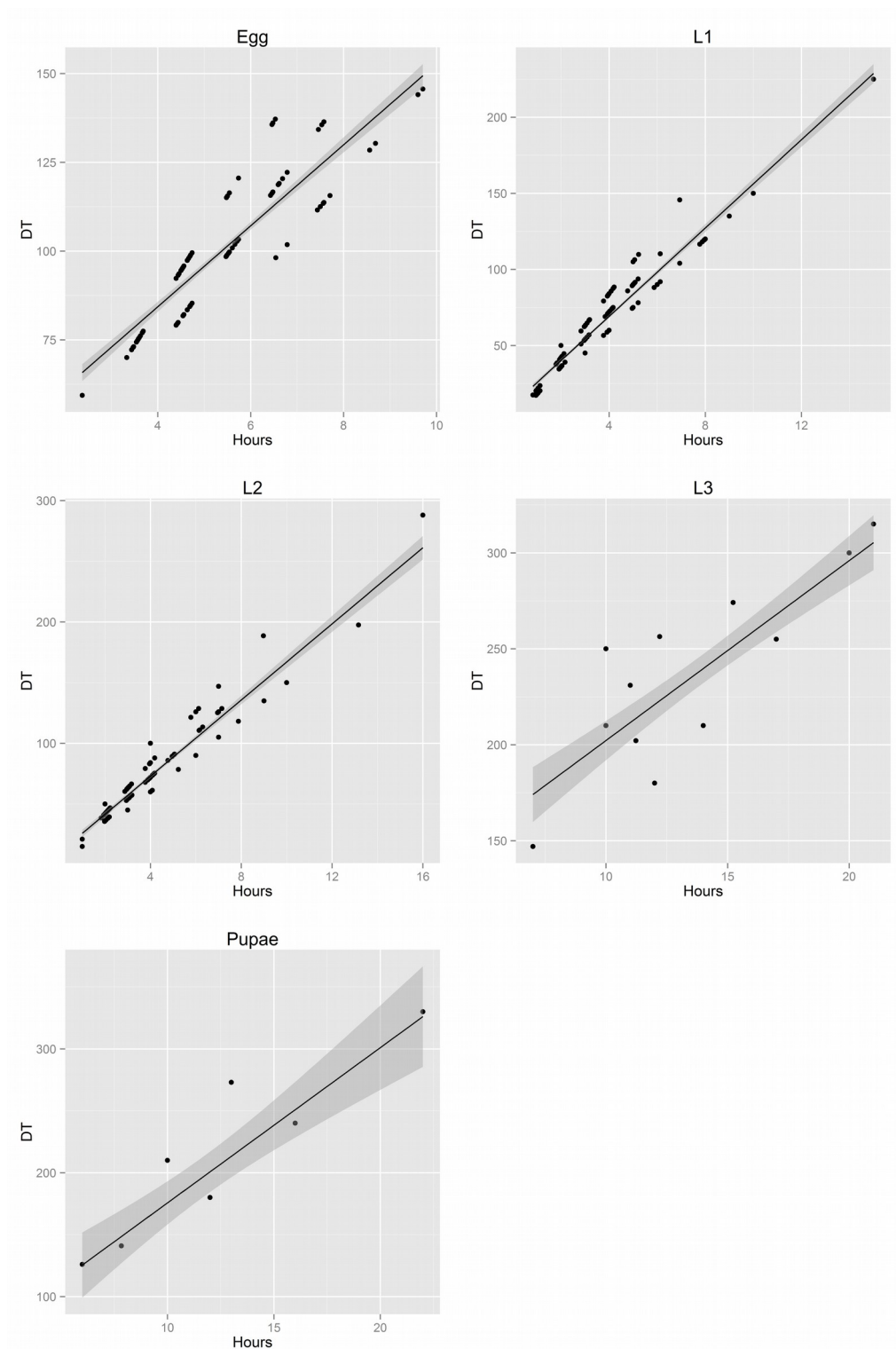
Stage	Temperature range	R^2	Df	p value	k	t
Egg	15-25	0.8134	220	2.20E-16	929.354 ±49.111	11.400 ±0.368
L1	15-25	0.9375	171	2.20E-16	233.683 ±27.031	15.437 ±0.305
L2	15-25	0.8768	206	2.20E-16	243.945 ±45.301	15.689 ±0.410
L3	15-25	0.8199	27	1.49E-11	2602.996 ±297.464	9.375 ±0.846
Pupae	15-21	0.8563	10	1.61E-05	1207.431 ±489.288	12.535 ±1.624

447 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

448   Fig. 1: Occurrence of *S. watsoni* in Europe based on observations and records from the GBIF
449   database (GBIF, 2015). Underlying map generated by package ggmap (Kahle & Wickham,
450   2013).Map data ©2015 Google.

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

452   Fig.3: Dorsal (A), lateral (B) and ventral (C) side of the third larval instar of *S. watsoni*. Point
453   where the head width was measured (the widest point of the head capsule) is shown (a).

454   Fig. 4: Mortality rates between developmental stages of *S. watsoni* over a range of experimental

455   temperatures, except for 12 and 28°C, where beetles did not breed successfully.

456   Fig. 5: Observed range of development times of *S. watsoni* over four experimental treatments (15,
457   18, 21, 25 °C) for each developmental stage (2012 data were excluded for egg and L1). The
458   horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the
459   75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile
460   ranges. Small, black dots are outliers. Small red dots are the mean values of development time.

461   Fig. 6: Bar plot of mean development time (in hours) of all observed stages (2012 data were
462   excluded for egg and L1) of *S. watsoni* over a range of experimental temperatures, except for 12
463   and 28°C, where beetles did not breed successfully.

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

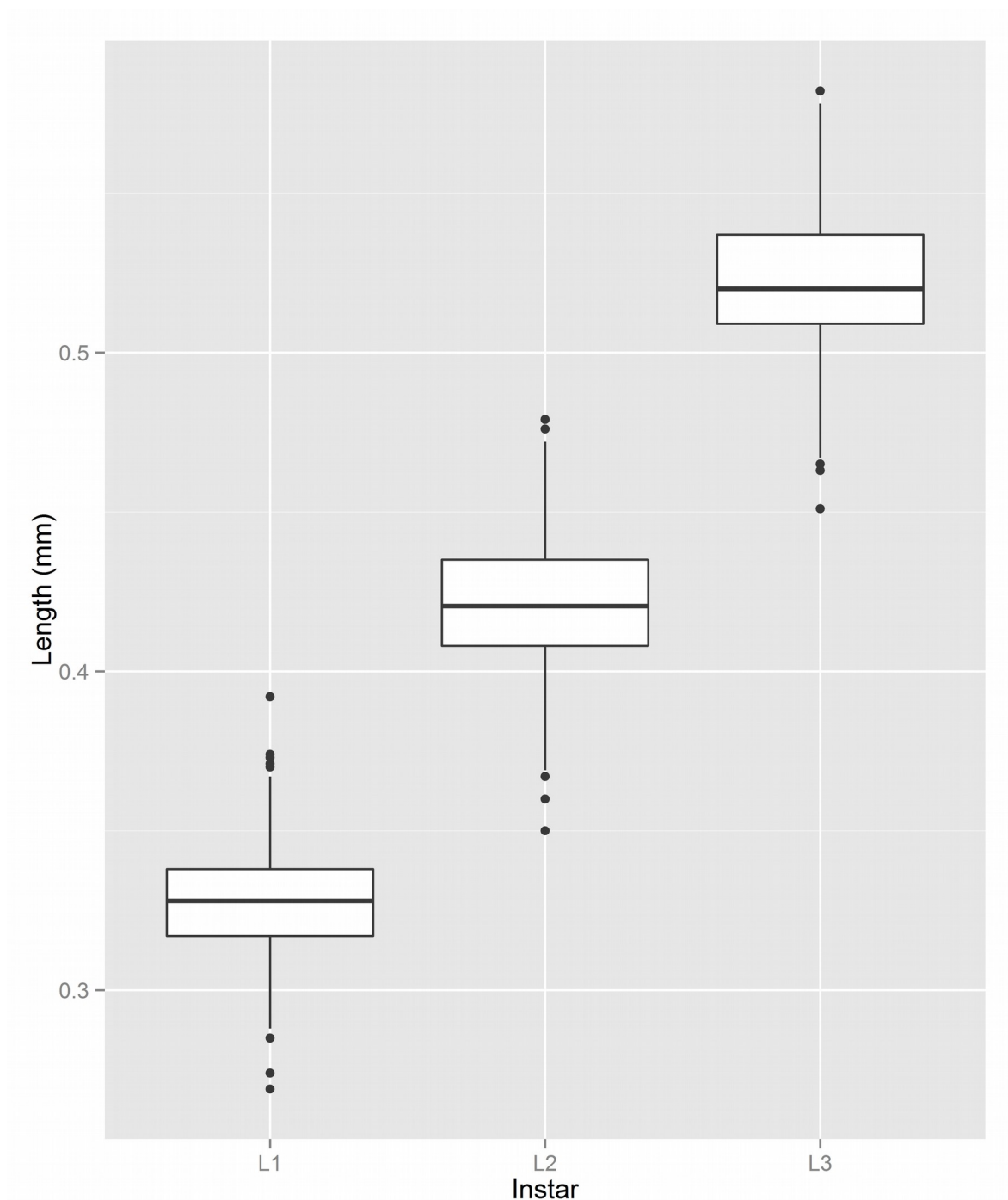



Fig. 8: Box plot graph of lengths of all three instars (L1, L2 and L3) of the *S. watsoni* larvae. The
horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the
75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile
ranges.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