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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 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.

Comment [O1]: I think it is not necessary in abstract

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3
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8
Abstract
9
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10
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information regarding developmental biology and instar determination of *Sciodrepoides*

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standard errors were calculated for each developmental stage. We also propose head width as a 19
new character for larval instar determination together with a new methodology for future studies 20
of size based characters. 21

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 (PMImin) 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.* ,

Comment [O2]: Is it one model or more?

Comment [O3]: There are many previous studies on larval morphology with head width measurements and it is clear that one of good characters for determining larval instars is head width ,especially in groups with poor chaetotaxy. It would be good to underline this well-known rule and support references. And it would be good better underline that for this species, there are other good characters, especially brown spot on head and chaetotoaxy.
Table 2 show that maximum head width L1 is bigger than minimum HWL2, similarly, maximum HWL2 is bigger than min HWL3 .the reason why should be better explained by author because this results show that head width is NOT good or enough for determining larval instar in this species. Mean value is , in determinig specimens of larva, practically useless. I think author should more underline importance of Thermal model than head width.

What's more, author does not provide detail information in which points, exactly, head width have been measured. It should be given in methods (the widest point, below antenna ?).
It is valuable that author provided a large data on head width, which enable better understanding changes among instars although this character is probably overrated.

Comment [O4]: One thermal models or others, it is not clear for me

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 PeerJ reviewing PDF | (2015:09:6548:0:0:NEW 2 Sep 2015)

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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 PMImin 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 PMImin
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 PMImin 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; Fratczak & 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

Comment [O5]: Examples, references

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 Klatovy (14 – 28 May 2013)).

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

Comment [O6]: I can't agree with this statement. Description of larval stages has the same main t purpose as description new species_ to analble other people to discriminate, determine. In most examples, onlu detailed observations make possible of determination of larval instars.

Comment [O7]: It is not true , please read description of second, and third instars.

Comment [O8]: I can't find in result or discussion exact explanation, summary of this method and providing combination of size based and morphological characters.

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~~observed 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~~For the reason 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) of breeding experiment was photographed every day,

Comment [O9]: ?

Comment [O10]: Freely and without intervention means the same

135
starting with their occurrence ~~as~~ from 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.

Comment [O11]: unclear

Comment [O12]: unclear, howtried to keep track?

Comment [O13]: Plenty of data? Pictures?if characters, it is unclear what exactly, why author chose only bead width

Comment [O14]: It is not good reason for omitting characters

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

Comment [O15]: Low resolution of camera is also not reasonable and scientific explanation of omitting some characters. Try to explain it in more scientific way.

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 (°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 and recorded, directly or indirectly, ~~and recorded~~ duration of the 180
development of all *S. watsoni* stages, namely eggs, 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.

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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
chambers 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 (L1 = 591, L2 = 500 and L3 = 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

Comment [O16]: See also comment below. Please, explain /resolve overlaping measurement of head width of instars and how you check when larva with head width 350 mm, is second or first. And how, that's why, use this character in legal investigation.

Comment [O17]: See comment above

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 & Mądra, 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; Fratczak & 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

Comment [O18]: The idea is good but practically, is it possible to observe individuals when you have hundred larvae in one petri dish? Maybe this is a reason of overlapping of head width and should be repeated/or larvae with border head width should be examined better(chaetotaxy, other measurements under light microscope) and then decided to which instar sthey belong.Author write about in 259-263 verse but I do not if he used this methods to check his own larvae with border measurements.

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 PeerJ reviewing PDF | (2015:09:6548:0:0:NEW 2 Sep 2015)

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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 PMImin in cases where it was not possible before. The instar 268
determination is the integral part of the PMImin 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.
272

Acknowledgements

273
I would like to thank A. Honěk and P. Saska for sharing their insight about beetle development 274
and construction of thermal summation models. I am also grateful to Jan Růžička and Max
275
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276
not have been possible without the help of my students from the Czech University of Life
277
Sciences Prague: T. Račáková, J. Pšajdl and M. Slachová, who took care of the experiment at 278
times I could not. The project was supported by the Internal Grant Agency of Faculty of
279

Comment [O19]: Better is using brown spot of head - is well visible in second and third instars. Did author observe this character during his study?

Environmental Sciences, CULS Prague (no. 421101312313020154221).

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Comment [O21]: Pages, give full name of journal

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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.

Temperature				
Stage				
range				
R2				
Df				

Comment [O22]: please

***p* value**	
k	
t	
22	
929.354 ±49.111	
11.400 ±0.368	
Egg	
15-25	
0.8134	
0	
2.20E-16	
17	
233.683 ±27.031	
15.437 ±0.305	
L1	
15-25	
0.9375	
1	
2.20E-16	
20	
243.945 ±45.301	
15.689 ±0.410	
L2	
15-25	
0.8768	
6	
2.20E-16	
2602.996	
9.375 ±0.846	
L3	
15-25	
0.8199	
27	
1.49E-11	
±297.464	
1207.431	
12.535 ±1.624	
Pupae	
15-21	
0.8563	
10	
1.61E-05	
±489.288	
395	

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

Instar

Comment [O23]: please, explain this abbreviations

Comment [O24]: se

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

Comment [O25]: see previous comments in the text

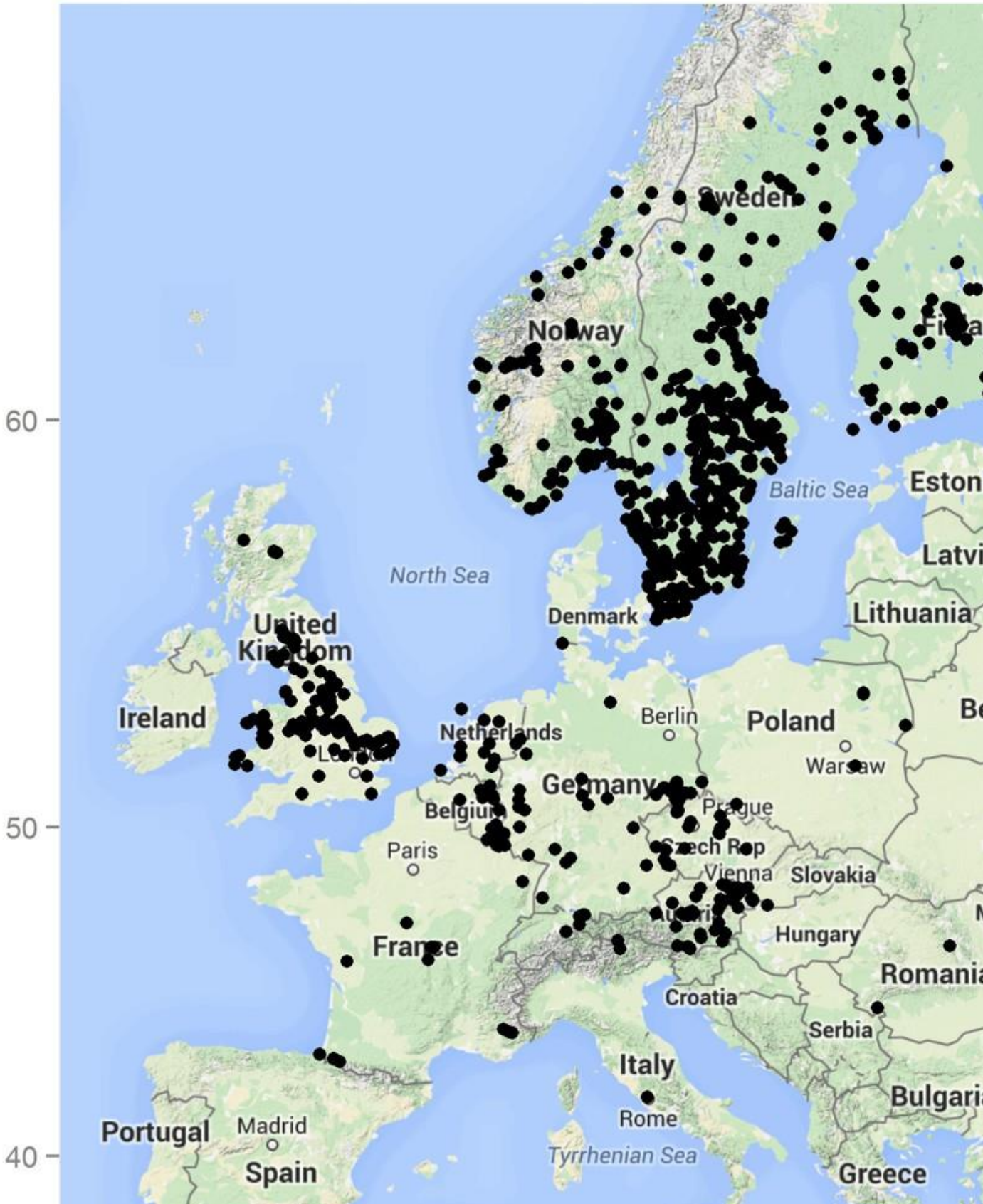

60
50
40
Sweden
Norway
Baltic Sea
North Sea
Denmark
United Kingdom
Ireland
Netherlands
Berlin
Poland
Warsaw
Germany
Belgium
Paris
Czech Rep
Vienna
Slovakia
France
Hungary
Croatia
Serbia
Italy
Rome
Portugal
Madrid
Spain
Tyrrhenian Sea
Greece
Lithuania

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).


1 mm

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

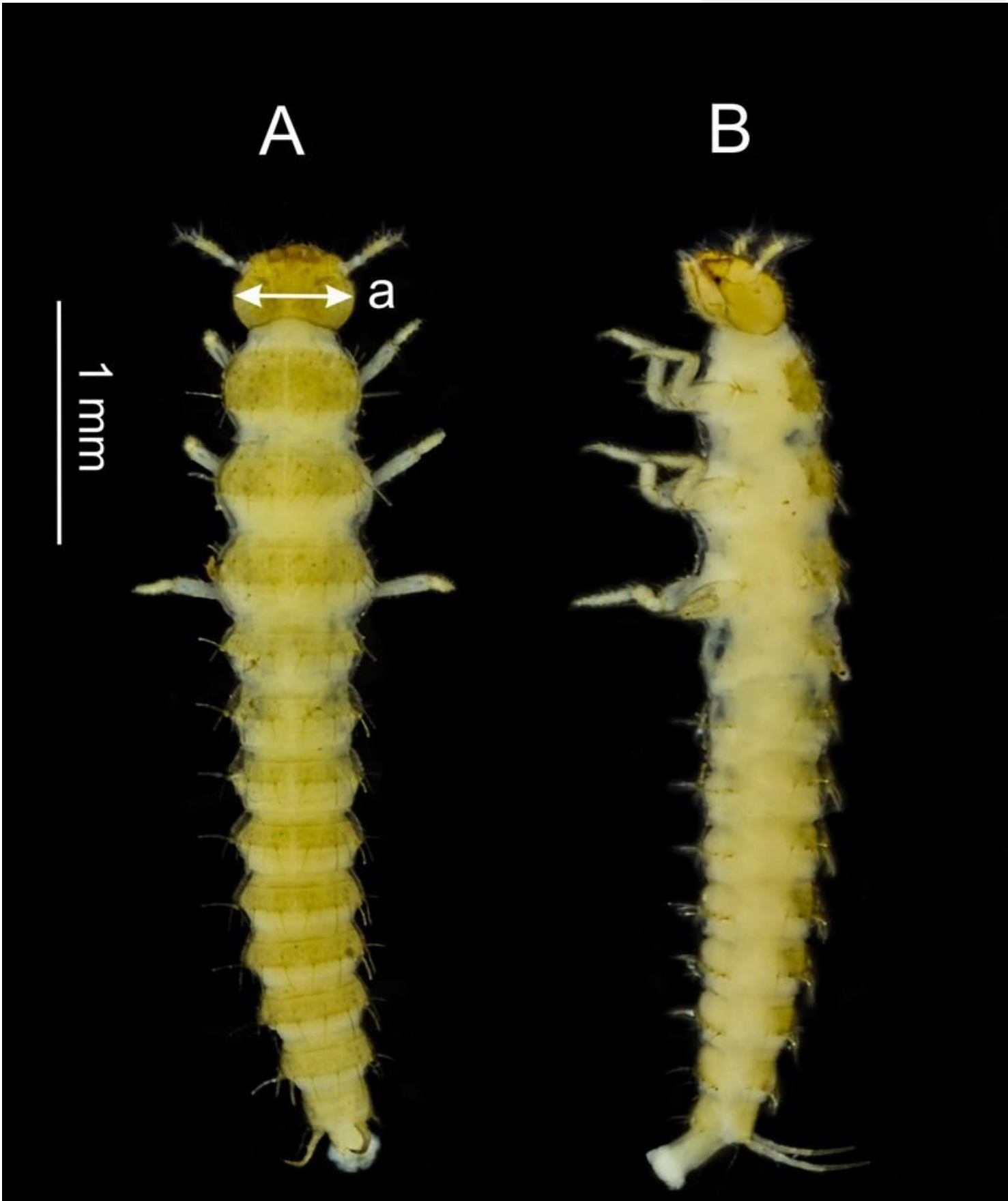

A
B
a
1 mm

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).

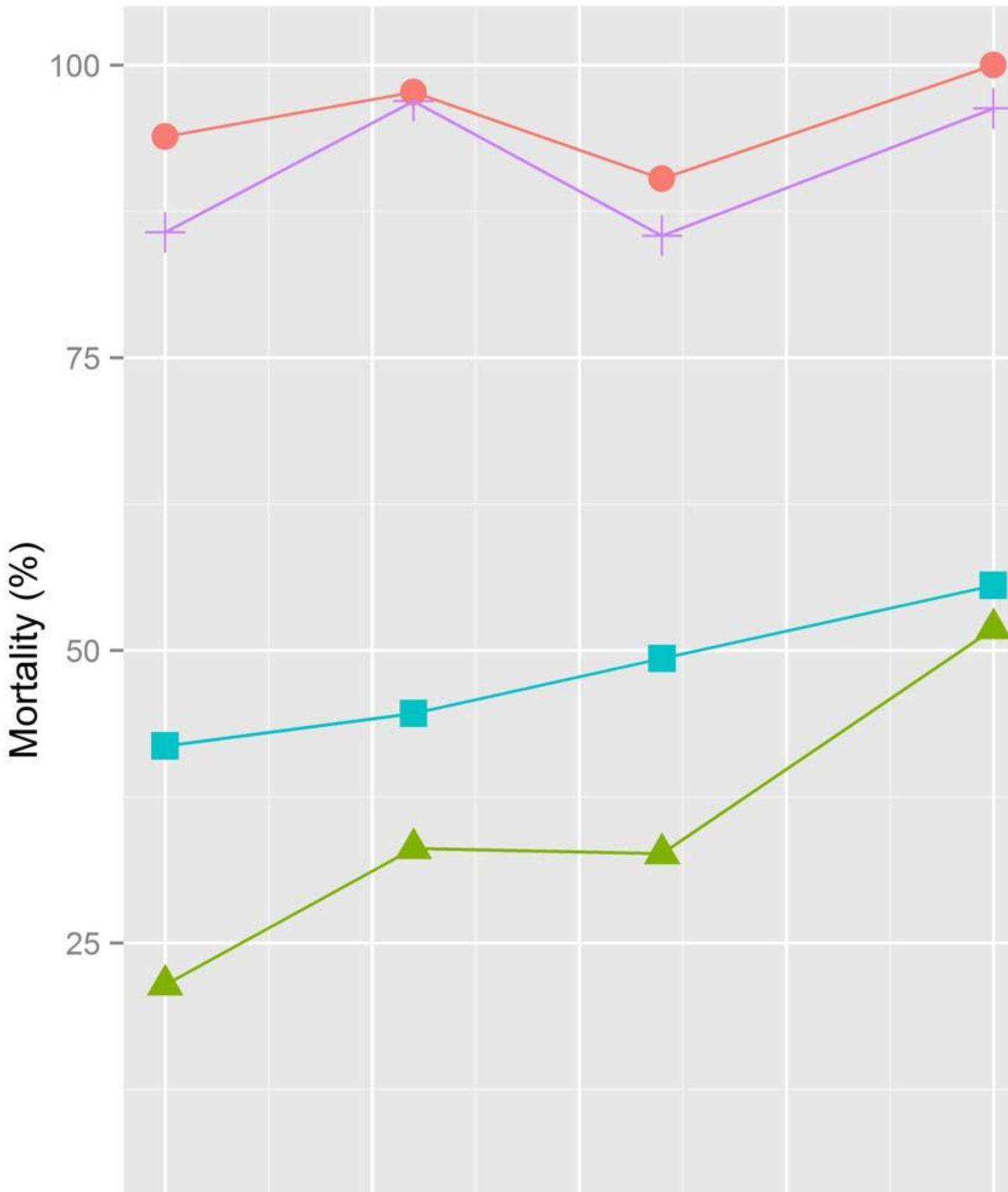
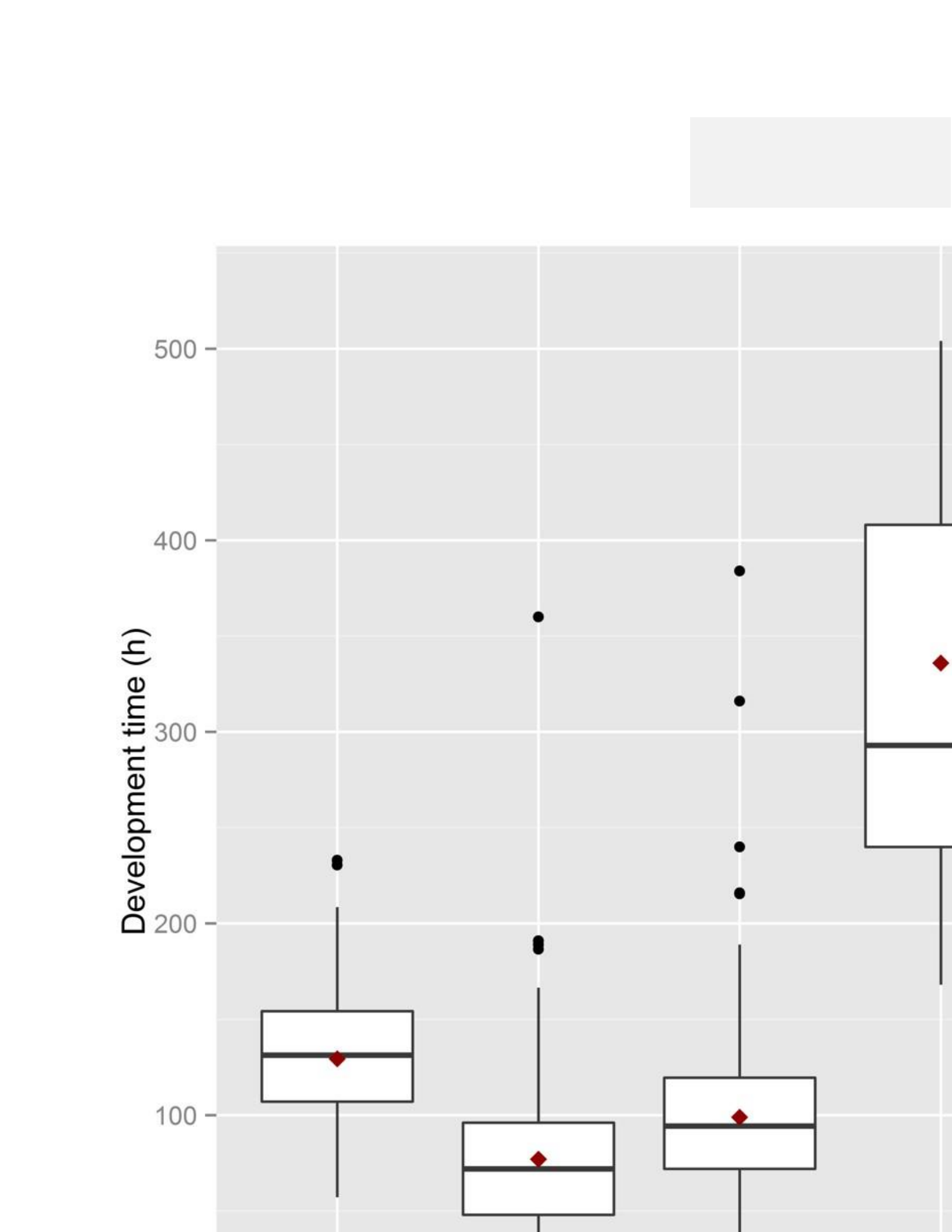

100
75
50
25
Mortality (%)

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


Development time (h)
500
400
300
200
100

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.

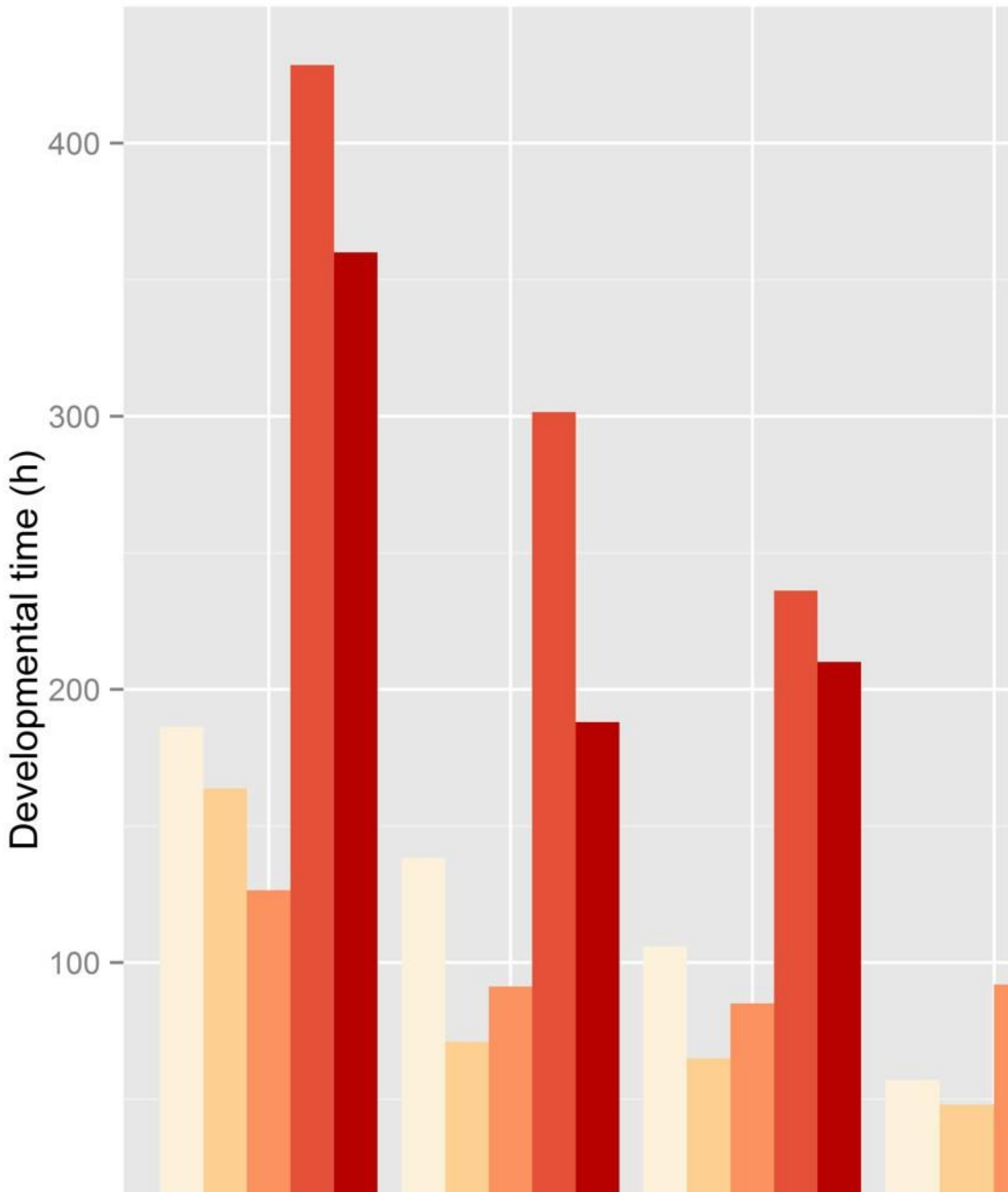
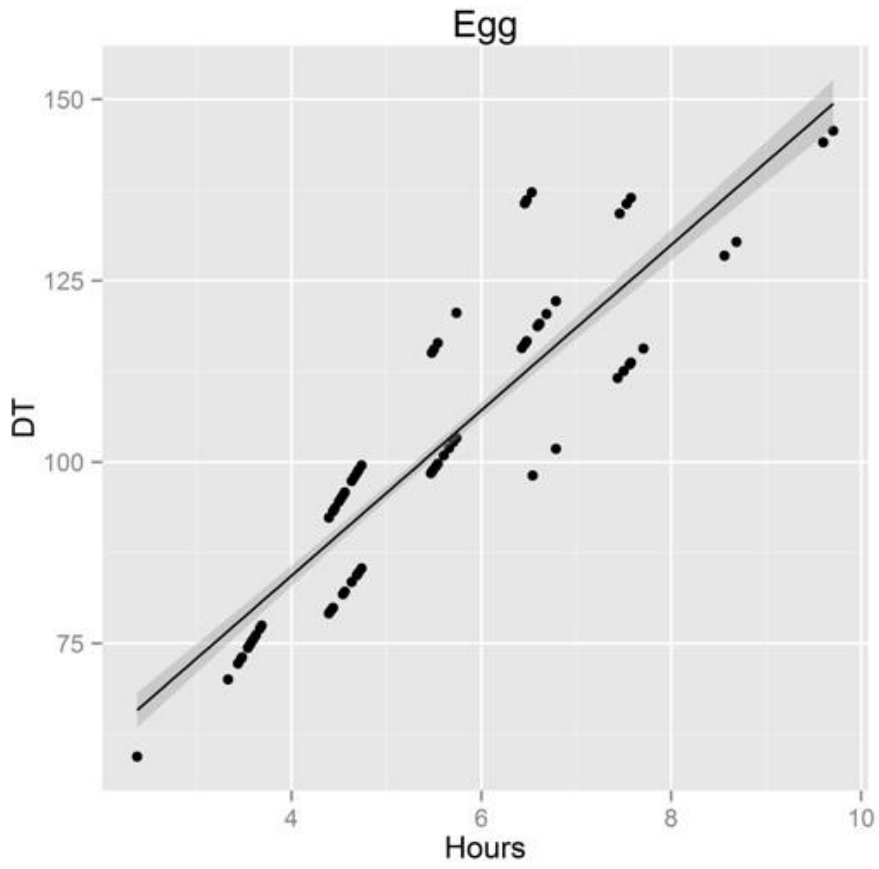
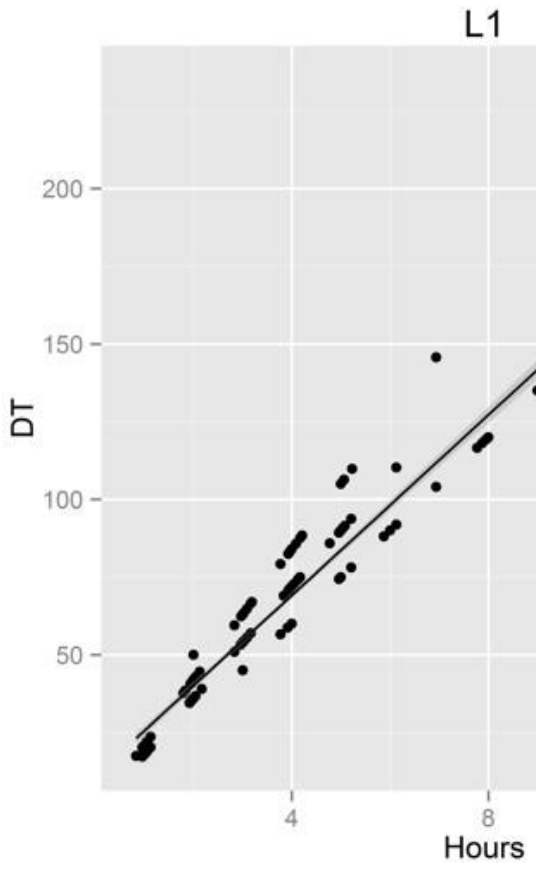
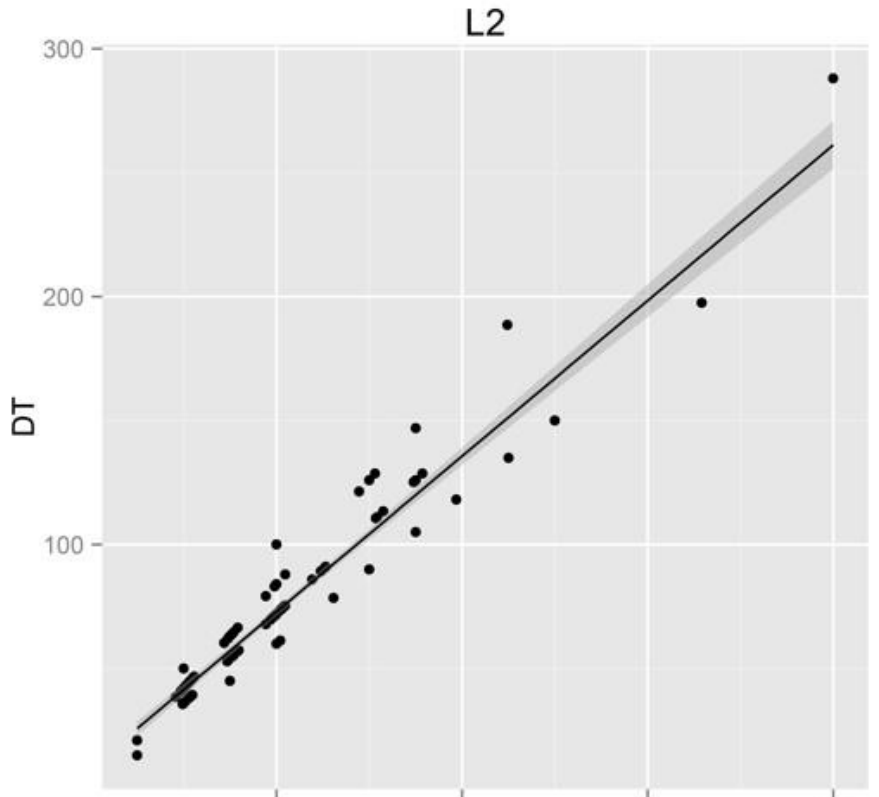
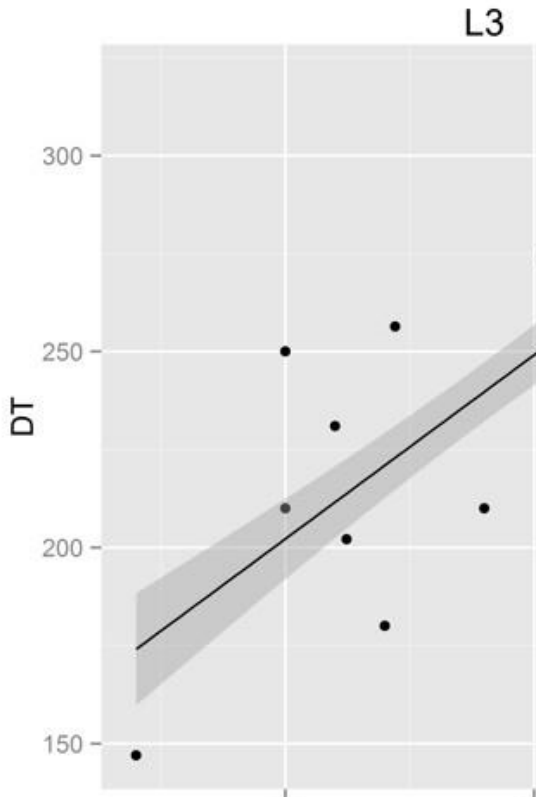

Developmental time (h)
400
300
200
100

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.

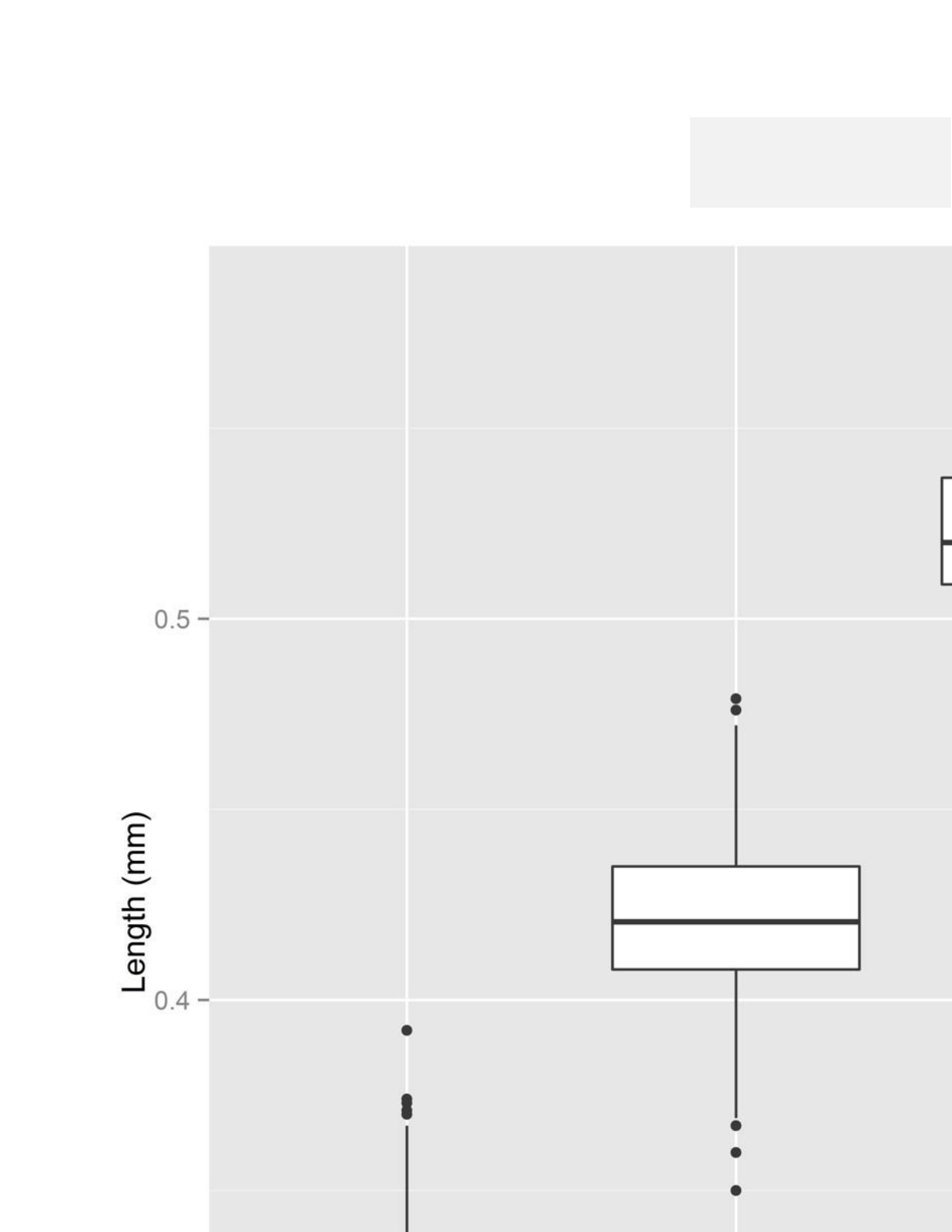

Egg
DT
150
125
100
75
4
6
8
10
Hours


L1
DT
200
150
100
50
4
8
Hours


L2
DT
300
200
100


L3
DT
300
250
200
150

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.


Length (mm)
0.5
0.4

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.