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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.

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2

developmental stages of necrophagous beetle, *Sciodrepoides watsoni*

3

(Spence) (Coleoptera: Leiodidae: Cholevinae)

4

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Abstract

9

Necrophagous beetles are underrepresented in forensic entomology studies despite their 10

undeniable utility for the field. In our article we would like to address this problem and 11

provide information regarding developmental biology and instar determination of *Sciodrepoides*

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

watsoni 12

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collected adult specimens from several localities across the Czech Republic to establish a 14

laboratory culture with constant temperature regime and long day photoperiod. These 15

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divided between five treatments that differed only in temperature (15, 18, 21, 25 and 28°C). 16

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documented every day until adulthood. Parameters of thermal summation **models** and their 18

standard errors were calculated for each developmental stage. We also propose **head width** 19
as a 19

new character for larval instar determination together with a new methodology for future 20
studies 20
of size based characters. 21

Introduction 22

Forensic entomology is a rapidly developing new field of science (Midgley *et al.* , 2010). 23

New 23
methods and models for estimation of minimum post mortem interval (PMI_{min}) are 24
developing 24

at a very rapid pace (e.g., pre-appearance interval, gene expression during larval 25
development, 25

quantile mixed effects models, generalized additive modeling or generalized additive 26
mixed 26

modeling) (Matuszewski, 2011; Tarone & Foran, 2011; Baqué *et al.* , 2015a, 2015b), but 27
even the 27

well-established models lack actual data for their further use and application. A good 28
example is 28

the commonly used thermal summation model (Richards & Villet, 2008). This model, 29
which is 29

based on the assumption that development of immature stages is linear, has been known for 30
30

several decades (Higley *et al.* , 1986), but it is still not established for the majority of 31
forensically 31

important species of invertebrates, which would be a great contribution to legal 32
investigations. 32

Currently **these models** are known for a number of fly species (Diptera) (Nabity *et al.* , 33

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 & Viloría, 2009; 37

Ridgeway *et al.* , 2014).

38

However, using beetles for PMIm 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 PMIm 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

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 & Viloría, 2009). *T.* 51

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 PMIm 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 & Vilorio, 2010; Fratzak & 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
Sciodreporides 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-~~
Sciodreporides 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



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74

found on decaying corpses of vertebrates in various types of habitats where they feed and develop

(Růžička, 1994; Peck & Cook, 2002; Topp, 2003).

76

Egg, all larval instars and pupae of this beetle were properly described recently by (Kilian & 77

Mađra, 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 & Mađra, 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 & Mađra, 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), Běstvína (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

Comment [O6]: I can't agree with this statement. Description of larval stages has the same main purpose as description new species, to enable other people to discriminate, determine. In most examples, only 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.
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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 Běstvína, 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.

Comment [O11]: unclear

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.

Comment [O12]: unclear, how tried to keep track?

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.
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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



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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.

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.

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, Běstvína - 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 overlapping 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 & Mađra, 2015), but it is possible
that we 221
missed it, because the estimated number of individuals in the box was close to one hundred.
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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* (Tellkamp, 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 approach differs from other studies with similar goals 251

(Velásquez & Vilorio, 2010; Fraczak & 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 they 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 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 discrimination of the first and second instar larvae. For additional differential diagnosis of those morphological characters, see (Kilian & Mađra, 2015).

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

264

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268

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271

Acknowledgements

272

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Sciences Prague: T. Račáková, J. Pšajdl and M. Slachová, who took care of the experiment times I could not. The project was supported by the Internal Grant Agency of Faculty of

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times I could not. The project was supported by the Internal Grant Agency of Faculty of

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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?

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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

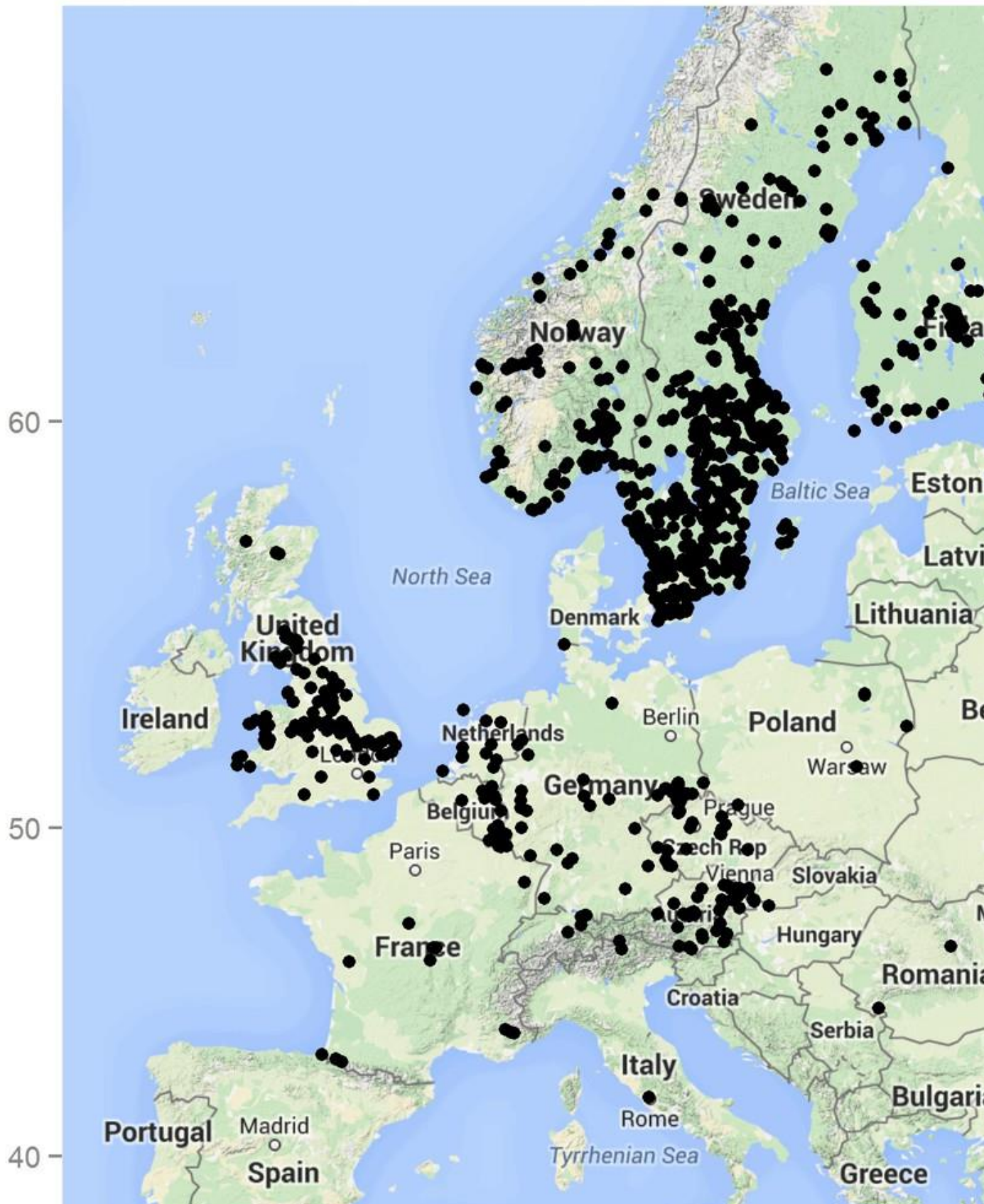
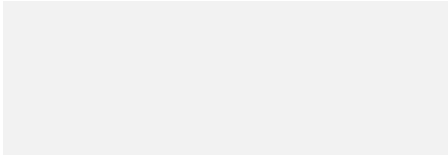
0.451

0.522

0.021

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Comment [O25]: see previous comments in the text





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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).

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1 mm



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399

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

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A

B

1 mm

a





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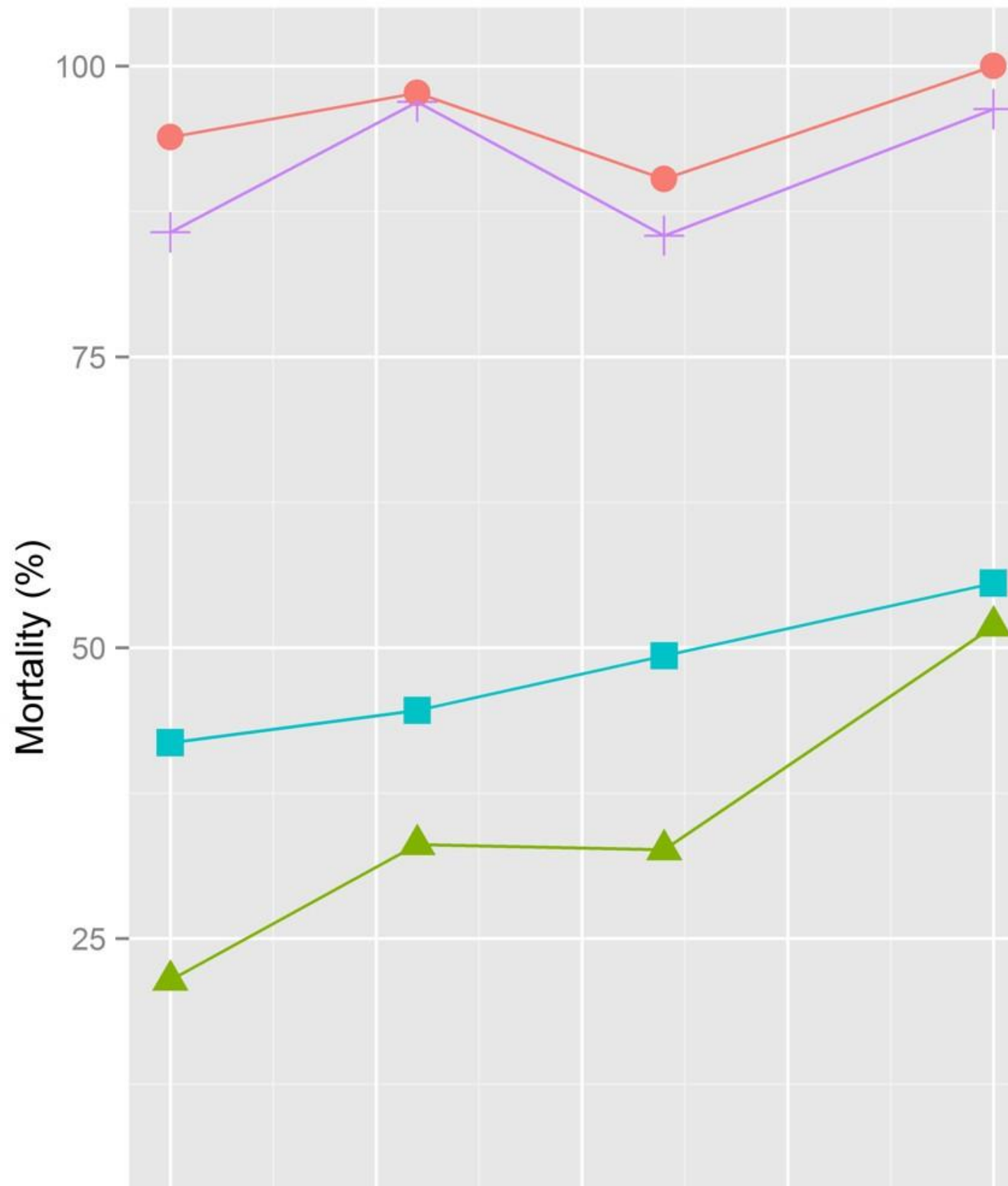
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Fig.3: Dorsal (A), lateral (B) and ventral (C) side of the third larval instar of *S. watsoni*.

Point 401

where the head width was measured is shown (a).

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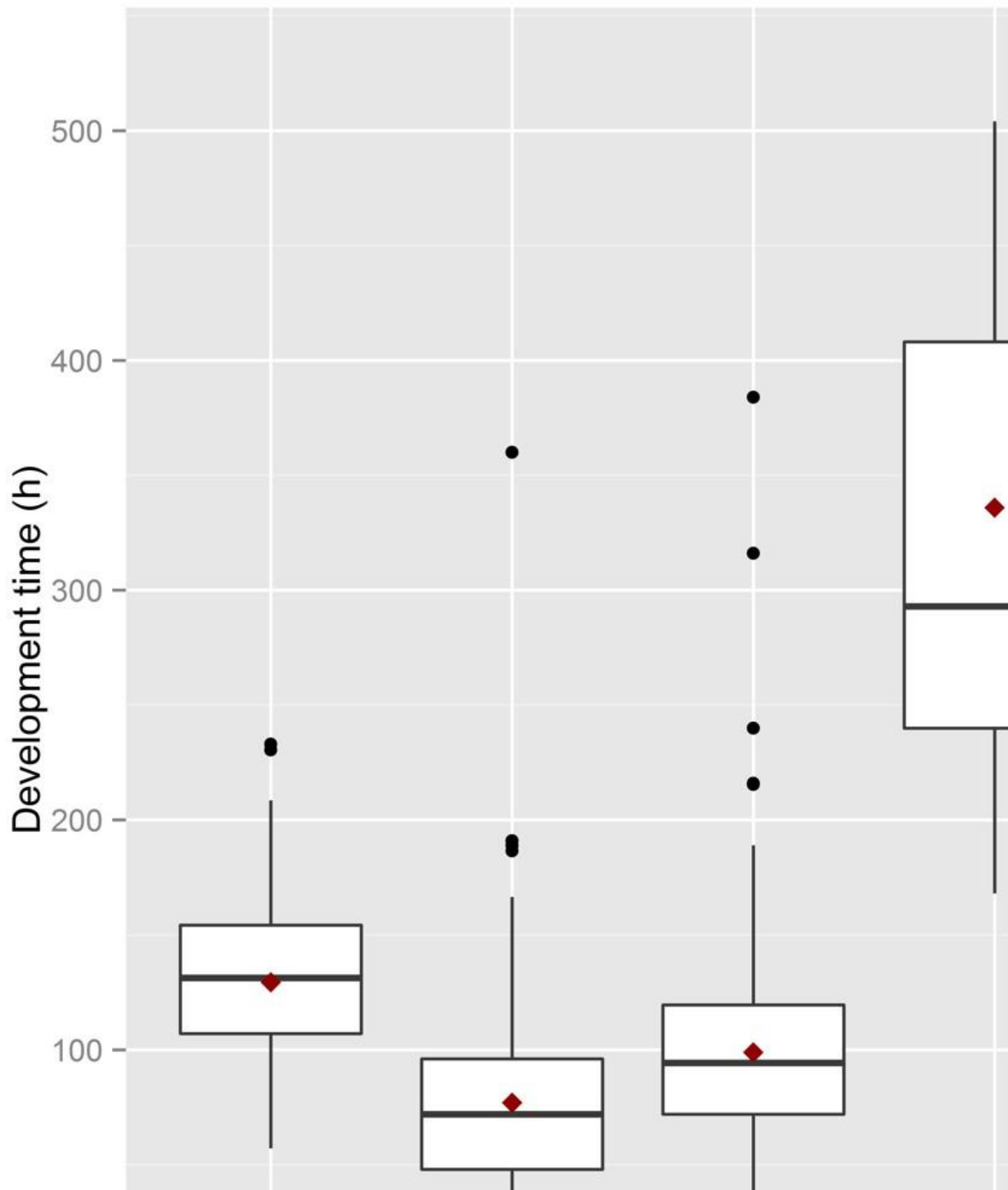
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Fig. 4: Mortality rates between developmental stages of *S. watsoni*. The 28°C treatment is not 403

shown, because breeding did not occur.

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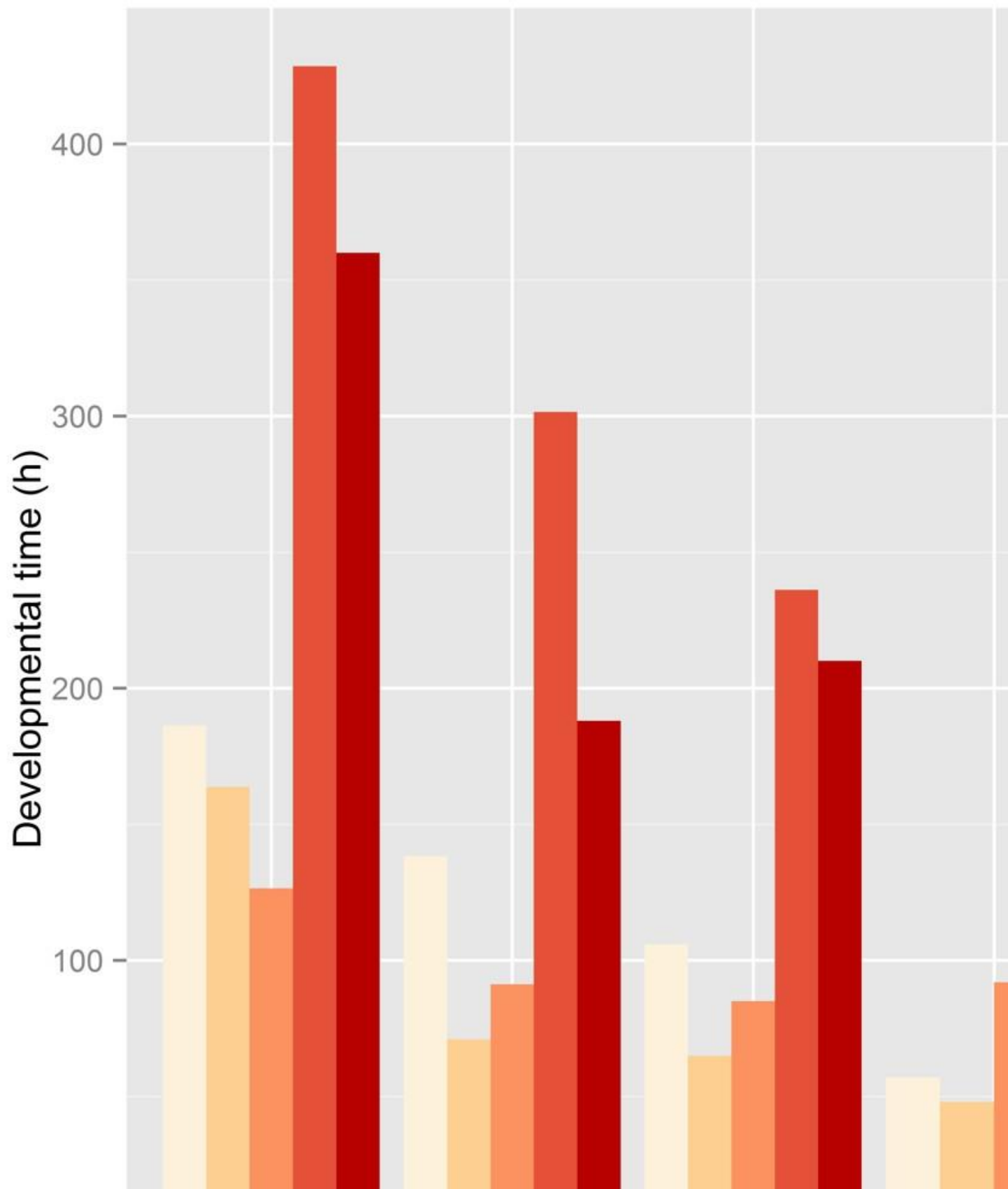


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404

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.

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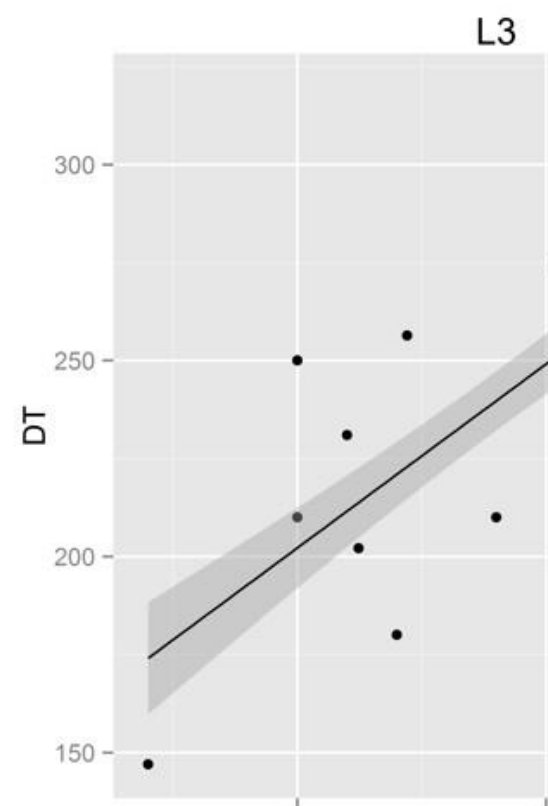
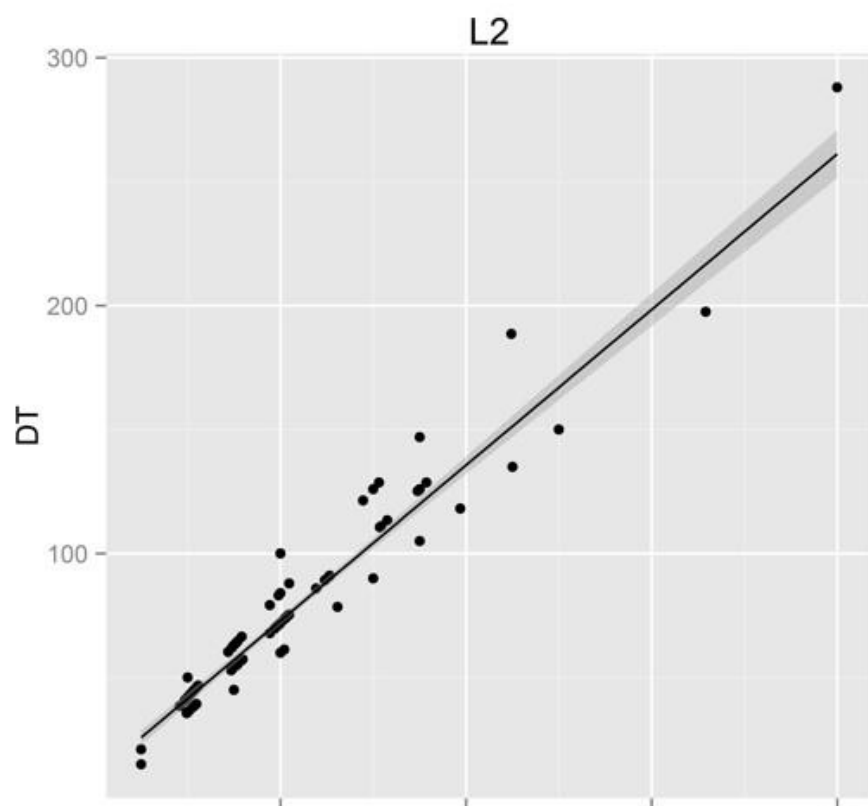
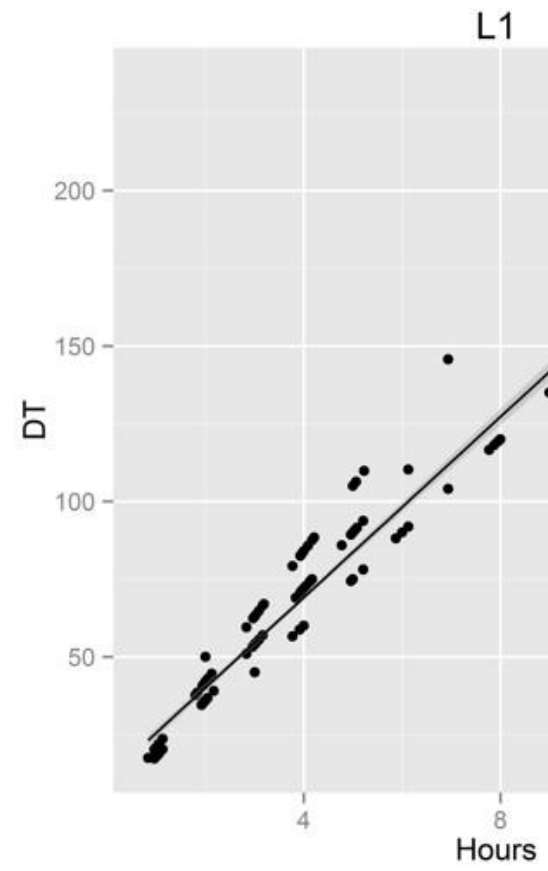
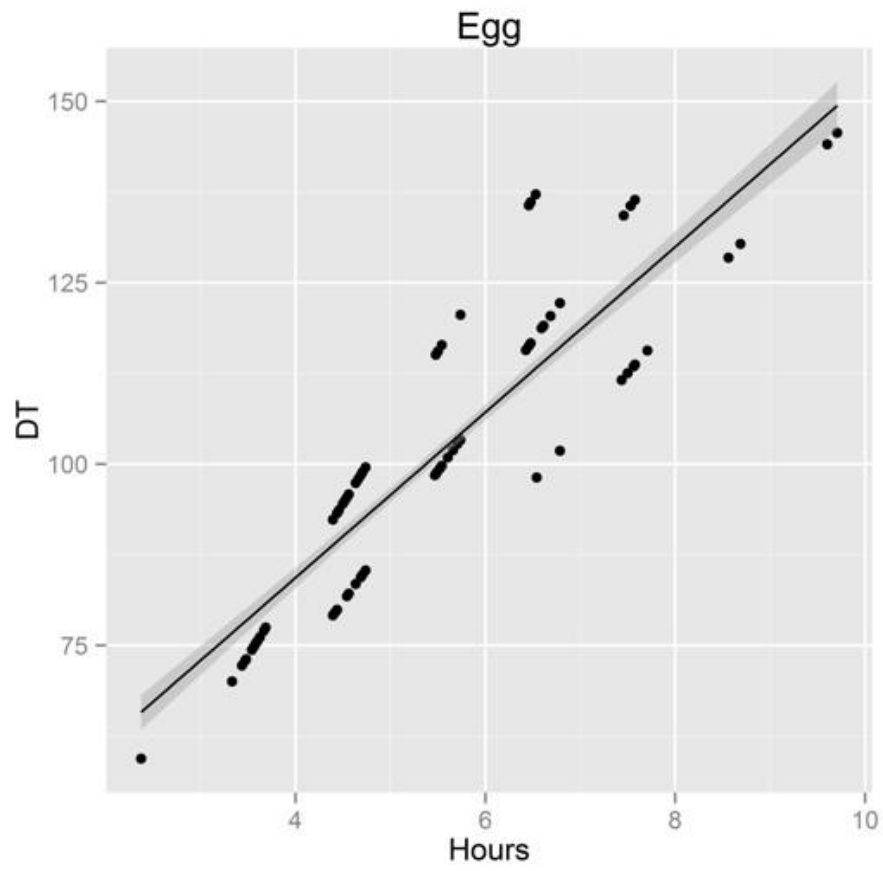




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409

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.
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Fig. 7: Major axis regression for all stages of development in *S. watsoni*. Black line shows

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median and grey area around is standard error. DT is the time in days to reach the stage

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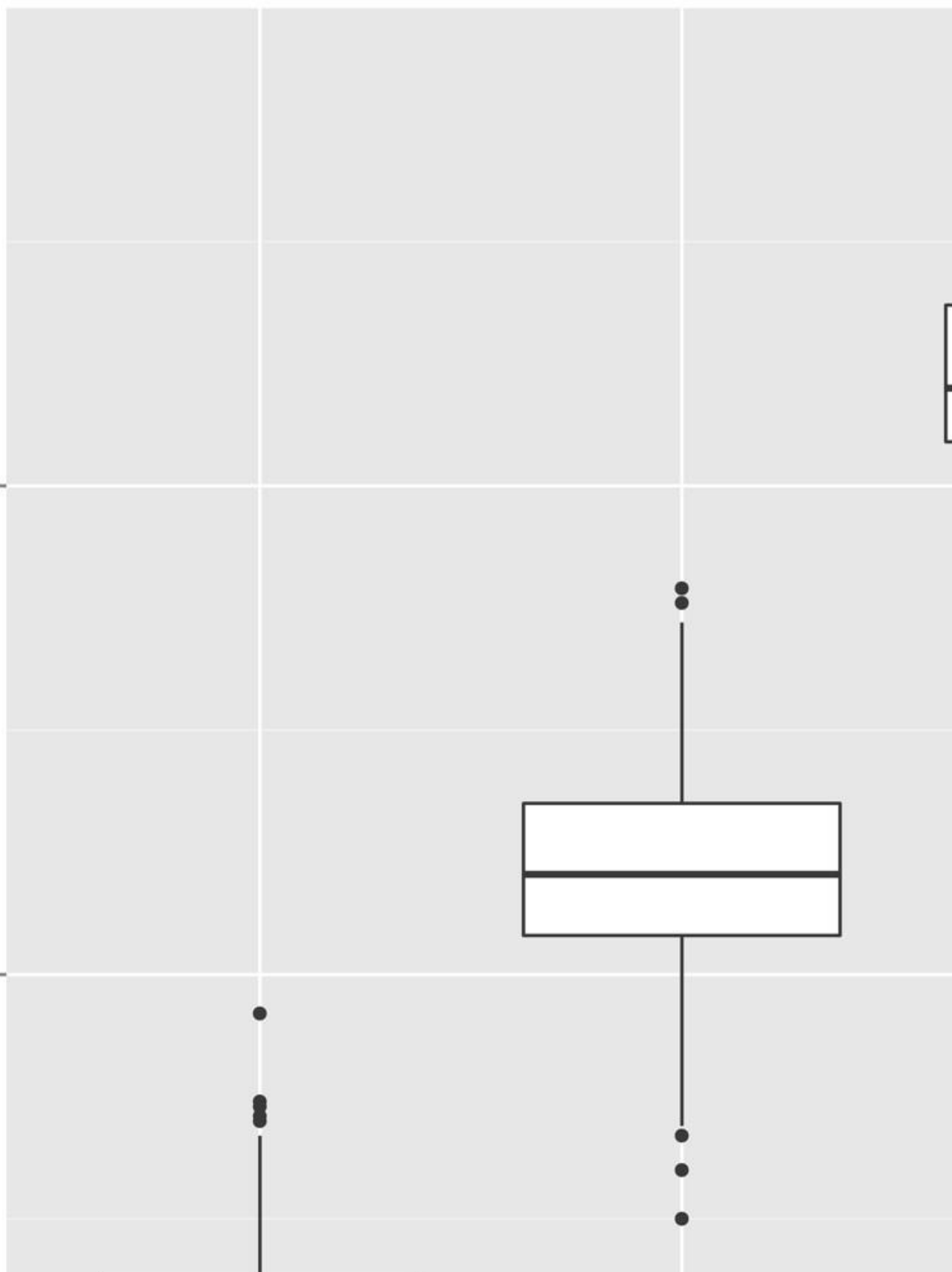
multiplied by the constant rearing temperature. 2012 data were excluded for egg and L1.

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Length (mm)

0.5

0.4





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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.

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