1   **Direct and transgenerational effects of an experimental heat wave on early life**

2   **stages in a freshwater snail**

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19   Running title: Direct and transgenerational effects of a heat wave

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25 **Abstract**

26 Global climate change imposes a serious threat to natural populations of many species. Estimates

27 of the effects of climate change are, however, typically based only on its direct effects on

28 organisms, completely neglecting the potential transgenerational effects. We investigated whether

29 high temperature (an experimental heat wave) that reduces adult performance, affects offspring

30 through maternal effects, which traits are affected, and how strong these effects are compared

31 with direct effects of high temperature in the freshwater snail *Lymnaea stagnalis*. We examined

32 the effect of maternal thermal environment ($15°C$ versus $25°C$) on per offspring investment (egg

33 size), and the role of both maternal and offspring thermal environments on offspring performance

34 (hatching success and developmental time of eggs, survival and size of hatched offspring after

35 five weeks). Exposure of mothers to high temperature benefitted offspring at very early stages of

36 their life cycles (hatching success, onset of hatching), but had negative effects at later stages

37 (survival, size). Direct effects of high temperature on offspring were largely negative.

38 Interestingly, the magnitude of transgenerational effects of high temperature on hatching success

39 and survival were similar to its direct effects. This indicates that heat waves cannot only impact

40 natural populations across generations, but that such effects can be equally strong to the direct

41 effects of temperature. Our results highlight the importance of considering transgenerational

42 effects of climate change when estimating its effects in the wild.

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44 Keywords: climate change, environmental stress, global warming, *Lymnaea stagnalis*, maternal

45 effects

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Introduction

Owing to global climate change, the average temperatures at the Earth's surface as well as the frequency and severity of extreme weather events such as summer heat waves are increasing (Easterling *et al.* 2000; Karl & Trenberth 2003; Meehl & Tebaldi 2004; Kirtman *et al.* 2013). These changes can have strong effects on organisms that escalate to higher levels of biological organization such as populations and communities (Walther *et al.* 2002; Parmesan & Yohe 2003; Walther 2010). Especially extreme weather events can dramatically influence population dynamics, species abundance, and species interactions (e.g. Easterling *et al.* 2000; Bruno *et al.* 2007; Hance *et al.* 2007). However, environmental conditions cannot only influence the fitness of the individuals exposed to them but also the fitness of their offspring through transgenerational maternal and/or paternal effects (reviewed in Bernardo 1996; Mousseau & Fox 1998a). Hence, for understanding the effects of climate change on natural populations, studies examining temperature effects not only within but also across generations are needed.

Especially transgenerational maternal effects after exposure to environmental stress can significantly alter offspring performance (e.g. Silbermann & Tatar 2000; Mitchell & Read 2005; Janhunen, Piironen & Peuhkuri 2010). Such effects can result from reduced physiological condition of the mother that limits the total amount of resources it invests in reproduction (Tessier *et al.* 1983; Steer *et al.* 2004), and/or exposure of offspring to hormones produced by the mother (McCormick 1999; Groothuis & Schwabl 2008). Maternal effects could also take place via altered resource allocation between reproduction and other traits depending on the environmental conditions the mother experiences. Challenging environmental conditions may, for example, reduce resource allocation to produced offspring to sustain self-maintenance, or increase per offspring investment when the reproductive value of individuals is changed so that investment in current reproduction increases at the expense of future reproduction (Fisher 1930;

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73   Williams 1966). Furthermore, maternal effects can be adaptations to prepare offspring for the

74   future conditions they are about to encounter (e.g. herbivory, parasitism, pollution; Agrawal

75   2002; Moret 2006; Marshall 2008).

76   To understand the consequences of such transgenerational effects in context of climate

77   change, it is essential to estimate their direction and magnitude compared with the direct effects

78   of the same environmental factors. Maternal effects are typically strongest in early stages of

79   organisms' life histories (Mousseau & Dingle 1991; Heath, Fox & Heath 1999; Pettay *et al.*

80   2008), but such stages are often also highly susceptible to the direct effects of environmental

81   variation (e.g. Jang 1991; Zhang *et al.* 2015; Klockmann, Günter & Fischer 2017). For example,

82   temperature determines the development of eggs and juveniles by altering their metabolic and

83   physiological processes in many species (Gillooly *et al.* 2001; Person-Le Ruyet *et al.* 2004; Zuo

84   *et al.* 2012). High temperature in particular can impose a serious challenge by reducing the

85   hatching success of eggs and early survival of hatched offspring (Janhunen *et al.* 2010; Zhang *et*

86   *al.* 2015; Klockmann *et al.* 2017). Despite of high interest on transgenerational effects of climate

87   change in natural populations (reviewed in Donelson *et al.* 2018), their relative importance

88   compared with direct effects of the same environmental factors is, however, often overlooked

89   (but see Burgess & Marshall 2011; Parker *et al.* 2012; Salinas & Munch 2012; Shama *et al.* 2014;

90   Wadgymar, Mactavish & Anderson 2018).

91   Here, we tested whether high temperature as it can occur during heat waves has

92   transgenerational effects on offspring performance, which traits they affect, and how strong they

93   are compared with direct effects of high temperature in the freshwater snail *Lymnaea stagnalis*.

94   We estimated the effect of maternal thermal environment (15°C versus 25°C) on per offspring

95   investment by adult snails (egg size), and the role of both maternal and offspring thermal

96   environments on offspring performance (hatching success and developmental time of eggs,

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97   survival of hatched offspring, offspring size at the age of five weeks) using a full-factorial design.

98   We used 25°C as a high (i.e. heat wave) temperature as it lies above the thermal optimum of the

99   snails (Vaughn 1953), reduces life history and immune defence traits in adult snails (Seppälä &

100   Jokela 2011; Leicht, Jokela & Seppälä 2013), and occurs intermittently in habitats of snails

101   during hot summers (T. Salo 2015, unpublished data). We chose 15°C as a control temperature as

102   it is close to the thermal optimum of *L. stagnalis* (Vaughn 1953) and common in ponds (T. Salo

103   2015, unpublished data). We found that high temperature affected offspring performance both

104   directly and through maternal effects. The relative importance and the direction of these effects

105   varied among traits, and maternal effects were equally strong to direct effects in some of the

106   examined traits. This highlights the importance of considering transgenerational effects when

107   estimating the consequences of climate change in natural populations.

108

Methods

109   **Methods**

110   *Study system and experimental animals*

111   *Lymnaea stagnalis* is a hermaphroditic freshwater snail that inhabits ponds and lakes in the

112   Holarctic region. In this species, exposure to high temperature initially increases growth and

113   reproduction, but prolonged exposure (one week or longer) ceases reproductive rate and reduces

114   immune function (Seppälä & Jokela 2011; Leicht *et al.* 2013). This indicates that high

115   temperature is physiologically challenging and has strong negative effects on adult snails.

116   The snails used in this study came from a laboratory stock population (F_4 generation)

117   originating from a pond in Zürich, Switzerland (47°22'N, 8°34'E). The summer water

118   temperature in ponds typically remains low (< 16°C) in this region, although it depends on pond

119   hydrology (T. Salo, 2015, unpublished data). However, during heat waves, water temperature can

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120   rapidly increase to 20–30°C and remain high for over two weeks (T. Salo, 2015, unpublished

121   data). We started the stock population using 45 adult snails collected from the pond. Since *L.*

122   *stagnalis* prefers outcrossing (Puurtinen *et al.* 2007; Nakadera *et al.* 2017), often engages in

123   multiple matings (Nakadera *et al.* 2017), and can store sperm from those matings for over two

124   months (Nakadera, Blom & Koene 2014), the stock population can be expected to reflect the

125   genetic variation in the source population well. We maintained the stock population in large

126   numbers at $15 \pm 2°C$ (control temperature used in the experiment; see the section about

127   experimental design below) for two years before the study (Leicht, Seppälä & Seppälä 2017). We

128   haphazardly collected 113 adult snails from the stock population and used them as a maternal

129   generation in the experiment. We placed the snails individually in 2 dl perforated plastic cups

130   sunk into a water bath (aged tap water at $15 \pm 1°C$) connected to a biological filter. We fed the

131   snails with fresh lettuce *ad libitum* and maintained them under these conditions for three days

132   prior to the experiment to acclimate them to the experimental conditions. Since *L. stagnalis* can

133   store allosperm from previous matings (Cain 1956; Nakadera *et al.* 2014), snails did not need a

134   mating partner to reproduce under these conditions.

135

136   *Experimental design*

137   Maternal treatments

138   At the beginning of the experiment, we randomly assigned the snails used as a maternal

139   generation (see the previous section) into two temperature treatments [$15 \pm 1°C$ (56 snails), $25 \pm$

140    $1°C$ (60 snails)]. We transferred the snails to their treatment temperatures in cups filled with aged

141   tap water at 15°C. This allowed a slow change (over 10 h) to the target temperature for snails

142   assigned to the high temperature treatment. We then transferred the snails into perforated plastic

143   cups (2 dl) sunk into similar water baths as above, and exposed them to their respective

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144   temperature treatments for seven days. During this period, survival of snails exposed to 25°C was

145   reduced (estimated marginal mean ± SE: 66.1 ± 6.2%) compared with snails exposed to 15°C

146   (estimated marginal mean ± SE: 94.4 ± 3.1%; generalized linear model: Wald Chi-Square =

147   10.940, d.f. = 1, p = 0.001). In those snails that survived, the probability of reproducing was

148   higher at 25°C (estimated marginal mean ± SE: 87.2 ± 5.4%) than at 15°C (estimated marginal

149   mean ± SE: 49.0 ± 7.0%; generalized linear model: Wald Chi-Square = 12.429, d.f. = 1, p <

150   0.001). We did not measure the number of oviposited eggs in this study as the effect of

151   temperature on reproduction has been described in detail earlier (Leicht *et al.* 2013; Leicht *et al.*

152   2017). It is important to note that because all parental snails did not survive or reproduce,

153   potential differences between the treatments in offspring generation could arise from selection in

154   parental population. This could be, for instance, if weak snails could not survive/reproduce under

155   environmental stress. This is, however, unlikely to explain our results since the effects of high

156   maternal temperature on offspring were mainly negative (see the results section below). If only

157   high quality individuals were able to produce offspring under high temperature this should

158   instead make our results more conservative.

159   After the initial exposure to temperature treatments, we removed all egg clutches

160   oviposited by the snails from the cups. Throughout the following ten days, we checked the cups

161   twice a day for new clutches to be collected for the next step of the experiment (see the next

162   section). This procedure ensured first, that the snails were exposed to their respective temperature

163   treatments long enough to induce strong effects on their performance before the clutches were

164   collected (see Leicht *et al.* 2013), and second, that the collected clutches were exposed to the

165   maternal temperature treatments only briefly. From each snail that oviposited (same individuals

166   as above reproduced), we collected the first clutch containing more than 20 eggs, or if only

167   smaller clutches were produced, the largest clutch. We placed each collected clutch on a

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168 millimetre paper and photographed it from 10 cm above with a Fujifilm FinePix F30 digital

169 camera (scene mode: close up, focal length: 35 mm, aperture: F/2.8, shutter speed: 1/85,

170 sensitivity: ISO-200, image size: 2848×2136 pixels, focus mode: auto focus). From the digital

171 images, we counted the eggs in each clutch. Furthermore, we measured the two-dimensional area

172 (mm^2) of five randomly chosen eggs in each clutch from the digital images using ImageJ

173 software (ImageJ 1.42q, Wayne Rasband, National Institute of Health, USA). After

174 photographing, we placed the clutches individually into plastic cups with 0.4 dl of aged tap water

175 to be transferred to the next step of the experiment (see the next section). It is important to note

176 that the time different snails needed for ovipositing after the initial exposure period varied

177 between one and ten days, which may have affected the thermal challenge imposed to them as

178 well as their offspring. However, to our knowledge, oviposition cannot be artificially induced in

179 this species.

180

181 Offspring treatments

182 We randomly assigned the clutches produced by the snails (see the previous section) into two

183 offspring temperature treatments (15°C, 25°C) so that in each maternal treatment the clutches

184 oviposited by half of the snails remained at the same temperature where they were produced and

185 the clutches from the rest of the snails were transferred to the other temperature. We slowly

186 warmed up or cooled down the clutches that were transferred to a different temperature as

187 described above to avoid a sudden change between temperatures. After that, we checked the

188 clutches daily, counted the number of hatched snails, and removed the hatchlings from the cups.

189 We placed 15 hatchlings from each clutch individually in plastic cups filled with 0.4 dl of aged

190 tap water. We fed the snails with Spirulina ad libitum, and changed the water in the cups twice a

191 week. We reared the isolated hatchlings for five weeks, and measured their survival and shell

192 length to the nearest 0.1 mm using a digital calliper at the end of the experiment.

193

194 **Statistical analyses**

195 We analysed the effect of temperature on the size of produced eggs (ln transformed) using an

196 analysis of variance (ANOVA). In the analysis, we used a model with maternal temperature

197 treatment as a fixed and the clutch each egg originated from (i.e. family) as a random factor

198 (nested within maternal temperature).

199   To estimate the effects of maternal and offspring temperature on offspring performance,

200 we first analysed the variation in hatching success of eggs using a generalized linear model. In

201 the analysis, we used the proportion of eggs that hatched from each clutch as a binomial response

202 variable (logit link function), and maternal temperature treatment and offspring temperature

203 treatment as fixed factors. Less than three snails hatched from two clutches. We excluded these

204 individuals from all the further analyses as they would not provide suitable replication within

205 those families.

206   After that, we calculated the developmental time until hatching for each egg as the

207 difference between the date the clutch was oviposited and the hatching date. We then analysed

208 the effects of temperature on developmental time using a multivariate analysis of variance

209 (MANOVA, with Pillai's trace test statistic for unequal sample sizes). We used the onset of

210 hatching (i.e. the first hatching day; square-root transformed), median developmental time (we

211 used the median rather than the mean as the distribution of hatching time within the clutches was

212 skewed), and the end of hatching (i.e. the last hatching day; ln transformed) for each clutch as

213 response variables. We used maternal temperature treatment and offspring temperature treatment

214 as fixed factors in the model. Since the MANOVA indicated effects of temperature on

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215   developmental time (see the results section), we conducted separate ANOVAs using a similar

216   model as above for the different parameters of developmental time to investigate whether their

217   responses to temperature were different.

218   We analysed the variation in survival of hatched offspring during the experiment using a

219   generalized linear model with the status of snails (survived, died) as a binomial response variable

220   (logit link function), maternal temperature treatment and offspring temperature treatment as fixed

221   factors, and family as a random factor (nested within the interaction between maternal

222   temperature and offspring temperature). From the offspring that survived until the end of the

223   experiment, we analysed the variation in size using an ANOVA with shell length (square-root

224   transformed) as a response variable, maternal temperature treatment and offspring temperature

225   treatment as fixed factors, and family as a random factor (nested within the interaction of

226   maternal temperature and offspring temperature). Survival and/or size could not be measured

227   from 30 juvenile snails (3.5% of all individuals) because of human errors. We excluded these

228   snails from the data. The assumptions of all the above analyses were fulfilled, and we performed

229   them using IBM SPSS Statistics Version 23.0 software (Armonk, NY: IBM Corp.).

230

231   **Results**

232   Eggs oviposited by snails at 25°C were smaller than those oviposited at 15°C (two-dimensional

233   area; estimated marginal mean ± SE: 25°C: 1.11 ± 0.01 mm^2; 15°C: 1.30 ± 0.01 mm^2; ANOVA:

234    $F_{1,57} = 26.275$, p < 0.001). Hatching success of eggs was affected by both the maternal

235   temperature treatment and the offspring temperature treatment (Table 1, Fig. 1). High maternal

236   temperature and low offspring temperature increased hatching success (Fig. 1). Maternal and

237   offspring temperature also affected the developmental time of eggs (MANOVA, maternal

238   temperature treatment: Pillai's trace = 0.310, $F_{3,51} = 7.654$, p < 0.001; offspring temperature

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239 treatment: Pillai's trace = 0.918, $F_{3,51}$ = 191.413, p < 0.001; maternal temperature treatment ×

240 offspring temperature treatment: Pillai's trace = 0.046, $F_{3,51}$ = 0.827, p = 0.485). Offspring started

241 to hatch earlier when mothers had been exposed to 25°C (ANOVA: $F_{1,53}$ = 15.806, p < 0.001;

242 Fig. 2). Furthermore, the onset, median, and end of hatching were earlier when offspring were

243 maintained at 25°C (ANOVA: first day of hatching: $F_{1,53}$ = 571.961, p < 0.001; median

244 developmental time: $F_{1,53}$ = 189.817, p < 0.001; last day of hatching: $F_{1,53}$ = 62.002, p < 0.001;

245 Fig. 2).

246

247 **Table 1.** Generalized linear model for hatching success of *L. stagnalis* eggs (proportion of eggs

248 that hatched per clutch) by maternal temperature treatment (15°C, 25°C) and offspring

249 temperature treatment (15°C, 25°C).

Effect	d.f.	Wald-Chi-Square	p
Maternal temperature treatment (M)	1	29.992	< 0.001
Offspring temperature treatment (O)	1	24.833	< 0.001
M × O	1	0.377	0.539

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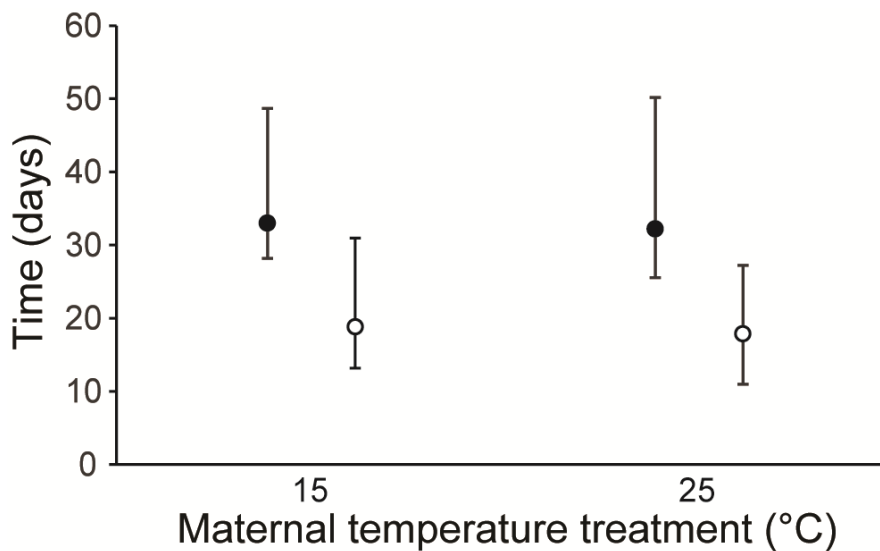255

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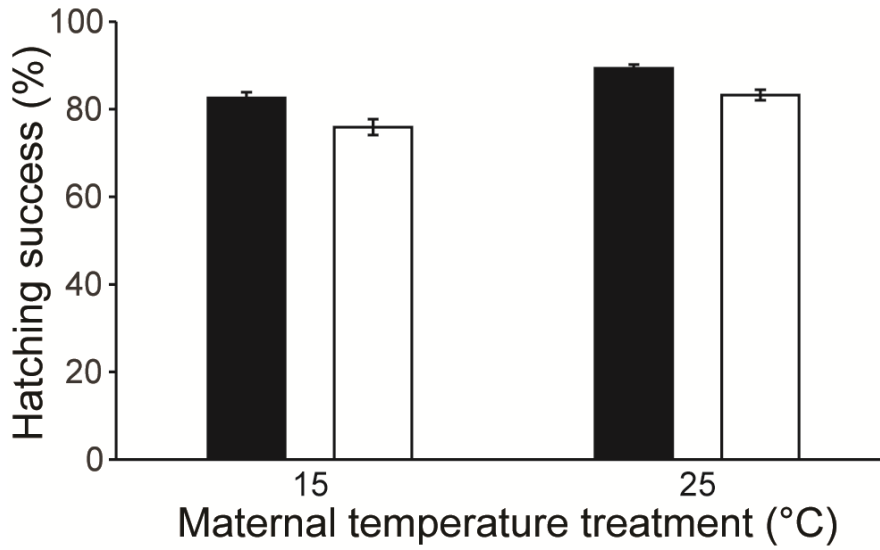
Figure 1. Hatching success of eggs [proportion of eggs that hatched (%; mean ± SE)] for egg

clutches produced at different maternal temperature treatments (15°C, 25°C) and maintained at

15°C (black bars) or at 25°C (white bars).

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Figure 2. Developmental time of eggs presented using the means for onset of hatching (lower

whisker), median developmental time (circles), and the end of hatching (upper whisker) for egg

clutches produced at different maternal temperature treatments (15°C, 25°C) and maintained at

15°C (black circles) or at 25°C (white circles).

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266

267   High maternal temperature reduced offspring survival (Table 2, Fig. 3). When offspring

268   were maintained at 25°C, they showed tendency towards lower survival (Table 2, Fig. 3).

269   Temperature affected the size of offspring so that high maternal temperature and low offspring

270   temperature reduced shell length at the end of the experiment (Table 3, Fig. 4).

271

272   **Table 2.** Generalized linear model for survival of juvenile *L. stagnalis* snails during the

273   experiment (survived/died) by maternal temperature treatment (15°C, 25°C), offspring

274   temperature treatment (15°C, 25°C), and family.

Effect	d.f.	F	Estimate	SD	Z	p
Maternal temperature treatment (M)	1	4.452				0.035
Offspring temperature treatment (O)	1	3.588				0.059
M × O	1	1.587				0.208
family (M × O)			0.582	0.184	3.165	0.002

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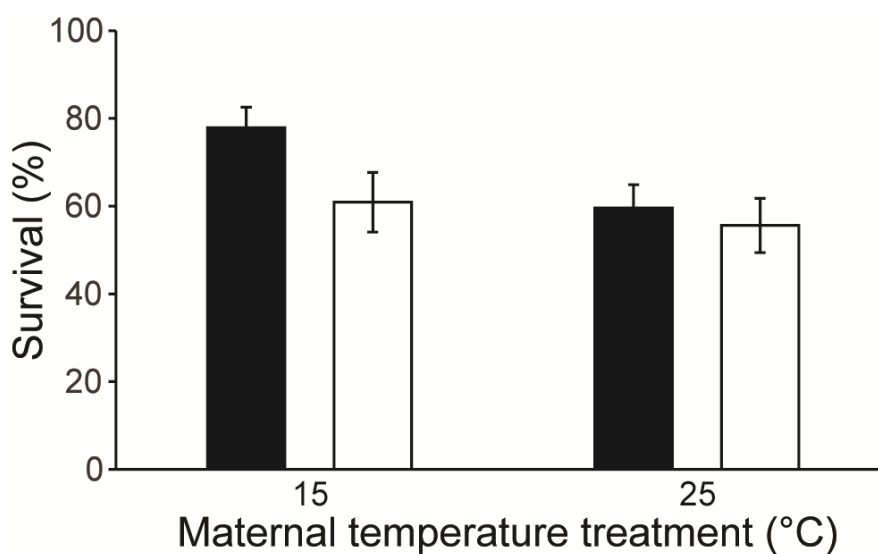
282 **Figure 3.** Proportion (%; estimated marginal mean ± SE) of offspring that survived until the end

283 of the experiment when produced at different maternal temperature treatments (15°C, 25°C) and

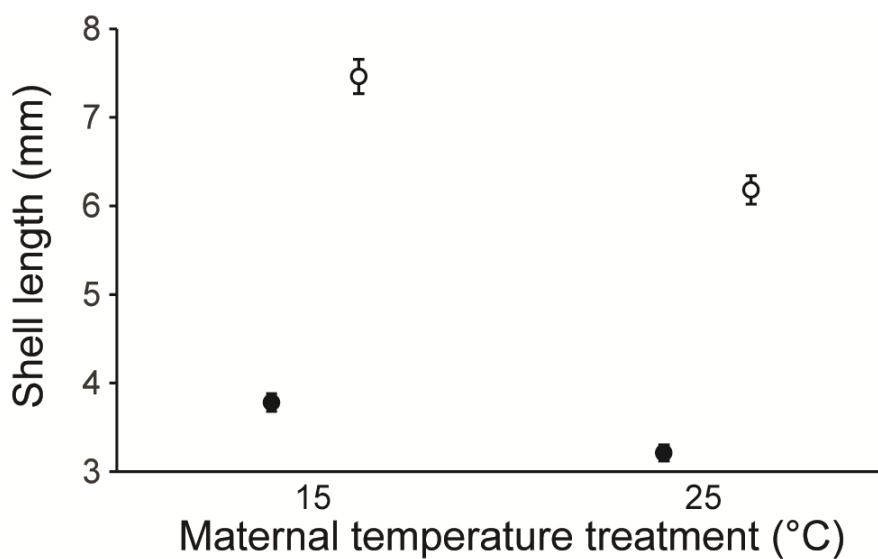284 maintained at 15°C (black bars) or at 25°C (white bars).

285

286 **Table 3.** Analysis of variance for shell length of juvenile *L. stagnalis* snails at the end of the

287 experiment by maternal temperature treatment (15°C, 25°C), offspring temperature treatment

288 (15°C, 25°C), and family.

Effect	d.f.	MS	F	p
Maternal temperature treatment (M)	1	3.926	15.012[a]	< 0.001
Offspring temperature treatment (O)	1	54.520	208.927[a]	< 0.001
M × O	1	0.216	0.827[a]	0.367
family (M × O)	53	0.302	3.370	< 0.001
error	455	0.090		

295 [a] family(M × O) as the error term

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14

298   **Figure 4.** Offspring shell length (mm; estimated marginal mean ± SE) at the end of the

299   experiment when produced at different maternal temperature treatments (15°C, 25°C) and

300   maintained at 15°C (black circles) or at 25°C (white circles).

301

302   **Discussion**

303   Exposure to an experimental heat wave affected eggs and hatchlings of *L. stagnalis* snails both

304   directly and via transgenerational maternal effects. Direct effects of high temperature were

305   largely negative. The magnitude and the direction of transgenerational effects when compared

306   with direct effects varied among examined traits. In general, high maternal temperature benefitted

307   offspring at very early life stages, but reduced performance at later stages. Hence, the potential

308   adaptive value of responding to high temperature by maternal effects may be limited only to very

309   early life stages (Mousseau & Dingle 1991; Heath *et al.* 1999; Pettay *et al.* 2008). Interestingly,

310   the magnitude of transgenerational effects on hatching success and survival of offspring were

311   similar to the direct effects of high temperature. These findings indicate that heat waves cannot

312   only impact natural populations across generations, but those effects can be equally strong to

313   their direct within generation effects.

314

315   *Effects of temperature on eggs*

316   Mothers exposed to high temperature produced smaller eggs that had higher hatching success

317   compared with snails that oviposited at benign temperature (14.6% reduction in size, 9.0%

318   increase in hatching success). Thus, exposure of the maternal generation to high temperature can

319   increase the quality of produced eggs despite their smaller size. High maternal temperature made

320   the onset of hatching earlier by 2.5 days (12.3% reduction). The magnitude of the direct effect of

321   high temperature on hatching success of eggs was similar to the observed transgenerational effect

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322   but negative (7.5% reduction). The direct effect of high temperature on development of eggs was

323   strong by shortening the median developmental time by two weeks (43.8% reductions). Faster

324   development and earlier hatching is suggested to be accompanied by higher fitness by increasing

325   future survival (Arcese & Smith 1985; Warner & Shine 2007) and allowing earlier maturiation

326   and increased reproductive output (Uller & Olsson 2010). However, early hatching may also

327   bring disadvantages by leading to less well developed offspring (e.g. Warkentin 1999; Buckley,

328   Michael & Irschick 2005).

329   Reduced egg size at high temperature may be adaptive to decreased oxygen concentration

330   in water and increased oxygen consumption of developing eggs at high temperature (Woods

331   1999; Moran & Woods 2007). Therefore, egg size might not be a good indicator for egg quality.

332   The observed transgenerational effect of high temperature on development of eggs

333   (developmental time and hatching success) may be due to two non-exclusive mechanisms. First,

334   high temperature can increase metabolic rate and resource level of adults that allow production of

335   high quality eggs (see Jann & Ward 1999; Saino *et al.* 2004). Second, it may increase resource

336   allocation towards reproduction rather than other traits when the residual reproductive value of

337   individuals decrease, for example, due to increased mortality (Fisher 1930; Williams 1966). The

338   effect of maternal temperature on developmental time of eggs was, however, limited to the onset

339   of hatching. This indicates that the possibly increased investment on oviposited eggs may be

340   rapidly depleted. On the other hand, smaller eggs are expected to develop more slowly (Levitan

341   2000). Therefore, it is possible that high maternal investment and small egg size overrode each

342   other's effects so that no net change in developmental time of eggs could be detected. The direct

343   effect of temperature on hatching success of eggs is likely to be due to high sensitivity of mollusc

344   embryos to high temperature (Vaughn 1953), which leads to mortality in several taxa probably

345   due to denaturation of proteins (reviewed in Pepin 1991; Noble, Stenhouse & Schwanz 2018).

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346   The direct effect of temperature on developmental time of eggs was most likely because

347   temperature determines the speed of biochemical processes of the developing embryos (e.g.

348   García-Guerrero, Villarreal & Racotta 2003; Sibert, Ouellet & Brêthes 2004).

349

350   *Effects of temperature on hatchlings*

351   Exposure of mothers to high temperature reduced the probability of offspring to survive until the

352   age of five weeks (17.8% reduction compared with 15°C). High maternal temperature also

353   reduced the size offspring reached (28.9% reduction). High temperature showed tendency to have

354   a negative direct effect on offspring survival (16.1% reduction). However, offspring that survived

355   grew larger at high temperature (97.4% increase in size). Hence, high maternal temperature

356   reduced offspring performance in the examined traits while the direct effects of high temperature

357   were both positive and negative. Reduced size of offspring due to high maternal temperature may

358   lead to delayed maturity as well as reduced mating success and fecundity (reviewed in Clutton-

359   Brock 1988), and also increase susceptibility to predators (e.g. Janzen 1993; Craig *et al.* 2006).

360   Instead, direct effect of high temperature can benefit those individuals that are able to survive

361   under such conditions.

362   Reduced offspring size when mothers experience high temperature is found across a wide

363   range of animal taxa (reviewed in Atkinson *et al.* 2001). The reason for this is not yet clear and

364   may either be an adaptation to maximize mother's life time fitness (Yampolsky & Scheiner 1996)

365   or due to physiological constraints under such conditions (Blanckenhorn 2000). The direct effect

366   of high temperature on offspring survival may be due to temperature-induced changes in, for

367   instance, protein structures and/or membrane fluidity (reviewed in Pörtner, Lucassen & Storch

368   2005), which can lead to body malfunctions and increased mortality. On the other hand, high

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369   temperature fastens metabolic rate and can increase growth of organisms (e.g. Iguchi & Ikeda

370   2005; Salo *et al.* 2017).

371

372   *General conclusions*

373   Our findings of equally strong direct and transgenerational effects of exposure to high

374   temperature contradict earlier studies that have examined their relative importance in determining

375   offspring performance and physiology (e.g. Groeters & Dingle 1988; Huey *et al.* 1995; Steigenga

376   & Fischer 2007; Burgess & Marshall 2011; Salinas & Munch 2012; Shama *et al.* 2014). In those

377   studies, transgenerational effects of temperature are typically reported to be weak compared with

378   its direct effects. To our knowledge, transgenerational effects of environmental change have been

379   found to be strong compared with its direct effects in a climate change context only in

380   germination probability of a perennial forb, *Boechera stricta*, when wintering conditions are

381   manipulated (Wadgymar *et al.* 2018). Together with that finding, our results indicate that climate

382   change mediated environmental changes cannot only impact natural populations across

383   generations, but those effects can be equally strong or even stronger than their direct effects

384   within generations.

385   Despite of high interest on transgenerational effects of climate change-mediated

386   environmental change on organisms (Donelson *et al.* 2018) many studies have not tested their

387   relative importance compared with direct within generation effects. This is because earlier studies

388   have focused, for example, on testing whether negative effects of environmental change are

389   reduced if parents experience the same environmental conditions (e.g. Donelson *et al.* 2012;

390   Miller *et al.* 2012). Testing this does not necessarily require a full-factorial design that is needed

391   for examining the relative importance of direct and transgenerational effects. This alternative

392   approach is relevant in systems where environment changes gradually and relatively slowly to a

18

393   predicted direction (e.g. in oceans). Such studies would, however, not be realistic in terrestrial

394   and freshwater systems that experience high and rapid fluctuations in several environmental

395   conditions owing to climate change. In marine species, exposing parents to altered environmental

396   conditions has been found to reduce the negative effects of increased temperature and CO_2-level

397   on offspring (Donelson *et al.* 2012; Miller *et al.* 2012; Shama *et al.* 2014). In our study, none of

398   the observed direct effects of temperature depended on the maternal environment. This may be

399   due to higher unpredictability of extreme weather events in freshwater systems compared with

400   marine environments that could limit the ability of such adaptive maternal effects to evolve (see

401   Mousseau & Fox 1998b).

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409   **Conflict of interest disclosure**

410   The authors of this preprint declare that they have no financial conflict of interest with the

411   content of this article.

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413   **References**

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