Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail

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Running title: Direct and transgenerational effects of a heat wave

Abstract

Global climate change imposes a serious threat to natural populations of many species. Estimates of the effects of climate change-mediated environmental stresses are, however, often based only on their direct effects on organisms, and neglect the potential transgenerational effects. We investigated whether high temperature (i.e. an experimental heat wave) that is known to reduce performance of adult *Lymnaea stagnalis* snails affects their offspring through maternal effects. Specifically, we tested whether eggs and hatched juveniles are affected by maternal thermal environment, and how strong these effects are compared with direct effects of temperature on offspring. We examined the effect of maternal thermal environment (15°C versus 25°C) on per offspring investment (egg size), and the role of both maternal and offspring thermal environments (15°C versus 25°C) on hatching success and developmental time of eggs, offspring survival after hatching, and hatchling size at the age of five weeks. Exposure of mothers to high temperature increased hatching success of eggs, and also made the onset of hatching earlier. However, high maternal temperature reduced the survival and the final size of hatched juveniles. Direct effects of high temperature on offspring survival were negative (both eggs and hatchlings), but increased the developmental rate and growth of those eggs and hatchlings that survived. Interestingly, the magnitude of transgenerational effects of high temperature on hatching success of eggs and hatchling survival were similar to its direct effects. This indicates that heat waves can affect natural populations through transgenerational effects, and that the magnitude of such effects can be equally strong to the direct effects of temperature, although this depends on the trait considered. Our results highlight the importance of considering transgenerational effects of climate warming when estimating its effects in the wild.

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

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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 can not 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 1998b). Hence, for understanding the effects of climate change on natural populations, studies examining such transgenerational effects 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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Williams 1966). Furthermore, maternal effects can be adaptations to prepare offspring for the

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

2002; Moret 2006; Marshall 2008).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Wadgymar, Mactavish & Anderson 2018).

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

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

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

(Gastropoda: Pulmonata). In this species, exposure of adult individuals to high temperature (≥

25°C) initially increases growth and reproduction, but prolonged exposure (one week or longer)

ceases reproductive rate and reduces immune function (Seppälä & Jokela 2011; Leicht, Jokela &

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Seppälä 2013). This indicates that high temperature is physiologically challenging and has strong negative effects on adult snails. We estimated the effect of maternal thermal environment (15°C versus 25°C) on per offspring investment by adult snails (egg size), and the role of both maternal and offspring thermal environments on offspring performance (hatching success and developmental time of eggs, survival of hatched offspring, offspring size at the age of five weeks) using a full-factorial design. We found that high temperature affected offspring performance both directly and through maternal effects. The relative importance and the direction of these effects varied among traits, and the magnitude of maternal effects were equally strong to direct effects in some of the examined traits. This highlights the importance of considering transgenerational effects when estimating the consequences of climate change in natural populations.

Methods

Experimental animals

The snails used in this study came from a laboratory stock population (F_4 generation) originating from a pond in Zurich, Switzerland (47°22'05''N, 8°34'41''E). The summer water temperature in ponds typically remains low (< 16°C) in this region, although it depends on pond hydrology (T. Salo, unpublished data). However, during heat waves, water temperature can rapidly increase to 20–30°C and remain high for over two weeks (T. Salo, unpublished data). We started the stock population using 45 adult snails collected from the pond. Since *L. stagnalis* prefers outcrossing (Puurtinen *et al.* 2007; Nakadera *et al.* 2017), often engages in multiple matings (Nakadera *et al.* 2017), and can store sperm from those matings for over two months (Nakadera, Blom & Koene 2014), the stock population can be expected to reflect the genetic variation in the source population well. We maintained the stock population in the approximate size of 400 individuals

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

144   design below) for two years before the study (see Leicht, Seppälä & Seppälä 2017).

145   We haphazardly collected 113 adult snails from the stock population and used them as a

146   maternal generation in the experiment. We placed the snails individually in 2 dl perforated plastic

147   cups sunk into a water bath (aged tap water at $15 \pm 1°C$) that was connected to a biological filter.

148   We used a water bath to provide maximal water quality for snails. This minimises the growth of

149   microorganisms in water that activate snail immune function (Seppälä & Leicht 2013). This is

150   important because immune challenge could potentially alter snail reproductive strategy and/or

151   affect the quality of produced offspring. We fed the snails with fresh lettuce *ad libitum* and

152   maintained them under these conditions for three days prior to the experiment to acclimate them

153   to the maintenance conditions. Because *L. stagnalis* snails can reproduce through self-fertilization

154   as well as through outcrossing using allosperm they have stored from previous matings (Cain

155   1956; Nakadera *et al.* 2014), experimental snails did not need a mating partner to oviposit eggs

156   under the used conditions.

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158   *Experimental design*

159   Maternal treatments

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

161   generation (see the previous section) into two temperature treatments [$15 \pm 1°C$ (54 snails), $25 \pm$

162    $1°C$ (59 snails); Fig. 1]. We used 25°C as a high (i.e. heat wave) temperature as it reduces

163   immune defence and life history traits in adult snails (Seppälä *et al.* 2011; Leicht *et al.* 2013), lies

164   above the thermal optimum for development and growth of juvenile snails (Vaughn 1953), and

165   occurs intermittently in habitats of snails during hot summers (T. Salo, unpublished data). We

166   chose 15°C as a control temperature as it is close to the thermal optimum of *L. stagnalis* (Vaughn

1953) and common in ponds (T. Salo, unpublished data). Note that we assigned more individuals into the high temperature treatment because we expected increased mortality in those snails. We transferred the snails to their treatment temperatures in cups filled with aged tap water at 15°C. This allowed a slow change (over 10 h) to the target temperature for snails assigned to the high temperature treatment. We then transferred the snails into perforated plastic cups (2 dl) sunk into similar water baths as above, and exposed them to their respective temperature treatments for seven days. At 15°C, 51 of these snails survived, and of those 25 oviposited eggs. At 25°C, 39 snails survived, and 34 of them reproduced. We did not measure the number of oviposited eggs in this experiment as the effect of temperature on snail fecundity has been described in detail in earlier studies (Leicht et al. 2013; 2017).
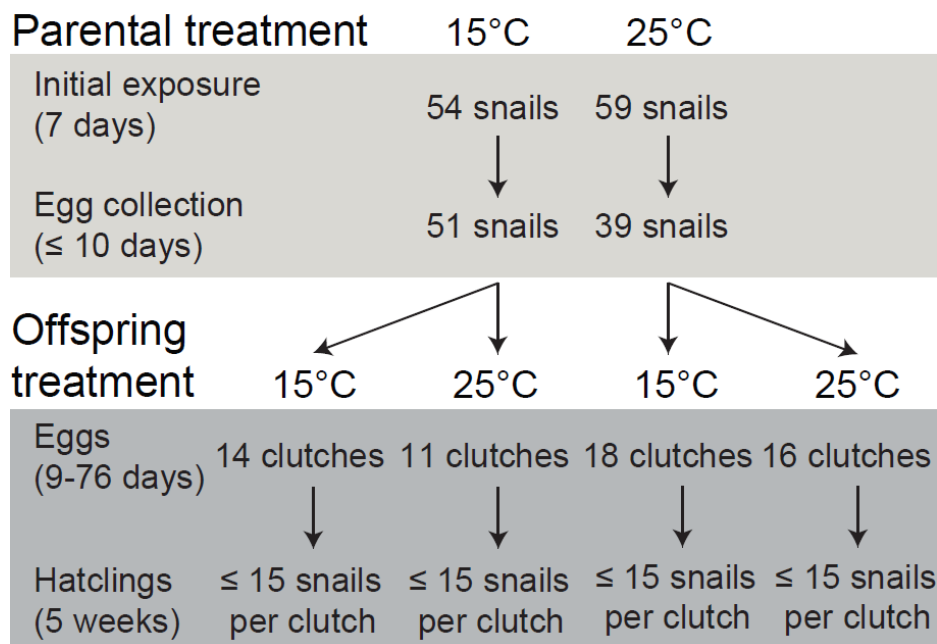

Figure 1. Summary of the full-factorial experimental design used to examine direct and transgenerational effects of exposure of *L. stagnalis* snails to two temperatures (benign: 15°C; heat wave: 25°C). One egg clutch per adult snail was used in offspring treatments. When available, 15 hatchlings per egg clutch were used. When fewer individuals were available, they were all used.

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184   After the initial seven-day exposure to temperature treatments, we removed all egg

185   clutches oviposited by the snails from the cups and continued maintaining the snails under the

186   same experimental conditions. During the following ten days, we checked the cups twice a day

187   for new clutches to be collected for the next step of the experiment (see the next section). This

188   procedure ensured first, that the snails were exposed to their respective temperature treatments

189   long enough to induce strong effects on their performance before the clutches were collected (see

190   Leicht *et al.* 2013), and second, that the collected clutches were exposed to the maternal

191   temperature treatments only briefly. From each snail that oviposited (same individuals as above

192   reproduced), we collected the first clutch containing more than 20 eggs, or if only smaller

193   clutches were produced, the largest clutch. We placed each collected clutch on a millimetre paper

194   and photographed it from 10 cm above with a Fujifilm FinePix F30 digital camera (scene mode:

195   close up, focal length: 35 mm, aperture: F/2.8, shutter speed: 1/85, sensitivity: ISO-200, image

196   size: 2848×2136 pixels, focus mode: auto focus). From the digital images, we counted the eggs

197   in each clutch. Furthermore, we measured the two-dimensional area (mm^2) of five randomly

198   chosen eggs in each clutch from the digital images using ImageJ software (ImageJ 1.42q, Wayne

199   Rasband, National Institute of Health, USA). After photographing, we placed the clutches

200   individually into plastic cups with 0.4 dl of aged tap water to be transferred to the next step of the

201   experiment (see the next section). It is important to note that the time different snails needed for

202   ovipositing after the initial exposure period varied between one and ten days, which may have

203   affected the thermal challenge imposed to them as well as their offspring. However, to our

204   knowledge, oviposition cannot be induced artificially in this species.

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206   Offspring treatments

207   We used a full-factorial design to expose egg clutches produced in both maternal temperature

208   treatments (see the previous section) to two offspring temperature treatments (15°C, 25°C; Fig.

209   1). In each maternal temperature treatment, we randomly assigned some of the oviposited egg

210   clutches to remain at the same temperature where they were produced and transferred the rest of

211   the clutches to the other temperature. The number of egg clutches per treatment combination

212   varied between 11-18, which was because of unequal mortality and probability to reproduce in

213   parental snails in different treatments (see the previous section). We slowly warmed up or cooled

214   down the clutches that were transferred to a different temperature as described above to avoid a

215   sudden change between temperatures. After that, we checked the clutches daily, counted the

216   number of hatched snails, and removed the hatchlings from the cups. When possible, we placed

217   15 hatchlings from each clutch individually in plastic cups filled with 0.4 dl of aged tap water.

218   We used all hatchlings when less than 15 individuals were available. We fed the snails with

219   Spirulina ad libitum, and changed the water in the cups twice a week. We reared the isolated

220   hatchlings for five weeks, and measured their survival and shell length to the nearest 0.1 mm

221   using a digital calliper at the end of the experiment.

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223   **Statistical analyses**

224   We analysed the effect of temperature on the survival of adult snails during the initial exposure to

225   experimental treatments (a seven-day period before the collection of egg clutches started) using a

226   generalized linear model. We used the status of snails (survived, died) as a binomial response

227   variable (logit link function) and temperature treatment as a fixed factor. Additionally, we

228   analysed variation in snail reproductive status (oviposited, did not oviposit) using a similar

229   generalised linear model. We analysed the effect of temperature on the size of produced eggs (ln

230   transformed to homogenize error variance) using a mixed-model analysis of variance (mixed-

231   model ANOVA). In the analysis, we used a model with maternal temperature treatment as a fixed

232   and the clutch each egg originated from as a random factor (nested within maternal temperature).

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

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

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

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

237   treatment as fixed factors. We included the interaction term between the factors into the model.

238   Less than three snails hatched from two clutches that were both produced and maintained at 25°C.

239   We excluded these individuals from all the further analyses as they would not provide sufficient

240   replication within those clutches.

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

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

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

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

245   hatching (i.e. the first hatching day; square-root transformed to homogenize error variance),

246   median developmental time (we used the median rather than the mean as the distribution of

247   hatching time within the clutches was skewed), and the end of hatching (i.e. the last hatching day;

248   ln transformed to homogenize error variance) for each clutch as response variables. We used

249   maternal temperature treatment and offspring temperature treatment as fixed factors in the

250   analysis, and included the interaction term between them into the model. Since the MANOVA

251   indicated effects of temperature on developmental time (see the results section), we conducted

252   separate ANOVAs using a similar model as above for the different parameters of developmental

253   time to investigate whether their responses to temperature were different.

254   We analysed the variation in the survival of hatched offspring during the experiment

255   using a generalized linear mixed-effects model with the status of snails (survived, died) as a

256   binomial response variable (logit link function). We used maternal temperature treatment and

257   offspring temperature treatment as fixed factors, and egg clutch each offspring originated from as

258   a random factor (nested within the interaction between maternal temperature and offspring

259   temperature). We included the interaction term between maternal and offspring temperature into

260   the model. From the offspring that survived until the end of the experiment, we analysed the

261   variation in size using a mixed-model ANOVA with shell length (square-root transformed to

262   homogenize error variance) as a response variable, and a similar model as for survival. Survival

263   and/or size could not be measured from 30 juvenile snails (3.5% of all individuals) because of

264   human errors. We excluded these snails from the data. The assumptions of all the above analyses

265   were fulfilled, and we performed them using IBM SPSS Statistics Version 23.0 software

266   (Armonk, NY: IBM Corp.).

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268   **Results**

269   During the initial exposure of adult snails to different temperature treatments (i.e. a seven-day

270   period before the collection of egg clutches started), the survival of snails exposed to 25°C was

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

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

273   10.940, d.f. = 1, p = 0.001). In those snails that survived, the probability of reproducing was

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

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

276   0.001). Note that the same individuals that reproduced during this initial exposure period also

277   produced the eggs that were exposed to offspring temperature treatments.

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

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

280   mm^2; ANOVA: $F_{1,57}$ = 26.275, p < 0.001). Hatching success of eggs was affected by both the

281   maternal temperature treatment and the offspring temperature treatment, and these effects were

282   independent of each other (Table 1, Fig. 2). High maternal temperature increased hatching

283   success by 9.0% whereas high offspring temperature reduced it by 7.5% (Fig. 2). Maternal and

284   offspring temperature also affected the developmental time of eggs, and these effects were

285   independent of each other (MANOVA, maternal temperature treatment: Pillai's trace = 0.310,

286   $F_{3,51}$ = 7.654, p < 0.001; offspring temperature treatment: Pillai's trace = 0.918, $F_{3,51}$ = 191.413, p

287   < 0.001; maternal temperature treatment × offspring temperature treatment: Pillai's trace = 0.046,

288   $F_{3,51}$ = 0.827, p = 0.485). The effects of temperature on developmental time were first because

289   offspring started to hatch 12.3% earlier when mothers had been exposed to 25°C (ANOVA: $F_{1,53}$

290   = 15.806, p < 0.001; Fig. 3a). Second, the onset, median, and the end of hatching were earlier

291   when offspring were maintained at 25°C (first day of hatching: 55.1% reduction; ANOVA: $F_{1,53}$

292   = 571.961, p < 0.001; median developmental time: 43.8% reduction; ANOVA: $F_{1,53}$ = 189.817, p

293   < 0.001; last day of hatching: 43.8% reduction; ANOVA: $F_{1,53}$ = 62.002, p < 0.001; Fig. 3).

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

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

297   temperature treatment (15°C, 25°C), and their interaction.

| 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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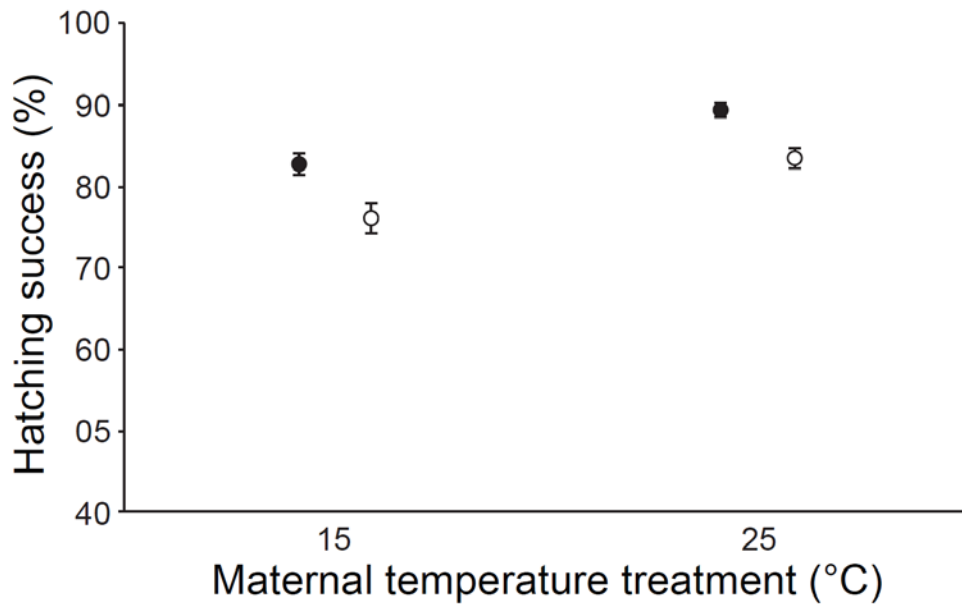
Figure 2. Hatching success of eggs [proportion of eggs that hatched (%; mean $\pm$ SE)] for egg

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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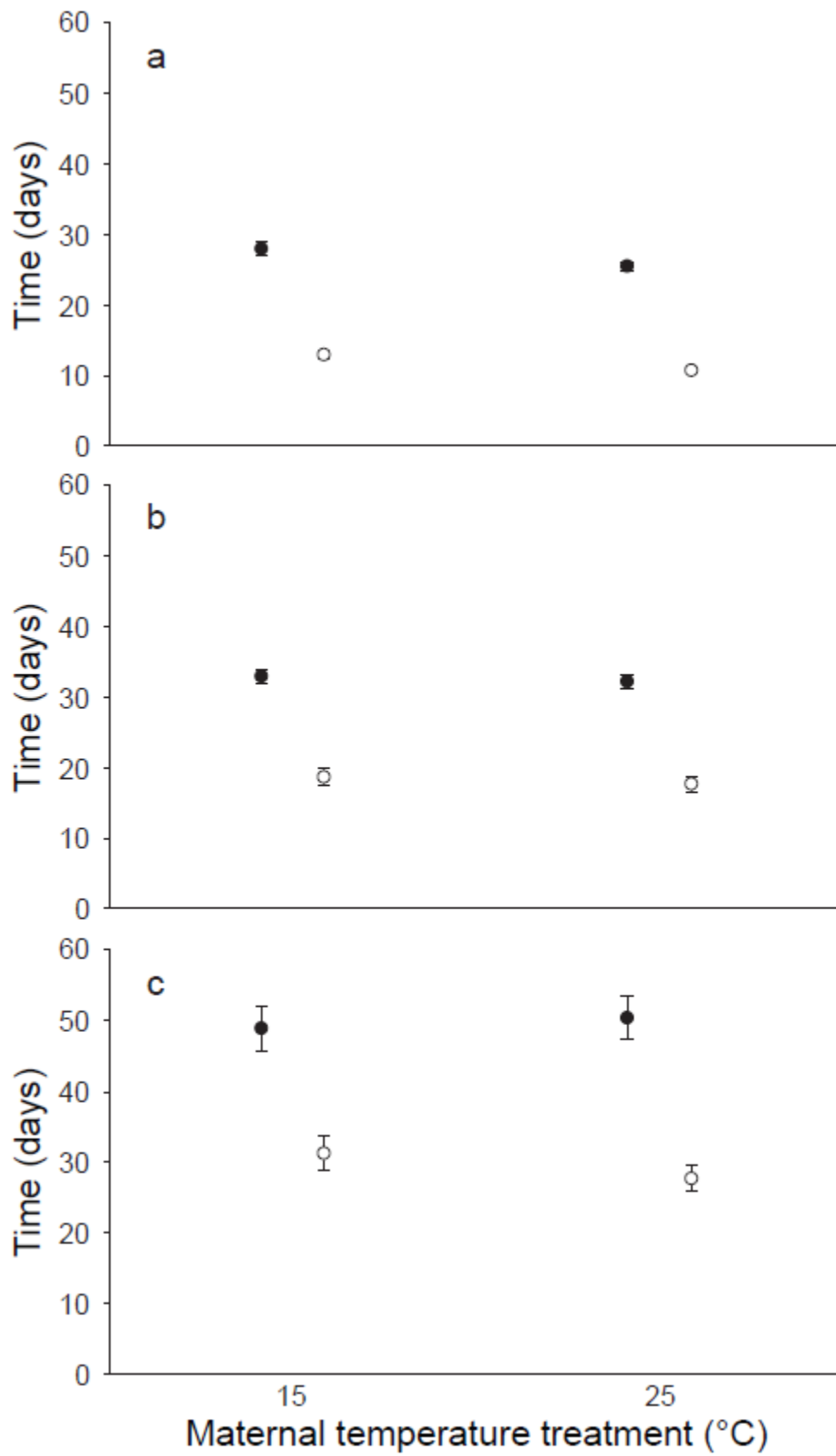
Figure 3. Developmental time of eggs presented using the mean ± SE of (a) onset of hatching, (b) median developmental time, and (c) end of hatching for egg clutches produced at different maternal temperature treatments (15°C, 25°C) and maintained at 15°C (black circles) or at 25°C (white circles).
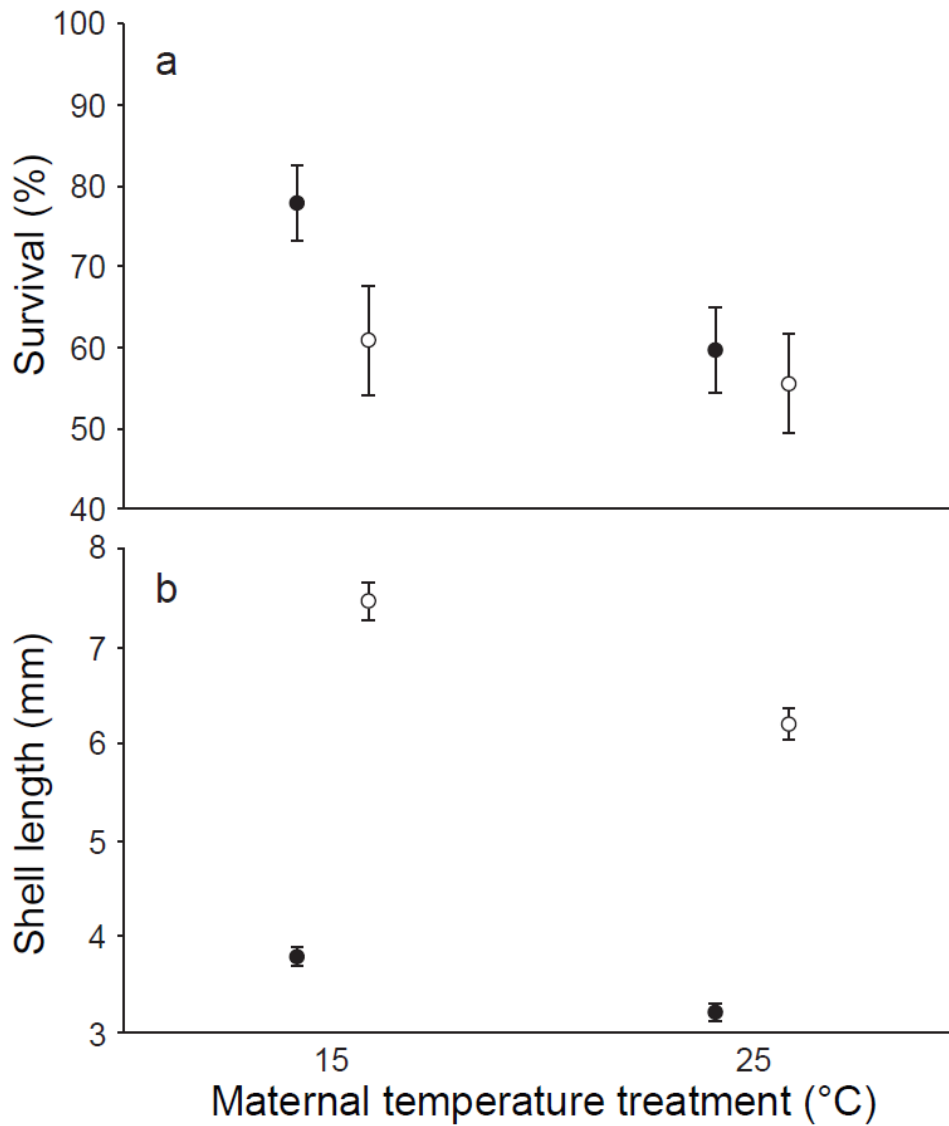
High maternal temperature reduced offspring survival by 17.8%, and this effect was independent of the temperature offspring were exposed to (Table 2, Fig. 4). When offspring were maintained at 25°C, they showed tendency towards lower survival (Table 2, Fig. 4). Temperature affected the size of offspring so that high maternal temperature and low offspring temperature reduced shell length at the end of the experiment (Table 3, Fig. 4).

Table 2. Generalized linear mixed-effects model for the survival of juvenile *L. stagnalis* snails during the experiment (survived/died) by maternal temperature treatment (15°C, 25°C), offspring temperature treatment (15°C, 25°C), their interaction, and the egg clutch each individual originated from.

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
Egg clutch (M × O)			0.582	0.184	3.165	0.002

Figure 4. (a) Proportion (%; estimated marginal mean ± SE) of offspring that survived until the end of the experiment, and (b) shell length (mm; estimated marginal mean ± SE) of those snails at the end of the experiment when produced at different maternal temperature treatments (15°C, 25°C) and maintained at 15°C (black circles) or at 25°C (white circles).

Table 3. Mixed-model analysis of variance for the shell length of juvenile *L. stagnalis* snails at the end of the experiment by maternal temperature treatment (15°C, 25°C), offspring temperature treatment (15°C, 25°C), their interaction, and the clutch each individual originated from.

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
Egg clutch (M × O)	53	0.302	3.370	< 0.001
Error	455	0.090		

[a] Egg clutch (M × O) as the error term

Discussion

Exposure to an experimental heat wave affected eggs and hatchlings of *L. stagnalis* snails both directly and via transgenerational maternal effects. Direct effects of high temperature were both negative and positive by reducing the hatching success of eggs, but by increasing their development rate and the subsequent growth of those individuals that hatched. The magnitude and the direction of transgenerational effects when compared with direct effects varied among examined traits. In general, high maternal temperature benefitted offspring at very early life stages (hatching success and the onset of hatching), but reduced performance at later stages (survival and final size of hatched snails). Hence, the potential adaptive value of responding to high temperature by maternal effects may be limited only to very early life stages. Interestingly, the magnitude of transgenerational effects on hatching success and survival of offspring were similar to the direct effects of high temperature, although in the case of hatching success the direction of the effects differed. These findings indicate that heat waves can not only impact

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natural populations through transgenerational effects, but that the magnitude of those effects can

be equally strong to the direct within generation effects of high temperature. The relative strength

of the direct and transgenerational effects, however, depends on the considered trait.

It is important to note that because all parental snails that we initially exposed to different

temperatures did not survive or reproduce, potential differences between the temperature

treatments in offspring generation could arise from selection in the parental population. This

could be, for instance, if weak snails could not survive under environmental stress. Similarly, not

all offspring hatched or they died before the end of the experiment. While we cannot exclude

neither estimate the potential role of selection in our experiment, it seems unlikely that selection

could explain our results on transgenerational effects. This is because the longest lasting effects

of high maternal temperature on offspring (i.e. hatchling survival and size at the end of the

experiment) were negative. If only high quality individuals were able to survive and reproduce at

high temperature this should lead to the opposite result. Because high temperature had a direct

negative effect on the hatching of juvenile snails, their increased size at the end of the experiment

could be not only due to phenotypic plasticity but also because of selection.

Effects of temperature on eggs

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

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

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

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

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

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

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

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388 eggs was strong by shortening the median developmental time by approximately two weeks

389 (43.8% reductions). Faster development and earlier hatching could reduce the risk of eggs being

390 exposed to natural enemies such as predators (see Warkentin 1995; Chivers *et al.* 2001), and it is

391 suggested to increase future survival of offspring (Arcese & Smith 1985; Warner & Shine 2007)

392 as well as allow earlier maturation and increased reproductive output (Uller & Olsson 2010).

393 However, early hatching may also bring disadvantages by leading to less well developed

394 offspring (e.g. Warkentin 1999; Buckley, Michael & Irschick 2005).

395   Large egg size is often beneficial for offspring by increasing their fitness (Hutchings

396 1991; Fox 1994; Krist 2011). This is likely to be because large eggs can provide more energy and

397 nutrients for developing embryos (reviewed in Williams 1994). In aquatic organisms, however,

398 reduced egg size may be beneficial by ensuring oxygen supply to embryos when oxygen

399 concentration in water decreases under high temperature (Woods 1999; Moran & Woods 2007).

400 Therefore, egg size might not be a good indicator of egg quality. The observed transgenerational

401 effect of high temperature on development of eggs (developmental time and hatching success)

402 may be due to two non-exclusive mechanisms. First, high temperature can increase metabolic rate

403 of adults that allow production of high quality eggs (see Jann & Ward 1999; Saino *et al.* 2004).

404 Second, it may increase resource allocation towards reproduction rather than other traits (e.g.

405 growth) when the residual reproductive value of individuals decrease, for example, due to

406 increased mortality (Fisher 1930; Williams 1966). The effect of maternal temperature on

407 developmental time of eggs was, however, limited to the onset of hatching. This indicates that the

408 possibly increased investment on oviposited eggs may be rapidly depleted. On the other hand,

409 smaller eggs are expected to develop more slowly (Levitan 2000). Therefore, it is possible that

410 high maternal investment and small egg size overrode each other's effects so that no net change

411 in developmental time of eggs could be detected. The direct effect of temperature on hatching

412 success of eggs is likely to be due to high sensitivity of mollusc embryos to high temperature

413 (Vaughn 1953), which leads to mortality in several taxa probably due to denaturation of proteins

414 (reviewed in Pepin 1991; Noble, Stenhouse & Schwanz 2018). The direct effect of temperature

415 on developmental time of eggs was possibly because temperature determines the speed of

416 biochemical processes of the developing embryos (e.g. García-Guerrero, Villarreal & Racotta

417 2003; Sibert, Ouellet & Brêthes 2004).

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419 *Effects of temperature on hatchlings*

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

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

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

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

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

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

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

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

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

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

430 under such conditions.

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

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

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

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

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

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

437   2005), which can lead to body malfunctions and increased mortality. On the other hand, high

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

439   2005; Salo *et al.* 2017). This, however, could also lead to faster use of energetic reserves that may

440   reduce survival.

441

442   *General conclusions*

443   Our finding that the potential adaptive value of responding to high temperature by maternal

444   effects was limited to very early life stages (i.e. eggs) is in line with earlier research (Mousseau *et*

445   *al.* 1991; Heath *et al.* 1999; Pettay *et al.* 2008). Instead, the result of equally strong direct and

446   transgenerational effects of exposure to high temperature on some of the examined traits

447   contradict earlier studies that have examined their relative importance in determining offspring

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

449   Fischer 2007; Burgess *et al.* 2011; Salinas *et al.* 2012; Shama *et al.* 2014). In those studies,

450   transgenerational effects of temperature are typically reported to be weak compared with its

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

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

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

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

455   change-mediated environmental changes can affect natural populations through transgenerational

456   effects, and that these effects may be as strong as or even stronger than the direct effects of

457   environmental change.

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

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

relative importance compared with direct within generation effects. This is because earlier studies have focused, for example, on testing whether negative effects of environmental change are reduced if parents have experienced the same environmental conditions (e.g. Donelson *et al.* 2012; Miller *et al.* 2012). Testing this does not necessarily require a full-factorial design that is needed for examining the relative importance of direct and transgenerational effects. The alternative approach is relevant in systems where environment changes gradually and relatively slowly to a predicted direction (e.g. in oceans). Such studies would, however, not be realistic in terrestrial and freshwater systems that experience high and rapid fluctuations in several environmental conditions owing to extreme weather events such as heat waves. In marine species, exposing parents to altered environmental conditions has been found to reduce the negative effects of increased temperature and CO_2-level on offspring (Donelson *et al.* 2012; Miller *et al.* 2012; Shama *et al.* 2014). In our study, none of the observed direct effects of temperature depended on the maternal environment, which was indicated by the lack of interactive effects between temperature treatments. This may be due to higher unpredictability of extreme weather events in freshwater systems compared with marine environments that could limit the ability of such adaptive maternal effects to evolve (see Mousseau & Fox 1998a).

Conflict of interest disclosure

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

485　content of this article.

486

487　**References**

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