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Heat waves during egg development alter maternal care and offspring quality in the European earwig

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HIGHLIGHTS

- Heat waves reduced the investment in earwig maternal care
- Heat waves reduced hatching success and accelerated egg development
- Heat waves reduced the upper thermal limit for earwig juveniles
- Overall, maternal care is not enough to offset the cost of heat waves in earwigs

SUMMARY

Climate change can disrupt animal fitness by reducing survival, fertility, fecundity and altering offspring development and survival. While parental care typically helps offspring cope with harsh environmental conditions, little is known about its role in buffering extreme temperature changes, such as heat waves. In this study, we tested whether parental care mitigates the impact of cold and heat waves on eggs and juveniles in the European earwig. In this insect, mothers provide obligatory egg care for about 50 days during winter, typically at temperatures around 10°C. We exposed mothers and their eggs to three-day thermal waves of 3°C, 10°C (control), 17°C or 24°C, both 15 and 30 days after oviposition. We then measured four maternal care behaviors, maternal weight variation, as well as eggs' developmental time, survival, and hatching rate. In the resulting juveniles, we measured weight, developmental time, thermal resistance, and the expression of six heat stress and immunity genes. We found that thermal waves reduced maternal care and induced maternal weight gain. High temperatures also decreased egg hatching success, accelerated egg and nymph development, reduced the upper thermal limit of juveniles and decreased the expression of a heat shock protein (Hsp68), while other traits remained unaffected. Overall, this study highlights that access to maternal care is not enough to alleviate the stress of exposure to non-optimal temperatures during egg development in the European earwig. It also suggests that species with maternal care do not necessarily have access to effective thermal protection and may not be better adapted to climate change.

Keywords: Heat wave; Gene expression; Egg care; Larvae; Immunity; Upper Thermal Limit

INTRODUCTION

Climate change affects all regions of the world, altering the environmental parameters essential for the survival and functioning of most living organisms (Coffel et al., 2018). Some of the most notable alterations are the increased likelihood, severity, duration and geographical extent of heat waves, defined as periods of localised heat accumulation during a succession of abnormally hot days and nights (Meehl and Tebaldi, 2004; Robinson, 2001). These heat waves are considered a major threat to biodiversity as they often lead to life cycle disruption and physiological stress in animals, altering a wide range of life history traits. For example, they can alter foraging behaviour, fecundity, hatching success, immune defence, and the condition of offspring and survival (Chirault et al., 2015; Fragueira et al., 2021; McCowan and Griffith, 2021; Melone et al., 2024; Miler et al., 2020; Pilakouta et al., 2023; Pilakouta and Baillet, 2022; Stiegler et al., 2023; Tobin et al., 2024; Vasudeva et al., 2021; Wild et al., 2019). Heat waves can also determine the ability of offspring to tolerate future exposure to extreme heat and cold stress, and their expression of heat shock proteins (HSPs) that play a critical role in responding to heat stress by stabilizing and refolding damaged proteins (Colinet et al., 2010; Elekonich, 2009; Huang et al., 2007).

Insects are expected to be particularly sensitive to changes in their thermal environment because they typically lack physiological processes to regulate their body temperature (Colinet et al., 2015a; Ma et al., 2021). As a result, they often need to rely on behavioural strategies to avoid excessive variations in their internal temperature (Heinrich, 2013; Kearney et al., 2009; May, 1979). These strategies may include selecting habitats and microhabitats with more suitable temperatures (Pincebourde and Woods, 2020) or shifting activity periods to moments when temperature variation is more optimal (Hamilton III, 1971; May, 1979). They may also involve manipulation of the thermal regulation of their biotic environment. For example, herbivorous insects that feed on the leaf in or on which they live alter the level of leaf transpiration, thereby affecting the temperature of their habitat (Pincebourde and Casas, 2019). Finally, in social insects such as honeybees and bumblebees, workers can express collective behaviours to increase or decrease nest temperature (Heinrich, 1981; Sepúlveda-Rodríguez et al., 2024).

In addition to the ability of some insects to regulate ambient temperature, others can alleviate the impact of excessive temperature fluctuations on their offspring by providing parental care. Parental care is a taxonomically widespread phenomenon that has typically evolved to limit the detrimental effects of harsh environmental conditions on eggs and juveniles (Royle et al., 2012; Wong et al., 2013). Accordingly, parents from mammals to birds and arthropods generally increase offspring care under extreme cold, limited food availability, or high risk of predation and pathogen infection (Klug and Bonsall, 2014; Meunier et al., 2022). However, the impact of warmer temperatures on parental care remains unclear, with studies showing widely varying results (Grew et al., 2019; Vincze et al., 2013). On the one hand, warmer temperatures may cause parents to increase their care to better protect their offspring. For instance, warmer temperatures cause males to increase egg fanning behaviour in the three-spined stickleback *Gasterosteus aculeatus* L. (Hopkins et al., 2011), and both parents to prolong egg incubation and increase the number of provisioning visits to the nest in the Kentish plover *Charadrius alexandrinus* (AlRashidi et al., 2011, 2010), and the Southern Pied Babbler *Turdoides bicolor* (Bourne et al., 2021), respectively. On the other hand, warmer temperatures can also cause parents to reduce their care. This is because parental care is energetically costly, and parents may therefore gain higher fitness benefits by reallocating resources to their own survival and the possibility of future reproduction (Boulton et al., 2010;

Clauser and McRae, 2017). This is the case in the waterbug *Belostoma flumineum*, where males maintained in warm temperatures reduce their egg brooding behavior (Kight et al., 2000), in the pied babbler *Turdoides bicolor*, where adults provision their young significantly less on hot days (Wiley and Ridley, 2016) and in the burrower bug *Sehirus cinctus*, where females housed in warmer temperatures terminate care earlier than females at cooler temperatures (Kight and Cseke, 1998). Interestingly, most of these studies have focused on the effects of long-term average temperature increases on parental care and few studies have focused on the effect of heat waves (Sidhu et al., 2024), leaving open the question of how parental care responds to these extreme temperature changes and how this affects the quality of the resulting offspring. Yet this information is crucial to determine whether parental care is a benefit or a potential burden for animal species facing this facet of global warming (Meehl and Tebaldi, 2004).

In this study, we tested whether exposure to extreme changes in temperature during egg development affects maternal care and the quality of the resulting offspring in the European earwig. In this insect, females typically lay their eggs at the beginning of winter, tend their clutch of eggs for two months over winter, remain with the resulting juveniles (called nymphs) for two weeks in early spring, and these juveniles then take about two months to become adults (Lamb, 1976, p. 197; Meunier, 2024). During winter, females provide obligatory forms of egg care including protection against predators and grooming behaviours to remove pathogens and apply chemical compounds on egg surface to improve resistance against desiccation (Boos et al., 2014; Diehl and Meunier, 2018; Lamb, 1976; Van Meyel et al., 2019). After hatching, females can provide non-obligatory forms of care to their nymphs, including food provisioning and protection against predators (Kölliker, 2007; Merleau et al., 2022; Staerke and Kölliker, 2008).

Two recent studies suggest that exposure to suboptimal temperatures is an important risk for eggs and mothers in this species. First, prolonged winter duration and the associated longer exposure to cold have detrimental long-term effects on the resulting adult offspring in terms of immunity (Körner et al., 2018). Second, mothers choose their oviposition site depending on the soil temperature and they can move their eggs to a warmer place if the temperature drops during the incubation period (Tourneur et al., 2022). Neglecting this behavioural thermoregulation leads to the death of eggs (Tourneur et al., 2022). Therefore, it has been suggested that this behaviour contributes to the species' adaptability to a variety of environments and has generally facilitated its establishment on almost all continents (Quarrell et al., 2018; Tourneur and Meunier, 2020). Whether the European earwig is resistant to extreme temperature changes and whether this resistance could be due to adaption in their maternal care remains, however, unclear.

To test the effects of extreme temperature change on maternal care and offspring quality, we first kept 207 female earwigs and their eggs under standard laboratory conditions at a constant temperature of 10°C to mimic natural temperatures at this stage of the life cycle (Honorio et al., 2023; Tourneur and Meunier, 2020; Van Meyel et al., 2019). We then exposed them to extreme temperature changes by shifting the nest temperature to either 3°C, 10°C (control), 17°C or 24°C for 3 days twice on the 15th and 30th day after egg laying during the two months of egg development. We then measured 1) four forms of maternal care towards the eggs and the resulting nymphs, 2) the level of maternal investment in egg care in terms of female weight variation (i.e., the more the female lost weight between oviposition and egg hatching, the more she invested in egg care, and vice versa), and 3) clutch quality in terms of hatching success, minimum egg development time, nymph weight and developmental time.

After hatching, we tested the ability of the resulting offspring to tolerate future exposure to extreme heat and cold stress by measuring 4) their upper thermal limit and chill coma recovery time, as well as 5) the expression level of heat stress and immune response-related genes after a (new) heat shock. Overall, we predicted that exposure to extreme temperature changes during egg development would lead to an increase in maternal care and that this increase would prevent us from detecting the detrimental effects of these temperatures on eggs and nymph survival and quality. We also predicted that exposure to extreme temperature change would increase the thermal resistance of the resulting nymphs through acclimation and/or selection.

MATERIAL AND METHODS

Animal rearing and thermal treatments

The experiment involved a total of 207 females belonging to *Forficula auricularia* Linneaus, 1758, also called *Forficula auricularia* clade A (González-Miguéns et al., 2020). We collected these females in the field in July 2022 using wooden traps placed on tree trunks for one week in an orchard in Saint-Marcel-lès-Valence, France (Lat 44.978526, Long 4.926383). Immediately after collection, we set up random groups consisting of about 50 females and 50 males (sampled simultaneously from the same location) in plastic containers. These containers were then kept under standard laboratory conditions at 20°C and under a 12:12 h light/dark photoperiod to allow for uncontrolled mating (Kramer et al., 2015; Sandrin et al., 2015). Five months later, we isolated the female to mimic her natural behaviour and provide conditions that closely resemble the natural winter environment for egg production. Using data from the reference period of 1961 to 1990, the average winter temperature in France is about 5°C above ground and 10°C at a depth of 20-30 cm. As earwigs typically nest at this depth, we used a temperature of 10°C as a normal baseline control temperature for our experiment, as in previous studies (Mauduit et al., 2021; Merleau et al., 2022; Van Meyel et al., 2019). We transferred each female to a petri dish filled with water-saturated sand (diameter 55 mm) placed in complete darkness and subjected them to a gradual decrease in temperature from 20°C to 10°C over a period of three weeks.

We then monitored each female twice a week to record the day of oviposition. After oviposition, we kept these females in constant darkness at 10°C until egg hatching (Tourneur & Meunier, 2020), except for the two periods of three days (starting 15 and 30 days after oviposition) during which each female and her eggs were randomly exposed to heat or cold thermal waves. To simulate ecologically relevant waves conditions, we varied the temperatures by $\pm 7^\circ\text{C}$ over a three-day period to reflect realistic fluctuations, with one extreme treatment reaching +14°C above the baseline. These thermal waves were applied by placing each petri dish (containing the female and her eggs) in an incubator under complete darkness at a constant temperature of either 3°C (N = 53), 10°C (control; N = 51), 17°C (N = 52) or 24°C (N = 51) for three days. Each female was exposed to the same temperature during the first and second wave. By incorporating two heatwaves, we were able to examine two key stages of embryonic development without prior determination of the specific phase in which sensitivity might be greatest. As soon as the first egg hatched, we transferred the entire family to a new petri dish (diameter 90 mm) maintained at 20°C under a 12:12 h light/dark photoperiod (Meunier et al., 2012). These families remained under these conditions until we measured nymph resistance to heat or cold seven days after hatching, and until we measured

the expression of heat stress and immune response-related genes 9 days after hatching (details below). All families were discarded from the experiment after this final measurement.

From field sampling until oviposition and after egg hatching, individuals were fed *ad libitum* on a standard diet consisting mainly of pollen, cat and bird food, wheat germ and agar (see details in Kramer et al., 2015). The food was replaced once a week. We removed food between egg laying and hatching, as earwig mothers typically stop their foraging activity during this time period (Kölliker, 2007). All plastic containers and petri dishes were covered with wet sand.

Maternal care

We first tested the effects of thermal waves on four forms of maternal care: egg collection after dispersal, frequency of mother-egg contact, and frequency of mother-nymph contact, and maternal investment in egg care. These behaviours are naturally performed by females to protect offspring against pathogens and predators (Meunier, 2024). Egg collection reflects the propensity of earwig mothers to collect their eggs after they have been experimentally scattered in the nest (Van Meyel et al., 2019). We measured egg collection the day after the second wave, i.e. 31 days after oviposition. We isolated each mother in a side petri dish, levelled the sand in the original petri dish and then evenly distributed 20 of the eggs in a 4.5 cm diameter circle at equal intervals. If more than 20 eggs were available, the remaining eggs were placed in another petri dish on a wet filter paper (Evadis intelligent systems) for the duration of the test. We then returned each mother to the centre of the circle and, 2 hours later, recorded whether all the eggs were gathered within a 1 cm diameter.

We measured the frequency of mother-egg contacts 3 days after the second wave (i.e. 36 days after egg deposition) and the frequency of mother-nymph contacts 4 days after egg hatching. These frequencies reflect the number of times a mother was in contact with her eggs or nymphs over a 30 min observation period (Merleau et al., 2022). We used a scan sampling process during which we recorded every minute whether females were touching/cleaning eggs or nymphs with their mouthparts (i.e. grooming) and/or with antennae (i.e. antennation). Finally, we measured the level of maternal investment in egg care by their weight variation during the period of egg care. Weight variation is an efficient proxy for this investment, because earwig mothers do not have access to food during this time (Koch and Meunier, 2014; Van Meyel et al., 2019). A loss of weight is interpreted as a higher investment, while a gain in weight indicates that the eggs have been cannibalized, as we do not feed the females during this period. For each mother, we obtained this value by subtracting the fresh weight measured 1 day after egg hatching from the fresh weight measured 3 days after oviposition. The weighing was done to the nearest 0.01 mg using a microbalance (Discovery DV215CD, OHAUS).

Clutch development and quality

We then tested the effects of thermal waves on clutch development and quality by measuring egg survival rate immediately after the second wave, minimum egg development time and hatching success, as well as mean nymph weight at hatching and date of second moult (the first moult occurs during egg hatching in this species; Tourneur et al., 2020). The egg survival immediately after the second wave was defined as the number of eggs still present in the petri dish one day after the second wave (i.e. day 34 after oviposition) divided by the number of eggs produced by the female. Note that filial egg cannibalism occurs in this species and typically explains egg loss (Van Meyel and Meunier, 2020). The minimum egg development

time was defined as the number of days between oviposition and the hatching of the first nymph. The egg hatching success was defined as the number of nymphs that hatched divided by the number of eggs laid. The mean weight of newly hatched nymphs was measured 24 h after the first nymph had hatched and was obtained by weighing a group of 10 random nymphs per clutch to the nearest 0.01 mg. Finally, the date of the second moult is a good proxy for clutch development (Gómez and Kölliker, 2013) and was obtained by checking each clutch daily and recording the date at which we observed the first newly moulted nymphs (Nymphs remain white for about 24h after moulting; Tourneur et al., 2020).

Thermal resistance of the nymphs

Third, we tested the effects of thermal waves on the subsequent capacity of nymphs to survive extreme temperatures by measuring their upper thermal limit (UTL), and chill coma recovery (CCR). The 24°C thermal waves produced virtually almost no nymphs (see results), so this treatment was excluded from the subsequent tests. We measured heat tolerance through ULT metrics seven days after hatching on 2 nymphs per family. We placed each nymph individually within heat-conducting wells arranged on a temperature-adjustable Peltier-effect module (Kellermann and Sgrò, 2018; Ma et al., 2021). The temperature was then increased at a rate of 1°C per minute from 25°C to 45°C. To prevent heat loss, each well was covered with transparent plastic paper. A temperature probe (HOBO UX120-014M) was placed at the bottom of one of the wells to record the temperature rise in the device over time. A second probe was placed between the well plate and the Peltier heater to obtain a second measurement of the device's temperature rise and check for any abnormal deviations from the well. The upper thermal limit (UTL) was then defined as the temperature at which the nymph was no longer able to move. We measured the chill coma recovery (CCR) eight days after hatching on two nymphs from each family (Mutamiswa et al., 2018). We isolated each nymph into a 1.5 mL Eppendorf tube immersed in ice at 0-1°C for 10 minutes. We then gently removed each nymph from the tube and placed it on a sheet of white paper at room temperature (20°C). We then recorded the time taken to wake up from the coma, which was defined as the time at which simultaneous movement of the legs, antennae and head were observed.

Nymph candidate gene expression after a thermal shock

Finally, we tested whether exposure to thermal waves during egg development influenced gene expression in the resulting nymphs after a new heat shock. We focused on the expression of 6 candidate genes possibly involved in heat stress response and immunity (details in Supplementary table 1). Nine days after hatching, two nymphs per family were individually exposed to either 20°C (control) or 30°C for one hour using the methods described above. Following the treatments, the nymphs were promptly frozen with liquid nitrogen and stored at -80°C for subsequent processing. We used nymphs from randomly selected families for a total of 24 families, 8 per thermal wave at 3°C, 10°C or 17°C, respectively. This provided us with a total of 48 pairs of nymphs for the qPCR gene expression experiment.

We isolated the total RNA from each pooled pair of nymphs using the Nucleospin RNA kit from Macherey Nagel. Initially, whole-body tissues were homogenized in lysis buffer through mechanical grinding using a Tissue Lyser (Qiagen) along with 2 tungsten beads (3mm). Subsequently, total RNA extraction was carried out following the manufacturer's protocol. RNAs were quantified using a Qubit fluorometer and the Qubit RNA assay Kit (Invitrogen), adhering to the supplier's instructions. Following quantification, each sample underwent

reverse transcription with the Qiagen Quantitech reverse transcriptase kit, utilizing 750 ng of total RNA from each sample.

We designed specific primers for *F. auricularia* qPCR reactions using the primer3 software via the primer3plus web interface (<https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). The *F. auricularia* sequences used for the primer design were obtained by performing a tblastx sequence query using the transcriptome shotgun assembly sequence database of *F. auricularia* with the selected homologous candidate genes of *Drosophila melanogaster* (Supplementary table 1). The first hit of each candidate was retained for primer design.

We then carried out RT-qPCRs to examine the candidate genes' expression level (Supplementary table 1). Reverse transcription-qPCR assays were set up using an Eppendorf EpMotion5070 Robotics system (Eppendorf). Triplicate first strand cDNA aliquots for each sample were used as templates for RT-qPCR using SYBR Green Master Mix MESA Blue qPCR Mastermix Plus (EurogenTech) on a QuantStudio TM 6Flex machine (Thermo Fisher) Real-Time PCR System. Amplification reactions were performed in 13 μ l total volumes with 3 μ l of cDNA (diluted 1:30) and 300 nM of each primer in 384-well optical plates (Applied Biosystems) under the following sequential conditions: incubation at 95 C $^{\circ}$ for 10 min, followed by 40 cycles of 95 C $^{\circ}$ for 15 s and 60 C $^{\circ}$ for 1 min. The primers used are shown in Table 1. Four housekeeping genes were designed and selected as candidate reference genes: Actine 5C (Act), Elongation factor 1-alpha (eEF1 α), Forkhead domain 68A (Mnf), and Ribosomal protein L11 (Rpl11). Expression levels were quantified based on the number of cycles required for amplification to reach a predefined threshold during the exponential phase of the PCR reaction, a method described by Walker (2002). This parameter is commonly known as the quantification cycle (Cq) value, in accordance with the RDML guidelines established by Lefever et al. (2009)

The housekeeping gene expression stability of the four genes analysed was evaluated with Bestkeeper (Pfaffl et al., 2004) and Genorm2 (Vandesompele et al., 2002) packages facilitated by the Ctrl Gene package in R (Zhong, 2019). Among them, the Actin gene demonstrated the lowest M average expression stability value (Avg.M) across various conditions (0.617), indicating greater stability, as calculated by the geNorm2 package. Furthermore, it exhibited a reduced standard deviation (0.755) of the critical threshold cycle (CP), indicating a reduced variability in expression levels across different treatments as determined by the BestKeeper package. Due to its superior stability characteristics, Actin was chosen as the reference gene for normalising the expression of the candidate genes in our study. The relative gene expression quantification was calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with $\Delta Ct = Ct \text{ target gene} - Ct \text{ housekeeping gene (Act)}$ and $\Delta\Delta Ct = \Delta Ct \text{ sample} - \Delta Ct \text{ standard}$. The ΔCt standard value corresponded to the average of Ct target gene across all 8 samples for control samples (i.e., 10 $^{\circ}$ C for thermal waves and 20 $^{\circ}$ C for heat shocks).

Statistical analyses

We carried out all the statistical analyses using the software R v4.3.3 (<https://www.r-project.org/>) loaded with the packages *car* (Fox and Weisberg, 2018), *ggplot2* (Villanueva and Chen, 2019), *ggpubr* (Kassambara, 2023), *emmeans* (Lenth, 2020) and *DHARMA* (Hartig, 2020). We analysed the minimum egg development time, mother-eggs contacts, nymph weight at hatching, mother-nymphs contacts, number of newly hatched nymphs and date of 1st moult using six linear models (LM). We analysed egg collection considering them as either collected (1) or not collected (0) using one generalized linear model (GLM) with binomial error

distribution. We analysed egg survival rate at day 34 and hatching success using two GLM with a binomial error distribution corrected to overdispersion. In these two models, rates were entered as odd ratios using the *cbind* function, which combines the number of newly hatched nymphs and the number of eggs that did not hatch, and the number of nymphs alive at day 34 and the number of nymphs that died before day 34, respectively. We analysed Ctmax, UTL and CCR using three linear models (LM) in which the mean values per family was entered as the response variable. In each of these 12 models, the thermal treatment was entered as the factorial explanatory variable. We then analysed the level of gene expression in the nymphs using a series of seven linear mixed-effects models (LMM) with the R package Lme4 (Bates, 2005), in which the level of expression of each gene ($2^{-\Delta\Delta Ct}$) was entered as the response variable, while the thermal treatment, the heat shock and the interaction between these two factors were entered as explanatory variables, and families were used as random factor. When necessary, we conducted pairwise comparisons using estimated marginal means of the models and with P-values corrected for multiple testing using Tukey methods (*emmeans* package). We checked all model residuals for homoscedasticity, normal distribution and overdispersion using *DHARMA* package. This required us to log+1 transform mother-egg contacts, square-root transform mother-nymphs contacts and nymph number, inverse-transform UTL and log-transform CCR, Hsp68, Hsp70, Hsp83, Hsp90 and PPO1.

RESULTS

Exposure to thermal waves affected two of the three forms of maternal care, and the level of investment in maternal care measured through mother weight variation. In particular, it influenced the likelihood of mothers to collect their eggs 2 h after they have been scattered (Figure 1A; LR $\chi^2_3 = 30.12$, $P < 0.0001$), the frequency of mother-egg contacts (Figure 1B; $F_{3,192} = 2.77$, $P = 0.043$), the weight variation by mothers during the period of egg care (Figure 1C; $F_{3,149} = 4.40$, $P = 0.005$), but not the frequency of mother-nymph contacts (Figure 1D; $F_{2,119} = 1.79$, $P = 0.172$). Mothers were less likely to collect their eggs in the treatment at 24°C compared to all other temperatures (all $P > 0.002$), and mothers gained the largest amount of weight at 24°C compared to all other temperatures (all $P > 0.031$). On the other hand, the number of contacts between mothers and eggs were lower in the 3°C compared to the 10°C (control) treatment (t-ratio = -2.7, $P = 0.037$), but comparable between the other treatments (all $P > 0.138$).

Exposure to thermal waves also affected six of the nine proxies measured for egg development and offspring quality. It affected egg survival rate at day 34, i.e. immediately after the second wave (Figure 2A; Likelihood Ratio $\chi^2_3 = 8.06$, $P = 0.045$), the minimum egg development time (Figure 2B; $F_{3,151} = 125.06$, $P < 0.0001$), egg hatching success (Figure 2C; LR $\chi^2_3 = 200.61$, $P < 0.0001$), the absolute number of newly hatched nymphs produced (Figure 2D; LR $\chi^2_3 = 89.45$, $P < 0.0001$), the date of the second moult in the resulting nymphs (Figure 2F; $F_{3,103} = 6.76$, $P = 0.0003$), and nymphs' capacity to survive extreme high temperatures (UTL; Figure 3A; $F_{2,122} = 8.11$, $P = 0.0005$). Eggs survival at day 34 tended to be lower in the 24°C compared to the 10°C (control) treatment (pairwise comparison, Z ratio = 2.56, $P = 0.051$), but not between the other treatments (all $P > 0.268$). Similarly, the mean hatching success was lower in the 24°C treatment compared to the three other treatments (24°C vs 3°C: Z ratio = 6.31, $P < 0.0001$; 24°C vs 10°C: Z ratio = 5.94, $P < 0.0001$; 24°C vs 17°C: Z ratio = 5.61, $P < 0.0001$), and lower in the 17°C treatment compared to the 3°C treatment (Z ratio = 2.53, $P = 0.056$). The minimum egg development time was shorter in the 24°C compared to the 17°C treatment (Z ratio = 4.3, $P = 0.0002$), shorter in the 17°C compared to the 10°C (control)

treatment (Z ratio = 12.7, $P < 0.0001$), and then shorter in the 10°C (control) compared to the 3°C treatment (Z ratio = 2.72, $P = 0.036$). Nymph development time was shorter in the 17°C treatment compared to both the 3°C and 10°C (control) treatments compared to the 17°C treatment (3°C: t-ratio = 4.01, $P = 0.0007$; 10°C: Z ratio = 3.78, $P = 0.0015$), with no difference between the other treatments (all $P > 0.74$). Finally, the upper thermal limit (UTL) was lower for nymphs of the 17°C compared to the 10°C (control) treatment (Z ratio = -2.84, $P = 0.015$) and 3°C (Z ratio = -3.9, $P = 0.0004$), whereas there was no difference between the 10°C (control) and 3°C treatments (Z ratio = -1.23, $P = 0.439$). In contrast to these effects, the exposure to thermal waves did not affect the mean weight of newly hatched nymphs (Figure 2E, $F_{3,146} = 0.56$, $P = 0.639$), nor their chill coma recovery time (CCR; Figure 3B; $F_{2,111} = 0.91$, $P = 0.406$).

Finally, exposure to thermal waves did not affect the overall gene expression in nymphs of any of the six tested heat shock proteins (Hsp60 : LR $\chi^2_2 = 3.98$, $P = 0.136$; Hsp68: LR $\chi^2_2 = 2.24$, $P = 0.326$; Hsp70: LR $\chi^2_2 = 1.32$, $P = 0.512$; Hsp83: LR $\chi^2_2 = 1.66$, $P = 0.437$; Hsp90: LR $\chi^2_2 = 0.06$, $P = 0.972$), the immune gene PPO1 (LR $\chi^2_2 = 4.19$, $P = 0.122$), or the heat shock-induced expression of five of these six genes (i.e. interaction between the treatments and the heat shock; Hsp60 : LR $\chi^2_2 = 5.85$, $P = 0.054$; Hsp70: LR $\chi^2_2 = 2.46$, $P = 0.292$; Hsp83: LR $\chi^2_2 = 0.42$, $P = 0.810$; Hsp90: LR $\chi^2_2 = 1.99$, $P = 0.370$; PPO1 : LR $\chi^2_2 = 2.37$, $P = 0.306$). However, there was a significant interaction between heat shock and thermal wave on the expression of Hsp68 (Figure 4B; LR $\chi^2_2 = 11.79$, $P = 0.003$). This interaction showed that at 20°C, the expression of Hsp68 was lower in the 17°C treatment than in the 10°C (control) treatment (t-ratio = 3.183, $P = 0.031$), with intermediate values in the 3°C treatment (t-ratio = -0.392, $P = 0.999$), whereas at 30°C there was no difference in the expression of Hsp68 between the three treatments (all $P > 0.841$). This notwithstanding, the expression levels of five of the six heat shock proteins tested and of PPO1 were overall higher after a new heat shock at 30°C than at 20°C (Figure 4; Hsp60 : LR $\chi^2_1 = 16.68$, $P < 0.0001$; Hsp68: LR $\chi^2_1 = 320.71$, $P < 0.0001$; Hsp70: LR $\chi^2_1 = 337.17$, $P < 0.0001$; Hsp83: LR $\chi^2_1 = 103.94$, $P < 0.0001$; PPO1 : LR $\chi^2_1 = 13.13$, $P = 0.0003$; but Hsp90: LR $\chi^2_1 = 0.03$, $P = 0.864$).

DISCUSSION

A growing body of research reveals that global warming can have major effects on animal reproduction (Bourne et al., 2021; Grew et al., 2019; Ma et al., 2021; Pilakouta et al., 2023; Vincze et al., 2013; Wiley and Ridley, 2016). However, there remains a surprising gap in our understanding of how parental care and the benefits of parental care respond to extreme temperature changes such as heat waves. In the present study, we addressed these gaps in knowledge by testing whether exposure to thermal waves of 3°C, 10°C (control), 17°C or 24°C during egg development affects maternal care and offspring quality in the European earwig. Our results first show that heat waves decreased maternal egg care and the level of maternal investment in egg care. Our results then show that heat waves reduced the rate of egg hatching and absolute number of nymphs produced, accelerated egg and nymph development, and reduced the maximal temperature they can withstand before they die. In contrast, heat or cold waves had no effect on the mean weight of newly hatched nymphs, and their speed of recovery from chill coma. Finally, our results show a significant interaction between heat shock and thermal waves on the expression of Hsp68 where heat waves reduced the basal level of Hsp68 gene expression only at 20°C in nymphs, but not that of five other heat shock proteins and immune components.

Our data first show that heat or cold waves subsequently changed the expression and investment in maternal care, and that the nature of these changes was trait specific. Compared to the other treatments, heat waves at 24°C reduced the speed of egg gathering and the level of maternal investment in egg care (i.e., induced a maternal weight gain during the period of egg care), while cold waves at 3°C reduced the frequency of mother-egg contacts. Overall, the detrimental effects of heat waves on care are consistent with results found in other animal species (e.g. Kight et al., 2000; Kight and Cseke, 1998; Wiley and Ridley, 2016). In earwigs, one possible explanation for this finding is that the heat waves at 24°C could have stimulated the metabolic activity in mothers (Hahn and Denlinger, 2011; Sinclair, 2015), so mothers were generally more active and less focused on egg care. The link between ambient temperature and activity levels is well known in insects (Huey and Kingsolver, 1989) and is also consistent with the reduced frequency of mother-egg contact observed in the 3°C treatment in earwigs. However, the frequency of mother-egg contact did not vary between the 10°C and 24°C treatments. This suggests that it is cold, not heat, that triggers changes in female activity, and therefore this change is unlikely to explain the reduced rate of egg collection and level of maternal investment in egg care observed at 24°C.

Another non-mutually exclusive hypothesis to explain the detrimental effects of heat waves on care is that 24°C reduces egg quality and that mothers stop caring for poor quality eggs (Filippi et al., 2009; Richardson et al., 2010). This prediction is consistent with previous studies showing that earwig mothers provide less care to poor quality nymphs (Mas et al., 2009; Mas and Kölliker, 2011) and the present study showing a general increase in mother body weight during this period (a proxy for reduced investment in care; Koch and Meunier, 2014; Van Meyel and Meunier, 2020). The weight gain also suggests that mother consumed some of their eggs. This could be an adaptive strategy that allows mothers to reallocate resources for future reproduction if current environmental conditions are harsh and/or current eggs are unlikely to develop and survive (Manica, 2002; Van Meyel and Meunier, 2020). Filial egg cannibalism may also be a non-adaptive behaviour triggered by thermal stress for mothers. Overall, these findings call for future studies to determine which of these hypotheses explain the effects of thermal waves on maternal care and determine whether these effects are primarily driven by the changes in the eggs and/or the parents. This notwithstanding, our results emphasize that thermal waves during egg care induce changes in maternal care after the thermal waves.

In addition to maternal care, we found that the temperature of the wave affected the fate of eggs in terms of development time and hatching success, even though this exposure lasted a total of 6 days over the more than 2 months of typical egg development. First, exposure to colder and warmer temperatures slowed down and accelerated the development of the eggs, respectively. No effect on female survival, with only one female dying during the egg care period was recorded. This is consistent with previous studies showing that a longer winter slows down egg development (Körner et al., 2018) and that eggs develop faster if they come from natural populations with warm winters (Tourneur and Meunier, 2020). Interestingly, the accelerated egg development in the warm treatments was associated with a reduction in hatching success, which resulted in almost complete clutch failure in the 24°C treatment. Ectothermic organisms like *F. auricularia* have metabolic rates highly dependent on ambient temperature (Angilletta, 2004; Howe, 1967; Taylor, 1981). As the mean environmental temperature increases, the enzymatic activities driving metabolism typically become more efficient (Kingsolver and Huey, 2008). However, heat exposure also induces dehydration and increases reactive oxygen compounds (ROS) (Colinet et al., 2015b), the

excessive accumulation of which can be toxic to organisms due to overoxidation and cellular damage (Sohal and Orr, 2012). Such metabolic damage processes could be responsible for the observed drop in nymphs viability under intense heat waves (24°C), as reported in other insects, such as the Asian corn borer and the Asian lady beetle (Harvey et al., 2020; Zhang et al., 2014; Zhou et al., 2018). Alternatively, falling oxygen supply and egg dehydration could also be drivers of egg mortality after extreme thermal events (Woods and Hill, 2004; Zhou et al., 2018). However, these are unlikely to explain our results, since the eggs were maintained in a water-saturated sand substrate. Overall, these results indicate that both the range and intensity of temperature changes can affect egg development in the European earwig and that these changes have a cost in terms of hatchability. More generally, they highlight that access to maternal egg care does not abolish the effects of egg exposure to thermal waves on these traits.

Our results then show that thermal waves at 17°C reduced the thermal resistance of the resulting nymphs in terms of UTL and reduced the basal expression of a heat shock protein (Hsp68). However, it had no effect on the CRC and no effect on the expression of four other heat shock proteins and one immune-related gene. Overall, these results contrast with data showing that heat stress during the egg and larval stages often induces heat tolerance (heat acclimation) in many other insects at different life stages (Kingsolver et al., 2011; Scharf et al., 2015; Terblanche and Chown, 2006). For example, higher temperatures during juvenile growth in *Tribolium castaneum* resulted in extended heat knockdown times for adult individuals (Scharf et al., 2015) and adult CT_{max} of *Culex pipiens* was higher after larval heat acclimation (Gray, 2013). Heat acclimatization has been related to the synthesis and accumulation of different protective products like polyols, sugars and heat shock proteins (Hahn and Denlinger, 2011; Sinclair, 2015; Storey and Storey, 2012). Further research is needed to investigate the molecular mechanisms underlying the differential expression response of Hsp68 and explore how it impacts the overall fitness and resilience of the nymphs to thermal stress throughout their development. Notwithstanding these underlying mechanisms, our results suggest that exposure of *F. auricularia* to thermal waves at the egg stage may result in sublethal performance at the nymph stage.

In conclusion, our study shows that relatively short and intense temperature changes during egg development can have negative effects on the expression of maternal care, egg development, hatching success and performance of newly hatched juveniles in the European earwig. These findings reveal that access to maternal care is not enough to counteract the stress of exposure to non-optimal temperatures during egg development, and thus that species with parental care do not necessarily have access to an efficient protection in case of significant increase in temperature during winter (AlRashidi et al., 2010; Bourne et al., 2021; Grew et al., 2019; Hopkins et al., 2011; Vincze et al., 2013). Our experimental design involved subjecting entire families to two thermal waves on days 15 and 30 after oviposition. An intriguing question remains whether the effects of both events are additive or if one event has a more pronounced detrimental effect than the other. Furthermore, our study prompts broader inquiries into the role of maternal care during such thermal waves, and whether the ability of mothers to move eggs to different locations could mitigate the effects of this exposure (Tourneur et al., 2022). Future research will be necessary to accurately assess the behavioral effects of heat waves on females during the heat wave and the relative contributions of reduced maternal care versus the direct impacts of heat waves on the apparent decline in fitness in *F. auricularia*.

CRedit Authorship Statement

LLR: Data curation; Investigation; Methodology; Validation; Writing - review & editing. JM & IV: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft.

Data accessibility

The datasets and the R script are available in a public repository at:
10.5281/zenodo.13918696

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Declaration of Competing Interests

The authors declare no competing interests.

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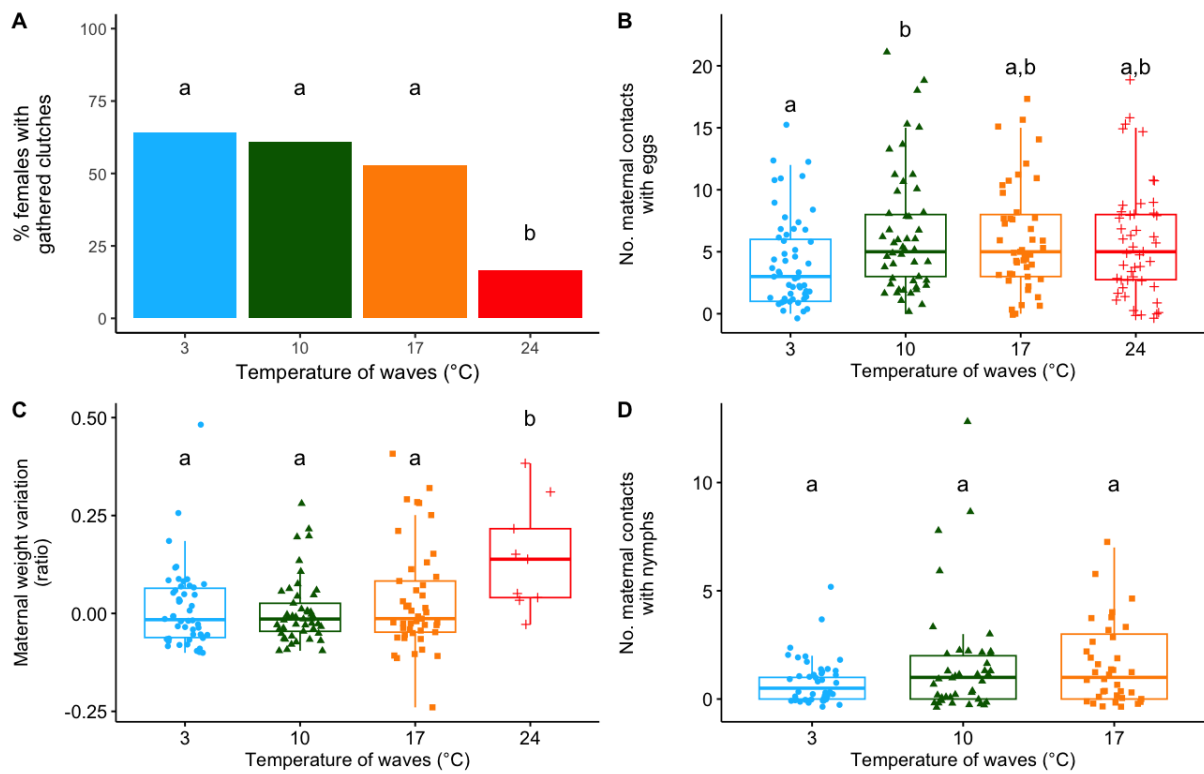


Figure 1 – Effects of waves temperature on maternal care. (A) Proportion of females that collected their clutch of eggs 2 h after dispersal. (B) Number of active contacts between mothers and eggs. (C) Maternal investment in egg care as measured by the relative weight variation of mothers from oviposition to hatching. (D) Number of active contacts between mother and nymphs. Boxplots depict median and interquartile ranges, with whiskers extending to 1.5 times the interquartile range and dots representing jittered experimental values. Different letters refer to $P < 0.05$.

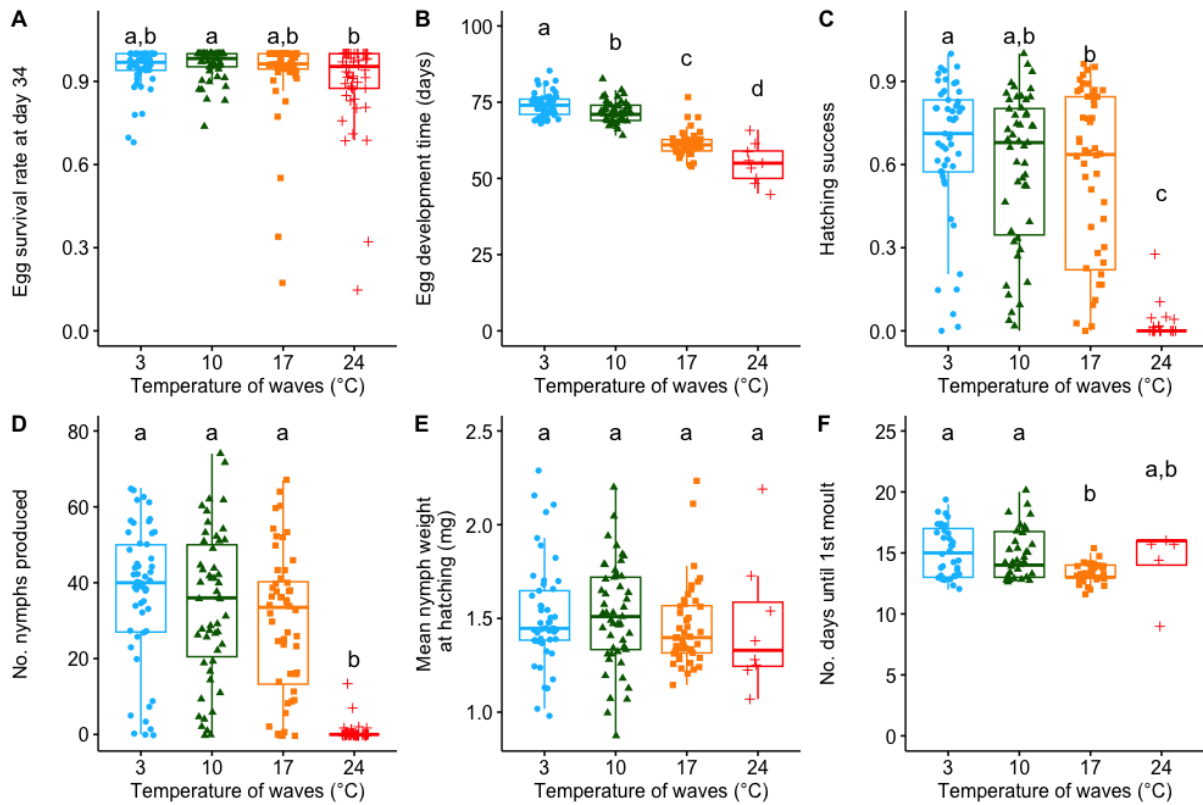


Figure 2 – Effects of waves temperature on eggs and nymphs. (A) Survival rate of eggs one day after the second thermal wave, i.e. day 34. (B) Number of days between oviposition and the first hatched egg per clutch. (C) Hatching success of the eggs. (D) Absolute number of nymphs produced. (E) Mean weight of the nymphs one day after hatching. (F) Number of days between hatching and nymphs reaching the second instar. The initial number of families (n) ranged from 51 to 53 across the different treatments. Boxplots depict median and interquartile ranges, with whiskers extending to 1.5 times the interquartile range and dots representing jittered experimental values. Different letters refer to $P < 0.05$.

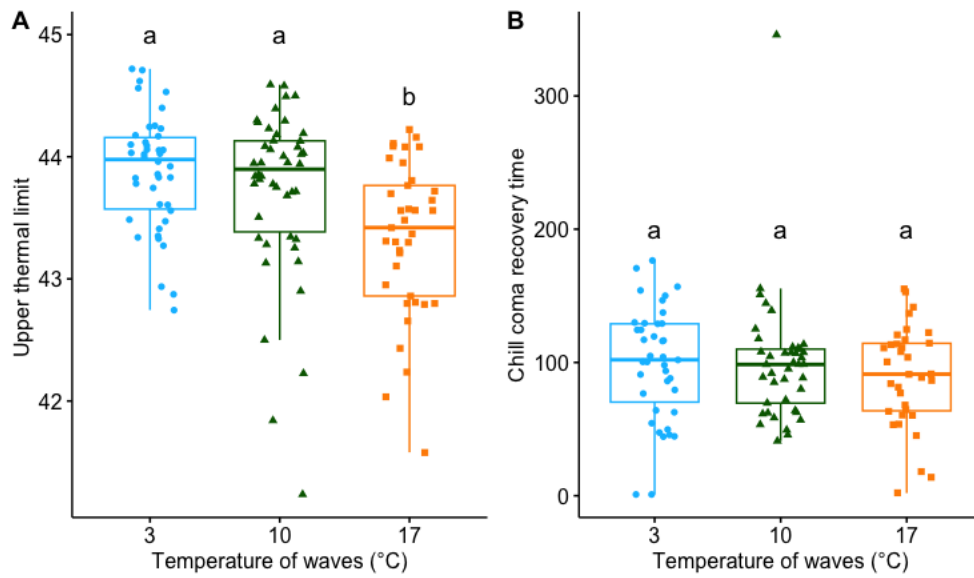


Figure 3 – Effects of waves temperature on nymphs' thermal resistance. (A) Upper thermal limit. (B) Chill coma recovery time. Boxplots depict median and interquartile ranges, with whiskers extending to 1.5 times the interquartile range and dots representing jittered experimental values. Different letters refer to $P < 0.05$.

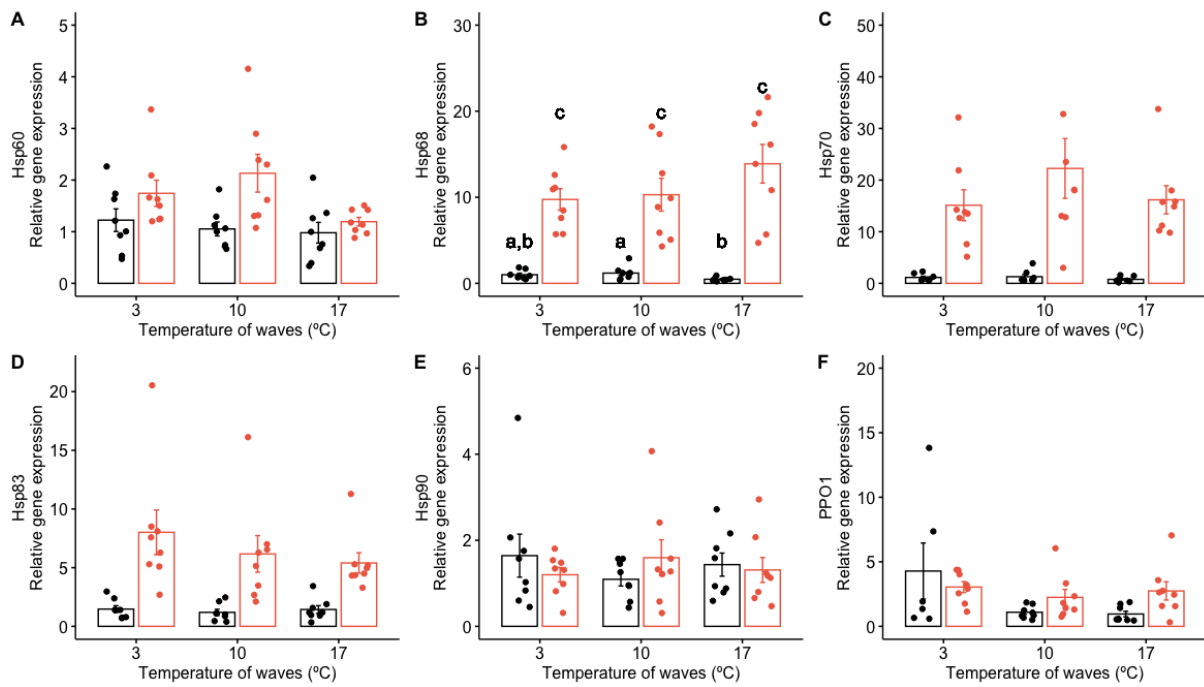


Figure 4 – Heat shock response of candidate genes. Relative mRNA expression levels of *Forficula auricularia* heat shock protein homologs Hsp60, Hsp68, Hsp70, Hsp83, Hsp90 and immune response PPO1 in response to 1 hour at 20°C (black, control) or 30°C (orange, heat shock) in 9-day-old nymphs from different thermal regimes. Expression in each treatment was calculated relative to the actine expression level of untreated (thermal wave 10°C) control (20°C) nymphs, which was arbitrarily set at 1. Expression values are shown as means \pm SE (n = 8 per treatment). Different letters refer to $P < 0.05$.

Supplementary table 1 - Candidate genes and primers selected for qPCRs to assess the heat shock response of nymphs from different thermal regimes.

Gene	Accession number	Oligo Name	Sequence (5' - 3')	Amplified fragment length (bp)	Function	<i>D. melanogaster</i> gene name (Annotation Symbol, FlyBase ID)
Hsp60	GAYQ02046497.1	F	GATGGAAAAACCCCTTCACGA	286	Molecular chaperone	Heat shock protein 60A (CG12101, FBgn0015245)
		R	CCACTTGGAGGCCAATCTTA	286		
HSP68	GAYQ02038622.1	F	CTGAACCCAGTCGAGAAAGC	265	Molecular chaperone	Heat shock protein 68 (CG5436, FBgn0001230)
		R	CCAAAGAGAGCGGAGTTACG	265		
Hsp70	GAYQ02038623.1	F	CCTTGAGGTGGATGCCCTTT	272	Molecular chaperone	Heat shock protein70(CG4264, FBgn0266599)
		R	CATAGGCAACAGCCTCGTCA	272		
Hsp83	GAAX01018341.1	F	TTGGCATGACAAAAGCTGAC	263	Molecular chaperone	Heat shock protein 83 (CG1242, FBgn0001233)
		R	ACCACGTCCAAGAGTTTAC	263		
Hsp90	GAYQ02036606.1	F	CACTCTGGCAAGCAAGACA	201	Molecular chaperone	Heat shock protein 90B1 (FBgg0000500)(CG5520, FBgn0039562)
		R	TCGGTTTGTGTCAATTGAGC	201		
PPO1	GAYQ02037981.1	F	CTCGTGGTGTCTTAGCA	346	Immune defense	Pro Phenol Oxidase (CG42639, FBgn0283437)
		R	CCACAGCCGAAAAGTTGAA	346		
Actin	MK756009.1	F	GCTGGTCGTGATCTGACTGA	226	Housekeeping	Actin 5C (CG4027,FBgn0000042)
		R	GGAATCGCTCATTCCGATA	226		
eEF1alpha1	MK756012.1	F	ATCGGCGTACCGAAAAACT	168	Housekeeping	Eukaryotic translation elongation factor 1 alpha 1 (CG8280, FBgn0284245)
		R	CAACAGCGACAGTTTGCCTC	168		
RpL11	GAYQ02038183.1	F	GCACTTTGGCAGCTCTTG TG	207	Housekeeping	<u>Ribosomal protein L11</u> (CG7726, FBgn0013325)
		R	TTTTCCGGTTCCAGTTCGCT	207		
Mnf	GAYQ02036296.1	F	ATGATCTGGAGTTGGGGAGC	231	Housekeeping	Forkhead box K (CG11799, FBgn0036134)
		R	CGAAGTCAAGAGGAACCAGG	231		