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Divergent survival and reproductive trajectories across three generations of warming in a small tropical cyclopoid copepod

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Divergent survival and reproductive trajectories across three generations of warming in a small tropical cyclopoid copepod

Running title: *Dioithona rigida* under multigenerational warming

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Abstract

Short-lived, small tropical ectotherms may experience temperatures near their upper limits for several generations, yet risk assessments often rely on short-term exposure and responses within a single generation. Whether survival, body size and reproduction respond in parallel to sustained warming across multiple generations is unclear, which limits predictions of ecological consequences. We exposed five replicate lineages of the small tropical cyclopoid copepod *Dioithona rigida* to constant 28, 30, 32 or 34 °C for three generations. Survival to adulthood was on average 52.6% lower at 32-34 °C than at 28-30 °C, with no significant change across generations. In contrast, body size decreased and clutch size and nauplii production increased across generations at all temperatures, indicating a generation-related shift largely independent of temperature. Temperature differences in reproduction appeared only in F3, with smaller clutches at 32-34 °C and lower nauplii production at 34 °C. Fewer individuals reaching adulthood could reduce the number of reproducing adults and the supply of copepods to fish and shrimp larvae in tropical coastal food webs.

Keywords: marine copepods; coastal warming; multigenerational exposure; reproductive performance; thermal sensitivity; tropical coastal ecosystems

1. Introduction

Whether organisms can maintain performance across generations under sustained warming is central to predicting biological responses to climate change (Donelson et al., 2012, 2018, Ryu et al., 2018, Doan et al., 2019, Dinh et al., 2020b, 2021). This is particularly relevant to tropical marine ectotherms where many tropical species are short-lived species and experience temperatures close to their upper physiological limits for weeks to months, which could be equal to several generations (Tewksbury et al., 2008, Nguyen et al., 2011). In copepods, a global synthesis identified smaller warming tolerances in populations from tropical environments and substantial geographical and taxonomic gaps in available thermal-tolerance measurements (Sasaki and Dam, 2021, Sasaki et al., 2022). As copepods transfer energy from phytoplankton and microzooplankton to higher trophic levels and contribute substantially to pelagic ecosystem functioning (Kiørboe, 1998, Chew and Chong, 2011, Kiørboe, 2011), changes in their survival, size, and offspring production could consequently alter both recruitment and the resources available to consumers (Chew and Chong, 2011).

Extreme temperatures can impair performance of tropical copepods (Lehette et al., 2016, Doan et al., 2018, Low et al., 2018). In *Pseudodiaptomus annandalei*, exposure to an extreme temperature (34 °C) across three generations prolonged development and reduced adult size, clutch size, hatching success, and nauplii production (Doan et al., 2019). Temperature-dependent feeding and metabolic responses provide energetic pathways for these effects (Lehette et al., 2016, Doan et al., 2018, Low et al., 2018). The changes in gut microbiome may also associate with the individual performance under warming (Vu et al., 2024). Exposure history can further change these relationships. Responses to warming and contaminants depend on life stages, response variables, and the number of exposed generations (Dinh et al., 2022). Chronic warming increased heat tolerance and reproductive performance at high temperatures in *Paracartia grani*, while warm-reared individuals were smaller and produced fewer offspring near the reproductive optimum (de Juan et al., 2023b). Recent work on *Nitocra spinipes* likewise found that the pace and duration of warming influenced reproductive and physiological responses across generations (Janssens et al., 2026).

Knowledge of how copepods respond to warming is highly unevenly distributed among taxa. Studies of thermal performance and heat tolerance have concentrated on calanoids and harpacticoids (Harada and Burton, 2019, Harada et al., 2019, Dinh et al., 2020a, Sasaki and Dam, 2021, Sasaki et al., 2024, Lutier et al., 2025), whereas cyclopoids have received much less attention (Nowakowski and Sługocki, 2024). This gap matters because small cyclopoids such as oithonids are widespread in coastal plankton (Gallienne and Robins,

2001, Hwang et al., 2010, Dahms et al., 2015, Madoui et al., 2017) and differ from calanoids in size and feeding mode, typically being ambush feeders (Kiørboe, 2011, Gréve et al., 2017). These traits could change how temperature affects their energy budget, growth and reproduction (Almeda et al., 2011, Kiørboe, 2024). The few cyclopoid studies have addressed short-term responses, such as acute heat shock or physiological and behavioural responses to temperature and salinity stress (Svetlichny et al., 2021, Nowakowski and Sługocki, 2024). It therefore remains unclear whether small tropical cyclopoids, which may experience near-limit temperatures for several generations, can sustain survival, body size and reproduction under such conditions.

Here, we exposed the tropical copepod *Dioithona rigida* to four temperatures (28, 30, 32, and 34 °C) across three consecutive generations, maintaining five replicate lineages per temperature. We measured survival to adulthood, male and female prosome length, clutch size, the proportion of females whose eggs hatched, and nauplii production. Based on studies of other tropical copepods, we hypothesised that survival would be lower at 32-34 °C than at 28-30 °C (Lehette et al., 2016, Doan et al., 2019, Nguyen et al., 2020), and that this difference would either persist or narrow across generations if plasticity or selection increased tolerance (Truong et al., 2022, de Juan et al., 2023b). We also predicted smaller adults at higher temperatures, as expected from the temperature-size rule (Atkinson, 1994, Horne et al., 2016), and lower reproductive output at high temperature (Doan et al., 2019).

2. Materials and methods

2.1 Study species and collection

Adult *Dioithona rigida* were collected from aquaculture ponds in Cam Ranh, Khanh Hoa province, Vietnam in September 2024. The experiments were conducted at the Cam Ranh Aquaculture Research Station, Nha Trang University. Copepods were collected using a zooplankton net and separated under a stereomicroscope using a plastic pipette. The isolated population was maintained in the laboratory before exposure to the experimental temperatures.

2.2 Experimental conditions and acclimation

Seawater was obtained from Cam Ranh Bay, treated with chlorine, and filtered after residual chlorine had dissipated. Freshwater used to adjust salinity was filtered, aerated, and left to stand for at least 48 h before use. Before the experiment, copepods were maintained at approximately 28 - 29 °C and a salinity of 22 PSU.

Salinity was gradually reduced to 20 PSU over one day. The parental copepods were acclimated to experimental temperatures over four days by increasing gradually approximately 2 °C per day for the higher-temperature treatments. Experimental bottles (1L borosilicate glass bottles) were placed in temperature-controlled water baths and provided with gentle aeration. Copepods were fed the diatom *Chaetoceros muelleri* at a concentration of 87,000 cells mL^{-1} once daily at around 08:00 am (Doan et al., 2018). The algae were cultured semi-continuously in sterilised seawater enriched with f/2 medium at approximately 25 °C under continuous illumination and aeration.

2.3 Temperature exposure across generations

We examined the effects of four temperatures (28, 30, 32 and 34 °C) across three consecutive generations (F1, F2 and F3). Each temperature treatment consisted of five replicate cultures. The offspring produced by each replicate culture were used to establish the corresponding culture in the next generation, maintaining separate lineages throughout the experiment. To initiate F1, 50 acclimated parental females carrying egg sacs were assigned to each 1-L glass bottle and maintained at the corresponding experimental temperature. After 30 h, adults were separated from the nauplii, and approximately 500 nauplii were placed in each 1-L rearing bottle. The nauplii were maintained at their assigned temperature with gentle aeration and fed *C. muelleri* as described above. Copepods were reared until adulthood, which lasted 7–10 days. When F1 reached adulthood, 50 females carrying egg sacs from each replicate were collected and allowed to reproduce at their respective temperature for 30 h. The resulting nauplii were used to establish F2 at the same initial density of 500 nauplii per bottle. The procedure was repeated to establish F3. At each generation, adults were used to determine survival, body size, clutch size, hatching success and nauplii production.

2.4 Response variables

Survival was determined from the number of adults recovered relative to the 500 nauplii used to establish each culture. At each temperature and generation, prosome length was measured for 10 adult males and 10 adult females from each replicate bottle. Measurements were made under a stereomicroscope fitted with an eyepiece scale. Prosome length was measured from the anterior end of the head to the posterior end of the prosome and converted to μm using a calibrated reference scale at the same magnification. Clutch size was determined for 10 females from the same females sampled for prosome length measurements from each bottle. Egg-carrying females were selected and preserved in 5% formalin. Their egg sacs were opened with a fine needle under a

stereomicroscope, and the total number of eggs carried by each female was counted. Clutch size was expressed as eggs per female.

Hatching success was assessed using 10 egg-carrying females per replicate culture. Females were placed individually in wells containing 3 mL of clean seawater and microalgal food. The plates were maintained at the corresponding experimental temperature. After 30 h, each female was examined under a stereomicroscope and recorded as having hatched eggs or no hatched eggs. Hatching success was expressed as the percentage of the 10 females whose eggs hatched. This measure describes the proportion of females with hatched eggs.

Nauplii production was measured using groups of 10 egg-carrying females. Each group was placed in a glass bottle containing 200 mL of clean seawater and maintained at its corresponding experimental temperature. After 30 h, nauplii were collected using a 25-µm mesh, preserved in 5% Lugol's solution, and counted under a stereomicroscope. Production was expressed as the total number of nauplii produced by 10 females over the incubation period.

2.5 Statistical analysis

All analyses were conducted in R version 4.5.2 (R Core Team 2025) using RStudio and the packages *lme4*, *lmerTest* and *emmeans*. Temperature (28, 30, 32 and 34 °C) and generation (F1–F3) were treated as categorical fixed factors. The culture bottle was the experimental unit, with five replicate lineages per temperature followed across three generations, yielding 60 bottle–generation observations. Individual measurements within bottles were treated as subsamples.

Survival to adulthood was analysed using a generalized linear model (GLM) with a quasibinomial error distribution and logit link. The response comprised the numbers of adults recovered and individuals that did not survive to adulthood out of the initial 500 nauplii per bottle. Temperature, generation and their interaction were included as explanatory variables. This model estimated dispersion from the data and did not include a lineage term. Hatching success was analysed using the same model structure, with the numbers of females with and without hatched eggs out of 10 females per bottle as the response. For hatching success, an initial beta-binomial mixed model failed to produce reliable parameter uncertainty estimates, while a subsequent binomial mixed model estimated the lineage variance as zero and indicated underdispersion. A quasibinomial GLM without a lineage term was therefore fitted. For both quasibinomial models, the

temperature × generation interaction was evaluated by comparing the full model with an additive model using an F test. The joint contribution of each factor, including its interaction with the other factor, was evaluated by comparing the full model with a model containing only the other factor.

Prosome length was analysed using a linear mixed-effects model (LMM) containing temperature, generation, sex and all two- and three-way interactions as fixed effects. Clutch size was analysed using an LMM containing temperature, generation and their interaction. Both models included random intercepts for lineage and bottle within generation; bottle identifiers represented unique lineage–generation combinations. Nauplii production was analysed using an LMM containing temperature, generation and their interaction as fixed effects and lineage as a random intercept. Models were fitted by restricted maximum likelihood. Fixed effects in these factorial analyses were evaluated using Type III F tests with sum-to-zero contrasts and Satterthwaite approximations for denominator degrees of freedom.

The correlation between female size and clutch size was examined using bottle-mean female prosome length and bottle-mean clutch size. The LMM included female prosome length, temperature, generation and the temperature × generation interaction as fixed effects, with lineage as a random intercept. Prosome length was centred and scaled so that its coefficient represented the difference in mean clutch size associated with a 50-µm increase in female mean length. A common size–clutch slope was estimated across experimental groups and evaluated using a Satterthwaite t test. For each bottle, we calculated mean female prosome length and mean clutch size. We then calculated Pearson correlations between these bottle means using all 60 observations together and separately for each generation (20 observations per generation).

Pairwise comparisons were obtained using *emmeans*. Temperatures were compared within each generation, and generations were compared within each temperature; for prosome length, these comparisons were additionally conditioned on sex. Tukey adjustment was applied separately within each comparison family. Male–female differences were evaluated within each temperature–generation combination, with Holm adjustment across the 12 comparisons. Comparisons from the quasibinomial models used asymptotic z tests on the log-odds scale, whereas comparisons from the LMMs used t tests with Satterthwaite degrees of freedom. Statistical significance was assessed at $P < 0.05$.

Model assessment included residual plots and checks for convergence problems and singular fits. The prosome-length and nauplii-production models estimated the lineage random-intercept variance at zero and were classified as singular fits; the prosome-length model retained a positive bottle-level variance. Residual

plots indicated unequal variance among some groups and departures from normality in the tails. Figures 1-3 and 5-6 show observed means ± 1 SE across five replicate cultures, with individual measurements averaged within bottles before calculating treatment summaries. Figure 4 shows bottle-mean female prosome length and clutch size alongside fitted relationships from the size–clutch model.

3. Results

3.1 Survival

Survival was more than 2 times lower at 32 and 34 °C than at 28 and 30 °C (main effect of temperature, quasibinomial model, $F_{9,48} = 21.57$, $P < 0.001$) and this effect was consistent across all three generations (temperature × generation interaction, $F_{6,48} = 0.32$, $P = 0.92$, Fig. 1). Within each generation, survival at both 32 and 34 °C was significantly lower than at either 28 or 30 °C (all Tukey-adjusted $P < 0.0001$). Survival did not differ significantly between 28 and 30 °C or between 32 and 34 °C in any generation (all adjusted $P \geq 0.62$). The overall contribution of generation was not significant ($F_{8,48} = 1.24$, $P = 0.295$), and comparisons among generations within each temperature detected no significant differences (all adjusted $P \geq 0.165$).

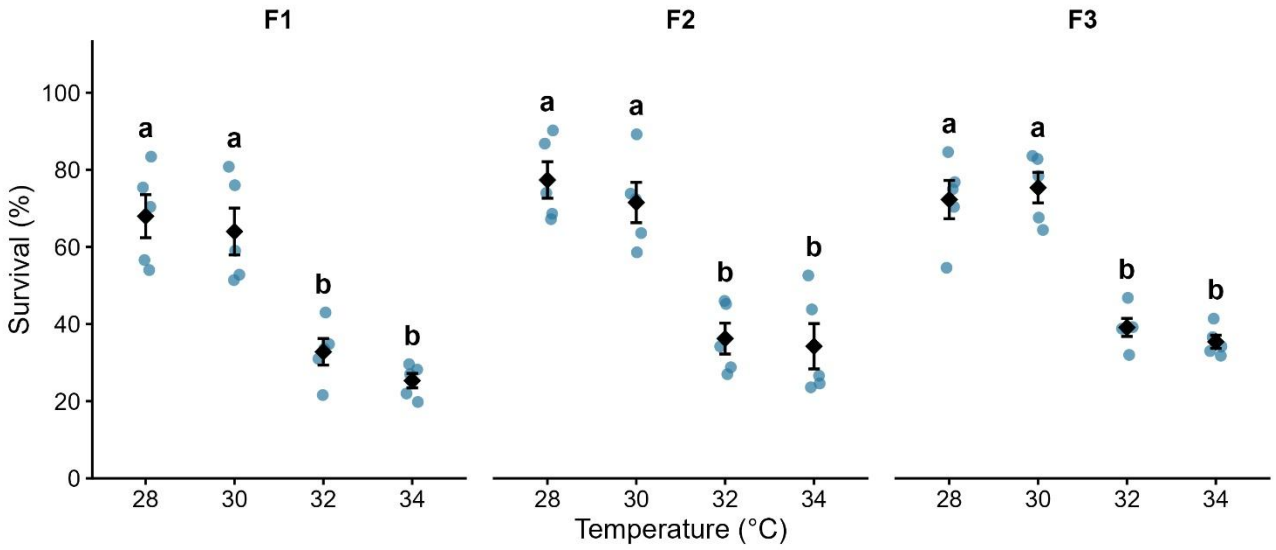


Figure 1. Survival to adulthood of *D. rigida* at four temperatures across three generations (F1–F3). Blue circles represent individual replicate cultures; black diamonds and error bars indicate means ± 1 SE across five replicates. Within each generation, groups sharing a lowercase letter do not differ significantly (Tukey-adjusted comparisons, $P < 0.05$). Letters indicate comparisons among temperatures within each generation only.

189

190 *3.2 Prosome length*

191 Prosome length differed significantly with temperature ($F_{3,48} = 9.30$, $P < 0.001$), generation ($F_{2,48} = 117.60$, P
192 < 0.001), and sex ($F_{1,1128} = 330.90$, $P < 0.001$; Fig. 2). Females were larger than males at every temperature in
193 all three generations (all Holm-adjusted $P \leq 0.016$). The difference between sexes varied among generations,
194 as indicated by a significant generation × sex interaction ($F_{2,1128} = 13.67$, $P < 0.001$). Neither the temperature
195 × generation interaction ($F_{6,48} = 0.61$, $P = 0.718$), the temperature × sex interaction ($F_{3,1128} = 0.10$, $P = 0.962$),
196 nor the three-way interaction ($F_{6,1128} = 0.43$, $P = 0.861$) was significant.

197 Mean prosome length decreased with increasing temperature in both sexes, although most temperature
198 comparisons within individual generations were not significant. F1 males were smaller at 34 °C than at 28 °C
199 (Tukey-adjusted $P = 0.0016$). Similarly, F2 females were smaller at 34 °C than at 28 °C ($P = 0.011$). No other
200 temperature comparisons within a generation and sex were significant (all adjusted $P \geq 0.071$).

201 Male prosome length decreased successively from F1 to F2 and F3 at all temperatures (all Tukey-
202 adjusted $P \leq 0.028$). Female prosome length did not differ significantly between F1 and F2 at any temperature
203 (all adjusted $P \geq 0.27$), but was smaller in F3 than in F1 at all temperatures (all adjusted $P \leq 0.004$). Females
204 were also smaller in F3 than in F2 at 28, 30, and 32 °C (all adjusted $P \leq 0.008$), whereas this difference was
205 not significant at 34 °C ($P = 0.087$).

206

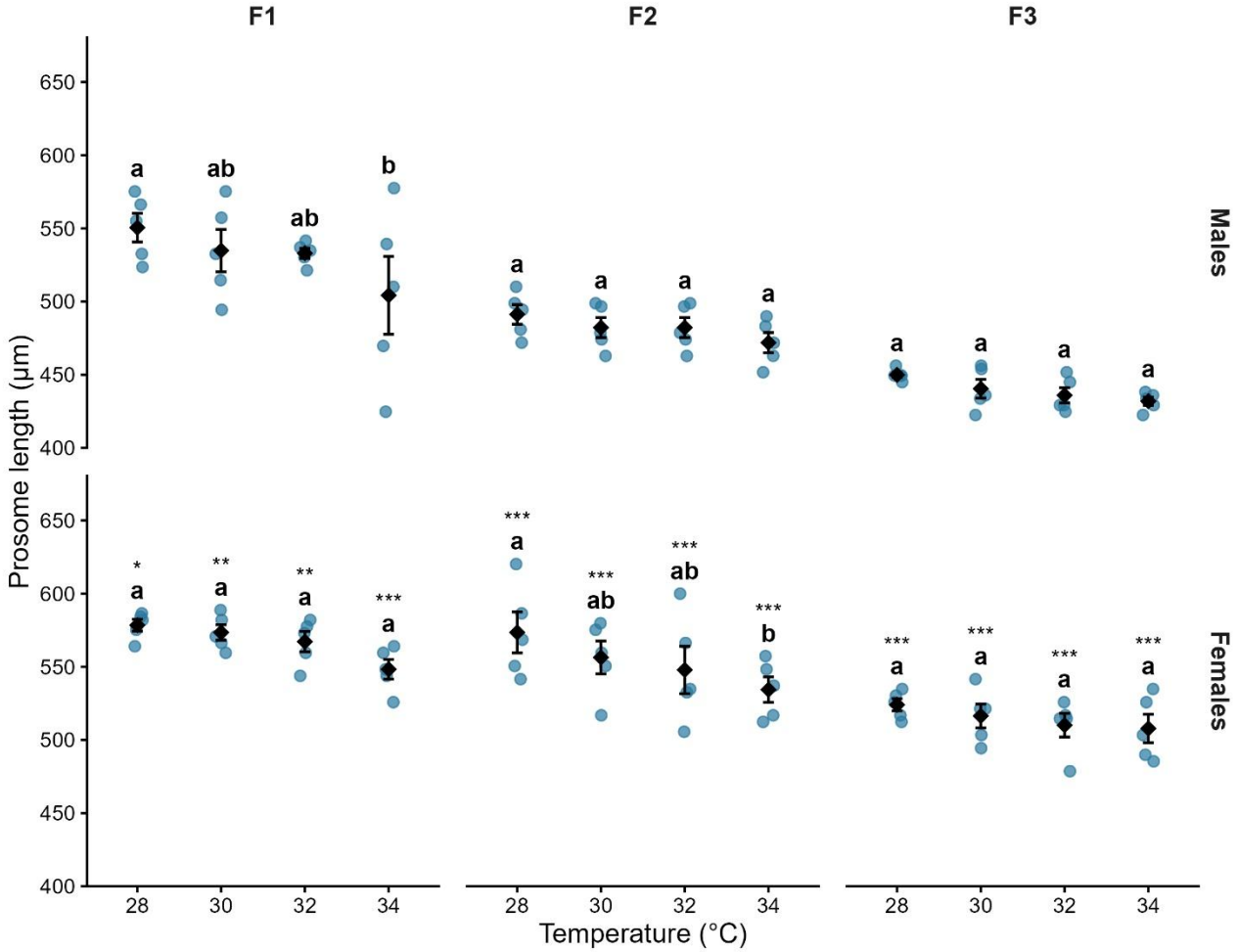


Figure 2. Prosome length of males (upper row) and females (lower row) at four temperatures across three generations (F1–F3). Blue circles represent bottle means (10 individuals per sex per bottle); black diamonds and error bars indicate means ± 1 SE across five replicate bottles. Within each panel, groups sharing a lowercase letter do not differ significantly (Tukey-adjusted comparisons, $P < 0.05$). Asterisks above female groups indicate significant differences from males at the same temperature and generation, with Holm adjustment across the 12 comparisons: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

3.3 Clutch size

The model detected significant overall effects of temperature ($F_{3,16} = 5.87$, $P = 0.0067$) and generation ($F_{2,32} = 77.43$, $P < 0.001$) while no significant effects of temperature on the clutch sizes of F1 or F2 females were observed (all adjusted $P \geq 0.097$, Fig. 3). In F3, females at 28 and 30 °C produced larger clutches than those at 32 and 34 °C (all Tukey-adjusted $P \leq 0.045$). Clutch size did not differ significantly between 28 and 30 °C or between 32 and 34 °C in F3 (both adjusted $P \geq 0.69$). Clutch size was larger in F3 than in either F1 or F2 at

every temperature (all Tukey-adjusted $P \leq 0.0078$), whereas F1 and F2 did not differ significantly at any temperature (all adjusted $P \geq 0.11$). Although temperature differences were greater in F3, the overall temperature × generation interaction was not statistically significant ($F_{6,32} = 1.93$, $P = 0.11$).

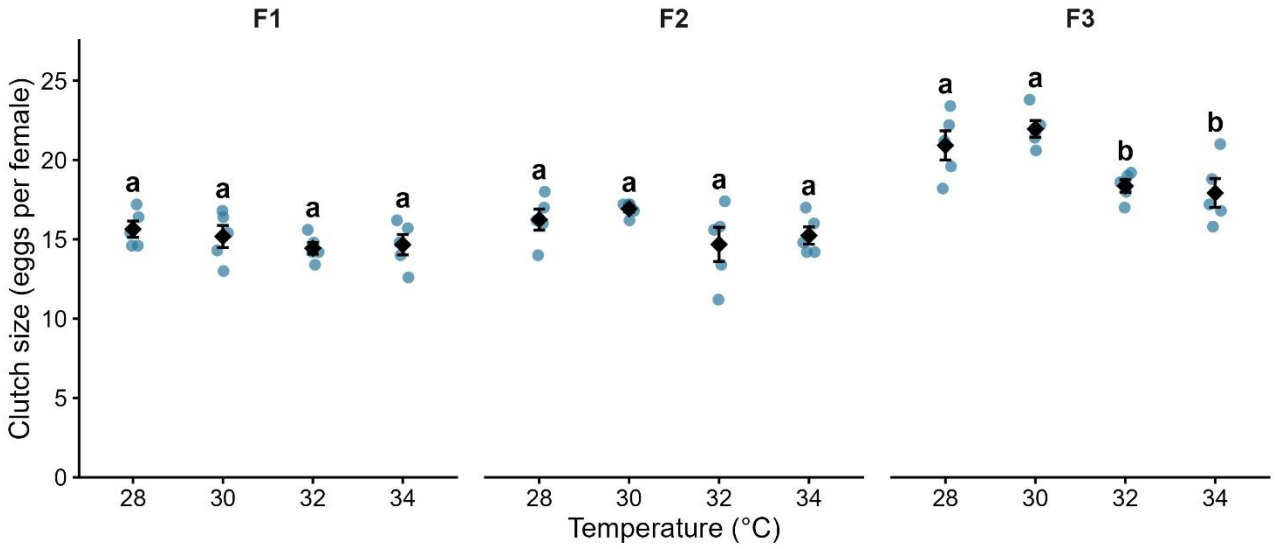


Figure 3. Clutch size at four temperatures across three generations (F1–F3). Blue circles represent bottle means (10 females per bottle); black diamonds and error bars indicate means ± 1 SE across five replicate bottles. Within each generation, groups sharing a lowercase letter do not differ significantly, whereas groups with no letter in common differ significantly (Tukey-adjusted comparisons, $P < 0.05$). Letters indicate comparisons among temperatures within each generation only.

3.4 Correlation between prosome length and clutch size

Female prosome length did not significantly predict clutch size after accounting for temperature, generation and lineage ($P = 0.49$; Fig. 6). The unadjusted correlation across all bottles was negative (r = −0.45), reflecting the opposing trends of size and clutch size across generations and not a relationship within generations (r = −0.002 to 0.32). The F3 vs F1 difference in clutch size remained after adjusting for size (+5.65 eggs at 28 °C; $P < 0.001$).

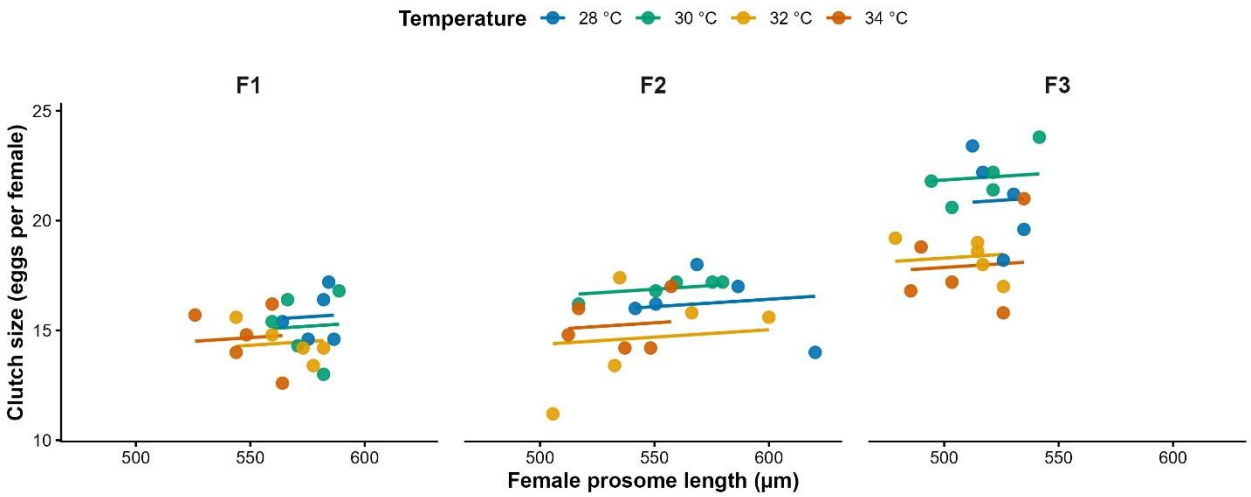


Figure 4. Relationship between female prosome length and clutch size in *Dioithona rigida* across three generations (F1-F3) at four constant temperatures. Each point is the bottle mean (10 females per bottle for each trait; n = 60 bottles from 20 lineages), coloured by temperature. Lines show the fitted common size effect from a linear mixed model of mean clutch size with female mean prosome length (per 50 µm), temperature, generation and their interaction as fixed effects and lineage as a random intercept. Female size did not significantly predict clutch size after adjustment (0.34 eggs per 50 µm; 95% CI −0.65 to 1.32; $P = 0.495$)

3.5 Hatching success

The overall contribution of temperature, including its interaction with generation on the percentage of females with hatched eggs, was significant (quasibinomial model, $F_{9,48} = 2.36$, $P = 0.027$, Fig. 5). However, Tukey-adjusted comparisons detected no significant differences among temperatures within any generation (all adjusted $P \geq 0.085$). The temperature × generation interaction was not significant ($F_{6,48} = 0.047$, $P = 0.99$). The overall contribution of generation was also not significant ($F_{8,48} = 0.49$, $P = 0.86$), and no significant differences among generations were detected within any temperature (all adjusted $P \geq 0.49$).

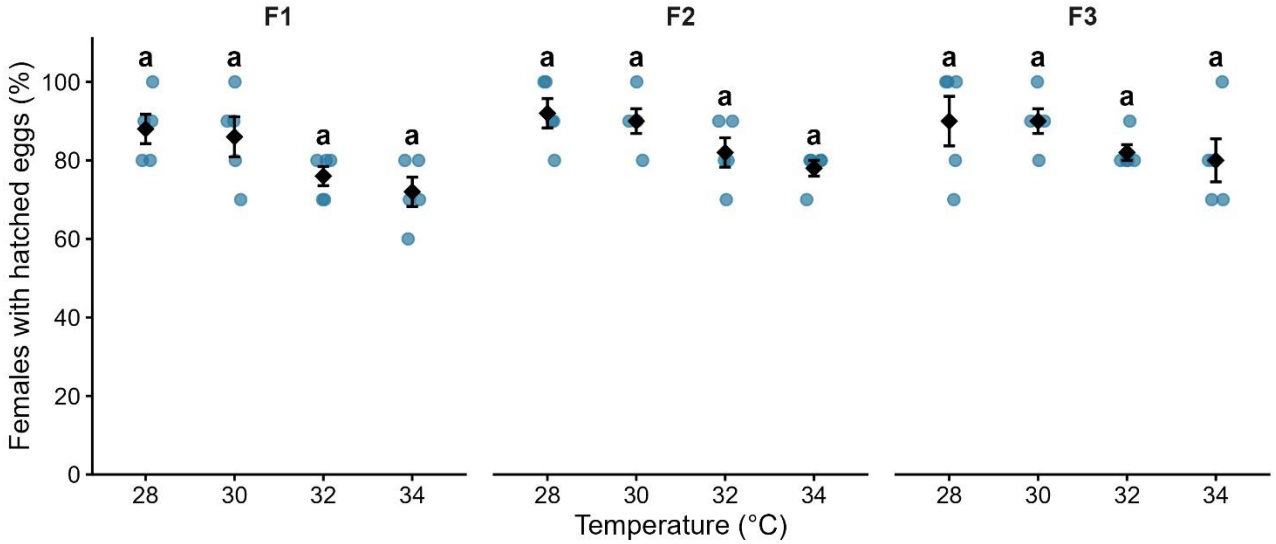


Figure 5. Hatching success of *D. rigida*, expressed as the percentage of 10 females whose eggs hatched, at four temperatures across three generations (F1–F3). Blue circles represent individual replicate percentages; black diamonds and error bars indicate means ± 1 SE across five replicates. Within each generation, groups sharing a lowercase letter do not differ significantly (Tukey-adjusted comparisons, $P < 0.05$). Letters indicate comparisons among temperatures within each generation only.

3.6 Nauplii production

Nauplii production differed significantly with temperature ($F_{3,48} = 4.23$, $P = 0.0099$) and generation ($F_{2,48} = 21.76$, $P < 0.001$), whereas the temperature × generation interaction was not significant ($F_{6,48} = 0.43$, $P = 0.85$). Specifically, nauplii production did not differ significantly among temperatures within F1 or F2 (all Tukey-adjusted $P \geq 0.35$; Fig. 6). In F3, production at 34 °C was approximately 30% lower than at 28 °C ($P = 0.034$) and 30 °C ($P = 0.036$). Production at 32 °C was intermediate and did not differ significantly from the other temperatures. Nauplii production was higher in F3 than in F1 at 28, 30, and 32 °C (all Tukey-adjusted $P \leq 0.0042$). No significant differences were detected between F1 and F2 or between F2 and F3 at any temperature (all adjusted $P \geq 0.053$), and no generation differences were significant at 34 °C.

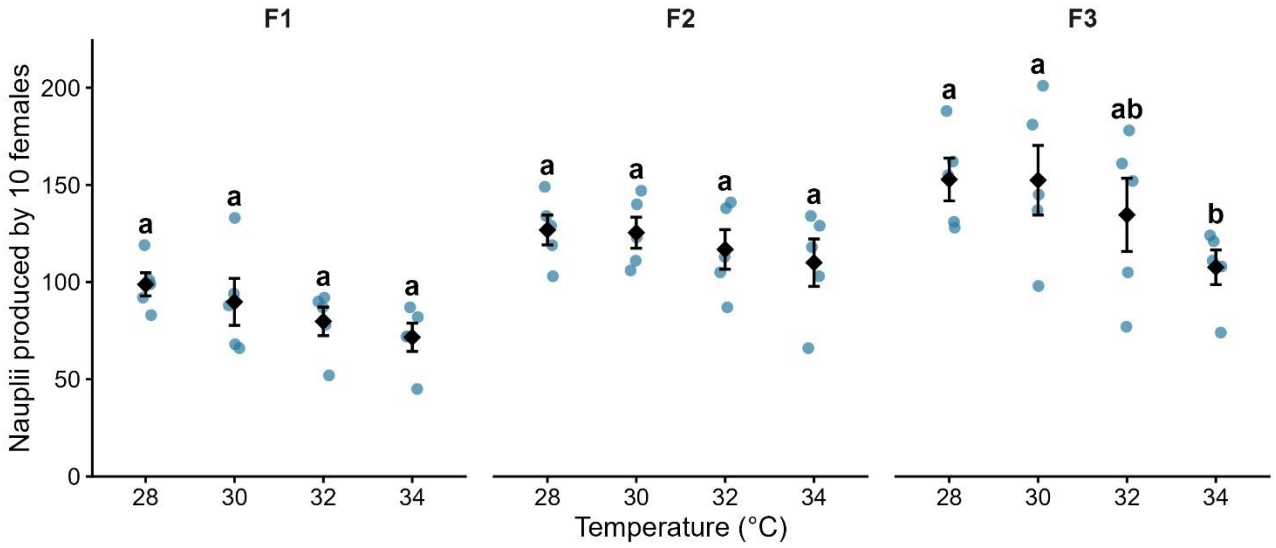


Figure 6. Total number of nauplii produced by 10 females at four temperatures across three generations (F1–F3). Blue circles represent individual replicates; black diamonds and error bars indicate means ± 1 SE across five replicates. Within each generation, groups sharing a lowercase letter do not differ significantly, whereas groups with no letter in common differ significantly (Tukey-adjusted comparisons, $P < 0.05$). Letters indicate comparisons among temperatures within each generation only.

4. Discussion

4.1 Survival and the potential for thermal adaptation

Survival to adulthood of *D. rigida* was substantially lower at 32 and 34 °C than at 28 and 30 °C across the three generations, suggesting that prolonged exposure to elevated temperatures impaired performance during development, which have often been observed in other tropical calanoid copepods exposed to comparable extreme temperatures (Doan et al., 2019, Dinh et al., 2020b, Nguyen et al., 2020). The reduced survival of *D. rigida* at 32 and 34 °C may reflect heat-induced physiological failure during development. One possible mechanism is impaired mitochondrial ATP production, which could compromise essential cellular functions and repair (Sokolova, 2023). In the copepod *Tigriopus californicus*, a decline in ATP synthesis at high temperatures was associated with reduced thermal tolerance (Harada et al., 2019, Healy and Burton, 2023). Heat-induced damage to proteins and membranes may also contribute to mortality (Somero, 1995, Somero et al., 2016), while reduced feeding could limit the energy available for maintenance, as observed at high temperature in the tropical copepod *Pseudodiaptomus annandalei* (Doan et al., 2018, Vu et al., 2026).

Importantly, the high mortality inferred at elevated temperatures could have imposed strong selection on traits associated with thermal tolerance. If individuals varied in tolerance, and if some of that variation was heritable, survivors could contribute disproportionately to the next generation (Krause et al., 2017). Experimental evolution studies demonstrate that copepod populations can show genetic changes during prolonged exposure to warming and other environmental stressors, but the direction and magnitude of their responses depend on the population and exposure conditions (Dam et al., 2021, Brennan et al., 2025). Despite continued exposure, survival at 32 and 34 °C remained lower than at 28 and 30 °C in F1–F3. The absence of a recovery in survival may be a result of a limited heritable variation available. Selection may also have changed traits or allele frequencies without producing a detectable improvement in the measured endpoint. More broadly, Dinh et al. (, 2022) emphasised that responses to environmental stress can depend on exposure history, life stage, and the number of generations examined.

The results contrast with those of Truong et al. (, 2022), who reported that parental exposure to a simulated marine heatwave ameliorated the heatwave's lethal effect on offspring of the tropical copepod *Pseudodiaptomus incisus*. The longer-term study of *Paracartia grani* by de Juan et al. (, 2023b) provides a further comparison: after more than 18 generations of warm rearing, copepods showed improved survival under acute thermal stress and higher fecundity at stressful temperatures, although warm-reared individuals were smaller and produced fewer offspring near the reproductive optimum. This suggests that a response to selection or acclimation can be trait-dependent and may involve costs under conditions other than those in which the lines were maintained. It does not establish that the same responses would emerge in *D. rigida* over a longer experiment.

4.2 Divergent survival, growth and reproductive responses

Across temperatures, survival and body size showed broadly consistent directional patterns: survival was lower at 32–34 °C, and mean prosome length tended to be smaller at higher temperatures. Similar reductions in adult size with warming have been reported for many copepods (Horne et al., 2016, Doan et al., 2019, de Juan et al., 2023b). The responses in our study were nevertheless not identical: the survival contrast between lower and higher temperatures was large in all generations, while most individual temperature comparisons for prosome length within a generation and sex were not significant. Several non-exclusive mechanisms could contribute to smaller adults. Temperature can affect growth and development differently, so individuals may reach

maturity at a smaller size when development proceeds faster relative to somatic growth (Horne et al., 2016). Energy limitation or thermal stress may also constrain growth, and food availability and population density can modify copepod body size (Corona et al., 2021). Furthermore, the adult-size data are also conditional on survival. At the higher temperatures, body size was measured in the individuals that reached adulthood. Consequently, the observed size response combines any effects of temperature on growth and development with possible differences in which individuals survived to be measured. Previous studies on various animal species have shown that larger body size animals are disproportionately vulnerable to heat stress than smaller ones (Burton and Einum, 2020, Hahn and Brennan, 2024) that may relate to constraints on oxygen supply relative to metabolic demand (Pörtner, 2010) and/or the tradeoff between energy allocation on growth and coping with stressors (Dinh et al., 2014, 2022).

Across generations, declining body size coincided with increased reproductive output but no detectable improvement in survival, suggesting that these traits differed in their capacity to respond to sustained warming. Body size is a potentially important link between thermal exposure and reproductive performance (Kiørboe and Sabatini, 1995, Sichlau and Kiørboe, 2011, Horne et al., 2016, Doan et al., 2019). Female size has been shown to positively correlate with fecundity in marine copepods, making size reduction a plausible contributor to lower reproductive output (Kiørboe and Sabatini, 1995, Krause et al., 2017). However, when warming occurs across multigenerations, physiological compensation can modify feeding and egg production beyond the effects attributable to smaller body size alone (de Juan et al., 2023a). The size–clutch analysis of *D. rigida* nevertheless provides no support for female size as the principal explanation for the generational increase in clutch size as it was not significantly associated with female size. This decoupling is consistent with evidence that multigenerational changes in feeding and egg production can involve physiological compensation beyond the effects of body-size reduction (de Juan et al., 2023b).

Hatching success showed a different pattern: its overall temperature contribution was significant, but neither within-generation temperature comparisons nor generational changes were significant. This comparatively weak response contrasts with reduced hatching success at 34 °C in tropical calanoid copepods from the same region (Grønning et al., 2019, Nguyen et al., 2020, Dinh et al., 2021), suggesting differences in reproductive thermal sensitivity among species. Moreover, the proportion of females producing hatched eggs describes the occurrence of successful hatching, whereas nauplii production integrates the number of offspring

346 released during the incubation period. A female can therefore remain reproductively successful by the first
347 measure while contributing fewer nauplii by the second.
348
349 *4.3 Conclusions and perspectives*
350 Sustained warming produced divergent responses across the life cycle of *D. rigida*: reproductive improvement
351 among surviving adults occurred alongside persistent losses during development. Multigenerational studies of
352 other copepods similarly show that changes in individual traits can conceal costs that become apparent when
353 survival and reproduction are integrated into population fitness (de Juan et al., 2023a, 2023b). Ecological risk
354 assessments should therefore evaluate whether increased offspring production translates into recruitment
355 through subsequent life stages, rather than treating improved adult reproduction as evidence of recovery.
356 Although warming-associated shifts towards smaller organisms are widespread in aquatic ecosystems,
357 the persistent warming-induced lower survival in *D. rigida* shows that small size can also suffer substantial
358 thermal vulnerability, which is highly relevant to small-sized ectotherms like oithonid copepods (Daufresne et
359 al., 2009). In tropical coastal food webs, where copepods support young fish and shrimp, reduced recruitment
360 could weaken prey replenishment even when surviving females maintain substantial reproductive output
361 (Chew and Chong, 2011, Doan et al., 2019).
362 The next logical step is linking multigenerational experiments to life-table models that would identify
363 which stages exert the strongest demographic constraints and whether those constraints shift with exposure
364 history (deMayo et al., 2023). Incorporating fluctuating temperatures, food availability, coastal contaminants
365 and seasonality would further connect these responses to the conditions experienced in tropical habitats, where
366 exposure timing and interacting stressors can alter biological outcomes (Dinh et al., 2022, 2023, Sasaki et al.,
367 2024, Janssens et al., 2026).
368
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Ethics declarations

This manuscript has not been published previously. This manuscript is not under consideration for publication elsewhere. The article's publication is approved by all authors. If accepted, the article will not be published elsewhere in the same form, in English, or any other language, including electronically, without the written consent of the copyright holder.

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Conflict of interest

None.

Declaration of generative AI use

During the preparation, Grammarly was used to check grammar and readability. The author reviewed and edited the content and takes full responsibility.

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