

# Multigenerational physiological compensation and body size reduction dampen the effects of warming on copepods

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## Abstract

Under the current ocean warming scenario, multigenerational studies are essential to address possible adaptive changes in phenotypic traits of copepod populations. In this study, we exposed the calanoid copepod *Paracartia grani*, reared in the laboratory at 19°C, to warmer conditions (22°C and 25°C) to investigate the changes in key phenotypic traits in the 1<sup>st</sup>, 10<sup>th</sup>, and 11<sup>th</sup> generations. Development rates and adult body size were inversely related to temperature in all generations. We also found a decline in copepod egg size at higher temperatures. Temperature had positive effects on the ingestion and egg production rate of females in the first generation, but the thermal response of the copepods diminished significantly in the consecutive generations. The decrease in thermal effects on feeding and egg production rates after multigenerational exposure cannot be explained only by the shrinkage in body size at warmer temperatures, but also involves the action of physiological compensation. These adaptive processes did not appear to have a significant cost on other traits, such as egg hatching success, gross growth efficiency, and sex ratio. Our findings have implications for the prediction of ocean warming effects on copepod activity rates and highlight the importance of physiological adaptation processes after multigenerational exposure.

The response of marine zooplankton to the forecasted rise in ocean temperature is of crucial interest for predicting the functioning of marine pelagic systems (Hays et al. 2005; Richardson 2008). Within zooplankton, copepods are the most abundant group (Longhurst 1985). They have key roles in marine food webs as secondary consumers (Banse 1995; Calbet 2001) and remineralizers (Alcaraz et al. 1994, 2010), influencing the energy and biochemical fluxes in marine ecosystems (Steinberg and Landry 2017).

As in most ectothermic animals, the physiological traits of zooplankton species are coupled with the environmental temperature in their habitats (Huey and Stevenson 1979; Angilletta et al. 2002). The effects of temperature on physiological activity have been extensively studied, particularly for copepods (Kjørboe et al. 1982; Uye 1988; Holste and

Peck 2006; Cruz et al. 2013). Within their thermal tolerance limits, vital rates of zooplankton respond to acute exposure to temperature according to the Arrhenius law (passive response), with  $Q_{10}$  coefficients (fold variation in rate for a 10°C increase) classically assumed to range between 2 and 4 (Saiz et al. 2022). Longer exposure allows the development of physiological compensatory processes (active plasticity) that diminish the phenotypic response of the organism to temperature, resulting in a decrease in  $Q_{10}$  values (Schulte et al. 2011; Scheffler et al. 2019; Havird et al. 2020). However, the acclimation capacity of an organism is limited and may have associated costs that challenge its persistence capacity (Angilletta et al. 2003; Alcaraz et al. 2014; Souissi et al. 2021). At longer timescales, comprising multiple generations, other compensatory mechanisms at phenotypic and genotypic levels can come into play and modify the thermal response of the species (Sanford and Kelly 2011; Dam 2013). Recent advances addressing the multigenerational effects of temperature on copepod traits (i.e., fecundity, recruitment, body size, and heat tolerance) suggest that adaptive shifts can be indeed rather rapid, within a few generations (Souissi et al. 2016; Kelly et al. 2017; Dam et al. 2021).

Temperature also affects body size owing to the different shapes of the thermal responses of developmental and growth rates (Forster and Hirst 2012). The shrinkage of body size driven by warming is generalized in nature (Sheridan and Bickford 2011) and has also been observed in copepods (Rice

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et al. 2015; Evans et al. 2020). These shifts in size associated with temperature can affect trophic interactions and have important ecological consequences (Sheridan and Bickford 2011; Brosset et al. 2016). Body size also influences metabolism (Gillooly et al. 2001). As body mass decreases, the metabolic rate declines and the mass-specific metabolic rate increases (Gillooly et al. 2001). In copepods, physiological rates such as feeding, egg production, and respiration scale with body mass (Runge 1984; Ban 1994; Saiz and Calbet 2011). Therefore, the direct effects of temperature on physiological rates are expected to overlap with those driven by the reduction in body size also induced by warming (Riemer et al. 2018).

In this study, we have addressed the multigenerational response of the marine calanoid copepod *Paracartia* (formerly *Acartia*) *grani* to temperature. *P. grani* is a well-studied small coastal species (ca. 1 mm; Calbet et al. 1999; Olivares et al. 2019) with broadcasting reproductive behavior whose distribution extends along a thermal gradient from cold northeast Atlantic waters to the warmer Mediterranean Sea (Boyer et al. 2012). Because *P. grani* has a short lifespan, its populations undergo a few generations annually (Ianora 1998), which make it suitable for multigenerational experiments. In our study, we exposed a parental population of *P. grani*, reared at 19°C in the laboratory, to warmer conditions (22°C and 25°C) for 11 generations and examined the direct effects of temperature on feeding, fecundity, development, population sex ratio, and somatic traits (adult and egg sizes and adult carbon content). The range of increased temperatures used was based on the Intergovernmental Panel on Climate Change's (IPCC) extreme prospects for long-term ocean warming (IPCC 2022) and also encompass shorter-period phenomena such as the severe, months-lasting marine heatwaves that have been increasingly present in the Mediterranean in recent years (Garrabou et al. 2022). We expected that the overall thermal response ( $Q_{10}$  coefficient) of two main physiological processes (ingestion and egg production rates) would be diminished after multigenerational exposure to warming. We hypothesized that the mechanisms of this dampening effect would be (1) a decrease in thermal sensitivity of these processes after multigenerational exposure and (2) a thermal-driven shrinkage of body size, which would result in a per capita decrease in metabolic rates. We also examined the possible costs of these adjustments in other life-history traits, such as sex ratio, hatching success, and gross growth efficiency.

## Materials and methods

### Experimental organisms, rearing conditions, and thermal exposure

Specimens of *P. grani* were originally collected in coastal waters near Barcelona (NW Mediterranean) and have been kept in culture at the Institut de Ciències del Mar (ICM-CSIC) for > 14 yr in 20- to 40-liter tanks (Saiz et al. 2015). The

copepod culture is kept in a temperature-controlled room at  $19 \pm 1^\circ\text{C}$  with a 10 : 14 light/dark cycle and routinely fed ad libitum three times a week with the cryptophyte *Rhodomonas salina* (strain K-0294, Scandinavian Culture Collection of Algae and Protozoa) grown in f/2 medium.

To start the F0 culture, we collected eggs spawned during the past 24 h from the stock culture and transferred 20,000 of them into a 20-liter tank submerged in a water bath at 19°C, where eggs were allowed to hatch and copepods to grow under excess *R. salina*. The number of eggs was established on the basis of previous experience (Saiz et al. 2015; Olivares et al. 2019) to ensure enough animals for sampling, experimentation, and future recruitment. The procedure for collecting the eggs consisted in siphoning the bottom of the tank and sieving the collected water through sieves of 200  $\mu\text{m}$  (to remove any adults present) and 40  $\mu\text{m}$  (to collect the eggs).

Once F0 reached adulthood, we collected eggs from the bottom of the tank and transferred them in batches of 20,000 eggs to duplicated 20-liter tanks filled with filtered seawater previously acclimated to the corresponding experimental temperatures (19°C, 22°C, and 25°C). These tanks were introduced into three 150-liter water baths provided with TECO water-temperature conditions set at 19°C, 22°C, and 25°C and diode lighting ( $25\text{--}30 \mu\text{E m}^{-2} \text{s}^{-1}$ , 10 : 14 light/dark cycle), where they were kept throughout the multigenerational rearing study. The copepods were routinely fed with *R. salina*, supplied in different amounts to maintain satiation during development (i.e., concentrations ranging from 2 ppm for early stage nauplii to 10 ppm for adults; Helenius and Saiz 2017; Olivares et al. 2019). We checked the prey concentration in the tanks three times per week using a Beckman Coulter Multisizer III particle counter and adjusted it to the desired concentrations according to parallel estimations of copepod abundance and stage composition. After the first thermal-exposed generation (F1) at each experimental temperature was raised and adults were present in the cohort, we repeated the aforementioned procedure to generate successive generations (F2, F3, ...) for each thermal treatment until the 11<sup>th</sup> generation was reached (5–6 months). Previous evidence suggests that 3–6 generations might be enough for copepods to adapt to a new temperature in terms of reproduction and foraging capacities (Souissi et al. 2016; Dam et al. 2021). Therefore, we assumed that full thermal adjustment was reached after 10 generations (F10) and repeated the experiments one generation later (F11) to better assess the differences from F1.

### Adult development time and adult sex ratio

Adult development times at 19°C, 22°C, and 25°C were assessed for the F1 and F10 generations. Throughout the development of the cohorts, we took water samples every 24 h from each rearing tank after thorough mixing; sample volumes ranged from 250 mL for nauplii to 500 mL for copepodites and 1000 mL for adults. The samples were sieved through a 40- $\mu\text{m}$  sieve, and the copepods were preserved in

4% formaldehyde. Later, the specimens were counted and photographed under an Olympus SZX12 stereomicroscope and identified from copepodite IV to the adult stage. We calculated the adult male and female median development times (MDT) by fitting a Hill sigmoidal function to the observed frequencies and then assessing the time required for 50% of the individuals to molt to that stage (Landry 1983). The adult sex ratio was calculated as the quotient between the mean abundance of adult males and adult females estimated from the last three samplings, when adult stages dominated the population.

### Feeding and fecundity experiments

Feeding and fecundity experiments were carried out on the F1, F10, and F11 generations. The experiments consisted in 24-h incubations to determine feeding and egg production rates and posterior incubation of the spawned eggs to assess hatching success. Prey concentrations and numbers of copepods in the incubations were chosen using previous feeding rate data to ensure feeding saturation conditions (i.e., maximum ingestion and egg production rates) and to produce a reduction of 20–30% of the prey concentration during the incubation (Olivares et al. 2019). To avoid aging effects (Saiz et al. 2015), we started every experiment 2–5 d after the copepods had molted into the adult stage; at that time, females were already fertilized and producing eggs. For each temperature treatment, we prepared four control and eight experimental 610-mL Pyrex bottles (four for females and four for males) filled with 7 ppm ( $\sim 1120 \mu\text{g C L}^{-1}$ ) of *R. salina*. We sorted the copepods under the stereomicroscope, and with the aid of a wide-bore Pasteur pipette we introduced either 18–28 females or 45–80 males into each experimental bottle, depending on the treatment (Olivares et al. 2019). All bottles were amended with 5 mL of f/2 medium per liter. Then, the bottles were placed in the corresponding temperature-controlled baths and regularly rotated by hand end-over-end (2–3 times per day) to homogenize their contents. After 24 h, the bottle contents were sequentially screened through 100- and 20- $\mu\text{m}$  sieves to retain the copepods and the eggs, respectively. Initial and final algae concentrations in the incubations were measured using the Coulter Multisizer. The copepods were checked for survival under the microscope and then preserved in 4% formaldehyde. Subsequently, we photographed random individuals ( $n = 28$  for each duplicated tank, treatment, generation, and sex; total  $n = 1008$ ) under the stereomicroscope and measured adult prosome length using FIJI ImageJ software. The egg samples were divided into four aliquots. One of them was immediately preserved in 2% Lugol's solution to determine egg production rates. To assess hatching success, we further incubated the remaining aliquots in 50-mL vials (filled with filtered seawater and 1 ppm of *R. salina*) at their respective temperatures and consecutively preserved them in 2% Lugol's solution after 24, 48, and 72 h. Eggs, egg shells, and nauplii were counted using an inverted microscope (Nikon Diaphot 200). Egg diameter was determined from pictures ( $n = 30$  per treatment and generation; total  $n = 270$ ) by fitting an ellipsoid using the ImageJ software and

calculating the average of the fitted major and minor axes (Saiz et al. 2020).

Copepod ingestion rates were calculated using Frost's equations (Frost 1972), which consider the disappearance and growth of prey in experimental and control bottles, respectively, assuming exponential models. Egg production rates were computed as the total number of eggs and hatched nauplii divided by the number of females in the incubation and the duration of the incubation. Hatching success was calculated as the percentage of nauplii in relation to the total number of eggs laid. For 22°C and 25°C, we used the data for the 48-h incubation, whereas for 19°C the samples after 72 h were used to ensure that the hatching plateau was reached (Supporting Information Fig. S1).

### Carbon-specific rates

Carbon ingestion rates were obtained by computing ingestion rates in prey biovolume units from the Coulter counter and then using *R. salina* carbon contents estimated from cell volume using the factor  $0.17 \text{ pg C } \mu\text{m}^{-3}$  (Saiz et al. 2020). Similarly, egg production rates were transformed into carbon units from egg volume (estimated from the measured diameter) by applying the factor  $0.129 \text{ pg C } \mu\text{m}^{-3}$  (C. de Juan, unpubl.; Saiz et al. 2020). To normalize physiological rates in terms of copepod body carbon, parallel to the feeding and fecundity incubations, we took additional copepod samples to assess the adult carbon content for each treatment and generation. The copepods were left for  $\sim 30$  min in 0.1- $\mu\text{m}$  filtered seawater to empty their guts, and then were narcotized with MS-222 (Saiz et al. 2015) and transferred into groups of 25–40 females and 60 males into precombusted 25-mm Whatman GF/C filters (450°C, 5 h). The filters were stored at  $-80^\circ\text{C}$  until analysis. Prior to analysis, the filters were thawed and then dried for 48 h at 60°C and finally processed with a Thermo Finnigan Flash EA1112 CHNS analyzer. Gross growth efficiency was obtained by dividing the carbon egg production rate by the respective carbon ingestion rate.

### Statistical analysis

Simple linear regression analysis was performed to assess the statistical significance of the relationship between copepod physiological traits and temperature. When the relationship was anticipated to follow the Arrhenius law and be exponential (MDT, feeding, and egg production; Almeda et al. 2010; Saiz et al. 2022), the dependent variable was ln-transformed, and  $Q_{10}$  coefficients were estimated from the slopes as  $\exp(10 \times \text{slope})$  (Ikeda 1985). When the slopes were not statistically different from 0, meaning that no thermal effect was observed, we assigned to the  $Q_{10}$  a value of 1. Statistical analyses were conducted in Prism v.7. Unless otherwise stated, mean values  $\pm$  SE are provided.

**Table 1.** MDT (d) and sex ratio (male/female) of *Paracartia grani* reared at 19°C, 22°C, and 25°C in F1 and F10 generations. Average  $\pm$  standard errors are shown.

Generation	T (°C)	MDT (d)		Sex ratio
		Female	Male	
F1	19°C	14.1 $\pm$ 0.08	13.9 $\pm$ 0.17	1.1 $\pm$ 0.07
	22°C	11.2 $\pm$ 0.02	10.9 $\pm$ 0.07	1.1 $\pm$ 0.09
	25°C	8.9 $\pm$ 0.01	8.8 $\pm$ 0.09	1.1 $\pm$ 0.05
F10	19°C	15.5 $\pm$ 0.55	15.4 $\pm$ 0.85	0.9 $\pm$ 0.06
	22°C	13.5 $\pm$ 0.20	13.2 $\pm$ 0.15	1.1 $\pm$ 0.08
	25°C	10.0	10.0	1.1 $\pm$ 0.06

## Results

MDTs decreased exponentially with temperature (linear regression on ln-transformed data,  $p < 0.01$  in all cases; Table 1); the 6°C difference between the lowest and the highest temperatures resulted in adult development times 4–5 d shorter. Covariance analysis showed that the rate of change with temperature did not differ between males and females in any of the generations (test for slope:  $p > 0.6$  for both F1 and F10; Table 1); in F1, male MDTs were slightly, but significantly, shorter (covariance analysis, test for intercept:  $p = 0.33$ ), whereas this difference disappeared in F10 ( $p > 0.7$ ; Table 1). Overall, MDTs for F10 were higher than those for F1 (on average 14–15% difference; covariance analysis, test for intercepts:  $p < 0.001$  for both male and female comparisons; Table 1). Sex ratios were close to 1 (Table 1;  $t$ -tests,  $p > 0.09$  in all cases) and did not show a significant relationship with temperature in either F1 or F10 (Table 1; linear regression,  $p > 0.07$  in both cases).

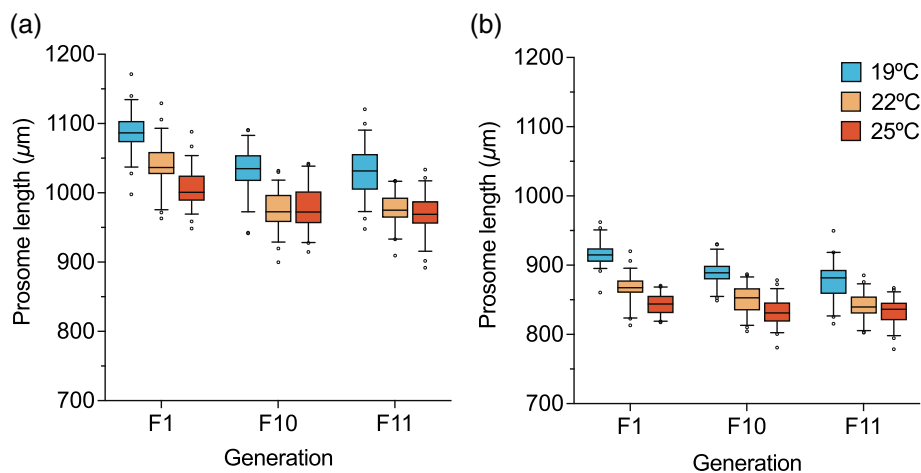
Figure 1 shows the prosome length of the female and male *P. grani* as a function of the experimental temperature and rearing generation. The copepods' prosome length was inversely related to temperature, both within gender and across

generations (linear regression,  $p < 0.001$  in all cases). On average, the 6°C temperature increase resulted in a body size decrease in F1 of 7.8% and 7.1% for males and females, respectively. The effect of temperature on size reduction decreased with time to 6.3% and 5.0% for males and to 5.1% and 5.9% for females in F10 and F11, respectively (Fig. 1a,b; covariance analysis, test for slopes:  $p = 0.012$  and  $p < 0.001$  for females and males, respectively). This pattern was linked to a generalized decrease in the adult prosome length over time, which was particularly evident when the male and female body sizes for the 19°C treatment were compared through generations (one-way ANOVA,  $p < 0.001$  in both cases).

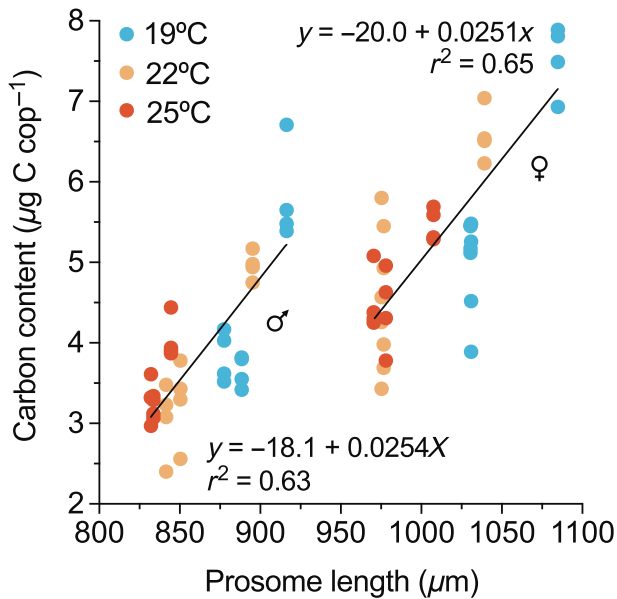
The mean carbon content of *P. grani* ranged from  $7.5 \pm 0.2 \mu\text{g C}$  at 19°C (F1) to  $4.4 \pm 0.3 \mu\text{g C}$  at 25°C (F10) for females and from  $5.8 \pm 0.3 \mu\text{g C}$  at 19°C (F1) to  $3.1 \pm 0.2 \mu\text{g C}$  at 22°C (F11) for males (Fig. 2). The differences in copepod carbon content were positively related to prosome length (linear regression analysis;  $p < 0.001$  for both males and females; Fig. 2).

For copepod ingestion rates, expressed as  $\mu\text{g C cop}^{-1} \text{d}^{-1}$ , only females in F1 showed a significant trend with temperature (linear regression on ln-transformed data,  $p = 0.034$ ), resulting in a  $Q_{10}$  coefficient of 1.28 (Fig. 3a). After multigenerational thermal exposure (F10, F11); however, neither female nor male feeding rates were related to temperature (linear regression on ln-transformed data,  $p > 0.12$  in all cases; Fig. 3a,b).

Like the per capita rates, carbon-specific ingestion rates for females in F1 showed a statistically significant relationship with temperature ( $Q_{10} = 2.15$ ; linear regression of ln-transformed data,  $p < 0.001$ ; Fig. 3c). After multigenerational rearing (F10 and F11), however, carbon-specific female feeding rates showed no significant trend with temperature (linear regression of ln-transformed data,  $p > 0.66$ ; Fig. 3c). For males, we found no statistically significant relationship with temperature in any generation (linear regression of ln-transformed data,  $p > 0.17$  for F1,  $p > 0.39$  for F10 and F11; Fig. 3d).

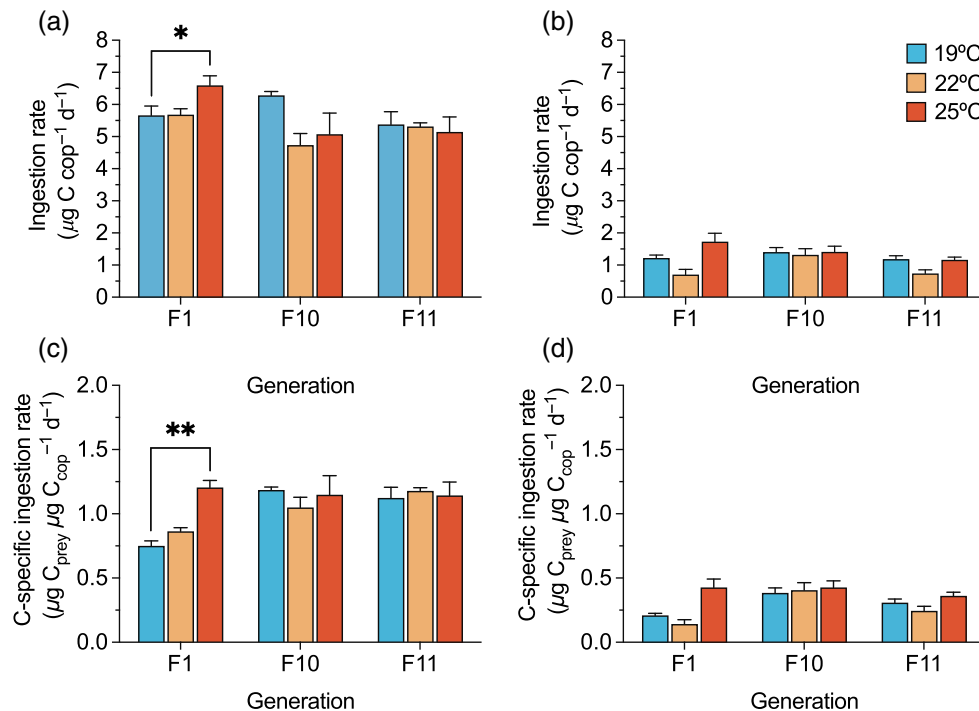


**Fig. 1.** Prosome length of adult female (a) and male (b) *Paracartia grani* as a function of temperature and generation. Box plots show median values and 25–75 percentiles, while the whiskers show the 5–95 percentiles.



**Fig. 2.** Relationship between prosome length and carbon content of adult female and male *Paracartia grani*. Regression equations are shown. Data from all generations are combined.

Egg production rates on a per capita basis showed no clear relationship with increasing temperature in any of the three generations (linear regression on ln-transformed data,  $p > 0.12$  in all cases; Fig. 4a); mean values were  $85 \pm 3.1$ ,  $61 \pm 3.3$ , and  $72 \pm 3.3$  eggs  $\text{cop}^{-1} \text{d}^{-1}$  for F1, F10, and F11, respectively.



**Fig. 3.** Per capita and carbon-specific ingestion rates of adult female (a,c) and male (b,d) *Paracartia grani*. Error bars show standard error. Asterisks highlight statistically significant relationships with temperature (linear regression of ln-transformed data; \* $p < 0.05$ , \*\* $p < 0.001$ ).

However, carbon-specific egg production rates in F1 showed a significant trend with increasing temperature ( $Q_{10} = 1.79$ ; linear regression on ln-transformed data,  $p < 0.001$ ; Fig. 4b). We found no statistically significant trend with temperature in the successive generations ( $p > 0.24$ ; Fig. 4b).

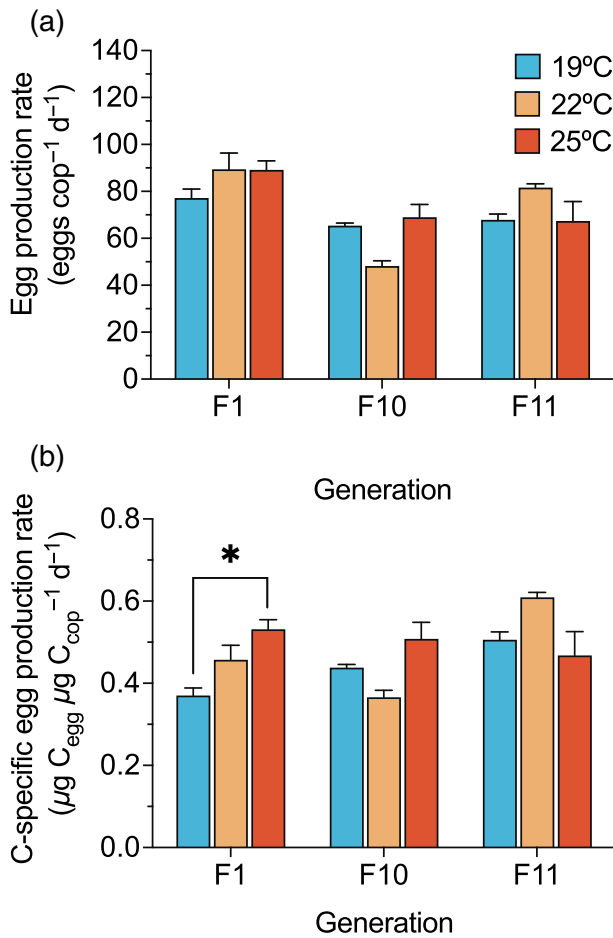
Egg diameter of *P. grani* declined overall with temperature, although differences were only statistically significant in F10 and F11 (linear regression analysis,  $p = 0.037$  and  $p = 0.034$  for F10 and F11, respectively; Fig. 5a). In addition to likely direct effects of temperature on egg diameter, we observed that egg diameter was positively related to female prosome length (linear regression analysis,  $p = 0.028$ ; Fig. 5b), suggesting an adult size effect. In F1, hatching success was influenced by temperature and decreased by 8% for the 6°C warming interval; this relationship with temperature was not observed in the successive generations (Table 2).

The carbon gross growth efficiency of female *P. grani* showed no statistically significant relationships with temperature in any of the generations (linear regression analysis,  $p > 0.27$  in all cases; Fig. 6), with a grand mean value of  $44\% \pm 1\%$  across temperatures and generations.

## Discussion

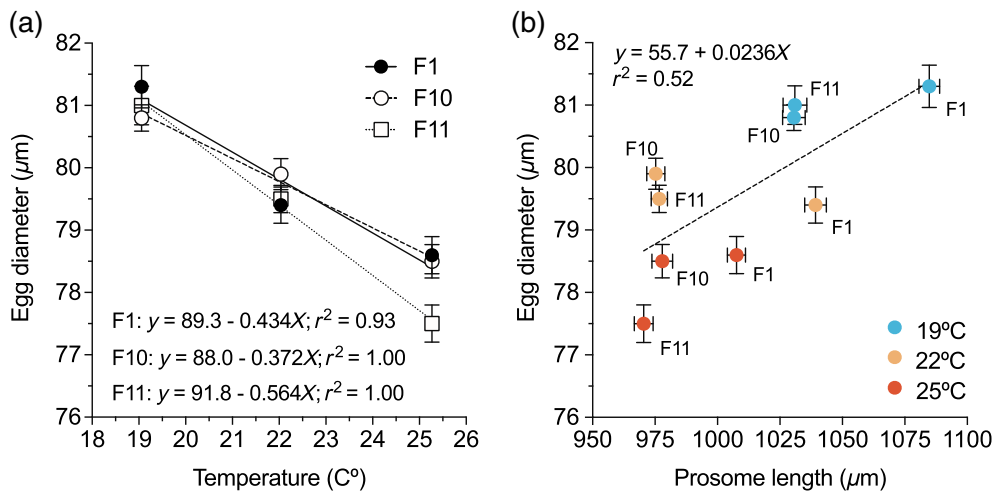
### Thermal effects on somatic traits and development times

The inverse relationship between body size and temperature in copepods has been well known for decades (McLaren 1965; Breteler and Gonzalez 1988), and it has



**Fig. 4.** Per capita (a) and carbon-specific egg production rates (b) of *Paracartia grani*. Error bars show SE. Asterisk highlights statistically significant relationships with temperature (linear regression of ln-transformed data; \* $p < 0.001$ ).

received renewed interest lately owing to ocean warming (Rice et al. 2015; Evans et al. 2020; Campbell et al. 2021). The decrease in copepod body size with warming is expected to



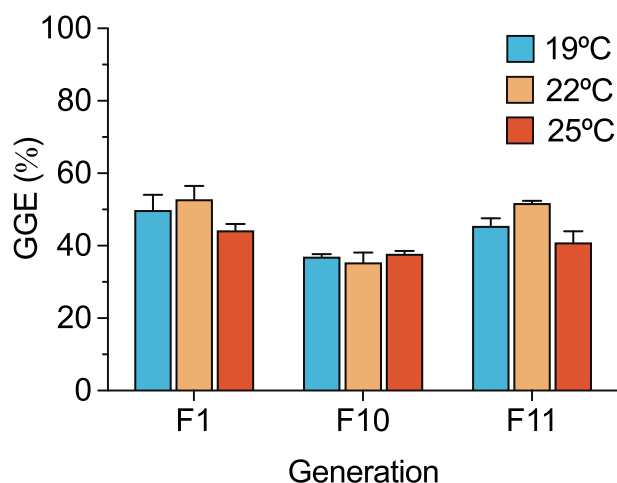
**Fig. 5.** (a) Egg diameter of *Paracartia grani* as a function of temperature and generation. (b) Relationship between egg diameter and adult female prosome length. Regressions equations are shown. Error bars show standard error.

**Table 2.** Hatching success of the eggs of *Paracartia grani* reared at 19°C, 22°C, and 25°C in F1, F10, and F11. At 19°C, the hatching incubations lasted 72 h, whereas at 22°C and 25°C, they lasted 48 h (see the text and Supporting Information Fig. S2). Average  $\pm$  standard errors are shown.

T (°C)	F1	F10	F11
19°C	91.1 $\pm$ 1.4	91.2 $\pm$ 2.0	89.9 $\pm$ 0.7
22°C	85.7 $\pm$ 1.6	84.3 $\pm$ 3.3	94.5 $\pm$ 0.8
25°C	83.8 $\pm$ 2.4	88.0 $\pm$ 2.6	88.6 $\pm$ 2.4

have a great effect on the performance of the organisms because of its close relationship with metabolism (Gillooly et al. 2001). In our experiments, as expected, we found a strong relationship between copepod body size and temperature. The observed reduction in prosome length with temperature was, on average, 1.2% °C<sup>-1</sup> and was associated with a decrease in body carbon content of 3.7% °C<sup>-1</sup>; these are similar to the values previously reported for calanoid copepods (Horne et al. 2016). The relationship between temperature and copepod body size is driven by the different thermal sensitivities between development and somatic growth rates (Forster and Hirst 2012). In our case, mean development times showed a similar negative relationship with temperature, with no differences in the slope between F1 and F10. The development times of *P. grani* in our experiments were comparable to those reported for the same or other closely related species at similar temperatures (Landry 1975; Saiz and Alcaraz 1991; Calbet and Alcaraz 1997).

It was rather unexpected to observe that copepod body size decreased overall after multigenerational rearing at all temperatures. However, in some previous studies, similar observations for body size changes over long timescales in laboratory reared copepods have been reported (Breteler and Gonzalez 1988; Breteler et al. 1990). Changes in food availability and crowding effects are among the main factors



**Fig. 6.** Gross growth efficiency of adult female *Paracartia grani* as a function of temperature and generation. Error bars show standard error.

attributed to the shifts in body size (Franco et al. 2017). In our multigenerational rearing, however, the food satiating conditions (Olivares et al. 2019) and low copepod density in the rearing tanks (from 1000 eggs per liter initially to 186–316 adults per liter) at the time of carrying out incubation experiments and recruiting eggs for the next generation (Jepsen et al. 2007; Chintada et al. 2021; Wilson et al. 2022) make these factors unlikely to have influenced copepod body size. Another plausible explanation could be that the overall experimental setup, with a very regular rearing routine (more stable temperatures and satiating food conditions), cumulatively affected the copepod size in all treatments. Nevertheless, given that this influence was homogeneous in all treatments, the effects of temperature were still evident, as demonstrated by the similarity of the slopes of the relationship of body size with temperature between generations.

The effects of temperature also affected egg size. The reduction in egg diameter ( $0.6\%^{\circ}\text{C}^{-1}$ ), however, was smaller than the  $1.2\%^{\circ}\text{C}^{-1}$  observed reduction in adult body size. Hansen et al. (2010), for a similar temperature range ( $17\text{--}24^{\circ}\text{C}$ ), also reported variations in copepod body and egg sizes of the order of  $1.4$  and  $0.4\%^{\circ}\text{C}^{-1}$ , respectively. It is worth noting, however, that temperature can also directly affect copepod egg size (Saiz et al. 2022), so the observed egg size is likely the result of the combined direct effects of temperature and adult size dependence.

### Thermal effects on physiological rates

The positive effects of warmer temperatures on the physiological rates of females of *P. grani* only appeared in the first generation, and this influence dampened at longer thermal exposures. In the case of males, there was also a positive trend of feeding rates with temperature in F1 (albeit not significant), which in successive generations also dampened. The drop in thermal response found in males and females in our

experiments was greater than that reported for the same species by Saiz et al. (2022), who found a decrease in the  $Q_{10}$  coefficients from 2.6–2.7 for an acute response (24 h) to 1.6–1.7 after a 7-d acclimation. It seems, therefore, that long-term exposure (multigenerational rearing) has deeper effects on the thermal compensation of physiological rates, leveling out the response to temperature and evidencing a notable phenotypic plasticity in the copepod species, which may be related to the coastal environments that constitute its habitat and the broad latitudinal distribution (Saiz et al. 2022).

Under a warming ocean scenario, chronic exposure to higher temperatures through multiple generations may allow other mechanisms, at phenotypic and genotypic levels, to modulate the thermal response (Angilletta et al. 2003; Dam 2013). Thus, within-generation and transgenerational (maternal effects) plasticity are increasingly examined for their role in evolutionary potential in the face of climate change (Donelson et al. 2018). For instance, the temperature experienced at earlier development stages can “carry over” to the next one (Donelson et al. 2018; Moore et al. 2019), and females may produce offspring better fitted for their environmental conditions (Vehmaa et al. 2012). It has been shown that phenotypic traits of adaptive value can be inherited in a few generations (Lee et al. 2013; Souissi et al. 2016). More recently, Dam et al. (2021) suggested that modifications at the genotype level that change the thermal response can occur quite rapidly, in just three generations. In this regard, the F1 copepods in our experiments experienced a temperature rise only during embryogenesis and development. This fact may explain some of the discrepancies found between F1 (raised from the stock culture F0) and later generations in our experiments.

### Combined effects of thermal compensation and body size reduction

Body size influences copepod ingestion and egg production rates (Runge 1984; Ban 1994; Saiz and Calbet 2011). Therefore, we would expect an interaction between the direct effects of temperature on metabolism and those mediated by the thermal-induced body size change (Riemer et al. 2018). Based on our results, we explored the relative contribution of body size change and physiological compensation to the dampening of physiological rates that we found in our experiments after multiple generational thermal exposure. To do this, we took the per capita female feeding rates at  $19^{\circ}\text{C}$  for each generation and applied a  $Q_{10}$  value from literature for *P. grani* ( $Q_{10} = 1.6$ ; Saiz et al. 2022) to estimate the expected temperature-mediated increase in ingestion. Subsequently, we corrected the obtained feeding rates for allometric effects applying a body weight scaling factor of 0.743 (Saiz and Calbet 2007), in order to take into account the decrease in body size (carbon content) at warmer temperatures. In F1, the body size reduction could largely explain (70% at  $22^{\circ}\text{C}$  and 129% at  $25^{\circ}\text{C}$ ) the copepod’s thermal response under

warming conditions (Supporting Information Fig. S2). In F10 and F11, however, the observed decline in body size at warmer temperatures could explain, respectively, only a 19–23% and 10–21% drop in the copepod feeding rates, the rest being attributed to the thermal compensation processes in the organism's physiology.

We must note that, whether plastic or adaptive, the underlying processes adjusting the thermal response to new conditions can be beneficial for some traits but may have a cost for the organism in other traits (Angilletta et al. 2003). A trade-off commonly considered in life-history theory is the expenditure of resources by the basal metabolism and reproductive effort (von Weissenberg et al. 2022). Here, we have reported an enhancement of feeding and fecundity rates caused by warming in the first generation reared. However, we found no long-term negative effect of temperature on gross growth efficiency or sex ratios, which were never significantly different from one, as expected for copepod species with no seminal receptacles that require frequent remating (Kiørboe 2006). Hatching success showed a significant negative trend with an 8% reduction in F1, but a recovery across generations. This result implied that thermal compensation after long-term exposure achieved homeostatic balance and that no detrimental effects appeared.

### Implications

The thermal plasticity in body size played a significant role in buffering the thermal effects on the per capita ingestion and egg production rates in the first generation of exposure to warming. After multigenerational exposure, however, other processes (either plastic or adaptive) came into play and further reduced the thermal effects on physiological rates, with no apparent cost for the life-history traits studied. It seems evident from our study that long-lasting exposure to warming such as that expected under future global scenarios will not necessarily imply higher rates of metabolic activity by copepods. Therefore, the use of  $Q_{10}$  coefficients of 2–3 for climate change models could overestimate the actual impacts of thermal change in copepod-mediated biogeochemical fluxes (Saiz et al. 2022). However, the variability between generations calls for caution in extrapolating our results. Also, our study was carried out with copepods from a long-term laboratory culture, and field animals may have higher genetic variability that allows other adaptive responses. Previous reports have shown that laboratory-reared copepods can lose some adaptive traits (Tiselius et al. 1995; Calbet et al. 1999), even in a few generations (Olivares et al. 2020). We must also keep in mind that in nature the effects of global warming on marine copepod populations will also encompass more complex responses, involving biogeographical and phenological changes and interactions with other components of the pelagic ecosystem, such as predators and prey, which in turn may also be affected by temperature (Beaugrand et al. 2002; Richardson 2008; Garzke et al. 2015).

### Data availability statement

The data that support the conclusions of this study are openly available in Digital.CSIC repository at <https://doi.org/10.20350/digitalCSIC/15130>.

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#### Conflict of Interest

None declared.

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