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RESEARCH ARTICLE

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Thermal plasticity has higher fitness costs among thermally tolerant genotypes of *Tigriopus californicus*

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Abstract

1. Under climate change, ectotherms will likely face pressure to adapt to novel thermal environments by increasing their upper thermal tolerance and its plasticity, a measure of thermal acclimation. Ectotherm populations with high thermal tolerance are often less thermally plastic, a trade-off hypothesized to result from (i) a phenotypic limit on thermal tolerance above which plasticity cannot further increase the trait, (ii) negative genetic correlation or (iii) fitness trade-offs between the two traits. Whether each hypothesis causes negative associations between thermal tolerance and plasticity has implications for the evolution of each trait.
2. We empirically tested the limit and trade-off hypotheses by leveraging the experimental tractability and thermal biology of the intertidal copepod *Tigriopus californicus*. Using populations from four latitudinally distributed sites in coastal California, six lines per population were reared under a laboratory common garden for two generations. Ninety-six full sibling replicates ($n=4$–5 per line) from a third generation were developmentally conditioned to 21.5 and 16.5°C until adulthood. We then measured the upper thermal tolerance and fecundity of sibships at each temperature.
3. We detected a significant trade-off in fecundity, a fitness corollary, between baseline thermal tolerance and its plasticity. *Tigriopus californicus* populations and genotypes with higher thermal tolerance were less thermally plastic. We detected negative directional selection on thermal plasticity under ambient temperature evidenced by reduced fecundity. These fitness costs of plasticity were significantly higher among thermally tolerant genotypes, consistent with the trade-off hypothesis. This trade-off was evident under ambient conditions, but not high temperature.
4. Observed thermal plasticity and fecundity were best explained by a model incorporating both the limit and trade-off hypotheses rather than models with parameters associated with one hypothesis. Effects of population and family on tolerance and plasticity negatively covaried, suggesting that a negative genetic correlation could not be ruled as contributing to negative associations between the traits. Our study provides a novel empirical test of the fitness trade-off

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hypothesis that leverages a strong inference approach. We discuss our results' insights into how thermal adaptation may be constrained by physiological limits, genetic correlations, and fitness trade-offs between thermal tolerance and its plasticity.

KEYWORDS
global change, natural selection, phenotypic plasticity, thermal acclimation, thermal tolerance, warming

1 | INTRODUCTION

Increasing annual temperatures and heat wave occurrences may impose positive directional selection on upper thermal tolerance and its plasticity, which drives acclimation to novel temperatures (Arnold et al., 2019; Huey et al., 2012). Positive directional selection on the two traits should occur when tolerance promotes survival under increasing thermal maxima and acclimation increases organismal performance and fitness under high temperature (Arnold et al., 2019; Huey et al., 2012). Stemming from observations that ectotherms with high upper thermal tolerance can be less plastic in their thermal tolerance, adaptive increases in tolerance are predicted to reduce thermal plasticity and vice versa (Barley et al., 2021; Stillman, 2003; van Heerwaarden & Kellermann, 2020). Multiple hypotheses may explain how negative correlations between thermal tolerance and its plasticity evolve (van Heerwaarden & Kellermann, 2020). One mechanism termed the 'fitness tradeoff hypothesis' states that this negative correlation arises from synergistic fitness costs shared by the two traits: The cost of thermal plasticity is significantly greater in tolerant genotypes (Roff & Fairbairn, 2007). The existence of a fitness trade-off between thermal tolerance and its plasticity would bear important constraints on adaptation to warming (Agrawal et al., 2010). Experiments selecting for upper thermal tolerance have observed reduced thermal plasticity and inferred that the reduction may result from a fitness trade-off (Kelly et al., 2011, 2017; Morgan et al., 2022; Sasaki & Dam, 2021). However, an empirical test of whether thermal plasticity's fitness costs are greater in tolerant genotypes remains unreported (van Heerwaarden & Kellermann, 2020).

Negative correlations between basal thermal tolerance and thermal plasticity have been observed across ectotherm genotypes and populations (Barley et al., 2021; Stillman, 2003; van Heerwaarden et al., 2016). The two traits can be defined as parameters of a thermal tolerance reaction norm (Figure 1a) where basal tolerance equals the intercept of tolerance's change across temperature and plasticity equals the slope of that change (Lachenicht et al., 2010). Because plasticity is derived from the difference between basal tolerance and tolerance under high temperature, it is statistically non-independent from basal tolerance (Kelly & Price, 2005). When their non-independence is controlled for, negative correlations between the traits persist in some species (Gunderson, 2023; Gunderson & Revell, 2022). Across populations, negative relationships between basal tolerance and plasticity can arise from adaptive canalization, where populations experiencing strong selection on upper thermal tolerance incur negative selection on plasticity because deviations from optimum tolerance induced by plasticity reduce fitness (Fairbairn, 2005). Across genotypes inhabiting a singular environment, reduced thermal plasticity in tolerant genotypes may result from evolutionary constraints that limit high phenotypic values for both traits (van Heerwaarden & Kellermann, 2020). These constraints have the potential to limit rates of thermal adaptation in environments positively selecting for tolerance and plasticity (Agrawal et al., 2010).

Fitness trade-offs between traits can arise when allocations towards their phenotypes draw from common resources (James, 1974; Riska, 1986) resulting in greater costs of one trait in genotypes expressing high levels of the other trait. In the case of thermal plasticity and tolerance, evidence of a fitness trade-off would be provided by genotypes with high baseline tolerance incurring greater costs of plasticity in a fitness correlating trait (Figure 1b). Fitness trade-offs between plasticity and other properties of phenotypes such as trait means or developmental stability have been observed (Couso & Fernández, 2012; Tonsor et al., 2013). The evolution of plasticity in thermal performance is proposed to be partially driven by fitness trade-offs associated with resource allocation (Angilletta et al., 2003). Simulations suggest that the fitness costs of plasticity in environmental tolerance should depend on baseline levels of that tolerance (Siljestam & Östman, 2017). Experiments selecting for thermal tolerance have observed subsequent declines in plasticity and inferred potential for this canalization to be caused by fitness trade-offs (Esperk et al., 2016; Kelly et al., 2017; Morgan et al., 2022; Sikkink et al., 2014), but empirical test of fitness trade-offs between thermal tolerance and plasticity remain unreported. Determining whether and how fitness trade-offs between thermal tolerance and plasticity shape intraspecific variation in thermal physiology will (i) aid understanding how the canalization of thermal traits evolves and (ii) help predict how ectotherms will adapt to warming (van Heerwaarden et al., 2016). These outcomes require that trade-off's effects be contrasted with alternative mechanisms that may also drive canalization in thermally tolerant individuals and populations.

Negative associations between basal thermal tolerance and thermal plasticity may also be caused by physiological limits to upper thermal tolerance and negative genetic correlations (Figure 1b). A

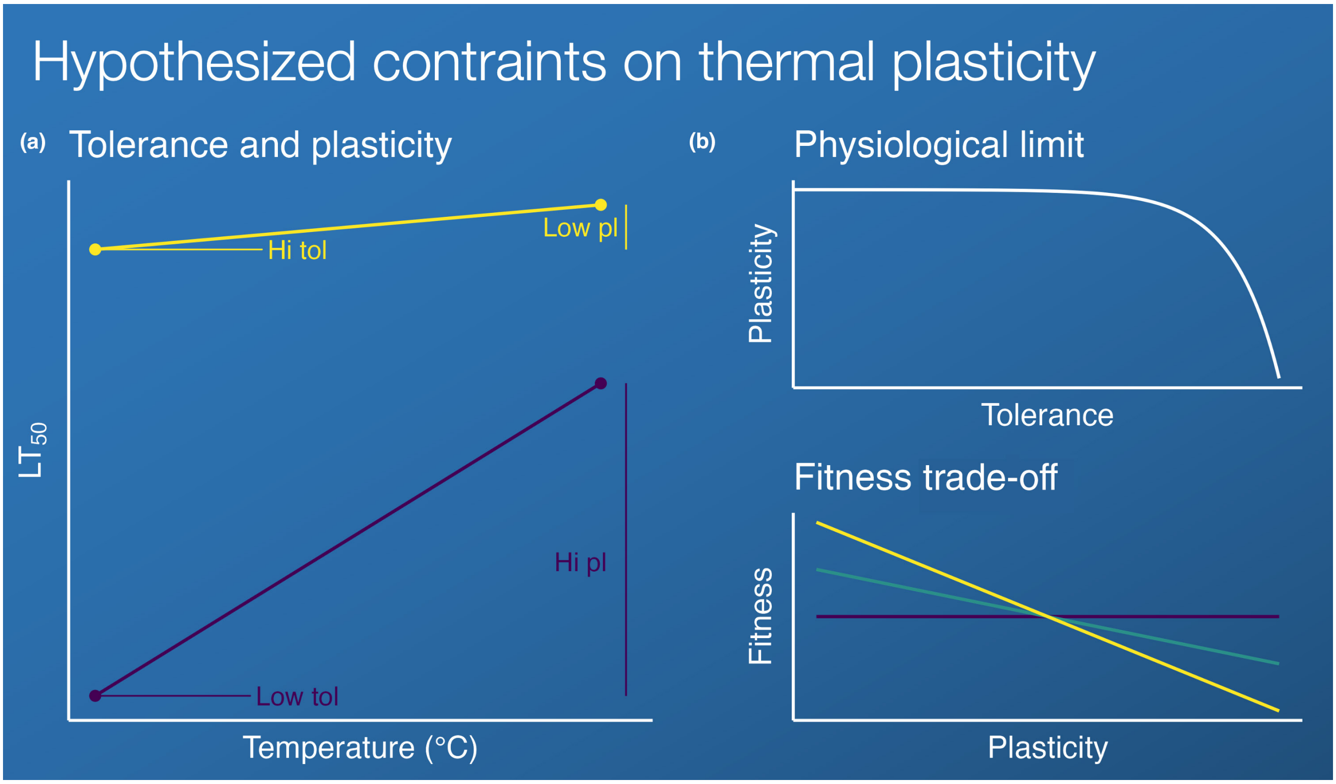

FIGURE 1 Hypothesized constraints on thermal plasticity. (a) Parameters associated with baseline upper thermal tolerance (Tol) and its plasticity (Pl) across a thermal reaction norm. (b) Visualizations of how thermal plasticity is constrained under the limit and trade-off hypotheses. Physiological limit: Evidence of a physiological limit can be derived from negative correlations between tolerance and plasticity arising at high levels of baseline tolerance such that thermal tolerance is limited by an asymptote to the sum of both traits. Fitness trade-off: Evidence of a fitness trade-off can be provided by genotypes with high baseline tolerance incurring greater costs of plasticity for a fitness correlating trait. Genotypes with high, medium, and low thermal tolerance are depicted using yellow, blue, and purple, respectively.

physiological limit is defined as a trait's maximum value that cannot be exceeded by the sum of a phenotypic intercept and phenotypic plasticity (Araújo et al., 2013). Because this threshold functions as an asymptote, physiological limits should impose a non-linear relationship between thermal tolerance and plasticity. The two traits would be uncorrelated or weakly correlated as basal tolerance increases until the sum of tolerance and plasticity approaches a limit, at which point plasticity will precipitously decline as shown in Figure 1b (Kempes et al., 2019). Negative genetic correlations result from antagonistic pleiotropy, where a single allele has different directional effects on basal tolerance and thermal plasticity, or linkage disequilibrium, where at least two co-inherited alleles independently affect tolerance and plasticity in opposing directions (Falconer, 1996; Lynch & Walsh, 1998). Positive and negative genetic correlations have been measured in quantitative genetic breeding designs as the co-variance of additive genetic effects on basal thermal limits and the plasticity of thermal limits (Blackburn et al., 2014; Ørsted et al., 2018).

Physiological limits, negative genetic correlations and fitness trade-offs each impose unique constraints on the evolution of traits they affect. Physiological limits constrain evolution by imposing maxima above which phenotypes cannot evolve (Kempes et al., 2019). By contrast, fitness trade-offs and negative genetic correlations can constrain evolutionary rates of the traits they affect. If tolerance and plasticity are under equal and positive directional selection, fitness trade-offs can reduce fitness in genotypes with independently optimal phenotypes resulting in selection for intermediate phenotypes and reduced evolutionary rates (Agrawal et al., 2010). Trade-offs can result in selection on phenotypes that is weaker than selection in their absence, slowing evolutionary rates (Weaver et al., 2020). Negative genetic correlations impose similar constraints on the extent and rate of evolution in two traits under equal directional selection because a genotype with high tolerance necessarily expresses lower plasticity and vice versa (Agrawal & Stinchcombe, 2008). Operating via distinct mechanisms, fitness trade-offs and negative genetic correlation can simultaneously constrain evolution (Garland et al., 2022). Their combined effect on evolution can be calculated with the Lande equation (Lande, 1975), where changes in each phenotype between generations ($\Delta\bar{z}$) equals the product of the additive genetic variance–covariance matrix of both traits (G) and the vector of selection gradients acting on each trait (β) such that $\Delta\bar{z} = G\beta$. Fitness trade-offs and negative genetic correlation jointly slow evolution by, respectively, reducing absolute β and negating elements of G. Because of their potential to constrain evolution, quantifying fitness trade-offs and negative genetic correlation between thermal tolerance and its plasticity is critical for predicting rates of adaptation to warming (van Heerwaarden & Kellermann, 2020).

Studying populations and family-level genotypes of the intertidal copepod *Tigriopus californicus*, we experimentally tested the hypothesis that thermal tolerance and plasticity possess a fitness trade-off. This species is well-poised to address knowledge gaps regarding evolutionary constraints that may drive negative correlations between thermal tolerance and its plasticity. *Tigriopus californicus* populations exhibit strong genetic differentiation and local adaptation across their distribution on the North American Pacific Coast such that genetically fixed thermal tolerance increases at lower latitudes (Barreto et al., 2018; Edmands, 2001; Hong & Shurin, 2015; Kelly et al., 2011; Sanford & Kelly, 2011). Populations also possess genetically fixed differences in the plasticity of thermal tolerance (Kelly et al., 2011; Pereira et al., 2017). Evolutionary experiments in this species are aided by a generation time as short as 21 days (Powlik et al., 1997), easily measured fitness correlates for fecundity and survival (Powers et al., 2020), and unipaternal sexual reproduction (Burton, 1985). We cultured lines from four populations distributed over 4°N of latitude under a laboratory common garden for two generations before splitting a third generation of 96 full-sibling genotypic replicates ($n=192$ cultures) across high and low temperature developmental conditions. Once matured, we measured the generation time, body size, upper thermal tolerance and fecundity of each sibship at high and low temperatures. We found in this experiment that populations and families exhibit a negative correlation between tolerance and plasticity. Using an expansion of the Lande & Arnold selection gradient model (Lande & Arnold, 1983), we measured the fecundity costs of thermal plasticity conditional upon basal tolerance (trade-off hypothesis) as shown in Figure 2. Alternative versions of this model included parameters associated with physiological limits and covariance between tolerance and plasticity's family and population-level effects. We then used marginal likelihoods to contrast support for the fitness trade-off hypothesis relative to physiological limits and evaluated evidence of a negative genetic correlation between tolerance and plasticity.

2 | MATERIALS AND METHODS

2.1 | Collection and common garden culturing of *Tigriopus californicus*

Tigriopus californicus were collected during summer and winter from four latitudinally distributed populations (Figure 3) in coastal California over a 4 day period between August 6th–9th, 2021 and February 16th–19th, 2022: the Bodega Marine Reserve (BMR)

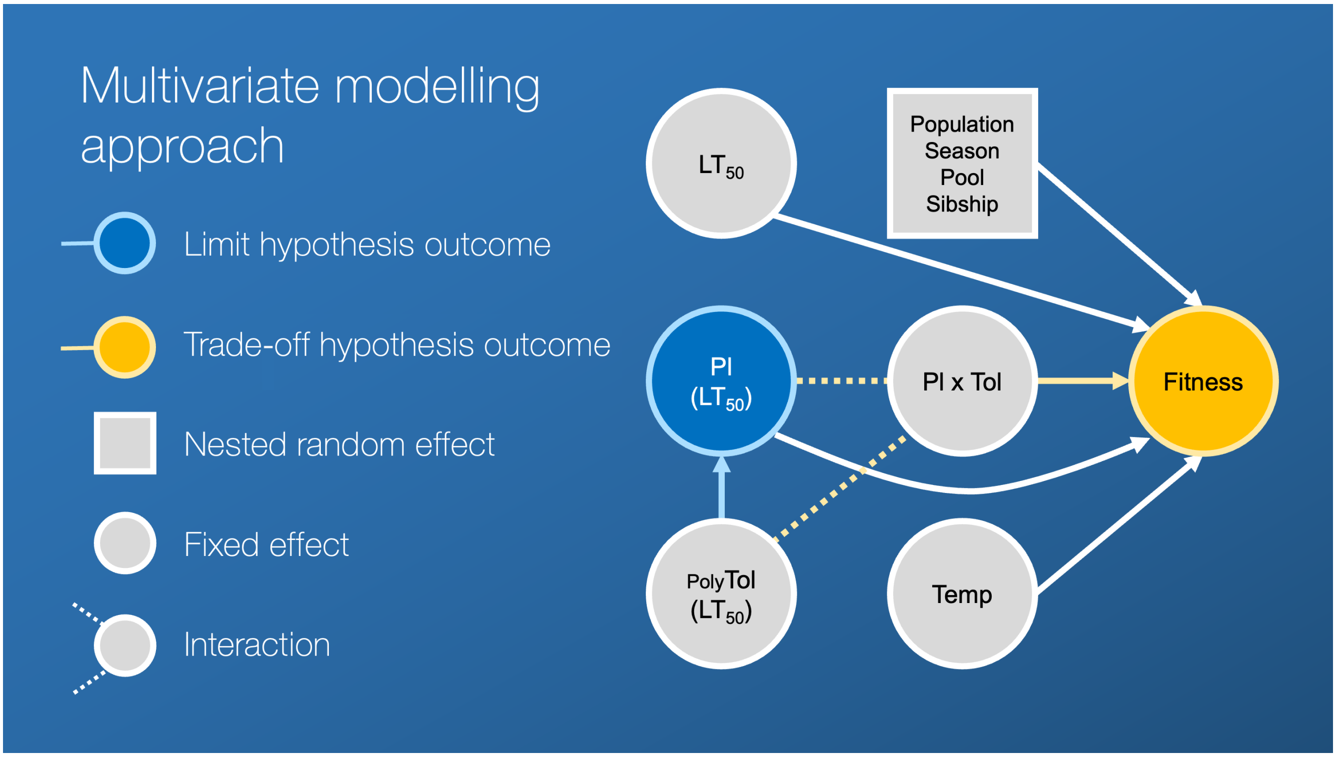

FIGURE 2 Visual representation of multivariate selection gradient model. A directed acyclic diagram generalizing the multivariate model testing the physiological limit (blue) and fitness trade-off (orange) hypotheses is shown. The limit hypothesis is evaluated by predicting the plasticity of thermal tolerance (PL) as a second order polynomial function of baseline thermal tolerance (e.g. the intercept of LT_{50}'s reaction norm; Tol). The polynomial (Poly) is constrained to be a negative, convex curve consistent with a physiological limit. The trade-off hypothesis is evaluated by fitting a selection gradient model that includes the fitness consequence of an interaction between LT_{50} pl and LT_{50} int. Not pictured are singular effects of season-of-collection, mean female body length, and interactive effects between 'Temp' and 'LT_{50}', 'PL' and 'PL × TOL' predicting 'Fitness'. These parameters were excluded to simplify this visual generalization of the model.

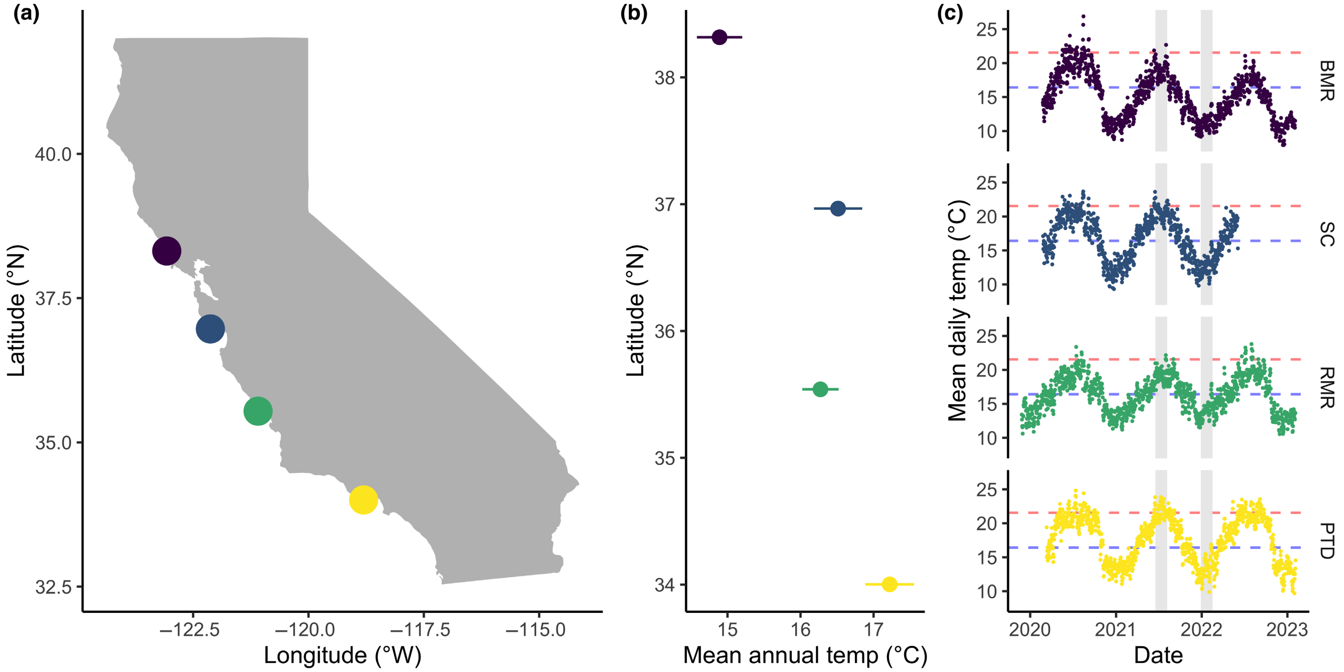

FIGURE 3 *Tigriopus californicus* collection sites and thermal environments. (a) Coordinates of four intertidal collection sites in coastal California, USA. (b) Mean in situ temperatures recorded in supralittoral pools inhabited by *T. californicus* at each collection site over a 2-year period between summer 2020 and summer 2022 using $n=3$ TidBit MX3000 loggers (Onset Computer Corp) per site. Error bars depict ±95% CI. (c) Mean daily temperature is plotted across time in each collection site. Colour depicts latitude in all panels. Horizontal dashed lines depict the high and low experimental temperatures used in this study. Vertical gay bars depict the 50-day period (the approximate minimum lifespan of *T. californicus*) prior to two field collections in August of 2021 and February of 2022.

in Bodega Bay, CA (38.316394°N, −123.071980°W), Four Mile Beach (SC) in Santa Cruz, CA (36.965262°N, −122.125983°W), the Kenneth S. Norris Rancho Marino Reserve (RMR) in Cambria, CA (35.540090°N, −121.092475°W), and Point Dume State Beach (PTD) in Malibu, CA (34.002035°N, −118.805029°W) as shown in Figure 3a. In situ temperatures were recorded at each site (Figure 3b,c) using methods described in Supporting Information. Adult animals were collected from three splash pools at each site, stored in 500-mL cups, and transported in coolers under a 12:12h photoperiod to laboratory facilities. During 4days of travel and 1week of incubation in the laboratory per collection, transport coolers and incubation maintained an average temperature of 19.96°C and 13.00°C during August and February, respectively. These transport temperatures were −0.24°C and −0.77°C away from mean in situ temperatures recorded at all sites 21days prior to August and February collections. During 4days of transport, polystyrene collection cups received daily seawater changes using aerated, 0.5µm filtered seawater and received an ad libitum diet of 20% spirulina fish food flakes (Handschumacher et al., 2010). Upon arrival at laboratory facilities, collection cups continued to receive daily seawater changes, were fed ad libitum and were held in an incubator set to 20.2±0.67°C (August) or 13.8±0.32°C (February) with a 12:12h photoperiod for 7days before the initiation of common garden lines. *Tigriopus californicus* were collected under California Department of Fish and Wildlife specific use permit S-192200007-19260-001 and California State Parks scientific research and collections permit 'Field Collections of Tigriopus californicus'. Collection of and research on *T. californicus* did not require ethics approval.

To remove in situ environmental effects from phenotypic variation, 12 common garden lines ($n=3$ lines per population) were reared in the laboratory for two generations before a third generation was split and conditioned to high and low temperatures until maturity. Phenotyping for upper thermal tolerance and fitness correlated traits, described below, was performed on temperature conditioned, third generation cultures. These generations are referred to as G1–G3 (laboratory-reared generations 1–3) from here forward. At initiation of new generations, $n=100$ gravid females per pool were added into new 500-mL polystyrene culture cups and incubated at 16.5°C, the mean annual temperature recorded in situ across all four collection sites between 2020 and 2021 (Figure 3). Gravid females were allowed to continuously hatch clutches of eggs in culture until the emergence of copepoid stage offspring at which time gravid females were removed. G1 and G2 cultures received seawater changes twice per week and continued to be fed ad libitum until cultures matured, mated, and females became gravid. G1 and G2 cultures maintained mean salinities of 33.54±0.53ppt standard deviation and 33.62±0.52ppt. Once 100 or more females within a culture became gravid, G2 was initiated using the same methods described for G1.

2.2 | Culturing of G3 sibships across high and low temperature

Twelve gravid G2 females per line were added to individual 20 mL of cultures in 12-well plates where they were allowed to continuously hatch broods ($n = 144$ total gravid females). Individual cultures were checked for hatchlings and split between developmental temperatures every 4 days. Resulting G3, full sibling nauplii were split between two 50 mL of cultures incubated at a target of 16.5 and 21.5°C that were recorded to be 16.42 ± 0.37°C standard deviation and 21.55 ± 0.40°C over the duration of G3 culture. Each gravid female was allowed to hatch offspring until mortality or a maximum number of 100 larvae had hatched. If a gravid female died, it was replaced with another gravid female from the same line which was used to initiate new G3 cultures ($n = 13$ out of 96 sibships; 13.54%). Seawater in 50 mL G3 cultures was changed every 4 weeks, resulting in an average salinity of 35.15 ± 2.20 and 34.87 ± 2.26 ppt under 16.5 and 21.5°C, respectively. Females were allowed to hatch over 4 weeks. The mean number of larvae per G3 culture equaled 40.52 ± 17.20 (Figure S1), 24.68x lower than the upper density of *T. californicus* in situ (Powlik, 1996).

The developmental progress of G3 cultures was scored by tracking the maximum life history stage of cultures during hatch checks: stages were scored as achieving the naupliar larval stage, the juvenile copepoid stage, or maturity upon the presence of sexually dimorphic morphology at stage-C6 moult as well as whether gravidity had been achieved by mature females. The generation time of each population × temperature group was then estimated using logit-transformed generalized linear models to determine the days post-hatch at which 50% of a population progenates the next generation at a given temperature. These generation times were then used to determine the relative ages of population × temperature groups (i.e. days post-gravidity).

2.3 | Assays of upper thermal tolerance, body size, and fecundity

When cultures achieved an average age of 16–26 days post-gravidity (Figure S2) and presented no evidence of mortality among stage-C6 adults, they were assayed for upper thermal tolerance and stored in 1% formalin-buffered seawater for morphometric measurements and counts of fecundity per brood. Upper thermal tolerance was measured using an LT_{50} assay, a measure of the temperature at which 50% of a population or group has died. LT_{50} was measured by adding mature males, mature females, and copepoids to 200-mL PCR tubes at an average density of 1–6 animals per tube. This density has been demonstrated to not result in an effect on LT_{50} in *T. californicus* via oxygen depletion (Kelly et al., 2011). Eight tubes per sibship × temperature group were randomly distributed across a thermal cycler plate where they were ramped up to a 3°C temperature gradient over 2 h at a ramping rate of 0.2°C per 1.5 min. BMR was exposed to a 34–37°C gradient, SC and RMR to 35–38°C, and PTD to 36–39°C. Animals were then allowed to recover at room temperature for 1 h. Following the recovery period, survival was scored for mature males, mature females, and copepoids from each sibship × temperature group. An average of 21.18 ± 9.8 animals per sibship × temperature group were assayed for LT_{50}. The LT_{50} parameter of each sibship × temperature group was measured as an inflection point randomly varying across sibship × temperature groups. This parameter was estimated by a nonlinear logistic regression model. The logistic regression predicted survival as a function of fixed effects for gradient temperature, age, season of collection and nested, random effects for pool of origin and population of origin. By accounting for sex, which was determined based on sexually dimorphic characters (Powlik et al., 1997), we estimated LT_{50} parameters controlling for the number of each sex in cultures. Logistic regressions predicting LT_{50} are described in greater detail in Supplemental Material. Correlation between baseline LT_{50} and its plasticity across sibships was modelled using a linear mixed model that included a nested random effect of population. Specification of the linear mixed model is further described in Supporting Information.

Total body length was measured in mature females via imaging on a compound light microscope under 40× magnification. Length equaled the total linear distance from the anterior end of the cephalon to the caudal radius. Fecundity per brood was measured as a fitness-correlated trait by dissecting and counting eggs in fully developed brood sacs from formalin-preserved gravid females. Only egg sacs with matured eggs exhibiting nauplii morphology were dissected in order to avoid underestimation of fecundity per brood. Brood sac dissections were conducted on females collected up to 7 days after LT_{50} assays to maximize the number of fecundity measurements per culture. Egg sac dissections were either performed on mature egg sacs immediately following the LT_{50} assays or on animals unexposed to the LT_{50} temperature gradient during the 7-day sampling window in order to avoid a confounding effect of the temperature gradient on reproduction. Similarly, body size was recorded in females sampled immediately after LT_{50} assays or in unexposed animals preserved during the 7 days fecundity sampling window. Mean fecundity andfemale body length were calculated for each sibship x developmental temperature group. Thermal tolerance and thermal plasticity data were measured for 96 sibships reared across two temperatures for a total of 192 experimental cultures. Fecundity measures were successfully obtained for 107 cultures representing 68 sibships (Table 1).

TABLE 1 Replication statement. This table describes the level of replication achieved for analyses of fecundity in the selection gradient model.

Scale of inference	Scale at which factor of interest is applied	Number of replicates at the appropriate scale
Genotype/family	Genotype/family	107 cultures from 68 family-level genotypes reared across low and high temperature

2.4 | Selection gradient modelling

Selection gradients acting on LT_{50} and its plasticity were estimated using a multivariate adaptation of the Lande and Arnold regression for measuring selection on correlating characters (Lande & Arnold, 1983). The multivariate model was fit using brms, an R interpreter of the Bayesian modelling language Stan (Bürkner, 2017; Gelman et al., 2015). A Bayesian approach was chosen due to its advantages in (i) modelling multivariate relationships via structural equations (Muthén & Asparouhov, 2012), (ii) constraining parameters via prior specification to evaluate specific hypothesis, and (iii) comparing the likelihoods of complex structural equation models via marginal likelihood (Garnier-Villarreal & Jorgensen, 2020; Moshagen, 2012). Models were run using 40,000 MCMC chains with 10,000 warm up iterations and a skew-normal distribution family, the family that best predicted variation in outcome variables. Models assumed uniform priors for fixed effects except in the case of nonlinear effects of tolerance on plasticity. This effect was constrained as negative and convex in order to test the physiological limit hypothesis (described below). Details regarding Bayesian model fitting and quality checking are described under Supporting Information.

The two outcome variables of the multivariate model were mean clutch size measured at high and low temperatures and the plasticity of thermal tolerance between temperatures. A multivariate approach was necessary to test both the physiological limit and fitness trade-off hypotheses in one singular model and perform quantitative hypothesis testing, which is described in the next Section 2. Mean egg clutch size of each sibship per temperature was modelled as a function of fixed effects for temperature, LT_{50} within a given temperature, the plasticity of LT_{50} across temperature, an interaction between plasticity and baseline LT_{50}, and nested random effects for sibship, pool of origin, population of origin and season of collection. To test the limit hypothesis, the multivariate model predicted LT_{50} plasticity as a second order polynomial function of baseline tolerance (Figure 2). By constraining the slope of this polynomial to be negative and convex, consistent with expectations under the limit hypothesis (Figure 1b), the marginal likelihood of the limit effect could be contrasted with alternative models. Statistical non-independence between LT_{50} intercepts (baseline thermal tolerance) and LT_{50} slopes (plasticity of thermal tolerance) was controlled for using an orthogonal polynomial transformation of the intercept value following guidance for regressing intercepts and slopes from a single function (Kline, 2015).

An additional transformation on slopes was necessary to control against the effect of regression to the mean in driving negative correlations between baseline tolerance and plasticity. A transformation controlling for regression to the mean for basal phenotypes' effects on plasticity was originally proposed by Kelly & Price and applied to thermal tolerance data by Gunderson & Revell (Gunderson, 2023; Gunderson & Revell, 2022; Kelly & Price, 2005). Our application of this transformation was conducted in concordance with Gunderson, 2023, and is detailed in Supplemental Material.

Equations 1–3 describe components of three Bayesian models predicting fecundity as a function of phenotypic costs, thermal plasticity as a function of basal tolerance, and thermal plasticity as a function of population and family-level effects. ω = mean fecundity of sibship i under temperature e, S—season of collection, L—mean body length per gravid female, X—LT_{50}, int—the intercept of LT_{50}, pl—the plasticity of LT_{50} and Z—random intercepts associated with nested effects (Figure 2) or population alone in the case of Z_2. Excluding fecundity as a predictor also avoided a recursive model structure whereby the fitness-correlating trait affected plasticity and plasticity affected fitness (Cortina, 2014; Kiiveri et al., 1984). Mean female body length was included as a fixed effect in order to control for its direct impact on egg clutch size (Voordouw et al., 2005).

One multivariate model sought to measure the potential for negative genetic correlation between tolerance and plasticity. This was achieved by modelling plasticity as a polynomial function of baseline tolerance as described above (Equation 2) and as a function of the hierarchical, nested random effect of family, pool, season and population (Equation 3). Baseline tolerance was also predicted to vary according to this random effect, enabling parameter estimation of covariance in family- and population-level effects on tolerance and plasticity (Equation 3). This covariance was not fitted in the larger model visualized in Figure 2 to prevent overfitting and sustain model convergence. While this approach measured covariance in random effects of family and population, this nested random effect does not permit measurement of additive genetic variance or covariance as a traditional pedigree or relatedness matrix would (Laine et al., 2022). For this reason, the reported covariances should be strictly interpreted as covariance in family-level effects rather than genetic covariance. The significance of random effect covariance was tested by performing a probability of direction test on bootstrapped estimates of the covariance (n = 10,000 iterations) using the R package 'boot' v1.3–28.1 (Canty & Ripley, 2022). A 95% interval of the bootstrap distribution below 0 constituted significant negative covariance.

$$\omega_{i,e} = \mu_1 + \beta_1 e_i + \beta_2 S_{i,e} + \beta_3 L_{i,e} + \beta_4 X_{i,e} + \beta_5 \mathrm{pl}X_{i,e} + \beta_6 \mathrm{int}X_i \mathrm{pl}X_i + Z_1 b_i + \varepsilon \tag{1}$$

$$plX_i = \mu_2 + \beta_7 \mathrm{int}X_i + \beta_8 \mathrm{int}X_i^2 + Z_2 b_i + \varepsilon \tag{2}$$

$$\begin{bmatrix} \mathrm{pl}X_i \\ \mathrm{int}X_i \end{bmatrix} = Z_1 b_i + \varepsilon \tag{3}$$

The significance of fixed effects was evaluated across all models using a probability of direction test, a Bayesian corollary of the p-value for determining effect existence. Fixed effects were deemed significant if 95% confidence intervals of their sampled posterior distributions did not overlap 0 (Makowski, Ben-Shachar, & Lüdecke, 2019).

2.5 | Quantitative hypothesis testing

Quantitative support was measured and compared for the physiological limit and fitness trade-off hypotheses in driving negative associations between thermal tolerance and its plasticity. Two distinct parameters were fit by the selection gradient model associated with the two hypotheses and are detailed in Equations 1 and 2: $\beta_7 \text{int}X_i plX_i$ (Equation 1; trade-off hypothesis) and $\beta_8 \text{int}X_i + \beta_9 \text{int}X_i^2$ (Equation 2 limit hypothesis). The limit hypothesis was tested by predicting the plasticity of thermal tolerance as a function of baseline LT_{50}. The trade-off hypothesis was tested by predicting mean clutch size as a function of interactive costs between the baseline thermal tolerance and its plasticity. The marginal likelihood of a model containing both parameters was compared to three alternative models that lacked one or both parameters using a Bayes factor test (Berger & Pericchi, 1996) in the R package bayestestR (Makowski, Ben-Shachar, Chen, et al., 2019).

3 | RESULTS

3.1 | Intraspecific variation in performance traits and their thermal plasticity

Populations and sexes exhibited differences in upper thermal tolerance (LT_{50}), organismal performance, and the plasticity of these traits across temperatures. Upper thermal tolerance exhibited a latitudinal cline such that baseline tolerance decreased towards higher latitudes at a rate of −0.44°C LT_{50} per °N latitude. Females had an average LT_{50} of 38.76±0.05°C, while males exhibited an LT_{50} of 38.43±0.08°C (Figure 4a). Controlling for population, this sex-specific effect equaled +0.19°C LT_{50}. As the thermal tolerance of populations increased toward lower latitudes, the plasticity of thermal tolerance decreased (Figure 4a). Scaling down from populations to genotypes, a significant negative correlation between upper thermal tolerance and its plasticity was observable among sibships as shown in Figure 4b (95% PI = −0.67 to −0.29). Plasticity is visualized in Figures 4 and 5 using Q_{10} of LT_{50}, the linear change in LT_{50} across 10°C. The negative association between sibships' thermal tolerance and its plasticity was significant after controlling against (i) non-independence between the intercept and slope of LT_{50} reaction norms and (ii) regression to the mean. Non-independence only explained 0.48% of baseline tolerance's negative effect on plasticity while regression to the mean explained 47.97% of the effect. Intraspecific variation in thermal physiology can be compared with in situ temperatures further described in Supplemental Material (Figure S3).

Body size increased toward higher latitudes (Figure S4A), while generation time did not exhibit a latitudinal pattern (Figure S4B). In response to high temperature, body size and generation time both decreased. Body size was less plastic among northern populations. The plasticity of generation time varied randomly across populations (Figure S4B).

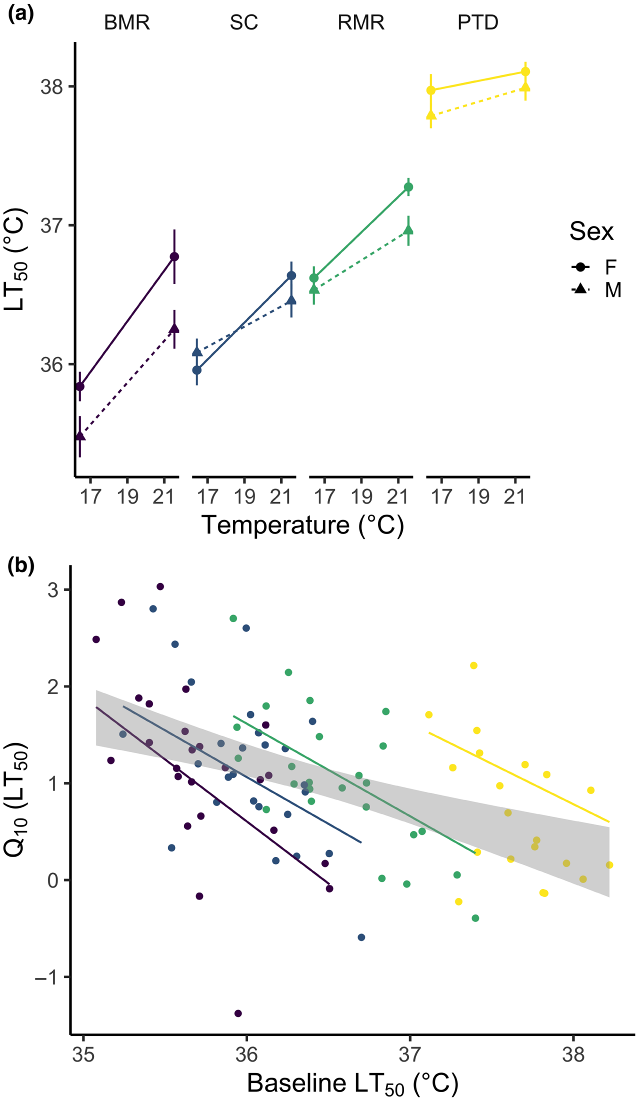

FIGURE 4 Negative correlation between baseline thermal tolerance and thermal plasticity at population and genotype levels. (a) Upper thermal tolerance measured as LT_{50} is plotted across developmental temperatures grouped by population (colour) and sex (shape and line type). Error bars represent 95% confidence intervals of LT_{50} parameter estimates. (b) Thermal plasticity, measured as the Q_{10} of LT_{50}, is plotted across baseline thermal tolerance measured as the intercept of LT_{50}'s reaction norm across developmental temperature. Each point depicts a single sibship whose population is represented by colour. The grey confidence interval depicts best fit to raw Q_{10} of LT_{50}. Solid lines depict fitted regressions to adjusted Q_{10} of LT_{50} for each population, which are represented by colour. This adjustment reduced the influence of regression to the mean on negative correlations between baseline thermal tolerance and its plasticity using the method described by Kelly & Price, 2005 and Gunderson, 2023. Model fitting and the plasticity adjustment are described under Supplemental Material. BMR slope = −1.26; $R^2 = 0.29$. SC slope = −0.97; $R^2 = 0.21$. RMR slope = −0.96; $R^2 = 0.38$. PTD slope = −0.84; $R^2 = 0.15$.

3.2 | Fitness costs of thermal plasticity and their dependence on tolerance

Using fecundity as a fitness-correlating character, neutral selection acted on upper thermal tolerance under high developmental temperature (selection gradient=0.05±0.06). Positive directional selection acted on thermal tolerance under low developmental temperature (Figure 5a; selection gradient=0.51±0.07). Differences in selection on thermal tolerance between treatments was evidenced by a significant interaction between temperature and LT_{50} in the selection gradient model. (95% PI=−0.215 to −0.04). Plasticity in thermal tolerance between treatments was under significant negative selection

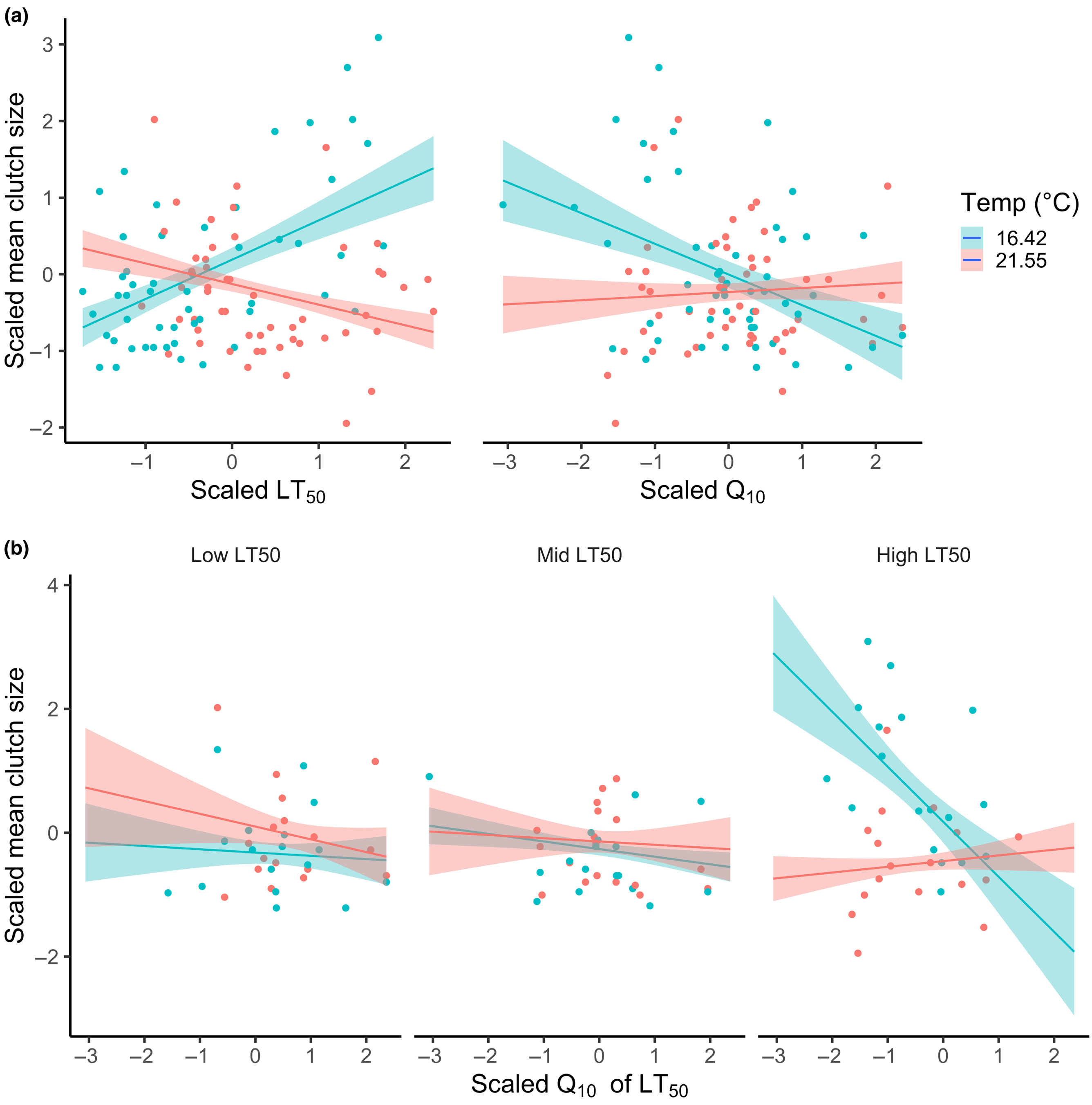

FIGURE 5 Selection gradients acting on upper thermal tolerance and its plasticity. Each point represents a sibship within a given developmental temperature. (a) On the left, mean-standardized average clutch sizes per sibship under low (blue) and high (red) developmental temperatures are plotted across mean-standardized upper thermal tolerance (LT_{50}). On the right, mean-standardized average clutch sizes per sibship × temperature culture are plotted across mean-standardized plasticity of upper thermal tolerance (Q_{10} of LT_{50}). (b) A fitness trade-off between baseline thermal tolerance (the intercept of an LT_{50} reaction norm) and the plasticity of thermal tolerance is visualized by plotting mean-standardized clutch sizes across the plasticity of thermal tolerance (Q_{10} of LT_{50}) grouped by terciles of baseline thermal tolerance (low – high LT_{50}).

regardless of temperature as shown in Figure 5a (95% PI = −2.23 to −0.14; selection gradient = −0.19 ± 0.05). Temperature and plasticity did not yield a significant interaction, but a trend of stronger negative selection under low temperature (selection gradient = −0.40 ± 0.08) relative to high temperature (selection gradient = −0.05 ± 0.06) was apparent (95% PI = −1.34e^{-3} to 0.11). Positive selection on tolerance and negative selection on plasticity under low temperature were consistent with canalization, where decreased plasticity reduces variance around a phenotypic optimum (Van Buskirk & Steiner, 2009). Season exhibited an insignificant effect on fecundity (95% PI = −0.41 to 0.83).

The costs of plasticity in thermal tolerance were significantly greater among more thermally tolerant genotypes while plasticity bore neutral fecundity effects among the least-tolerant genotypes. This trade-off between basal tolerance and plasticity was strongest under low temperature as evidenced by a significant two-way interaction between baseline tolerance and plasticity (95% PI = −2.83 to −0.80) and a three-way interaction between temperature, baseline tolerance, and plasticity (Figure 5b; 95% PI = 0.14–3.95). Selection gradients acting on thermal plasticity in the lowest and highest terciles of baseline thermal tolerance equaled −0.053 ± 0.084. and −0.888 ± 0.163, respectively, under low temperature. These binned selection coefficients were calculated as the slopes of the selection gradient models' fitted fecundity values regressed against observed values in each tercile of baseline tolerance. The dependence of thermal plasticity's costs on baseline tolerance was consistent with the fitness trade-off hypothesis (Figure 5b).

Female body length significantly affected sibships' mean egg clutch size per brood (95% PI = 5.89–0.36). Including this parameter in the selection gradient model controlled for its effect on fecundity, permitting robust estimation of the fitness-correlating costs of LT_{50} and its plasticity. Temperature was not a singular fixed effect in the top performing model. However, high temperature treatment reduced egg clutch sizes by an average of 15.4% or 3.25 eggs (Figure 5). Female body length had a positive but weak effect on fecundity (Figure S5). Summaries of model fitting, probability of direction tests, and model quality checks are available in Figures S6–S13 and Tables S1 and S2.

3.3 | Quantitative support for fitness trade off and competing hypotheses

A ‘limit hypothesis’ model incorporating parameters for a negative and convex nonlinear effect of baseline thermal tolerance on thermal plasticity possessed a marginal likelihood 1.60× greater than a null model assuming neither hypothesis. A ‘tradeoff hypothesis’ model including parameters predicting fitness-correlating costs of plasticity conditional upon basal tolerance had a relative likelihood of 3.96×. The ‘limit + tradeoff hypotheses’ model outperformed iterations incorporating singular hypotheses and achieved a relative likelihood of 6.02×. Thus, our observations of thermal plasticity and fecundity were best explained by models jointly accounting for

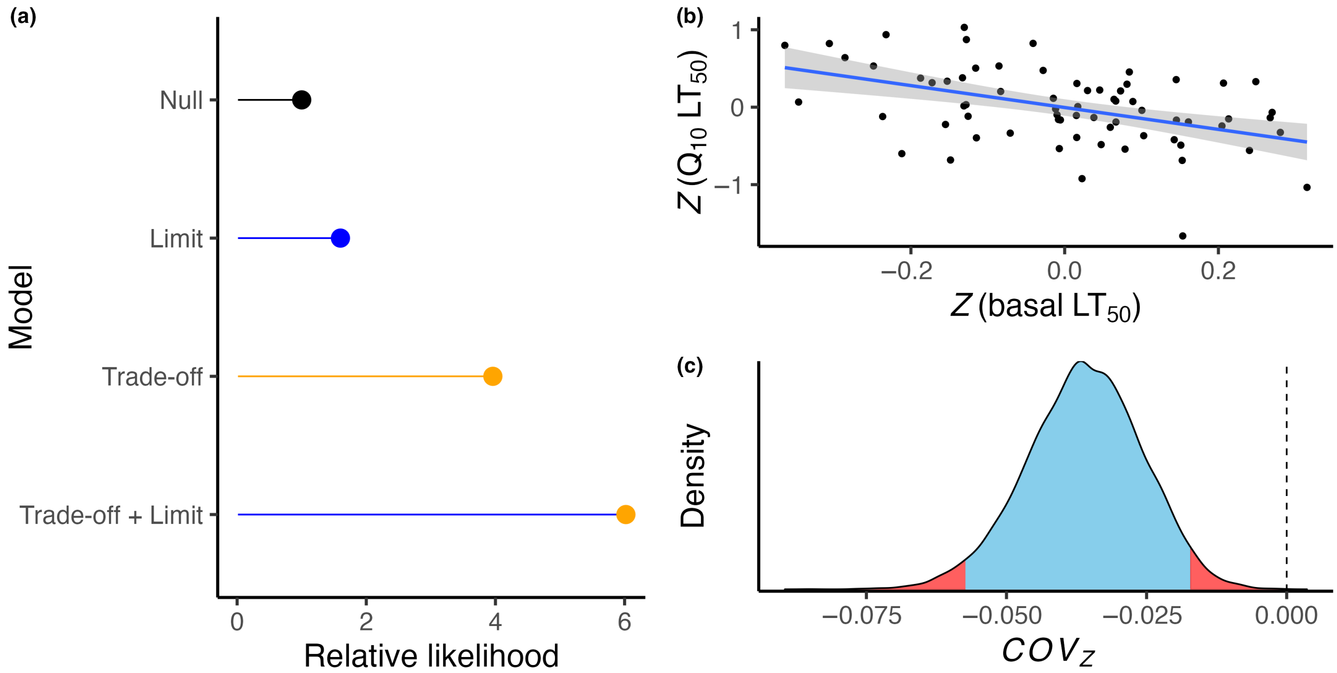

FIGURE 6 Evidence for alternative hypotheses. (a) The marginal likelihoods of three models containing parameters associated with the fitness trade-off (orange) and/or the physiological limit hypothesis (blue) are plotted relative to a null model lacking these parameters. Marginal likelihoods relative to the null model were computed using the Bayes factor method. (b) Correlation in random intercepts associated with nested random effects (Z) of family and population on basal LT_{50} and the plasticity (Q_{10}) of LT_{50}. (c) Covariance (COV) in family- and population-level effects (Z) on basal LT_{50} and LT_{50} plasticity is visualizes as a distribution of bootstrapped parameter estimates (blue = 95% distribution interval; red = 2.5% and 97.5% bounds).

both limits and trade-offs (Figure 6a). Significant negative covariance in family- and population-level effects on basal tolerance and thermal plasticity was detected (bootstrapped 95% CI < 0), which equaled −0.1213 ± 0.1034 SE (Figure 6b,c). The marginal likelihoods of models fitting parameters for limit, trade-off, and covariance in family-level effects or trade-off and family covariance could not be measured due to overfitting and poor convergence. Thus, there is some likelihood that genetic correlation shaped the negative relationship between basal tolerance and plasticity.

4 | DISCUSSION

Whether and how thermal plasticity evolves via natural selection has remained an open question since first being discussed by Feder et al. (1987) and Huey and Kingsolver (1989). Addressing this issue stands as a challenge in studies of adaptation to climate change and novel thermal environments (Arnold et al., 2019; van Heerwaarden & Kellermann, 2020). We provide the first empirical support, to our knowledge, for the fitness trade-off hypothesis in shaping negative associations between ectotherms' upper thermal tolerance and its plasticity. We find that costs of thermal plasticity for a fitness-correlating trait are greater among thermally tolerant genotypes. Here we discuss our results as they relate to (i) the evolution of phenotypic plasticity at large, (ii) theory and prior empirical research regarding the co-evolution of thermal tolerance and its plasticity, and (iii) forecasts of adaptation by ectotherms to warmer, more variable climates. Discussion of plasticity in body size and generation time can be found under Supplemental Material.

4.1 | Evolution and costs of phenotypic plasticity

Measuring and detecting fitness costs or benefits to phenotypic plasticity has proven challenging (Hendry, 2016; Van Buskirk & Steiner, 2009), and the plasticity of thermal performance is no exception (Arnold et al., 2019). While plasticity should theoretically incur fitness effects, selection gradients acting on plasticity are frequently neutral (Van Buskirk & Steiner, 2009). It has been suggested that one source of plasticity's seemingly neutral fitness effects is that they are conditional on traits and/or environmental variables untested in selection gradient studies (Hendry, 2016; Van Buskirk & Steiner, 2009). In fact, it was suggested as early as 1992 that dependency of plasticity's costs on other traits may contribute to the difficulty of their detection (Newman, 1992). With respect to the plasticity of thermal performance, these conditional variables may include (i) how predictable and variable temperature is during an experiment, such that moderate variation may dampen directional selection (Bitter et al., 2021), and (ii) intraspecific variation in fitness consequences attributed to sex, morphotypes or other phenotypically distinct groups (Hangartner et al., 2022; Svensson et al., 2020).

The dependence of plasticity's costs on environmental context has been more frequently studied than interactive costs between plasticity and additional traits. However, such interactions can bear critical impacts on how plasticity both evolves and affects evolution. For example, reproductive benefits of plasticity in flowering time in *Arabidopsis* depends on telomere length, a trait known to impact environmental stress response in this system (Campitelli et al., 2022). In maize, selection against maladaptive plasticity improved fitness and multiple performance traits (Choquette et al., 2023). In other plants, plasticity has exhibited fitness trade-offs with phenotypic properties such as trait means and developmental stability. Fewer studies exploring fitness trade-offs involving phenotypic plasticity have been explored in animals. We show in an invertebrate ectotherm that a trade-off exists between the plasticity of a trait and its phenotypic intercept.

Fitness trade-offs between a trait's phenotypic plasticity and intercept not only influence the evolution of plasticity, but may effect evolution itself via plasticity-first evolution or plastic buffering. Plasticity first evolution states that plasticity exposes an alternative phenotype to selection after which continuous selection on the alternative state drives its canalization (Levis & Pfennig, 2016). Buffering suggests that plasticity reduces genetic adaptation by improving the survival of genotypes that, without plasticity, would be exposed to selection (Price et al., 2003). If a fitness trade-off exists between a trait's plasticity and intercept, this relationship should facilitate faster canalization and drive plasticity-first evolution. Plastic buffering is less likely to occur under a fitness trade-off. Under a fitness trade-off, an interactive cost of plasticity and a trait intercept requires that the two traits bear fitness effects. Under plastic buffering, selection for plasticity can reduce absolute selection on the trait intercept to 0.

Our study evaluated fitness tradeoffs between baseline thermal tolerance and its plasticity. It was not designed to quantify such tradeoffs for body size or generation time, which also exhibited thermal plasticity. Interpopulation variation in the thermal plasticity of body size and generation time has been observed previously in *T. californicus* and other copepods (Hong & Shurin, 2015; Sasaki & Dam, 2020; Scheffler et al., 2019). However, Hong & Shurin, 2015 observed that thermal plasticity in developmental rate increased in southern populations of *T. californicus* while we observed no latitudinal effect. Also inconsistent with our findings are the results of Scheffler et al., 2019 who found that interpopulation variation in thermal plasticity of *T. californicus* body size did not scale with latitude.

4.2 | Fitness trade-offs shaping thermal plasticity

Fitness trade-offs between thermal tolerance and its plasticity have become increasingly more discussed in global change biology and evolution, but experiments directly measuring interactive fitness effects of each trait have not been reported (van Heerwaarden & Kellermann, 2020) and would be limited by traditional, univariate selection gradient models (Lande & Arnold, 1983). Experimental evolution studies exposing lines to positive selection for upper thermal tolerance have subsequently observed decreases in the plasticity of thermal tolerance (Kelly et al., 2011, 2017; Morgan et al., 2022; Sasaki & Dam, 2021). However, it cannot be determined from these studies whether fitness trade-offs or physiological limits caused reductions to plasticity without measuring (i) the fitness costs of plasticity and tolerance and (ii) the direct effect of tolerance on plasticity, controlling for the traits' fitness effects. Furthermore, studies measuring thermal plasticity's fitness costs have largely detected neutral effects (Arnold et al., 2019). In instances where significant costs or benefits were detected, traditional selection gradient models prohibited the fitting of interactive costs between thermal tolerance and its plasticity because their non-independence must be statistically controlled (Kline, 2015). Our expansion of the Lande & Arnold regression enabled us to determine, for the first time, whether significant costs of plasticity in thermal tolerance changed as baseline tolerance increased. This modification of the Lande & Arnold regression can be applied to other selection gradient studies to begin evaluating the prevalence of fitness trade-offs between thermal tolerance and its plasticity.

Thermally tolerant genotypes of *T. californicus* in the top tercile of baseline LT_{50} exhibited 2.29× greater negative directional selection on plasticity relative to the lowest tercile of baseline tolerance (Figure 5b). This difference in gradient exceeded a threshold of 0.2 representing significant variation in selection gradients (Scheiner & Berrigan, 1998; Van Buskirk & Steiner, 2009). Among studies on nine species including plants, birds and insects, directional or stabilizing selection on thermal plasticity was significant in four out of 14 reported traits and also greater than a gradient of 0.2 in 2 out of 14 traits (Arnold et al., 2019; Choi et al., 2019; De Lisle et al., 2022; Svensson et al., 2020; Valdés et al., 2019). Not only was the interactive effect of thermal plasticity and baseline tolerance on fecundity significant in our study, but it drove large increases in fitness costs relative to reported effects of thermal plasticity on fitness corollaries in other systems. It is important to underscore that fecundity is one component of fitness, which is a multivariate function of adult and offspring survival, egg viability and offspring reproduction.

Fitness trade-offs between thermal tolerance and its plasticity may arise via two mechanisms termed 'resource acquisition allocation' (or 'resource allocation') and 'genetic trade-off'. Under the resource allocation model, energy is limiting and divided between biological processes such that investment in thermal tolerance and plasticity come at the cost of investment in fitness-correlated traits like reproduction (Roff & Fairbairn, 2007; van Noordwijk & de Jong, 1986). Under a genetic trade-off, high thermal tolerance and high plasticity are underpinned by alleles with negative epistatic effects on fitness. These epistatic effects can result in negative genetic correlation that manifests as (i) antagonistic pleiotropy where an allele increasing one phenotype has a decreasing effect on the other or (ii) linkage disequilibrium where distinct alleles have independent effects on tolerance and plasticity but are frequently co-inherited (Williams, 1957). Indeed, it has been long established that thermal tolerance and thermal plasticity/acclimation capacity can share negative genetic correlations (Debes et al., 2021; Ushakov, 1977). While we did not explicitly quantify genetic correlations, our observed negative covariance between family and population-level effects on plasticity and tolerance support the potential for genetic trade-off. Our study's design was also unable to determine whether significant fitness trade-offs between thermal tolerance and its plasticity were attributed to resource allocation versus genetic trade-off. Pursuing this question and explicitly quantifying genetic correlations is essential for determining how the fitness trade-off hypothesis may shape the evolution of thermal physiology. This is largely because genetic trade-offs being less prohibitive to the effects of drift and selection on phenotypic variation (Roff & Fairbairn, 2007).

4.3 | Implications for adaptation to novel thermal environments

Under environments positively selecting for both upper thermal tolerance and thermal plasticity, adaptation may be constrained by physiological limits to tolerance (Kempes et al., 2019) and slowed by fitness trade-offs between the two traits as visualized in Figure 1c (Agrawal & Stinchcombe, 2008; Stearns, 1989). We found evidence that both limits and trade-offs may drive a negative association between thermal tolerance and its plasticity in *T. californicus*. The most likely model of thermal plasticity and fecundity incorporated parameters associated with both hypotheses rather than one or none (Figure 6a). Under Supplemental Material we discuss the potential confounding effects of non-linear reaction norms on the negative correlation between thermal tolerance and its plasticity.

Numerous ectotherms exhibit negative associations between thermal tolerance and its plasticity that are threatened by climate change and/or are commercially significant. The domestication of Atlantic salmon (*Salmo salar*), a fisheries species threatened by warming (Thorstad et al., 2021), has reduced CT_{max} and increased thermal plasticity (Debes et al., 2021). Similarly, Olympia oysters (*Ostrea lurida*) experiencing local extinctions attributed to heatwaves and other factors (Raymond et al., 2022; Zu Ermgassen et al., 2013) exhibit reduced thermal plasticity in thermally tolerant populations (Bible et al., 2020). If fitness trade-offs between thermal tolerance and plasticity are prevalent across other ectotherms, the breeding of 'heat hardened' aquaculture stocks and assisted evolution efforts for species at risk under climate change must balance selection for basal thermal tolerance and plasticity (Gibbin et al., 2017).

Negative correlations between ectotherms' upper thermal tolerance and thermal plasticity, though often observed (Barley et al., 2021; van Heerwaarden & Kellermann, 2020), have received little mechanistic investigation. Due to a lack of statistically robust methods for accounting for the effect of regression-to-the-mean on this negative correlation across ectotherms (Gunderson, 2023; Gunderson & Revell, 2022), the significance of this phenomenon during thermal adaptation has remained an open question. The presence of a fitness trade-off between thermal tolerance and its plasticity and their possible negative genetic correlation in *T. californicus* signifies that these two traits are unlikely to evolve independently of one another. Rather than simultaneous selection on thermal tolerance and its plasticity driving optimal changes in both traits, thermal tolerance and plasticity may evolve in a three-legged race by which adaptive change in one trait comes at the cost of the other.

AUTHOR CONTRIBUTIONS

SNB and GEH conceived of the scope and aim of the study. SNB and MJM developed field-based and experimental protocols. SNB and OIP conducted collections, executed culturing experiments, and performed phenotypic assays. SNB analysed all data and wrote the manuscript. All authors have edited and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All in situ temperature times series and phenotypic data reported in this paper and code used for data processing, statistical analyses, and plotting can be accessed on GitHub under the repository https://github.com/snbogan/Plasticity_Selection archived on Zenodo under https://doi.org/10.5281/zenodo.10939101 (Bogan, 2024).

STATEMENT OF INCLUSION

Our study was conducted on the Pacific coast of the United States, and our field sites were a combination of nature reserves associated with the University of California and sites within a California State Park. We communicated our results and research outcomes with the representatives and stakeholders associated with the reserves and the state park. In addition, through inclusion of undergraduates in the project and engagement with the public while at the field sites, we were able to disseminate the study to a broader community accessing the public sites. All authors reside and work in the country where research activities took place.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Mean density of Tigriopus californicus per culture.

Figure S2. Mean culture ages at time of LT_{50} assay.

Figure S3. Monthly latitudinal temperature gradients measured in situ.

Figure S4. Thermal plasticity of generation time and body size.

Figure S5. Weak but positive effect of mean female body size on fecundity per brood.

Figure S6. Posterior predictive check for model of baseline LT_{50}'s effect on the plasticity of LT_{50}.

Figure S7. Outlier prediction (threshold = 0.5) for model of baseline LT_{50}'s effect on the plasticity of LT_{50}.

Figure S8. Posterior probabilities and associated MCMC chains for model of baseline LT_{50}'s effect on the plasticity of LT_{50}.

Figure S9. Posterior predictive check of mean fecundity per brood in multivariate selection gradient model.

Figure S10. Posterior predictive check of the plasticity of LT_{50} in multivariate selection gradient model.

Figure S11. Prediction of high leverage observations (threshold = 0.5) for multivariate selection gradient model.

Figure S12. Posterior probabilities and associated MCMC chains for multivariate selection gradient model.

Figure S13. Selection of multivariate selection gradient models with and without parameters associated with mean culture age, culture density, and mean gravid female length.

Table S1. Probability of direction test: adjusted plasticity of LT_{50} ~ baseline LT_{50}.

Table S2. Probability of direction test: multivariate selection gradient model.

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