**Temperature variability does not influence phenotypic plasticity in ectotherms – a meta-analysis**

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**Abstract**

Phenotypic plasticity can allow individuals to compensate for potentially negative changes in their thermal environment. It is important, therefore, to understand the impacts of changes in both mean and fluctuations in temperature on plastic responses. Our aim was to establish the current state-of-knowledge regarding the influence of thermal variability on the capacity for phenotypic plasticity in ectothermic vertebrates and invertebrates. We conducted a quantitative synthesis of 44 studies (212 effect sizes across 40 species) to compare the effects of constant and fluctuating temperatures with the same mean on plasticity in biological responses, across different ecosystems (terrestrial and aquatic) and type of phenotypic plasticity (acclimation and developmental plasticity). We found that most studies implemented diel temperature fluctuations, and that phenotypic plasticity does not differ between constant and fluctuating thermal environments. We conclude that plasticity and its attendant compensation for thermal variability is driven by changes in longer-term mean temperatures.

**Introduction**

Climate change is increasing both mean temperatures and the amplitudes of thermal fluctuations (IPCC 2023). Ectotherms are particularly susceptible to thermal fluctuations as their body temperature is closely tied to environmental conditions (Angilletta 2009; Huey *et al.* 2012). The thermal sensitivity of phenotypic traits follows a non-linear thermal performance curve (TPC) as a function of body temperature (Huey and Kingsolver, 1989). TPCs vary between species and traits (Bozinovic *et al.* 2020), but generally incorporate three common features: an initial increase in performance as temperature increases from a minimum critical temperature; a thermal optimum where maximum performance is reached; and a rapid decline in performance to a critical thermal maximum (Huey & Kingsolver 1989). Thermal changes therefore can be both beneficial and detrimental to the performance of an organism depending on their location within the TPCs (Denny 2019; Marshall *et al.* 2021). However, ectotherms can exhibit plastic responses that adjust TPCs to allow relatively constant function despite climate variability (Kingsolver et al., 2015).

Phenotypic plasticity can allow individuals to at least partially compensate for changes in their environment (Schulte 2014). Developmental plasticity and acclimation are manifestations of within-individual phenotypic plasticity that can reduce variance in phenotypic trait values across environmental gradients (Beaman *et al.* 2016; Burggren 2020) . Developmental plasticity involves relatively persistent phenotypic changes in response to the thermal environment experienced during early development (Burggren 2018; Loughland *et al.* 2021). Acclimation is a reversible phenotypic shift induced by environmental changes lasting from days to weeks (Guderley 2004). The capacity for phenotypic plasticity has been tested mainly in response to changes in constant temperatures. However, in natural environments temperatures fluctuate, calling into question whether constant temperature experiments are representative of natural conditions (Marshall *et al.* 2021).

Fluctuating environmental conditions may dampen the capacity for phenotypic plasticity because they increase the variation in temperatures experienced by organisms and may thereby dilute longer-term signals such as temperature trends during development and seasonal changes. Unpredictable thermal variability can also increase the probability of organisms experiencing damaging temperature extremes (Dowd & Denny 2020; Vázquez *et al.* 2017). In addition, phenotypic plasticity can come at the cost of a mismatch between phenotype and environment, and plastic responses to short-term variation (e.g., daily variation) could exacerbate that cost especially if the environment changes rapidly (Beaman *et al.* 2016; Gabriel 2006). Filtering out frequent short-term environmental signals may render plastic responses more efficient, and compensation for longer-term changes may be the principal benefit of plasticity (Leung *et al.* 2020). At present, empirical evidence is equivocal, leaving the relationship between temperature fluctuations and phenotypic plasticity unresolved (Jacob *et al.* 2024). To predict the impacts of climate change, it is important therefore to understand whether short-term fluctuations around mean temperatures influence the capacity for phenotypic plasticity compared to constant mean temperatures (Dowd *et al.* 2015).

Here, our aim was to conduct a quantitative synthesis that establishes the current state-of-knowledge regarding the influence of thermal variability on the capacity for phenotypic plasticity in ectotherms. Specifically, we conducted a meta-analysis that tested whether the capacity for phenotypic plasticity differed in constant and fluctuating thermal environments with the same mean temperature. The potential impacts of constant and fluctuating thermal environments were also investigated in subset analyses of (i) ecological levels (individual and population), (ii) ecosystems (aquatic and terrestrial organisms), and (iii) the form of phenotypic plasticity (acclimation or developmental plasticity).

**Materials and Methods**

We followed, as closely as possible, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.* 2015) guidelines, modified for Ecology and Evolution (PRISMA-Eco Evo) (O’Dea *et al.* 2021) (Supplementary Materials Fig. S1). Question formulation, literature searching and screening were performed according to (Foo *et al.* 2021).

*Systematic Literature Search and Screening*

We adhered to the PECO (Population, Exposure, Comparator, Outcome) framework (Morgan *et al.* 2018) in the development of focus questions and the literature search and screening processes (Supplementary Materials Table S1). The systematic literature search was conducted on the 5th of April 2022 in Scopus, Web of Science and ScienceDirect databases. Search terms included synonyms for ‘plasticity’, ‘acclimation’ and ‘developmental effects’ to identify studies that conducted treatments during the lifespan of an individual (Noble *et al.* 2018). To limit the search string to temperature treatments ‘thermal’ and ‘temperature’ were added as search terms (Noble *et al.* 2018) . Synonyms for ‘fluctuating’ or ‘varying’ were added to explicitly look for studies with a fluctuating temperature treatment. The exact search strings used in each database are given in Supplementary Materials Search Strings.

These searches returned a total of 13,549 unique studies (Supplementary Materials Fig. S1). Of these, we identified a total of 57 studies that were written in English and experimentally compared plastic responses in both constant and fluctuating temperature treatments in ectothermic organisms under controlled laboratory conditions. In addition, we used Scopus, Web of Science, ScienceDirect and Google Scholar to conduct a forward (papers citing the original study) and backward (papers that were cited in the original study) search on the 19th of October 2022, and identified an additional 87 studies. One species was removed from the compiled data set due to an unresolved phylogeny in the Open Tree of Life database (Michonneau *et al.* 2016). We conducted an abstract and full-text screening (in Rayyan Software) (Ouzzani *et al.* 2016) of all identified papers following our exclusion and inclusion criteria (Supplementary Materials Fig. S2). Following this search, 44 studies were included in the final analysis (Supplementary Materials Studies included in the analysis).

*Data Extraction*

All studies included in the analysis had at least four chronic (days to weeks) treatments: two constant temperature treatments at a "high" and "low" temperature, and two fluctuating temperature treatments with mean temperatures corresponding to those of the constant treatments (Fig. 1). The acute test temperatures at which phenotypic traits were measured had to coincide with the mean temperatures of the corresponding chronic thermal treatments (Seebacher *et al.* 2015). If there were more than two treatment temperatures, we selected the two experimental temperatures that coincided best with the natural temperature range of each study species (Seebacher *et al.* 2015). In experiments that had multiple fluctuating treatments with different amplitudes or types of fluctuations (e.g., sinusoidal, stepwise, alternating, or stochastic), we compared each to the corresponding constant treatment to calculate separate effect size. We extracted data (sample sizes, means, and errors) from these treatments from texts, tables, supplementary materials, and figures (using R package *metaDigitise* to extract data from figures)(Pick *et al.* 2019). We used only biological replicates as the sample size.

In addition, we recorded associated data which may influence phenotypic plasticity and used these in subset analyses and as moderators in meta-regression analyses. Hence, we recorded taxonomic information, preferred ecosystems, and life-history stages. We also recorded descriptors of the experimental fluctuations (mean temperature and the fluctuations amplitude, type, and period) that could influence phenotypic trait values (Raynal *et al.* 2022; Stocker *et al.* 2024). Phenotypic responses were sorted into trait category and specific phenotypic trait measurement, and we noted details of the experimental design including the type and duration of treatment exposure. Investigators involved in data extraction were not permitted to screen or extract data from studies they had a previous association with (Macartney *et al.* 2022).

*Data Processing and Transformations*

Standard errors (SE) and 95% confidence internals (CI) were converted to standard deviations (SD), which were used for effect size calculations (Quinn & Keough 2002). Bounded data such as proportions (e.g., survival) were transformed before calculating effect sizes to obtain means and variances that were more likely to satisfy normality assumptions (Macartney *et al.* 2022):

|  |  |
| --- | --- |
|  | (1) |

|  |  |
| --- | --- |
|  | (2) |

where and are the transformed means and SD, respectively, and are the bounded (proportional) means and SD. We transformed means and variances that were reported as the natural logarithm using Equation 3 and 4 (Higgins *et al.* 2008) :

|  |  |
| --- | --- |
|  | (3) |

|  |  |
| --- | --- |
|  | (4) |

where and are the transformed means and SD, respectively, and are the means and SD on the natural log scale. A constant of 0.5 was added to all means and SD to avoid taking the natural log of zero during effect size calculations.

Taxonomic information was retrieved from the Open Tree of Life (Michonneau *et al.* 2016). Amphibians were considered aquatic organisms for the analysis.

*Effect Size Calculations*

We developed and used a standardised interaction-based plasticity response ratio difference (PRRDS; Equation 7) and its corresponding sampling variance (Equation 8) as our effect. The PRRDS is derived by first calculating log response ratios (lnRR) within treatments (Noble *et al.* 2022) to estimate the change in phenotypic traits following either acclimation or developmental exposure to different temperatures. lnRR indicates the capacity for phenotypic plasticity. The PRRDS captures the difference in phenotypic plasticity between (constant and fluctuating) treatments by subtracting the lnRR of the constant from the fluctuating thermal environment. To control for the fact that temperature gradients differed between studies, the PRRDS standardises the differences in lnRR to a 1oC change in treatment temperature (Noble *et al.* 2022; Pottier *et al.* 2021). PRRDS = 0 indicates that there is no difference in the capacity for phenotypic plasticity between constant and fluctuating thermal environments (PRRDS > 0, phenotypic plasticity increases in fluctuating temperatures; PRRDS < 0, phenotypic plasticity increases in constant temperatures).

|  |  |
| --- | --- |
|  | (7) |

|  |  |
| --- | --- |
|  | (8) |

where is the mean response, T is temperature, subscripted F and C denote the fluctuating and constant thermal treatments, respectively, and subscripted H and L denote the high and low temperatures, respectively. Note that Equation 8 assumes that constant and fluctuating treatments are independent.

*Statistical Analysis*

We used R.4.2.2 in RStudio version 2022.12.0 for all calculations, analyses, and figures (using R Package *ggplot2* for figures) (Wickham 2011) . All data and code are available at https://github.com/daniel1noble/plasticity\_fluc\_meta (metadata given in Supplementary Materials Table S2). Data are presented as the mean PRRDS ± 95% CIs. For ease of comparison, the mean PRRDs are transformed to give the percentage change of phenotypic plasticity from constant to fluctuating thermal environments (Pustejovsky 2018). We visualised mean and raw effects using orchard and bubble plots (R package *orchard* version. 2.0) (Nakagawa *et al.* 2023), which also provide 95% prediction intervals. Prediction intervals provide important information about effect heterogeneity (Noble *et al.* 2022).

*Meta-analyses and non-independence*

Multi-level meta-analyses (MLMA) were fitted with frequentist models using restricted maximum likelihood (REML) and an adjusted convergence threshold (1e-8). Inferential tests were done using a t-distribution (Nakagawa *et al.* 2022b). The model was fitted with a modified sampling covariance matrix to account for multiple effect sizes using the same control treatment (Noble *et al.* 2022). We assumed that effect sizes sharing a common control were correlated by r = 0.5, and the shared sampling covariance was calculated using the sampling variances for each effect (Noble *et al.* 2017).

Dependencies in our data set were accounted for by including several random effects in the model (Nakagawa *et al.* 2017). A Study ID random effect was included to account for multiple effect sizes from the same study. A Shared Animal ID random effect was included to account for the same set of animals used for several measured responses. To control for phylogenetic relatedness, a phylogenetic tree was created using the Open Tree of Life database and converted into a correlation matrix that was included in the model when estimating phylogenetic variance. Polytomies were randomly resolved (using R Package *Ape*) (Paradis & Schliep 2018) and branch lengths were calculated with Grafen’s method (Power = 1) (Grafen 1989). A species-level random effect was included to account for the repeated use of the same species across effect sizes (Cinar *et al.* 2022). To reduce the likelihood of specific traits dominating the analysis, a trait-level random effect was added to the model (except in meta-regressions that analysed specific phenotypic traits as the moderator). Lastly, an observational-level random effect was added to estimate the final ‘residual’ / ‘within-study’ variation (Nakagawa & Santos 2012).

*Multi-level Meta-analytic Models*

MLMA models (using the function *rma.mv* in the R Package *metafor*) (Viechtbauer 2010) were implemented to estimate the overall meta-analytic mean on data subsets (population- and individual-level traits) (Figure 2). We took a holistic approach to calculating and presenting heterogeneity statistics following recommendations by Yefeng *et al*. 2023. In addition to prediction intervals, we report relative heterogeneity (I2 , i.e., *I2Total*) as well as two magnitude measures of heterogeneity (i.e., CV and M, following Yefeng *et al.* 2023). CVTotal expresses the magnitude of heterogeneity relative to the overall meta-analytic mean. While CVTotal is a useful metric, its interpretation can become problematic when the meta-analytic mean approaches zero. A more robust measure of the magnitude of heterogeneity is therefore to use MTotal (Yefeng *et al.* 2023). We also decompose these total measures of heterogeneity into their various partitions/strata corresponding to each of the random effects (e.g., *I2Animal*, *I2Obs*, *I2Phylo*, *I2Species*, *I2Study*, and *I2Trait*,) (Yefeng *et al.* 2023). Heterogeneity statistics were calculated from our overall MLMA model.

*Meta-regression Models*

We conducted separate meta-regression models to compare moderators that had the potential to explain effect size variation. Categorical moderators included type of plasticity (acclimation and developmental plasticity), fluctuation type (sinusoidal, alternating, stepwise, and stochastic), phenotypic trait categories (behavioural, biochemical assay, gene expression, life-history, morphological, and physiological), the specific phenotypic trait, and broad taxonomic group (invertebrate vs vertebrate) (see Supplementary Materials Fig. S3 for phylogenetic tree). Categorical moderators and their levels were only included in analyses if the number of effect sizes were > 10 (O’Dea *et al.* 2021). We analysed the amplitude of fluctuations, and the number of fluctuations (treatment exposure duration/period of fluctuations; range = 4 – 546 fluctuations) as continuous moderators.

*Publication Bias and Sensitivity Analysis*

Publication bias was first investigated by visually examining funnel plots for asymmetry (Sterne *et al.* 2005). Secondly, our overall MLMA model was then altered to include z-transformed publication year and precision (inverse of sampling variance) as moderators to assess the presence of a time-lag effect (or decline effect) (Nakagawa *et al.* 2022a). There was no evidence of small-study effects (i.e., no relationship with precision) in our overall data set, however, mean PRRDs, unsurprisingly, did show evidence of time-lag bias where average effects have decreased towards zero through time (Supplementary Materials Publication bias and Sensitivity analysis) coinciding with our overall meta-analytic means for most analyses.

A sample of effect sizes showed higher than expected precision. We carefully checked these extreme values to ensure that data was correctly extracted. To understand how ‘robust’ our overall MLMA model was to influential data points, we also conducted a Cook’s distance sensitivity analysis (using the function *cooks.distance* in the R Package *metafor*). Results suggest that our conclusions are robust and free from significant influential outliers (Supplementary materials Fig. S4). We also confirmed that our results were consistent with robust variance estimators (Nakagawa *et al.* 2022b), which better account for any sources of non-independence within studies that our models may not have accounted for well enough. All results were also largely consistent between our main analyses and those using robust variance estimators.

**Results**

The final data set included 212 effect sizes from 40 species derived from 44 studies. The dataset was dominated by invertebrates (n = 32 species) but also included vertebrate ectotherms (n = 8 species). Overall, 98% of the studies used diurnal temperature fluctuations (24 h cycles).

In the overall MLMA analysis (effect size estimate = 0.0019; 95% CIs = [-0.009, 0.0128]; p = 0.73; n = 212, Figure 2a) and individual trait subset analysis (Figure 2b), the mean plastic responses were not significantly different between constant and fluctuating thermal environments (Figure 2). Total heterogeneity was high overall (*I2Total* rounded to 99.35%, CVTotal = 18.56 and MTotal = 0.95), with substantial between study (*I2study* =38%, CVstudy = 11.49, Mstudy = 0.59) and within-study heterogeneity (*I2obs* = 52%, *CVobs* = 13.37, *Mobs* = 0.68). Little between species heterogeneity was identified (see full heterogeneity statistics in Supplementary Materials Table S13).

We explored a priori factors which we expected would explain the high heterogeneity. There were no significant differences in phenotypic plasticity between fluctuating and constant thermal environments across broad trait categories (Figure 3a), but specific trait categories where enough data existed did explain a substantial amount of variation (R2 = 0.30) (Figure 3b). More specifically, plasticity in development time increased in fluctuating environments. In contrast, plasticity in body mass was greater in constant environments (Figure 3b, Supplement Table S7). In each case, however, effect size magnitude was still small despite being significant (0.97-1.57% change) and the upper and lower bound of the 95% prediction intervals still predict small effects (~9 – 10%). Constant and fluctuating temperatures did not influence phenotypic plasticity differently between aquatic or terrestrial species or vertebrates and invertebrates (Figure 4). Neither the fluctuation type (Figure 5), plasticity type (Figure 6) or the amplitude of fluctuating temperature regimes (Figure 7) explained differences in phenotypic plasticity between fluctuating and constant temperature conditions (Supplementary Materials Tables S3-S12).

**Discussion**

Our meta-analysis shows that experiencing constant or fluctuating environmental conditions does not generally impact the expression of phenotypic plasticity in ectothermic organisms. It is important to note, however, that there was substantial heterogeneity in effects overall. Our estimates of *I2Total*, *CVTotal* and *MTotal* all fall well above the 75th percentile of empirically derived heterogeneity estimates (Yang et al. 2023), with most variation being attributable to within and between study factors. However, our findings were remarkably consistent across species with low species-level heterogeneity in effects suggesting that the small overall effects we observed are likely generalisable across species when between and within study sources of variation have been controlled (Yang et al. 2023). Analyses of key moderators predicted to drive between and within study level variation (e.g., type of plasticity measured, fluctuation type and amplitude) mostly failed to explain variability aside from the specific trait being measured (marginal R2 = 30%). Importantly, while we saw differences in plasticity between constant and fluctuating temperatures for two specific trait categories, development time and body mass (in opposite directions), these average effects were still small overall. Trait variation likely reflects how thermal fluctuations differently impact traits with different thermal performance curves. For example, development time is known to exhibit a non-linear exponential decline with temperature. Developing under fluctuating or constant temperatures will therefore impact the magnitude of responses of plastic responses. Nonetheless, the findings of our study suggest that the ability to maintain relatively constant phenotypes across environmental gradients is not influenced by regular, short-term fluctuations around the temperature mean with the impacts of fluctuations on plasticity mostly depending on the type of trait measured.

Plasticity can be beneficial by allowing organisms to compensate for potentially negative environmental effects (Loughland *et al.* 2021; Schulte 2014). However, the remodelling of phenotypes can be detrimental if inducing environments do not match current environmental conditions (Beaman *et al.* 2016). A mismatch cost can arise from a lag in the time to complete the compensatory response relative to the frequency of the variation in temperature (Pfab *et al.* 2016). Phenotype-environment mismatches occur for some time after the environment changes because environments induce phenotypes so that there necessarily is a lag between environmental and phenotypic change (Gabriel 2005). The occurrence of such mismatches would increase if phenotypic plasticity was induced by short term temperature fluctuations that would cause increasing frequencies of lag periods (Ghalambor *et al.* 2007). Our analysis instead indicates that short-term variability in temperatures does not impact plastic responses, and we conclude that plastic responses are driven by longer-term mean temperatures.

An interesting question that remains unresolved, is what the characteristics of the inducing environment are that lead to plastic responses (O’Connor *et al.* 2019; Zimmer *et al.* 2022). Our analysis indicates that diel fluctuations do not induce plasticity. However, in the natural environment, animals experience thermal fluctuations around the mean at different spatial- and temporal scales (e.g., diurnal, seasonal, and annual) (Marshall *et al.* 2021). The ability to filter frequent, short-term thermal signals could be an advantageous mechanism that increases the efficacy of plastic responses. A ‘band-pass filter’ could explain this phenomenon, in which regular thermal noise around the mean is filtered out and does not contribute to the capacity for phenotypic plasticity (Lattin & Kelly 2020). The efficient compensation for the effects of long-term changes in mean temperature could be the primary benefit of phenotypic plasticity that increases the resilience and persistence of ectotherms to future climate changes (Leung *et al.* 2020).

Increases in the amplitude of thermal fluctuations can have detrimental impacts on phenotypic trait values by reaching extreme ranges that damage cellular function and structures (Raynal *et al.* 2022; Stocker *et al.* 2024; Stoks *et al.* 2017). Our meta-analysis indicates that increases in the magnitude of diel fluctuations do not impact plasticity and are therefore unlikely to affect the vulnerability of ectotherms. However, we do not know whether the fluctuation reported in the studies analysed here were actually damaging, and frequent damaging temperature spikes could alter the molecular mechanisms underlying plasticity (Murray *et al.* 2022). These dynamics are important in assessing the impact of climate change-induced increases in mean temperature and heat waves, but require more experimental studies. With that caveat in mind, our findings support the validity of experiments using different constant temperature treatments to assess thermal plasticity and suggest that findings from such experiments are transferable to environments with diel temperature variation. Our conclusions pertain to phenotypic plasticity within the lifetime of an organism (acclimation and developmental plasticity). In the literature there are few data showing the impact of fluctuations with longer periods, and it will be important to determine the periodicity at which fluctuations become sufficient to induce or impact plastic responses. We suggest that future research should explore the impacts of transgenerational thermal variability and temporal scales on the capacity for phenotypic plasticity.

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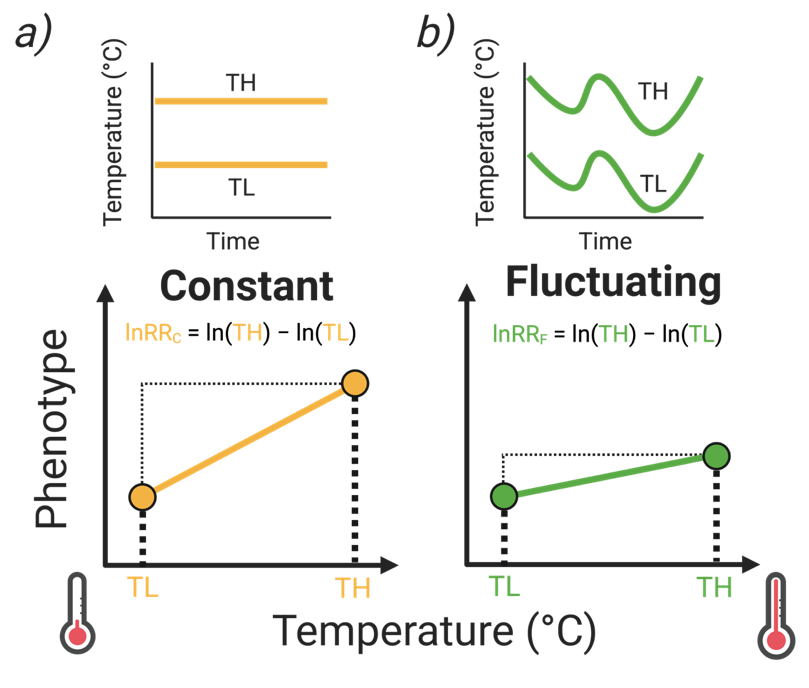
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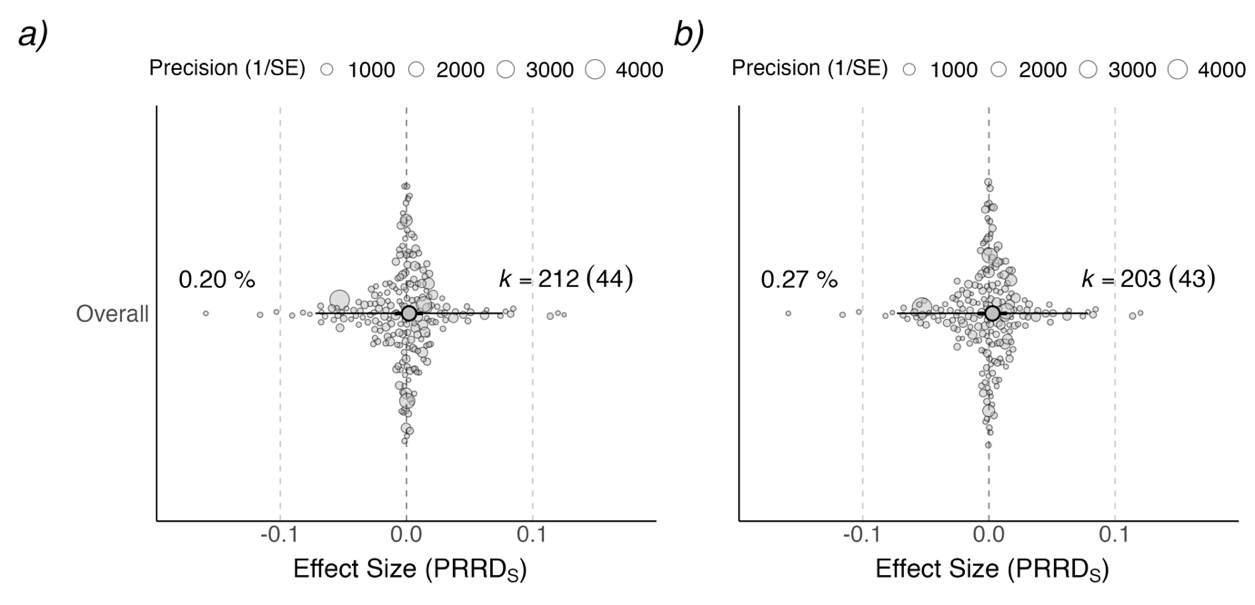
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**Figures**



**Fig. 1**

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**Fig. 2**

**A graph of different numbers and a number of numbers

AI-generated content may be incorrect.**

**Fig. 3**

**A diagram of a graph

AI-generated content may be incorrect.**

**Fig. 4**

**A diagram of different sizes and colors

AI-generated content may be incorrect.**

**Fig. 5**

A diagram of a diagram of a diagram

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**Fig. 6**

A graph of different sizes of blue dots

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**Fig. 7**

**Figure captions**

**Figure 1 Quantifying changes in plasticity resulting from acclimation to a constant or thermally fluctuating environment.** a) Measurement temperatures taken for two samples of organisms at low temperature (TL) and high temperature (TH) when each group is acclimated at a constant temperature. Calculating the plastic response ratio for the constant treatment (lnRRC) can be done by subtracting the natural logarithm of the sample means for the two measurement temperatures. b) Treatment groups where the temperatures fluctuated through the course of acclimation. The plastic response ratio for the fluctuating treatment (lnRRF)can also be computed by subtracting the natural logarithm of the sample means for the two measurement temperatures in the fluctuating treatment. From these effect sizes the PRRDS can be calculated according to equation 7 and 8.

**Figure 2 Multi-level meta-analytic results for the overall data set (a) and only studies measuring at the individual level (b).** There were no significant differences in phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Orchard plots depict mean effect size (plasticity response ratio difference; PRRDS) estimates ± 95% CIs (solid circles and horizontal bars, respectively) as well as distributions of individual effect sizes. Effect sizes are weighted (sized) by their precision which is inverse of their sampling error (1/SE). Percentage labels are the mean PRRDS estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of studies in brackets. x-axis limits are cropped for presentation.

**Figure 3 Meta-regression results for the overall data set with broad (a) and specific (b) phenotypic trait category as the moderator**. There were no significant differences in phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Presentation and abbreviations as in Figure 2.

**Figure 4 Meta-regression results for the overall data set across major habitat types (a) and broad taxonomic group (b) as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Presentation and abbreviations as in Figure 2.

**Figure 5 Meta-regression results for the overall data set with fluctuation type as the moderator.** There were no significant differences in phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Presentation and abbreviations as in Figure 2.

**Figure 6** **Meta-regression results for the individual subset data set with plasticity type as the moderator.** There were no significant differences in phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Presentation and abbreviations as in Figure 2.

**Figure 7 Relationship between effect size and the amplitude of fluctuation.** There were no significant relationships between the amplitudes of fluctuations and the difference in phenotypic plasticity between the constant and fluctuating temperature treatments. Dashed line = PRRDS estimate from the MLMA models. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis and Y-axis limits are cropped for presentation.