**Temperature variability has limited effects on phenotypic plasticity in ectotherms – a meta-analysis**

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**Abstract**

Phenotypic plasticity can allow individuals to compensate for potentially adverse effects 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 46 studies (241 effect sizes across 41 species) to compare the effects of constant and fluctuating temperatures with the same mean on plasticity in biological responses. We compared different traits across different ecosystems (terrestrial and aquatic) and types of phenotypic plasticity (acclimation and developmental plasticity). We found that 98% of studies implemented diel temperature fluctuations, and most data were derived from invertebrates. Our analysis shows that phenotypic plasticity does not differ between constant and fluctuating thermal environments except for weak effects on developmental time. We conclude that plasticity and its attendant compensation for thermal variability is more likely to be 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 fluctuations therefore can be both beneficial and detrimental to the performance of an organism depending on their location within the TPCs and the shape of the TPC (Denny 2019; Marshall *et al.* 2021). A previous meta-analysis has shown that exposure to diel temperature fluctuations in ectotherms has limited overall effects on the means and variances of trait values compared to constant temperature treatments with the same mean and for the same duration (Stocker *et al.* 2024). Increasing fluctuation amplitudes, however, appeared to be stressful and increased heat shock protein expression, reduced longevity, and had negative impacts on populations of aquatic animals. These effects were likely to be the result of reaching damaging high temperatures as amplitudes increased (Stocker *et al.* 2024). In the special case of egg incubation temperatures in reptiles, the effects of fluctuating temperatures depended on mean temperatures and the trait. Compared to constant temperatures, fluctuations increased hatching success and decreased incubation time at cooler mean temperatures, while fluctuations had a negative effect at warmer mean temperatures (Raynal *et al.* 2022). Negative effects of fluctuations at high mean temperatures were likely to be due to reaching damaging high temperatures.

Importantly, ectotherms can exhibit plastic responses that adjust TPCs to at least partially compensate for potentially damaging effects of temperature variation in their environment (Noble *et al.* 2025; Schulte 2014; Seebacher *et al.* 2015). For example, thermal acclimation shifted the temperature at which maximal heart rates occurred from 28oC in 5oC-acclimated fish (*Fundulus heteroclitus*) to 37oC in 33oC-acclimated fish; in the absence of warm acclimation, 5oC-acclimated fish would have suffered heart failure at 37oC (Safi *et al.* 2019).  Acclimation and developmental plasticity are manifestations of 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. The extent to which phenotypic plasticity is expressed has been tested mainly in response to changes in constant temperatures (Schulte *et al.* 2011). 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 plastic responses 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, thereby limiting the benefits of plasticity and reduce selection for plastic phenotypes (Dowd & Denny 2020; Jørgensen *et al.* 2021; Vázquez *et al.* 2017). Alternatively, plastic responses could shift TPCs so that fluctuations with increased amplitudes become less damaging, thereby promoting selection for plasticity (Scheuffele *et al.* 2021). In addition, in fluctuating environments phenotypic plasticity can come at the cost of a mismatch between phenotype and environment (Beaman *et al.* 2016) . Plastic responses to short-term variation (e.g., daily variation) could exacerbate that cost, especially if the environment changes rapidly and the lag time between phenotypic and environmental change increases (Beaman *et al.* 2016; Gabriel 2006; Jacob *et al.* 2024). 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). As we briefly summarised above, temperature fluctuations could promote or constrain plasticity but whether this occurs generally, and how it varies between traits, is unknown. It is important to understand whether and how short-term fluctuations around mean temperatures influence the expression of phenotypic plasticity to predict the impacts of climate change (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 expression of phenotypic plasticity in ectotherms. The analysis we present here follows from our earlier meta-analyses that considered the impacts of temperature fluctuations on mean trait values (Raynal *et al.* 2022; Stocker *et al.* 2024), and we here make the conceptual advance of investigating the impact of fluctuating temperatures on the *plasticity* of mean trait values. Specifically, we conducted a meta-analysis that tested whether the expression of phenotypic plasticity differed in constant and fluctuating thermal environments with the same mean temperature. Hence, we analysed data from studies that conducted fully factorial experiments with at least two developmental or acclimation temperature treatments crossed with constant and fluctuating conditions. We also analysed the potential impacts of constant and fluctuating thermal environments across different (i) ecological levels (individual and population), (ii) ecosystems (aquatic and terrestrial organisms), and (iii) forms 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 and Table 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 S2). The initial literature search was conducted on 5 April 2022 in Scopus, Web of Science (Core Collection including Current Contents, BIOSIS Previews, CAB Abstracts, Medline, Agricola and Pubmed) and ScienceDirect databases. We acknowledge that we were limited to including only publications in English, which may lead to bias (Konno *et al.* 2020). A section of the results from this search was published in (Stocker *et al.* 2024), although the data on plasticity used in the present analysis were not published in the earlier paper. Additionally, we updated the search in Scopus and Web of Science on 17 July 2025. 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. All authors (except DWAN) were involved in the search, screening, and data extraction process. We ensured consistency between authors by screening a common set of 100 papers and extracting data collectively from a subset of five papers.

The initial search (2022) returned a total of 13,549 studies after removing duplicates (Supplementary Materials Fig. S1). Of these, we identified a total of 57 studies that fulfilled our inclusion criteria (Supplementary Material Fig. S2). 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 19 October 2022, and identified an additional 87 studies. (Michonneau *et al.* 2016)We conducted 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 full-text screening, 43 studies were included in the final analysis (Supplementary Materials Studies included in the analysis). The updated search (2025) returned an additional 1475 studies after removing duplicates. Following title and abstract screening we identified three studies that met our inclusion criteria and from which we extracted data. Hence, the meta-analysis includes data from a total number of 46 studies, 41 species (20.9% vertebrates, 79.1 % invertebrates), and 241 effect sizes.

*Data Extraction*

All studies included in the analysis had at least four chronic (≥ days) (Seebacher *et al.* 2015) 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 to minimise analysis of data from potentially damaging temperature ranges when studies included exposures to relatively extreme temperatures (Seebacher *et al.* 2015; Wilson & Franklin 2002). 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* version 1.0.1 to extract data from figures)(Pick *et al.* 2019). We used only biological replicates as the sample size, rather than technical replicates from repeated measures using the same samples or animals if they were performed. In addition, we recorded taxonomic information, preferred ecosystems, and life-history stages to include as moderators in subset analyses. 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). None of the screened studies were excluded because of missing information stipulated in our inclusion criteria (Fig. S2), but for one study (Breitenbach *et al.* 2020) we accessed the published dataset to calculate standard deviations. 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.

*Data Processing and Transformations*

Standard errors (SE) and 95% confidence internals (CI) were converted to standard deviations (SD) (Quinn & Keough 2002) , which were used for effect size calculations. Bounded data such as proportions (e.g., survival) were transformed before calculating effect sizes (23 effect sizes) to obtain means and variances that were more likely to satisfy normality assumptions (Quinn & Keough 2002):

|  |  |
| --- | --- |
|  | (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. We transformed these data because considering the measurement scale is important when deriving interaction-based effect sizes (Catford *et al.* 2022; Macartney *et al.* 2022) . For percentages, for example, we are working on the transformed scale, not the percentage scale.

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*

To quantify changes in plasticity between constant and fluctuation experiments, we derived a standardised effect size called PRRDS (an interaction-based, Plasticity Response Ratio Difference) (Equation 7) and its corresponding sampling variance (Equation 8).

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

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

PRRDS is derived in the exact same way other interaction-based effect sizes for SMD (Gurevitch *et al.* 2000) and log response ratios (lnRR)(Macartney *et al.* 2022; Noble *et al.* 2022) have been calculated for application in other contexts. In our case, we use it to compare the plastic responses to temperature between constant and fluctuating treatments while also standardising the effect of temperature differences across studies. Given PRRDS is a response ratio-type effect statistic, following Macartney et al. 2022, we first calculated lnRR within treatments to estimate the change in phenotypic traits following either acclimation or developmental exposure to different temperatures (Macartney *et al.* 2022). lnRR quantifies the expression of phenotypic plasticity because it measures the change in phenotype across two different temperatures (Figure 1a & b). Unlike, Macartney et al. 2022, we do not focus on within-treatment effects as they have been the focus of other studies (Noble *et al.* 2018; Stocker *et al.* 2024) . Our question was specifically focused on how plastic responses are affected by temperature fluctuations, which requires the use of PRRDS. To calculate PRRDs we subtracted the lnRR of the constant from that of the fluctuating thermal environment (Figure 1c). To control for the fact that temperature gradients differed between studies, the PRRDS standardises the differences in plastic responses to a 1oC change in treatment temperature following approaches described by (Noble *et al.* 2022; Pottier *et al.* 2021). A PRRDS = 0 indicates that there is no difference in the expression of phenotypic plasticity between constant and fluctuating thermal environments whereas a PRRDS > 0, indicates that the plastic response is stronger in fluctuating temperatures. In contrast, PRRDS < 0, indicates that the response is stronger under constant conditions (Figure 1c). Importantly, depending on the direction of plastic responses to temperature (positive, negative or opposite) this impacts the sign of PRRDS (Supplementary Materials Table S2). We ensured that the sign of PRRDS remained consistent across the different responses to temperature by multiplying effects that were opposite in meaning (e.g., mortality vs survival) by -1.

*Statistical Analysis*

We used R.4.2.2 in RStudio version 2022.12.0 for all calculations, analyses, and figures (using R Package *ggplot2* version 3.4.2 for figures) (Wickham 2011). All data and code are available at https://github.com/daniel1noble/plasticity\_fluc\_meta (metadata given in Supplementary Materials Table S3). Data are presented as the mean PRRDS ± 95% CIs. For ease of comparison, the mean PRRDs are transformed (i.e., (exp(meanPRRD)-1)\*100%) 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 (IntHout *et al.* 2016) . Prediction intervals provide important information about effect heterogeneity by establishing what the expected effect size would be from future studies 95% of the time (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 (46 levels) was included to account for multiple effect sizes from the same study. A Shared Animal ID random effect (93 levels) 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* version 5.8-1) (Paradis & Schliep 2018) and branch lengths were calculated with Grafen’s method (Power = 1) (Grafen 1989). A species-level random effect (41 levels) 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 (52 levels) 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* version 4.8) (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 Yang *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., CVH2 and M2, following Yang *et al.* 2023). CVH2Total expresses the magnitude of heterogeneity relative to the overall meta-analytic mean. While CVH2Total 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 M2Total (Yang *et al.* 2023). We also decomposed 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*). 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). Levels of a given categorical moderator were only included in MLMR models if the number of effect sizes within the relevant level was > 10 (O’Dea *et al.* 2021). We analysed the amplitude of fluctuations as continuous moderator.

*Publication Bias and Sensitivity Analysis*

Publication bias was investigated by altering out MLMA model to include z-transformed publication year to assess the presence of a time-lag effect or decline effect, and the inverse effective sample size (and its square root), which approximates the inverse of sampling variance, to test for small-study effects, as moderators (Nakagawa *et al.* 2022a). We used inverse sample size (and its square root) to investigate small-study effects because it avoids correlations between an effect sampling variance and the effect size itself when working with log response ratios. 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. Decreasing effect size through time is a common form of publication bias resulting from studies with weaker effects taking longer to be published contributing to a decline in average effect through time (Jennions & Mller 2002).

A sample of effect sizes showed higher than expected precision. We carefully checked these extreme values to ensure that data were 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* version 4.8). 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 241 effect sizes from 41 species derived from 46 studies. The dataset was dominated by invertebrates (80%, 37/46 studies) but also included vertebrate ectotherms (20%, 9/46 studies). Overall, 98% of the studies used diurnal temperature fluctuations (24 h cycles). 47% (k = 114) traits had negative slopes with increasing temperature in both the constant and fluctuation treatments, 25% (k = 61) had positive slopes in both treatments, and in 27% (k = 66) slopes were in opposite directions in the constant and fluctuating thermal treatments.

In the overall MLMA analysis (effect size estimate = 0.0041; 95% CIs = [-0.0020, 0.0103]; p = 0.19; n = 241, Figure 2a) and individual trait subset analysis (effect size estimate = 0.0041; 95% CIs = [-0.0023, -0.0105]; p = 0.20; n = 234, Figure 2b), the mean plastic responses were not significantly different between constant and fluctuating thermal environments and effect sizes were small in magnitude (0.41% different) (Figure 2). Total heterogeneity, however, was high overall (*I2Total* rounded to 99.25%, CVH2Total = 50.92 and M2Total = 0.98). Between-study heterogeneity was low (*I2study* =1.51%, CVH2study = 0.77, M2study = 0.01) and within-study heterogeneity high (*I2obs* = 90.29%, *CVH2obs* = 46.32, *M2obs* = 0.89). There was also low between-trait (*I2trait* = 7.46%, *CVH2trait* = 3.83, *M2trait*  = 0.07) and between-species heterogeneity (see full heterogeneity statistics in Supplementary Materials Table S15).

We explored *a priori* factors which we expected would explain the high within study heterogeneity. Differences in phenotypic plasticity between fluctuating and constant thermal environments across broad trait categories were rather small and non-significant (0.05-0.28%, Figure 3a), but specific trait categories where enough data existed did explain some variation (R2 = 0.22) (Figure 3b). More specifically, plasticity in development time significantly increased (shallower slope = lower thermal sensitivity) in fluctuating environments (-1.01%). In contrast, at a one-tailed significance level plasticity in metabolic rate was greater in constant environments (2.11%, Figure 3b, Supplement Table S8). In each case, however, effect size magnitude was still small and the upper and lower bound of the 95% prediction intervals predict small effects (~5-8%. Figure 3b). Constant and fluctuating temperatures did not significantly influence phenotypic plasticity differently between aquatic or terrestrial species or vertebrates and invertebrates (Figure 4). In addition, neither the fluctuation type (Figure 5), plasticity type (Figure 6) or the amplitude of fluctuating temperature regimes (Figure 7) revealed significant differences in phenotypic plasticity between fluctuating and constant temperature conditions, and in all cases effect sizes were small (Supplementary Materials Tables S4-S13).

**Discussion**

Our meta-analysis shows that experiencing constant or fluctuating environmental conditions does not strongly impact the expression of phenotypic plasticity in ectothermic organisms. It is important to note, however, that there was substantial within-study heterogeneity in effects overall and PRRDs, as an interaction-based effect size, will likely require larger sample sizes to detect statistically significant differences particularly with small effects (Catford *et al.* 2022) . Our estimates of *I2Total*, *CVH2Total* and *M2Total* all fall well above the 75th percentile of empirically derived heterogeneity estimates (Yang et al. 2023), with most variation explained by within study factors. Our findings were relatively consistent across studies and species, with low between-study and species-level heterogeneity in effects, which suggests that the small overall effects we observed are similar across species and studies when within-study sources of variation have been controlled (Yang et al. 2023). We note, however, that the number of species and studies were very similar in our dataset (i.e., each study tended to use a unique species) so that we may not be able to disentangle study- and species-specific effects very robustly. Additionally, the data in the analysis stem from a relatively small number of species that are unevenly distributed phylogenetically so that the generality of the current state-of-knowledge is not well established. Taxonomic coverage is likely to be correlated with geographic coverage, and many habitats - particularly biodiverse areas outside Europe and North America - are poorly represented by physiological studies (White *et al.* 2021). Future work is needed to extend understanding of how temperature fluctuations affect plasticity to a broader taxonomic and geographic range of animals, with particular emphasis on those taxa that are likely to be affected by climate change-induced increases in fluctuations (Cooke *et al.* 2021).

Analyses of key moderators predicted to drive effect size variation (e.g., type of plasticity measured, fluctuation type and amplitude) mostly did not explain variability aside from the specific trait being measured (marginal R2 = 21%). Importantly, even though there were differences in plasticity between constant and fluctuating temperatures for development time and to a lesser extent metabolic rate (in opposite directions), these average effects were small. Trait variation is likely to reflect how thermal fluctuations impact traits with different thermal performance curves. In both cases, development time and metabolic rate respond exponentially to temperature, but in opposite directions. For example, development time exhibited a non-linear exponential decline with increasing temperature (Fischer *et al.* 2011) while metabolic rates followed a Gaussian (inverted U-shape) curve as temperature increased (Bozinovic *et al.* 2020). Fluctuating or constant temperatures are therefore likely to have different impacts on mean trait values and the magnitude of plastic responses. However, we hesitate to overinterpret our results given the small effects. The findings of our study overall indicate that there is weak support in the current literature that phenotypic plasticity is influenced by regular diel fluctuations around mean temperatures.

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; Nilsson-Örtman & Brönmark 2022). 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 (diel) fluctuations in temperatures do not impact plastic responses, and we suggest that plastic responses are driven by mean temperatures across more than one day.

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 strong differences in 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 plastic responses (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 significantly 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 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 those caveats 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. However, the data available in the literature provide a very limited picture of how fluctuations in environments affect plastic responses. Diel variations are ubiquitous in natural environments but, in addition, temperatures also vary at different temporal and spatial scales. Very little is known about the impact of these more complex environments, and it will be important in future work to determine the periodicity at which fluctuations become sufficient to impact plastic responses. Additionally, oWe suggest that future research should explore the impacts of transgenerational thermal variability at different temporal scales on phenotypic plasticity.

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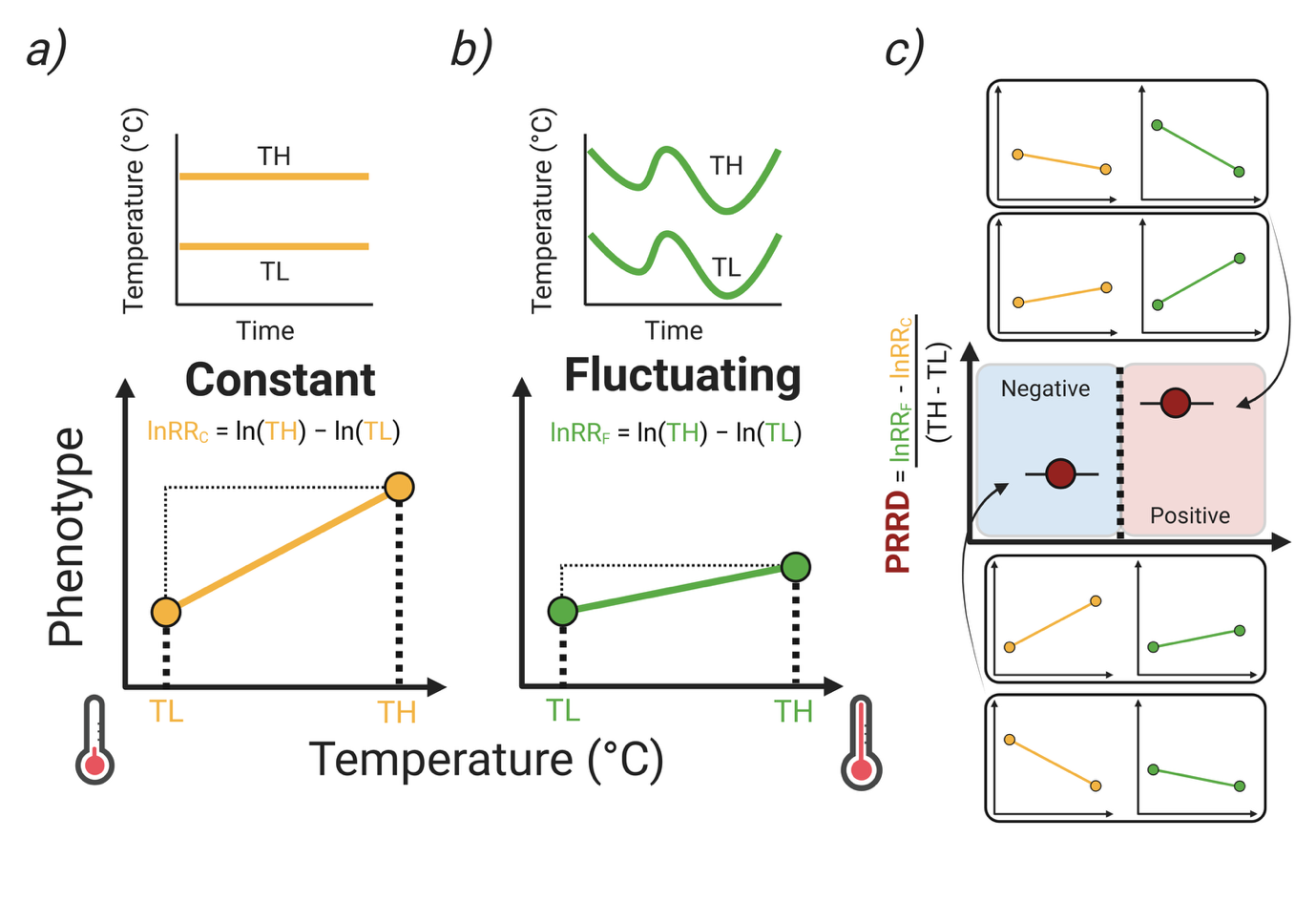
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**Figures**

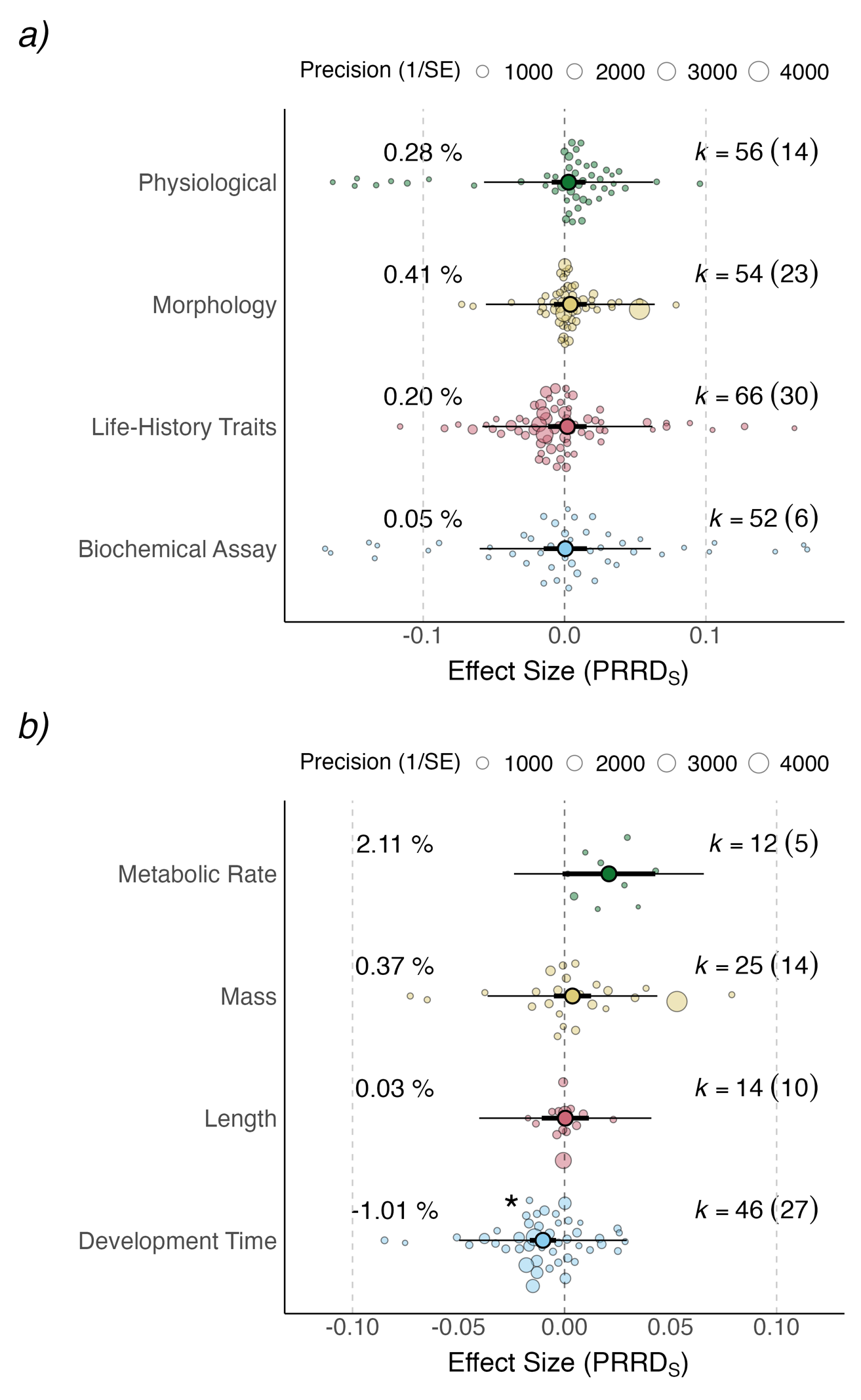
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**Fig. 1**

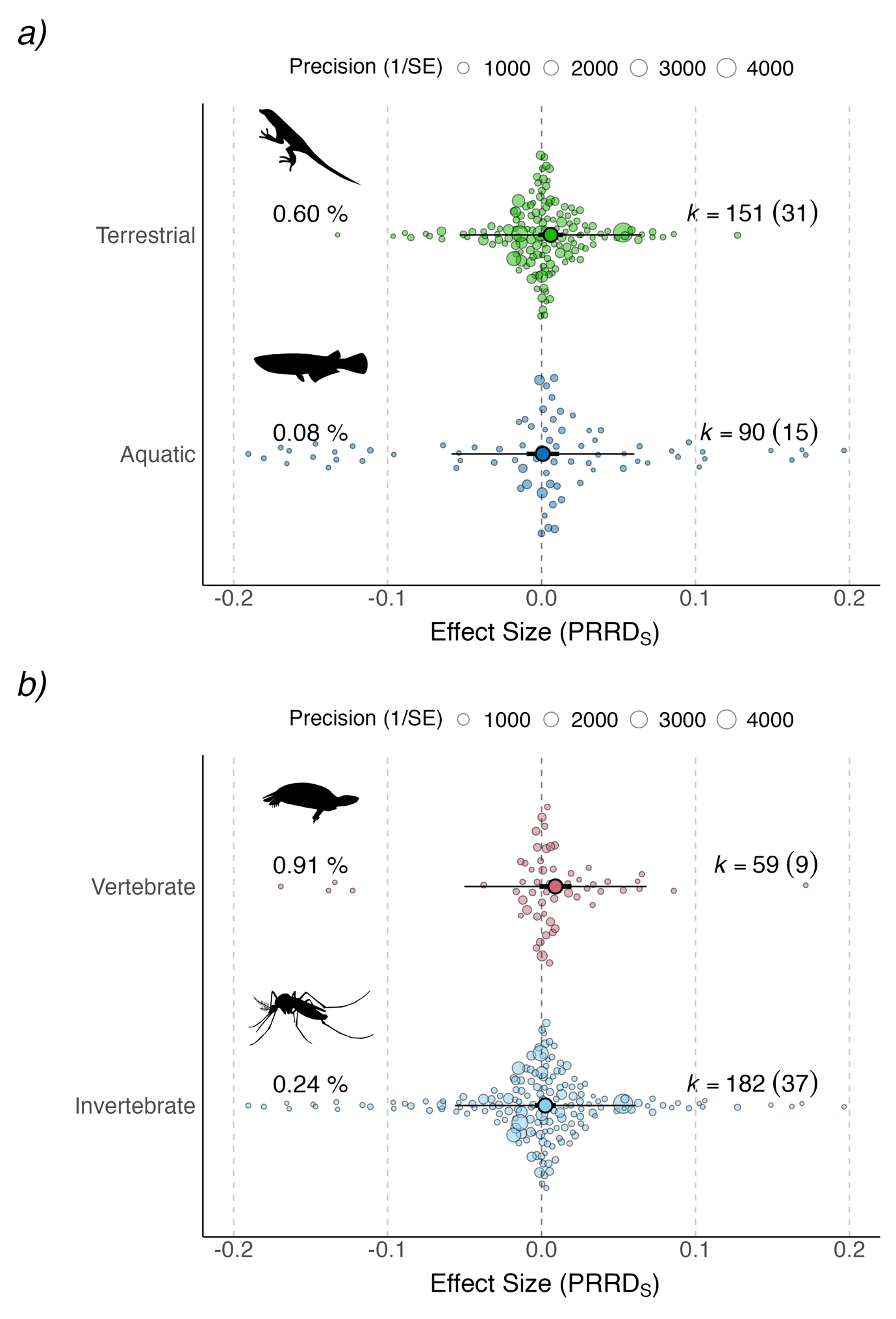
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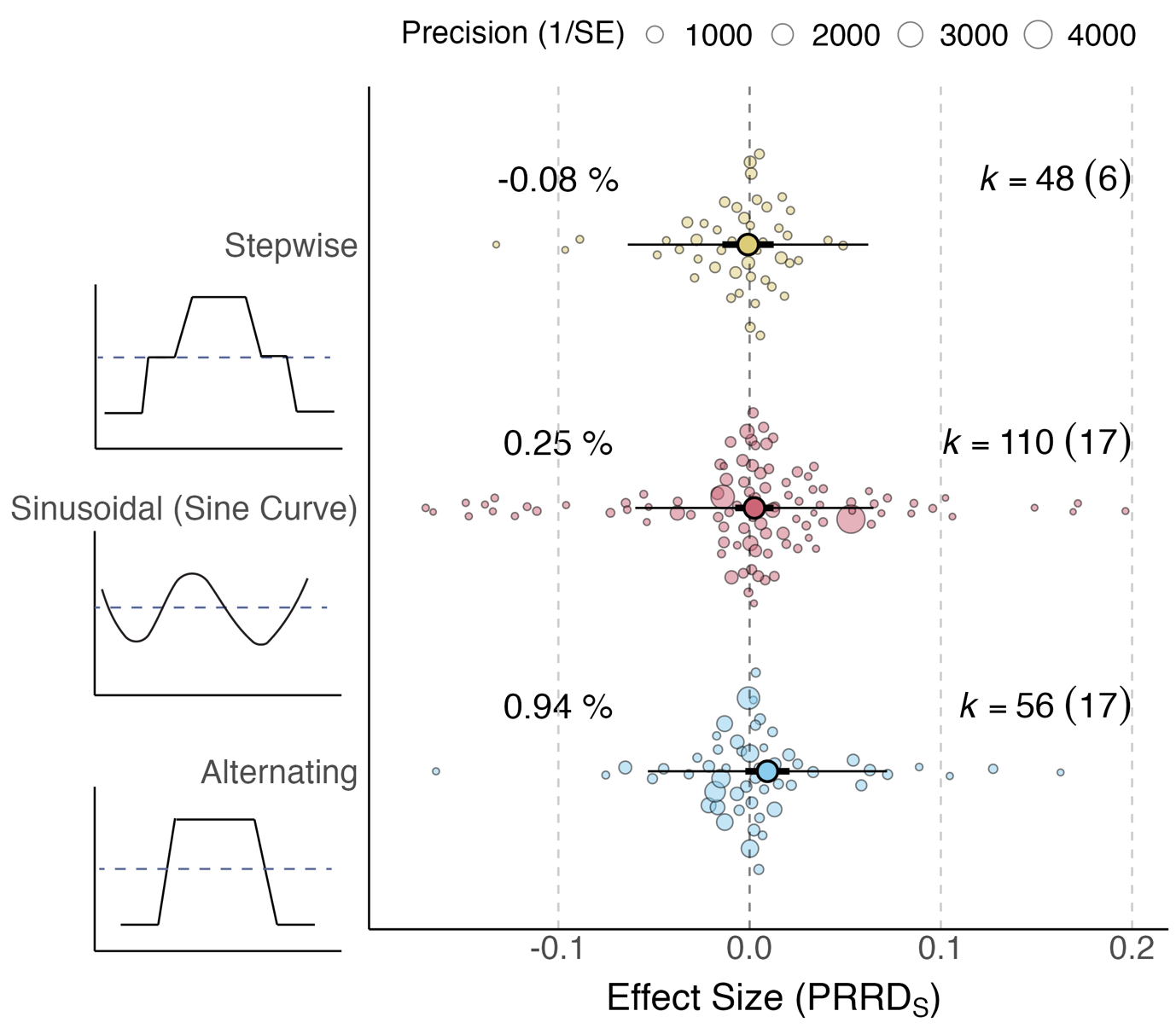
**Fig. 2**

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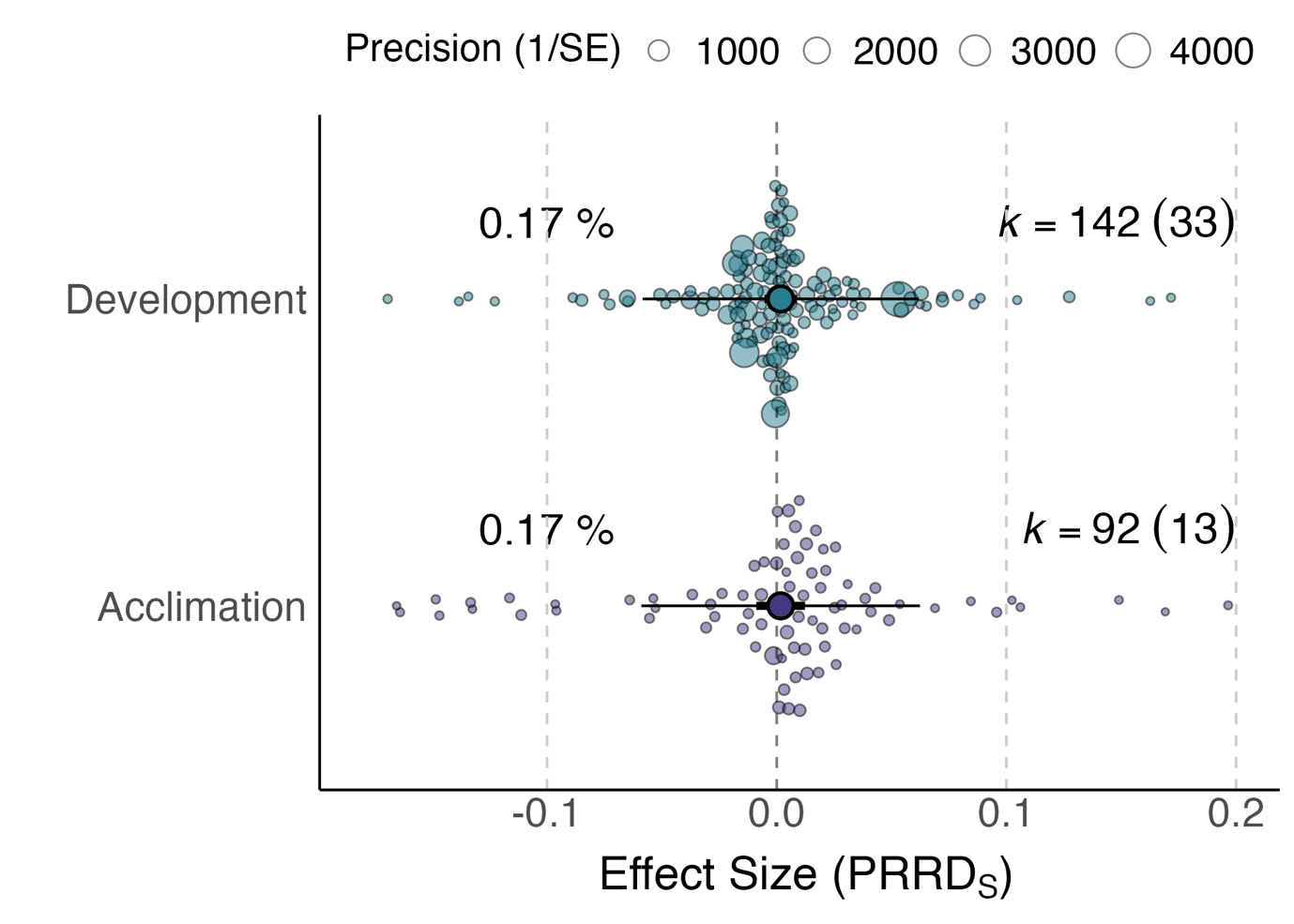
**Fig. 3**

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**Fig. 4**

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**Fig. 5**

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**Fig. 6**

**A graph of different sizes of data

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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 log-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 log-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. c) From these response ratios the effect size (plasticity response ratio difference; PRRD) can be calculated according to equation 7 and 8. Note that there is no a priori assumption that phenotypic responses are higher at TH. Traits where temperature results in either negative or positive plastic responses can result in the same effect size (both in direction and magnitude) if their slopes are the same, as demonstrated with hypothetical examples c) Proportions of different phyla represented in the data set. Arthropods (particularly insects) dominate the data-set (79.1%), followed by Chordates (particulalry reptiles; 20.9%). Created in https://BioRender.com

**Figure 2 Multi-level meta-analytic results for the overall data set (a) and only studies measuring traits 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 thick 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.