# Sources of variation in phenotypic plasticity across ectotherms

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## Statement of Originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree of other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Clayton W. Stocker

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# Author Attribution

The work in this thesis is a result of my own work, unless otherwise acknowledged. Members of the Evolutionary and Ecological Physiology Lab including, Stephanie M. Bamford, Miki Jahn, Geoffrey P. F. Mazué, Amanda K. Pettersen, Daniel Ritchie, Alexander M. Rubin, and Frank Seebacher were involved in the data screening and extraction for Chapter 2 and 3. The meta-analytical skills required for Chapter 2, 3, and 4 were learned from Dan Noble.

In addition to the statement above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

Clayton W. Stocker

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statement above is correct.

Frank Seebacher

# Abstract

Anthropogenic changes including pollution, artificial light-at-night, and climate change can influence physiological and ecological functions at all levels of organisation. The aim of this thesis was to investigate the consequences of these anthropogenic drivers on ectotherms and understand the factors that undermine compensatory responses. A fully factorial experiment was conducted to determine the interactive effects of chemical pollution and artificial light-at-night on physiological performance. Meta-analyses were also conducted to determine the difference in phenotypic traits and their plastic responses between constant and fluctuating thermal environments, and the extent of population-level differences in the capacity for phenotypic plasticity. Here, we found that BPS exposure, but not photoperiod, had an inhibitory effect on metabolism and swimming performance in zebrafish (*Danio rerio*). However, there was no interactive effect between plastic pollution and artificial light. The thesis also shows that phenotypic responses of ectotherms to thermal change is mostly driven by long-term mean temperatures, and only in certain contexts by temperature fluctuations particularly at high amplitudes. Negative consequences of extreme fluctuations did not affect plastic responses, and the capacity for phenotypic plasticity was not different in constant and fluctuating thermal environments. Lastly, it was found that there are differences in the capacity for phenotypic plasticity among populations of the same species. The disparity between populations was particularly evident in physiological responses, and in the developmental plasticity of morphological traits. The work presented in this thesis advances our understanding of the impacts of anthropogenic changes and the factors that influence the compensatory responses of ectotherms, which can have implications for conservation and management of natural resources.

# General Introduction

Anthropogenic changes have produced novel environmental signals that have left very few ecosystems unaffected. The three most prominent anthropogenic drivers are pollution, artificial light-at-night (ALAN), and climate change. All three are rapidly increasing and can influence many biological, ecological, and evolutionary functions across all levels of organisation (Borrelle et al., 2020; IPCC, 2022; Sanders et al., 2021). An understanding of how animals respond to these environmental changes is crucial for effective conservation and management of natural resources.

Global increases in plastic manufacturing have resulted in the extensive release of plastic wastes into aquatic ecosystems (Geyer et al., 2017). The entanglement or physical damage from discarded plastics and the ingestion of macro- or micro plastics are significant threats to aquatic wildlife (Li et al., 2016). Additionally, endocrine disrupting chemicals (EDCs) can leach out of plastic pollutants and quickly disperse through river and ocean currents (Hahladakis et al., 2018; Windsor et al., 2018). There is a growing concern regarding the conservation and health impacts of EDCs due to their widespread contamination, high commercial and industrial demand, and capacity to interfere with hormone-signalling pathways (Corrales et al., 2015; Gore et al., 2015; Sutherland et al., 2021). The most produced EDC worldwide, bisphenol A (BPA), has received an increasingly negative reputation due to its endocrine disrupting effects (BPA; Huang et al., 2017). For example, the binding of BPAs to thyroid hormone receptors and the resulting inhibitory effects on physiology have been extensively reported (Seebacher, 2022). To offset the potentially harmful effects of BPA, there has been an increase in the use of derivatives such as bisphenol S (BPS) in plastic manufacturing (Flint et al., 2012). Current research has suggested that BPS

could be equally detrimental as an endocrine disruptor to aquatic organisms (Lee et al., 2019; Zhang et al., 2017).

In recent years, there has been an increase in anthropogenic light pollution leading to the occurrence of ALAN (Kyba et al., 2017). Urbanisation is the primary cause as it has increased the presence of artificial light sources, including streetlights, buildings, and vehicles (Falchi et al., 2016). Increased light levels are not restricted to major cities and can travel to less developed areas through a process known as skyglow, where dust, water and gas molecules scatter artificial light throughout the atmosphere (Gaston, 2018). The novel photoperiods that are produced differ greatly to the natural environment and can interfere with circadian rhythms. Responses to light are regulated by hormones, principally melatonin, and the extension of daylight hours can therefore act as an endocrine disruptor (Ouyang et al., 2018; Russart and Nelson, 2018). Anthropogenic environmental signals from chemical and light pollution can disrupt endocrine-mediated functions and act as agonists that reduce or eliminate hormone signals and alter gene expression (Gentilcore et al., 2013; Zhang et al., 2019). The interaction between anthropogenic drivers is often unpredictable from the individual effects (Orr et al., 2020; Wu et al., 2022) and it may be that the combined effect of BPS and ALAN on the endocrine system causes unexpected endocrine-mediated physiological responses (Hale et al., 2017; Paine et al., 1998). Effective conservation and management of natural resources will require an understanding of the relationships between multiple anthropogenic drivers (Barouki et al., 2022; Santos et al., 2011).

Changes to the thermal environment can have profound impacts on the biodiversity, distribution, and phenology of organisms (Lavergne et al., 2010; Parmesan, 2006). Global climate change models predict that anthropogenic activities will increase mean temperatures and the amplitudes of thermal fluctuations (e.g., diurnal, seasonal, and annual) in the coming decades (IPCC, 2021; Vasseur et al., 2014). Unpredictable thermal variability at different

spatial- and temporal scales, increases the probability of organisms experiencing temperature extremes (Wedler et al., 2023; Xu et al., 2020). Variations to the external environment are particularly important for ectotherms as their internal body temperatures are determined by their surroundings (Chown et al., 2010; Huey et al., 2012). To inform effective policy and conservation decisions it is imperative to understand the phenotypic responses of ectotherms to variable temperatures (IPCC, 2022). The sensitivity of phenotypic traits to thermal signals can be characterised by non-linear thermal performance curves (TPC; Huey and Kingsolver, 1989). An individual's performance can be positively or negatively impacted by natural or anthropogenic temperature changes depending on their relative position within the TPC (Denny, 2019; Marshall et al., 2021). The inability to compensate for adverse conditions could have negative implications for the survival of an organism through environmental variability (Nunez et al., 2019; Pacifici et al., 2017).

Ectotherms can relocate to align their geographic range with more favourable conditions, or attempt to alleviate the impacts of changes to their environment (Bellard et al., 2012; Guisan and Zimmermann, 2000). Organisms can mitigate the impacts of persistent environmental signals over many generations through genetic adaptation (Chevin et al., 2010; Hoffmann and Sgro, 2011). However, anthropogenic drivers are expected to impose rapid environmental changes with unpredictable directionality that can cause genetic adaptation to be ineffective (Alley et al., 2003; Kawecki, 2000). Instead, organisms can exhibit plastic responses that adjust TPCs to allow relatively constant function despite climate variability (Kingsolver et al., 2015). This phenotypic plasticity remodels phenotypes independently from the genotypes and allows organisms to at least partially mitigate the impacts of harmful conditions (Beaman et al., 2016; Loughland et al., 2021).

An adult's phenotypes can be influenced by the environments they are exposed to during early life history stages through developmental plasticity (Burggren, 2018; Le Roy et al., 2017; Warner et al., 2018). Adults and juveniles can also respond to relatively long-term intragenerational variation by inducing reversible and repeatable phenotypic modifications via acclimation (Guderley, 2004; Loughland and Seebacher, 2020). There can be a mechanistic link between intragenerational mechanisms of phenotypic plasticity (developmental plasticity and acclimation) that allows them to function conjointly in response to environmental change (Beaman et al., 2016). Phenotypic plasticity can also occur across generations (transgenerational effects) where offspring phenotypes are shaped by the (grand)parental environment (Le Roy et al., 2017). Each of these mechanisms of phenotypic plasticity can mitigate the negative impacts of climate change by maintaining relatively stable phenotypes across environmental gradients (Lagerspetz, 2006; Schulte et al., 2011). The vulnerability of ectotherms is, therefore, influenced by an individual's capacity for phenotypic plasticity (Botero et al., 2015; Kawecki, 2000).

Perfect plasticity would shift the TPCs so that optimal performance coincides with the novel conditions (Wilson and Franklin, 2002). It is rare for ectotherms to maintain optimal performance completely across environmental gradients, which suggests that there are sources of variation in the capacity for phenotypic plasticity. Previous studies have identified differences in the capacity for phenotypic plasticity across phylogenetic lineages, habitats, and phenotypic traits (Seebacher et al., 2012; Seebacher et al., 2015). However, empirical research into the relationships between thermal fluctuations and plastic responses, and the extent to which populations differ in their capacity for phenotypic plasticity, have been equivocal. Understanding the factors that influence compensatory responses is essential for accurate predictions of the impacts of climate change and for identifying areas where conservation efforts should be prioritised (Kefford et al., 2022; Pearman et al., 2010).

The aim of this thesis was to investigate the consequences of anthropogenic changes on ectotherms and understand the factors that undermine compensatory responses. Covid-19 significantly restricted field and laboratory research that caused extensive delays to my PhD. These delays changed the focus of my PhD from empirical to mostly meta-analytical approaches. In the first chapter, we conducted a fully factorial experiment to determine the interactive effects of BPS exposure and photoperiod on the metabolism and swimming performance of zebrafish (*Danio rerio*). For the second chapter, we conducted a meta-analysis to provide a quantitative synthesis of studies that addressed whether phenotypic traits (mean and variance) differed between exposure to constant versus fluctuating thermal environments with the same mean temperature. The third chapter focuses on a meta-analysis that tested whether the capacity for phenotypic plasticity differed in constant versus fluctuating thermal environments with the same mean temperature. In the final chapter, we conducted a meta-analysis that investigated whether the capacity for phenotypic plasticity differed between populations of the same ectothermic species.

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# Chapter 1

## Interactive effects of plastic pollution and artificial light on the metabolism and swimming performance of zebrafish (*Danio rerio*)

# 1 | Abstract

Anthropogenic changes have produced novel environments that can undermine hormone-mediated signalling and impact the physiology of animals. The increase in plastic manufacturing has resulted in the extensive release of endocrine disrupting chemicals (EDCs) into the environment. Bisphenol A (BPA) is one of the leading EDCs produced worldwide, and to mitigate its harmful impacts, alternatives such as bisphenol S (BPS) have been increasingly used. Concurrently, urbanisation has led to artificial light-at-night (ALAN) which disrupts natural photoperiods. Extending the hours of daylight can interfere with natural circadian rhythms and act as an endocrine disruptor. The aim of this study was to determine the interactive effects of BPS exposure and photoperiod on the metabolism and swimming performance of zebrafish (*Danio rerio*). We found that BPS exposure, but not photoperiod, had an inhibitory effect on metabolism and swimming performance with a relatively high confidence (95% CI). There was no significant interactive effect between plastic pollution and artificial light. Therefore, the release of BPS from plastic manufacturing and pollution can have implications for both conservation and the management of natural resources. Future studies should focus on identifying additional BPA alternatives for plastic manufacturing and experimentally assessing the functional consequences of EDCs across different environmental conditions.

# 2 | Introduction

Anthropogenic changes have produced novel environmental signals that have left very few ecosystems unaffected. Plastic pollution releases endocrine disrupting compounds (EDCs), and urban environments have generated artificial light-at-night (ALAN) that affect even the most remote areas (Meijer et al., 2021; Sanders et al., 2021). EDCs and ALAN undermine hormone-mediated signalling and impact the physiology of animals (Besson et al., 2020; Russart and Nelson, 2018). For example, EDCs and ALAN affect thyroid and glucocorticoid hormones, which are important in regulating fish metabolisms and swimming performance (Mullur et al., 2014; Sacta et al., 2016). Interactions between human-induced changes can cause unpredictable and compounding effects that occur concurrently with natural variation (Buma and Wessman, 2012; Paine et al., 1998; Warner et al., 2021).

Increases in plastic manufacturing have resulted in the extensive release of macro-plastics, micro-plastics, and EDCs into the natural environment (Landrigan et al., 2023; Naveira et al., 2021). EDCs are particularly prolific in aquatic ecosystems as they are released through effluents and transported through river and ocean currents (Hahladakis et al., 2018; Windsor et al., 2018). There is a growing concern regarding the conservation and health impacts of EDCs due to the widespread contamination, high commercial and industrial demand, and capacity to interfere with hormone signalling pathways (Corrales et al., 2015; Gore et al., 2015; Sutherland et al., 2021). Bisphenol A (BPA) is one of the leading EDCs produced worldwide (Huang et al., 2017). To mitigate the potentially harmful impacts of BPA, derivatives such as bisphenol S (BPS) are used in plastic manufacturing (Flint et al., 2012). Recent studies, however, have indicated that BPS, too, disrupts thyroid hormone signalling and impacts fish physiological responses (Lee et al., 2019; Zhang et al., 2017).

The increase in urbanisation has produced novel photoperiods that are fundamentally different to the natural environment (Kyba et al., 2017). For example, streetlights, buildings, and vehicles produce near-constant light in urban areas (Falchi et al., 2016). Increased light levels are also extended to remote areas via skyglow, a process where artificial light is scattered in the atmosphere by dust, water, and gas molecules (Gaston, 2018). It is predicted that with continued urbanisation there will be an increase in ALAN globally (Seto et al., 2012). Extending the hours of daylight can interfere with natural circadian rhythms and act as an endocrine disruptor that can have inhibitory effects on physiological responses (Ouyang et al., 2018; Russart and Nelson, 2018).

Previous studies have investigated the separate impacts of plastic pollution and different photoperiods on the physiological performance of fish. BPS exposure at an environmentally relevant concentration negatively impacted swimming performance and metabolism (Pelaia et al., 2023; Wu and Seebacher, 2021). Manipulations of photoperiod influenced thermal acclimation of swimming performance and metabolic rates (Le Roy and Seebacher, 2020). However, there is limited empirical research conducted on the interactive effects of BPS and ALAN (Seebacher, 2022). Interactions of anthropogenically altered environments can induce outcomes that are unpredictable from the individual effects (Orr et al., 2020; Wu et al., 2022). The combined disruption of BPS and ALAN on the endocrine system could cause unexpected endocrine-mediated physiological responses (Hale et al., 2017; Paine et al., 1998). An understanding of the interaction between multiple environmental drivers is essential for effective conservation efforts and management of natural resources (Barouki et al., 2022; Santos et al., 2011).

The aim of this study was to determine the interactive effects of BPS exposure and photoperiod on the metabolism and swimming performance of zebrafish (*Danio rerio*). We conducted a fully factorial experiment that tested this interaction by manipulating both BPS (BPS exposed and BPS control) and photoperiod (control, extended and continuous light regimes). Specifically, we tested the hypotheses (i) that BPS exposure will reduce metabolism and swimming performance, (ii) that increased daylight will have an inhibitory effect on metabolism and swimming performance, and (iii) that there is an interactional effect between BPS exposure and photoperiod manipulation.

# 3 | Materials and Methods

## 3.1 | Animal husbandry

We commercially sourced (Livefish, Bundaberg, Australia) adult *D. rerio* (mixed sex; mean mass = 0.5142 ± 0.0107 [se] g, mean standard length = 3.1593 ± 0.0195 [se] cm) and housed them in large communal tanks (60 x 40 x 35cm) for two weeks. The tanks were filled with aged water (dechlorinated) and maintained at a similar temperature (25°C ± 0.5°C) and photoperiod (14L:10D; expressed as hours; L = Light; D = Dark) as at the supplier. The fish were then distributed haphazardly to 10L experimental glass tanks (30 x 19 x 21cm). Each tank was fitted with sponge filters that connected to air pumps, and water changes were conducted bi-weekly. Fish were fed daily with fish flakes (Tetramin Tropical Flakes, Tetramin, USA) and supplemented with live Artemia once a week, for the duration of the experiment. All procedures were performed in accordance with the University of Sydney Animal Ethics Committee (approval number: 2021/1897).

### 3.2 | Experimental design

A fully factorial design was implemented with BPS exposure and photoperiod as independent factors that resulted in six experimental treatments (BPS-exposed and control light regime; BPS-exposed and extended light regime; BPS-exposed and continuous light regime; BPS control and control light regime; BPS control and extended light regime; BPS control and continuous light regime). There were six replicate tanks per treatment, with (n = 6-8 fish per tank). The experiment was conducted in three blocks with two replicate tanks per treatment and block. Fish were exposed to each treatment for three weeks.

BPS-exposed fish were exposed to 10μg $l^{-1}$ of BPS (Sigma Aldrich, Castle Hill, Australia) in tank water dissolved in DMSO (0.0005%; Ajax Finechem, Taren Point, Australia). Final concentrations of DMSO were below toxicological limits (Hutchinson et al., 2006). BPS control fish were exposed to the same concentration of DMSO with no BPS. BPS and DMSO were administered following bi-weekly water changes (50%; Wu and Seebacher, 2021). Photoperiod treatments exposed fish to one of three light regimes; control light regime typical for winter in south east Australia, 10L:14D; extended light regime typical for summer, 16L:8D; and an continuous light regime representing ALAN, 24L:0D (Abdollahpour et al., 2020). Experiments were conducted in three identical environment-controlled rooms that each had a different photoperiod treatment. Light was provided by white, fluorescent lights (Crompton Triphosphor 36W 4000K, Bradford, UK) connected to automated timers (HPM, Zetland, Australia).

Resting metabolic rate (RMR), active metabolic rate (AMR), and swimming performance were measured in each fish following the treatment exposures. A subset of fish was measured from each replicate tank. Phenotypic responses were measured on consecutive days to allow a sufficient recovery period following each trial. To track individuals but allow for continued visual and olfactory cues, fish were separated with 1L cylindrical perforated baskets within their treatment tanks (Le Roy et al., 2021). Fish were photographed following measurements of swimming performance to determine their standard body length using ImageJ (Schneider et al., 2012). After the conclusion of the experiment, fish were euthanised by anaesthesia in Aqui-S (Aqui-S, New Zealand) followed by cervical dislocation and weighed on an electronic balance (Sartorius, Australia).

### 3.3 | Oxygen consumption

We used established protocols (Le Roy et al., 2021) to measure RMR and AMR by intermittent flow respirometry. Fish were placed within a clear Perspex cylindrical respirometer (15mm diameter x 100mm length: 27ml volume) that was housed within a water bath (set to 25°C) and attached to a peristaltic pump that maintained water circulation (i150, iPumps, Tewkesbury, UK). Oxygen concentration inside respirometers was measured using a fibre optic cable that connected to a sensor spot (Pt3, Loligo Systems, Denmark) placed at the midline of the respirometer, and to a $O_2$ meter (Wiltrox, Loligo Systems, Denmark) at the other end. Fish were left undisturbed in the respirometers for 2 hours to recover from handling stress (Seebacher et al., 2016). The peristaltic pump was then remotely turned off and the resulting decrease in oxygen concentration was recorded for approximately 20 minutes.

AMR was measured using a glass respirometer (130ml volume) placed within a water bath (set to 25℃ ± 0.5℃) that was on top of a magnetic stir plate. Water flow was generated with a magnetic stir bar (adjusted by the magnetic stir plate) placed at the bottom of the respirometer and separated from the fish by aquarium mesh. To reduce turbulence in the respirometer, a plastic column was attached to the lid that extended down to the aquarium mesh. The oxygen consumption in the respirometer was measured by connecting a sensor spot (Pt3, Loligo Systems, Denmark) in the respirometer to an $O_2$ meter (Wiltrox, Loligo Systems, Denmark) with a fibre optic cable. A fish was placed in the respirometer and the water flow speed was gradually increased until it could no longer maintain its position in the water column. The water flow speed was then decreased slightly to allow the fish to swim in the respirometer at near maximal effort (Rubin and Seebacher, 2023). Oxygen consumption was then recorded for approximately 10 minutes (oxygen concentration remained above 90% saturation).

The slopes of the decline in oxygen concentration within respirometers were used to calculate rates of oxygen consumption (RMR and AMR; using R package *RespR*; Harianto et al., 2019). Background measurements were recorded each day to determine the microbial respiration in the systems and these values were subtracted from the respective oxygen consumption rates (Rubin and Seebacher, 2023). The difference between RMR and AMR were used to calculate the aerobic scope (AS = AMR – RMR).

### 3.4 | Swimming performance

We determined swimming performance as the critical sustained swimming speed ($U_{crit}$; Kolok, 1999) according to a previously published protocol (Seebacher et al., 2015). Briefly, trials were conducted in Blazka-type swimming flumes that consisted of a clear plastic (Perspex) cylinder (150mm length and 26 mm diameter) fitted over the intake end of a submersible inline pump (12V DC, iL500, Rule, Hertfordshire, UK). The flume was separated from the pump with a plastic grid, and a bundle of hollow straws was located at the inlet to maintain a laminar flow. The speed of flow (measured with a flow meter; DigiFlow 6710 M, Savant Electronics, Taichung, Taiwan) was adjusted using a variable DC power source (NP9615; Manson Engineering Industrial, Hong Kong, China). The flume and pump were contained within a tank (60 x 40 x 35cm) filled with aged water at 25°C ± 0.5°C (measured with a digital thermometer). Individual *D. rerio* were added to the flume and swam for 10 minutes at 0.1 m $s^{-1}$ water flow. Flow rate was increased by 0.06 m $s^{-1}$ every 10 minutes until the fish could no longer keep its position in the water column. The trial was then paused for 10 seconds to allow the fish to rest, and then the water flow was returned to the previous settings. When the fish reached exhaustion a second time the trial was ended. A variation of the Brett, (1964) equation (Equation 1) was used to calculate $U_{crit}$:

$$U_{crit} = U_f + \left(T_f / T_i\right) \times U_i \quad (1)$$

where $U_f$ is the highest speed maintained for an entire interval, $T_f$ is the duration until exhaustion in the final speed interval, $T_i$ is the prescribed period of swimming at each speed, and $U_i$ is the speed increments. $U_{crit}$ is reported as body lengths per second (BL $s^{-1}$).

### 3.5 | Statistical analysis

All calculations, analyses, and figure production (using R package *ggplot2*; Wickham, 2016) were completed with R.4.2.2 in RStudio version 2022.12.0. Each phenotypic trait was analysed separately, using identical models that had BPS exposure and photoperiod as fixed effects, and the experimental block as a random error. A permutational analysis was run on each model (using function *aovp* in the R package *ImPerm*; Wheeler and Torchiano, 2016) as it does not depend on underlying frequency distributions and the associated assumptions (Drummond and Vowler, 2012). Statistical results are presented as permutation p-values (Ludbrook and Dudley, 1998).

Additionally, effect sizes were calculated as Cohen's D ± 95% bootstrapped confidence intervals (CI); 95% CIs were calculated in the R package *boot* (Canty and Ripley, 2022; Davison and Hinkley, 1997). Effect sizes and 95% CIs provide estimates of the magnitude of responses and their precision, respectively (Nakagawa and Cuthill, 2007). The directionality of effect size estimates and the overlap of CIs with zero are informative in assessing biological effects. For example, a small overlap of CIs with zero can suggest a biological effect with relatively high confidence (Nakagawa and Cuthill, 2007). Effect sizes were calculated to determine the standardised marginal mean differences between BPS-exposed and BPS control treatments, and between the increased daylight regimes (extended light regime and continuous light regime) and the control photoperiod.

# 4 | Results

## 4.1 | Impact of BPS exposure and photoperiod on metabolic rate

There was no significant effect of either BPS exposure, photoperiod, or their interaction on resting metabolic rate (Figure 1A), active metabolic rate (Figure 1B) or aerobic scope (Figure 1C; Table 1).

**Table 1 Permutational probabilities.** Results of the effect of BPS exposure, photoperiod, and their interaction on metrics of metabolic rate (resting and active metabolic rate, and aerobic scope) and swimming performance ($U_{crit}$). Subscripts indicate *df*.

| Phenotypic Trait | BPS Exposure | Photoperiod | Interaction |
|---|---|---|---|
| Resting Metabolic Rate | 0.9804 (1, 61) | 0.7083 (2, 61) | 0.3644 (2, 61) |
| Active Metabolic Rate | 0.1300 (1, 68) | 0.7273 (2, 68) | 0.4384 (2, 68) |
| Aerobic Scope | 0.1023 (1, 61) | 0.5023 (2, 61) | 0.1965 (2, 61) |
| Swimming Performance | 0.1160 (1, 69) | 0.1643 (2, 69) | 0.6667 (2, 69) |

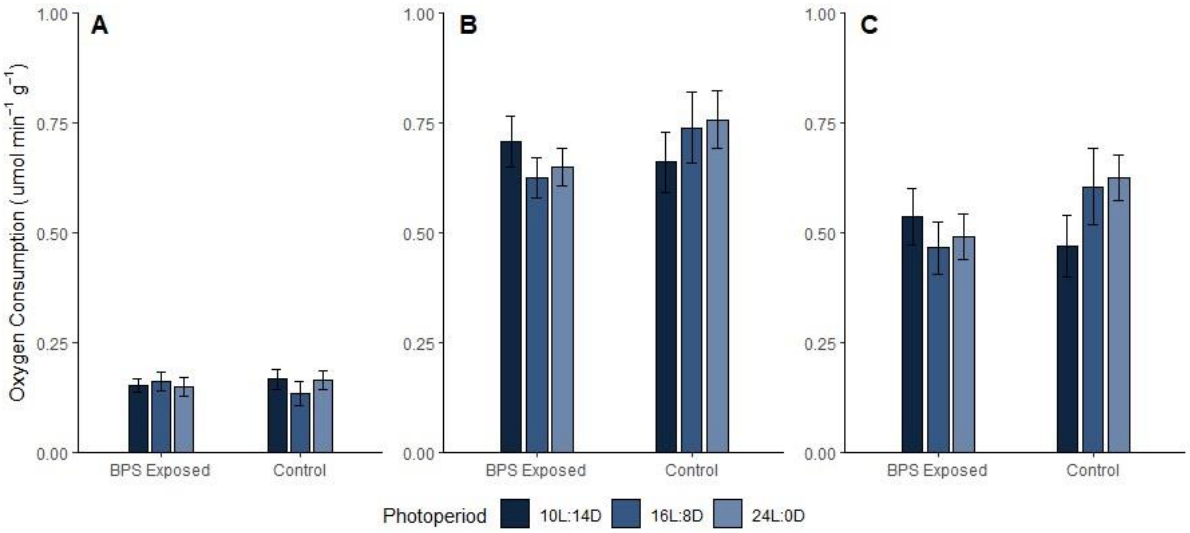


**Figure 1 Metabolic rate.** Resting metabolic rate (A), active metabolic rate (B) and aerobic scope (C) in response to BPS exposure (BPS Exposed and Control), at different photoperiods (10L:14D, 16L:8D, 24L:0D; L = Light and D = Dark). Means ± standard errors are shown, and sample sizes were 11-14 fish per treatment group.

### 4.2 | Impact of BPS exposure and photoperiod on swimming performance

The swimming performance of *D. rerio* was not affected significantly by either BPS exposure, photoperiod, or their interaction (Table 1; Figure 2).

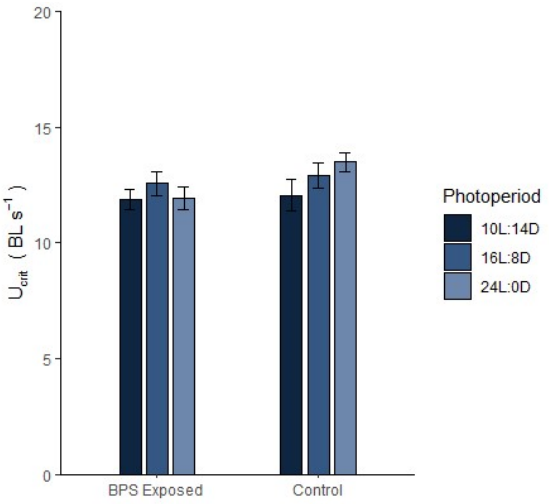


**Figure 2 Swimming performance.** Swimming performance in response to BPS exposure (BPS Exposed and Control) and different photoperiods (10L:14D, 16L:8D, 24L:0D; L = Light and D = Dark). Means ± standard errors are shown, and sample sizes were 12-14 fish per treatment group.

### 4.3 | Effect sizes

Exposure to BPS decreased AMR, AS and swimming performance, with a relatively high confidence (CIs have small overlap with zero; Figure 3A). Photoperiods with increased light (extended and continuous light regimes) had no significant effect on the phenotypic responses of *D. rerio* (Figure 3B, C).

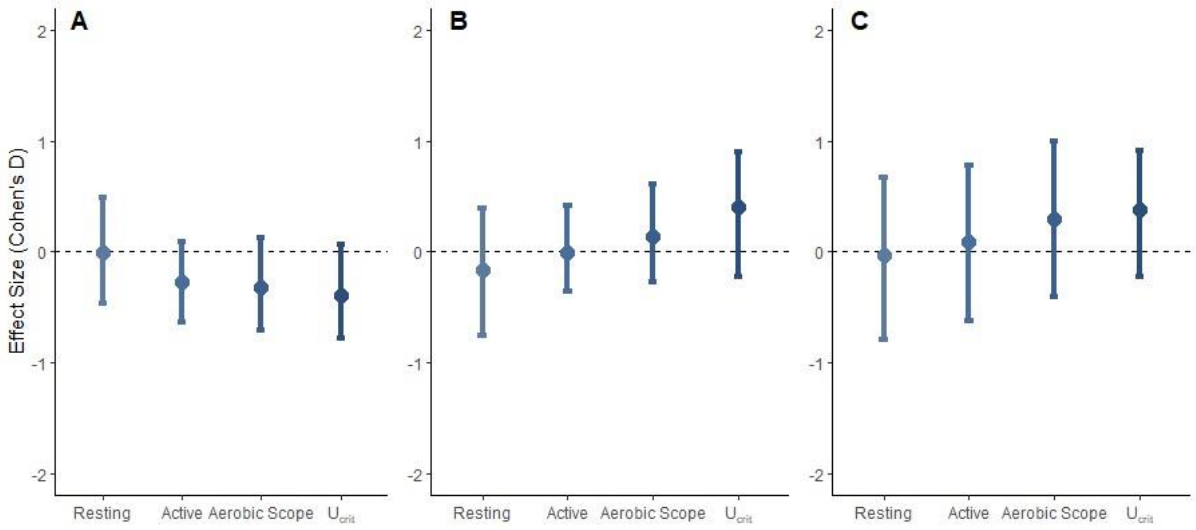


**Figure 3 Effect sizes.** Results indicate the difference in resting metabolic rate, active metabolic rate, aerobic scope, and swimming performance ($U_{crit}$) between: (A) BPS-exposed and BPS control; (B) 16L:8D and 10L:14D photoperiods (L = light; D = dark); (C) 24L:0D and 10L:14D photoperiods. Effect sizes presented as Cohen's D ± bootstrapped 95% CIs. BPS exposure and photoperiods with increased daylight were considered the 'high' treatment for effect size calculations.

## 5 | Discussion

We have shown that exposure to ecologically relevant concentrations of BPS disrupt physiological function in adult *D. rerio*. Decreases in AMR, AS, and swimming performance were observed in BPS-exposed fish with a relatively high confidence. There was no evidence to support our hypothesis that, extended and continuous light regimes have an inhibitory effect on metabolism and swimming performance. Furthermore, we reject our hypothesis that there is an interactive effect between plastic pollution and artificial light. The findings of our analysis suggest that the disruption of EDCs on the physiological responses of fish are unaltered by anthropogenically inflated ALAN.

There has been an uptake in the use of BPS in plastic manufacturing to offset the potentially harmful impacts of BPA (Flint et al., 2012). BPS was identified as a less toxic alternative to BPA, that produced lower rates of mortality and deformity in developmental life-history stages of *D. rerio* (Moreman et al., 2017). However, our data suggests that BPS can instead have an inhibitory effect on adult physiological responses. These findings align with previous studies that also found BPS exposure to negatively impact swimming performance and metabolism (Pelaia et al., 2023; Wu and Seebacher, 2021). The reduction in physiological performance could be mechanistically explained by the disruption EDCs inflict on the signalling pathways of most hormones (Caporale et al., 2022; Toporova and Balaguer, 2020). Thyroid hormones play a significant role in regulating locomotor performance and energy metabolism (Little et al., 2013; Mullur et al., 2014). Bisphenols can bind to thyroid hormone receptors and thereby block or induce hormone signals and alter gene expression (Gentilcore et al., 2013; Zhang et al., 2019). Therefore, the effect of bisphenol exposure on thyroid function could be responsible for the observed decrease in swimming performance and metabolism. The combined decrease in metabolism and swimming performance resulting from BPS exposure can have important implications for many ecological and evolutionary processes, including migration, dispersal, foraging, and social interactions (Lailvaux and Husak, 2014; Norin and Metcalfe, 2019).

Circadian rhythms of melatonin release are determined by photoperiod regimes of both absolute light levels and wavelengths (Brüning et al., 2016; Sánchez-Vázquez et al., 2019). Thyroid and glucocorticoid hormones interact with melatonin release to dictate daily physiological cycles (Falcón et al., 2010; Jaikumar et al., 2020). ALAN can interfere with the natural circadian rhythms and act as an endocrine disruptor that has the potential to impact physiological responses (Ouyang et al., 2018; Russart and Nelson, 2018). Extending daylight hours can influence the thermal sensitivity of physiological responses (Le Roy and

Seebacher, 2020). In contrast, we found that extended and continuous light regimes alone do not affect the swimming performance and metabolism of *D. rerio*. Our results are somewhat unexpected and suggest that physiological responses remain consistent despite anthropogenic increases in ALAN.

Interactions between anthropogenically altered environments are not necessarily additive and can result in biological outcomes that are unpredictable from the individual effects (Caporale et al., 2022; Orr et al., 2020). The interactions of EDCs with environmental variables are important to consider as they can predict the impacts of plastic pollution in a changing world (Wu and Seebacher, 2021). The extent that EDCs interfere with physiological responses has been previously found to be temperature dependent (Wu and Seebacher, 2021). In this study, there was no interaction between photoperiod and BPS exposure.

Our study emphasises the inhibitory effects of BPS on physiological responses. BPS as an alternative to BPA in plastic manufacturing may need to be reconsidered due to its negative implications for ecological and evolutionary processes. Furthermore, our findings combined with previous studies highlight that the interactive effect between EDCs and climate change can vary depending on the altered environment. Predictions of the ecological impacts of increased pollution and future climate changes, therefore, require an understanding of endocrine disruptors in the context of environmental factors. Future studies should focus on identifying additional BPA alternatives for plastic manufacturing and experimentally assessing the functional consequences of EDCs across different environmental conditions.

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# Chapter 2

## Temperature variability has limited effect on phenotypic traits in ectotherms – a meta-analysis

In review as:

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# 1 | Abstract

Anthropogenic climate change is increasing the mean and variation in temperature globally. In theory, fluctuating temperatures should produce different phenotypic outcomes for ectotherms compared to stable temperatures. Hence, climate change can have qualitatively different impacts, and predictions underpinning conservation and management may need to incorporate both mean and variability in temperature. Our aim was to establish the current state-of-knowledge of how temperature fluctuations impact phenotypes of ectothermic animals. We conducted a meta-analysis of 143 studies (1492 effect sizes across 118 species) comparing the effects of constant and fluctuating temperature treatments with the same mean on individual phenotypic traits of ectotherms (behavioural, biochemical, gene expression, life-history, morphological, and physiological), and on population responses (e.g., survival). Most studies in the literature implemented diurnal temperature fluctuations (24 h cycles). Our analyses indicate that these periodic temperature fluctuations have surprisingly little effect overall on mean and variance of phenotypic traits. In certain contexts, temperature fluctuations appeared to be stressful: temperature fluctuations had a positive effect on "gene expression" in aquatic animals, which was mainly driven by increased hsp70. Fluctuating temperature also decreased longevity, and increased amplitudes of fluctuations had a negative effect on mean traits values, particularly on population-level traits in aquatic organisms. Variance in developmental time of aquatic animals was significantly reduced in fluctuating treatments. We conclude that in a conservation context, mean temperatures, and extreme events such as heatwaves are important to consider, but daily temperature fluctuations are of lesser importance. However, the effects of fluctuating temperatures may interact with other anthropogenic drivers such as pollution, and such interactions are an important area for future research.

## 2 | Introduction

The thermal environment influences many biological and ecological processes (Kingsolver and Buckley, 2017). Anthropogenic activities are increasing the magnitude and frequency of extreme climatic events (IPCC, 2021), leading to an increase in both mean and amplitude of temperature fluctuations (Angélil et al., 2017; Vázquez et al., 2017; Verheyen and Stoks, 2019; Xu et al., 2020). In the natural environment, these changes manifest as unpredictable thermal variability at different timescales (Mora et al., 2013; Wedler et al., 2023). Ectotherms are particularly susceptible to such thermal variability because their internal body temperatures are directly influenced by their surrounding environment (Chown et al., 2010; Huey and Kingsolver, 1989). Understanding responses of ectothermic organisms to thermal variability is crucial for predicting impacts of climate change that can inform policy and conservation decisions (IPCC, 2022).

Much of our knowledge regarding the phenotypic responses of ectotherms to thermal change originates from manipulative laboratory experiments conducted at different constant temperatures (Colinet et al., 2015; Massey and Hutchings, 2021; Noble et al., 2018). Different constant temperature treatments are often implemented to reduce ‘noise’, and this method provides an effective approach to compare phenotypic outcomes from exposure to different mean temperatures (Kroeker et al., 2020). However, in natural environments animals also experience thermal fluctuations around the mean at different timescales (Marshall et al., 2021; Stoks et al., 2017). It is important therefore to determine whether relatively short-term fluctuations around mean temperatures influence the phenotypic outcome compared to constant mean temperatures (Dowd et al., 2015; Kefford et al., 2022; Warner et al., 2023).

Responses of ectotherms to short-term temperature change are characterised by non-linear thermal performance curves (Huey and Kingsolver, 1989). Thermal performance curves vary between species and traits, but common features include an initial increase in performance as temperatures increase from a minimum critical temperature until maximum performance is reached at a thermal optimum. Beyond the thermal optimum there is a rapid decline in performance to a critical thermal maximum (Little and Seebacher, 2021; Sinclair et al., 2016). This non-linear phenotypic response to temperature variation indicates that thermal variability can be either beneficial or detrimental to organism performance (Denny, 2019; Marshall et al., 2021; Stoks et al., 2017). Jensen's inequality predicts that constant and fluctuating temperatures will produce different phenotypic outcomes depending on their location within the thermal performance curves (Denny, 2019; Foray et al., 2014; Pásztor et al., 2000). Despite these mathematical predictions, empirical evidence is equivocal (Raynal et al., 2022), leaving the relationship between thermal variability and phenotypic outcomes unresolved.

Our aim was to establish the current state-of-knowledge regarding the consequences of thermal variability for ectotherms. We conducted a meta-analysis to provide a quantitative synthesis of studies that addressed whether phenotypic traits (mean and variance) differed between exposure to constant versus fluctuating thermal environments with the same mean temperature. We determined whether there are differences between (i) ecological levels (individual and population), (ii) ecosystems (aquatic and terrestrial organisms), and (iii) exposure periods (acclimation or developmental treatments).

# 3 | Materials and Methods

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), were adhered to throughout this analysis. Question formulation, literature searching and screening were performed according to Foo et al., (2021).

## 3.1 | Systematic Literature Search and Screening

To establish the focus questions and assist in our literature search and screening processes, we followed the PECO (Population, Exposure, Comparator, Outcome) framework (Supplementary Materials 7.1; Morgan et al., 2018). On the 5$^{th}$ of April 2022, we conducted systematic literature searches of the Scopus, Web of Science and ScienceDirect databases. We searched for studies that investigated the impacts of fluctuating temperatures on ectothermic organisms. Our search included synonyms of 'plasticity', 'acclimation' and 'developmental effects' as we were explicitly looking for treatments imposed within the lifespan of an individual (Noble et al., 2018; Pottier et al., 2021). We added 'thermal' and 'temperature' to each search string to limit treatment manipulations to temperature (Barley et al., 2021; Noble et al., 2018). Synonyms for 'fluctuating' or 'varying' were then used to specify that one of the treatments must include a thermal variation. The exact search strings used in each database can be found in the Supplementary Materials 7.2.

The search of the three databases returned a total of 13,549 unique studies (Supplementary Materials 7.3). An initial screening of the abstracts was conducted following the exclusion and inclusion criteria (Supplementary Materials 7.4). Briefly, the study had to be in English and experimentally compared the effects of a constant and a fluctuating temperature treatment on an ectothermic organism in controlled-laboratory conditions. There

were 202 studies that matched these criteria. We conducted a full-text screening of these studies (Supplementary Materials 7.4).

For inclusion in the analysis, each study had to have: (a) treatments with identical housing and habituation periods; (b) fluctuating temperatures imposed during the lifespan of an individual (acclimation and developmental effects); (c) constant and fluctuating treatments with the same mean temperature and all other conditions identical; (d) report of a phenotypic response. Metrics of thermal performance such as critical thermal minima and maxima were excluded. Studies had to have all relevant statistical information (including sample size, mean, and error metrics). Principal components and factor loadings were excluded (Tarka et al., 2018). In total, there were 57 studies that adhered to both screening protocols and were included in the analysis. All authors conducted screening of the studies and completed both stages using Rayyan software (Ouzzani et al., 2016).

Next, we conducted forward and backward searches from the 57 studies selected above. Papers citing the original study (forward search), and papers that were cited in the original study (backward search), were identified in Scopus, Web of Science, ScienceDirect and Google Scholar on the 19$^{th}$ of October 2022. This process yielded a total of 3,932 unique studies that also underwent abstract and full-text screenings. As a result, an additional 87 studies were included. There was one species in the compiled data set with an unresolved phylogeny in the Open Tree of Life database, which required the exclusion of the study (Michonneau et al., 2016; Slein et al., 2023). At the conclusion of the systematic literature search and screening process, there were a total of 143 included studies (Supplementary Materials 7.5).

### 3.2 | Data Extraction

We extracted data (sample size, mean and error metric) manually from texts, tables, supplementary materials, and figures (using R package *metaDigitise* to extract data from figures; Pick et al., 2019) from papers in the final selection. Biological replicates were used as sample size, rather than technical replicates, to avoid non-independence (Wu and Seebacher, 2020). Constant temperature treatments were considered the 'control' that was compared to a fluctuating treatment. When there were multiple fluctuating treatments, each treatment was compared to its appropriate control (Raynal et al., 2022). For studies that conducted paired control and fluctuating treatments across multiple mean temperatures, all comparisons were recorded (Raynal et al., 2022). If there were multiple acute test temperatures at which phenotypic traits were measured, we selected those that coincided with the treatment mean (Seebacher et al., 2015). In addition, we recorded taxonomic information and habitat, experimental design (exposure type, length, and life-history stage), details of the fluctuation treatment (mean temperature, amplitude, number of fluctuations, fluctuation type and length), and phenotypic outcome (trait category, trait measurement, and units). All authors were involved in the data extraction process. To remove any conflict of interest, authors were not permitted to screen or extract studies they had a previous association with (Macartney et al., 2022).

### 3.3 | Data Processing and Transformations

Standard errors (SE; Equation 1) and 95% confidence internals (CI; Equation 2) were converted to standard deviations (SD), as required for effect size calculations (Quinn and Keough, 2002):

$$SD = SE \times \sqrt{n} \quad (1)$$

$$SD = \sqrt{n} \times \frac{CI_{High} - CI_{Low}}{3.92} \quad (2)$$

where n is sample size and $CI_{High}$ and $CI_{Low}$ represent the upper and lower 95% CIs respectively.

Data reported as percentages (e.g., survival) were transformed using Equation 3 and 4 for the means and variances respectively (Macartney et al., 2022):

$$\bar{x} = \arcsin\left(\sqrt{\bar{z}}\right) \quad (3)$$

$$SD_{\bar{x}} = \sqrt{\frac{SD_z^2}{4\bar{z}(1 - \bar{z})}} \quad (4)$$

where $\bar{x}$ and $SD_{\bar{x}}$ are the transformed means and SD, $\bar{z}$ and $SD_{\bar{z}}$ are the percentage means and SD. Means and variances that were reported on the natural log scale were transformed using Equation 5 and 6 respectively (Higgins et al., 2008):

$$\bar{x} = \exp\left(\bar{z} + \frac{SD_{\bar{z}}^2}{2}\right) \quad (5)$$

$$SD_{\bar{x}} = \sqrt{\left(\exp\left(SD_{\bar{z}}^2\right) - 1\right)\exp\left(2\bar{z} + SD_{\bar{z}}^2\right)} \quad (6)$$

where $\bar{x}$ and $SD_{\bar{x}}$ are the transformed means and SD, $\bar{z}$ and $SD_{\bar{z}}$ are the means and SD on the natural log scale. To avoid taking the natural log of zero during calculations, a constant of 0.5 was added to all means and SD (Rubin and Seebacher, 2022; Wu and Seebacher, 2020).

In cases where different terminology was used in different publications, we categorised developmental exposure period (Supplementary Materials 7.6), acclimation life-history stages (Supplementary Materials 7.7) and trait measurements (Supplementary Materials 7.8). Species names were adjusted to match the Open Tree of Life database (Michonneau et al., 2016), from which taxonomic information was retrieved. Amphibians were considered as aquatic organisms for the analysis.

### 3.4 | Effect Size Calculations

To investigate differences in the phenotypic response following constant and fluctuating temperature treatments we calculated the log response ratio (Equation 7) as the effect size (lnRR; Hedges et al., 1999; Lajeunesse, 2011) and the sampling variance (Equation 8) as in Nakagawa et al., (2017):

$$\mathrm{lnRR} = \ln\left(\frac{\bar{x}_F}{\bar{x}_C}\right) \quad (7)$$

$$v_{\mathrm{lnRR}} = \frac{SD_F^2}{n_F\bar{x}_F^2} + \frac{SD_C^2}{n_C\bar{x}_C^2} \quad (8)$$

where n is sample size, $\bar{x}$ is mean, and subscripts C and F denote the constant and fluctuating temperature treatments, respectively. For studies that had a low-sample size (n = 2) we applied the Lajeunesse Delta method (Equation 9 for effect size and Equation 10 for the sampling variance; Lajeunesse, 2015):

$$\ln RR^{\Delta} = \ln RR + \frac{1}{2}\left(\frac{SD_F^2}{n_F \bar{x}_F^2} + \frac{SD_C^2}{n_C \bar{x}_C^2}\right) \quad (9)$$

$$v_{\ln RR^{\Delta}} = v + \frac{1}{2}\left(\frac{SD_F^4}{n_F^2 \bar{x}_F^4} + \frac{SD_C^4}{n_C^2 \bar{x}_C^4}\right) \quad (10)$$

where notation is the same as Equation 7 and 8. lnRR was used rather than the standardised mean differences (SMD), as it holds no assumptions regarding heteroscedasticity between treatment groups and often provides greater statistical power (Macartney et al., 2022; Nakagawa et al., 2015; Yang et al., 2022). Effect sizes were calculated so that a negative result represents a negative impact of fluctuating temperatures on the organism (multiplying the lnRR of traits identified in Supplementary Materials 7.8 by -1; Hasik et al., 2023; Slein et al., 2023).

To investigate differences in phenotypic variation between the two treatments, we calculated and analysed the log coefficient of variation ratio (lnCVR; Equation 11 for effect size and Equation 12 for the sampling variance; Senior et al., 2020):

$$\ln CVR = \ln\left(\frac{CV_F}{CV_C}\right) + \frac{1}{2}\left(\frac{1}{n_F\text{-}1} - \frac{1}{n_C\text{-}1}\right) + \frac{1}{2}\left(\frac{SD_C^2}{n_C\bar{x}_C^2} - \frac{SD_F^2}{n_F\bar{x}_F^2}\right) \quad (11)$$

$$v_{\ln CVR} = \frac{SD_C^2}{n_C\bar{x}_C^2} + \frac{SD_C^4}{2n_C^2\bar{x}_C^4} + \frac{n_C}{(n_C\text{-}1)^2} + \frac{SD_F^2}{n_F\bar{x}_F^2} + \frac{SD_F^4}{2n_F^2\bar{x}_F^4} + \frac{n_F}{(n_F\text{-}1)^2} \quad (12)$$

where notation is the same as Equation 7 and 8.

### 3.5 | Statistical Analysis

All calculations, analyses, and figure production were completed with R.4.2.2 in RStudio version 2022.12.0 (using R Package *ggplot2* for figures; Wickham, 2016). All data and code are available at https://github.com/ClaytonStocker/Fluctuation_Meta (Supplementary Materials 7.9). Data are presented as both the mean effect sizes ± 95% CIs and as a percentage difference between treatments (exponentially transformed mean lnRR or lnCVR; Pustejovsky, 2018).

In the analyses, we included the following random effects to account for dependencies in our data set (Nakagawa et al., 2017; Sánchez-Tójar et al., 2020): Study ID, to account for several effect sizes coming from the same experiment; and Shared Animal, to account for multiple responses measured in the same individuals. To control for phylogenetic relatedness, a phylogenetic tree (Supplementary Materials 7.10) was created using the Open Tree of Life database (Michonneau et al., 2016), where polytomies were randomly resolved (using R Package *Ape*; Paradis and Schliep, 2019) and Grafen's method was used to calculate branch lengths (Power = 1; Grafen, 1989). For inclusion in the model, the phylogenetic tree was converted into a correlation/covariance matrix. Additionally, the repeated use of a species across studies was accounted for with a species-level random effect. We also added a trait-

level random effect to reduce the chance of certain traits dominating the analysis (excluding meta-regressions with specific phenotypic traits as the moderator). To estimate the final 'residual' variation we added an observation-level random effect (Nakagawa and Santos, 2012).

Frequentist models were employed, using the restricted maximum likelihood (REML) approach with a t-statistic containing adjusted degrees of freedom based on the number of studies, and an adjusted convergence threshold ($1e^{-8}$; Nakagawa et al., 2022b). Models were fitted with a modified sampling covariance matrix to account for instances where several fluctuating treatments were compared to the same constant temperature treatment (Noble et al., 2022).

### 3.6 | Multi-level Meta-analytic Models

Multi-level meta-analytic (MLMA) models were fitted using the *metafor* package in R (using the function *rma.mv*; Viechtbauer, 2010). Firstly, a MLMA model was implemented to estimate the overall meta-analytic mean of the complete data set. Subsequently, we conducted a number of subset analyses to estimate the meta-analytic means for population- and individual- level traits, in terrestrial and aquatic organisms, and after acclimation or developmental exposure (Figure 1). The definitions of included terms in each subset can be found in Table 1. From the MLMA models, the extended heterogeneity statistic ($I^2$; 2.d.p) was calculated to quantify unexplained variation after removing the known sampling variance. The total heterogeneity ($I^2_{Total}$) was partitioned to correspond with each random effect ($I^2_{Animal}$, $I^2_{Obs}$, $I^2_{Phylo}$, $I^2_{Species}$, $I^2_{Study}$, and $I^2_{Trait}$; Borenstein et al., 2021; Nakagawa and Santos, 2012). $I^2$ of 75% are considered high, 50% medium, and 25% small (Higgins et al., 2003). However, in multispecies meta-analysis it is not uncommon to achieve an $I^2$ that is greater than 90% (Senior et al., 2016).

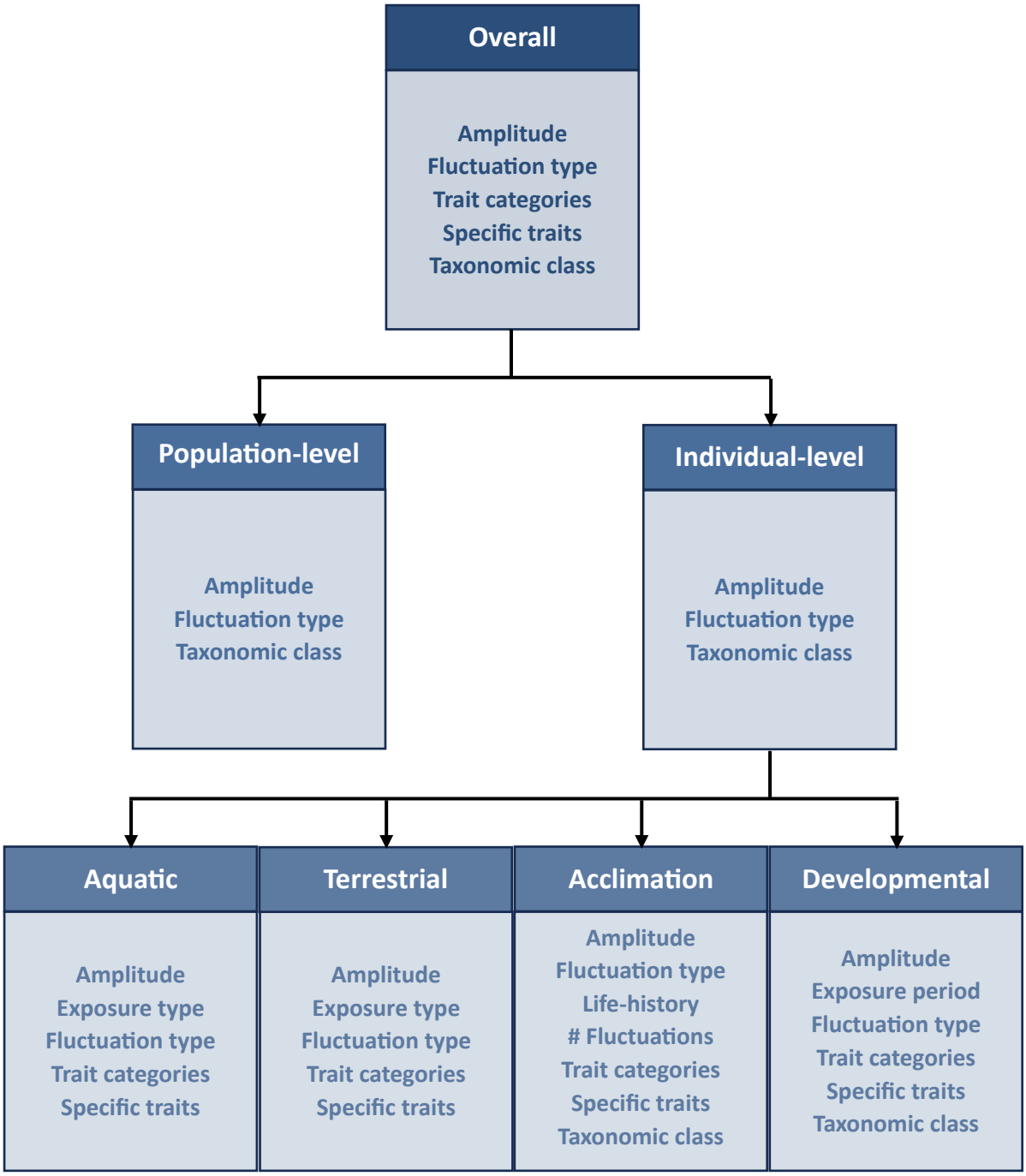


**Figure 1 The order of subset MLMA analyses and the corresponding meta-regression analyses.** White text = MLMA model. Coloured text = meta-regression model. "Amplitude" refers to the amplitude experienced during the fluctuation treatment. "Fluctuation type" denotes the thermal regime implemented in the experimental design (Figure 2). "Specific traits" and "Trait Categories" refer to the individual traits and categorised groups of traits measured following treatment exposure. "Taxonomic class" is the class for each experiment's

model species. “Exposure type” distinguishes between fluctuations that were imposed during developmental periods or acclimation treatments. Acclimation “Life-history” and Developmental “Exposure periods” refer to the life-history stage during treatment exposure. “Number of Fluctuations” is the treatment exposure duration divided by the period of each fluctuation. Definitions of MLMA and meta-regression terms are found in Table 1.

**Table 1 Definitions of included terms in each subset.** An outline of the requirements for the inclusion of effect sizes into each subset analysis.

| Subset Analyses | Definitions |
|---|---|
| **Population-level Traits** | Phenotypic traits measured for an entire population (e.g., Survival). |
| **Individual-level Traits** | Phenotypic traits measured in an individual (e.g., Food Consumption, Cortisol Levels, hsp70, Development Time, Length, Locomotor Performance etc.). |
| **Aquatic Organisms** | Organisms that spend the predominant amount of their lifecycle in aquatic ecosystems, including amphibians. |
| **Terrestrial Organisms** | Organisms that spend the predominant amount of their lifecycle in terrestrial ecosystems. |
| **Acclimation** | A treatment that does not encompass a significant period of development, and a reversible phenotypic trait is measured. This generally appears in an experimental design with a treatment and trait measurement within the same life-history stage. |
| **Developmental** | A treatment exposure that encompasses a significant developmental period. This generally appears in an experimental design with treatments and trait measurements across life-history stages. |

### 3.7 | Meta-regression Models

Meta-regression models were conducted using a variety of moderators that had the potential to influence the observed mean and variance effect size estimates. Categorical moderators that were explored include developmental exposure period (embryo, larva, pupa and juvenile), exposure type (acclimation and developmental), fluctuation type (sinusoidal, alternating, stepwise and stochastic; Figure 2), acclimation life-history stages (embryo, larva, pupa, juvenile and adult), phenotypic trait categories (behavioural, biochemical assay, gene expression, life-history, morphological and physiological), specific phenotypic traits (see Supplementary Materials 7.8 for full list of phenotypic traits), and taxonomic class (see Supplementary Materials 7.10 for phylogenetic tree). Levels within categorical moderators were only included if there were > 10 effect sizes (O'Dea et al., 2021). Additionally, we investigated several continuous moderators (Figure 1), including the amplitude of fluctuations (range = 1℃ – 45.5℃), and the number of fluctuations (treatment exposure duration/period of fluctuations; range = 4 – 546 fluctuations).

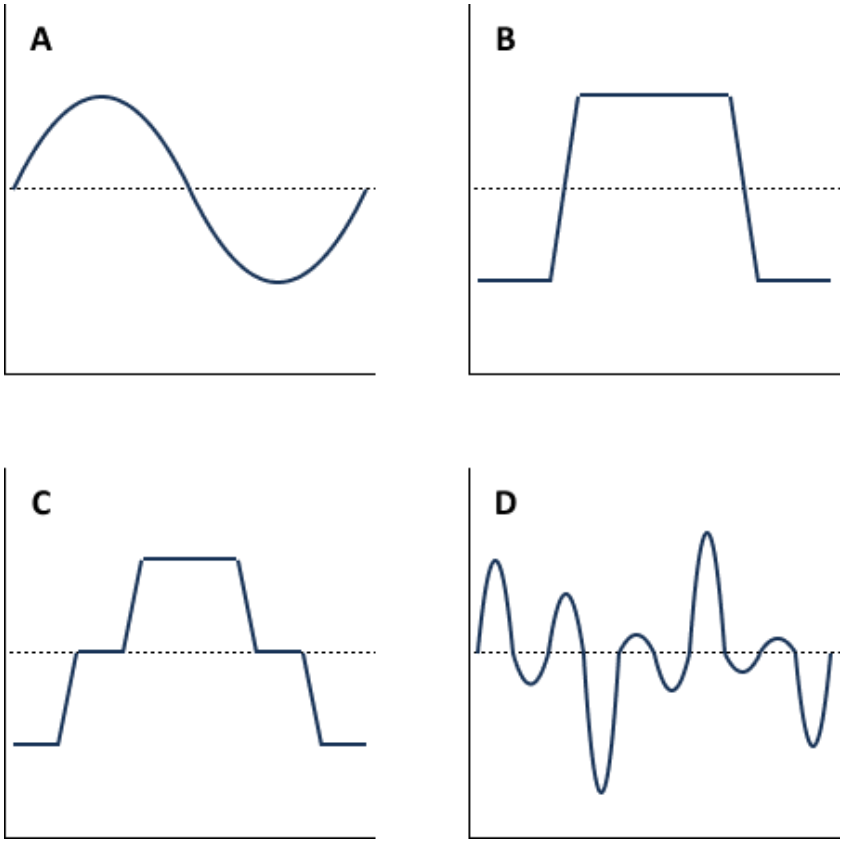


**Figure 2 Examples of different fluctuation types in the experimental designs of included studies.** Fluctuation patterns included sinusoidal regimes (A), alternating regimes (B), stepwise regimes (C), and stochastic regimes (D).

### 3.8 | Publication Bias and Sensitivity Analysis

To investigate significant publication bias in our results, we firstly conducted a visual inspection of funnel plots for indications of asymmetry (Sterne et al., 2005). The presence of a time-lag effect (or decline effect) was assessed by adding z-transformed publication year and precision (inverse of variance) as moderators to our overall MLMA model (Nakagawa et al., 2022a). A time-lag bias would be evident in our data sets if 95% confidence intervals of the slope were to exclude zero (Wu and Seebacher, 2020). The investigation into the publication bias of the lnRR data set found evidence of a time-lag bias, where mean phenotypic responses significantly increased in more recent years. There was no evidence of publication bias in our lnCVR data set (Supplementary Materials 7.11).

A sensitivity analysis was conducted to understand the 'robustness' of our results through estimating the influence of data points on our overall MLMA model (using the function *cooks.distance* in the R Package *metafor*; Viechtbauer, 2010). The results of the overall MLMA model were also compared to models fitted with a comparable effect size (Hedge's g; Gurevitch et al., 2000) and untransformed data (Knol et al., 2011; Macartney et al., 2022). The findings of the sensitivity analysis suggested that both the lnRR and lnCVR data sets were robust and free from significantly influential outliers (Supplementary Materials 7.11).

In addition to these analyses, robust variance estimations (using the function *robust* in the R package *metafor*; Viechtbauer, 2010) were investigated to understand the 'robustness' of the significant results after accounting for unknown non-independencies and complex within study covariances (Nakagawa et al., 2022b). Except for gene expression traits in the overall, aquatic, and developmental lnRR data sets, the significant results of our analyses did not hold true following the robust variance estimation (Supplementary Materials 7.11). This suggests that there is insufficient data available to support robust inference on these moderators and future experimental investigations are required to strengthen the model-based inferences reported in this study.

## 4| Results

### 4.1 | Are the mean phenotypic traits different in constant versus fluctuating temperatures?

The final data set included 1492 effect sizes from 143 studies (Supplementary Materials 7.5) across 118 unique species (Supplementary Materials 7.10). Overall, the means of the phenotypic traits in constant and fluctuating thermal environments were not significantly different (Figure 3). Total heterogeneity was high ($I^2_{Total}$ = 99.69%), with little to no variation being explained by the phylogeny ($I^2_{Phylo}$ = 0.00%), species ($I^2_{Species}$ = 0.00%), and phenotypic trait ($I^2_{Trait}$ = 1.57%). Together, shared animal ($I^2_{Animal}$ = 25.70%), observation ($I^2_{Obs}$ = 56.48%), and study ($I^2_{Study}$ = 15.94%) random effects explained most of the heterogeneity observed (heterogeneity statistics for each subset analyses in Supplementary Materials 7.12).

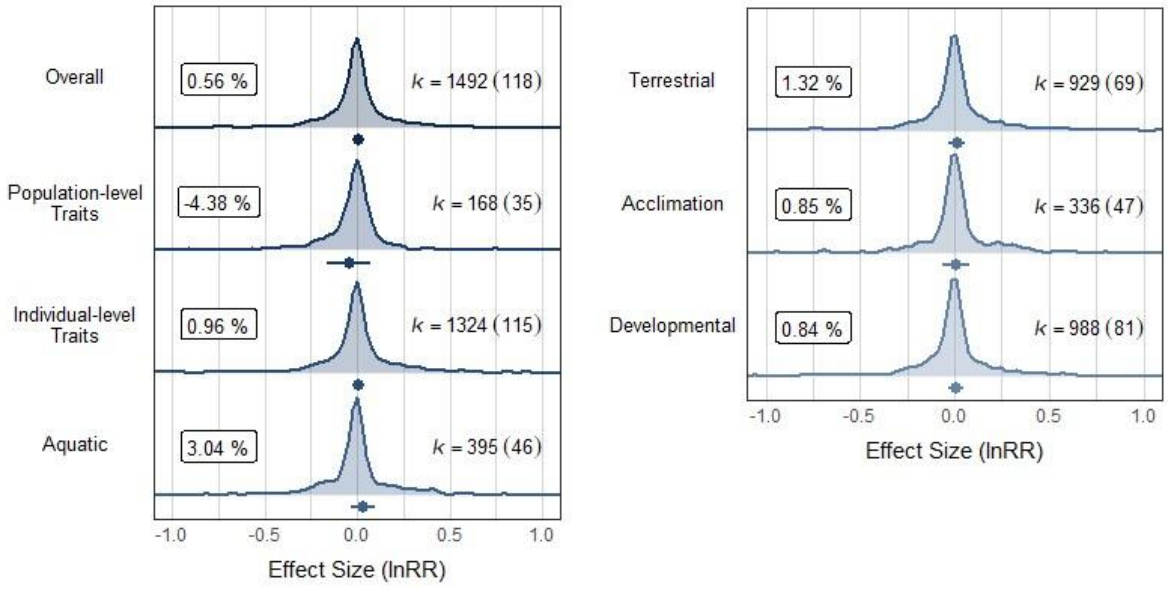


**Figure 3 Multi-level meta-analytic results for the overall data set and subsets.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

However, meta-regression analyses showed that measurements of gene expression were significantly different between the two thermal treatments (moderator = trait category; effect size estimate = 0.24; 95% CIs = [0.11, 0.38]; p = 0.0004; n = 50; Figure 4), driven mostly by the expression of the hsp70 gene (moderator = specific phenotypic trait; effect size estimate = 0.40; 95% CIs = [0.14, 0.66]; p = 0.0025; n = 12; Figure 5). The increase in gene expression in fluctuating treatments was significant for aquatic organisms (38.14%) and during developmental treatments (23.35%; Table 2). The longevity of an organism was significantly less in the fluctuating compared to constant temperature treatments (moderator = specific phenotypic trait; effect size estimate = -0.10; 95% CIs = [-0.17, -0.02]; p = 0.0092; n

= 93; Figure 5). Meta-regression analyses testing for the effect of taxonomic class (Figure 6) and fluctuation type (Figure 7) as moderators showed no significant differences between constant and fluctuating treatments.

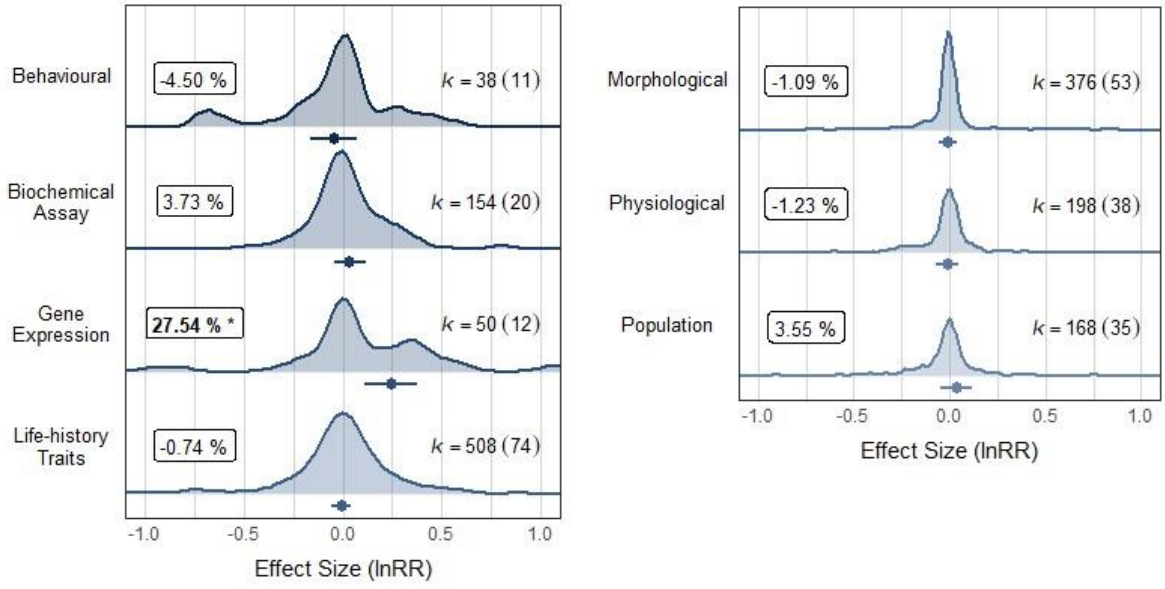


**Figure 4 Meta-regression results for the overall data set with phenotypic trait category as the moderator.** There was a significant increase in gene expression found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

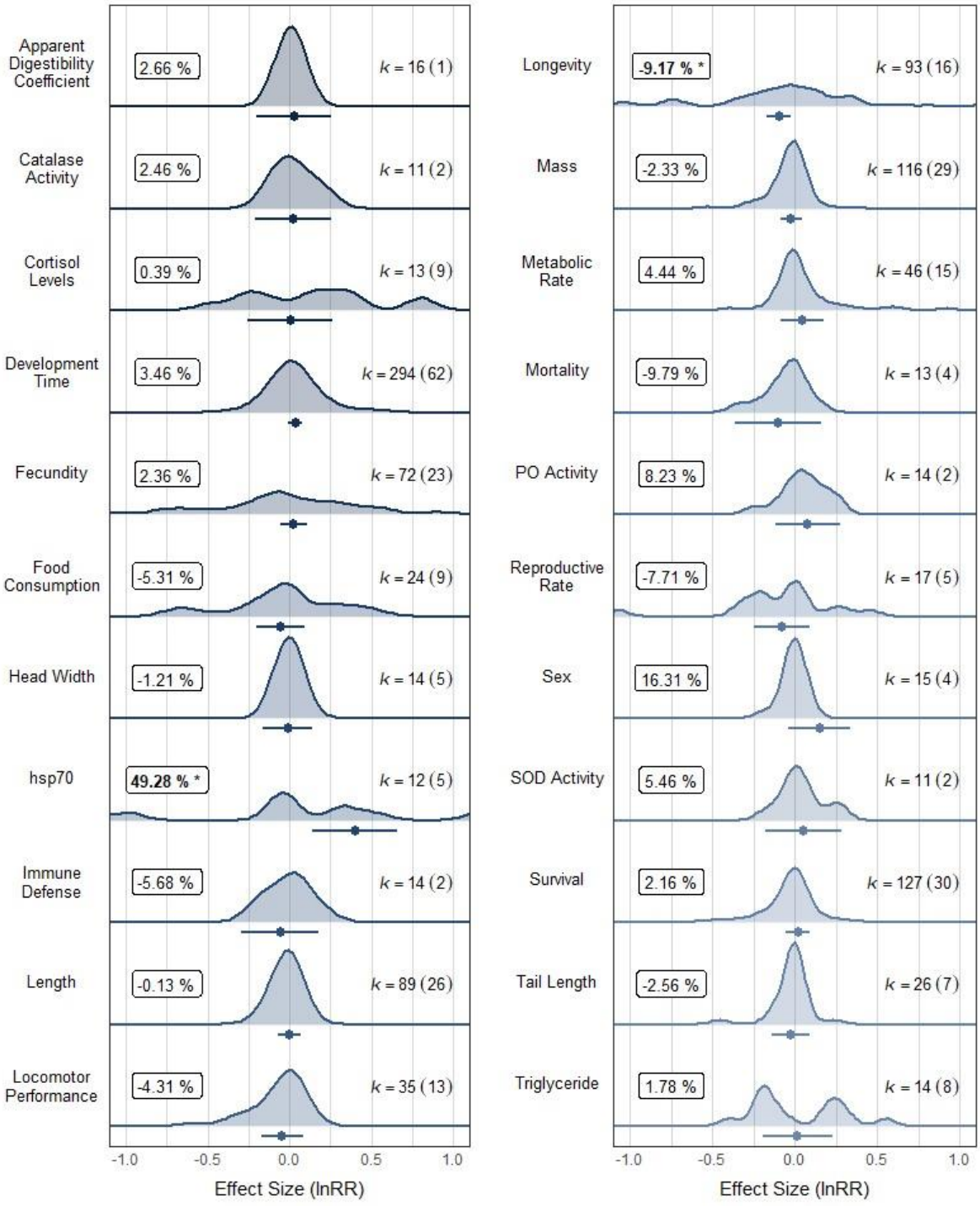


**Figure 5 Meta-regression results for the overall data set with specific phenotypic traits as the moderator.** There was a significant increase in the expression of the hsp70 mRNA and decrease in longevity found in the fluctuating temperature treatment, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual

effect sizes. Percentage labels are mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

**Table 2 Results of the subset meta-regression analyses that found significant differences in mean phenotypic trait values between the constant and fluctuating thermal treatments.** Effect size = lnRR.

| Subset | Moderator | Estimate | CI Low | CI High | p value |
|---|---|---|---|---|---|
| **Population-level** | **Fluctuation Amplitude** | -0.0289 | -0.0382 | -0.0195 | 0.0000 |
| **Aquatic** | **Alternating Regime** | 0.1002 | 0.0164 | 0.1841 | 0.0203 |
| | **Fluctuation Amplitude** | -0.0041 | -0.0080 | -0.0002 | 0.0416 |
| | **Food Consumption** | -0.1559 | -0.2988 | -0.0131 | 0.0333 |
| | **Gene Expression** | 0.3231 | 0.1996 | 0.4465 | 0.0000 |
| | **Mass** | -0.0900 | -0.1419 | -0.0381 | 0.0008 |
| **Developmental** | **Gene Expression** | 0.2099 | 0.0504 | 0.3694 | 0.0106 |

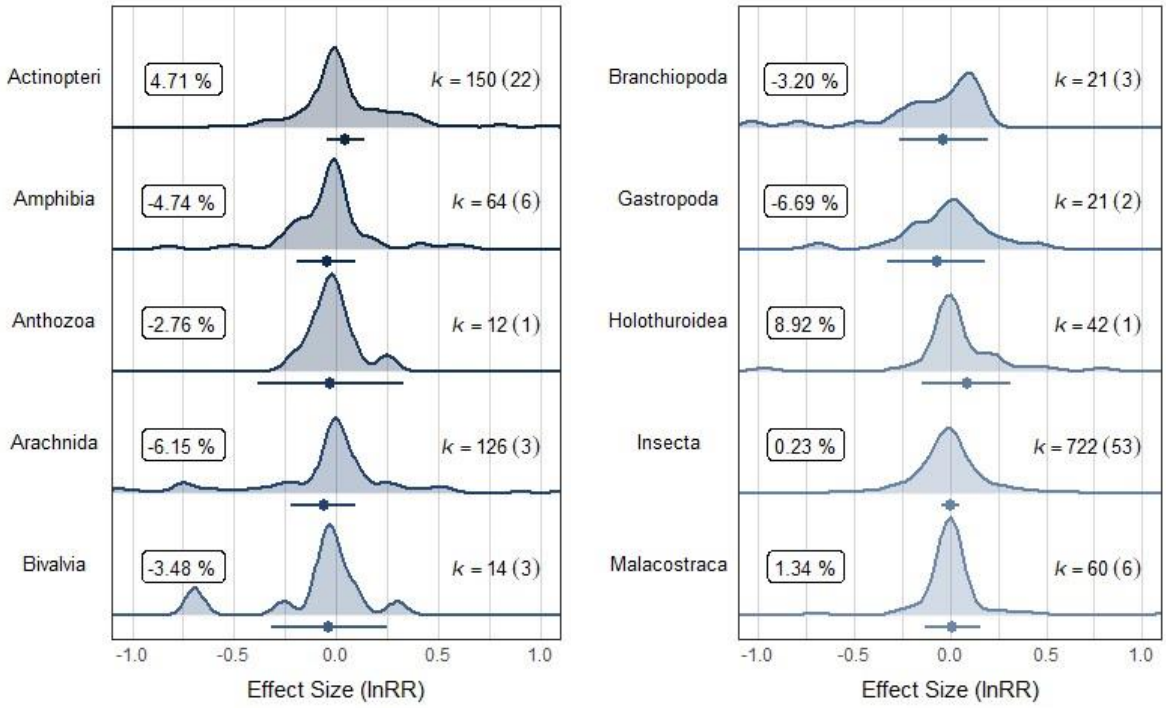


**Figure 6 Meta-regression results for the overall data set with taxonomic class as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

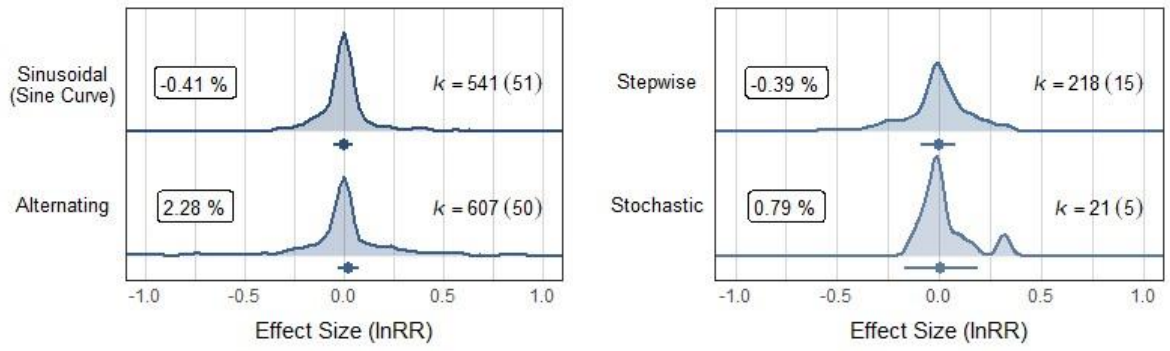


**Figure 7 Meta-regression results for the overall data set with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

The amplitude of the fluctuations had a significant effect on the mean phenotypic outcomes overall (moderator = fluctuation amplitude; effect size estimate = -0.01; 95% CIs = [-0.01, -0.00]; p = 0.0053; n = 1492;). As the amplitude of the fluctuations increased, the mean phenotypic trait values decreased relative to the stable treatment (Figure 8), particularly in population-level traits, and in aquatic organisms (Table 2). Unlike the overall data set, subset analyses of aquatic organisms (10.54%) indicated a significant increase in mean phenotypic trait values under the fluctuation type "alternating thermal regime" compared to stable temperatures (moderator = fluctuation type; Table 2). Additionally, there was a decrease in food consumption (-14.44%) and mass (-8.61%) in fluctuating compared to constant temperature treatments in aquatic organisms (moderator = specific phenotypic traits; Table 2). All other subset meta-regression analyses found no significant differences in mean

trait values between thermal treatments. Full results of all analyses are shown in the Supplementary Materials 7.13 and 7.14.

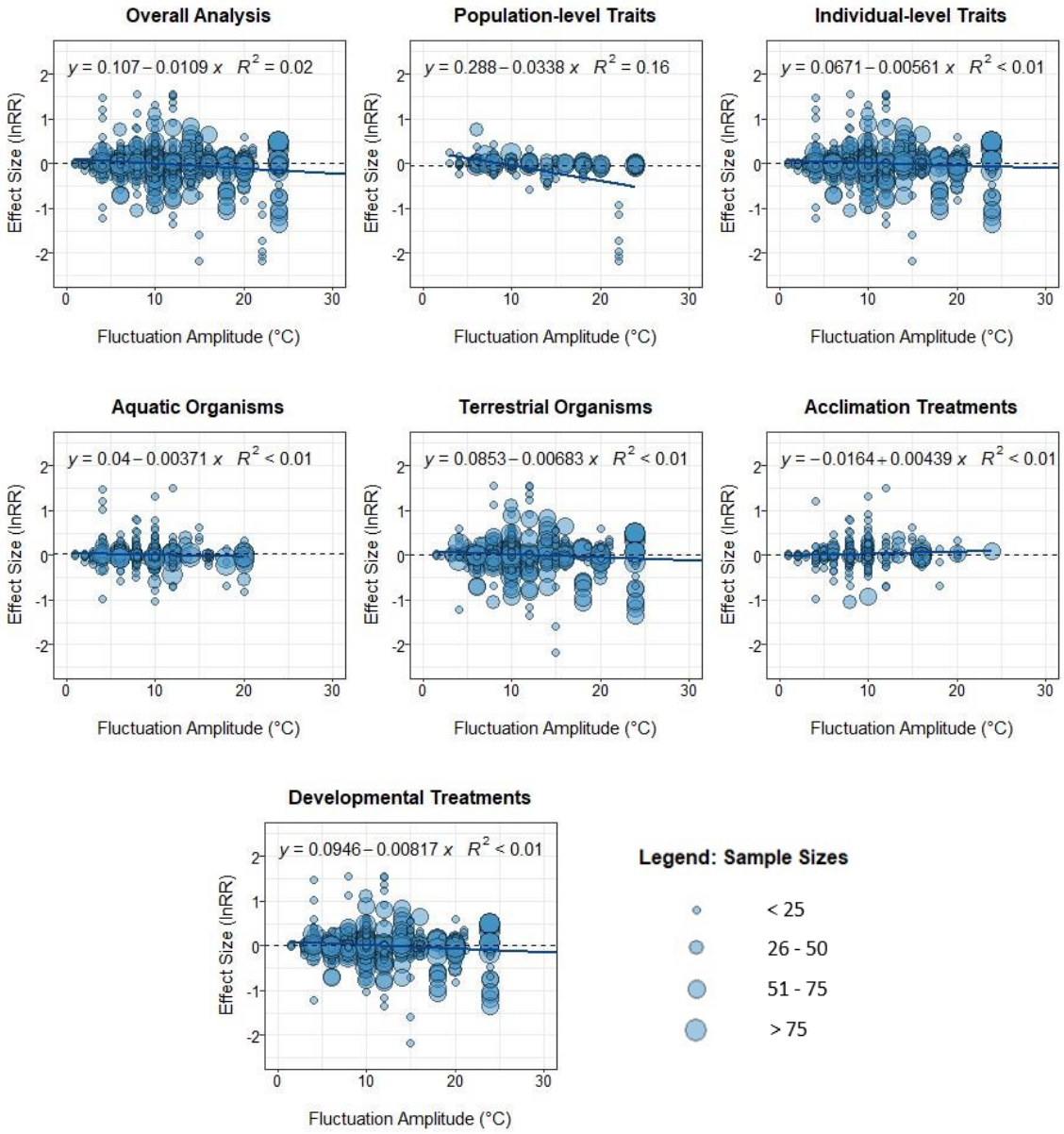


**Figure 8 Relationship between effect size (lnRR) and the amplitude of fluctuation.** The overall analysis and subset analyses for population-level traits and aquatic organisms showed that an increase in fluctuation amplitude was significantly associated with a decrease in mean phenotypic trait value. Dashed line = lnRR estimate from the overall MLMA model. 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.

### 4.2 | Are the variances of phenotypic traits different in constant versus fluctuating temperatures?

Both the overall and subset MLMA models indicated no significant differences in phenotypic trait variance between constant and fluctuating thermal environments (Figure 9). There was a medium level of total heterogeneity ($I^2_{Total}$ = 69.32%), with little to no variation being explained by the shared animal ($I^2_{Animal}$ = 0.00%), phylogeny ($I^2_{Phylo}$ = 0.00%), species ($I^2_{Species}$ = 0.00%), study ($I^2_{Study}$ = 4.86%), and phenotypic trait ($I^2_{Trait}$ = 0.00%) random effects. Most heterogeneity was explained by the final 'residual' variation ($I^2_{Obs}$ = 64.45%; heterogeneity statistics for each subset analyses in Supplementary Materials 7.12). However, meta-regression analyses found that the variance in life-history traits of aquatic organisms differed significantly between the two thermal treatments (moderator = trait category; effect size estimate = -0.16; 95% CIs = [-0.25, -0.07]; p = 0.0009; n = 67), mostly driven by developmental time (moderator = specific phenotypic trait; effect size estimate = -0.17; 95% CIs = [-0.28, -0.06]; p = 0.0037; n = 35). Life-history traits (Figure 10) and development time (Figure 11) were respectively, 14.87% and 15.64% less variable in fluctuating compared to constant aquatic temperatures. All other meta-regression analyses of the overall and subset data sets showed no significant effect on phenotypic trait variance (Supplementary Materials 7.15 and 8.16).

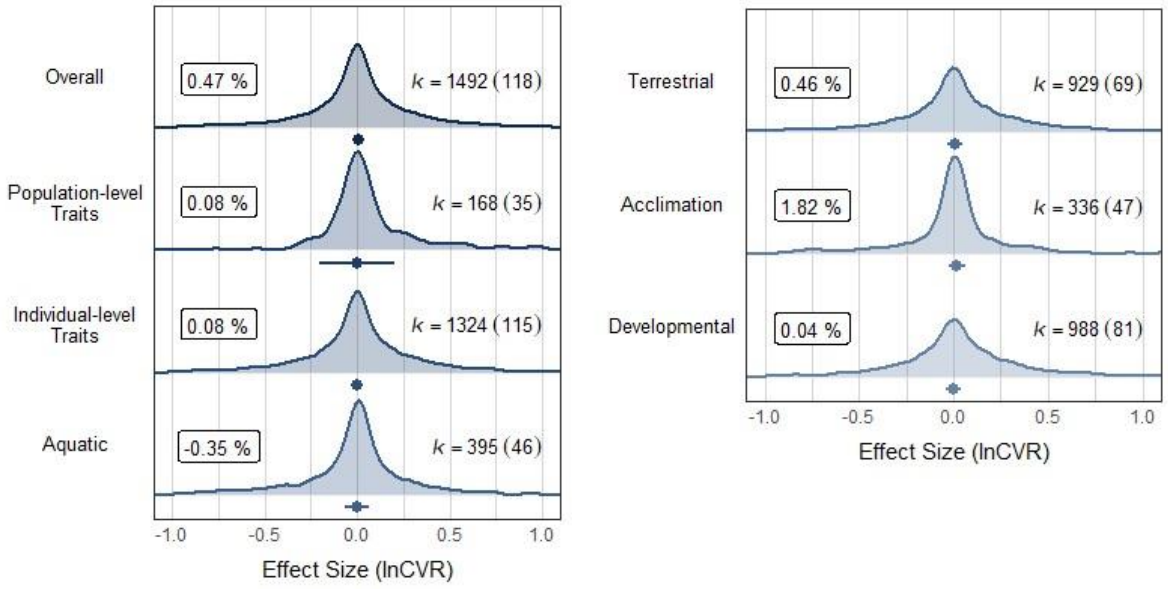


**Figure 9 MLMA results for the overall data set and subsets.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

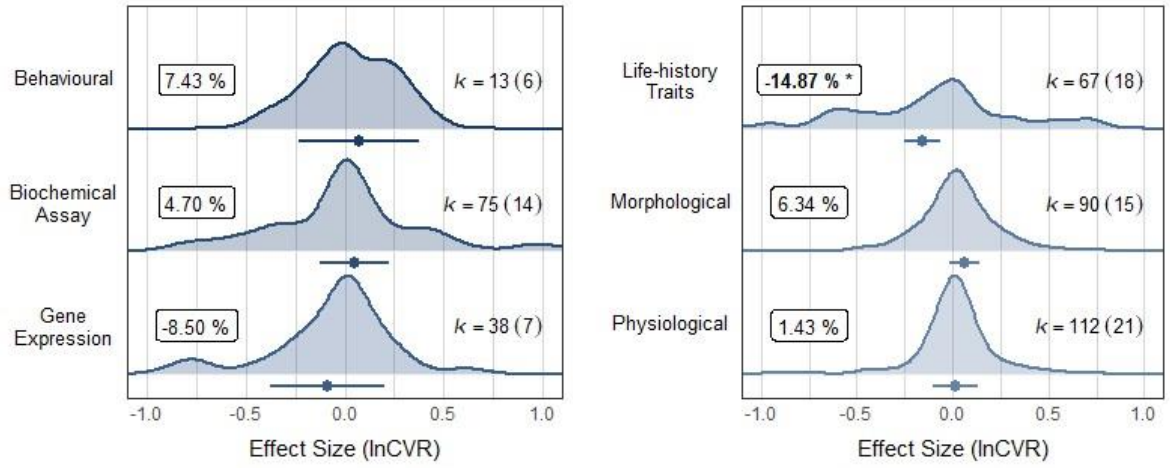


**Figure 10 Meta-regression results for the aquatic subset with phenotypic trait category as the moderator.** There was a significant decrease in the variance of life-history traits found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

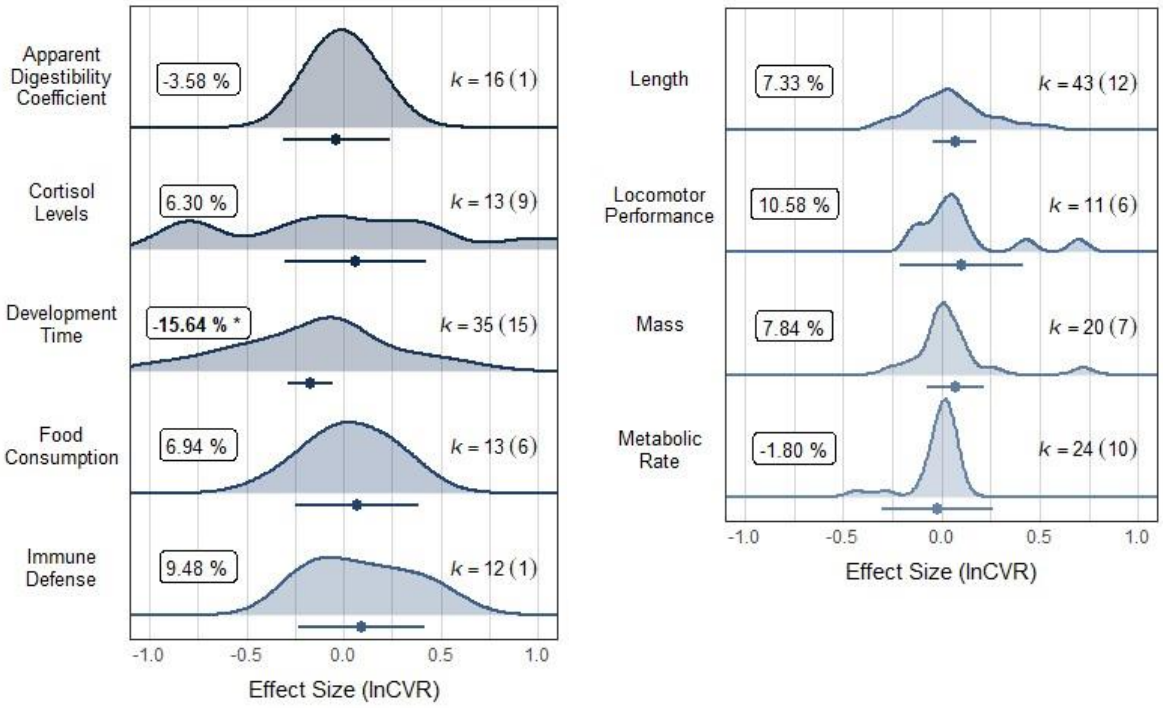


**Figure 11 Meta-regression results for the aquatic subset with specific phenotypic traits as the moderator.** There was a significant decrease in the variance of development time found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are mean lnCVR estimates transformed to a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

## 5 | Discussion

Our meta-analysis of the entire data set shows that fluctuating temperatures do not alter the mean or variance of phenotypic traits compared to constant temperatures. The data set contained experimental manipulations of thermal variation across a range of ecological levels, ecosystems, and types of exposure. Subset analyses of these more specific contexts also showed limited effects of temperature fluctuations on mean and variance of ectothermic traits. However, there are special cases where temperature fluctuations did make a difference and we discuss these below. Short term thermal variation such as heat waves can impact the phenotypes of ectotherms (Bauerfeind and Fischer, 2014; Breitenbach et al., 2020; Johansen et al., 2021), but our findings indicate that longer-term responses are not influenced by regular thermal fluctuations around the mean and are instead more likely to be determined by mean temperatures.

Climate change is predicted to manifest as an increase in temperature means, in fluctuation amplitudes, and in the frequency of extreme climatic events (IPCC, 2021; Pachauri et al., 2014; Vasseur et al., 2014). Manipulative experiments conducted at constant laboratory temperatures are the foundation of understanding how ectothermic organisms respond to thermal change (Colinet et al., 2015; Massey and Hutchings, 2021; Noble et al., 2018). The state-of-knowledge we established here supports the validity of constant temperature experiments, and the literature as a whole indicates that longer term (i.e., over 4+ diurnal cycles) phenotypic responses of ectotherms are primarily driven by mean environmental temperatures. Our analysis indicates that diurnal temperature fluctuations are filtered out so that they do not contribute to mean phenotypic responses. Regulating phenotypes in response to regular, short thermal fluctuations such as daily variation, could be disadvantageous for ectotherms by producing excessive phenotypic variation. Instead, a time lag could reflect a beneficial response that reduces phenotypic variation.

However, the nature of the temperature fluctuations can make a difference. Subset analyses for aquatic organisms showed that an alternating fluctuating thermal regime led to an increase in trait values compared to constant temperatures. Diurnal patterns in natural environments resemble most closely a sinusoidal regime in laboratory conditions (Bowden and Paitz, 2018; Marshall and Burgess, 2015; Spanoudis et al., 2015). It may be that the rate of environmental change is important in inducing phenotypic responses, and rates may differ between the different thermal regimes reported in the literature. If that were the case, it would have implications for interpretations and extrapolations of laboratory results, and for predicting the impacts of environmental change. A change in the phenotypic traits of ectothermic organisms can be induced by environmental cues, and phenotypic plasticity is a regulated response that can remodel phenotypes to mitigate the immediate impacts from environmental stresses (Loughland et al., 2021; Schulte, 2014; Seebacher et al., 2015). However, these reversible phenotypic adjustments can be dependent on the timescale between receiving the thermal signal and initiating a response, creating a time-lag effect (Gabriel, 2006). The induction of phenotypic change depends on the relationship between the rate of the environmental input and the timescale of the phenotypic adjustments (Loughland et al., 2022). Short periods of exposure to temperatures outside of the thermal mean can be insufficient to induce a change in the phenotypic traits (Gabriel, 2006).

Importantly, the magnitude of fluctuation matters for some traits (i.e., population-level traits). As the amplitude of the fluctuations increased, the mean phenotypic trait values decreased relative to the constant thermal treatment. Previous syntheses investigated thermal variation in various forms of acclimation (Slein et al., 2023), and in the developmental period of reptiles (Raynal et al., 2022). Both studies demonstrated that detrimental effects are exacerbated during warm temperatures with more extreme variability. The impact of absolute temperature depends on the evolutionary history and thermal sensitivity of each species

(Deutsch et al., 2008). However, the amplitude of temperature fluctuations around the same mean is relevant within species. We show that increasing amplitudes of fluctuations decreased mean phenotypic trait values relative to the constant thermal treatment. Extreme fluctuations could expose organisms to body temperatures near the critical thermal limits of their performance curves and therefore have a detrimental impact on their performance (Amarasekare and Savage, 2012; Schulte et al., 2011). Hence, the negative impacts of fluctuating temperatures are mediated by the intensity of the fluctuation and the resulting body temperatures in relation to the population- or species specific thermal performance curve (Amarasekare and Savage, 2012; Kellermann et al., 2019; Thompson et al., 2013).

Our analysis also showed that variance in phenotypic traits was not affected by thermal fluctuations. This result is commensurate with our suggestion that short term fluctuations are more or less filtered out so that there is no variation between individual responses to fluctuations. The only exception was developmental time in aquatic organisms which decreased somewhat in fluctuating environments. A decrease in developmental time may reflect greater energy efficiency and investment into growth by embryos in challenging environments (Pettersen et al., 2023; Vangansbeke et al., 2015). Even though the overarching trend in the literature shows that there is no effect of temperature fluctuations on phenotypes, there are some exceptions. We found that mRNA levels ("gene expression") increased in fluctuating temperatures (overall data set, aquatic organisms, and developmental exposure) which was mostly driven by the expression of the hsp70. Increased hsp70 is indicative of a stress response, and functions in facilitating protein re-folding and preventing protein degradation in stressful environments (Shamovsky and Nudler, 2008; Wu et al., 2019), while the continued over-expression or dysregulation of hsp70 can result in pathological conditions (Peraçoli et al., 2013; Wu et al., 2019).

Ectotherms are particularly susceptible to thermal variability and may experience thermal stress as a result of temperature fluctuations, particularly at higher amplitudes (Chown et al., 2010; Huey and Kingsolver, 1989), and increased hsp70 expression may stabilise phenotypes. Increased hsp70 in fluctuating environments can indicate thermal stress, which may also be reflected in the decrease in longevity (overall data set), food consumption (aquatic organisms) and body mass (aquatic organisms) we observed in the literature. Stressful fluctuations in temperature may influence the optimal allocation of resources that can impact life-history and physiological processes (McNamara and Buchanan, 2005; Monaghan et al., 2009). Trade-offs in resource allocation could compromise growth and ageing (Monaghan et al., 2009; Zera and Harshman, 2001), and these relationships are an important direction for future research.

The quantitative synthesis of the literature shows that the phenotypic response of ectotherms to thermal change is mostly driven by long-term mean temperatures, and only in certain contexts by the temperature fluctuations particularly at high amplitude. A major conclusion is that outside of extremes, temperature fluctuations do not impact the phenotypes of ectothermic organisms. Anthropogenic activities are expected to increase both temperature means, and the frequency of extreme climatic events (IPCC, 2021). We identified both as key mediators of the long-term responses of ectotherms. These findings provide crucial insight into the temporal relationship between phenotypic outcomes and fluctuating environments, with the potential to inform conservation approaches: mean temperatures and extreme events such as heat waves (Bauerfeind and Fischer, 2014; Breitenbach et al., 2020) are important to consider, but daily temperature fluctuations are of lesser importance.

These conclusions pertain to the comparative effects of stable and fluctuating temperatures on single traits. However, temperature fluctuations can modify biotic interactions (Stoks et al., 2017), and environmental temperature profiles can interact with other anthropogenic drivers such as pollution (Chang et al., 2022). The phenotypic effects of microplastics and of endocrine disrupting compounds, for example, are sensitive to environmental temperatures (Chang et al., 2022; Little and Seebacher, 2015; Wu and Seebacher, 2021), and their effects can be transmitted across generations epigenetically (Meng et al., 2021; Zhu et al., 2021). We suggest that future research should focus on these more complex effects of temperature fluctuations (Verheyen et al., 2019).

## 6 | References


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# 7 | Supplementary Materials

## 7.1 | PECO Framework

**Table S1 PECO framework table.** Populations, Exposures, Comparator and Outcomes that were the foundation for the focus question of the meta-analysis, the database search strings (Supplementary Materials 7.2) and the decision tree (Supplementary Materials 7.4).

| PECO | Description |
|---|---|
| **Population** | Ectothermic Metazoans |
| **Exposure** | Fluctuating temperature treatment with a mean matching the control (minimum of 1°C amplitudes; maximum of 1°C differences in means). |
| **Comparator** | Constant temperature treatment (maximum of 0.5°C amplitude). |
| **Outcome** | Any phenotypic trait measurement that:<br>- was measured at a similar temperature to the treatment mean (< 4°C difference acceptable);<br>- treatment conditions were maintained through to trait measurement;<br>- was not a metric of thermal performance (e.g., Critical Thermal Maximum, Critical Thermal Minimum and Temperature Preference). |

## 7.2 | Search Strings and Protocols

Scopus

TITLE-ABS-KEY((plasticity OR "plastic response" OR acclimat* OR "develop* effect") AND (thermal OR temperature*) AND (fluctuat* OR var* OR regime OR chang* OR irregular OR shift* OR inconstant OR diel OR unstable OR alter* OR vacill* OR oscill* OR period*) AND NOT (tolerance OR ctmax OR ctmin OR endotherm* OR bacter* OR fung* OR alga* OR unicellular OR protist OR microorganism OR micro-organism OR plant* OR photosyn* OR tree OR grass OR bird OR avia* OR aves OR mammal OR rodent OR rat OR mouse OR mice OR cattle OR livestock* OR cow OR pig OR sheep OR goat OR house OR rabbit OR chicken OR duck OR turkey OR cat OR dog OR human OR woman OR man OR "parental care" OR child* OR infant OR educat* OR industr* OR commerc* OR domest* OR cancer* OR medic*)) AND (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (LANGUAGE, "English")) AND (LIMIT-TO (SUBJAREA, "AGRI") OR LIMIT-TO (SUBJAREA, "BIOC") OR LIMIT-TO (SUBJAREA, "ENVI"))

Web of Science

TS = ((plasticity OR "plastic response" OR acclimat* OR "develop effect" OR "development effect" OR "developmental effect") AND (thermal OR temperature*) AND (fluctuat* OR var* OR regime OR chang* OR irregular OR shift* OR inconstant OR diel OR unstable OR alter* OR vacill* OR oscill* OR period*)) NOT TS = (tolerance OR ctmax OR ctmin OR endotherm* OR bacter* OR fung* OR alga* OR unicellular OR protist OR microorganism OR micro-organism OR plant* OR photosyn* OR tree OR grass OR bird OR avia* OR aves OR mammal OR rodent OR rat OR mouse OR mice OR cattle OR livestock* OR cow OR pig OR sheep OR goat OR house OR rabbit OR chicken OR duck OR turkey OR cat OR dog OR human OR woman OR man OR "parental care" OR child* OR infant OR educat* OR industr* OR commerc* OR domest* OR cancer* OR medic*)

Further refinements:

- Document Types: Articles
- Languages: English
- Not Web of Science Categories:
    - Metallurgy Metallurgical Engineering
    - Mechanics
    - Engineering Mechanical

ScienceDirect

* The search engine has a max of 8 Boolean connectors per field, so multiple searches were conducted.

** All searches were conducted in the 'Title, abstract or author-specified keyword' field.

**1.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (fluctuate OR regime OR diel) NOT (endotherm)

**2.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (vary OR change OR irregular) NOT (endotherm)

**3.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (shift OR inconsistent OR unstable) NOT (endotherm)

**4.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (alter OR vacillate OR oscillate) NOT (endotherm)

**5.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (period) NOT (endotherm)

**6.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (fluctuate OR regime OR diel) NOT (endotherm)

**7.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (vary OR change OR irregular) NOT (endotherm)

**8.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (shift OR inconsistent OR unstable) NOT (endotherm)

**9.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (alter OR vacillate OR oscillate) NOT (endotherm)

**10.** (“development effect" OR "developmental effect" OR "develop effect”) AND (thermal OR temperature) AND (period) NOT (endotherm)

Further refinements to the searches:

- Article Type: Research Articles
- Subject Areas:
    - Agricultural and Biological Sciences
    - Biochemistry, Genetics and Molecular Biology
    - Environmental Science

### 7.3 | PRISMA Flow Diagram

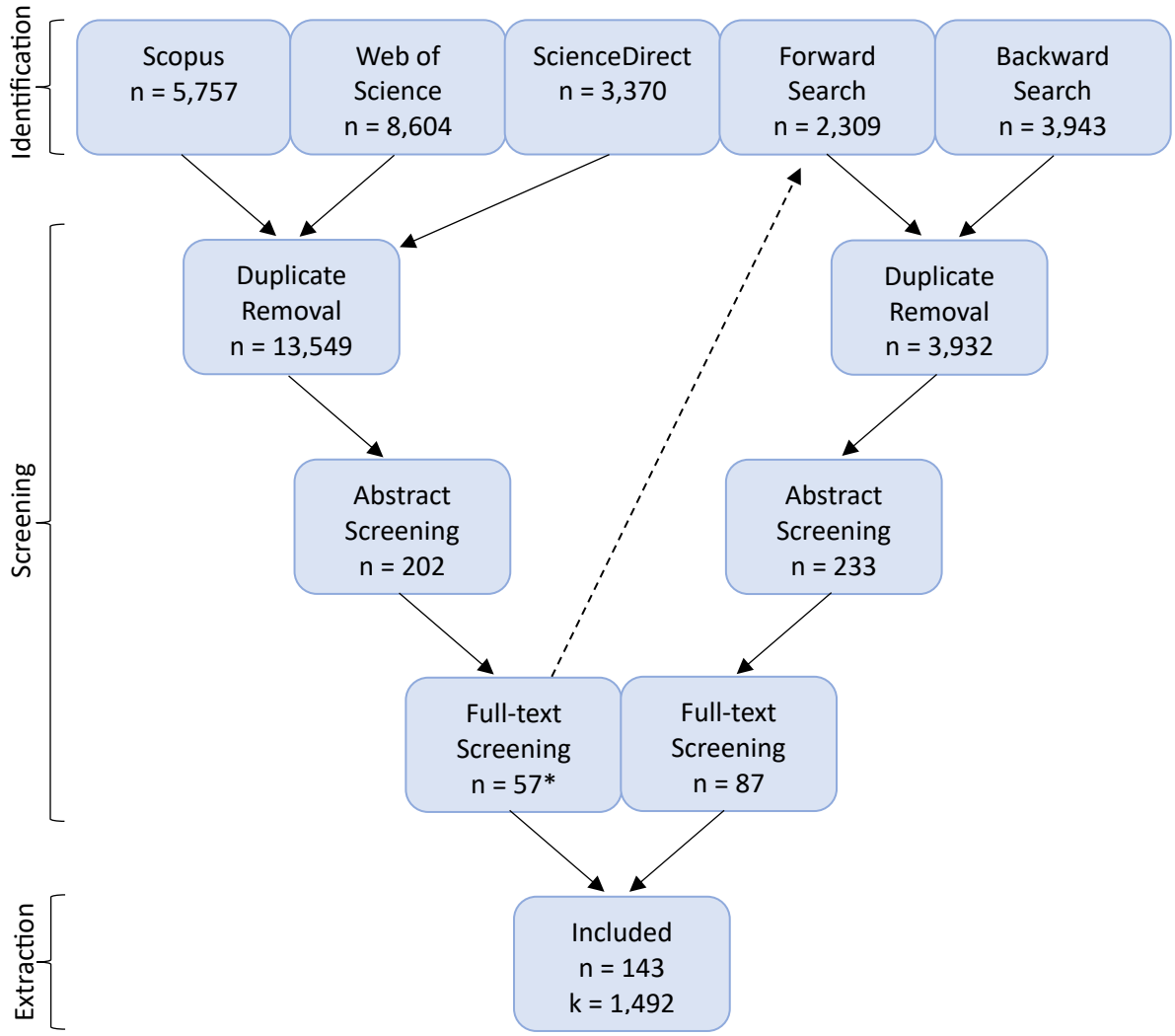


**Figure S1 PRISMA flow diagram for the systematic search and screening processes.** n = number of studies remaining after that respective stage. k = number of effect sizes. Dashed line = studies that the forward and backward search was conducted upon. * One study removed due to an unresolved phylogeny in the Open Tree of Life database during the data analysis stage.

## 7.4 | Decision Tree

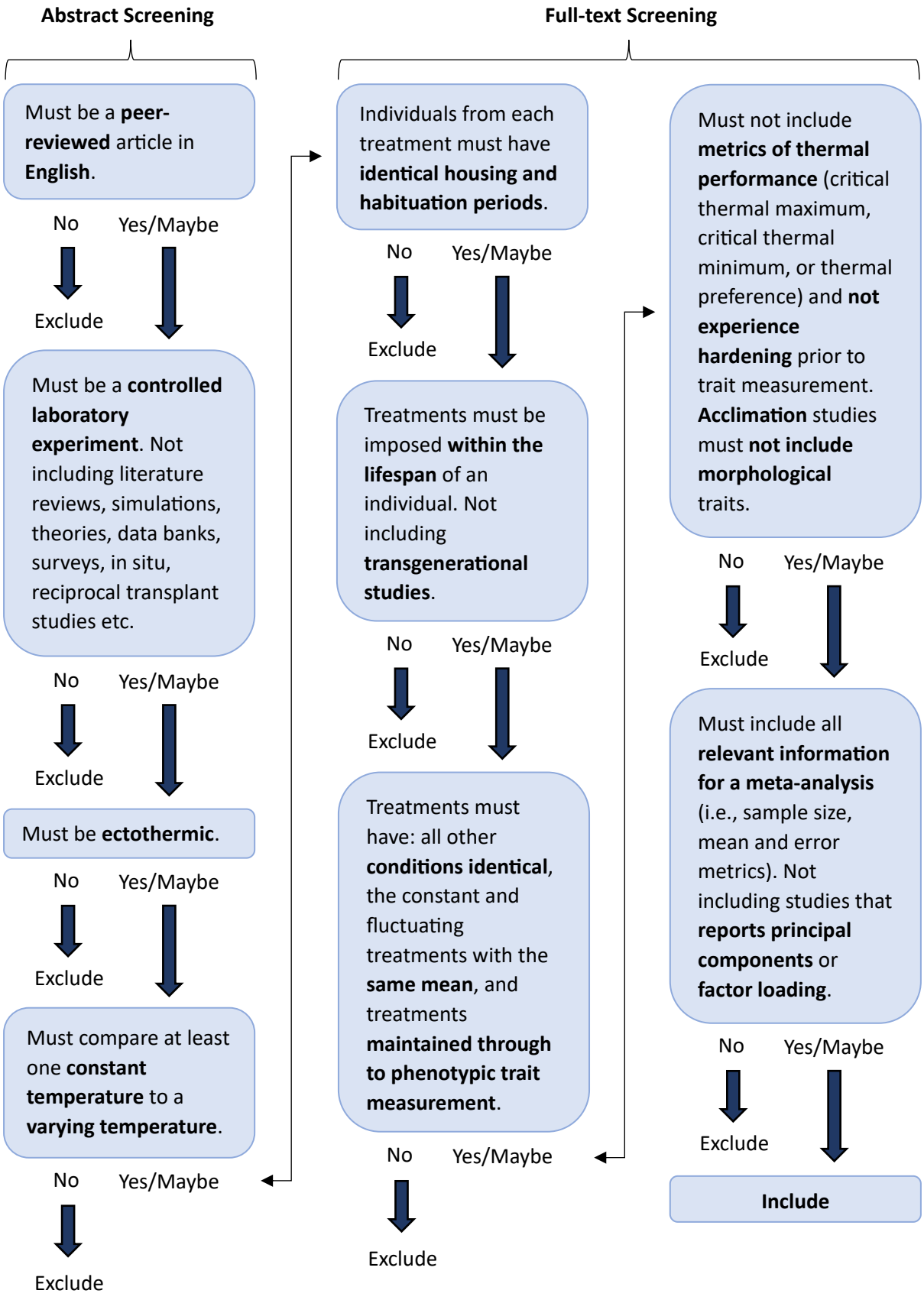


**Figure S2 Decision tree and inclusion criteria.**

## 7.5 | List of Included Studies


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### 7.6 | Developmental Exposure Period Adjustments and Categorisation

**Table S2 Embryo development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Embryo development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental treatment exposures.

| Embryo Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Egg Incubation | Egg Incubation | 41 | 40 | 464 |
| | Egg to Adult | 2 | 4 | 48 |
| | Egg to Larvae | 3 | 3 | 61 |
| | **Adjusted Record Totals** | **46** | **46** | **573** |
| Embryo Development | Embryo Development | 1 | 1 | 6 |
| | Embryo to Hatching | 1 | 1 | 13 |
| | Embryo to Hatchling | 1 | 1 | 5 |
| | Late Embryonic Phase to Yntema Stage 25 | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **4** | **4** | **25** |
| | **Category Totals** | **50** | **50** | **598** |

**Table S3 Larval development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Larva development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental treatment exposures.

| Larva Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Final Larvae Period | Final Larvae Period | 1 | 1 | 14 |
| Larvae Period | Larvae Development | 2 | 2 | 44 |
| | Larvae Period | 10 | 12 | 89 |
| | Larvae to Pupae | 1 | 1 | 2 |
| | Larval Development | 1 | 1 | 20 |
| | Larval Period | 9 | 8 | 97 |
| | **Adjusted Record Totals** | 23 | 20 | 252 |
| Stage 0 - 21 | Stage 0 - 21 | 1 | 1 | 6 |
| Stage 0 - 24 | Stage 0 - 24 | 1 | 1 | 6 |
| Third Instar Larvae Period | Third Instar Larvae Period | 1 | 1 | 3 |
| Until Gosner Stage 35 - 37 | Until Gosner Stage 35 - 37 | 2 | 3 | 30 |
| | **Category Totals** | **28** | **25** | **311** |

**Table S4 Pupal development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Pupa development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental treatment exposures.

| Pupa Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Pupae Period | Pupae Period | 2 | 4 | 14 |
| | Pupation | 1 | 1 | 15 |
| | **Adjusted Record Totals** | **3** | **5** | **29** |
| | **Category Totals** | **3** | **5** | **29** |

**Table S5 Juvenile development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Juvenile development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental treatment exposures.

| Juvenile Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Juvenile Period | Juvenile Period | 2 | 2 | 14 |
| | Newborn to Maturity | 1 | 1 | 14 |
| | Until Adult | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **4** | **4** | **30** |
| Neonate Period | Neonate to Maturity | 1 | 1 | 4 |
| | Neonate to Pupation | 1 | 2 | 8 |
| | **Adjusted Record Totals** | **2** | **3** | **12** |
| Nymphal Period | Nymphal to Adult | 1 | 1 | 8 |
| | **Category Totals** | **7** | **8** | **50** |

### 7.7 | Acclimation Life-history Stages Adjustments and Categorisation

**Table S6 Embryo stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Embryo stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures.

| Embryo Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Egg | Eggs | 2 | 2 | 10 |
| Embryo | Embryo | 1 | 1 | 1 |
| | **Category Totals** | **3** | **3** | **11** |

**Table S7 Larval stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Larva stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures.

| Larva Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Cocoon | Cocoons | 1 | 1 | 3 |
| Fourth Instar | Fourth Instar Nymphs | 1 | 1 | 3 |
| Larvae | Larvae | 8 | 6 | 75 |
| | Larval | 1 | 1 | 10 |
| | **Adjusted Record Totals** | **9** | **6** | **85** |
| Tadpole | Tadpole | 1 | 1 | 1 |
| | **Category Totals** | **12** | **9** | **92** |

**Table S8 Pupa stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Pupa stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures.

| Pupa Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Pupae | Pupa | 1 | 1 | 5 |
| | **Category Totals** | **1** | **1** | **5** |

**Table S9 Juvenile stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Juvenile stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures.

| Juvenile Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Juvenile | Juvenile | 10 | 8 | 97 |
| | Juveniles | 2 | 2 | 4 |
| | **Adjusted Record Totals** | **12** | **9** | **101** |
| | **Category Totals** | **12** | **9** | **101** |

**Table S10 Adult stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Adult stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures.

| Adult Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Adult | Adult | 9 | 10 | 36 |
| | Adults | 18 | 22 | 89 |
| | **Adjusted Record Totals** | **27** | **29** | **125** |
| | **Category Totals** | **27** | **29** | **125** |

### 7.8 | Phenotypic Trait Measurements Adjustments and Categorisation

**Table S11 Behavioural traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Behavioural traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Behavioural Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Exploration Speed | Exploration Speed | 1 | 1 | 2 |
| Food Consumption | Consumption | 1 | 1 | 2 |
| | Feeding Activity Rate | 1 | 1 | 1 |
| | Feeding Rate | 2 | 2 | 4 |
| | Food Consumed | 1 | 1 | 1 |
| | Food Consumption | 2 | 3 | 13 |
| | Frass | 1 | 1 | 1 |
| | Ingestion Rate | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **8** | **9** | **24** |
| Latency * | Latency | 1 | 1 | 2 |
| Righting Time * | Righting Time | 1 | 1 | 2 |
| | Roll-over Speed | 1 | 1 | 4 |
| | Rollover Speed | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **3** | **2** | **8** |
| Tortuosity * | Tortuosity | 1 | 1 | 2 |
| | **Category Totals** | **12** | **11** | **38** |

**Table S12 Biochemical assay traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Biochemical Assay traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Biochemical Assay | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| AChE Activity | AChE Activity | 1 | 1 | 2 |
| Alkadienes | Alkadienes | 1 | 1 | 1 |
| Alkanes | Dimethyl Alkanes | 1 | 2 | 2 |
| | n-Alkanes | 1 | 2 | 2 |
| | **Adjusted Record Totals** | **1** | **2** | **4** |
| Alkenes | Alkenes | 1 | 2 | 2 |
| Ash Content | Ash Content | 1 | 1 | 3 |
| Bleaching Index * | Bleaching Index | 1 | 1 | 2 |
| Calcium | Ca2+ | 1 | 1 | 2 |
| Catalase Activity | CAT Activity | 1 | 1 | 8 |
| | Catalase Activity | 1 | 1 | 3 |
| | **Adjusted Record Totals** | **2** | **2** | **11** |
| Chlorine | Cl- | 1 | 1 | 2 |
| Cortisol Levels | Cortisol | 2 | 8 | 11 |
| | Cortisol Concentrations | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **3** | **9** | **13** |
| CytP450 Activity | CytP450 Activity | 1 | 1 | 2 |
| Enzyme Activity | Enzyme Activity | 1 | 1 | 2 |

| | | | | |
|---|---|---|---|---|
| Erythrocytes | Erythrocytes | 1 | 1 | 1 |
| ETS Activity | ETS Activity | 1 | 1 | 8 |
| Fat Content | Fat Content | 1 | 1 | 8 |
| | Total Fat Content | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **1** | **10** |
| Glucose | Blood Glucose | 1 | 1 | 2 |
| | Glucose | 1 | 1 | 1 |
| | Glucose Concentrations | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **3** | **3** | **5** |
| Glycogen | Glycogen | 1 | 1 | 2 |
| Haemocytes | Number of Haemocytes | 1 | 1 | 4 |
| Haemoglobin | Haemoglobin | 1 | 1 | 1 |
| | Haemoglobin Content | 1 | 1 | 1 |
| | Hemoglobin | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **3** | **1** | **3** |
| Haemolymph Phenoloxidase Activity | Haemolymph Phenoloxidase Activity | 1 | 1 | 2 |
| Hexokinase Activity | Hexokinase Activity | 1 | 1 | 3 |
| Lipid Content | Crude Lipid Content | 1 | 1 | 3 |
| MDA Levels * | MDA | 1 | 1 | 8 |
| | MDA Levels | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **1** | **10** |
| Monounsaturated Fatty Acids | Monounsaturated Fatty Acids | 1 | 1 | 2 |
| NKA | NKA | 1 | 1 | 2 |
| PO Activity | PO Activity | 2 | 1 | 10 |
| | Total PO Activity | 1 | 1 | 4 |
| | **Adjusted Record Totals** | **3** | **2** | **14** |

| | | | | |
|---|---|---|---|---|
| Polyunsaturated Fatty Acids | Polyunsaturated Fatty Acids | 1 | 1 | 2 |
| Potassium | k+ | 1 | 1 | 2 |
| Protein Content | Crude Protein Content | 1 | 1 | 3 |
| Pyruvate Kinase Activity | Pyruvate Kinase Activity | 1 | 1 | 3 |
| Saturated Fatty Acids * | Saturated Fatty Acids | 1 | 1 | 2 |
| SOD Activity | SOD Activity | 1 | 1 | 8 |
| | Superoxide Dismutase Activity | 1 | 1 | 3 |
| | **Adjusted Record Totals** | **2** | **2** | **11** |
| Sodium | Na+ | 1 | 1 | 2 |
| Triglyceride | Triglyceride | 1 | 7 | 10 |
| | Triglyceride Content | 1 | 1 | 4 |
| | **Adjusted Record Totals** | **2** | **8** | **14** |
| | **Category Totals** | **18** | **20** | **154** |

**Table S13 Gene expression traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Gene Expression traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Gene Expression Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| agrp | agrp | 1 | 1 | 1 |
| cck8 | cck8 | 1 | 1 | 1 |
| COX | COX | 1 | 2 | 2 |
| crh | crh | 1 | 1 | 1 |
| CS | CS | 1 | 3 | 4 |
| Cyp19A1 | Cyp19A1 | 1 | 1 | 2 |
| Dmrt1 | Dmrt1 | 1 | 1 | 2 |
| Foxl2 | Foxl2 | 1 | 1 | 2 |
| gh | gh | 2 | 2 | 2 |
| ghrelin | ghrelin | 1 | 1 | 1 |
| grp | grp | 1 | 1 | 1 |
| hsp70 | hsp70 | 2 | 2 | 5 |
| | HSP70 | 1 | 1 | 2 |
| | Hsp70 | 2 | 2 | 5 |
| | **Adjusted Record Totals** | **5** | **5** | **12** |
| hsp90 | hsp90 | 1 | 2 | 4 |
| igf1 | igf1 | 1 | 1 | 1 |
| igf1a | igf1a | 1 | 1 | 1 |

| | | | | |
|---|---|---|---|---|
| igf2a | igf2a | 1 | 1 | 1 |
| Kdm6b | Kdm6b | 1 | 1 | 2 |
| LDH | LDH | 1 | 2 | 2 |
| mch1 | mch1 | 1 | 1 | 1 |
| mch2 | mch2 | 1 | 1 | 1 |
| npy | npy | 1 | 1 | 1 |
| orexin | orexin | 1 | 1 | 1 |
| Sox4 | Sox4 | 1 | 1 | 2 |
| Sox9 | Sox9 | 1 | 1 | 2 |
| | **Category Totals** | **9** | **12** | **50** |

**Table S14 Life-history traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Life-history traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Life-history Traits Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Brood Duration * | Brood Duration | 1 | 1 | 2 |
| | Brood Interval | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **2** | **2** | **3** |
| Brood Size | Brood Number | 1 | 1 | 2 |
| | Brood Size | 2 | 2 | 3 |
| | **Adjusted Record Totals** | **2** | **2** | **5** |
| Budding Rate | Budding Rate | 1 | 1 | 2 |
| Calcification Rate | Calcification Rate | 1 | 1 | 2 |
| Cannibalism | Cannibalism | 1 | 1 | 3 |
| Development Time * | Adult Development Time | 1 | 1 | 2 |
| | Age at Maturity | 1 | 1 | 2 |
| | Age at Reproduction | 1 | 1 | 2 |
| | Development Rate | 3 | 3 | 10 |
| | Development Time | 38 | 39 | 201 |
| | Development time | 1 | 1 | 6 |
| | Developmental Rate | 1 | 1 | 2 |
| | Developmental time | 1 | 3 | 6 |
| | Developmental Time | 1 | 1 | 2 |

| | | | | |
|---|---|---|---|---|
| | Feeding Instar Duration | 1 | 1 | 1 |
| | First Brood | 1 | 1 | 1 |
| | First Instar Development Time | 1 | 1 | 2 |
| | Gosner Stage | 1 | 1 | 5 |
| | Hatching Time | 1 | 1 | 1 |
| | Incubation Duration | 1 | 1 | 4 |
| | Incubation Length | 1 | 1 | 2 |
| | Incubation Period | 2 | 2 | 4 |
| | Larvae Development Time | 1 | 1 | 2 |
| | Larvae Duration | 1 | 2 | 4 |
| | Larval Development | 2 | 2 | 8 |
| | Ovisposition Period | 1 | 1 | 2 |
| | Post Feeding Development Time | 1 | 1 | 2 |
| | Pre-Adult Period | 1 | 1 | 3 |
| | Preoviposition Period | 1 | 1 | 2 |
| | Pupae Development Time | 2 | 2 | 4 |
| | Pupal Development | 1 | 1 | 4 |
| | Pupal Development Time | 1 | 3 | 5 |
| | Reproductive Instar Duration | 1 | 1 | 1 |
| | Second Instar Development Time | 1 | 1 | 2 |
| | Third Instar Development Time | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **62** | **62** | **294** |
| Egg Production | Egg Production | 1 | 1 | 1 |
| Fecundity | Fecundity | 18 | 22 | 70 |
| | Lifetime Fecundity | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **19** | **23** | **72** |
| Gonotrophic Cycles | Gonotrophic Cycles | 1 | 1 | 2 |

| | | | | |
|---|---|---|---|---|
| Individual Growth Rate | Individual Growth Rate | 5 | 4 | 10 |
| Longevity | Adult Longevity | 1 | 1 | 10 |
| | Lifespan | 2 | 2 | 4 |
| | Longevity | 14 | 13 | 79 |
| | **Adjusted Record Totals** | **17** | **16** | **93** |
| Ovarian Development Grade | Ovarian Development Grade | 1 | 1 | 2 |
| Reproductive Rate | Life-time Reproductive Output | 1 | 1 | 1 |
| | Mating Frequency | 1 | 1 | 2 |
| | Mating Percentage | 1 | 1 | 2 |
| | Net Reproductive Rate | 1 | 1 | 1 |
| | Ovipositing | 1 | 1 | 5 |
| | Reproductive Output | 1 | 1 | 6 |
| | **Adjusted Record Totals** | **5** | **5** | **17** |
| Retained Unfertilized Eggs * | Retained Unfertilized Eggs | 1 | 1 | 1 |
| Spermatophore Production | Spermatophore Production | 1 | 1 | 1 |
| | **Category Totals** | **76** | **74** | **508** |

**Table S15 Morphological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Morphological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Morphological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Abdomen Length | Abdomen Length | 1 | 1 | 6 |
| Background brightness of eyespot | Background brightness of eyespot | 1 | 1 | 4 |
| Carapace Length | Carapace Length | 1 | 1 | 2 |
| Carapace Width | Carapace Width | 1 | 1 | 2 |
| Collar Volume | Collar Volume | 1 | 1 | 3 |
| Femur Length | Femur Length | 1 | 1 | 1 |
| Forelimb Length | Forelimb Length | 2 | 2 | 8 |
| Forewing Length | Forewing Length | 1 | 2 | 8 |
| Forewing Spot Area | Forewing Spot Area | 1 | 1 | 4 |
| Head Depth | Head Depth | 1 | 1 | 3 |
| Head Length | Head Length | 1 | 1 | 6 |
| Head Width | Head Width | 5 | 5 | 14 |
| Hindlimb Length | Hind Limb Length | 1 | 1 | 2 |
| | Hindlimb Length | 1 | 1 | 6 |
| | **Adjusted Record Totals** | **2** | **2** | **8** |
| Hindwing Band | Hindwing Band | 1 | 2 | 8 |
| Hindwing Band Area | Hindwing Band Area | 1 | 1 | 4 |
| Hindwing Spot Area | Hindwing Spot Area | 1 | 1 | 4 |

| | | | | |
|---|---|---|---|---|
| Length | Body Length | 1 | 1 | 1 |
| | Body Size | 2 | 2 | 4 |
| | Hatching Length | 1 | 3 | 3 |
| | Length | 10 | 10 | 31 |
| | Size | 3 | 4 | 19 |
| | Snout Vent Length | 2 | 2 | 11 |
| | Snout-vent Length | 5 | 5 | 14 |
| | Total Length | 1 | 3 | 6 |
| | **Adjusted Record Totals** | **24** | **26** | **89** |
| Lip Volume | Lip Volume | 1 | 1 | 3 |
| Mass | Body Mass | 4 | 4 | 9 |
| | Final Weight | 1 | 1 | 1 |
| | Mass | 23 | 20 | 80 |
| | Weight | 3 | 7 | 26 |
| | **Adjusted Record Totals** | **31** | **29** | **116** |
| Plastron Colouration | Plastron Colouration | 1 | 1 | 2 |
| Plastron Length | Plastron Length | 3 | 3 | 8 |
| Relative eyespot size | Relative eyespot size | 1 | 1 | 4 |
| Tail Length | Tail length | 7 | 7 | 26 |
| Thorax Size | Thorax Size | 1 | 1 | 4 |
| Tibia Size | Tibia Size | 1 | 1 | 1 |
| Total Boutons | Total Boutons | 1 | 1 | 3 |
| Wing Area | Wing Area | 1 | 1 | 4 |
| Wing Aspect | Wing Aspect | 1 | 1 | 4 |
| Wing Centroid | Wing Centroid | 1 | 1 | 2 |
| Wing Length | Wing length | 2 | 2 | 7 |
| Wing Load | Wing Load | 1 | 1 | 4 |

| | | | | |
|---|---|---|---|---|
| Wing Size | Wing Size | 1 | 3 | 6 |
| Yolk Dry Mass | Yolk Dry Mass | 1 | 1 | 1 |
| Yolk Free Mass | Yolk Free Mass | 1 | 1 | 1 |
| Yolk Sac Volume | Hatching Yolk Sac Volume | 1 | 3 | 3 |
| | Yolk Sac Volume | 1 | 3 | 3 |
| | **Adjusted Record Totals** | **1** | **3** | **6** |
| | **Category Totals** | **53** | **53** | **376** |

**Table S16 Physiological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Physiological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Physiological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Apparent Digestibility Coefficient | Apparent Digestability Coefficient | 2 | 1 | 16 |
| Byssal Thread Regeneration | Byssal Thread Regeneration | 1 | 1 | 2 |
| Byssal Thread Strength | Byssal Thread Strength | 1 | 1 | 2 |
| Cellular Energy Allocation | Cellular Energy Allocation | 1 | 1 | 8 |
| Desiccation Tolerance | Desiccation Tolerance | 1 | 1 | 2 |
| Energy Content | Energy Content | 1 | 1 | 3 |
| Erythrocytic Haemoglobin | Erythrocytic Haemoglobin | 1 | 1 | 1 |
| Erythrocytic Volume | Erythrocytic Volume | 1 | 1 | 1 |
| Excretion Rate | Excretion Energy | 1 | 1 | 4 |
| | Excretion Rate | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **2** | **2** | **5** |
| Exuvia Energy | Exuvia Energy | 1 | 1 | 4 |
| Faeces Energy | Faeces Energy | 1 | 1 | 4 |
| Feeding Efficiency | Feed Conversion Ratio | 1 | 1 | 1 |
| | Feeding Efficiency | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **2** | **2** | **2** |
| Filtration Rate | Filtration Rate | 1 | 1 | 2 |
| Flight Distance | Flight Distance | 1 | 1 | 4 |

| | | | | |
|---|---|---|---|---|
| Flight Velocity | Flight Velocity | 1 | 1 | 4 |
| Haematocrit | Blood Haematocrit | 1 | 1 | 2 |
| | Haematocrit | 1 | 1 | 1 |
| | Haematocrit Content | 1 | 1 | 1 |
| | Hematocrit | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **4** | **2** | **5** |
| Heart Rate | Cardiac Rate | 1 | 1 | 1 |
| | Heart Rate | 2 | 2 | 4 |
| | **Adjusted Record Totals** | **3** | **3** | **5** |
| Immune Defense | Antibacterial Activity | 1 | 1 | 2 |
| | Immune Defense | 1 | 1 | 12 |
| | **Adjusted Record Totals** | **2** | **2** | **14** |
| Locomotor Performance | Absolute Speed | 1 | 1 | 2 |
| | Average Speed | 1 | 1 | 4 |
| | Critical Swimming Speed | 1 | 1 | 2 |
| | Locomotor Performance | 1 | 1 | 1 |
| | Maximal Swimming Speed | 1 | 1 | 1 |
| | Running Speed | 1 | 1 | 1 |
| | Sprint Speed | 3 | 3 | 12 |
| | Swimming Performance | 3 | 4 | 6 |
| | Swimming Speed | 1 | 1 | 1 |
| | Walking Speed | 2 | 2 | 5 |
| | **Adjusted Record Totals** | **13** | **13** | **35** |
| Maximum Excitation Pressure | Maximum Excitation Pressure | 1 | 1 | 2 |

| | | | | |
|---|---|---|---|---|
| Metabolic Rate | Carbon Dioxide Production | 1 | 1 | 2 |
| | Metabolic Rate | 4 | 4 | 7 |
| | Metabolic rate | 1 | 1 | 2 |
| | Oxygen Consumption | 6 | 7 | 15 |
| | Oxygen Consumption Rate | 1 | 1 | 6 |
| | Oxygen Utilisation | 1 | 1 | 1 |
| | Respiration Energy | 1 | 1 | 4 |
| | Respiration Rate | 1 | 1 | 9 |
| | **Adjusted Record Totals** | **15** | **15** | **46** |
| Muscular Strength | Muscular Strength | 1 | 1 | 2 |
| Osmolality | Osmolality | 1 | 1 | 2 |
| PHA Response | PHA Response | 1 | 1 | 2 |
| Plasma Protein Levels | Plasma Protein Levels | 1 | 1 | 2 |
| Red Cell Size | Red Cell Size | 1 | 1 | 1 |
| Starvation Tolerance | Starvation Resistance | 1 | 3 | 6 |
| | Starvation Tolerance | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **4** | **8** |
| Stroke Volume | Stroke Volume | 1 | 1 | 1 |
| Swelling | Swelling | 1 | 1 | 2 |
| Travel Duration | Flight Duration | 1 | 1 | 4 |
| | Maximal Length | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **2** | **6** |
| Ventilatory Flow | Ventilatory Flow | 1 | 1 | 1 |
| Ventilatory Rate | Ventilatory Rate | 1 | 1 | 1 |
| Water Content | Water Content | 1 | 1 | 3 |
| | **Category Totals** | **46** | **38** | **198** |

**Table S17 Population-level traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Population-level Traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set. * Detrimental trait measurements where effect sizes were inverted.

| Population-level Traits Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Growth Rate | Growth | 1 | 1 | 5 |
| | Relative Growth Rate | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **2** | **7** |
| Hatchling Abnormalities * | Hatchling Abnormalities | 1 | 3 | 3 |
| Mortality * | Mortality | 4 | 4 | 13 |
| Sex | Gender | 3 | 4 | 15 |
| Sex Reversal | Sex Reversal | 1 | 1 | 3 |
| Survival | Emergence Rate | 1 | 1 | 5 |
| | Hatchability | 1 | 1 | 8 |
| | Hatching Rate | 1 | 1 | 1 |
| | Hatching Success | 2 | 4 | 7 |
| | Pupation Rate | 2 | 3 | 9 |
| | Settlement Success | 1 | 1 | 2 |
| | Success of Parasitism | 1 | 1 | 2 |
| | Survival | 22 | 21 | 85 |
| | Survival Rate | 1 | 1 | 8 |
| | **Adjusted Record Totals** | **30** | **30** | **127** |
| | **Category Totals** | **36** | **35** | **168** |

### 7.9 | Codebook and Metadata

**Table S18 Codebook and Metadata for the Raw Data Set.** Raw data descriptors for the meta-analysis.

| Column Name | Description |
|---|---|
| Effect_Size_ID | Unique identifiers for individual effect sizes. |
| Study_ID | Unique identifiers for each paper. |
| Species_ID | Identifier for each species that a study investigates. |
| Treatment_ID | Identifier for each treatment that a study imposes. |
| Trait_ID | Identifier for each phenotypic traits a study measures. |
| First_Author | Initials and surname for the first author of the study. |
| Title | Title of the study. |
| Year | Year of publication. |
| Year_Z | Z-transformed year of publication. |
| Journal | Name of the journal the study was published in. |
| Journal_Impact_Factor-2021 | Journal impact factor as of 2021 (most recent records at time of data collection). |
| Kingdom | Kingdom for each species that a study investigates. |
| Phylum | Phylum for each species that a study investigates. |
| Class | Class for each species that a study investigates. |
| Order | Order for each species that a study investigates. |
| Family | Family for each species that a study investigates. |
| Scientific_Name | Binomial nomenclature for species. |
| Ecosystem | Ecosystem the species is naturally observed (Aquatic and Terrestrial). Amphibians considered aquatic. |
| Plasticity_Mechanism | Exposure type of the temperature treatments (Acclimation or Development). |
| Developmental_Exposure_Time_Category | Categorisation of Developmental Exposure Time. |
| Developmental_Exposure_Time | The period of exposure for treatments imposed during development. |
| Acclimation_Exposure_Time | The duration of exposure for acclimation treatments. |

| | |
|---|---|
| Exposure_Units | Units of Acclimation Exposure Time (Days). |
| T1_constant | Temperature of the constant temperature treatments. |
| T2_mean | Mean temperature of the fluctuating temperature treatments. |
| T2_Magnitude | Magnitudes converted to amplitudes of the fluctuating temperature treatments. |
| Fluctuation_Category | Type of fluctuations imposed (Sinusoidal, Alternating, Stepwise, Stochastic). |
| Fluctuation_Period | Period of one fluctuation oscillation. |
| Fluctuation_Unit | Units of Fluctuation Period (Days). |
| Number_Of_Fluctuations | Acclimation Exposure Time/Fluctuation Period for acclimation treatments. |
| Acclimation_Life-History_Stage | Life-history stage of organisms for acclimation treatments. |
| Acclimation_Life-History_Stage_Category | Categorisation of Acclimation Life-history Stages. |
| Trait_Category | Categorisation of Trait Measurements. |
| Measurement | Phenotypic traits measured following treatment exposure. |
| Trait_Unit | Units for measurements. |
| Performance_Curve | Whether a performance curve was recorded in the study (Yes, No). |
| Complex_Design | Whether a comparison between constant and fluctuating treatments was made at multiple temperatures (Yes, No). |
| Species_Overlap | Identifier for effect sizes that use the same species within a study |
| Animal_Overlap_T1 | Identifier for effect sizes where the constant treatment was conducted on the same animals within a study. |
| Animal_Overlap_T2 | Identifier for effect sizes where the fluctuating treatment was conducted on the same animals within a study. |
| Animal_Overlap | Identifier for effect sizes that measure phenotypic traits on the same animal within a study. |
| Animal_Code | Study ID: Species Overlap: Animal Overlap T1: Animal Overlap T2: Animal Overlap. |
| Shared_Animal_Number | Unique identifier for shared animal codes across effect sizes. |
| Shared_Control | Identifier for effect sizes that use the same control treatment within a study. |

| | |
|---|---|
| Shared_Control_Code | Study ID: Species Overlap: Shared Control: Trait ID. |
| Shared_Control_Number | Unique identifier for shared control codes across effect sizes. |
| n_R1-1 | Sample size of the control treatment. |
| R1-1_Mean | Mean response of the control treatment. |
| R1-1_SD_Final | Standard deviation of the control treatment. |
| n_R2-2 | Sample size of the fluctuating treatment. |
| R2-2_Mean | Mean response of the fluctuating treatment. |
| R2-2_SD_Final | Standard deviation of the fluctuating treatment. |
| Percentage_Transformation | Whether the recorded mean was a percentage (Yes, No). |
| Proportion_Transformation | Whether the recorded mean was a proportion (Yes, No). |
| In_Transformation | Whether the recorded mean was a natural log (Yes, No). |
| R1-1_Mean_Transformed | Control mean transformed for percentages, proportions or natural logs, and with a constant of 0.5 added. |
| R1-1_Mean_Add | Untransformed control mean with a constant 0.5 added. |
| R1-1_SD_Final_Transformed | Control standard deviation transformed for percentages, proportions or natural logs, and with a constant 0.5 added. |
| R1-1_SD_Final_Add | Untransformed control standard deviation with a constant 0.5 added. |
| R2-2_Mean_Transformed | Fluctuating mean transformed for percentages, proportions or natural logs, and with a constant of 0.5 added. |
| R2-2_Mean_Add | Untransformed fluctuating mean with a constant 0.5 added. |
| R2-2_SD_Final_Transformed | Fluctuating standard deviation transformed for percentages, proportions or natural logs, and with a constant 0.5 added. |
| R2-2_SD_Final_Add | Untransformed fluctuating standard deviation with a constant 0.5 added. |
| InRR | Log response ratio. |
| v_InRR | Log response ratio sampling variance. |
| Precision | Z-transformed inverse of log response ratio sampling variance. |
| InCVR | Log coefficient of variation ratio. |
| v_InCVR | Log coefficient of variation ratio sampling variance. |

| | |
|---|---|
| Precision_CVR | Z-transformed inverse of log coefficient of variation ratio sampling variance. |
| Reciprocal_Transformation | Whether phenotypic traits need to be multiplied by -1 so that a negative result represents a negative impact (Yes, No). |
| InRR_Transformed | Log response ratio transformed to account for reciprocal transformation. |
| InRR_Untransformed | Log response ratio calculated from untransformed data. |
| v_InRR_Untransformed | Log response ratio sampling variance calculated from untransformed data. |
| InCVR_Untransformed | Log coefficient of variation ratio calculated from untransformed data. |
| v_InCVR_Untransformed | Log coefficient of variation ratio sampling variance calculated from untransformed data. |
| SMD | Hedge's g. |
| v_SMD | Hedge's g sampling variance. |

## 7.10 | Phylogenetic Tree

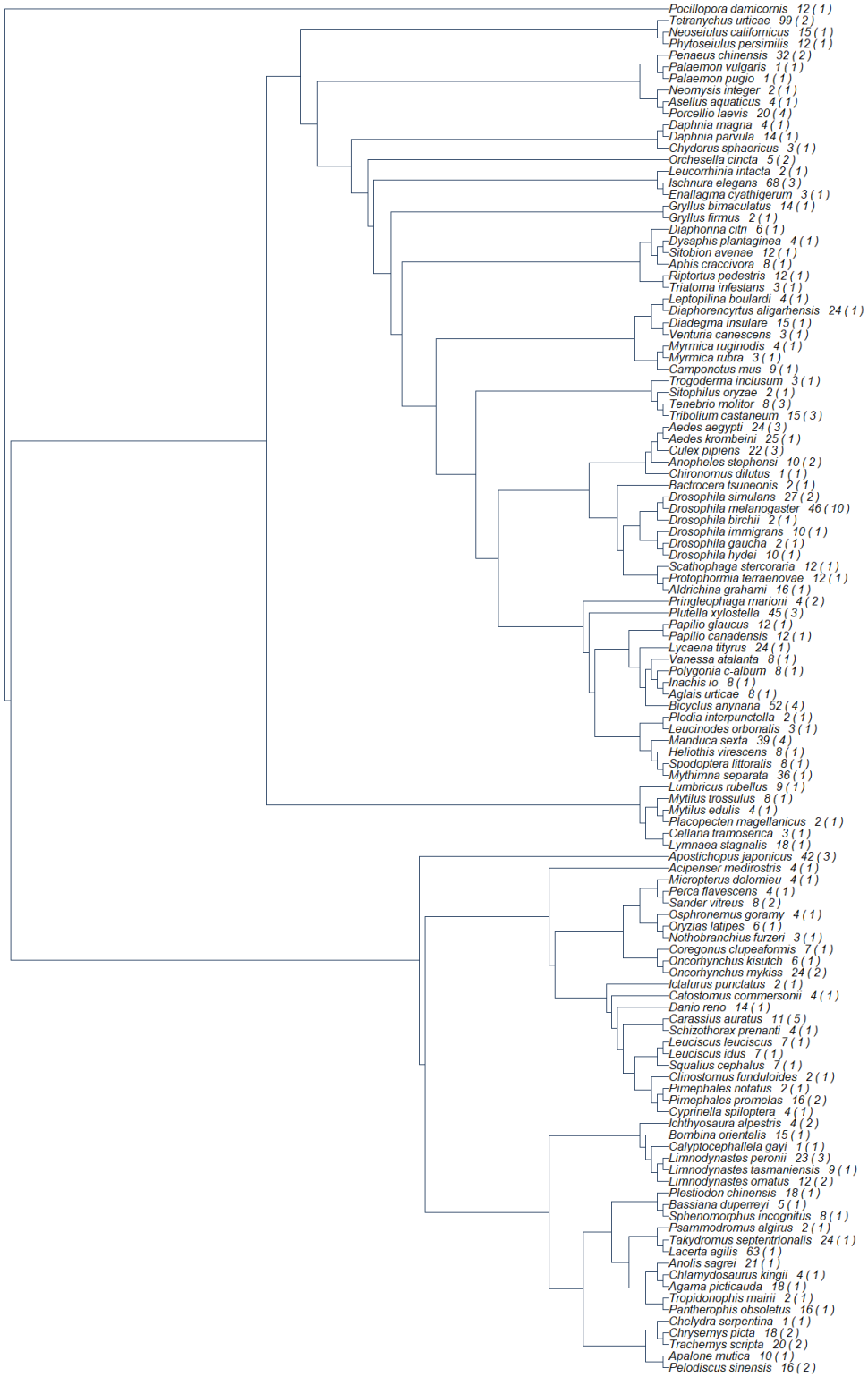


**Figure S3 Phylogenetic tree of species present in the meta-analysis.** Node labels are scientific names followed by the number of effect sizes with the number of studies in brackets.

### 7.11 | Publication Bias and Sensitivity Analysis

lnRR

A visual inspection did not indicate the presence of asymmetry in the funnel plot of the meta-analytic residuals of the overall MLMA model (Figure S4). The overall MLMA model with z-transformed publication year (effect size estimate = -0.0275; 95% CIs = [-0.0526, -0.0024]; p = 0.0319) and precision (effect size estimate = -0.0009; 95% CIs = [-0.0159, 0.0140]; p = 0.9031) as moderators, found a significant relationship between the year of publication and lnRR (Figure S5). Although there was no evidence of funnel plot asymmetry, a significant decrease in lnRR was observed in more recent years, suggesting the presence of time-lag bias in our data set. A potential reasoning for this decline is the preference in earlier years to publish only significant findings but not non-significant results (Koricheva and Kulinskaya, 2019; Trikalinos and Ioannidis, 2005).

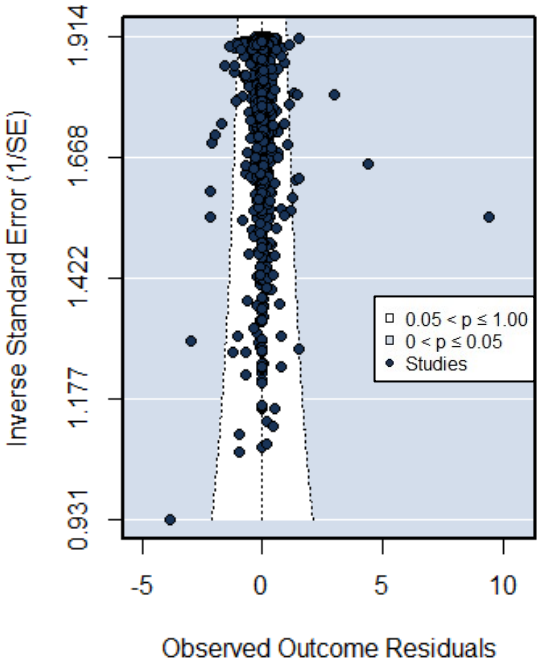


**Figure S4 Funnel plot.** Showing the observed outcome residuals from the overall MLMA model against the inverse standard error (precision). Effect size = lnRR. Dotted lines = 95% pseudo confidence intervals.

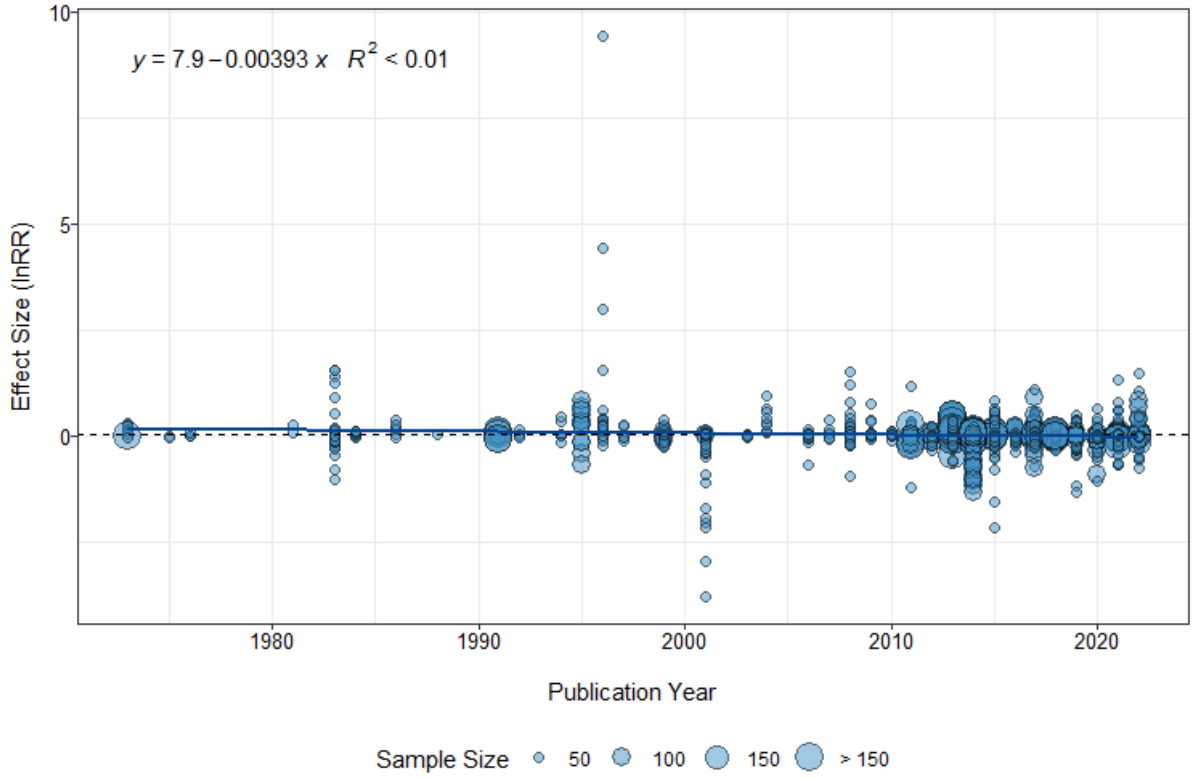


**Figure S5 The relationship between effect sizes (lnRR) and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = lnRR estimate from the overall MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

The sensitivity analysis showed no implausible data points with the Cook's distance never exceeding 1. Results from the models containing both a comparable effect size and untransformed data were quantitatively similar to the overall MLMA model (Table S19). These two results combined suggest that our lnRR data set is both robust and free from significantly influential outliers.

**Table S19 MLMA Outputs.** Comparison of the overall MLMA model with models fitted with untransformed data and Hedge's g effect sizes. Effect size = lnRR. $I^2$ values reported as percentages.

| Model | Estimate | CI Low | CI High | df | p-value | $I^2_{Total}$ |
|---|---|---|---|---|---|---|
| **Overall (lnRR)** | 0.0056 | -0.0264 | 0.0375 | 117 | 0.7314 | 99.69 |
| **Untransformed Data** | 0.0061 | -0.0258 | 0.0379 | 117 | 0.7066 | 99.71 |
| **Hedge's g** | -0.0207 | -0.1431 | 0.1017 | 117 | 0.7384 | 96.42 |

Measurements of gene expression remained significantly different between fluctuating and constant thermal environments following the robust variance estimations, specifically in aquatic environments and during developmental treatments (Table S20). All other significant results from the lnRR analyses did not hold true, suggesting that there is insufficient data available to support the robust inference on these moderators and future experimental investigations are required to strengthen model-based inferences.

**Table S20 Robust Variance Estimation Outputs.** Results from the robust variance estimation conducted on significant results from this study. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Subset | Moderator | Estimate | CI Low | CI High | p value |
|---|---|---|---|---|---|
| **Overall** | **Fluctuation Amplitude** | -0.0052 | -0.0136 | 0.0033 | 0.2290 |
| | **Gene Expression** | 0.2433 | 0.0934 | 0.3932 | **0.0017** |
| | **hsp70** | 0.4007 | -0.2160 | 1.0174 | 0.2005 |
| | **Longevity** | -0.0962 | -0.3666 | 0.1741 | 0.4819 |
| **Population-level** | **Fluctuation Amplitude** | -0.0289 | -0.0696 | 0.0119 | 0.1593 |
| **Aquatic** | **Alternating Regime** | 0.1002 | -0.0214 | 0.2219 | 0.1037 |
| | **Fluctuation Amplitude** | -0.0041 | -0.0084 | 0.0003 | 0.0646 |
| | **Food Consumption** | -0.1559 | -0.3825 | 0.0706 | 0.1704 |
| | **Gene Expression** | 0.3231 | 0.2137 | 0.4324 | **0.0000** |
| | **Mass** | -0.0900 | -0.1905 | 0.0104 | 0.0773 |
| **Developmental** | **Gene Expression** | 0.2099 | 0.0474 | 0.3723 | **0.0120** |

lnCVR

There was no indication of the presence of funnel asymmetry during visual inspection of the plots created from the meta-analytic residuals of the overall MLMA model (Figure S6). The overall MLMA model with z-transformed publication year (effect size estimate = 0.0302; 95% CIs = [-0.0003, 0.0606]; p = 0.0519) and precision (effect size estimate = 0.0011; 95% CIs = [-0.0150, 0.0171]; p = 0.8954) as moderators found no significant relationships with lnCVR (Figure S7). These results together indicate no evidence of funnel plot asymmetry or time-lag bias in our data set.

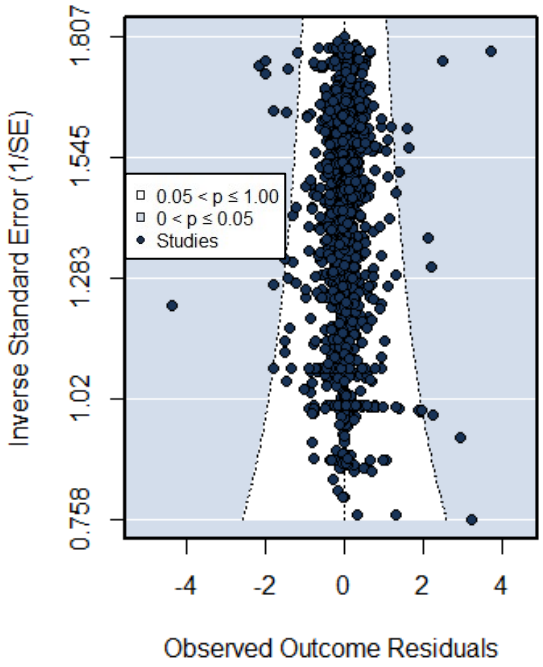


**Figure S6 Funnel plot.** Showing the observed outcome residuals from the overall MLMA model against the inverse standard error (precision). Effect size = lnCVR. Dotted lines = 95% pseudo confidence intervals.


**Figure S7 The relationship between effect sizes (lnCVR) and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = lnCVR estimate from the overall MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

The sensitivity analysis showed no implausible data points as the Cook's distance never exceeded 1. A comparison of the overall MLMA model with a model containing untransformed data showed quantitatively similar results (Table S21). These two results combined suggest that our lnCVR data set is both robust and free from significantly influential outliers.

**Table S21 MLMA Outputs.** Comparison of the overall MLMA model with a model fitted with untransformed data. Effect size = lnCVR. $I^2$ values reported as percentages.

| Model | Estimate | CI Low | CI High | df | p-value | $I^2_{Total}$ |
|---|---|---|---|---|---|---|
| **Overall (lnCVR)** | 0.0047 | -0.0269 | 0.0363 | 117 | 0.7685 | 69.32 |
| **Untransformed Data** | 0.0064 | -0.0275 | 0.0404 | 117 | 0.7087 | 73.54 |

All the significant results from the lnCVR analyses were found to not be significant following the robust variance estimations (Table S22). This suggests that there is insufficient data available to support the robust inference on these moderators and future experimental investigations are required to strengthen model-based inferences.

**Table S22 Robust Variance Estimation Outputs.** Results from the robust variance estimation conducted on significant results from this study. Effect size = lnCVR. Bold p-value = significant result (< 0.05).

| Subset | Moderator | Estimate | CI Low | CI High | p value |
|---|---|---|---|---|---|
| **Aquatic** | **Developmental Time** | -0.1701 | -0.3826 | 0.0425 | 0.1130 |
| | **Life-History Traits** | -0.1609 | -0.3295 | 0.0076 | 0.0608 |

*References*

Koricheva, J. and Kulinskaya, E. (2019). Temporal instability of evidence base: A threat to policy making? *Trends in Ecology and Evolution* **34**(10), 895-902. doi: 10.1016/j.tree.2019.05.006

Trikalinos, T. A. and Ioannidis, J. P. A. in *Publication bias in meta-analysis: Prevention, assessment and adjustments. Assessing the evolution of effect sizes over time* (ed John Wiley & Sons Ltd) Ch. 13, 241-259 (2005).

### 7.12 | Heterogeneity Results

**Table S23 Heterogeneity results for the MLMA models.** Effect size = lnRR. $I^2$ values reported as percentages.

| MLMA Models (lnRR) | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|
| **Overall** | 25.70 | 56.48 | 0.00 | 0.00 | 15.94 | 1.57 | 99.69 |
| **Population-level Traits** | 85.02 | 0.00 | 1.51 | 0.00 | 9.96 | 0.00 | 96.48 |
| **Individual-level Traits** | 21.86 | 59.59 | 0.00 | 0.00 | 16.69 | 1.57 | 99.71 |
| **Aquatic** | 1.75 | 16.42 | 1.82 | 13.67 | 0.71 | 64.26 | 98.62 |
| **Terrestrial** | 25.77 | 57.18 | 0.00 | 0.00 | 15.98 | 0.90 | 99.83 |
| **Acclimation** | 0.77 | 0.00 | 0.00 | 56.71 | 2.21 | 30.02 | 89.71 |
| **Developmental** | 25.30 | 59.20 | 0.00 | 0.00 | 14.06 | 1.27 | 99.83 |

**Table S24 Heterogeneity results for the MLMA models.** Effect size = lnCVR. $I^2$ values reported as percentages.

| MLMA Models (lnCVR) | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|
| **Overall** | 0.00 | 64.45 | 0.00 | 0.00 | 4.86 | 0.00 | 69.32 |
| **Population-level Traits** | 0.12 | 0.00 | 28.51 | 0.00 | 22.76 | 0.00 | 51.39 |
| **Individual-level Traits** | 0.00 | 61.22 | 0.00 | 0.00 | 3.68 | 0.00 | 64.90 |
| **Aquatic** | 11.62 | 11.93 | 0.00 | 0.00 | 5.55 | 3.90 | 33.00 |
| **Terrestrial** | 0.00 | 68.36 | 0.00 | 0.00 | 2.92 | 0.00 | 71.28 |
| **Acclimation** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Developmental** | 0.00 | 68.16 | 0.00 | 0.00 | 4.64 | 0.00 | 72.80 |

### 7.13 | Raw MLMA and Meta-regression Outputs (lnRR)

**Table S25 Results of the Overall MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR.

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 143 | 118 | 1492 | 0.0056 | -0.0264 | 0.0375 | 117 | 0.7314 |

**Table S26 Results of the meta-regression of the overall data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 143 | 118 | 1492 | -0.0052 | -0.0088 | -0.0015 | 536 | **0.0053** |

**Table S27 Results of the meta-regression of the overall data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR.

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 61 | 51 | 541 | -0.0041 | -0.0518 | 0.0436 | 481 | 0.8665 |
| **Alternating** | 51 | 50 | 607 | 0.0226 | -0.0284 | 0.0736 | 481 | 0.3848 |
| **Stepwise** | 17 | 15 | 218 | -0.0039 | -0.0867 | 0.0788 | 481 | 0.9257 |
| **Stochastic** | 5 | 5 | 21 | 0.0079 | -0.1733 | 0.1892 | 481 | 0.9317 |

**Table S28 Results of the meta-regression of the overall data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 12 | 11 | 38 | -0.0461 | -0.1672 | 0.0750 | 145 | 0.4533 |
| **Biochemical Assay** | 18 | 20 | 154 | 0.0366 | -0.0432 | 0.1164 | 145 | 0.3659 |
| **Gene Expression** | 9 | 12 | 50 | 0.2433 | 0.1108 | 0.3758 | 145 | **0.0004** |
| **Life-history Traits** | 76 | 74 | 508 | -0.0074 | -0.0588 | 0.0440 | 145 | 0.7770 |
| **Morphological** | 53 | 53 | 376 | -0.0110 | -0.0578 | 0.0358 | 145 | 0.6441 |
| **Physiological** | 46 | 38 | 198 | -0.0124 | -0.0702 | 0.0455 | 145 | 0.6732 |
| **Population** | 36 | 35 | 168 | 0.0348 | -0.0443 | 0.1140 | 145 | 0.3859 |

**Table S29 Results of the meta-regression of the overall data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | 0.0263 | -0.2036 | 0.2561 | 1064 | 0.8225 |
| **Catalase Activity** | 2 | 2 | 11 | 0.0243 | -0.2064 | 0.2550 | 1064 | 0.8362 |
| **Cortisol Levels** | 3 | 9 | 13 | 0.0039 | -0.2583 | 0.2661 | 1064 | 0.9766 |
| **Development Time** | 62 | 62 | 294 | 0.0341 | -0.0120 | 0.0801 | 1064 | 0.1469 |
| **Fecundity** | 19 | 23 | 72 | 0.0234 | -0.0581 | 0.1049 | 1064 | 0.5739 |
| **Food Consumption** | 8 | 9 | 24 | -0.0546 | -0.2010 | 0.0918 | 1064 | 0.4644 |
| **Head Width** | 5 | 5 | 14 | -0.0122 | -0.1628 | 0.1384 | 1064 | 0.8739 |
| **hsp70** | 5 | 5 | 12 | 0.4007 | 0.1416 | 0.6598 | 1064 | **0.0025** |
| **Immune Defense** | 2 | 2 | 14 | -0.0585 | -0.2919 | 0.1749 | 1064 | 0.6231 |
| **Length** | 24 | 26 | 89 | -0.0013 | -0.0716 | 0.0690 | 1064 | 0.9715 |
| **Locomotor Performance** | 13 | 13 | 35 | -0.0441 | -0.1739 | 0.0857 | 1064 | 0.5053 |
| **Longevity** | 17 | 16 | 93 | -0.0962 | -0.1686 | -0.0238 | 1064 | **0.0092** |
| **Mass** | 31 | 29 | 116 | -0.0235 | -0.0886 | 0.0415 | 1064 | 0.4780 |
| **Metabolic Rate** | 15 | 15 | 46 | 0.0435 | -0.0866 | 0.1736 | 1064 | 0.5120 |
| **Mortality** | 4 | 4 | 13 | -0.1030 | -0.3640 | 0.1580 | 1064 | 0.4389 |
| **PO Activity** | 3 | 2 | 14 | 0.0791 | -0.1161 | 0.2743 | 1064 | 0.4268 |
| **Reproductive Rate** | 5 | 5 | 17 | -0.0802 | -0.2492 | 0.0888 | 1064 | 0.3518 |
| **Sex** | 3 | 4 | 15 | 0.1511 | -0.0381 | 0.3403 | 1064 | 0.1175 |
| **SOD Activity** | 2 | 2 | 11 | 0.0531 | -0.1759 | 0.2822 | 1064 | 0.6491 |
| **Survival** | 30 | 30 | 127 | 0.0213 | -0.0517 | 0.0943 | 1064 | 0.5666 |
| **Tail Length** | 7 | 7 | 26 | -0.0260 | -0.1396 | 0.0876 | 1064 | 0.6538 |
| **Triglyceride** | 2 | 8 | 14 | 0.0176 | -0.1945 | 0.2297 | 1064 | 0.8707 |

**Table S30 Results of the meta-regression of the overall data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnRR.

| Overall Data (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 20 | 22 | 150 | 0.0460 | -0.0483 | 0.1403 | 90 | 0.3349 |
| **Amphibia** | 8 | 6 | 64 | -0.0486 | -0.1916 | 0.0944 | 90 | 0.5012 |
| **Anthozoa** | 1 | 1 | 12 | -0.0280 | -0.3840 | 0.3280 | 90 | 0.8762 |
| **Arachnida** | 3 | 3 | 126 | -0.0634 | -0.2203 | 0.0934 | 90 | 0.4239 |
| **Bivalvia** | 3 | 3 | 14 | -0.0354 | -0.3176 | 0.2468 | 90 | 0.8037 |
| **Branchiopoda** | 3 | 3 | 21 | -0.0326 | -0.2633 | 0.1982 | 90 | 0.7799 |
| **Gastropoda** | 2 | 2 | 21 | -0.0693 | -0.3217 | 0.1832 | 90 | 0.5869 |
| **Holothuroidea** | 3 | 1 | 42 | 0.0854 | -0.1487 | 0.3195 | 90 | 0.4704 |
| **Insecta** | 70 | 53 | 722 | 0.0023 | -0.0458 | 0.0504 | 90 | 0.9255 |
| **Malacostraca** | 9 | 6 | 60 | 0.0133 | -0.1296 | 0.1563 | 90 | 0.8534 |

**Table S31 Results of the population-level traits subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnRR.

| Population-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 36 | 35 | 168 | -0.0448 | -0.1622 | 0.0727 | 5 | 0.3721 |

**Table S32 Results of the meta-regression of the population-level traits data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Population-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 36 | 35 | 168 | -0.0289 | -0.0382 | -0.0195 | 134 | **0.0000** |

**Table S33 Results of the meta-regression of the population-level traits data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnRR.

| Population-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 18 | 19 | 60 | 0.0185 | -0.1222 | 0.1592 | 30 | 0.7901 |
| **Alternating** | 9 | 10 | 74 | -0.1441 | -0.2961 | 0.0079 | 120 | 0.0629 |
| **Stepwise** | 7 | 8 | 22 | 0.0127 | -0.2056 | 0.2309 | 120 | 0.9087 |

**Table S34 Results of the meta-regression of the population-level traits data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnRR.

| Population-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 7 | 9 | 19 | -0.0256 | -0.2630 | 0.2118 | 23 | 0.8253 |
| **Arachnida** | 1 | 1 | 24 | 0.0106 | -0.3105 | 0.3317 | 23 | 0.9462 |
| **Insecta** | 21 | 16 | 108 | -0.0966 | -0.1974 | 0.0041 | 23 | 0.0592 |

**Table S35 Results of the individual-level traits subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnRR.

| Individual-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 135 | 115 | 1324 | 0.0095 | -0.0229 | 0.0420 | 114 | 0.5618 |

**Table S36 Results of the meta-regression of the individual-level traits data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnRR.

| Individual-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 135 | 115 | 1324 | -0.0003 | -0.0030 | 0.0023 | 472 | 0.8191 |

**Table S37 Results of the meta-regression of the individual-level traits data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnRR.

| Individual-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 58 | 48 | 481 | -0.0073 | -0.0548 | 0.0401 | 423 | 0.7612 |
| **Alternating** | 48 | 49 | 533 | 0.0370 | -0.0144 | 0.0884 | 423 | 0.1574 |
| **Stepwise** | 16 | 14 | 196 | -0.0057 | -0.0890 | 0.0776 | 423 | 0.8932 |
| **Stochastic** | 5 | 5 | 20 | 0.0011 | -0.1720 | 0.1743 | 423 | 0.9897 |

**Table S38 Results of the meta-regression of the individual-level traits data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnRR.

| Individual-level Traits (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 20 | 22 | 131 | 0.0490 | -0.0460 | 0.1441 | 87 | 0.3078 |
| **Amphibia** | 8 | 6 | 64 | -0.0490 | -0.1899 | 0.0918 | 87 | 0.4910 |
| **Arachnida** | 3 | 3 | 102 | -0.1060 | -0.2613 | 0.0493 | 87 | 0.1786 |
| **Bivalvia** | 3 | 3 | 12 | -0.0536 | -0.3512 | 0.2440 | 87 | 0.7211 |
| **Branchiopoda** | 3 | 3 | 21 | -0.0387 | -0.2650 | 0.1876 | 87 | 0.7349 |
| **Gastropoda** | 2 | 2 | 21 | -0.0673 | -0.3162 | 0.1815 | 87 | 0.5921 |
| **Holothuroidea** | 3 | 1 | 39 | 0.0923 | -0.1405 | 0.3251 | 87 | 0.4329 |
| **Insecta** | 63 | 52 | 614 | 0.0155 | -0.0352 | 0.0662 | 87 | 0.5445 |
| **Malacostraca** | 8 | 4 | 56 | 0.0145 | -0.1325 | 0.1616 | 87 | 0.8446 |

**Table S39 Results of the aquatic subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR.

| Aquatic Organisms (InRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 51 | 46 | 395 | 0.0300 | -0.0360 | 0.0960 | 45 | 0.3651 |

**Table S40 Results of the meta-regression of the aquatic data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 51 | 46 | 395 | -0.0041 | -0.0080 | -0.0002 | 135 | **0.0416** |

**Table S41 Results of the meta-regression of the aquatic data set with exposure type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR.

| Aquatic Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 30 | 28 | 197 | 0.0368 | -0.0421 | 0.1156 | 393 | 0.3600 |
| **Developmental** | 22 | 21 | 198 | 0.0213 | -0.0613 | 0.1040 | 393 | 0.6124 |

**Table S42 Results of the meta-regression of the aquatic data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 33 | 24 | 267 | 0.0388 | -0.0219 | 0.0995 | 40 | 0.2039 |
| **Alternating** | 9 | 16 | 98 | 0.1002 | 0.0164 | 0.1841 | 40 | **0.0203** |

**Table S43 Results of the meta-regression of the aquatic data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 6 | 6 | 13 | -0.1523 | -0.4770 | 0.1724 | 86 | 0.3538 |
| **Biochemical Assay** | 12 | 14 | 75 | 0.0302 | -0.0654 | 0.1259 | 86 | 0.5313 |
| **Gene Expression** | 5 | 7 | 38 | 0.3231 | 0.1996 | 0.4465 | 86 | **0.0000** |
| **Life-history Traits** | 17 | 18 | 67 | -0.0095 | -0.1297 | 0.1108 | 86 | 0.8761 |
| **Morphological** | 15 | 15 | 90 | -0.0432 | -0.1500 | 0.0636 | 86 | 0.4233 |
| **Physiological** | 29 | 21 | 112 | 0.0068 | -0.0714 | 0.0851 | 86 | 0.8626 |

**Table S44 Results of the meta-regression of the aquatic data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | 0.0109 | -0.1003 | 0.1220 | 178 | 0.8472 |
| **Cortisol Levels** | 3 | 9 | 13 | -0.0005 | -0.1544 | 0.1535 | 32 | 0.9950 |
| **Development Time** | 14 | 15 | 35 | 0.0052 | -0.0322 | 0.0426 | 178 | 0.7835 |
| **Food Consumption** | 6 | 6 | 13 | -0.1559 | -0.2988 | -0.0131 | 32 | **0.0333** |
| **Immune Defense** | 1 | 1 | 12 | 0.0167 | -0.0991 | 0.1324 | 32 | 0.7712 |
| **Length** | 11 | 12 | 43 | -0.0332 | -0.0713 | 0.0048 | 178 | 0.0865 |
| **Locomotor Performance** | 6 | 6 | 11 | -0.0845 | -0.1941 | 0.0251 | 178 | 0.1299 |
| **Mass** | 8 | 7 | 20 | -0.0900 | -0.1419 | -0.0381 | 178 | **0.0008** |
| **Metabolic Rate** | 10 | 10 | 24 | -0.0164 | -0.1169 | 0.0841 | 178 | 0.7474 |

**Table S45 Results of the terrestrial subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 84 | 69 | 929 | 0.0131 | -0.0316 | 0.0579 | 68 | 0.5600 |

**Table S46 Results of the meta-regression of the terrestrial data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 84 | 69 | 929 | 0.0000 | -0.0035 | 0.0034 | 336 | 0.9795 |

**Table S47 Results of the meta-regression of the terrestrial data set with exposure type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 22 | 19 | 139 | 0.0068 | -0.0832 | 0.0968 | 927 | 0.8825 |
| **Developmental** | 66 | 60 | 790 | 0.0145 | -0.0330 | 0.0619 | 927 | 0.5491 |

**Table S48 Results of the meta-regression of the terrestrial data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 25 | 24 | 214 | -0.0130 | -0.0935 | 0.0674 | 295 | 0.7502 |
| **Alternating** | 39 | 33 | 435 | 0.0332 | -0.0333 | 0.0996 | 295 | 0.3263 |
| **Stepwise** | 13 | 11 | 186 | -0.0079 | -0.1113 | 0.0955 | 295 | 0.8805 |
| **Stochastic** | 4 | 4 | 16 | 0.0170 | -0.2155 | 0.2495 | 295 | 0.8857 |

**Table S49 Results of the meta-regression of the terrestrial data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 6 | 5 | 25 | -0.0123 | -0.1639 | 0.1392 | 65 | 0.8714 |
| **Biochemical Assay** | 6 | 6 | 79 | 0.0473 | -0.0838 | 0.1783 | 65 | 0.4741 |
| **Gene Expression** | 4 | 5 | 12 | 0.1848 | -0.0824 | 0.4520 | 65 | 0.1720 |
| **Life-history Traits** | 59 | 56 | 441 | 0.0202 | -0.0404 | 0.0809 | 65 | 0.5076 |
| **Morphological** | 38 | 38 | 286 | 0.0032 | -0.0562 | 0.0627 | 65 | 0.9136 |
| **Physiological** | 17 | 17 | 86 | -0.0122 | -0.1003 | 0.0759 | 65 | 0.7829 |

**Table S50 Results of the meta-regression of the terrestrial data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnRR.

| Terrestrial Organisms (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 48 | 47 | 259 | 0.0457 | -0.0149 | 0.1062 | 659 | 0.1389 |
| **Fecundity** | 16 | 20 | 67 | 0.0665 | -0.0352 | 0.1683 | 659 | 0.1995 |
| **Food Consumption** | 2 | 3 | 11 | 0.0039 | -0.2179 | 0.2258 | 659 | 0.9721 |
| **Head Width** | 5 | 5 | 14 | 0.0045 | -0.1786 | 0.1877 | 659 | 0.9613 |
| **Length** | 13 | 14 | 46 | 0.0199 | -0.0916 | 0.1313 | 659 | 0.7261 |
| **Locomotor Performance** | 7 | 7 | 24 | -0.0121 | -0.1893 | 0.1651 | 659 | 0.8933 |
| **Longevity** | 14 | 13 | 86 | -0.0643 | -0.1561 | 0.0276 | 659 | 0.1700 |
| **Mass** | 23 | 22 | 96 | -0.0067 | -0.0937 | 0.0803 | 659 | 0.8801 |
| **Metabolic Rate** | 5 | 5 | 22 | 0.0974 | -0.1066 | 0.3014 | 659 | 0.3489 |
| **PO Activity** | 3 | 2 | 14 | 0.0435 | -0.1956 | 0.2827 | 659 | 0.7209 |
| **Reproductive Rate** | 4 | 4 | 11 | -0.1059 | -0.3338 | 0.1220 | 659 | 0.3618 |
| **Tail Length** | 6 | 6 | 21 | 0.0165 | -0.1375 | 0.1706 | 659 | 0.8332 |

**Table S51 Results of the acclimation subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 52 | 47 | 336 | 0.0084 | -0.0647 | 0.0816 | 46 | 0.8175 |

**Table S52 Results of the meta-regression of the acclimation data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 52 | 47 | 336 | 0.0023 | -0.0011 | 0.0058 | 158 | 0.1809 |

**Table S53 Results of the meta-regression of the acclimation data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 25 | 22 | 151 | 0.0202 | -0.0653 | 0.1057 | 131 | 0.6407 |
| **Alternating** | 13 | 15 | 75 | -0.0247 | -0.1418 | 0.0924 | 42 | 0.6726 |
| **Stepwise** | 8 | 6 | 76 | 0.0383 | -0.0676 | 0.1442 | 131 | 0.4752 |

**Table S54 Results of the meta-regression of the acclimation data set with life-history stage as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Adult** | 27 | 29 | 125 | 0.0189 | -0.0711 | 0.1088 | 148 | 0.6794 |
| **Embryo** | 3 | 3 | 11 | 0.0019 | -0.2649 | 0.2688 | 41 | 0.9885 |
| **Juvenile** | 12 | 9 | 101 | 0.0605 | -0.0482 | 0.1693 | 148 | 0.2733 |
| **Larva** | 12 | 9 | 92 | -0.0755 | -0.2285 | 0.0774 | 148 | 0.3308 |

**Table S55 Results of the meta-regression of the acclimation data set with the number of fluctuations as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Number of Fluctuations** | 50 | 45 | 329 | 0.0005 | -0.0003 | 0.0012 | 152 | 0.2007 |

**Table S56 Results of the meta-regression of the acclimation data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 8 | 6 | 24 | -0.1151 | -0.3267 | 0.0965 | 65 | 0.2813 |
| **Biochemical Assay** | 15 | 17 | 140 | 0.0060 | -0.0709 | 0.0829 | 65 | 0.8763 |
| **Life-history Traits** | 9 | 10 | 28 | 0.0202 | -0.0975 | 0.1380 | 65 | 0.7324 |
| **Physiological** | 31 | 23 | 134 | -0.0095 | -0.0835 | 0.0645 | 65 | 0.7988 |

**Table S57 Results of the meta-regression of the acclimation data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | 0.0255 | -0.1702 | 0.2211 | 111 | 0.7968 |
| **Catalase Activity** | 2 | 2 | 11 | 0.1110 | -0.1541 | 0.3761 | 111 | 0.4084 |
| **Cortisol Levels** | 3 | 9 | 13 | -0.0219 | -0.2289 | 0.1850 | 19 | 0.8269 |
| **Food Consumption** | 5 | 5 | 12 | -0.1552 | -0.4179 | 0.1076 | 19 | 0.2314 |
| **Immune Defense** | 2 | 2 | 14 | -0.0304 | -0.3653 | 0.3044 | 19 | 0.8511 |
| **Metabolic Rate** | 13 | 12 | 41 | 0.0028 | -0.1416 | 0.1472 | 111 | 0.9696 |
| **SOD Activity** | 2 | 2 | 11 | 0.1572 | -0.1064 | 0.4208 | 111 | 0.2398 |

**Table S58 Results of the meta-regression of the acclimation data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnRR.

| Acclimation Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 16 | 17 | 86 | 0.0181 | -0.8102 | 0.8463 | 35 | 0.9649 |
| **Bivalvia** | 3 | 3 | 12 | -0.0472 | -0.9477 | 0.8532 | 35 | 0.9158 |
| **Gastropoda** | 2 | 2 | 21 | -0.0683 | -0.9648 | 0.8282 | 35 | 0.8780 |
| **Holothuroidea** | 3 | 1 | 39 | 0.1161 | -0.7906 | 1.0227 | 35 | 0.7965 |
| **Insecta** | 14 | 14 | 103 | -0.0638 | -0.7836 | 0.6560 | 35 | 0.8583 |
| **Malacostraca** | 7 | 4 | 48 | 0.0222 | -0.8590 | 0.9033 | 35 | 0.9596 |

**Table S59 Results of the developmental subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 88 | 81 | 988 | 0.0084 | -0.0337 | 0.0505 | 80 | 0.6921 |

**Table S60 Results of the meta-regression of the developmental data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 88 | 81 | 988 | -0.0021 | -0.0061 | 0.0019 | 326 | 0.3033 |

**Table S61 Results of the meta-regression of the developmental data set with exposure period as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Embryo** | 50 | 50 | 598 | -0.0070 | -0.0605 | 0.0464 | 84 | 0.7938 |
| **Juvenile** | 7 | 8 | 50 | 0.0041 | -0.1389 | 0.1471 | 84 | 0.9543 |
| **Larva** | 28 | 25 | 311 | 0.0339 | -0.0350 | 0.1027 | 84 | 0.3307 |
| **Pupa** | 3 | 5 | 29 | 0.0308 | -0.1586 | 0.2202 | 84 | 0.7474 |

**Table S62 Results of the meta-regression of the developmental data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 37 | 33 | 330 | -0.0239 | -0.0879 | 0.0401 | 300 | 0.4637 |
| **Alternating** | 35 | 36 | 458 | 0.0478 | -0.0156 | 0.1112 | 300 | 0.1390 |
| **Stepwise** | 10 | 10 | 120 | -0.0070 | -0.1225 | 0.1084 | 300 | 0.9048 |
| **Stochastic** | 5 | 5 | 17 | 0.0048 | -0.2042 | 0.2138 | 300 | 0.9639 |

**Table S63 Results of the meta-regression of the developmental data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR. Bold p-value = significant result (< 0.05).

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 4 | 5 | 14 | -0.0134 | -0.1849 | 0.1581 | 79 | 0.8768 |
| **Biochemical Assay** | 3 | 3 | 14 | 0.0715 | -0.0923 | 0.2353 | 79 | 0.3875 |
| **Gene Expression** | 6 | 8 | 40 | 0.2099 | 0.0504 | 0.3694 | 79 | **0.0106** |
| **Life-history Traits** | 69 | 69 | 480 | 0.0130 | -0.0462 | 0.0721 | 79 | 0.6637 |
| **Morphological** | 53 | 53 | 376 | -0.0065 | -0.0590 | 0.0461 | 79 | 0.8068 |
| **Physiological** | 17 | 18 | 64 | -0.0309 | -0.1275 | 0.0656 | 79 | 0.5258 |

**Table S64 Results of the meta-regression of the developmental data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 60 | 61 | 287 | 0.0401 | -0.0102 | 0.0905 | 720 | 0.1177 |
| **Fecundity** | 16 | 18 | 67 | 0.0490 | -0.0440 | 0.1421 | 720 | 0.3014 |
| **Food Consumption** | 3 | 4 | 12 | 0.0066 | -0.1879 | 0.2010 | 720 | 0.9471 |
| **Head Width** | 5 | 5 | 14 | -0.0075 | -0.1734 | 0.1584 | 720 | 0.9293 |
| **Length** | 24 | 26 | 89 | 0.0055 | -0.0725 | 0.0836 | 720 | 0.8895 |
| **Locomotor Performance** | 9 | 10 | 27 | -0.0128 | -0.1688 | 0.1432 | 720 | 0.8721 |
| **Longevity** | 16 | 15 | 91 | -0.0729 | -0.1540 | 0.0081 | 720 | 0.0778 |
| **Mass** | 31 | 29 | 116 | -0.0243 | -0.0951 | 0.0465 | 720 | 0.5004 |
| **Tail Length** | 7 | 7 | 26 | -0.0207 | -0.1480 | 0.1066 | 720 | 0.7495 |

**Table S65 Results of the meta-regression of the developmental data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnRR.

| Developmental Exposure (lnRR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 5 | 7 | 45 | 0.0365 | -0.1601 | 0.2330 | 58 | 0.7118 |
| **Amphibia** | 6 | 5 | 61 | -0.0493 | -0.2226 | 0.1241 | 58 | 0.5717 |
| **Arachnida** | 3 | 3 | 102 | -0.1066 | -0.2833 | 0.0701 | 58 | 0.2321 |
| **Branchiopoda** | 3 | 3 | 21 | -0.0398 | -0.3065 | 0.2270 | 58 | 0.7665 |
| **Insecta** | 51 | 45 | 511 | 0.0342 | -0.0293 | 0.0976 | 58 | 0.2857 |

## 7.14 | Graphical Representation of the MLMA and Meta-regression Results (lnRR)

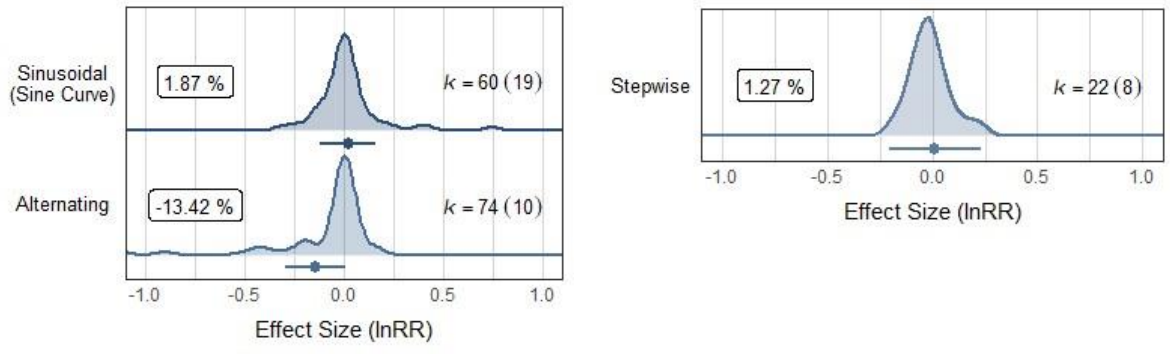


**Figure S8 Meta-regression results for the population-level traits subset with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

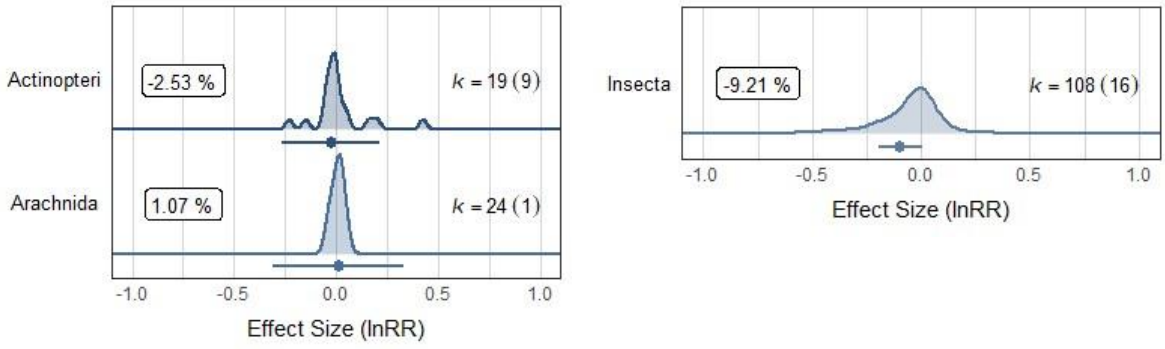


**Figure S9 Meta-regression results for the population-level traits subset with taxonomic class as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

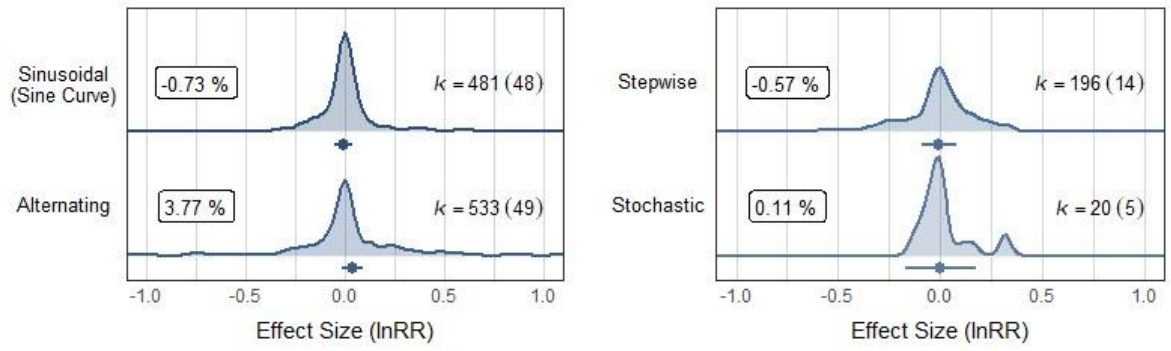


**Figure S10 Meta-regression results for the individual-level traits subset with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

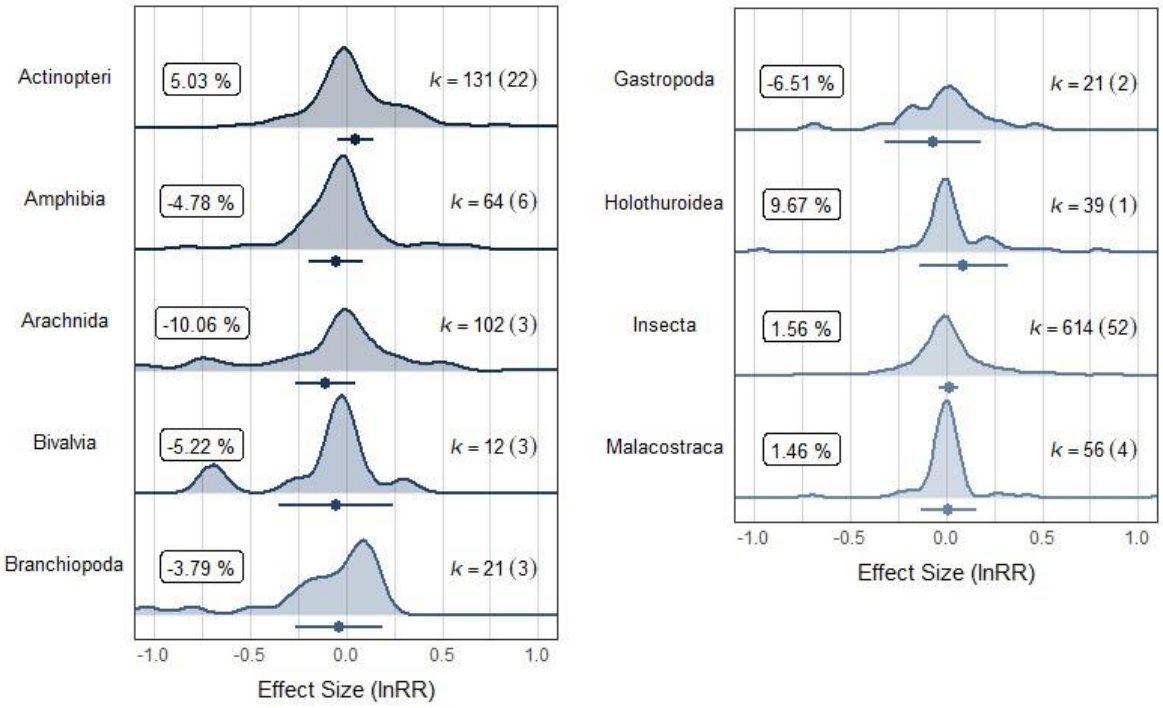


**Figure S11 Meta-regression results for the individual-level traits subset with taxonomic class as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

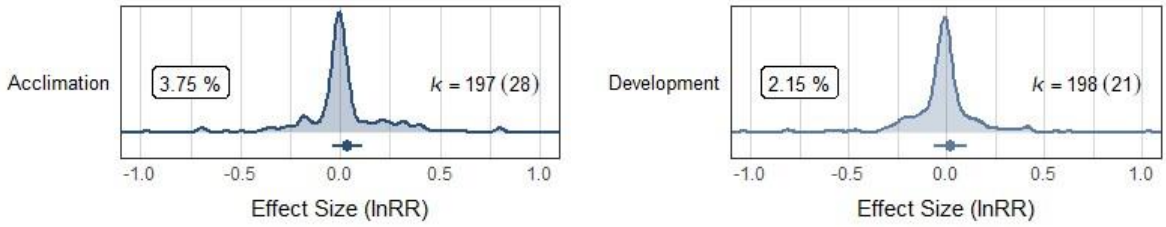


**Figure S12 Meta-regression results for the aquatic subset with exposure type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.


**Figure S13 Meta-regression results for the aquatic subset with fluctuation type as the moderator.** There was a significant increase in mean phenotypic trait values found in alternating fluctuating regime treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

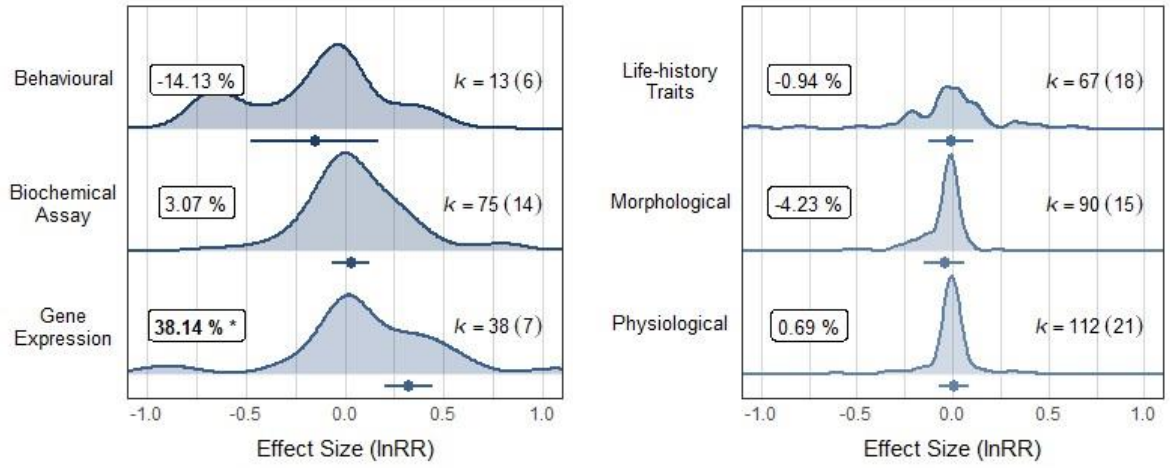


**Figure S14 Meta-regression results for the aquatic subset with phenotypic trait category as the moderator.** There was a significant increase in gene expression found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

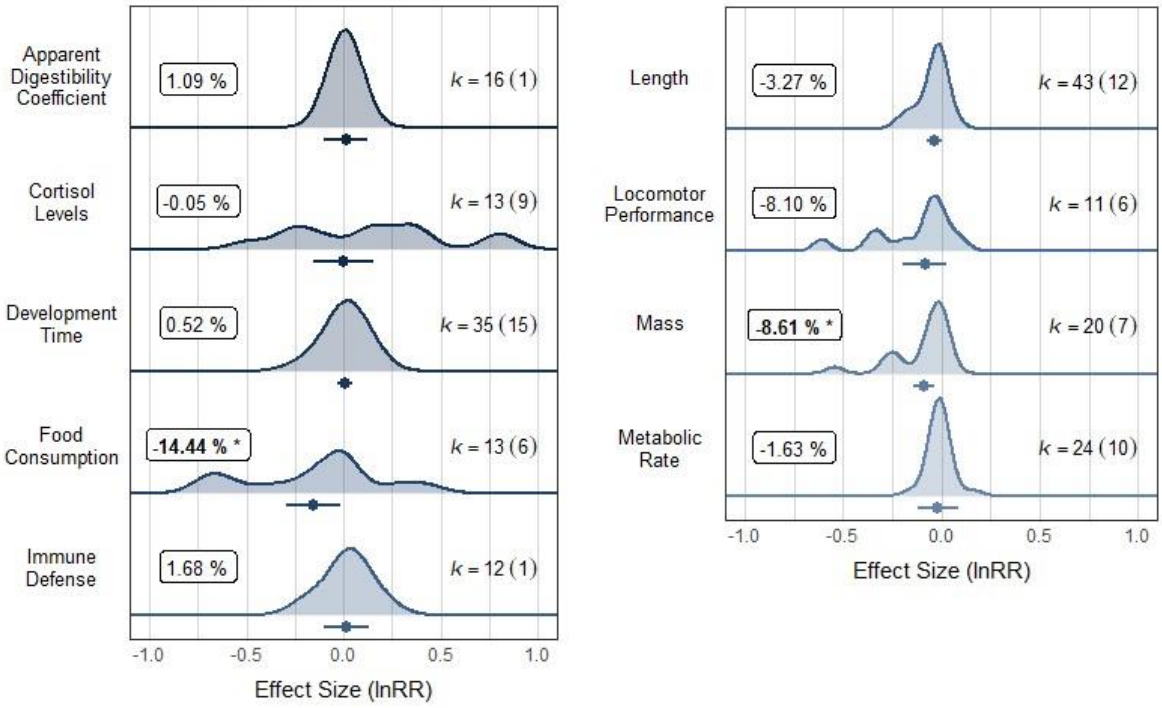


**Figure S15 Meta-regression results for the aquatic subset with specific phenotypic traits as the moderator.** There was a significant decrease in both food consumption and mass found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.


**Figure S16 Meta-regression results for the terrestrial subset with exposure type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

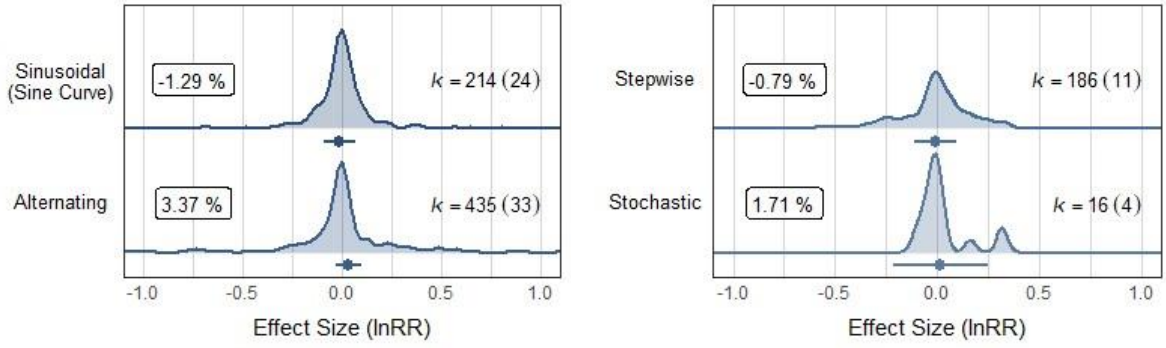


**Figure S17 Meta-regression results for the terrestrial subset with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

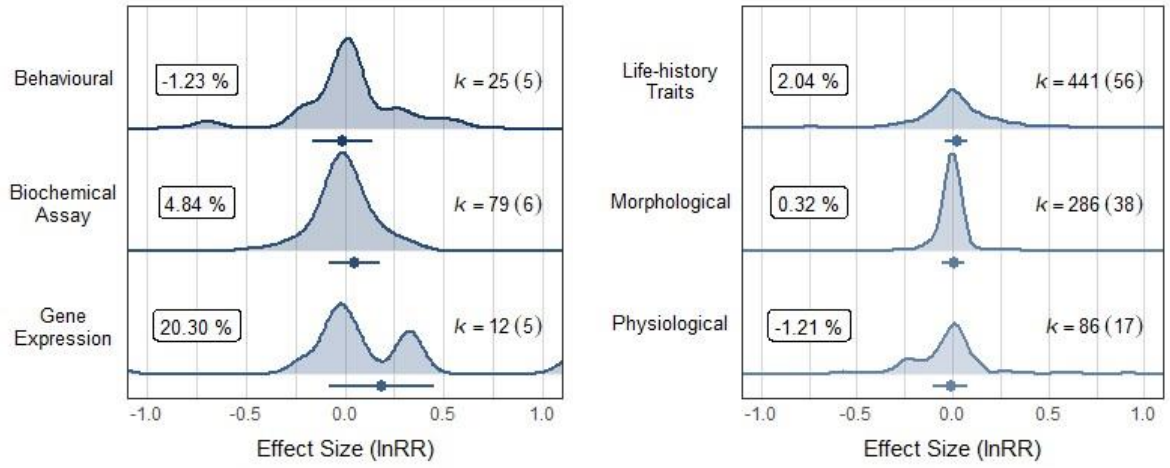


**Figure S18 Meta-regression results for the terrestrial subset with phenotypic trait category as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

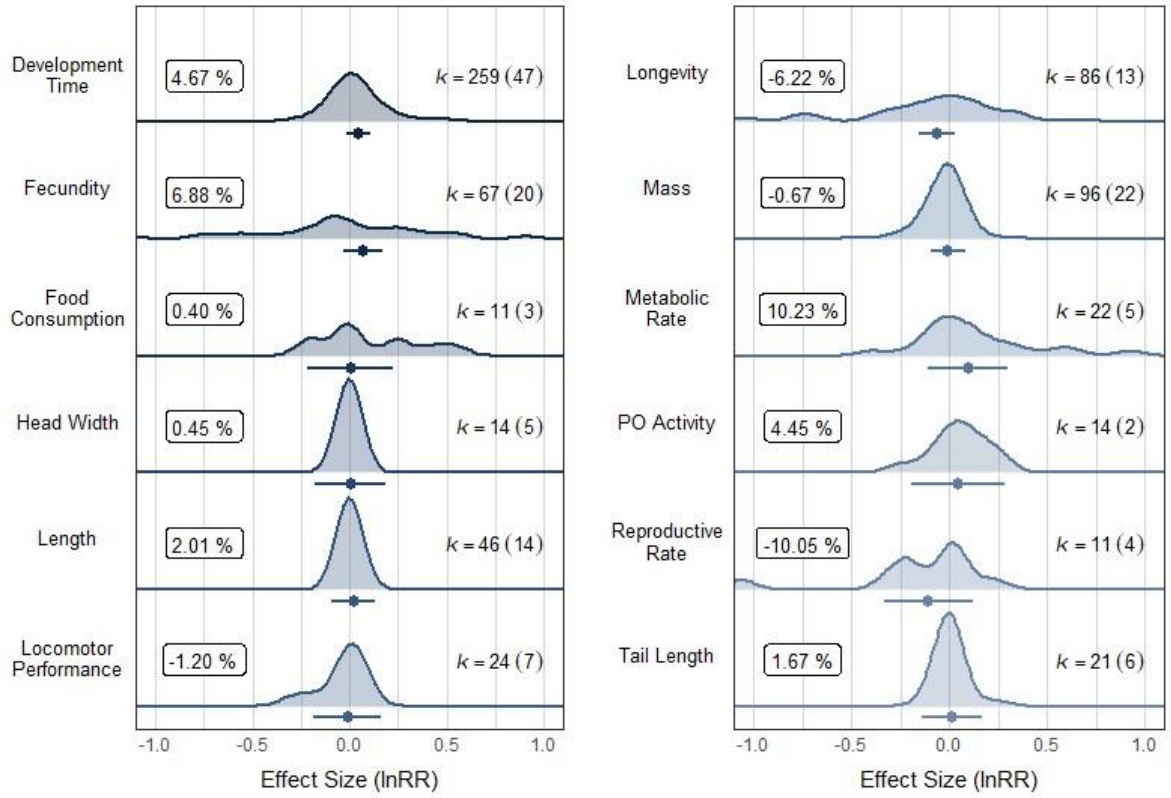


**Figure S19 Meta-regression results for the terrestrial subset with specific phenotypic traits as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

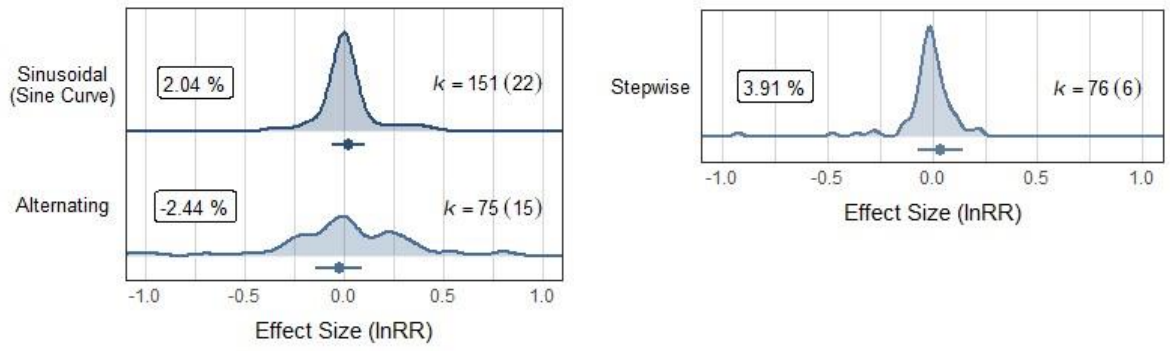


**Figure S20 Meta-regression results for the acclimation subset with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

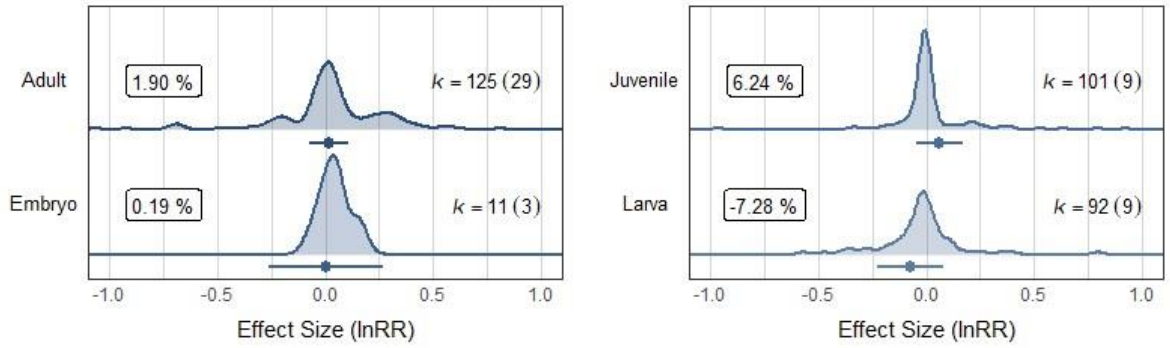


**Figure S21 Meta-regression results for the acclimation subset with life-history stage as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

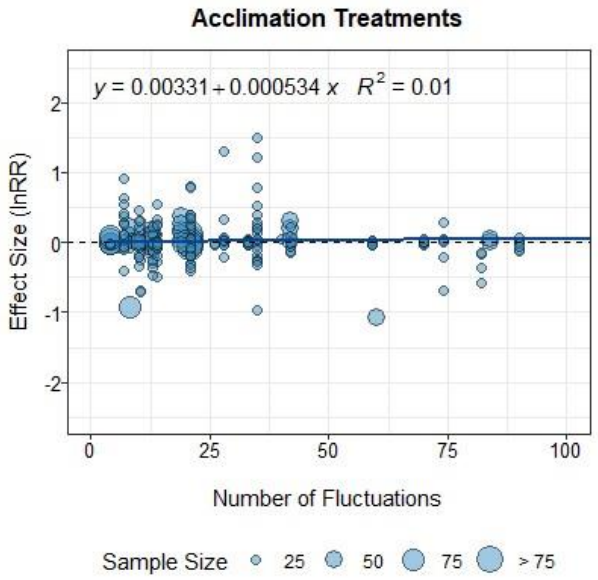


**Figure S22 Relationship between effect size (lnRR) and the number of fluctuations for the acclimation subset.** There was no significant relationship between the number of fluctuations and the mean phenotypic trait value. Dashed line = lnRR estimate from the acclimation subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis and Y-axis limits are cropped for presentation.

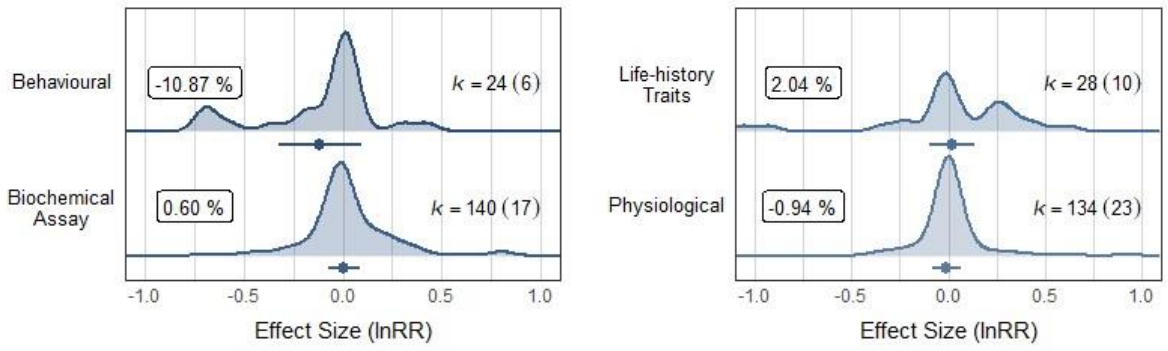


**Figure S23 Meta-regression results for the acclimation subset with phenotypic trait category as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

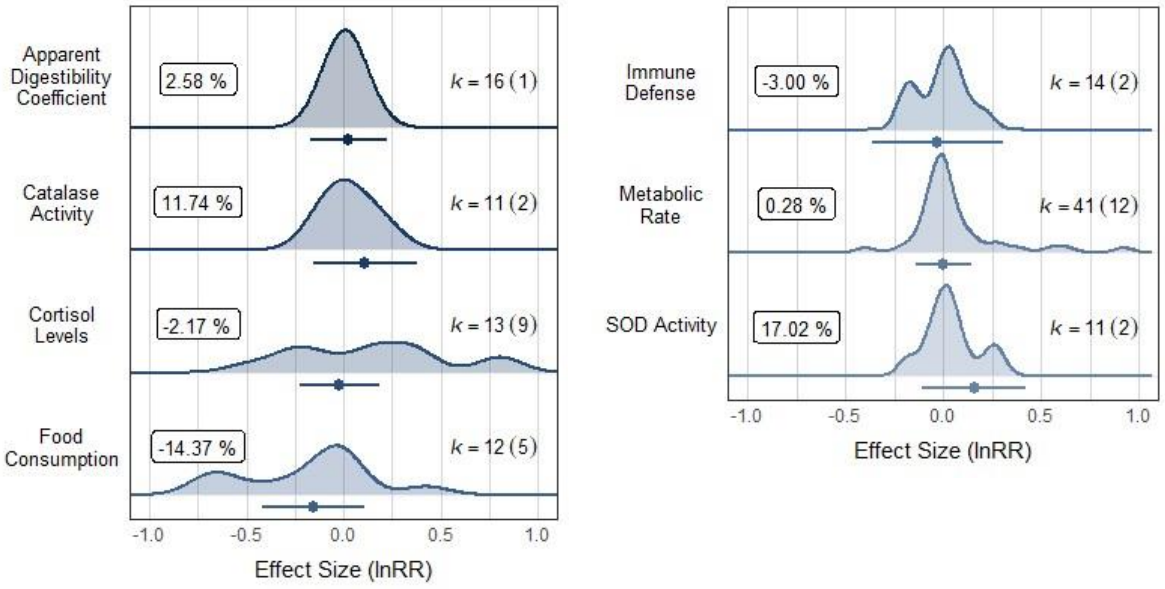


**Figure S24 Meta-regression results for the acclimation subset with specific phenotypic traits as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

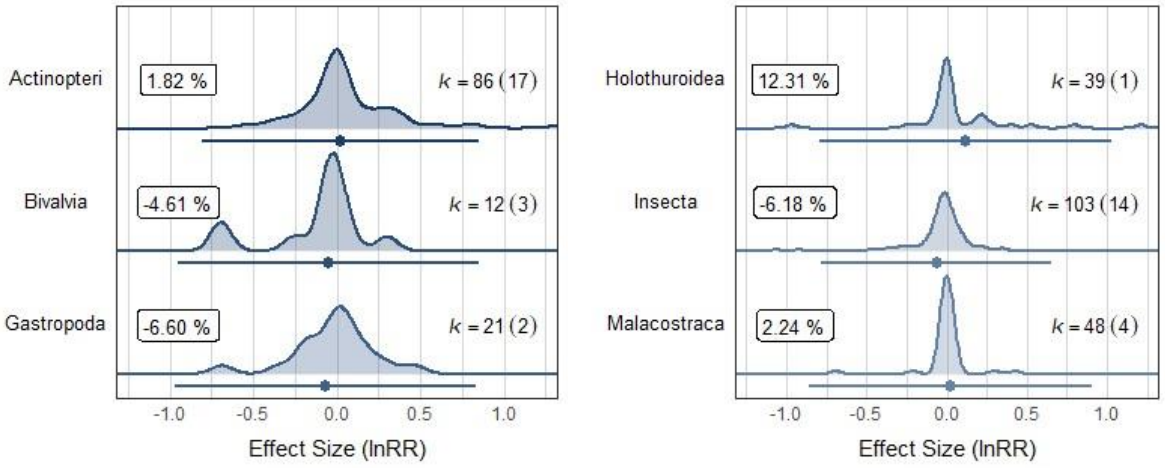


**Figure S25 Meta-regression results for the acclimation subset with taxonomic class as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

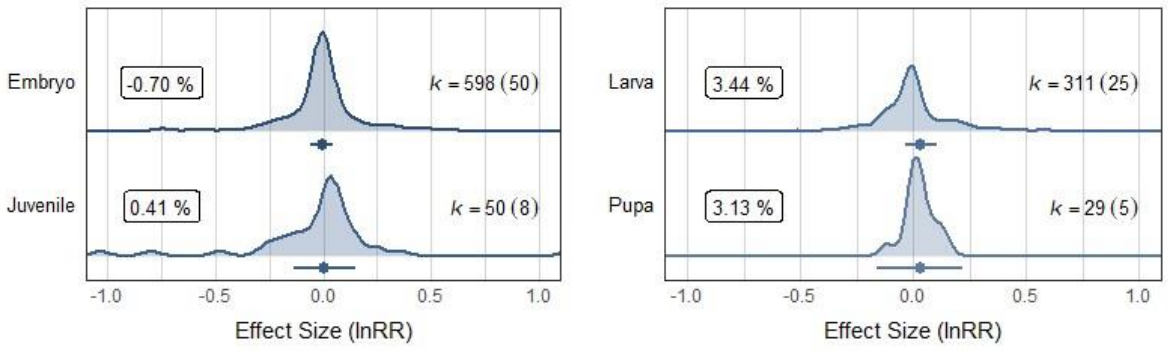


**Figure S26 Meta-regression results for the developmental subset with exposure period as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

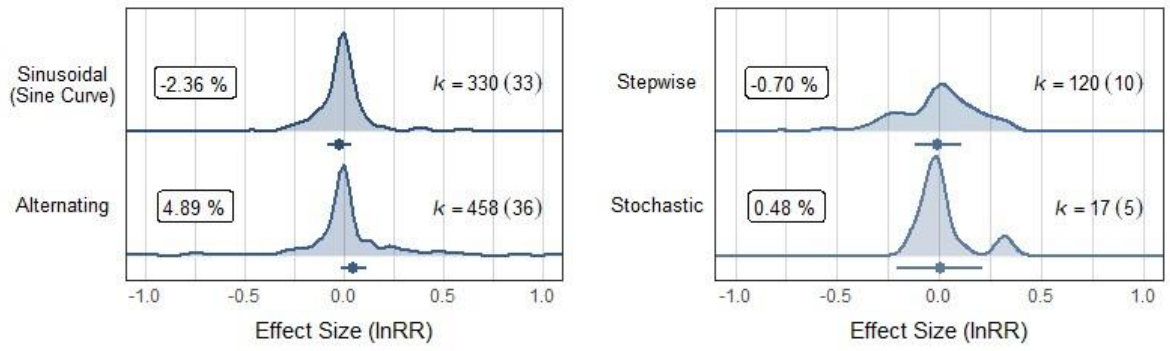


**Figure S27 Meta-regression results for the developmental subset with fluctuation type as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

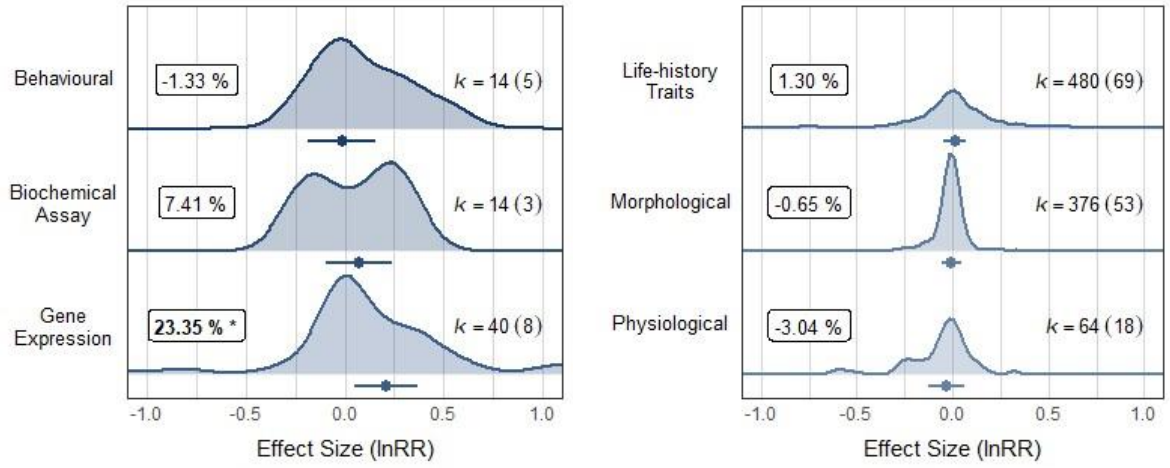


**Figure S28 Meta-regression results for the developmental subset with phenotypic trait category as the moderator.** There was a significant increase in gene expression found in fluctuating treatments, indicated in the figure by an asterisk next to the bold percentage label ($p < 0.05$). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

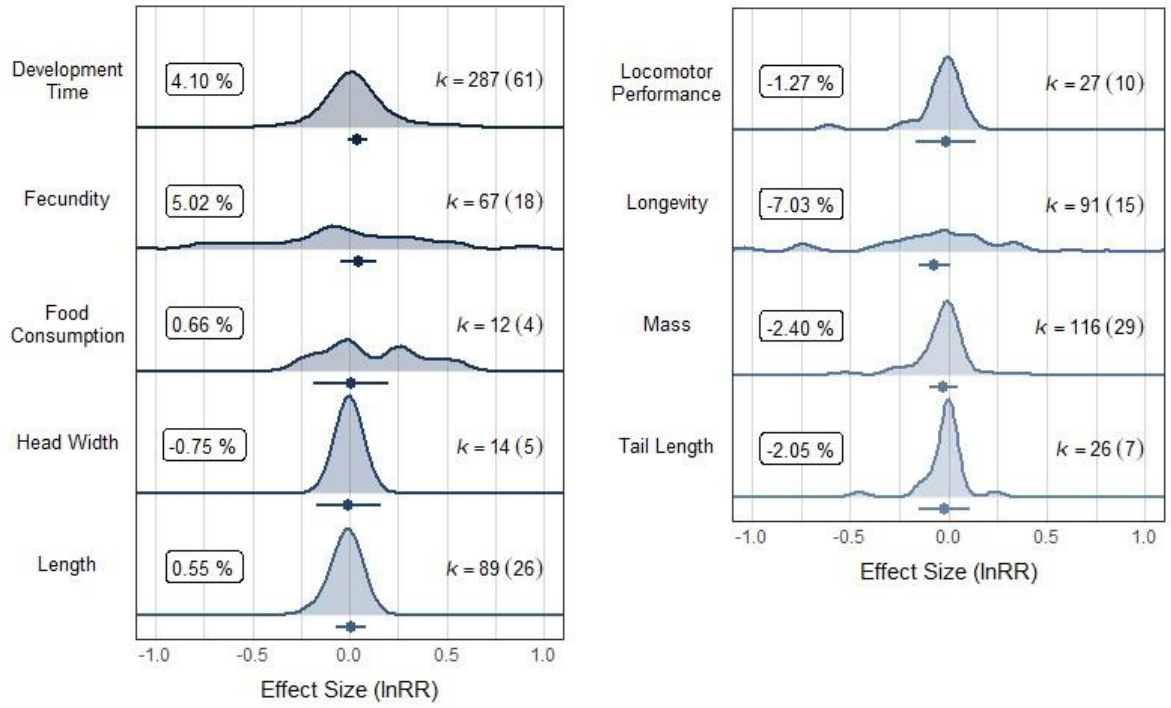


**Figure S29 Meta-regression results for the developmental subset with specific phenotypic traits as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between the fluctuating and constant temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

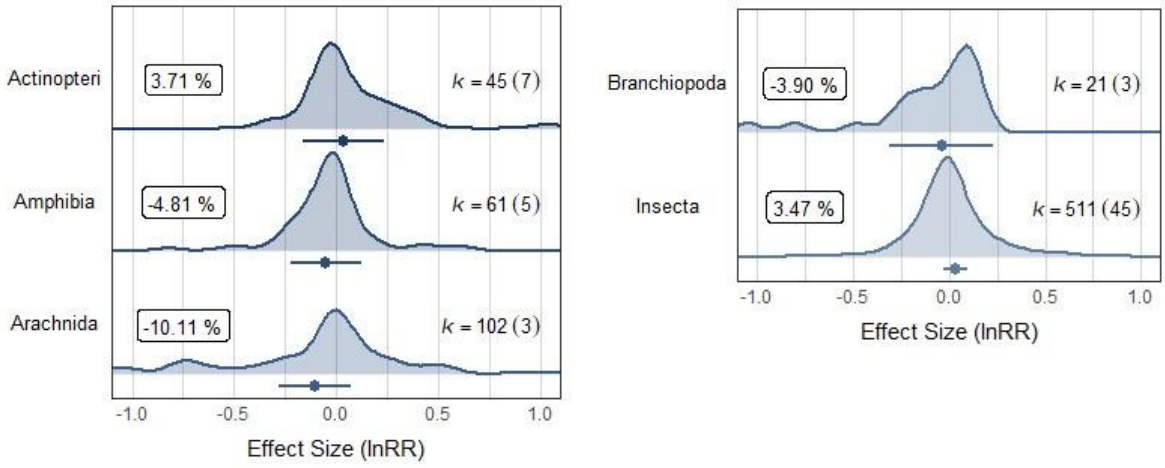


**Figure S30 Meta-regression results for the developmental subset with taxonomic class as the moderator.** There were no significant differences in mean trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnRR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnRR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

### 7.15 | Raw MLMA and Meta-regression Outputs (lnCVR)

**Table S66 Results of the Overall MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 143 | 118 | 1492 | 0.0047 | -0.0269 | 0.0363 | 117 | 0.7685 |

**Table S67 Results of the meta-regression of the overall data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 143 | 118 | 1492 | 0.0012 | -0.0013 | 0.0038 | 536 | 0.3394 |

**Table S68 Results of the meta-regression of the overall data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 61 | 51 | 541 | 0.0076 | -0.0422 | 0.0574 | 481 | 0.7652 |
| **Alternating** | 51 | 50 | 607 | -0.0057 | -0.0575 | 0.0461 | 481 | 0.8291 |
| **Stepwise** | 17 | 15 | 218 | 0.0066 | -0.0710 | 0.0843 | 481 | 0.8667 |
| **Stochastic** | 5 | 5 | 21 | 0.1138 | -0.0792 | 0.3067 | 481 | 0.2471 |

**Table S69 Results of the meta-regression of the overall data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 12 | 11 | 38 | -0.0140 | -0.1872 | 0.1592 | 145 | 0.8733 |
| **Biochemical Assay** | 18 | 20 | 154 | 0.0459 | -0.0632 | 0.1549 | 145 | 0.4074 |
| **Gene Expression** | 9 | 12 | 50 | -0.0888 | -0.3260 | 0.1484 | 145 | 0.4604 |
| **Life-history Traits** | 76 | 74 | 508 | -0.0057 | -0.0528 | 0.0413 | 145 | 0.8102 |
| **Morphological** | 53 | 53 | 376 | 0.0093 | -0.0447 | 0.0633 | 145 | 0.7333 |
| **Physiological** | 46 | 38 | 198 | -0.0025 | -0.0909 | 0.0858 | 145 | 0.9549 |
| **Population** | 36 | 35 | 168 | 0.0304 | -0.0701 | 0.1308 | 145 | 0.5512 |

**Table S70 Results of the meta-regression of the overall data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | -0.0322 | -0.3547 | 0.2903 | 1064 | 0.8446 |
| **Catalase Activity** | 2 | 2 | 11 | 0.0072 | -0.3858 | 0.4001 | 1064 | 0.9715 |
| **Cortisol Levels** | 3 | 9 | 13 | 0.0578 | -0.3424 | 0.4581 | 1064 | 0.7768 |
| **Development Time** | 62 | 62 | 294 | -0.0201 | -0.0796 | 0.0395 | 1064 | 0.5087 |
| **Fecundity** | 19 | 23 | 72 | -0.0019 | -0.1355 | 0.1316 | 1064 | 0.9773 |
| **Food Consumption** | 8 | 9 | 24 | -0.0285 | -0.2572 | 0.2001 | 1064 | 0.8067 |
| **Head Width** | 5 | 5 | 14 | 0.0690 | -0.2656 | 0.4035 | 1064 | 0.6860 |
| **hsp70** | 5 | 5 | 12 | -0.1196 | -0.5393 | 0.3000 | 1064 | 0.5760 |
| **Immune Defense** | 2 | 2 | 14 | 0.1065 | -0.2337 | 0.4468 | 1064 | 0.5391 |
| **Length** | 24 | 26 | 89 | 0.0491 | -0.0581 | 0.1564 | 1064 | 0.3691 |
| **Locomotor Performance** | 13 | 13 | 35 | 0.0251 | -0.1777 | 0.2279 | 1064 | 0.8079 |
| **Longevity** | 17 | 16 | 93 | 0.0588 | -0.0527 | 0.1703 | 1064 | 0.3010 |
| **Mass** | 31 | 29 | 116 | -0.0030 | -0.0960 | 0.0900 | 1064 | 0.9496 |
| **Metabolic Rate** | 15 | 15 | 46 | -0.0620 | -0.2895 | 0.1655 | 1064 | 0.5928 |
| **Mortality** | 4 | 4 | 13 | -0.0044 | -0.3333 | 0.3245 | 1064 | 0.9792 |
| **PO Activity** | 3 | 2 | 14 | 0.0007 | -0.2804 | 0.2818 | 1064 | 0.9963 |
| **Reproductive Rate** | 5 | 5 | 17 | 0.0474 | -0.2133 | 0.3081 | 1064 | 0.7214 |
| **Sex** | 3 | 4 | 15 | -0.0339 | -0.3710 | 0.3032 | 1064 | 0.8435 |
| **SOD Activity** | 2 | 2 | 11 | -0.0061 | -0.3651 | 0.3528 | 1064 | 0.9732 |
| **Survival** | 30 | 30 | 127 | 0.0444 | -0.0726 | 0.1614 | 1064 | 0.4565 |
| **Tail Length** | 7 | 7 | 26 | 0.0295 | -0.1736 | 0.2326 | 1064 | 0.7757 |
| **Triglyceride** | 2 | 8 | 14 | 0.0136 | -0.3211 | 0.3483 | 1064 | 0.9366 |

**Table S71 Results of the meta-regression of the overall data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = lnCVR.

| Overall Data (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 20 | 22 | 150 | -0.0001 | -0.1114 | 0.1112 | 90 | 0.9991 |
| **Amphibia** | 8 | 6 | 64 | 0.0398 | -0.0875 | 0.1672 | 90 | 0.5358 |
| **Anthozoa** | 1 | 1 | 12 | 0.0023 | -0.3319 | 0.3365 | 90 | 0.9893 |
| **Arachnida** | 3 | 3 | 126 | 0.0809 | -0.0336 | 0.1955 | 90 | 0.1638 |
| **Bivalvia** | 3 | 3 | 14 | 0.2645 | -0.1102 | 0.6392 | 90 | 0.1643 |
| **Branchiopoda** | 3 | 3 | 21 | -0.1070 | -0.3439 | 0.1300 | 90 | 0.3721 |
| **Gastropoda** | 2 | 2 | 21 | 0.0784 | -0.2003 | 0.3570 | 90 | 0.5778 |
| **Holothuroidea** | 3 | 1 | 42 | -0.1053 | -0.4398 | 0.2291 | 90 | 0.5332 |
| **Insecta** | 70 | 53 | 722 | 0.0044 | -0.0388 | 0.0476 | 90 | 0.8390 |
| **Malacostraca** | 9 | 6 | 60 | 0.0178 | -0.1327 | 0.1682 | 90 | 0.8149 |

**Table S72 Results of the population-level traits subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnCVR.

| Population-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 36 | 35 | 168 | 0.0008 | -0.2027 | 0.2042 | 5 | 0.9927 |

**Table S73 Results of the meta-regression of the population-level traits data set with the amplitude of the fluctuation as the moderator.**

Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnCVR.

| Population-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 36 | 35 | 168 | 0.0013 | -0.0038 | 0.0063 | 134 | 0.6183 |

**Table S74 Results of the meta-regression of the population-level traits data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnCVR.

| Population-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 18 | 19 | 60 | -0.0444 | -0.1706 | 0.0819 | 30 | 0.4785 |
| **Alternating** | 9 | 10 | 74 | 0.0415 | -0.0579 | 0.1409 | 120 | 0.4101 |
| **Stepwise** | 7 | 8 | 22 | -0.0510 | -0.1852 | 0.0832 | 120 | 0.4536 |

**Table S75 Results of the meta-regression of the population-level traits data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with population-level phenotypic traits. Effect size = lnCVR.

| Population-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 7 | 9 | 19 | -0.0435 | -0.2420 | 0.1549 | 23 | 0.6542 |
| **Arachnida** | 1 | 1 | 24 | -0.0185 | -0.2736 | 0.2365 | 23 | 0.8818 |
| **Insecta** | 21 | 16 | 108 | 0.0167 | -0.0651 | 0.0985 | 23 | 0.6769 |

**Table S76 Results of the individual-level traits subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnCVR.

| Individual-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 135 | 115 | 1324 | 0.0008 | -0.0317 | 0.0333 | 114 | 0.9601 |

**Table S77 Results of the meta-regression of the individual-level traits data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnCVR.

| Individual-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 135 | 115 | 1324 | 0.0007 | -0.0020 | 0.0033 | 472 | 0.6347 |

**Table S78 Results of the meta-regression of the individual-level traits data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnCVR.

| Individual-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 58 | 48 | 481 | 0.0086 | -0.0427 | 0.0600 | 423 | 0.7411 |
| **Alternating** | 48 | 49 | 533 | -0.0143 | -0.0670 | 0.0384 | 423 | 0.5947 |
| **Stepwise** | 16 | 14 | 196 | 0.0106 | -0.0702 | 0.0913 | 423 | 0.7970 |
| **Stochastic** | 5 | 5 | 20 | 0.1216 | -0.0792 | 0.3225 | 423 | 0.2347 |

**Table S79 Results of the meta-regression of the individual-level traits data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = lnCVR.

| Individual-level Traits (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 20 | 22 | 131 | -0.0005 | -0.1147 | 0.1137 | 87 | 0.9931 |
| **Amphibia** | 8 | 6 | 64 | 0.0400 | -0.0857 | 0.1657 | 87 | 0.5286 |
| **Arachnida** | 3 | 3 | 102 | 0.0948 | -0.0157 | 0.2053 | 87 | 0.0919 |
| **Bivalvia** | 3 | 3 | 12 | 0.0682 | -0.4124 | 0.5487 | 87 | 0.7787 |
| **Branchiopoda** | 3 | 3 | 21 | -0.1160 | -0.3536 | 0.1217 | 87 | 0.3349 |
| **Gastropoda** | 2 | 2 | 21 | 0.0788 | -0.1952 | 0.3528 | 87 | 0.5689 |
| **Holothuroidea** | 3 | 1 | 39 | -0.0369 | -0.3998 | 0.3261 | 87 | 0.8404 |
| **Insecta** | 63 | 52 | 614 | -0.0030 | -0.0476 | 0.0416 | 87 | 0.8945 |
| **Malacostraca** | 8 | 4 | 56 | 0.0157 | -0.1376 | 0.1689 | 87 | 0.8395 |

**Table S80 Results of the aquatic subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR.

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 51 | 46 | 395 | -0.0035 | -0.0701 | 0.0631 | 45 | 0.9164 |

**Table S81 Results of the meta-regression of the aquatic data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR.

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 51 | 46 | 395 | 0.0018 | -0.0044 | 0.0080 | 135 | 0.5728 |

**Table S82 Results of the meta-regression of the aquatic data set with exposure type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR.

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 30 | 28 | 197 | 0.0112 | -0.0911 | 0.1136 | 393 | 0.8291 |
| **Developmental** | 22 | 21 | 198 | -0.0134 | -0.0975 | 0.0708 | 393 | 0.7553 |

**Table S83 Results of the meta-regression of the aquatic data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR.

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 33 | 24 | 267 | -0.0074 | -0.0864 | 0.0716 | 40 | 0.8508 |
| **Alternating** | 9 | 16 | 98 | -0.0489 | -0.1879 | 0.0901 | 40 | 0.4813 |

**Table S84 Results of the meta-regression of the aquatic data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 6 | 6 | 13 | 0.0716 | -0.2341 | 0.3774 | 86 | 0.6426 |
| **Biochemical Assay** | 12 | 14 | 75 | 0.0460 | -0.1279 | 0.2198 | 86 | 0.6006 |
| **Gene Expression** | 5 | 7 | 38 | -0.0889 | -0.3776 | 0.1999 | 86 | 0.5422 |
| **Life-history Traits** | 17 | 18 | 67 | -0.1609 | -0.2543 | -0.0676 | 86 | **0.0009** |
| **Morphological** | 15 | 15 | 90 | 0.0614 | -0.0200 | 0.1429 | 86 | 0.1374 |
| **Physiological** | 29 | 21 | 112 | 0.0142 | -0.1030 | 0.1314 | 86 | 0.8107 |

**Table S85 Results of the meta-regression of the aquatic data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = lnCVR. Bold p-value = significant result (< 0.05).

| Aquatic Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | -0.0365 | -0.3143 | 0.2413 | 178 | 0.7959 |
| **Cortisol Levels** | 3 | 9 | 13 | 0.0611 | -0.3065 | 0.4287 | 32 | 0.7371 |
| **Development Time** | 14 | 15 | 35 | -0.1701 | -0.2842 | -0.0559 | 178 | **0.0037** |
| **Food Consumption** | 6 | 6 | 13 | 0.0671 | -0.2514 | 0.3857 | 32 | 0.6707 |
| **Immune Defense** | 1 | 1 | 12 | 0.0906 | -0.2350 | 0.4163 | 32 | 0.5748 |
| **Length** | 11 | 12 | 43 | 0.0707 | -0.0409 | 0.1824 | 178 | 0.2130 |
| **Locomotor Performance** | 6 | 6 | 11 | 0.1006 | -0.2132 | 0.4144 | 178 | 0.5279 |
| **Mass** | 8 | 7 | 20 | 0.0755 | -0.0701 | 0.2211 | 178 | 0.3077 |
| **Metabolic Rate** | 10 | 10 | 24 | -0.0182 | -0.3029 | 0.2665 | 178 | 0.8998 |

**Table S86 Results of the terrestrial subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 84 | 69 | 929 | 0.0045 | -0.0346 | 0.0437 | 68 | 0.8177 |

**Table S87 Results of the meta-regression of the terrestrial data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 84 | 69 | 929 | 0.0007 | -0.0024 | 0.0038 | 336 | 0.6525 |

**Table S88 Results of the meta-regression of the terrestrial data set with exposure type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 22 | 19 | 139 | -0.0201 | -0.1262 | 0.0861 | 927 | 0.7109 |
| **Developmental** | 66 | 60 | 790 | 0.0079 | -0.0336 | 0.0494 | 927 | 0.7090 |

**Table S89 Results of the meta-regression of the terrestrial data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 25 | 24 | 214 | 0.0182 | -0.0566 | 0.0930 | 295 | 0.6330 |
| **Alternating** | 39 | 33 | 435 | -0.0013 | -0.0593 | 0.0568 | 295 | 0.9657 |
| **Stepwise** | 13 | 11 | 186 | 0.0109 | -0.0734 | 0.0953 | 295 | 0.7987 |
| **Stochastic** | 4 | 4 | 16 | 0.0430 | -0.1962 | 0.2821 | 295 | 0.7237 |

**Table S90 Results of the meta-regression of the terrestrial data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 6 | 5 | 25 | -0.0415 | -0.2542 | 0.1711 | 65 | 0.6979 |
| **Biochemical Assay** | 6 | 6 | 79 | 0.0401 | -0.0977 | 0.1778 | 65 | 0.5630 |
| **Gene Expression** | 4 | 5 | 12 | -0.0183 | -0.4519 | 0.4153 | 65 | 0.9332 |
| **Life-history Traits** | 59 | 56 | 441 | 0.0145 | -0.0391 | 0.0680 | 65 | 0.5909 |
| **Morphological** | 38 | 38 | 286 | -0.0144 | -0.0831 | 0.0543 | 65 | 0.6771 |
| **Physiological** | 17 | 17 | 86 | -0.0166 | -0.1472 | 0.1139 | 65 | 0.8001 |

**Table S91 Results of the meta-regression of the terrestrial data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = lnCVR.

| Terrestrial Organisms (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 48 | 47 | 259 | -0.0024 | -0.0712 | 0.0665 | 659 | 0.9457 |
| **Fecundity** | 16 | 20 | 67 | 0.0235 | -0.1238 | 0.1708 | 659 | 0.7542 |
| **Food Consumption** | 2 | 3 | 11 | -0.0947 | -0.4170 | 0.2277 | 659 | 0.5645 |
| **Head Width** | 5 | 5 | 14 | 0.0610 | -0.2916 | 0.4136 | 659 | 0.7343 |
| **Length** | 13 | 14 | 46 | -0.0031 | -0.1753 | 0.1691 | 659 | 0.9719 |
| **Locomotor Performance** | 7 | 7 | 24 | -0.0085 | -0.2657 | 0.2486 | 659 | 0.9481 |
| **Longevity** | 14 | 13 | 86 | 0.0806 | -0.0421 | 0.2033 | 659 | 0.1974 |
| **Mass** | 23 | 22 | 96 | -0.0176 | -0.1308 | 0.0956 | 659 | 0.7597 |
| **Metabolic Rate** | 5 | 5 | 22 | -0.1141 | -0.4499 | 0.2216 | 659 | 0.5047 |
| **PO Activity** | 3 | 2 | 14 | 0.0044 | -0.2936 | 0.3023 | 659 | 0.9770 |
| **Reproductive Rate** | 4 | 4 | 11 | 0.0416 | -0.2893 | 0.3726 | 659 | 0.8050 |
| **Tail Length** | 6 | 6 | 21 | -0.0353 | -0.2989 | 0.2283 | 659 | 0.7925 |

**Table S92 Results of the acclimation subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 52 | 47 | 336 | 0.0180 | -0.0236 | 0.0596 | 46 | 0.3879 |

**Table S93 Results of the meta-regression of the acclimation data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 52 | 47 | 336 | 0.0016 | -0.0015 | 0.0048 | 158 | 0.3142 |

**Table S94 Results of the meta-regression of the acclimation data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 25 | 22 | 151 | 0.0013 | -0.0797 | 0.0823 | 131 | 0.9745 |
| **Alternating** | 13 | 15 | 75 | 0.0449 | -0.0126 | 0.1025 | 42 | 0.1226 |
| **Stepwise** | 8 | 6 | 76 | -0.0147 | -0.1069 | 0.0775 | 131 | 0.7536 |

**Table S95 Results of the meta-regression of the acclimation data set with life-history stage as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Adult** | 27 | 29 | 125 | 0.0302 | -0.0210 | 0.0813 | 148 | 0.2456 |
| **Embryo** | 3 | 3 | 11 | 0.0530 | -0.3312 | 0.4373 | 41 | 0.7818 |
| **Juvenile** | 12 | 9 | 101 | 0.0006 | -0.1164 | 0.1175 | 148 | 0.9924 |
| **Larva** | 12 | 9 | 92 | -0.0092 | -0.0980 | 0.0797 | 148 | 0.8387 |

**Table S96 Results of the meta-regression of the acclimation data set with the number of fluctuations as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Number of Fluctuations** | 50 | 45 | 329 | -0.0005 | -0.0012 | 0.0002 | 152 | 0.1698 |

**Table S97 Results of the meta-regression of the acclimation data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 8 | 6 | 24 | 0.0398 | -0.1502 | 0.2298 | 65 | 0.6769 |
| **Biochemical Assay** | 15 | 17 | 140 | 0.0317 | -0.0191 | 0.0826 | 65 | 0.2170 |
| **Life-history Traits** | 9 | 10 | 28 | -0.0261 | -0.1772 | 0.1250 | 65 | 0.7310 |
| **Physiological** | 31 | 23 | 134 | -0.0110 | -0.1000 | 0.0781 | 65 | 0.8067 |

**Table S98 Results of the meta-regression of the acclimation data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Apparent Digestibility Coefficient** | 2 | 1 | 16 | -0.0374 | -0.2752 | 0.2005 | 111 | 0.7562 |
| **Catalase Activity** | 2 | 2 | 11 | -0.0017 | -0.2867 | 0.2834 | 111 | 0.9908 |
| **Cortisol Levels** | 3 | 9 | 13 | 0.0591 | -0.2759 | 0.3942 | 19 | 0.7159 |
| **Food Consumption** | 5 | 5 | 12 | 0.0942 | -0.2049 | 0.3932 | 19 | 0.5177 |
| **Immune Defense** | 2 | 2 | 14 | 0.1030 | -0.1520 | 0.3581 | 19 | 0.4082 |
| **Metabolic Rate** | 13 | 12 | 41 | -0.0755 | -0.2722 | 0.1212 | 111 | 0.4483 |
| **SOD Activity** | 2 | 2 | 11 | -0.0113 | -0.2415 | 0.2190 | 111 | 0.9228 |

**Table S99 Results of the meta-regression of the acclimation data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = lnCVR.

| Acclimation Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 16 | 17 | 86 | 0.0147 | -0.1096 | 0.1390 | 35 | 0.8120 |
| **Bivalvia** | 3 | 3 | 12 | 0.0661 | -0.3713 | 0.5035 | 35 | 0.7608 |
| **Gastropoda** | 2 | 2 | 21 | 0.0809 | -0.1250 | 0.2867 | 35 | 0.4305 |
| **Holothuroidea** | 3 | 1 | 39 | -0.0372 | -0.3799 | 0.3054 | 35 | 0.8267 |
| **Insecta** | 14 | 14 | 103 | 0.0217 | -0.0296 | 0.0731 | 35 | 0.3961 |
| **Malacostraca** | 7 | 4 | 48 | -0.0041 | -0.1309 | 0.1227 | 35 | 0.9478 |

**Table S100 Results of the developmental subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 88 | 81 | 988 | 0.0004 | -0.0391 | 0.0399 | 80 | 0.9855 |

**Table S101 Results of the meta-regression of the developmental data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 88 | 81 | 988 | 0.0009 | -0.0023 | 0.0040 | 326 | 0.5837 |

**Table S102 Results of the meta-regression of the developmental data set with exposure period as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Embryo** | 50 | 50 | 598 | -0.0114 | -0.0620 | 0.0392 | 84 | 0.6561 |
| **Juvenile** | 7 | 8 | 50 | 0.0145 | -0.1404 | 0.1693 | 84 | 0.8530 |
| **Larva** | 28 | 25 | 311 | 0.0051 | -0.0692 | 0.0794 | 84 | 0.8927 |
| **Pupa** | 3 | 5 | 29 | 0.1844 | -0.0661 | 0.4349 | 84 | 0.1470 |

**Table S103 Results of the meta-regression of the developmental data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 37 | 33 | 330 | 0.0144 | -0.0495 | 0.0782 | 300 | 0.6588 |
| **Alternating** | 35 | 36 | 458 | -0.0243 | -0.0865 | 0.0380 | 300 | 0.4436 |
| **Stepwise** | 10 | 10 | 120 | 0.0181 | -0.0886 | 0.1248 | 300 | 0.7383 |
| **Stochastic** | 5 | 5 | 17 | 0.1358 | -0.0922 | 0.3638 | 300 | 0.2421 |

**Table S104 Results of the meta-regression of the developmental data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Behavioural** | 4 | 5 | 14 | -0.0735 | -0.3451 | 0.1982 | 79 | 0.5919 |
| **Biochemical Assay** | 3 | 3 | 14 | 0.1267 | -0.1353 | 0.3886 | 79 | 0.3387 |
| **Gene Expression** | 6 | 8 | 40 | -0.0803 | -0.3429 | 0.1822 | 79 | 0.5443 |
| **Life-history Traits** | 69 | 69 | 480 | -0.0057 | -0.0584 | 0.0471 | 79 | 0.8316 |
| **Morphological** | 53 | 53 | 376 | 0.0078 | -0.0510 | 0.0665 | 79 | 0.7934 |
| **Physiological** | 17 | 18 | 64 | 0.0132 | -0.1317 | 0.1581 | 79 | 0.8561 |

**Table S105 Results of the meta-regression of the developmental data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 60 | 61 | 287 | -0.0256 | -0.0898 | 0.0386 | 720 | 0.4342 |
| **Fecundity** | 16 | 18 | 67 | 0.0157 | -0.1299 | 0.1612 | 720 | 0.8327 |
| **Food Consumption** | 3 | 4 | 12 | -0.0949 | -0.4045 | 0.2148 | 720 | 0.5478 |
| **Head Width** | 5 | 5 | 14 | 0.0664 | -0.2832 | 0.4160 | 720 | 0.7093 |
| **Length** | 24 | 26 | 89 | 0.0464 | -0.0676 | 0.1605 | 720 | 0.4245 |
| **Locomotor Performance** | 9 | 10 | 27 | 0.0463 | -0.1968 | 0.2895 | 720 | 0.7084 |
| **Longevity** | 16 | 15 | 91 | 0.0690 | -0.0509 | 0.1889 | 720 | 0.2588 |
| **Mass** | 31 | 29 | 116 | -0.0038 | -0.1031 | 0.0954 | 720 | 0.9395 |
| **Tail Length** | 7 | 7 | 26 | 0.0302 | -0.1868 | 0.2472 | 720 | 0.7846 |

**Table S106 Results of the meta-regression of the developmental data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = lnCVR.

| Developmental Exposure (lnCVR) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Actinopteri** | 5 | 7 | 45 | -0.0112 | -0.1984 | 0.1759 | 58 | 0.9048 |
| **Amphibia** | 6 | 5 | 61 | 0.0400 | -0.1050 | 0.1850 | 58 | 0.5833 |
| **Arachnida** | 3 | 3 | 102 | 0.0948 | -0.0339 | 0.2234 | 58 | 0.1458 |
| **Branchiopoda** | 3 | 3 | 21 | -0.1237 | -0.3822 | 0.1348 | 58 | 0.3422 |
| **Insecta** | 51 | 45 | 511 | -0.0033 | -0.0571 | 0.0505 | 58 | 0.9027 |

## 7.16 | Graphical Representation of the MLMA and Meta-regression Results (lnCVR)

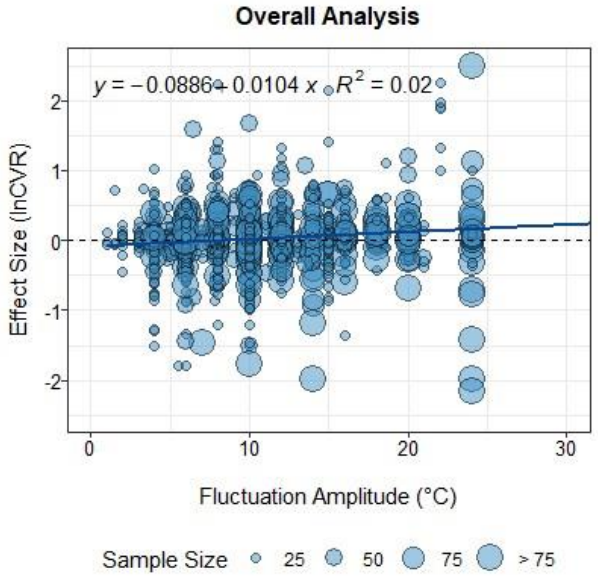


**Figure S31 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the overall data set.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the overall MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis and Y-axis limits are cropped for presentation.

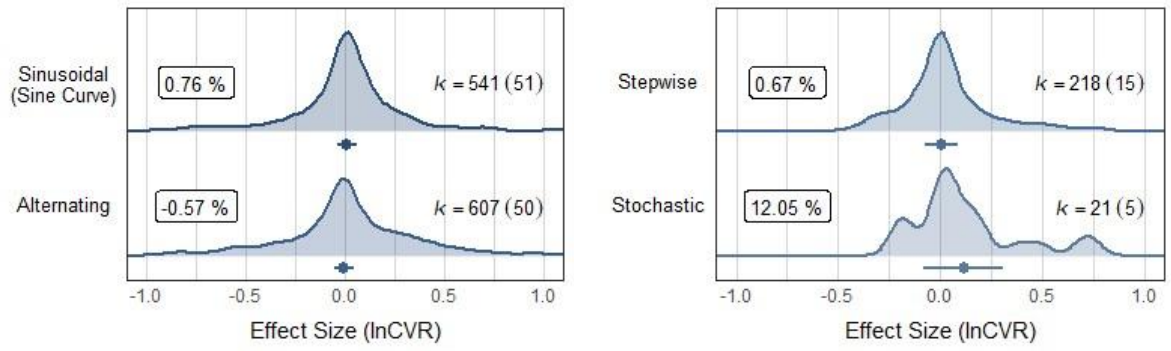


**Figure S32 Meta-regression results for the overall data set with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. Distribution curve created from raw effect sizes. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

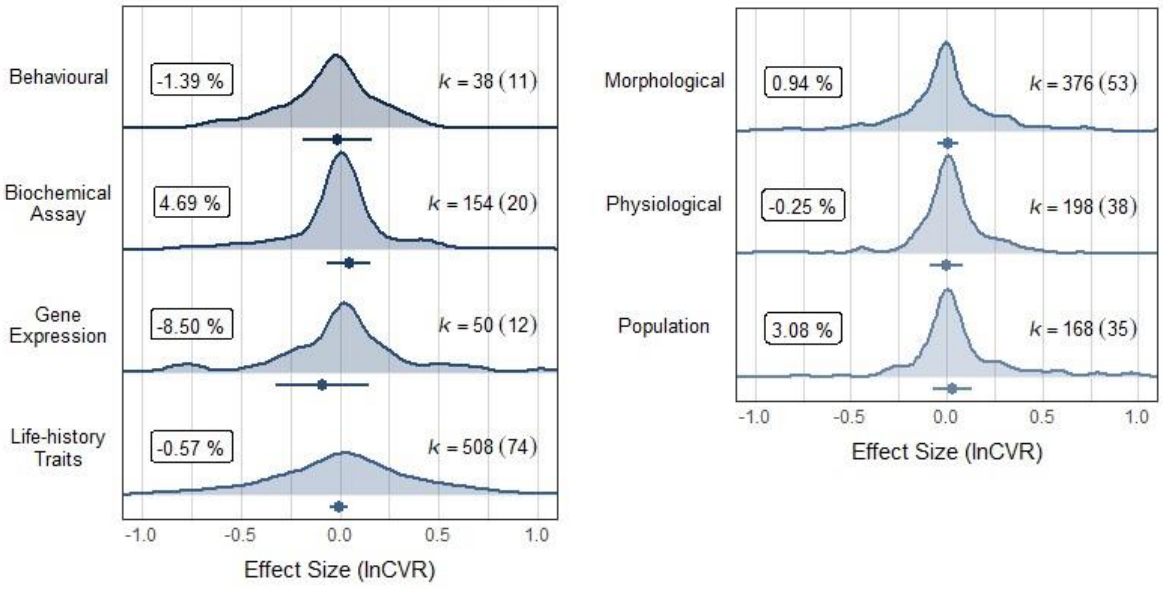


**Figure S33 Meta-regression results for the overall data set with phenotypic trait category as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

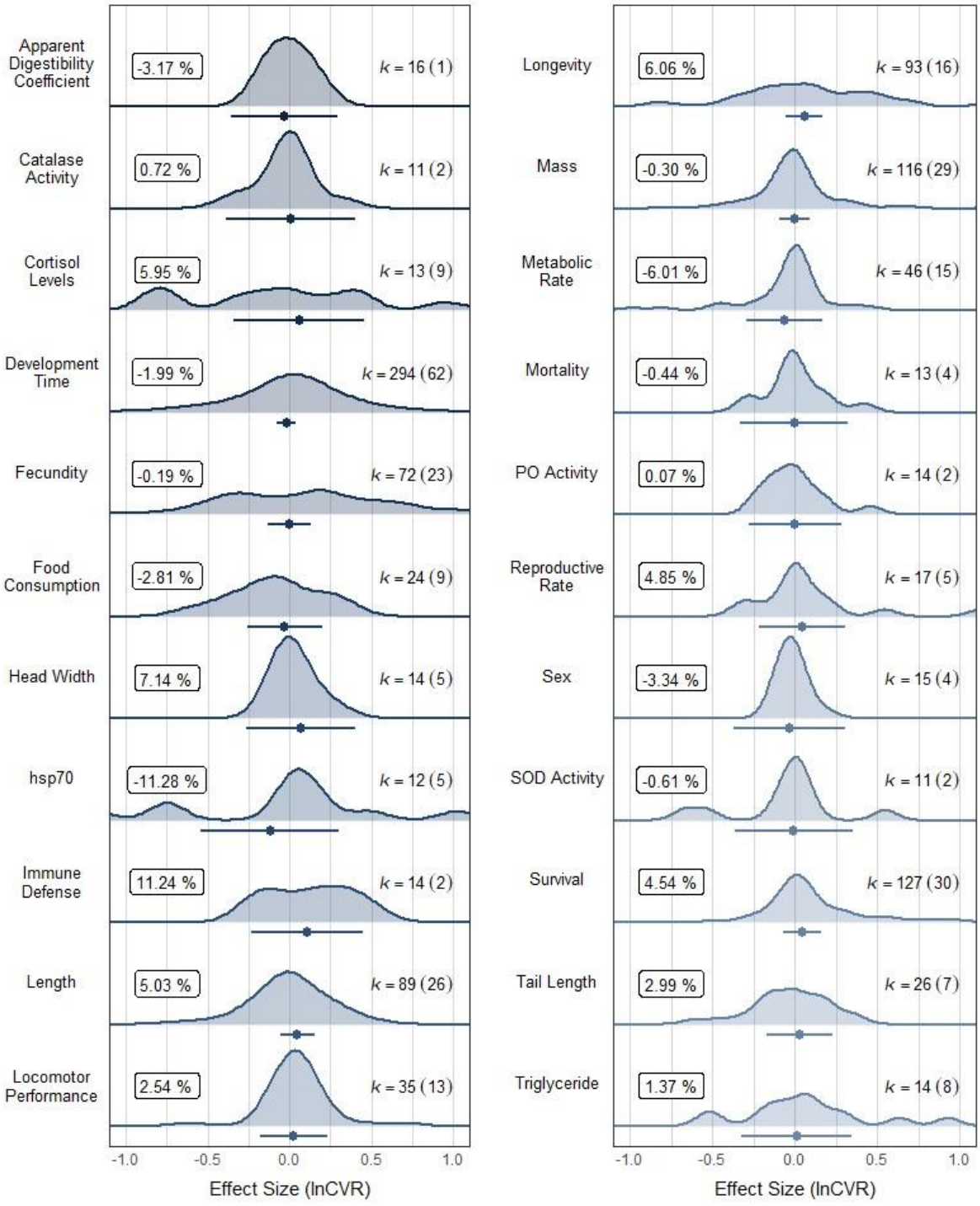


**Figure S34 Meta-regression results for the overall data set with specific phenotypic traits as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates

transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

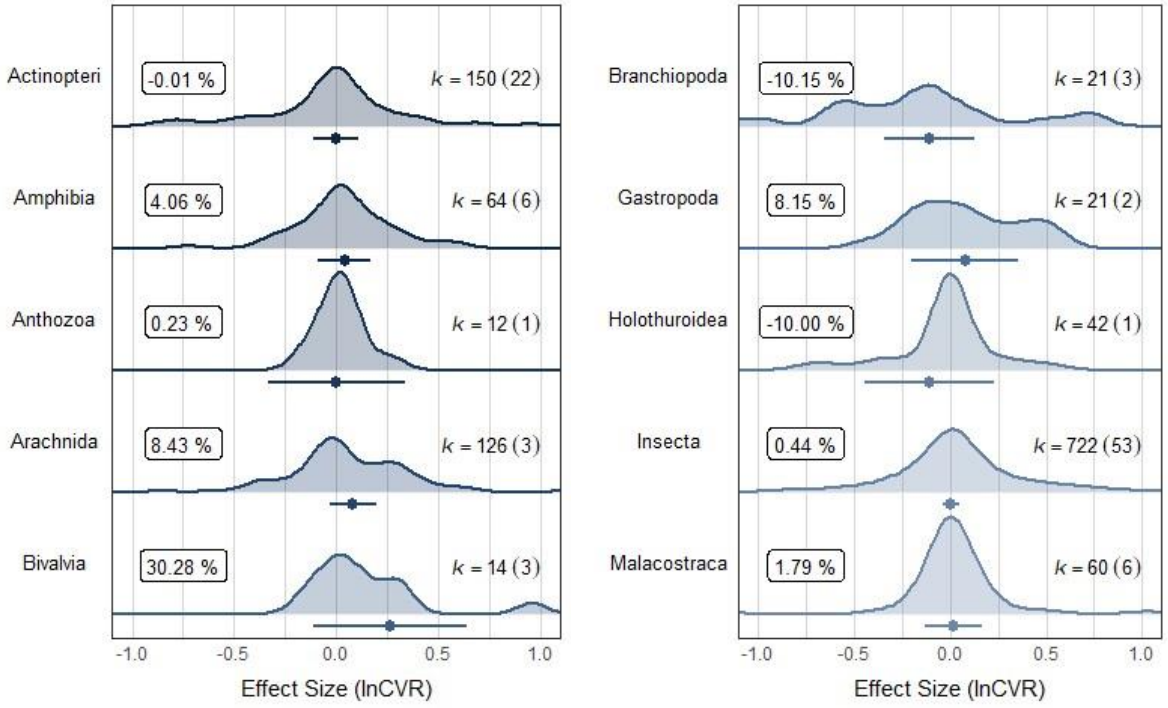


**Figure S35 Meta-regression results for the overall data set with taxonomic class as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

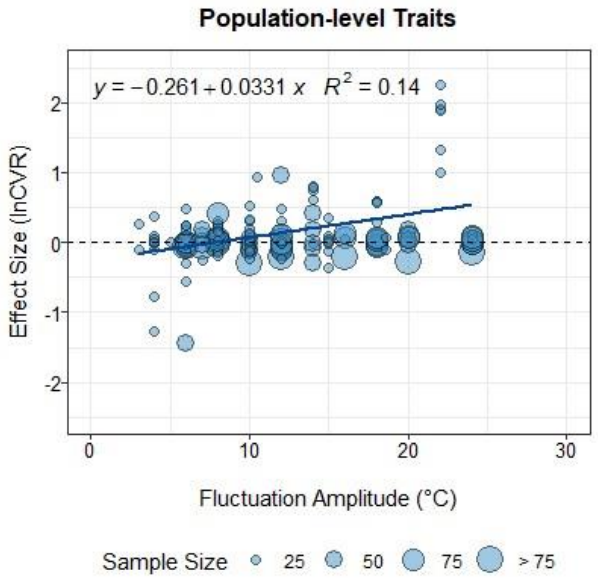


**Figure S36 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the population-level traits subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the population-level traits MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

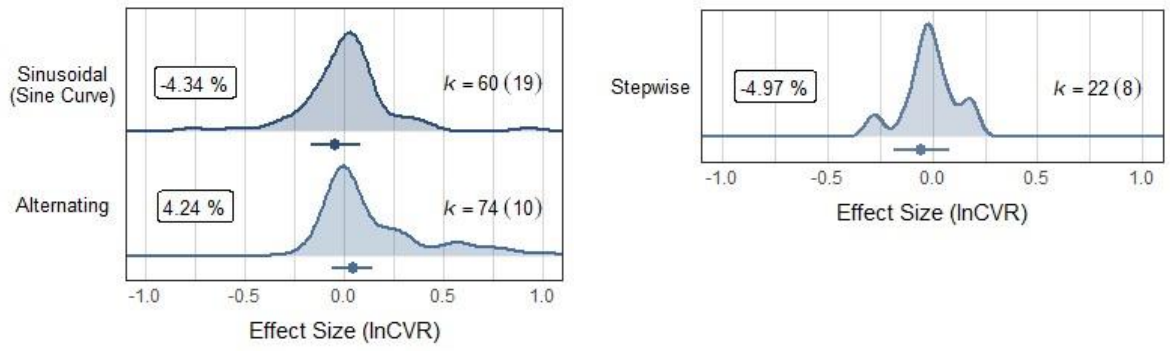


**Figure S37 Meta-regression results for the population-level traits subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

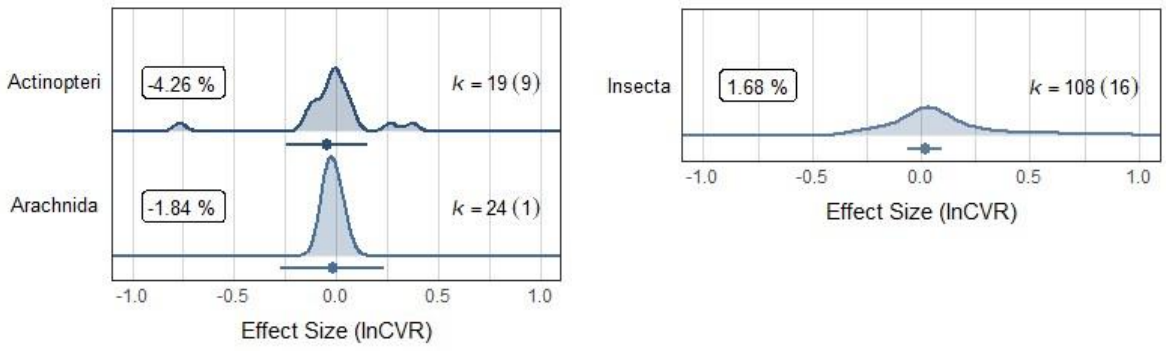


**Figure S38 Meta-regression results for the population-level traits subset with taxonomic class as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

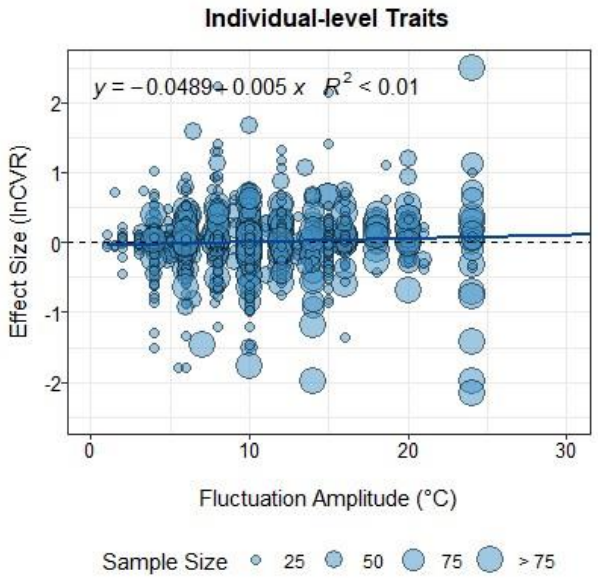


**Figure S39 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the individual-level traits subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the individual-level traits MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis limits are cropped for presentation.

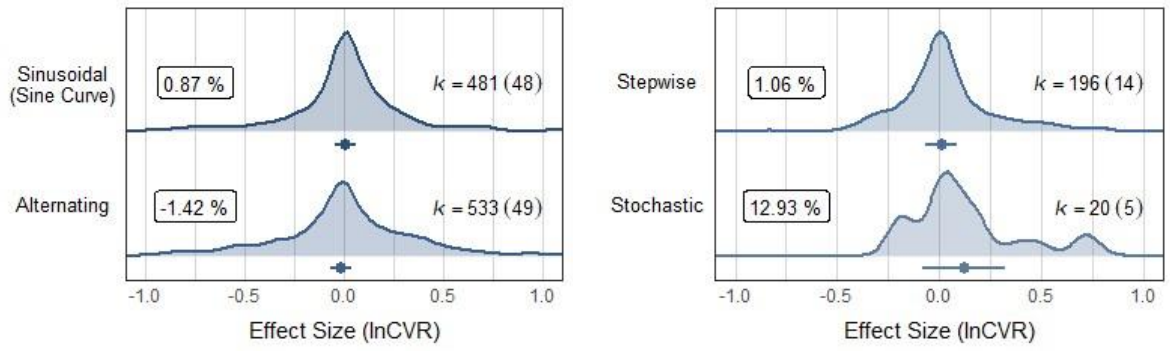


**Figure S40 Meta-regression results for the individual-level traits subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

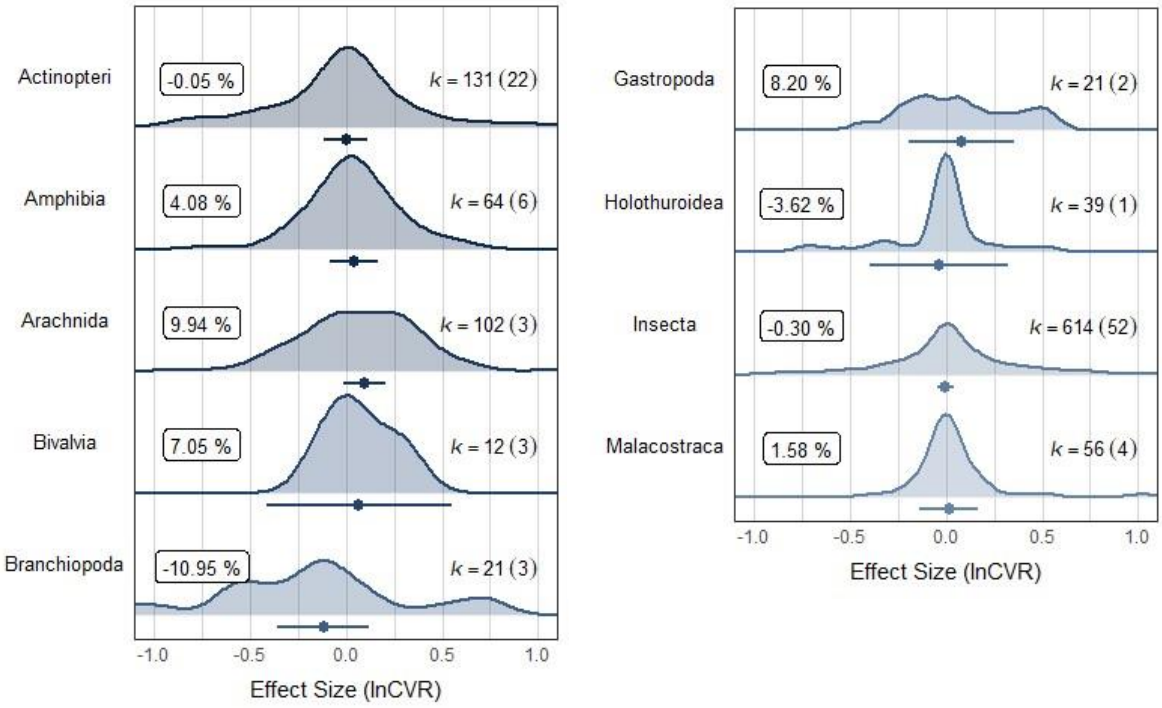


**Figure S41 Meta-regression results for the individual-level traits subset with taxonomic class as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

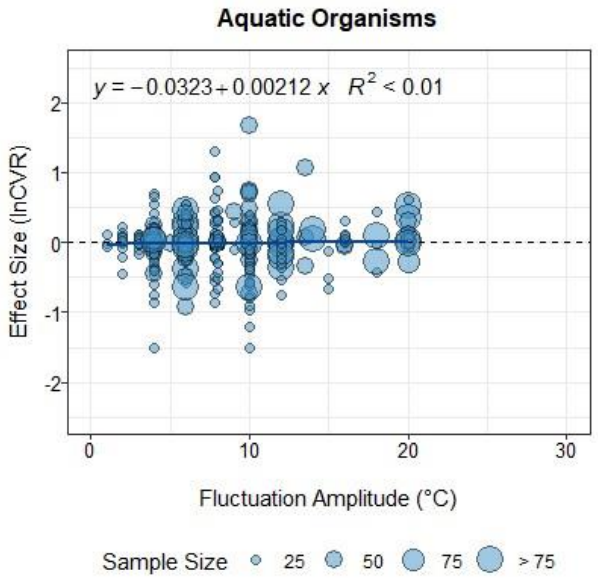


**Figure S42 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the aquatic subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the aquatic subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

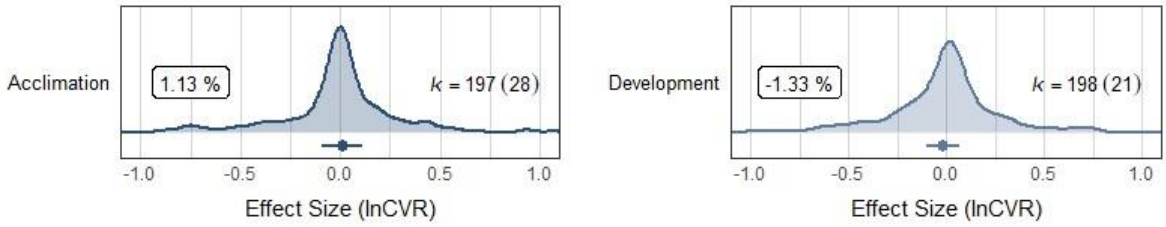


**Figure S43 Meta-regression results for the aquatic subset with exposure type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

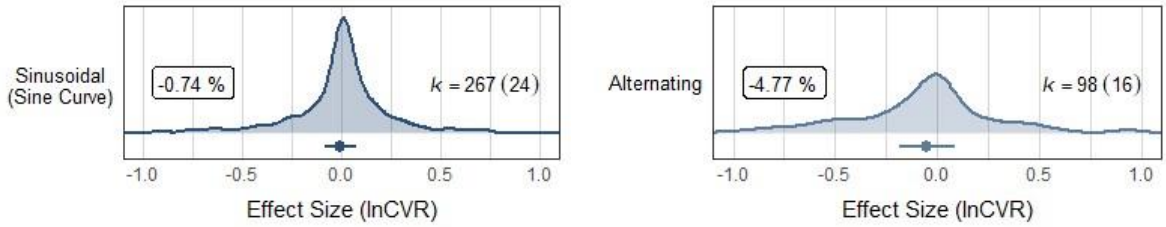


**Figure S44 Meta-regression results for the aquatic subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

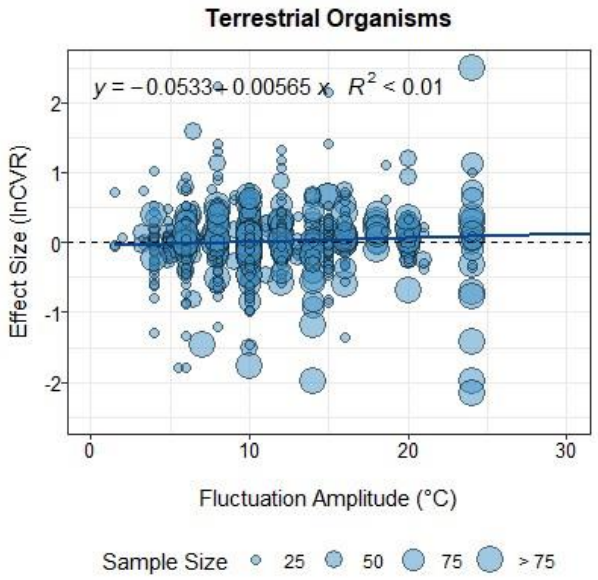


**Figure S45 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the terrestrial subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the terrestrial subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis limits are cropped for presentation.


**Figure S46 Meta-regression results for the terrestrial subset with exposure type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

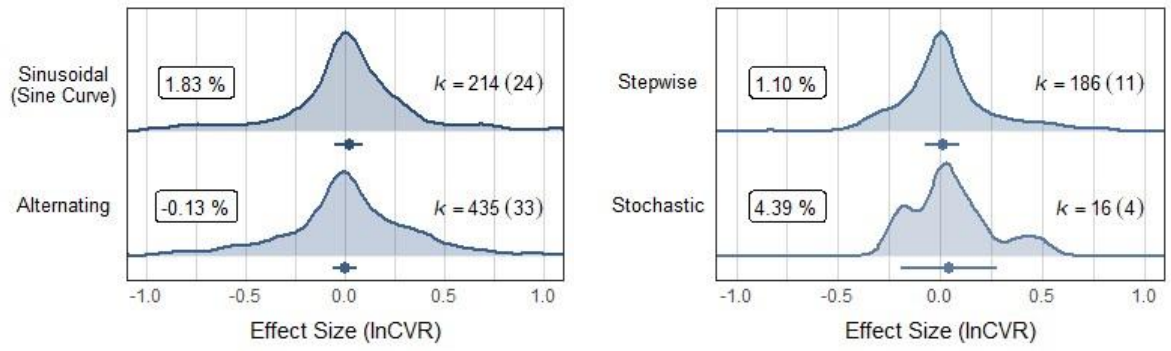


**Figure S47 Meta-regression results for the terrestrial subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

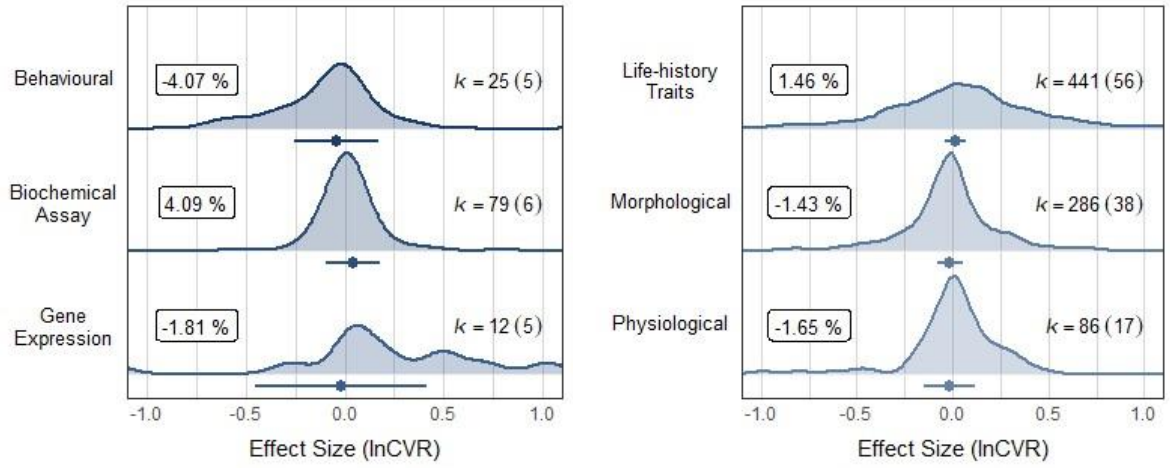


**Figure S48 Meta-regression results for the terrestrial subset with phenotypic trait category as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

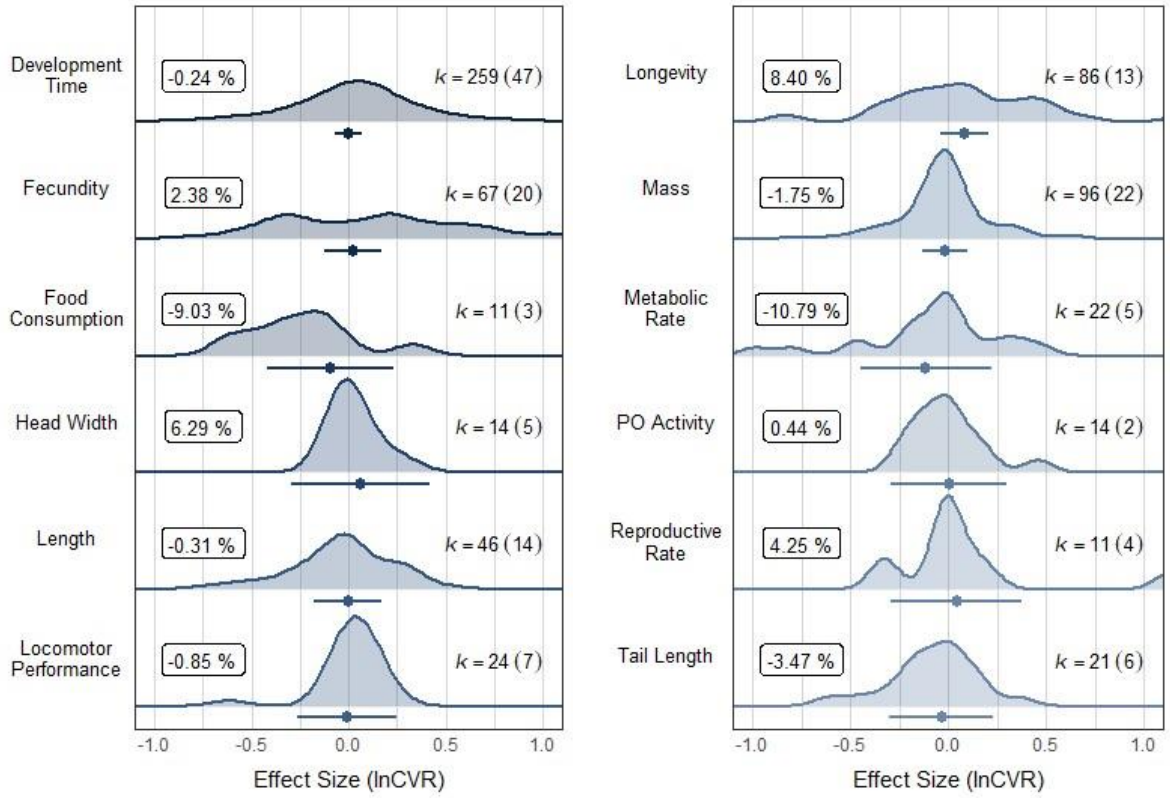


**Figure S49 Meta-regression results for the terrestrial subset with specific phenotypic traits as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

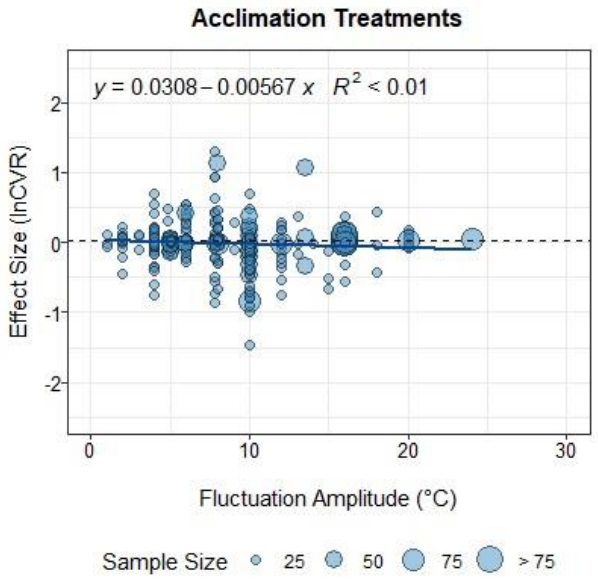


**Figure S50 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the acclimation subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the acclimation subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

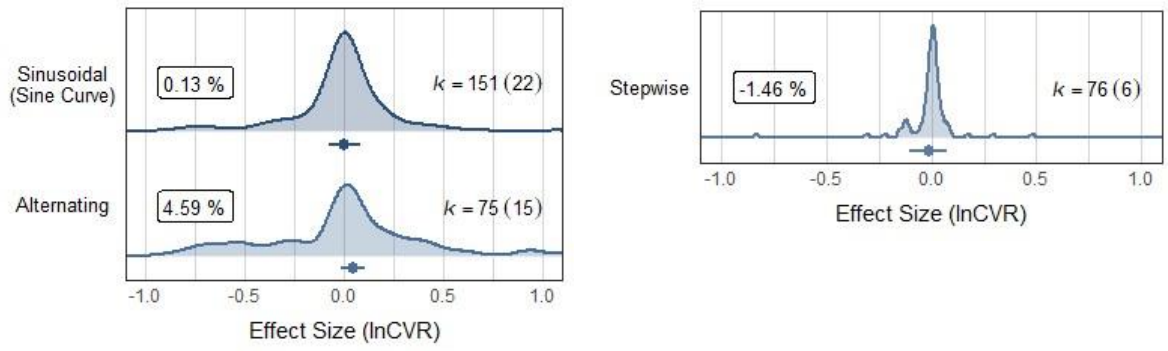


**Figure S51 Meta-regression results for the acclimation subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

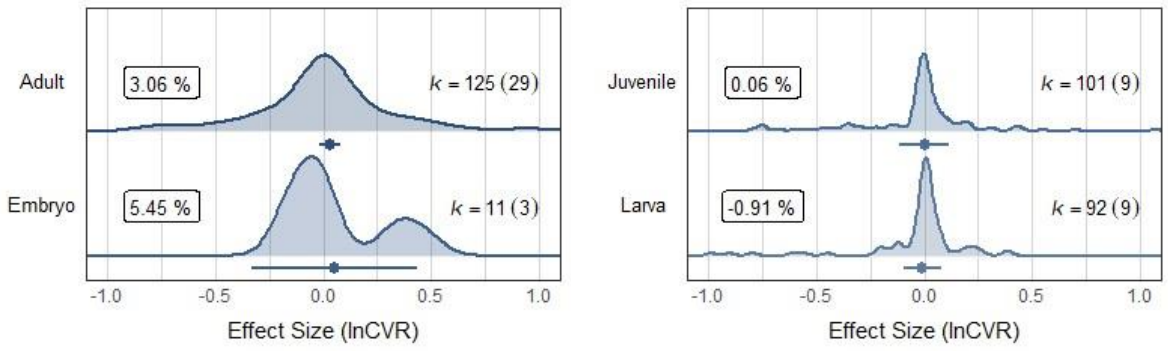


**Figure S52 Meta-regression results for the acclimation subset with life-history stage as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

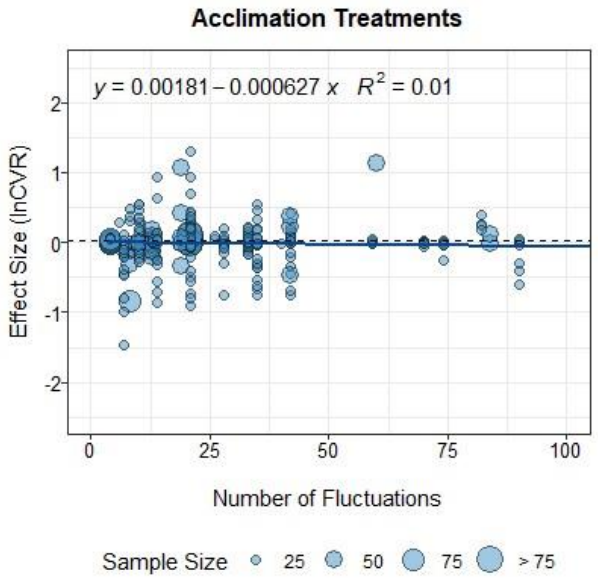


**Figure S53 Relationship between effect size (lnCVR) and the number of fluctuations for the acclimation subset.** There was no significant relationship between the number of fluctuations and the phenotypic trait variance. Dashed line = lnCVR estimate from the acclimation subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis limits are cropped for presentation.

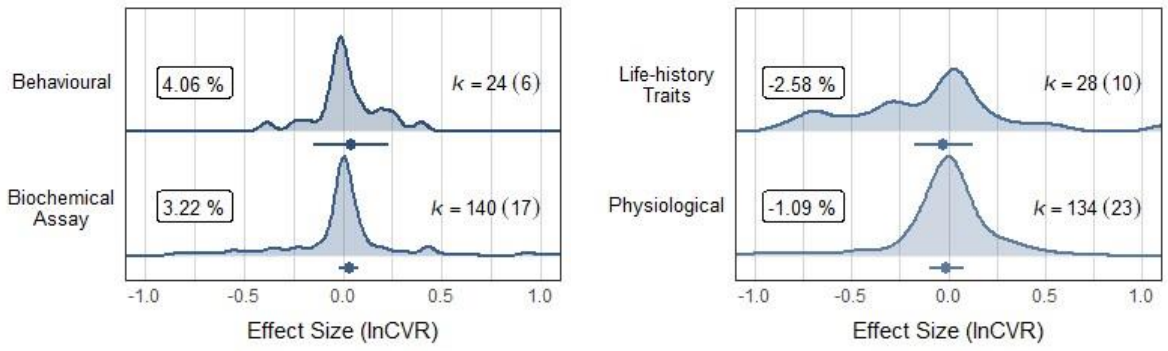


**Figure S54 Meta-regression results for the acclimation subset with phenotypic trait category as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

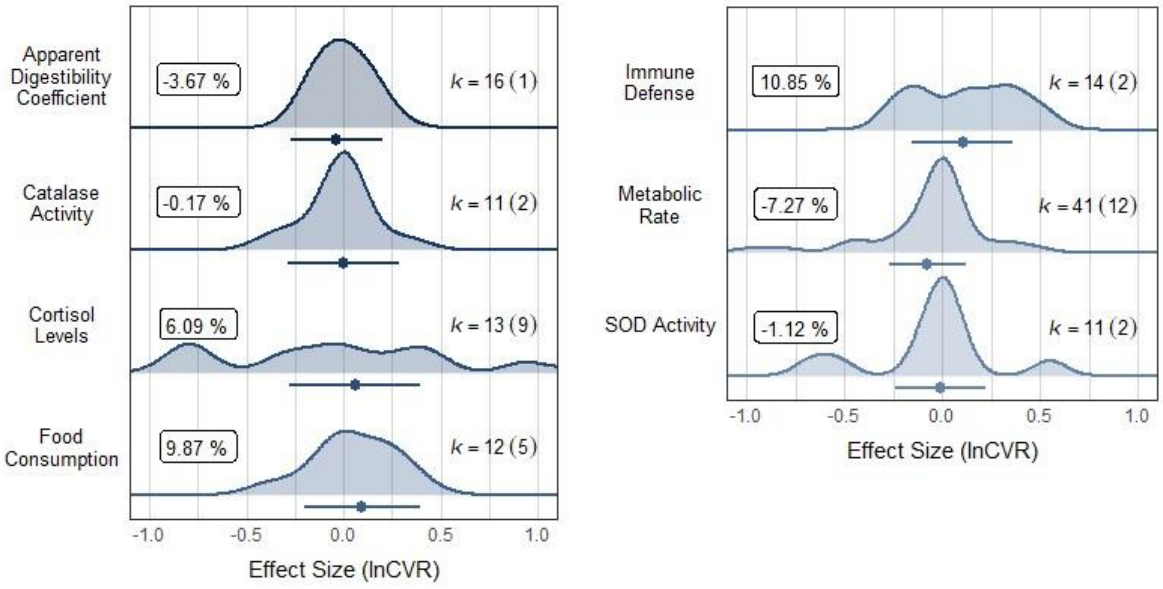


**Figure S55 Meta-regression results for the acclimation subset with specific phenotypic traits as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

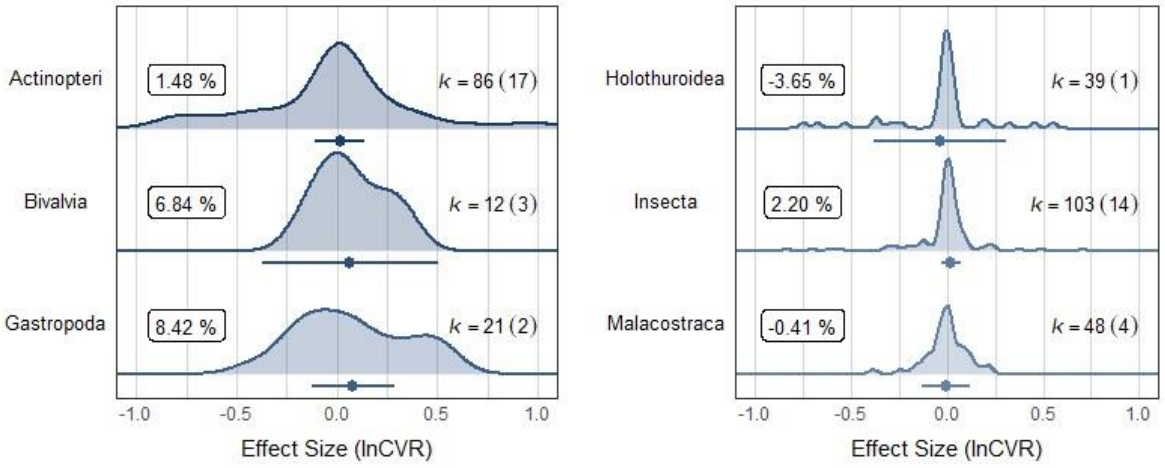


**Figure S56 Meta-regression results for the acclimation subset with taxonomic class as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

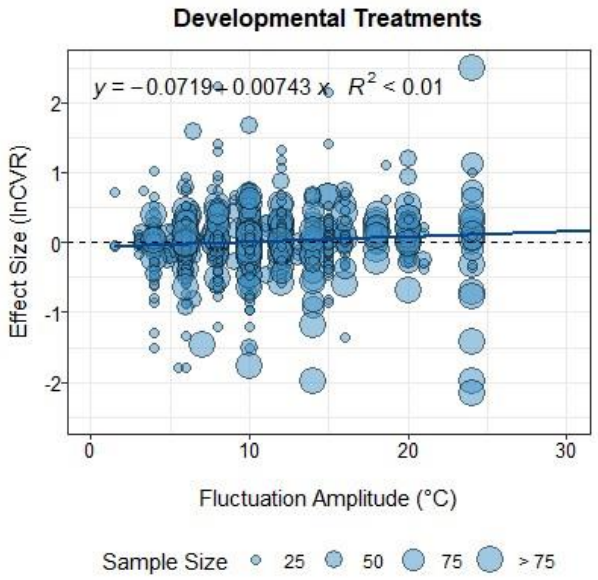


**Figure S57 Relationship between effect size (lnCVR) and the amplitude of fluctuation for the developmental subset.** There was no significant relationship between the fluctuation amplitude and the phenotypic trait variance. Dashed line = lnCVR estimate from the developmental subset MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size. X-axis limits are cropped for presentation.

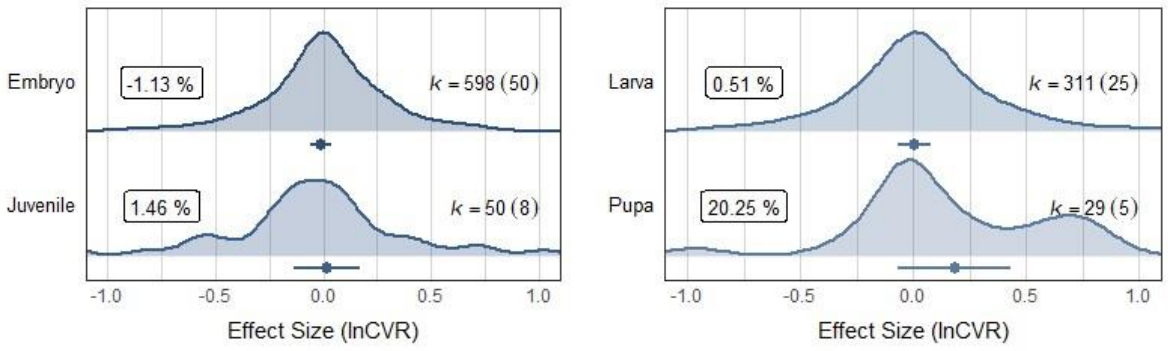


**Figure S58 Meta-regression results for the developmental subset with exposure period as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

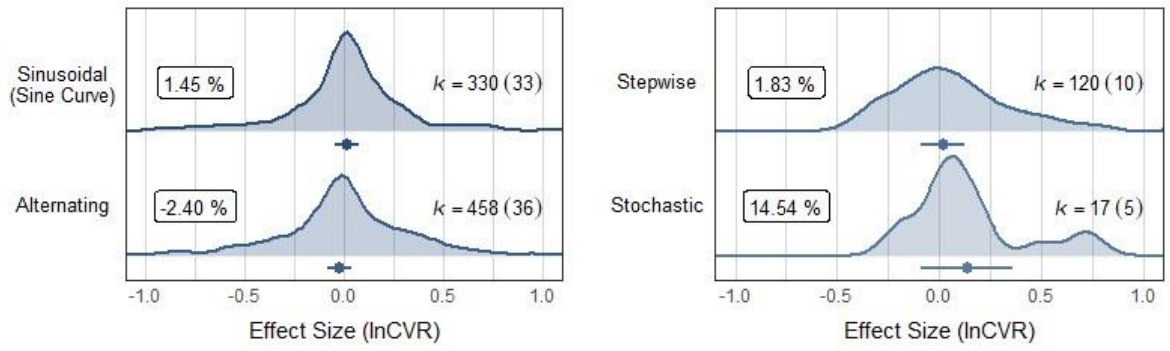


**Figure S59 Meta-regression results for the developmental subset with fluctuation type as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimate ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

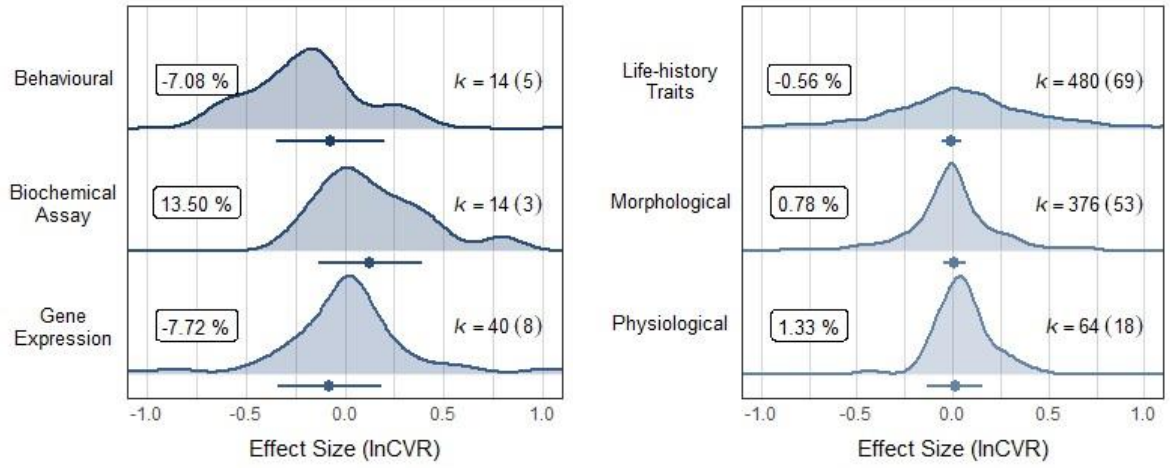


**Figure S60 Meta-regression results for the developmental subset with phenotypic trait category as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

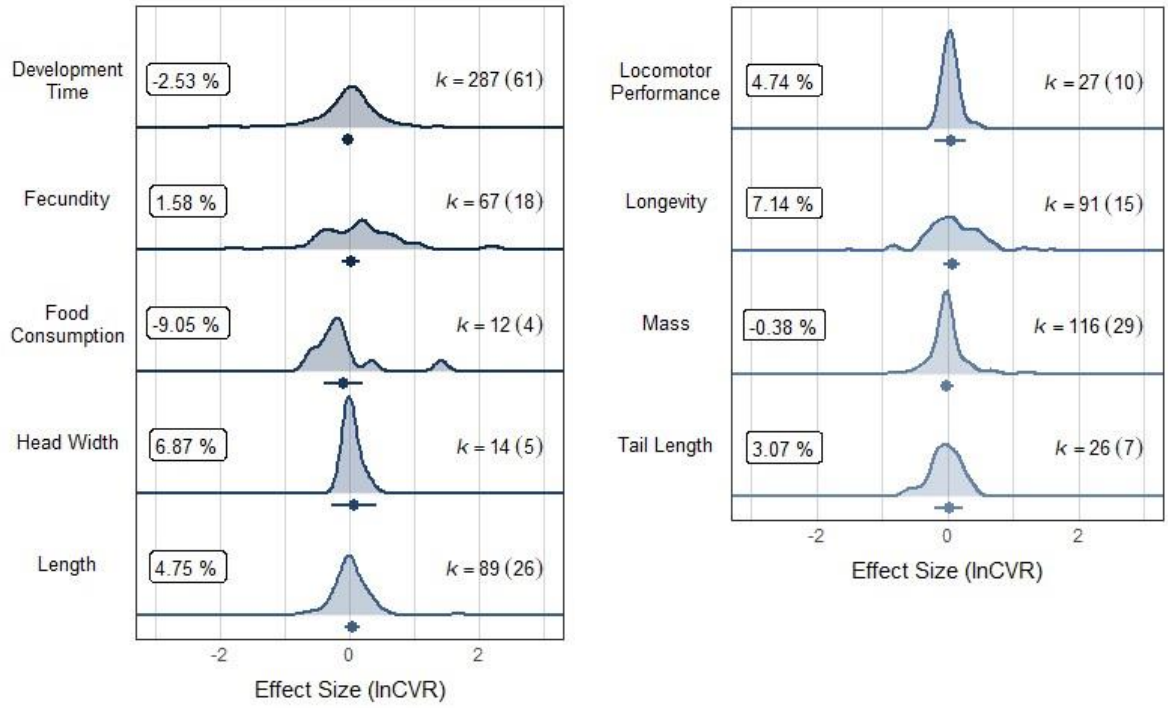


**Figure S61 Meta-regression results for the developmental subset with specific phenotypic traits as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

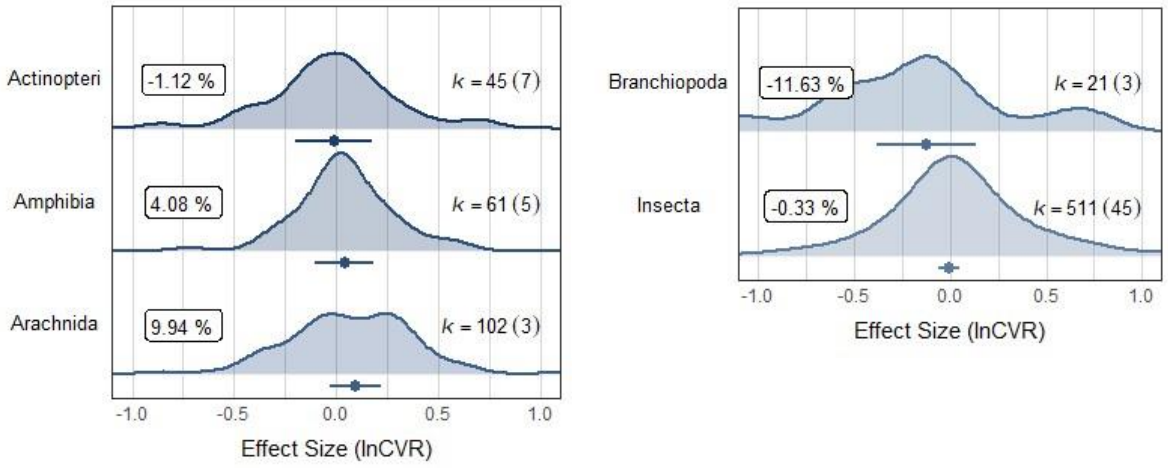


**Figure S62 Meta-regression results for the developmental subset with taxonomic class as the moderator.** There were no significant differences in the variance of phenotypic trait values between the thermal treatments (effect size 95% CIs crossing zero). Mean effect size (lnCVR) estimates ± 95% CIs (solid circles and horizontal bars) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean lnCVR estimates transformed to show a proportional difference between fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

# Chapter 3

## Temperature variability has no effect on phenotypic plasticity in ectotherms – a meta-analysis

# 1 | Abstract

Anthropogenic climate change can have profound impacts across all ecological levels. Phenotypic plasticity allows individuals to compensate for changes to their thermal environment and may increase resilience to environmental variability. It is predicted that thermal changes will occur to both the mean temperature and the amplitudes of fluctuations at different temporal-scales. Therefore, it is imperative to understand the impacts of thermal mean and fluctuation changes on the plastic responses of ectotherms. Our aim was to establish the current state-of-knowledge regarding the influence of thermal variability on the capacity for phenotypic plasticity in ectothermic organisms. We conducted a quantitative synthesis of 44 studies (212 effect sizes across 40 species) that compares the effects of constant and fluctuating temperatures with the same mean on plastic responses (behavioural, biochemical assay, gene expression, life-history, morphological and physiological) across different ecosystems (terrestrial and aquatic) and mechanisms of phenotypic plasticity (acclimation and developmental plasticity). Most studies in the literature implemented diurnal temperature fluctuations (24 h cycles). Our analysis shows that phenotypic plasticity in ectothermic organisms does not differ between constant and fluctuating thermal environments. We conclude that changes in diurnal fluctuations do not alter the resilience of ectotherms to temperature change. Instead, plasticity and its attendant compensation for thermal variability is driven by changes in longer-term mean temperatures.

## 2 | Introduction

The thermal environment has profound impacts on biological, ecological, and evolutionary functions across all levels of organisation (Angilletta, 2009; Hochachka and Somero, 2002; Hoffmann et al., 2003). 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, 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 (Little and Seebacher, 2021; Sinclair et al., 2016). 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; Stoks et al., 2017). However, ectotherms can exhibit plastic responses that adjust TPCs to allow relatively constant function despite climate variability (Kingsolver et al., 2015).

Phenotypic plasticity allows individuals to at least partially compensate for changes in their environment (Loughland et al., 2021; Schulte, 2014). Both developmental plasticity and acclimation are examples of phenotypic plasticity that allow organisms to maintain relatively constant phenotypic trait function across environmental gradients, independently from the genotype (Beaman et al., 2016). Developmental plasticity involves relatively persistent phenotypic changes in response to the thermal environment experienced during development (Burggren, 2018; Warner et al., 2018). 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 (Arrighi et al., 2013). These experiments provide a logistically simple approach to explore the phenotypic response of ectotherms. However, in natural environments temperatures can

fluctuate (Chen et al., 2013), and it is unclear whether results from constant temperature experiments are transferable to natural conditions.

Climate change is increasing mean temperatures and the amplitudes of thermal fluctuations at different periods (e.g., diurnal, seasonal, and annual; IPCC, 2021). Unpredictable thermal variability can increase the probability of organisms experiencing temperature extremes (Guo et al., 2018; Vázquez et al., 2017; Xu et al., 2020). Ectotherms are particularly susceptible to such thermal change as their body temperatures are closely tied to environmental conditions (Huey et al., 2012; Karl et al., 2009). It is imperative to understand whether short-term fluctuations around mean temperatures influence the capacity for phenotypic plasticity compared to constant mean temperatures, to predict the impacts of climate change (Dowd et al., 2015; Kefford et al., 2022; Warner et al., 2023).

Phenotypic plasticity can come at a cost to the organism (e.g., energetic cost; Angilletta, 2009) and plastic responses to short-term variation could exacerbate that cost. Instead, 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). Thermal changes could also affect plastic responses differently depending on the relationship between the period of fluctuations and the lifespan of the organisms, or the response time of different phenotypic traits. At present, empirical evidence is equivocal, leaving the relationship between temperature fluctuations and phenotypic plasticity unresolved.

Our aim was to provide a quantitative synthesis that establishes the current state-of-knowledge regarding the influence of thermal variability on 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) mechanisms of phenotypic plasticity (acclimation or developmental plasticity).

## 3 | Materials and Methods

We followed 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 7.1) in our study. Question formulation, literature searching and screening were performed according to Foo et al., (2021).

### 3.1 | Systematic Literature Search and Screening

The PECO (Population, Exposure, Comparator, Outcome) framework was adhered to in the development of focus questions and the literature search and screening processes (Supplementary Materials 7.1; Morgan et al., 2018).

A systematic literature search was conducted for a parallel study that investigated whether phenotypic traits differed between exposure to constant versus fluctuating thermal environments (Chapter 2). The search was conducted on the 5$^{th}$ of April 2022 and used the Scopus, Web of Science and ScienceDirect databases. Search terms included synonyms for ‘plasticity’, ‘acclimation’ and ‘developmental effects’ to identify studies that imposed treatments during the lifespan of an individual (Noble et al., 2018; Pottier et al., 2021). To

limit the search string to temperature treatment manipulations ‘thermal’ and ‘temperature’ were added as search terms (Barley et al., 2021; 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 can be found in the Supplementary Materials 7.2.

These searches returned a total of 13,549 unique studies (Supplementary Materials 7.3). A total of 57 studies were identified that were in English and experimentally compared the effect of constant and fluctuating temperature treatments on the phenotypic traits of ectothermic organisms in controlled-laboratory conditions. Scopus, Web of Science, ScienceDirect and Google Scholar were used to conduct a forward (papers citing the original study) and backward (papers that were cited in the original study) search on the 19$^{th}$ of October 2022. An additional 87 studies were identified. There was one species that was removed from the compiled data set due to an unresolved phylogeny in the Open Tree of Life database (Michonneau et al., 2016; Slein et al., 2023). A total of 143 studies were included in the original study (Supplementary Materials 7.3).

This meta-analysis was conducted on a subset of these studies that experimentally compared the phenotypic plasticity of ectothermic organisms across fluctuating and constant temperature treatments. To identify eligible studies, we conducted an additional abstract and full-text screening (screenings in Rayyan Software; Ouzzani et al., 2016) of the original included studies that followed an exclusion and inclusion criteria (Supplementary Materials 7.4). For a study to be included in the analysis, experiments had to have: (a) identical housing and habituating periods across treatments; (b) acclimation or developmental plasticity treatments (within the lifespan of an individual); (c) fluctuating and constant temperature treatments pairs with the same mean temperature; (d) all other treatment conditions identical (e.g., photoperiod and salinity); (e) measurements of plastic phenotypic responses (excluding

metrics of thermal performance including critical thermal minima and maxima). Phenotypic response measurements could be either at the population-level (measured for an entire population, e.g., survival) or individual-level (measured in an individual, e.g., development time or locomotor performance). All relevant statistical information needed to be available (including sample size, mean, and error metrics) and principal components and factor loadings were excluded (Tarka et al., 2018). This process identified 44 studies that were included in the final analysis (Supplementary Materials 7.5).

### 3.2 | Data Extraction

There were four essential thermal treatments: two constant temperature treatments at a high and low temperature, and two fluctuating temperature treatments with mean temperature corresponding to those of constant treatments. We extracted data (sample sizes, means, and errors) for individuals kept in these treatments to calculate effect sizes. The acute test temperatures at which phenotypic traits were measured had to coincide with the mean temperatures of the corresponding thermal treatment (Seebacher et al., 2015). If there were more than two treatment temperatures, we selected the two experimental temperatures that coincided best with the study species' natural temperature range (Seebacher et al., 2015). In experiments that had fluctuating thermal treatments at multiple amplitudes or types of fluctuations (e.g., sinusoidal, stepwise, alternating, or stochastic; Figure 1), we compared each to the constant thermal treatments as a separate effect size. The required statistical information was manually extracted from texts, tables, supplementary materials, and figures (using R package *metaDigitise* to extract data from figures; Pick et al., 2019). To avoid non-independence, we extracted biological replicates as the sample size, instead of technical replicates (Wu and Seebacher, 2020).

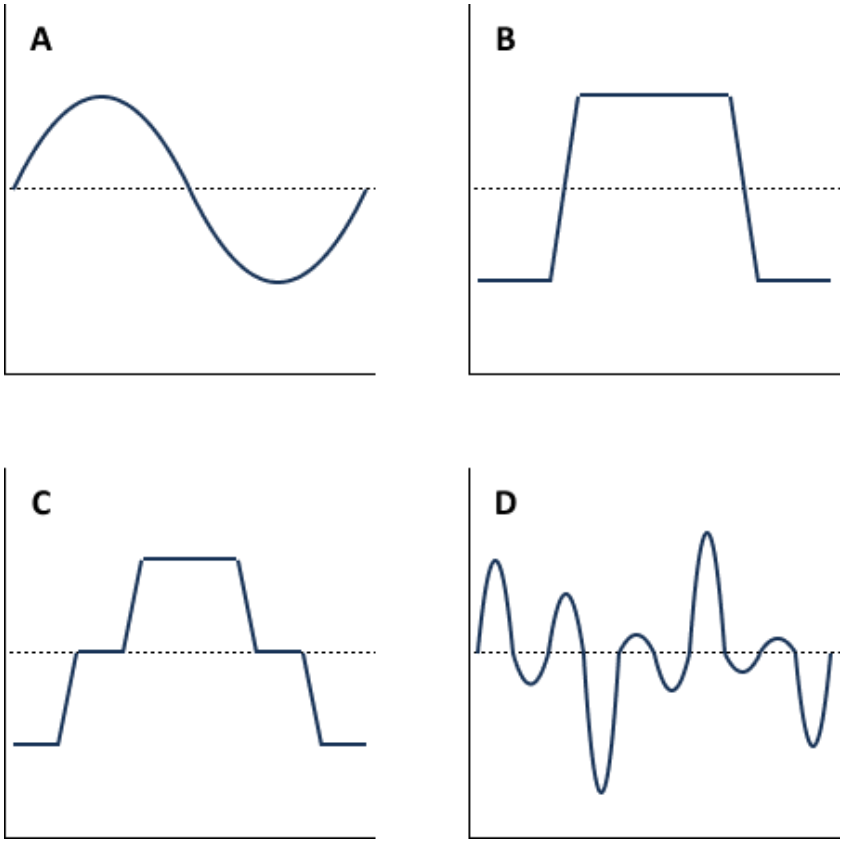


**Figure 1 Examples of different fluctuation types in the experimental designs of included studies.** Fluctuation patterns included sinusoidal regimes (A), alternating regimes (B), stepwise regimes (C), and stochastic regimes (D).

In addition, we recorded descriptive statistics for use in subset analyses and as moderators in meta-regression analyses, that had the potential to influence differences in the capacity for phenotypic plasticity. We recorded taxonomic information, preferred ecosystems, and life-history stages, as the capacity for phenotypic plasticity can differ across phylogenetic lineages, habitats, and developmental periods (Le Roy et al., 2017; Seebacher et al., 2012; Seebacher et al., 2015). Parameters of the fluctuation treatment were recorded (mean temperature and the fluctuations amplitude, type, and period) because a previous meta-analysis had indicated that the extremity of fluctuations can influence phenotypic trait values in reptiles (Raynal et al., 2022). Information on the phenotypic outcome were recorded (trait category and specific phenotypic trait measurement) as phenotypic plasticity can differ across

traits (Le Roy et al., 2017). Lastly, details of the experimental design were recorded including the type and duration of treatment exposure. All authors were involved in the data extraction process and were not permitted to screen or extract studies they had a previous association with (Macartney et al., 2022). Collectively we conducted an initial screen and data extraction of a subset of studies to ensure that each process was consistent across all authors.

### 3.3 | Data Processing and Transformations

Standard errors (SE; Equation 1) and 95% confidence internals (CI; Equation 2) were converted to standard deviations (SD), as required for effect size calculations (Quinn and Keough, 2002):

$$SD = SE \times \sqrt{n} \quad (1)$$

$$SD = \sqrt{n} \times \frac{CI_{High} - CI_{Low}}{3.92} \quad (2)$$

where n is sample size and $CI_{High}$ and $CI_{Low}$ represent the upper and lower 95% CIs respectively.

Data reported as percentages (e.g., survival) are bounded between 0 and 1. As such, they were transformed prior to effect size calculations using Equation 3 and 4 to obtain means and variances that were more likely to satisfy normality assumptions (Macartney et al., 2022):

$$\bar{x} = \arcsin\left(\sqrt{\bar{z}}\right) \quad (3)$$

$$SD_{\bar{x}} = \sqrt{\frac{SD_z^2}{4\bar{z}(1 - \bar{z})}} \quad (4)$$

where $\bar{x}$ and $SD_{\bar{x}}$ are the transformed means and SD, $\bar{z}$ and $SD_{\bar{z}}$ are the percentage means and SD. To integrate data presented on different scales we transformed means and variances that were reported as the natural logarithm using Equation 5 and 6 (Higgins et al., 2008):

$$\bar{x} = \exp\left(\bar{z} + \frac{SD_{\bar{z}}^2}{2}\right) \quad (5)$$

$$SD_{\bar{x}} = \sqrt{\left(\exp\left(SD_{\bar{z}}^2\right) - 1\right)\exp\left(2\bar{z} + SD_{\bar{z}}^2\right)} \quad (6)$$

where $\bar{x}$ and $SD_{\bar{x}}$ are the transformed means and SD, $\bar{z}$ and $SD_{\bar{z}}$ 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 (Rubin and Seebacher, 2022; Wu and Seebacher, 2020).

We categorised the developmental exposure period (Supplementary Materials 7.6), acclimation life-history stages (Supplementary Materials 7.7) and trait measurements (Supplementary Materials 7.8) to ensure consistency across different studies. Taxonomic information was retrieved from the Open Tree of Life (Michonneau et al., 2016), and species scientific names were adjusted to match the database. Amphibians were considered aquatic organisms for the analysis.

### 3.4 | Effect Size Calculations

To quantify the difference in phenotypic plasticity between constant and fluctuating thermal environments, we employed a standardised interaction-based plasticity response ratio difference ($PRRD_S$; Equation 7) and its corresponding sampling variance (Equation 8). The $PRRD_S$ is derived by first calculating log response ratios (lnRR; Equation 9; Hedges et al., 1999; Lajeunesse, 2011) for the change in phenotypic traits following either acclimation or developmental exposure to different temperatures (Seebacher et al., 2015). This is indicative of an organism's capacity for phenotypic plasticity, with a lnRR closer to 0 indicating that phenotypic traits are less likely to be affected by changes to temperature (lnRR of 0 = perfect compensation by phenotypic plasticity). The $PRRD_S$ captures the difference in phenotypic plasticity by subtracting the lnRR of the constant from the fluctuating thermal environment (Macartney et al., 2022). To control for the fact that temperature gradients varied among effect sizes within and across studies, $PRRD_S$ standardises the differences in lnRRs to a one-degree change in temperature (Noble et al., 2022; Pottier et al., 2021). A $PRRD_S = 0$ would indicate that there is no variation in the capacity for phenotypic plasticity between constant and fluctuating thermal environments ($PRRD_S > 0$, phenotypic plasticity increases in fluctuating temperatures; $PRRD_S < 0$, phenotypic plasticity increases in constant temperatures).

$$\text{PRRD}_S = \frac{lnRR_F - lnRR_C}{T_H - T_L} \quad (7)$$

$$v_{\text{PRRD}_S} = \left(\frac{1}{T_H - T_L}\right)^2 \left(\frac{SD_{FH}^2}{n_{FH}\bar{x}_{FH}^2} + \frac{SD_{FL}^2}{n_{FL}\bar{x}_{FL}^2} + \frac{SD_{CH}^2}{n_{CH}\bar{x}_{CH}^2} + \frac{SD_{CL}^2}{n_{CL}\bar{x}_{CL}^2}\right) \quad (8)$$

$$\text{lnRR} = \ln\left(\frac{\bar{x}_H}{\bar{x}_L}\right) \quad (9)$$

where $\bar{x}$ is the mean response, t is the treatments complimenting constant and fluctuating mean temperature, subscripts for F and C denote the fluctuating and constant thermal treatments, and subscripts H and L denote the high and low temperatures.

### 3.5 | Statistical Analysis

All calculations, analyses, and figure production were completed with R.4.2.2 in RStudio version 2022.12.0 (using R Package *ggplot2* for figures; Wickham, 2016). All data and code are available at https://github.com/ClaytonStocker/Plasticity_Fluctuation_Meta (Supplementary Materials 7.9). Data are presented as the mean PRRDs ± 95% CIs. The mean PRRDs are transformed to give the percentage change of phenotypic plasticity from constant to fluctuating thermal environments (Pustejovsky, 2018).

### 3.6 | Meta-analyses and Non-independence

Multi-level meta-analyses (MLMA) were conducted with frequentist models fitted with the restricted maximum likelihood (REML) approach and an adjusted convergence threshold ($1e^{-8}$). The t-statistic of the model contained an adjusted degrees of freedom based on the number of studies (Nakagawa et al., 2022b). To account for multiple effect sizes using the same control treatment, the model was also fitted with a modified sampling covariance matrix (Noble et al., 2022). Here, 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 into the model (Nakagawa et al., 2017; Sánchez-Tójar et al., 2020). A Study ID random effect was included to account for multiple effect sizes from the same study. Instances where the same set of animals were used for several measured responses were accounted for with a Shared Animal ID random effect. To control for phylogenetic relatedness, a phylogenetic tree was created using the Open Tree of Life database (Michonneau et al., 2016), and converted into a correlation/covariance matrix that was included in the model when estimating for phylogenetic variance. Polytomies were randomly resolved (using R Package *Ape*; Paradis and Schliep, 2019) and branch lengths calculated with Grafen's method (Power = 1; Grafen, 1989). In addition, a species-level random effect was included to account for the repeated use of the same species across effect sizes. 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 and Santos, 2012).

### 3.7 | Multi-level Meta-analytic Models

MLMA models (using the function *rma.mv* in the R Package *metafor*; Viechtbauer, 2010) were implemented to estimate the meta-analytic mean of the overall data set. In addition, analyses were conducted on a variety of data subsets to estimate the meta-analytic means for population- and individual-level traits, terrestrial and aquatic organisms, and acclimation or developmental plasticity treatments (Figure 2). The definitions of the subset terms can be found in Table 1. The total extended heterogeneity statistic ($I^2_{Total}$; 2.d.p) and partitions corresponding to the random effects ($I^2_{Animal}$, $I^2_{Obs}$, $I^2_{Phylo}$, $I^2_{Species}$, $I^2_{Study}$, and $I^2_{Trait}$,), were calculated for each of the MLMA models to quantify unexplained variation after removing the known sampling variance (high $I^2$ = 75%, medium $I^2$ = 50%, small $I^2$ = 25%; Borenstein et al., 2021; Higgins et al., 2003; Nakagawa and Santos, 2012). It is common to achieve an $I^2_{Total}$ greater than 90% in multispecies meta-analyses (Senior et al., 2016).

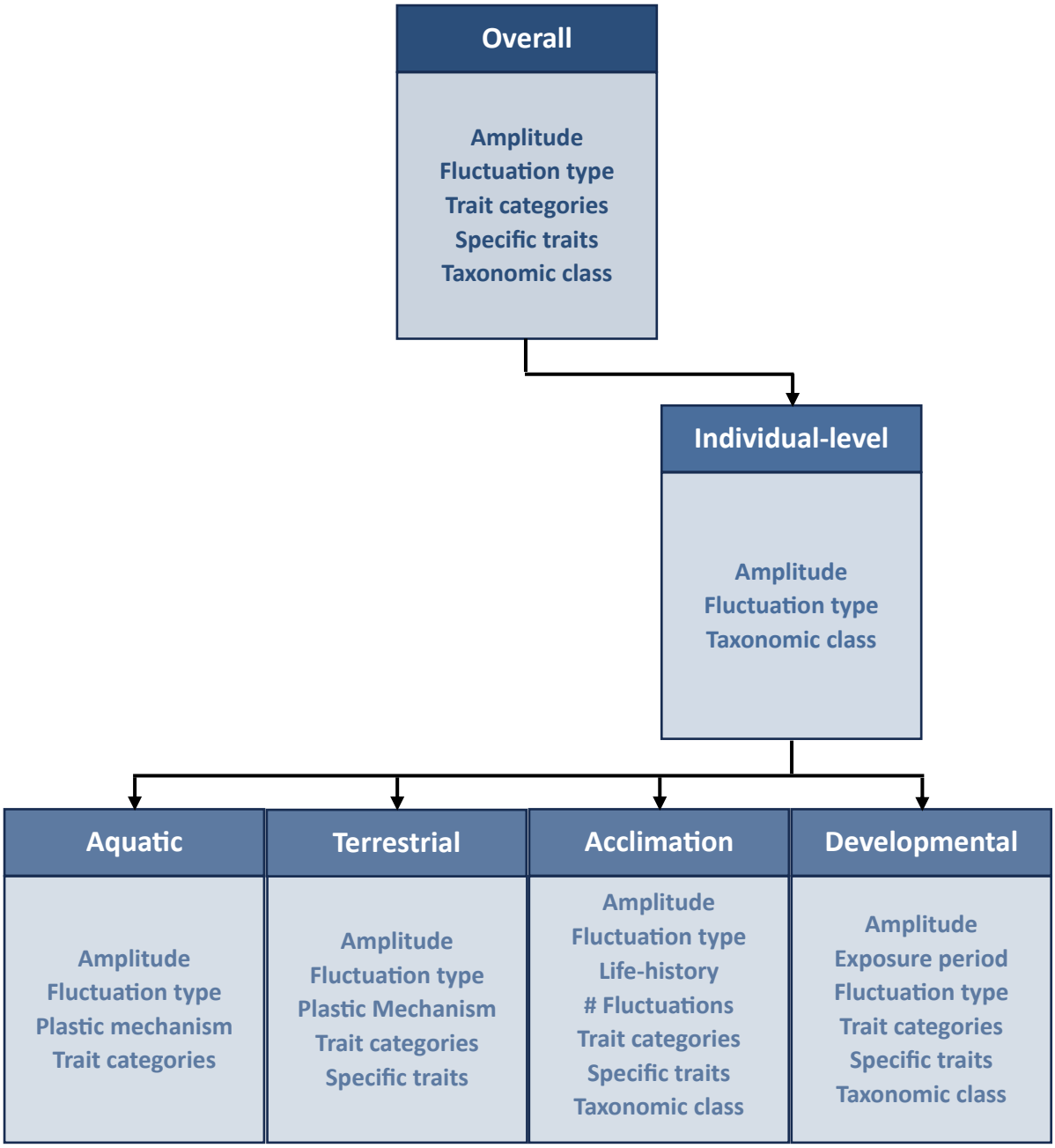


**Figure 2 The order of subset MLMA analyses and the corresponding meta-regression analyses.** White text = MLMA model. Blue text = meta-regression model. "Amplitude" refers to the amplitude experienced during the fluctuation treatments. "Fluctuation type" denotes the thermal regime implemented in the experimental design (Figure 1). "Specific traits" and "Trait categories" refer to the individual traits and categorised groups of traits measured following treatment exposure. "Taxonomic class" is the class for each experimental species. "Plastic mechanisms" distinguishes acclimation from developmental plasticity

treatments. “Life-history” is the life-history stage during an acclimation treatment exposure. “Exposure period” refers to the life-history stage that a developmental plasticity treatment encompassed. “Number of Fluctuations” is the treatment exposure duration divided by the period of each fluctuation. Subset analyses and meta-regressions were included if the number of effect sizes > 10 (e.g., Population-level traits excluded). Definitions of MLMA and meta-regression terms are found in Table 1.

**Table 1 Definitions of included terms in each subset.** An outline of the requirements for the inclusion of effect sizes into each of the subset analyses.

| Subset Analyses | Definitions |
|---|---|
| **Population-level Traits** | Phenotypic traits measured for an entire population (e.g., Survival). |
| **Individual-level Traits** | Phenotypic traits measured in an individual (e.g., Food Consumption, Cortisol Levels, hsp70, Development Time, Length, Locomotor Performance etc.). |
| **Aquatic Organisms** | Organisms that spend the predominant amount of their lifecycle in aquatic ecosystems, including amphibians. |
| **Terrestrial Organisms** | Organisms that spend the predominant amount of their lifecycle in terrestrial ecosystems. |
| **Acclimation** | A treatment that does not encompass a significant period of development, and a reversible phenotypic trait is measured. This generally appears in an experimental design with a treatment and trait measurement within the same life-history stage. |
| **Developmental** | A treatment exposure that encompasses a significant developmental period. This generally appears in an experimental design with treatments and trait measurements across life-history stages. |

### 3.8 | Meta-regression Models

For each data subset we conducted separate meta-regression models to compare moderators that had the potential to influence the observed mean effect size estimate. Categorical moderators that were explored include developmental exposure period (embryo, larva, pupa and juvenile), mechanism of plasticity (acclimation and developmental plasticity), fluctuation type (sinusoidal, alternating, stepwise and stochastic; Figure 1), acclimation life-history stages (embryo, larva, pupa, juvenile and adult), phenotypic trait categories (behavioural, biochemical assay, gene expression, life-history, morphological and physiological), specific phenotypic traits (see Supplementary Materials 7.8 for full list of phenotypic traits), and taxonomic class (see Supplementary Materials 7.10 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 (range = 1℃ – 45.5℃), and the number of fluctuations (treatment exposure duration/period of fluctuations; range = 4 – 546 fluctuations) as continuous moderators (Figure 2).

### 3.9 | 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 variance) as moderators to assess the presence of a time-lag effect (or decline effect; Nakagawa et al., 2022a). There was no evidence of publication bias found in our overall data set (Supplementary Materials 7.11).

To understand how 'robust' our overall MLMA model was to influential data points, we conducted Cook's distance sensitivity analysis (using the function *cooks.distance* in the R Package *metafor*; Viechtbauer, 2010). An overall MLMA model was also compared to a model fitted with untransformed data (Knol et al., 2011; Macartney et al., 2022). These two results combined suggest that our data set is both robust and free from significant influential outliers (Supplementary materials 7.11).

## 4 | Results

The final data set included 212 effect sizes from 44 studies (Supplementary Materials 7.5) across 40 species (Supplementary Materials 7.10), that predominantly looked at diurnal temperature fluctuations (24 h cycles). In the overall MLMA analysis (effect size estimate = -0.00; 95% CIs = [-0.02, 0.03]; p = 0.8732; n = 212) and the subsequent subset analyses (Supplementary Materials 7.12), the mean plastic responses were not significantly different between constant and fluctuating thermal environments (Figure 3). Total heterogeneity was extremely low ($I^2_{Total}$ rounded to 0.00%), and the random effects in the MLMA models did not explain a significant proportion of variation (heterogeneity statistics for each subset analyses in Supplementary Materials 7.13). An extremely low $I^2_{Total}$ could indicate that heterogeneity in our data is predominantly due to within-study rather than between-study variability.

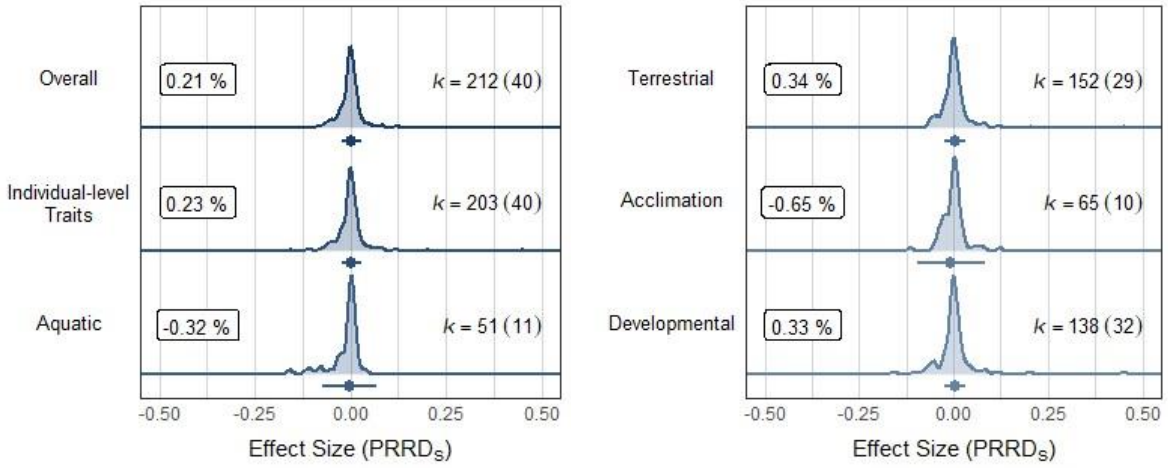


**Figure 3 Multi-level meta-analytic results for the overall data set and subsets.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

There were no significant differences in phenotypic plasticity between fluctuating and constant thermal environments in different trait categories (Figure 4) or in specific phenotypic traits (Figure 5). Similarly, there were no differences in phenotypic plasticity within different taxonomic classes (Figure 6). Neither the type (Figure 7) nor the amplitude (Figure 8) of fluctuating temperature regimes affected the differences in phenotypic plasticity. The results of the meta-regression analyses for all data subsets paralleled those of the overall data set and are shown in the Supplementary Materials 7.12 and 7.14.

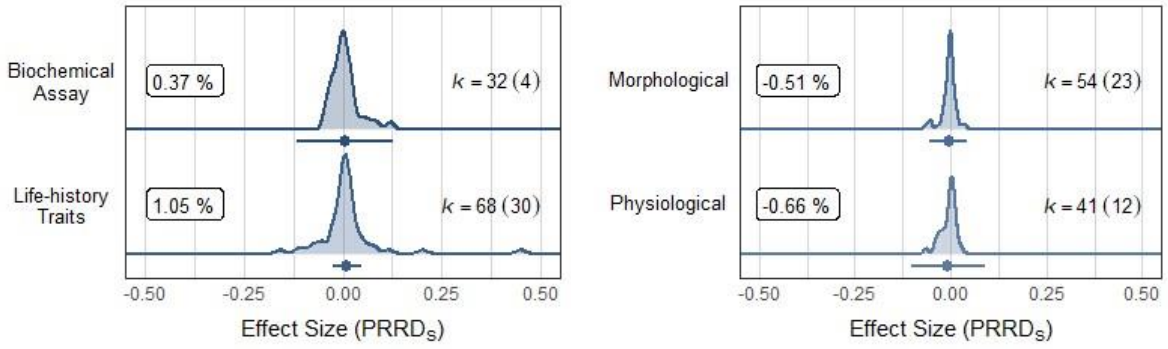


**Figure 4 Meta-regression results for the overall data set with phenotypic trait category as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

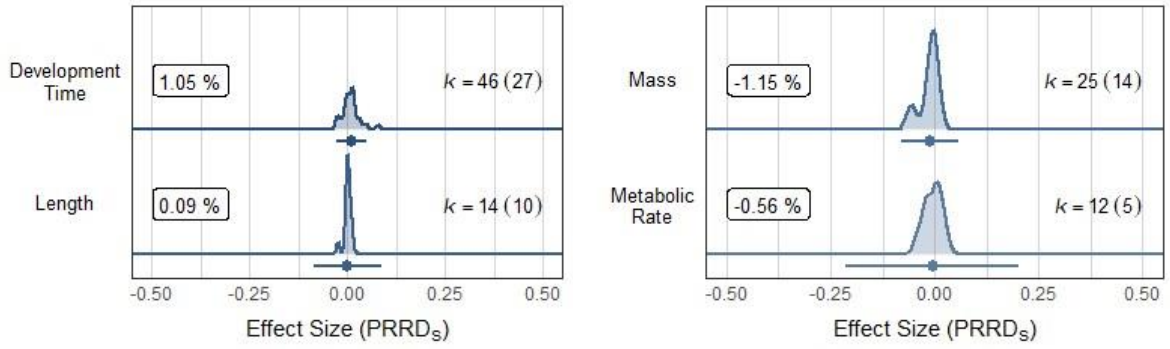


**Figure 5 Meta-regression results for the overall data set with specific phenotypic traits as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

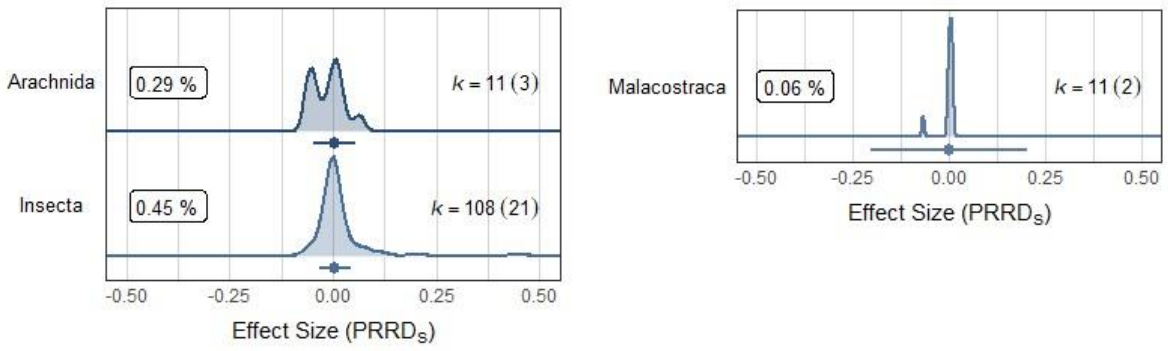


**Figure 6 Meta-regression results for the overall data set with taxonomic class as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

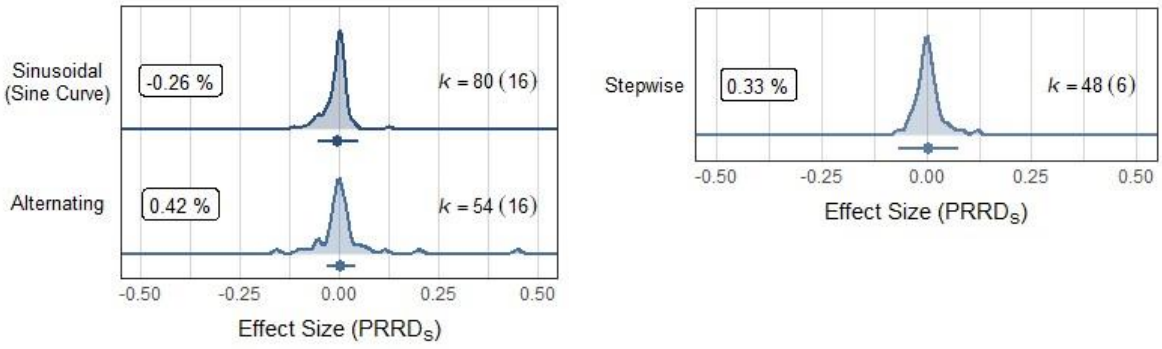


**Figure 7 Meta-regression results for the overall data set with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

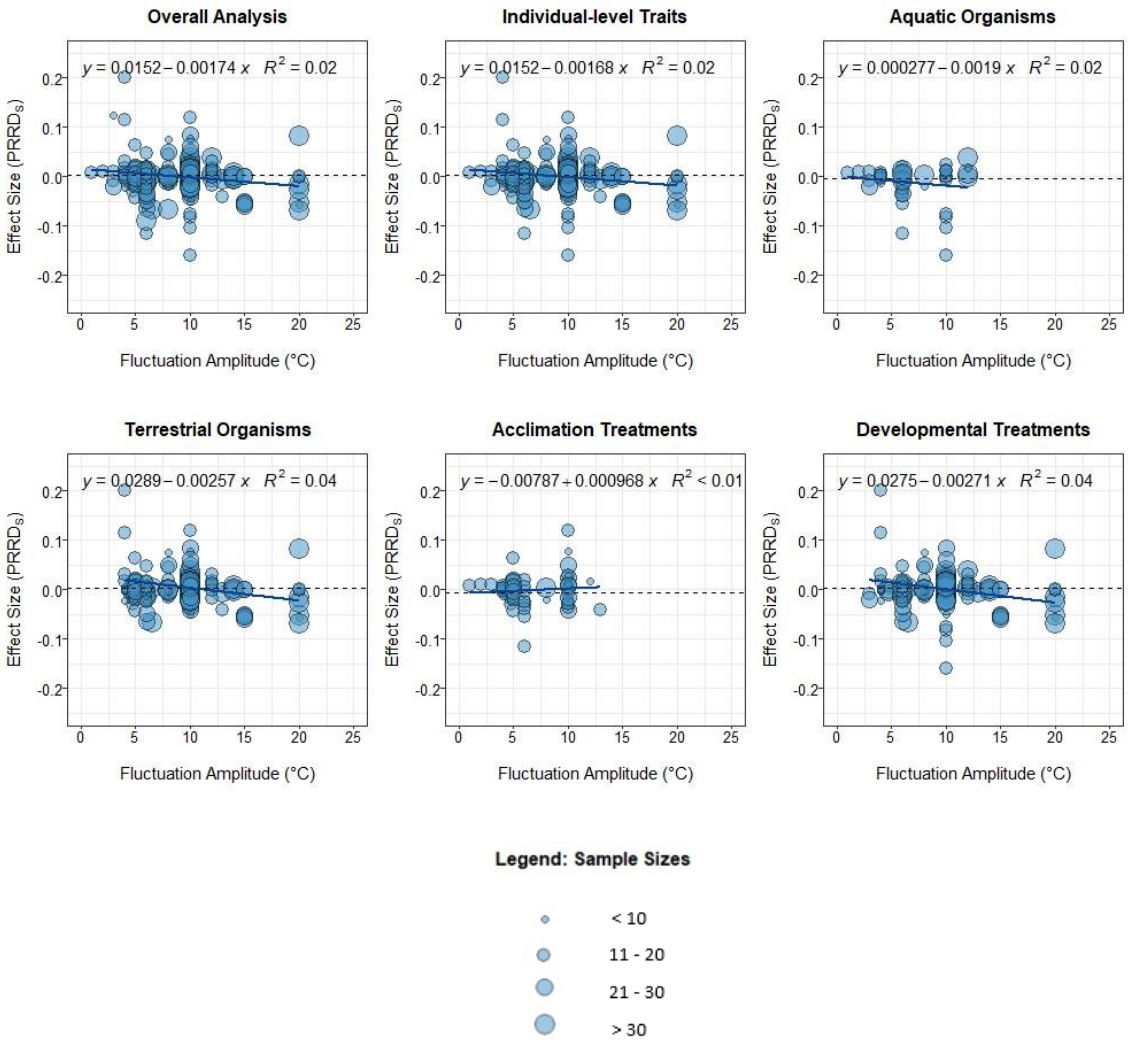


**Figure 8 Relationship between effect size ($PRRD_S$) and the amplitude of fluctuation.** There were no significant relationships between the amplitudes of fluctuations and the difference in the phenotypic plasticity between the constant and fluctuating temperature treatments. Dashed line = PRRDs estimate 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.

## 5 | Discussion

Our meta-analysis of the entire data set showed a consensus in the literature that the capacity for phenotypic plasticity in ectothermic organisms does not differ between constant and fluctuating thermal environments. The quantitative synthesis was multifaceted, with a range of ecological levels, ecosystems, and mechanisms of phenotypic plasticity present in the data. Analyses of these subsets were, remarkably, in agreement that the capacity for phenotypic plasticity remains unaltered by thermal variability. 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 thermal mean.

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). Phenotypic plasticity that is continually induced by small frequent temperature fluctuations could increase the occurrence of such phenotype-environment mismatches (Chevin and Lande, 2015; Ghalambor et al., 2007). A mismatch cost could 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). Findings from our analysis instead indicate that short periods of exposure to temperatures beyond the thermal mean are insufficient to impact plastic responses. Therefore, we conclude that plastic responses are driven by longer-term mean temperatures.

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; Stoks et al., 2017). Phenotypic plasticity can come at an energetic cost to ectotherms (Angilletta, 2009) and plastic responses to frequent diurnal fluctuations could exacerbate that cost. 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 and Kelly, 2020; Pérez et al., 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 (Raynal et al., 2022). The findings of our meta-analysis indicate that increases in the magnitude of diurnal variations do not impact plasticity and are therefore unlikely to affect the vulnerability of ectotherms. Plastic responses to environmental changes are often investigated through experiments manipulating constant temperatures in controlled laboratory conditions (Colinet et al., 2015; Massey and Hutchings, 2021; Noble et al., 2018). Our findings support the validity of constant temperature experiments and suggest that our current understanding of phenotypic plasticity is transferable to environments with diurnal temperature variation.

Anthropogenic climate change is predicted to increase temperature means, fluctuation amplitudes, and the severity and frequency of extreme climatic events (IPCC, 2021; Pachauri et al., 2014). The outcomes of our quantitative synthesis shed light on the temporal relationship between phenotypic plasticity and fluctuating environments and have the potential to influence conservation approaches: mean temperatures are important to consider, but changes to fluctuations around the thermal mean will not affect the vulnerability of ectotherms to environmental change. Our conclusions pertain to mechanisms of phenotypic plasticity within the lifetime of an organism (acclimation and developmental plasticity) and in the literature there are few data showing the periodicity at which fluctuations become sufficient to 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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## 7 | Supplementary Materials

### 7.1 | PECO Framework

**Table S1 PECO framework table.** Populations, Exposures, Comparator and Outcomes that were the foundation for the focus question of the meta-analysis, the database search strings (Supplementary Materials 7.2) and the decision tree (Supplementary Materials 7.4).

| PECO | Description |
|---|---|
| **Population** | Ectothermic Metazoans |
| **Exposure** | Two fluctuating acclimation or developmental treatments (minimum of 1°C amplitudes), used to calculate the phenotypic plasticity in fluctuating thermal environments. The mean of each treatment matches the corresponding constant temperature treatment (maximum of 1°C different in means). |
| **Comparator** | Two acclimation or developmental treatments (maximum of 0.5°C amplitudes), used to calculate the phenotypic plasticity in constant thermal environments. |
| **Outcome** | The phenotypic plasticity of any phenotypic trait measurement that:<br>- was measured at a similar temperature to the corresponding constant thermal treatment or mean of the fluctuating treatment (< 4°C difference acceptable);<br>- treatment conditions were maintained through to trait measurement;<br>- was not a metric of thermal performance (e.g., Critical Thermal Maximum, Critical Thermal Minimum and Temperature Preference). |

## 7.2 | Search Strings and Protocols

Scopus

TITLE-ABS-KEY((plasticity OR "plastic response" OR acclimat* OR "develop* effect") AND (thermal OR temperature*) AND (fluctuat* OR var* OR regime OR chang* OR irregular OR shift* OR inconstant OR diel OR unstable OR alter* OR vacill* OR oscill* OR period*) AND NOT (tolerance OR ctmax OR ctmin OR endotherm* OR bacter* OR fung* OR alga* OR unicellular OR protist OR microorganism OR micro-organism OR plant* OR photosyn* OR tree OR grass OR bird OR avia* OR aves OR mammal OR rodent OR rat OR mouse OR mice OR cattle OR livestock* OR cow OR pig OR sheep OR goat OR house OR rabbit OR chicken OR duck OR turkey OR cat OR dog OR human OR woman OR man OR "parental care" OR child* OR infant OR educat* OR industr* OR commerc* OR domest* OR cancer* OR medic*)) AND (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (LANGUAGE, "English")) AND (LIMIT-TO (SUBJAREA, "AGRI") OR LIMIT-TO (SUBJAREA, "BIOC") OR LIMIT-TO (SUBJAREA, "ENVI"))

Web of Science

TS = ((plasticity OR "plastic response" OR acclimat* OR "develop effect" OR "development effect" OR "developmental effect") AND (thermal OR temperature*) AND (fluctuat* OR var* OR regime OR chang* OR irregular OR shift* OR inconstant OR diel OR unstable OR alter* OR vacill* OR oscill* OR period*)) NOT TS = (tolerance OR ctmax OR ctmin OR endotherm* OR bacter* OR fung* OR alga* OR unicellular OR protist OR microorganism OR micro-organism OR plant* OR photosyn* OR tree OR grass OR bird OR avia* OR aves OR mammal OR rodent OR rat OR mouse OR mice OR cattle OR livestock* OR cow OR pig OR sheep OR goat OR house OR rabbit OR chicken OR duck OR turkey OR cat OR dog OR human OR woman OR man OR "parental care" OR child* OR infant OR educat* OR industr* OR commerc* OR domest* OR cancer* OR medic*)

Further refinements:

- Document Types: Articles
- Languages: English
- Not Web of Science Categories:
    - Metallurgy Metallurgical Engineering
    - Mechanics
    - Engineering Mechanical

ScienceDirect

* The search engine has a max of 8 Boolean connectors per field, so multiple searches were conducted.

** All searches were conducted in the 'Title, abstract or author-specified keyword' field.

**1.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (fluctuate OR regime OR diel) NOT (endotherm)

**2.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (vary OR change OR irregular) NOT (endotherm)

**3.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (shift OR inconsistent OR unstable) NOT (endotherm)

**4.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (alter OR vacillate OR oscillate) NOT (endotherm)

**5.** (plasticity OR "plastic response" OR acclimation) AND (thermal OR temperature) AND (period) NOT (endotherm)

**6.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (fluctuate OR regime OR diel) NOT (endotherm)

**7.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (vary OR change OR irregular) NOT (endotherm)

**8.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (shift OR inconsistent OR unstable) NOT (endotherm)

**9.** ("development effect" OR "developmental effect" OR "develop effect") AND (thermal OR temperature) AND (alter OR vacillate OR oscillate) NOT (endotherm)

**10.** (“development effect" OR "developmental effect" OR "develop effect”) AND (thermal OR temperature) AND (period) NOT (endotherm)

Further refinements to the searches:

- Article Type: Research Articles
- Subject Areas:
    - Agricultural and Biological Sciences
    - Biochemistry, Genetics and Molecular Biology
    - Environmental Science

### 7.3 | PRISMA Flow Diagram

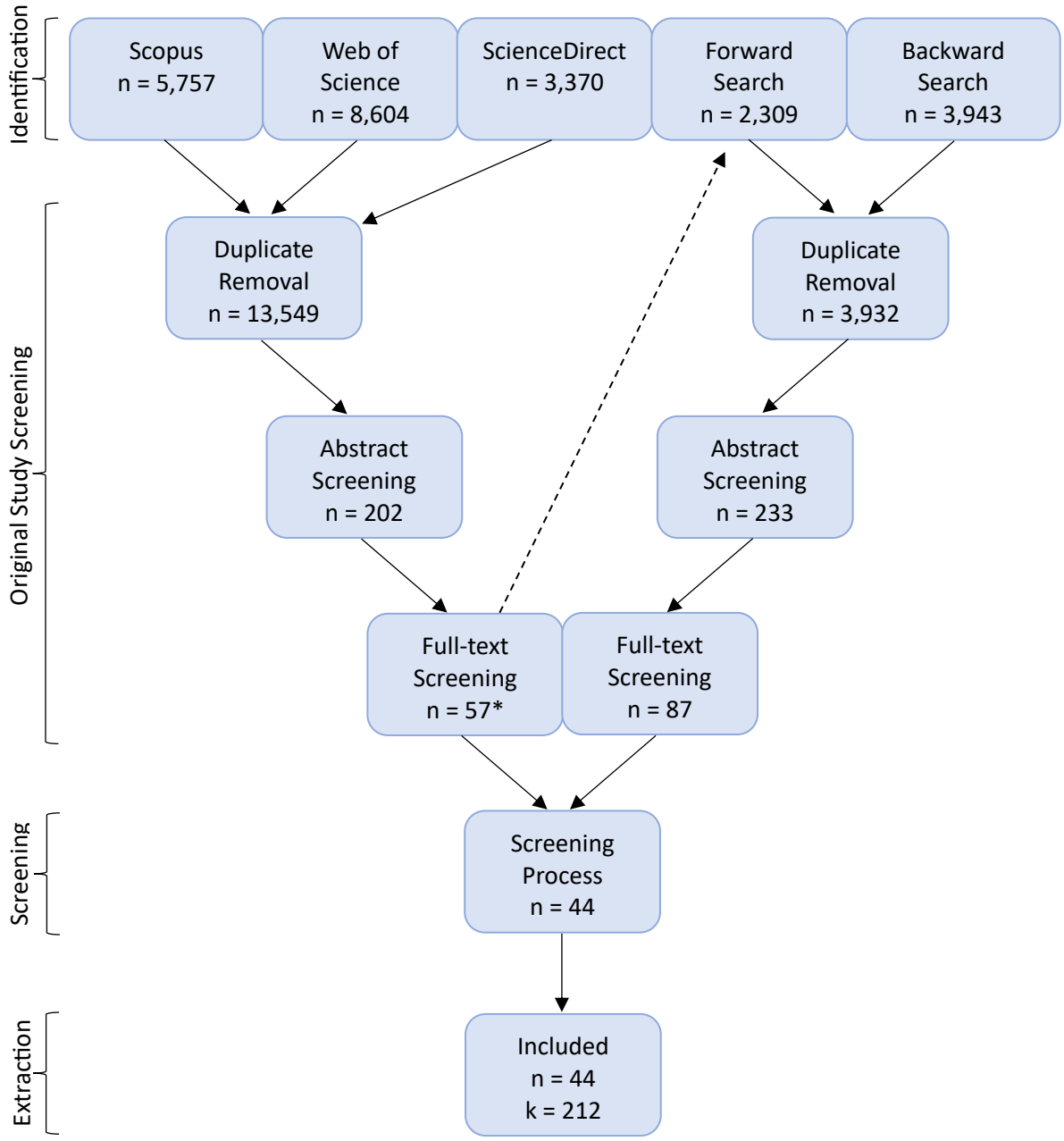


**Figure S1 PRISMA flow diagram for the systematic search and screening processes.** n = number of studies remaining after that respective stage. k = number of effect sizes. Dashed line = studies that the forward and backward search was conducted upon. * One study removed due to unresolved phylogeny in the Open Tree of Life database during the data analysis stage of the original study (Chapter 2).

## 7.4 | Decision Tree

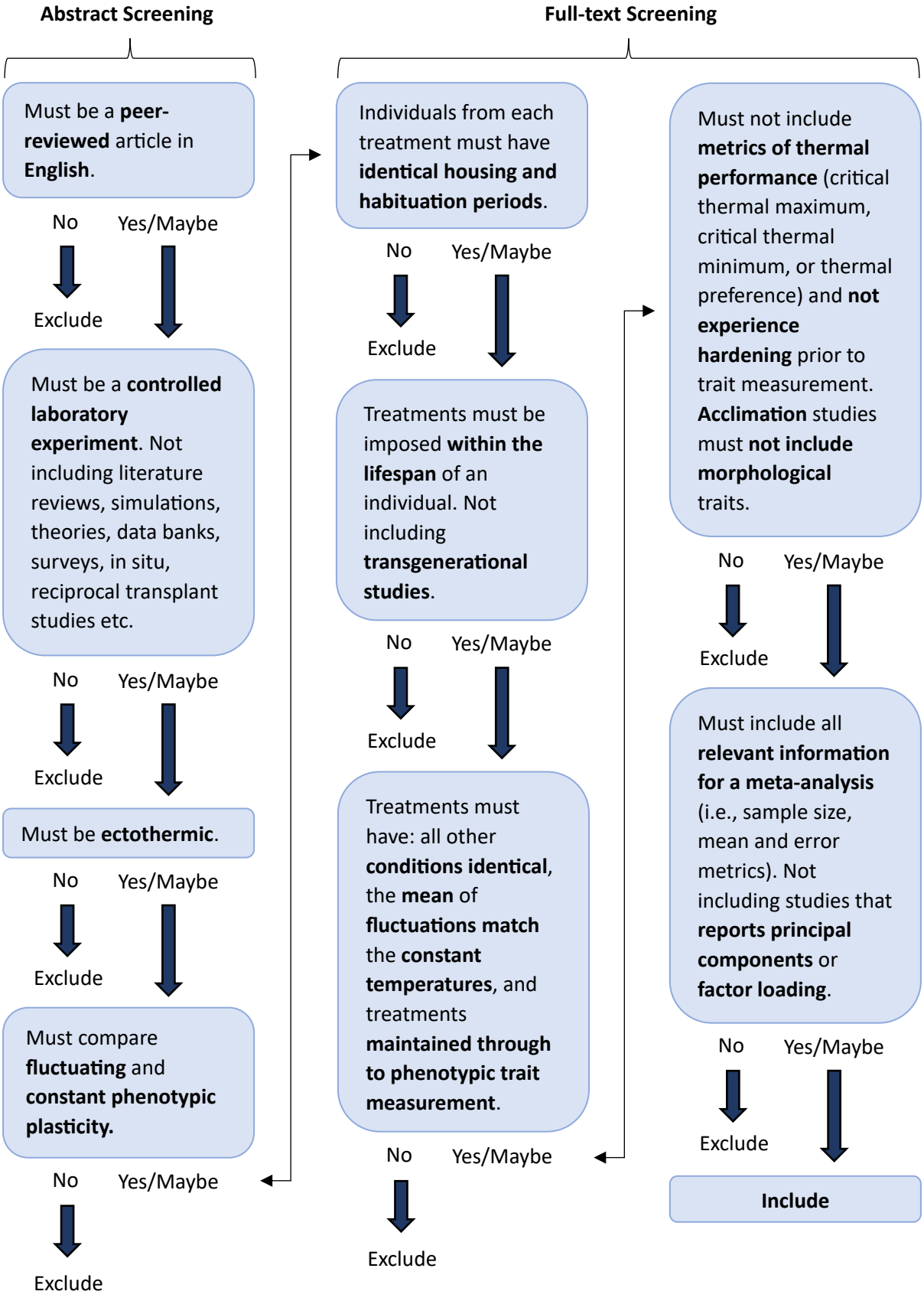


**Figure S2 Decision tree and inclusion criteria.**

## 7.5 | List of Included Studies


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### 7.6 | Developmental Exposure Period Adjustments and Categorisation

**Table S2 Embryo development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Embryo development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental plasticity treatments.

| Embryo Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Egg Incubation | Egg Incubation | 15 | 16 | 68 |
| | Egg to Larvae | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **16** | **17** | **70** |
| | **Category Totals** | **16** | **17** | **70** |

**Table S3 Larval development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Larva development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental plasticity treatments.

| Larva Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Larvae Period | Larvae Development | 1 | 1 | 12 |
| | Larvae Period | 6 | 9 | 18 |
| | Larval Development | 1 | 1 | 10 |
| | Larval Period | 4 | 4 | 15 |
| | **Adjusted Record Totals** | 12 | 12 | 55 |
| | **Category Totals** | **12** | **12** | **55** |

**Table S4 Juvenile development.** The consistency adjustments (if any) for treatment exposure periods that were categorised as occurring during Juvenile development. These records were used for the meta-regression analysis of the developmental subset with exposure period as a moderator. Number of studies, species and effects sizes are totals from the subset of data with developmental plasticity treatments.

| **Juvenile Category** | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Juvenile Period | Juvenile Period | 1 | 1 | 3 |
| | Newborn to Maturity | 1 | 1 | 7 |
| | Until Adult | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **3** | **3** | **11** |
| Nymphal Period | Nymphal to Adult | 1 | 1 | 2 |
| | **Category Totals** | **4** | **4** | **13** |

### 7.7 | Acclimation Life-history Stages Adjustments and Categorisation

**Table S5 Embryo stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Embryo stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatments.

| Embryo Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Egg | Eggs | 1 | 1 | 3 |
| | **Category Totals** | **1** | **1** | **3** |

**Table S6 Larval stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Larva stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatments.

| Larva Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Cocoon | Cocoons | 1 | 1 | 1 |
| Larvae | Larvae | 3 | 2 | 29 |
| | **Category Totals** | **4** | **3** | **30** |

**Table S7 Juvenile stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Juvenile stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatments.

| Juvenile Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Juvenile | Juvenile | 3 | 2 | 14 |
| | Juveniles | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **4** | **3** | **15** |
| | **Category Totals** | **4** | **3** | **15** |

**Table S8 Adult stage.** The consistency adjustments (if any) for the life-history stages of individuals that were categorised as experiencing the acclimation treatments during the Adult stage. These records were used for the meta-regression analysis of the acclimation subset with life-history stage as a moderator. Number of studies, species and effect sizes are totals from the subset of data with acclimation treatments.

| Adult Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Adult | Adult | 1 | 1 | 9 |
| | Adults | 3 | 4 | 7 |
| | **Adjusted Record Totals** | **4** | **5** | **16** |
| | **Category Totals** | **4** | **5** | **16** |

### 7.8 | Phenotypic Trait Measurements Adjustments and Categorisation

**Table S9 Behavioural traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Behavioural traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| **Behavioural Category** | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Food Consumption | Feeding Rate | 1 | 1 | 1 |
| | Food Consumption | 1 | 2 | 2 |
| | **Adjusted Record Totals** | **2** | **3** | **3** |
| Righting Time | Righting Time | 1 | 1 | 1 |
| | **Category Totals** | **3** | **4** | **4** |

**Table S10 Biochemical traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Biochemical Assay traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| Biochemical Assay | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Ash Content | Ash Content | 1 | 1 | 1 |
| Bleaching Index | Bleaching Index | 1 | 1 | 1 |
| Catalase Activity | CAT Activity | 1 | 1 | 4 |
| ETS Activity | ETS Activity | 1 | 1 | 4 |
| Fat Content | Fat Content | 1 | 1 | 4 |
| Haemocytes | Number of Haemocytes | 1 | 1 | 2 |
| Lipid Content | Crude Lipid Content | 1 | 1 | 1 |
| MDA Levels | MDA | 1 | 1 | 4 |
| PO Activity | PO Activity | 1 | 1 | 4 |
| | Total PO Activity | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **2** | **2** | **6** |
| Protein Content | Crude Protein Content | 1 | 1 | 1 |
| SOD Activity | SOD Activity | 1 | 1 | 4 |
| | **Category Totals** | **4** | **4** | **32** |

**Table S11 Gene expression traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Gene Expression traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| Gene Expression Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| hsp70 | hsp70 | 1 | 1 | 2 |
| hsp90 | hsp90 | 1 | 2 | 2 |
| | **Category Totals** | **2** | **3** | **4** |

**Table S12 Life-history traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Life-history traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| Life-history Traits Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Brood Duration | Brood Duration | 1 | 1 | 1 |
| Brood Size | Brood Number | 1 | 1 | 1 |
| | Brood Size | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **1** | **1** | **2** |
| Development Time | Age at Maturity | 1 | 1 | 1 |
| | Age at Reproduction | 1 | 1 | 1 |
| | Development Rate | 1 | 1 | 1 |
| | Development Time | 17 | 19 | 33 |
| | Development time | 1 | 1 | 1 |
| | Developmental Rate | 1 | 1 | 1 |
| | Gosner Stage | 1 | 1 | 1 |
| | Incubation Duration | 1 | 1 | 2 |
| | Incubation Period | 1 | 1 | 1 |
| | Larval Development | 1 | 1 | 2 |
| | Pupal Development | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **26** | **27** | **46** |
| Fecundity | Fecundity | 6 | 7 | 7 |
| Individual Growth Rate | Individual Growth Rate | 1 | 1 | 2 |
| Longevity | Adult Longevity | 1 | 1 | 2 |

| | | | | |
|---|---|---|---|---|
| | Lifespan | 1 | 1 | 1 |
| | Longevity | 3 | 3 | 4 |
| | **Adjusted Record Totals** | **5** | **5** | **7** |
| Reproductive Rate | Reproductive Output | 1 | 1 | 3 |
| | **Category Totals** | **28** | **30** | **68** |

**Table S13 Morphological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Morphological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| Morphological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Head Depth | Head Depth | 1 | 1 | 1 |
| Head Width | Head Width | 1 | 1 | 1 |
| Length | Body Size | 1 | 1 | 1 |
| | Length | 3 | 3 | 4 |
| | Size | 2 | 3 | 3 |
| | Snout Vent Length | 2 | 2 | 4 |
| | Snout-vent Length | 1 | 1 | 2 |
| | **Adjusted Record Totals** | **9** | **10** | **14** |
| Mass | Body Mass | 1 | 1 | 2 |
| | Mass | 9 | 8 | 17 |
| | Weight | 2 | 5 | 6 |
| | **Adjusted Record Totals** | **12** | **14** | **25** |
| Plastron Length | Plastron Length | 1 | 2 | 2 |
| Tail Length | Tail length | 4 | 4 | 7 |
| Thorax Size | Thorax Size | 1 | 1 | 2 |
| Wing Length | Wing length | 1 | 1 | 2 |
| | **Category Totals** | **20** | **23** | **54** |

**Table S14 Physiological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Physiological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| Physiological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Apparent Digestibility Coefficient | Apparent Digestability Coefficient | 2 | 1 | 5 |
| Cellular Energy Allocation | Cellular Energy Allocation | 1 | 1 | 4 |
| Energy Content | Energy Content | 1 | 1 | 1 |
| Excretion Rate | Excretion Energy | 1 | 1 | 1 |
| Exuvia Energy | Exuvia Energy | 1 | 1 | 1 |
| Faeces Energy | Faeces Energy | 1 | 1 | 1 |
| Immune Defense | Immune Defense | 1 | 1 | 6 |
| Locomotor Performance | Average Speed | 1 | 1 | 2 |
| | Sprint Speed | 1 | 1 | 2 |
| | Swimming Performance | 1 | 1 | 1 |
| | Walking Speed | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **3** | **3** | **6** |
| Maximum Excitation Pressure | Maximum Excitation Pressure | 1 | 1 | 1 |
| Metabolic Rate | Metabolic Rate | 1 | 1 | 1 |
| | Oxygen Consumption | 2 | 2 | 4 |
| | Oxygen Consumption Rate | 1 | 1 | 3 |
| | Respiration Energy | 1 | 1 | 1 |
| | Respiration Rate | 1 | 1 | 3 |

| | | | | |
|---|---|---|---|---|
| | **Adjusted Record Totals** | **6** | **5** | **12** |
| Muscular Strength | Muscular Strength | 1 | 1 | 1 |
| PHA Response | PHA Response | 1 | 1 | 1 |
| Water Content | Water Content | 1 | 1 | 1 |
| | **Category Totals** | **14** | **12** | **41** |

**Table S15 Population-level traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Population-level Traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, phenotypic trait category moderator and/or the specific phenotypic trait moderator. Number of studies, species and effects sizes are totals from the overall data set.

| **Population-level Traits Category** | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Mortality | Mortality | 2 | 2 | 2 |
| Survival | Settlement Success | 1 | 1 | 1 |
| | Survival | 5 | 6 | 6 |
| | **Adjusted Record Totals** | **6** | **7** | **7** |
| | **Category Totals** | **8** | **9** | **9** |

### 7.9 | Codebook and Metadata

**Table S16 Codebook and Metadata for the Raw Data Set.** Raw data descriptors for the meta-analysis.

| Column Name | Description |
|---|---|
| Effect_Size_ID | Unique identifiers for individual effect sizes. |
| Study_ID | Unique identifiers for each paper. |
| Species_ID | Identifier for each species that a study investigates. |
| Treatment_ID_T1 | Identifier for the studies' first constant and fluctuating temperature treatment pair (fluctuating mean matching constant temperature). |
| Treatment_ID_T2 | Identifier for the studies' second constant and fluctuating temperature treatment pair (fluctuating mean matching constant temperature). |
| Trait_ID | Identifier for each phenotypic trait a study measures. |
| First_Author | Initials and surname for the first author of the study. |
| Title | Title of the study. |
| Year | Year of publication. |
| Journal | Name of the journal the study was published in. |
| Journal_Impact_Factor-2021 | Journal impact factor as of 2021 (most recent records at time of data collection). |
| Kingdom | Kingdom for each species that a study investigates. |
| Phylum | Phylum for each species that a study investigates. |
| Class | Class for each species that a study investigates. |
| Order | Order for each species that a study investigates. |
| Family | Family for each species that a study investigates. |
| Scientific_Name | Binomial nomenclature for species. |
| Ecosystem | Ecosystem the species is naturally observed (Aquatic and Terrestrial). Amphibians considered aquatic. |
| Plasticity_Mechanism | Mechanism of plasticity the treatments were exposed to (Acclimation or Developmental Plasticity). |
| Developmental_Exposure_Time_Category | Categorisation of Developmental Exposure Time. |
| Developmental_Exposure_Time | The period of exposure for treatments imposed during development. |
| Acclimation_Exposure_Time | The duration of exposure for acclimation treatments. |
| Exposure_Units | Units of Acclimation Exposure Time (Days). |

| | |
|---|---|
| T1_constant | Temperature of the first constant temperature treatment. |
| T1_fluctuation | Mean temperature of the first fluctuating temperature treatment. |
| T2_constant | Temperature of the second constant temperature treatment. |
| T2_fluctuation | Mean temperature of the second fluctuating temperature treatment. |
| High | Whether the first or second constant and fluctuating temperature treatment pair is around the higher temperature (T1 or T2). |
| Fluctuation_Magnitude | Amplitude of the two fluctuating temperature treatments. |
| Fluctuation_Category | Type of fluctuations imposed (Sinusoidal, Alternating, Stepwise, Stochastic). |
| Fluctuation_Period | Period of one fluctuation oscillation. |
| Fluctuation_Unit | Units of Fluctuation Period (Days). |
| Number_Of_Fluctuations | Acclimation Exposure Time/Fluctuation Period for acclimation treatments. |
| Acclimation_Life-History_Stage | Life-history stage of organisms for acclimation treatments. |
| Acclimation_Life-History_Stage_Category | Categorisation of Acclimation Life-history Stages. |
| Trait_Category | Categorisation of Trait Measurements. |
| Measurement | Phenotypic traits measured following treatment exposure. |
| Trait_Unit | Units for measurements. |
| Sex | Sex of the organisms being investigated (Both, Female or Male). NA = sex not specified. |
| Performance_Curve | Whether a performance curve was recorded in the study (Yes, No). |
| Complex_Design | Whether a comparison between constant and fluctuating treatments was made at multiple temperatures (Yes). |
| Species_Overlap | Identifier for effect sizes that use the same species within a study |
| Animal_Overlap_T1_Constant | Identifier for effect sizes where the constant treatment was conducted on the same animals within a study (first constant and fluctuating temperature treatment pair). |

| | |
|---|---|
| Animal_Overlap_T1_Fluctuation | Identifier for effect sizes where the fluctuating treatment was conducted on the same animals within a study (first constant and fluctuating temperature treatment pair). |
| Animal_Overlap_Trait_T1 | Identifier for effect sizes that measure phenotypic traits on the same animal within a study (first constant and fluctuating temperature treatment pair). |
| Animal_Overlap_T2_Constant | Identifier for effect sizes where the constant treatment was conducted on the same animals within a study (second constant and fluctuating temperature treatment pair). |
| Animal_Overlap_T2_Fluctuation | Identifier for effect sizes where the fluctuating treatment was conducted on the same animals within a study (second constant and fluctuating temperature treatment pair). |
| Animal_Overlap_Trait_T2 | Identifier for effect sizes that measure phenotypic traits on the same animal within a study (second constant and fluctuating temperature treatment pair). |
| Animal_Code | Study ID: Species Overlap: Animal Overlap T1 Constant: Animal Overlap T1 Fluctuation: Animal Overlap T2 Constant: Animal Overlap T2 Fluctuation: Animal Overlap Trait T1; Animal Overlap Trait T2 |
| Shared_Animal_Number | Unique identifier for shared animal codes across effect sizes. |
| Shared_Control_T1 | Identifier for effect sizes that use the same constant temperature treatment within a study (first constant and fluctuating temperature treatment pair). |
| Shared_Control_T2 | Identifier for effect sizes that use the same constant temperature treatment within a study (second constant and fluctuating temperature treatment pair). |
| Shared_Control_Code | Study ID: Species Overlap: Shared Control T1: Shared Control T2: Trait ID. |
| Shared_Control_Number | Unique identifier for shared control codes across effect sizes. |
| n_T1_C | Sample size of the constant treatment (first constant and fluctuating temperature treatment pair). |
| Mean_T1_C | Mean response of the constant treatment (first constant and fluctuating temperature treatment pair). |

| | |
|---|---|
| SD_Final_T1_C | Standard deviation of the constant treatment (first constant and fluctuating temperature treatment pair). |
| n_T1_F | Sample size of the fluctuating treatment (first constant and fluctuating temperature treatment pair). |
| Mean_T1_F | Mean response of the fluctuating treatment (first constant and fluctuating temperature treatment pair). |
| SD_Final_T1_F | Standard deviation of the fluctuating treatment (first constant and fluctuating temperature treatment pair). |
| n_T2_C | Sample size of the constant treatment (second constant and fluctuating temperature treatment pair). |
| Mean_T2_C | Mean response of the constant treatment (second constant and fluctuating temperature treatment pair). |
| SD_Final_T2_C | Standard deviation of the constant treatment (second constant and fluctuating temperature treatment pair). |
| n_T2_F | Sample size of the fluctuating treatment (second constant and fluctuating temperature treatment pair). |
| Mean_T2_F | Mean response of the fluctuating treatment (second constant and fluctuating temperature treatment pair). |
| SD_Final_T2_F | Standard deviation of the fluctuating treatment (second constant and fluctuating temperature treatment pair). |
| Percentage_Transformation_T1 | Whether the recorded means for the first constant and fluctuating temperature treatment pair were recorded as a percentage (Yes, No). |
| Proportion_Transformation_T1 | Whether the recorded means for the first constant and fluctuating temperature treatment pair were recorded as a proportion (Yes, No). |
| In_Transformation_T1 | Whether the recorded means for the first constant and fluctuating temperature pair were recorded as the natural log (Yes, No). |
| Percentage_Transformation_T2 | Whether the recorded means for the second constant and fluctuating temperature treatment pair were recorded as a percentage (Yes, No). |

| | |
|---|---|
| Proportion_Transformation_T2 | Whether the recorded means for the second constant and fluctuating temperature treatment pair were recorded as a proportion (Yes, No). |
| In_Transformation_T2 | Whether the recorded means for the second constant and fluctuating temperature pair were recorded as the natural log (Yes, No). |
| Mean_Transformed_T1_C | Constant treatment means transformed for percentages, proportions, or natural logs, and with a constant of 0.5 added (first constant and fluctuating temperature treatment pair). |
| SD_Final_Transformed_T1_C | Constant treatment standard deviations transformed for percentages, proportions, or natural logs, and with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |
| Mean_Transformed_T1_F | Fluctuating treatment means transformed for percentages, proportions, or natural logs, and with a constant of 0.5 added (first constant and fluctuating temperature treatment pair). |
| SD_Final_Transformed_T1_F | Fluctuating treatment standard deviations transformed for percentages, proportions, or natural logs, and with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |
| Mean_Transformed_T2_C | Constant treatment means transformed for percentages, proportions, or natural logs, and with a constant of 0.5 added (second constant and fluctuating temperature treatment pair). |
| SD_Final_Transformed_T2_C | Constant treatment standard deviations transformed for percentages, proportions, or natural logs, and with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| Mean_Transformed_T2_F | Fluctuating treatment means transformed for percentages, proportions, or natural logs, and with a constant of 0.5 added (second constant and fluctuating temperature treatment pair). |
| SD_Final_Transformed_T2_F | Fluctuating treatment standard deviations transformed for percentages, proportions, or natural logs, and with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| Mean_T1_C_Add | Untransformed constant treatment means with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |

| | |
|---|---|
| SD_Final_T1_C_Add | Untransformed constant standard deviations with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |
| Mean_T1_F_Add | Untransformed fluctuating treatment means with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |
| SD_Final_T1_F_Add | Untransformed fluctuating treatment standard deviations with a constant 0.5 added (first constant and fluctuating temperature treatment pair). |
| Mean_T2_C_Add | Untransformed constant treatment means with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| SD_Final_T2_C_Add | Untransformed constant standard deviations with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| Mean_T2_F_Add | Untransformed fluctuating treatment means with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| SD_Final_T2_F_Add | Untransformed fluctuating treatment standard deviations with a constant 0.5 added (second constant and fluctuating temperature treatment pair). |
| InRR | Plasticity response ratio difference. Represents the difference in plastic responses in constant and fluctuating temperatures, standardised to a one-degree change in treatment temperature. |
| InRR_Transformed | Plasticity response ratio difference transformed to account for reciprocal transformation. |
| v_InRR | Plasticity response ratio difference sampling variance. |
| InRR_Untransformed | Plasticity response ratio difference calculated from untransformed data. |
| v_InRR_Untransformed | Plasticity response ratio difference sampling variance calculated from untransformed data. |
| Year_Z | Z-transformed year of publication. |
| Precision | Z-transformed inverse of plasticity response ratio difference sampling variance. |

## 7.10 | Phylogenetic Tree

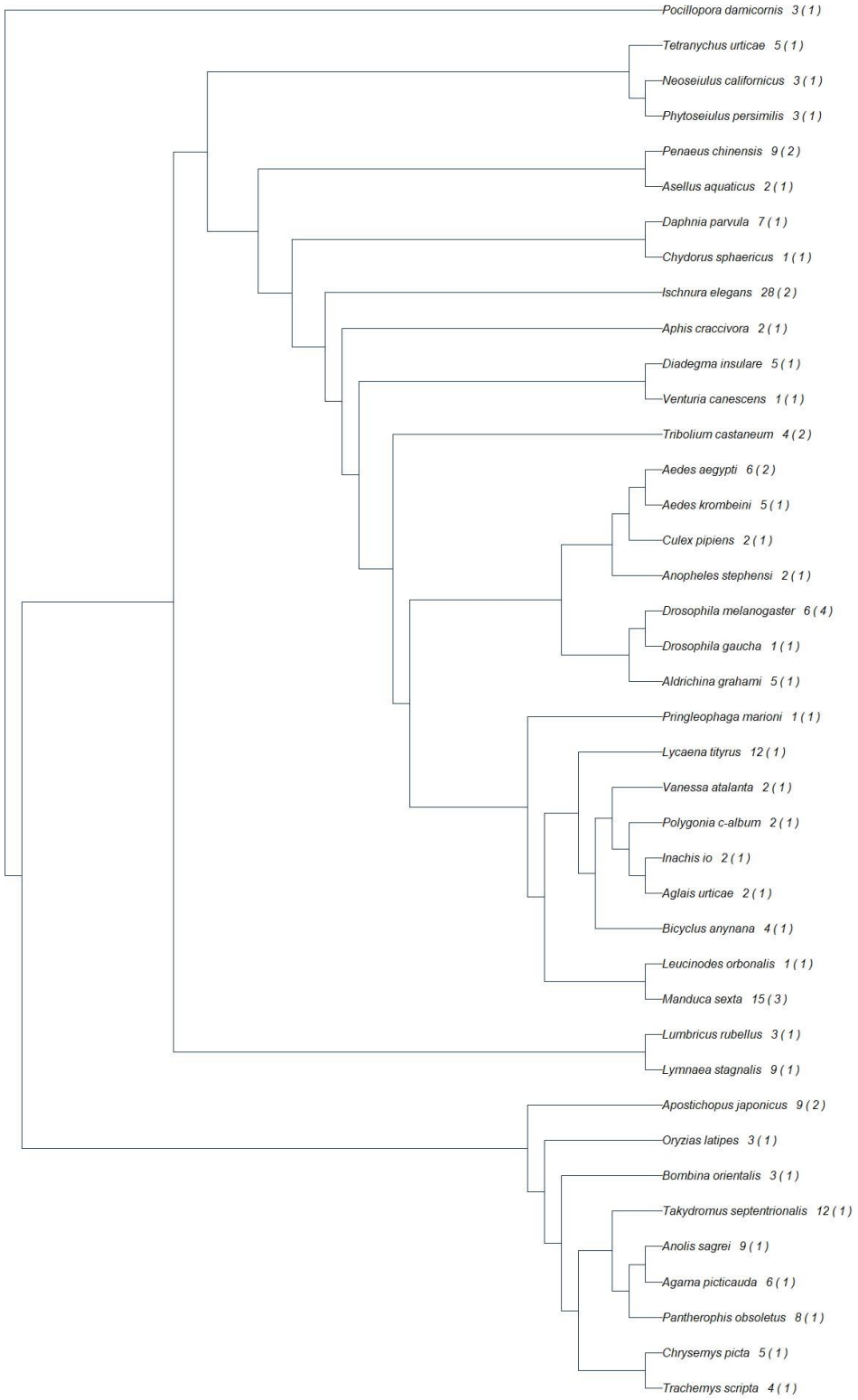


**Figure S3 Phylogenetic tree of species present in the meta-analysis.** Node labels are scientific names followed by the number of effect sizes with the number of studies in brackets.

### 7.11 | Publication Bias and Sensitivity Analysis

Visual inspection of the funnel plot of the meta-analytic residuals of the overall MLMA model did not indicate the presence of asymmetry (Figure S4). The overall MLMA model fitted with z-transformed publication year (effect size estimate = -0.0036; 95% CIs = [-0.0294, 0.0221]; p = 0.7771) and precision (effect size estimate = 0.0023; 95% CIs = [-0.0075, 0.0122]; p = 0.6388) as moderators, found no significant relationships with the PRRDs (Figure S5). These results together indicate no evidence of funnel plot asymmetry or time-lag bias in our data set.

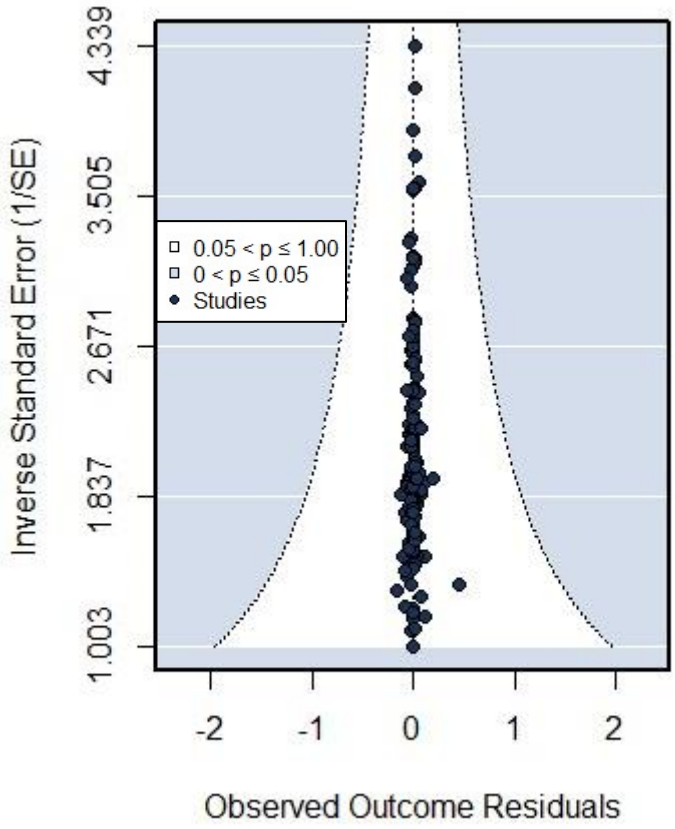


**Figure S4 Funnel plot.** Showing the observed outcome residuals from the overall MLMA model against the inverse standard error (precision). Effect size = PRRDs. Dotted lines = 95% pseudo confidence intervals.

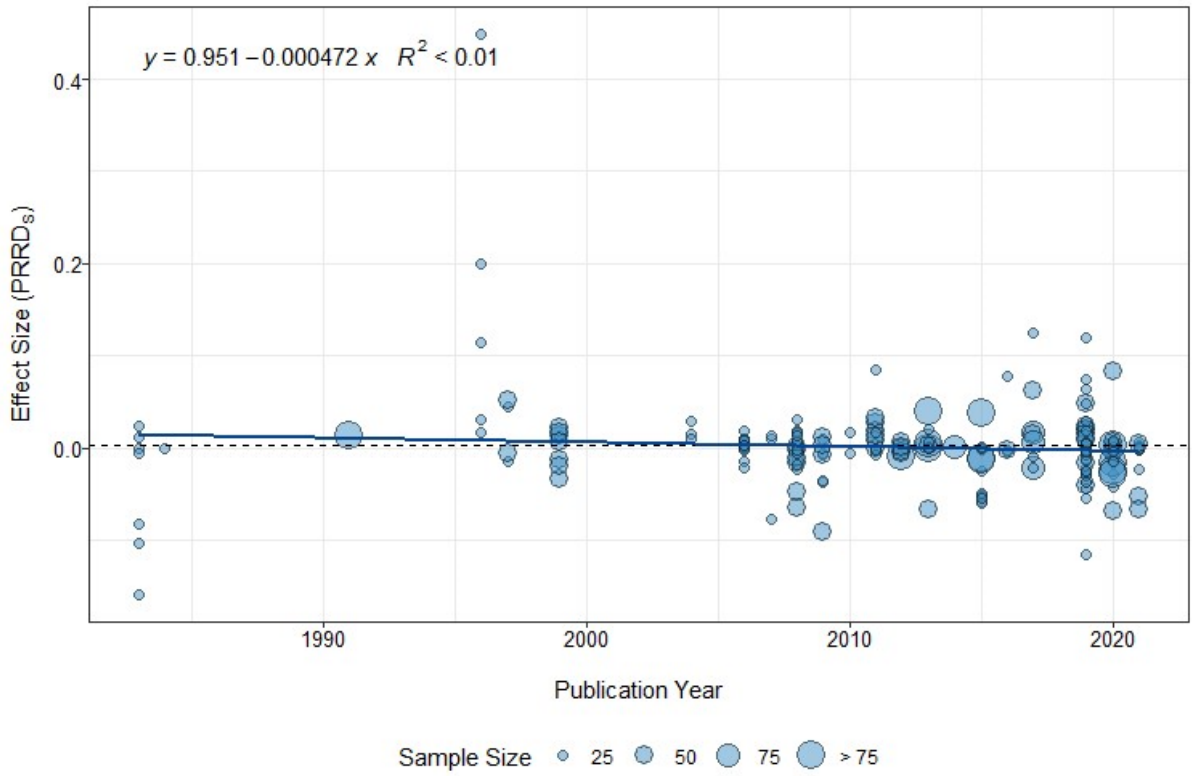


**Figure S5 The relationship between effect sizes (PRRDs) and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = PRRDs estimate from the overall MLMA model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

No implausible data points were identified in the sensitivity analysis, with the Cook's distant never exceeding 1. The model containing untransformed data was quantitatively similar to the overall MLMA model (Table S17). These two results combined suggest that our data set is both robust and free from significantly influential outliers.

**Table S17 MLMA outputs.** Comparison of the overall MLMA model with a model fitted with untransformed data. Effect size = PRRDs. $I^2$ values reported as percentages.

| Model | Estimate | CI Low | CI High | df | p-value | $I^2_{Total}$ |
|---|---|---|---|---|---|---|
| **Overall (PRRDs)** | 0.0021 | -0.0241 | 0.0282 | 39 | 0.8732 | 0.00 |
| **Untransformed Data** | 0.0022 | -0.0235 | 0.0278 | 39 | 0.8648 | 0.00 |

### 7.12 | Raw MLMA and Meta-regression Outputs

**Table S18 Results of the overall MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 44 | 40 | 212 | 0.0021 | -0.0241 | 0.0282 | 39 | 0.8732 |

**Table S19 Results of the meta-regression of the overall data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 44 | 40 | 212 | 0.0000 | -0.0024 | 0.0025 | 80 | 0.9771 |

**Table S20 Results of the meta-regression of the overall data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 18 | 16 | 80 | -0.0026 | -0.0528 | 0.0476 | 63 | 0.9187 |
| **Alternating** | 14 | 16 | 54 | 0.0042 | -0.0318 | 0.0402 | 35 | 0.8145 |
| **Stepwise** | 7 | 6 | 48 | 0.0033 | -0.0684 | 0.0750 | 63 | 0.9265 |

**Table S21 Results of the meta-regression of the overall data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Biochemical Assay** | 4 | 4 | 32 | 0.0037 | -0.1178 | 0.1251 | 35 | 0.9513 |
| **Life-history Traits** | 28 | 30 | 68 | 0.0105 | -0.0248 | 0.0457 | 35 | 0.5503 |
| **Morphological** | 20 | 23 | 54 | -0.0051 | -0.0550 | 0.0447 | 35 | 0.8352 |
| **Physiological** | 14 | 12 | 41 | -0.0066 | -0.1040 | 0.0908 | 35 | 0.8917 |

**Table S22 Results of the meta-regression of the overall data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 26 | 27 | 46 | 0.0105 | -0.0281 | 0.0490 | 93 | 0.5917 |
| **Length** | 9 | 10 | 14 | 0.0009 | -0.0868 | 0.0886 | 93 | 0.9832 |
| **Mass** | 12 | 14 | 25 | -0.0115 | -0.0801 | 0.0571 | 93 | 0.7394 |
| **Metabolic Rate** | 6 | 5 | 12 | -0.0056 | -0.2132 | 0.2020 | 93 | 0.9575 |

**Table S23 Results of the meta-regression of the overall data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Overall Data (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Arachnida** | 2 | 3 | 11 | 0.0029 | -0.0485 | 0.0544 | 23 | 0.9069 |
| **Insecta** | 25 | 21 | 108 | 0.0045 | -0.0335 | 0.0424 | 23 | 0.8093 |
| **Malacostraca** | 3 | 2 | 11 | 0.0006 | -0.2023 | 0.2034 | 23 | 0.9956 |

**Table S24 Results of the individual-level traits subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = PRRDs.

| Individual-level Traits (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 43 | 40 | 203 | 0.0023 | -0.0240 | 0.0286 | 39 | 0.8616 |

**Table S25 Results of the meta-regression of the individual-level traits data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = PRRDs.

| Individual-level Traits (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 43 | 40 | 203 | 0.0000 | -0.0024 | 0.0025 | 78 | 0.9687 |

**Table S26 Results of the meta-regression of the individual-level traits data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = PRRDs.

| Individual-level Traits (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 18 | 16 | 74 | -0.0023 | -0.0532 | 0.0486 | 61 | 0.9291 |
| **Alternating** | 13 | 16 | 53 | 0.0043 | -0.0319 | 0.0405 | 34 | 0.8109 |
| **Stepwise** | 7 | 6 | 47 | 0.0037 | -0.0682 | 0.0756 | 61 | 0.9187 |

**Table S27 Results of the meta-regression of the individual-level traits data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with individual-level phenotypic traits. Effect size = PRRDs.

| Individual-level Traits (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Arachnida** | 2 | 3 | 11 | 0.0029 | -0.0486 | 0.0545 | 22 | 0.9070 |
| **Insecta** | 24 | 21 | 104 | 0.0046 | -0.0338 | 0.0430 | 22 | 0.8048 |

**Table S28 Results of the aquatic subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = PRRDs.

| Aquatic Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 12 | 11 | 51 | -0.0033 | -0.0737 | 0.0672 | 10 | 0.9201 |

**Table S29 Results of the meta-regression of the aquatic data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = PRRDs.

| Aquatic Organisms (PRRDS) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 12 | 11 | 51 | -0.0002 | -0.0085 | 0.0081 | 20 | 0.9550 |

**Table S30 Results of the meta-regression of the aquatic data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = PRRDs.

| Aquatic Organisms (PRRDS) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 8 | 8 | 39 | -0.0023 | -0.0813 | 0.0766 | 9 | 0.9483 |
| **Alternating** | 3 | 3 | 11 | -0.0078 | -0.1792 | 0.1636 | 9 | 0.9198 |

**Table S31 Results of the meta-regression of the aquatic data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = PRRDs.

| Aquatic Organisms (PRRDS) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 6 | 4 | 28 | -0.0117 | -0.1388 | 0.1154 | 9 | 0.8400 |
| **Developmental** | 6 | 7 | 23 | 0.0007 | -0.0858 | 0.0871 | 9 | 0.9868 |

**Table S32 Results of the meta-regression of the aquatic data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from aquatic environments. Effect size = PRRDs.

| Aquatic Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Life-history Traits** | 6 | 7 | 15 | -0.0066 | -0.1143 | 0.1011 | 14 | 0.8973 |
| **Physiological** | 7 | 5 | 23 | -0.0057 | -0.1347 | 0.1233 | 14 | 0.9262 |

**Table S33 Results of the terrestrial subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 31 | 29 | 152 | 0.0034 | -0.0260 | 0.0328 | 25 | 0.8131 |

**Table S34 Results of the meta-regression of the terrestrial data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 31 | 29 | 152 | 0.0001 | -0.0026 | 0.0027 | 57 | 0.9521 |

**Table S35 Results of the meta-regression of the terrestrial data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 10 | 8 | 35 | -0.0022 | -0.0774 | 0.0730 | 40 | 0.9529 |
| **Alternating** | 10 | 13 | 42 | 0.0050 | -0.0330 | 0.0430 | 22 | 0.7873 |
| **Stepwise** | 6 | 5 | 46 | 0.0037 | -0.0694 | 0.0767 | 40 | 0.9193 |

**Table S36 Results of the meta-regression of the terrestrial data set with mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Acclimation** | 6 | 6 | 37 | -0.0012 | -0.1152 | 0.1129 | 150 | 0.9840 |
| **Developmental** | 26 | 25 | 115 | 0.0037 | -0.0254 | 0.0328 | 150 | 0.8016 |

**Table S37 Results of the meta-regression of the terrestrial data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Biochemical Assay** | 2 | 2 | 28 | 0.0040 | -0.1237 | 0.1318 | 18 | 0.9479 |
| **Life-history Traits** | 22 | 23 | 53 | 0.0128 | -0.0261 | 0.0516 | 18 | 0.4983 |
| **Morphological** | 16 | 18 | 46 | -0.0071 | -0.0643 | 0.0502 | 18 | 0.7984 |
| **Physiological** | 7 | 7 | 18 | -0.0082 | -0.1753 | 0.1590 | 18 | 0.9193 |

**Table S38 Results of the meta-regression of the terrestrial data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with organisms from terrestrial environments. Effect size = PRRDs.

| Terrestrial Organisms (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 21 | 21 | 40 | 0.0113 | -0.0299 | 0.0525 | 71 | 0.5863 |
| **Length** | 6 | 7 | 11 | -0.0001 | -0.0955 | 0.0953 | 71 | 0.9983 |
| **Mass** | 11 | 12 | 23 | -0.0127 | -0.0854 | 0.0601 | 71 | 0.7296 |

**Table S39 Results of the acclimation subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 12 | 10 | 65 | -0.0066 | -0.0976 | 0.0845 | 9 | 0.8742 |

**Table S40 Results of the meta-regression of the acclimation data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 12 | 10 | 65 | -0.0010 | -0.0120 | 0.0101 | 30 | 0.8604 |

**Table S41 Results of the meta-regression of the acclimation data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 5 | 4 | 25 | -0.0128 | -0.1266 | 0.1010 | 19 | 0.8165 |
| **Stepwise** | 4 | 3 | 30 | -0.0008 | -0.1297 | 0.1281 | 19 | 0.9893 |

**Table S42 Results of the meta-regression of the acclimation data set with life-history stage as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Adult** | 4 | 5 | 16 | -0.0155 | -0.1519 | 0.1210 | 24 | 0.8169 |
| **Juvenile** | 4 | 3 | 15 | 0.0037 | -0.1984 | 0.2058 | 24 | 0.9702 |
| **Larva** | 4 | 3 | 30 | 0.0003 | -0.1315 | 0.1321 | 24 | 0.9965 |

**Table S43 Results of the meta-regression of the acclimation data set with the number of fluctuations as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Number of Fluctuations** | 11 | 8 | 63 | -0.0001 | -0.0037 | 0.0035 | 10 | 0.9569 |

**Table S44 Results of the meta-regression of the acclimation data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Biochemical Assay** | 2 | 2 | 27 | 0.0003 | -0.1472 | 0.1479 | 16 | 0.9965 |
| **Physiological** | 9 | 7 | 32 | -0.0054 | -0.1165 | 0.1057 | 16 | 0.9195 |

**Table S45 Results of the meta-regression of the acclimation data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Metabolic Rate** | 6 | 5 | 12 | -0.0056 | -0.2959 | 0.2847 | 4 | 0.9599 |

**Table S46 Results of the meta-regression of the acclimation data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with acclimation treatment exposures. Effect size = PRRDs.

| Acclimation Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p-value |
|---|---|---|---|---|---|---|---|---|
| **Insecta** | 4 | 4 | 31 | 0.0008 | -0.2025 | 0.2041 | 3 | 0.9905 |

**Table S47 Results of the developmental subset MLMA model.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **MLMA** | 32 | 32 | 138 | 0.0033 | -0.0251 | 0.0317 | 23 | 0.8117 |

**Table S48 Results of the meta-regression of the developmental data set with the amplitude of the fluctuation as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Amplitude** | 32 | 32 | 138 | 0.0001 | -0.0025 | 0.0027 | 50 | 0.9346 |

**Table S49 Results of the meta-regression of the developmental data set with exposure period as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Embryo** | 16 | 17 | 70 | 0.0018 | -0.0330 | 0.0366 | 29 | 0.9174 |
| **Juvenile** | 4 | 4 | 13 | -0.0155 | -0.1876 | 0.1567 | 29 | 0.8554 |
| **Larva** | 12 | 12 | 55 | 0.0080 | -0.0416 | 0.0575 | 29 | 0.7445 |

**Table S50 Results of the meta-regression of the developmental data set with fluctuation type as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Sinusoidal (Sine Curve)** | 14 | 13 | 49 | 0.0007 | -0.0576 | 0.0590 | 39 | 0.9807 |
| **Alternating** | 12 | 15 | 50 | 0.0043 | -0.0323 | 0.0409 | 26 | 0.8111 |
| **Stepwise** | 4 | 4 | 17 | 0.0059 | -0.0837 | 0.0956 | 39 | 0.8941 |

**Table S51 Results of the meta-regression of the developmental data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Life-history Traits** | 27 | 29 | 65 | 0.0113 | -0.0267 | 0.0493 | 12 | 0.5292 |
| **Morphological** | 20 | 23 | 54 | -0.0051 | -0.0586 | 0.0484 | 12 | 0.8375 |

**Table S52 Results of the meta-regression of the developmental data set with specific phenotypic traits as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Development Time** | 26 | 27 | 46 | 0.0105 | -0.0282 | 0.0491 | 82 | 0.5919 |
| **Length** | 9 | 10 | 14 | 0.0009 | -0.0869 | 0.0888 | 82 | 0.9832 |
| **Mass** | 12 | 14 | 25 | -0.0115 | -0.0802 | 0.0572 | 82 | 0.7395 |

**Table S53 Results of the meta-regression of the developmental data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the subset of data with developmental treatment exposures. Effect size = PRRDs.

| Developmental Exposure (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High | df | p value |
|---|---|---|---|---|---|---|---|---|
| **Arachnida** | 2 | 3 | 11 | 0.0029 | -0.0491 | 0.0550 | 19 | 0.9072 |
| **Insecta** | 20 | 18 | 73 | 0.0050 | -0.0355 | 0.0455 | 19 | 0.7995 |

### 7.13 | Heterogeneity Results

**Table S54 Heterogeneity results for the MLMA models.** Effect size = PRRDs. $I^2$ values reported as percentages.

| MLMA Models (PRRDs) | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|
| **Overall** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Individual-level Traits** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Aquatic** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Terrestrial** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Acclimation** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Developmental** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

## 7.14 | Graphical Representation of the MLMA and Meta-regression Results

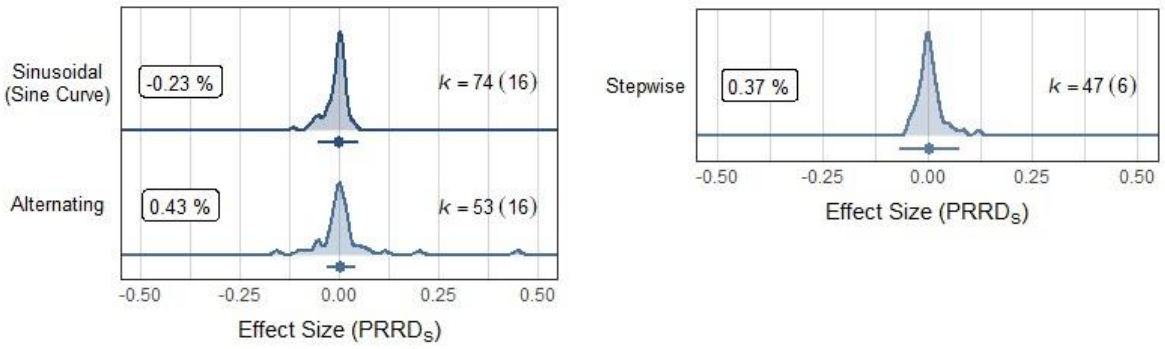


**Figure S6 Meta-regression results for the individual-level traits subset with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

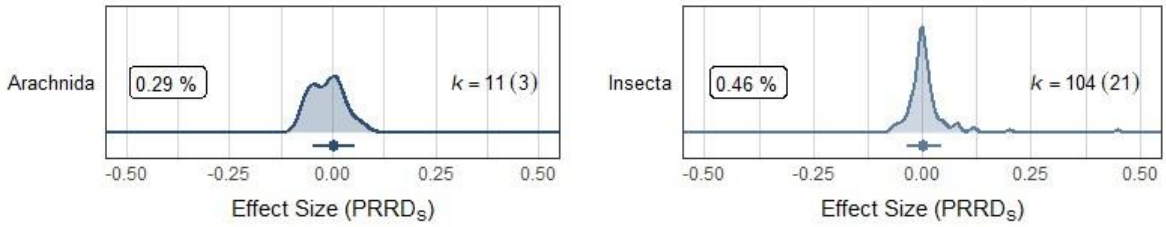


**Figure S7 Meta-regression results for the individual-level traits subset with taxonomic class as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

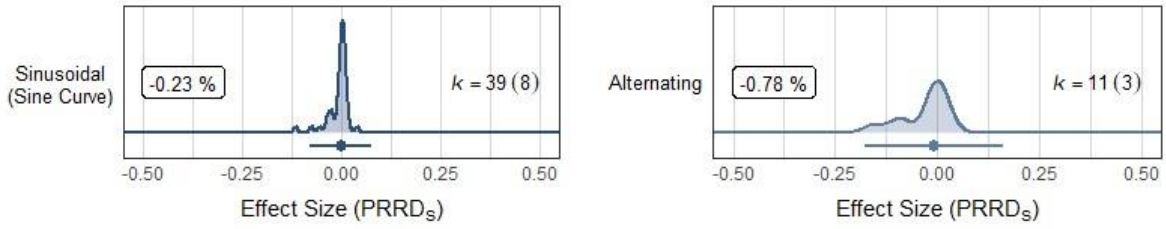


**Figure S8 Meta-regression results for the aquatic subset with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

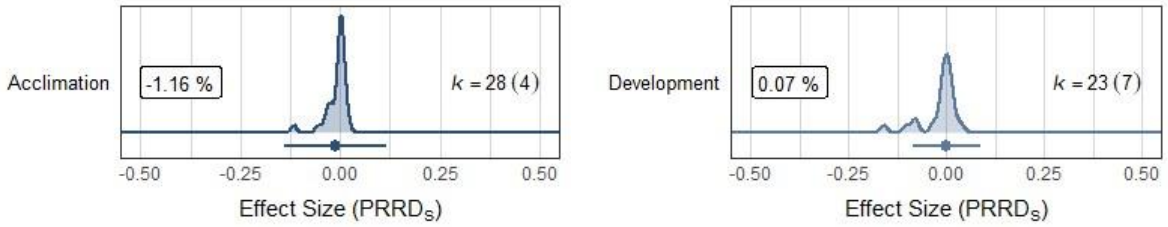


**Figure S9 Meta-regression results for the aquatic subset with the mechanism of plasticity as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean $PRRD_S$ estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

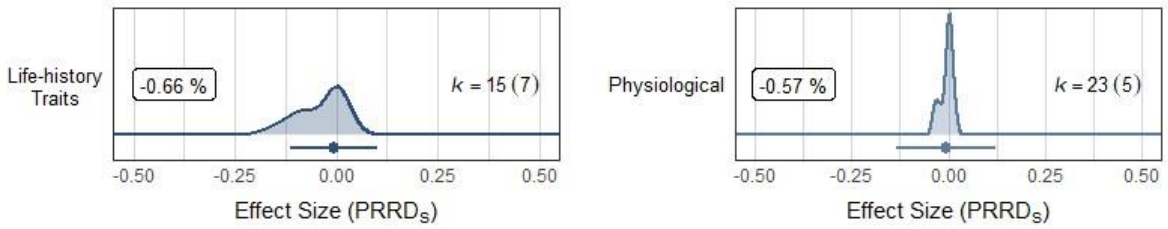


**Figure S10 Meta-regression results for the aquatic subset with phenotypic trait category as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean $PRRD_S$ estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

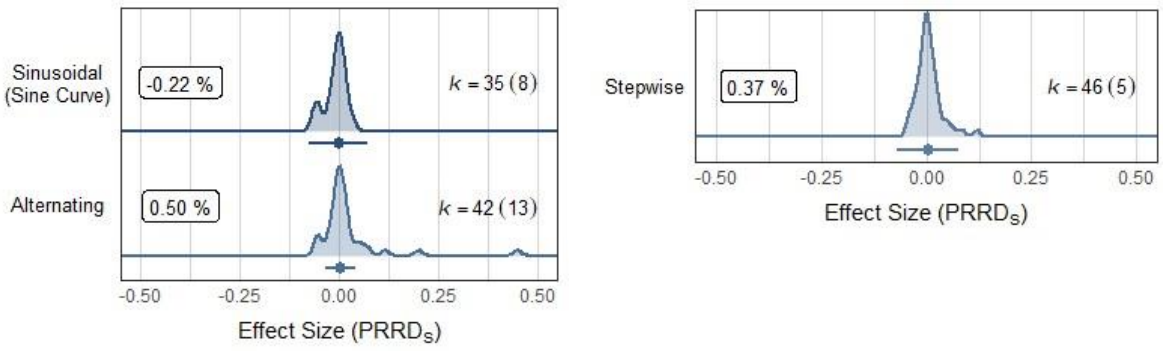


**Figure S11 Meta-regression results for the terrestrial subset with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

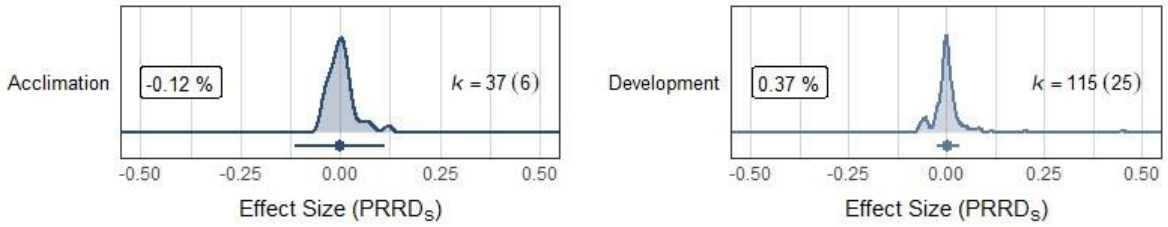


**Figure S12 Meta-regression results for the terrestrial subset with the mechanism of plasticity as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean $PRRD_S$ estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

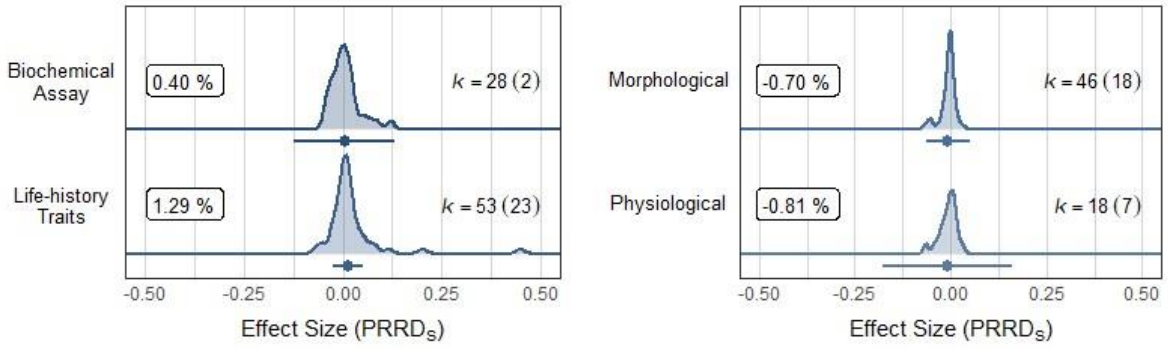


**Figure S13 Meta-regression results for the terrestrial subset with phenotypic trait category as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

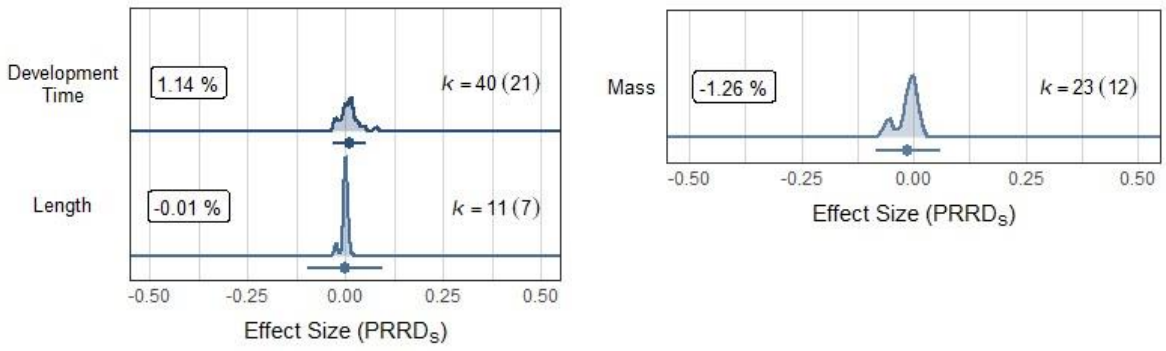


**Figure S14 Meta-regression results for the terrestrial subset with specific phenotypic traits as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

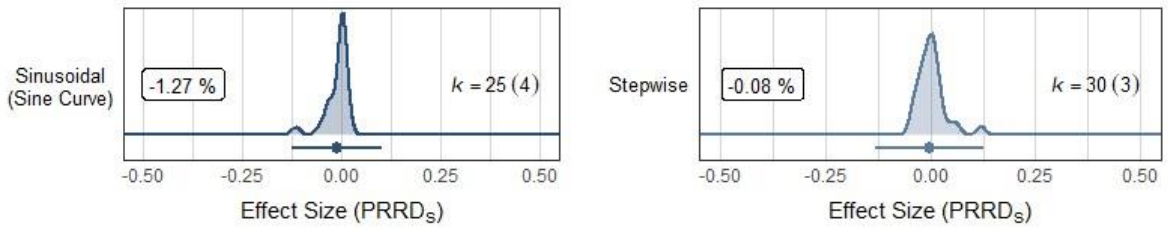


**Figure S15 Meta-regression results for the acclimation subset with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean $PRRD_S$ estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

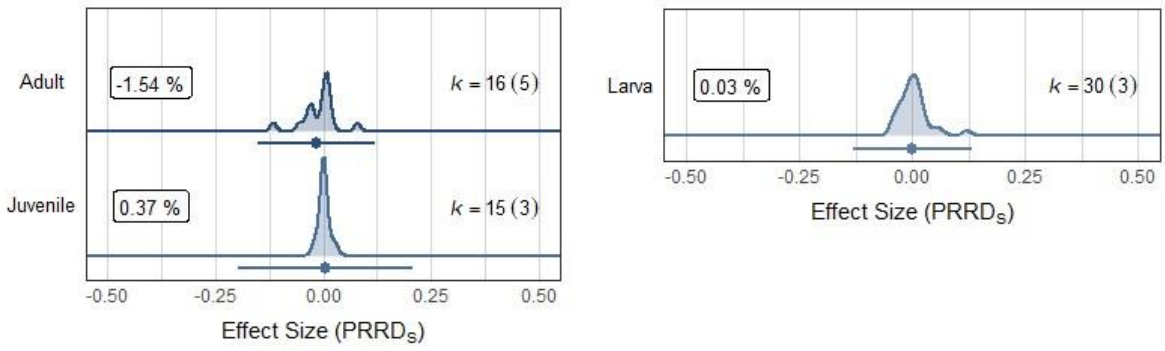


**Figure S16 Meta-regression results for the acclimation subset with life-history stage as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

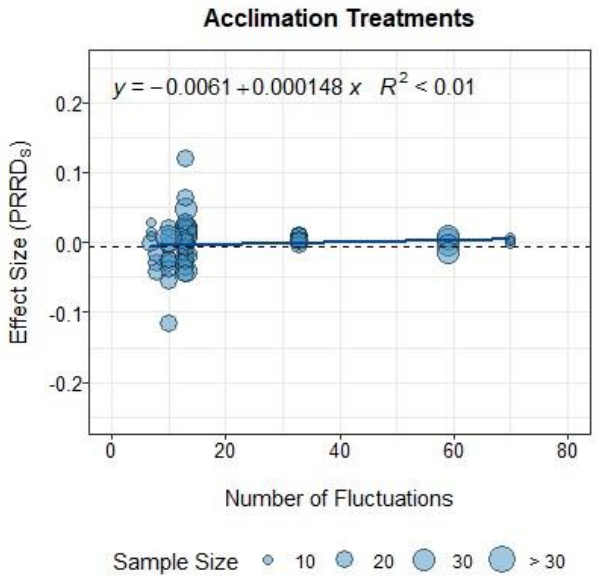


**Figure S17 Relationship between effect size ($PRRD_S$) and the number of fluctuations for the acclimation subset.** There was no significant relationship between the number of fluctuations and the difference in the phenotypic plasticity between the constant and fluctuating temperature treatments. Dashed line = $PRRD_S$ 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.

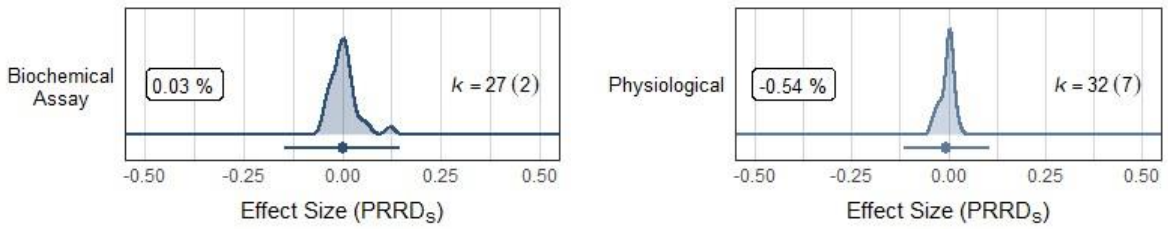


**Figure S18 Meta-regression results for the acclimation subset with phenotypic trait category as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

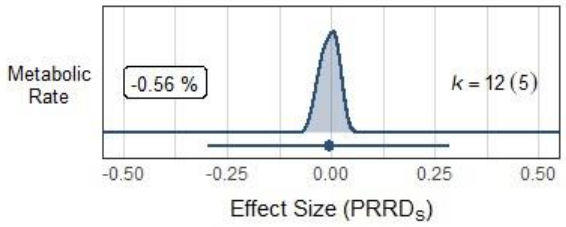


**Figure S19 Meta-regression results for the acclimation subset with specific phenotypic traits as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

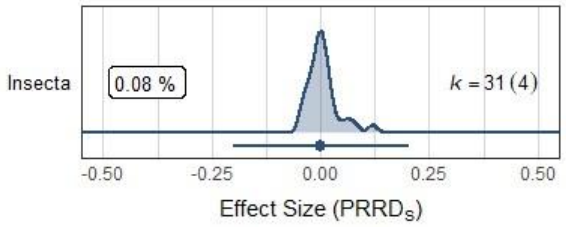


**Figure S20 Meta-regression results for the acclimation subset with taxonomic class as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

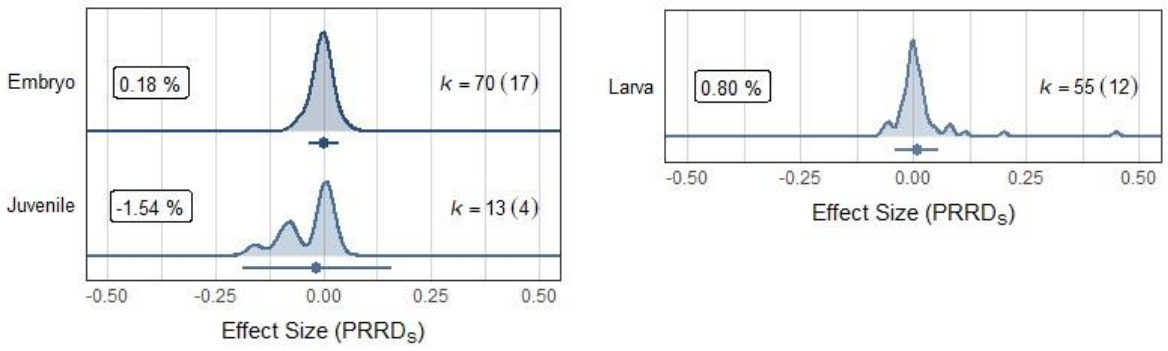


**Figure S21 Meta-regression results for the developmental subset with exposure period as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

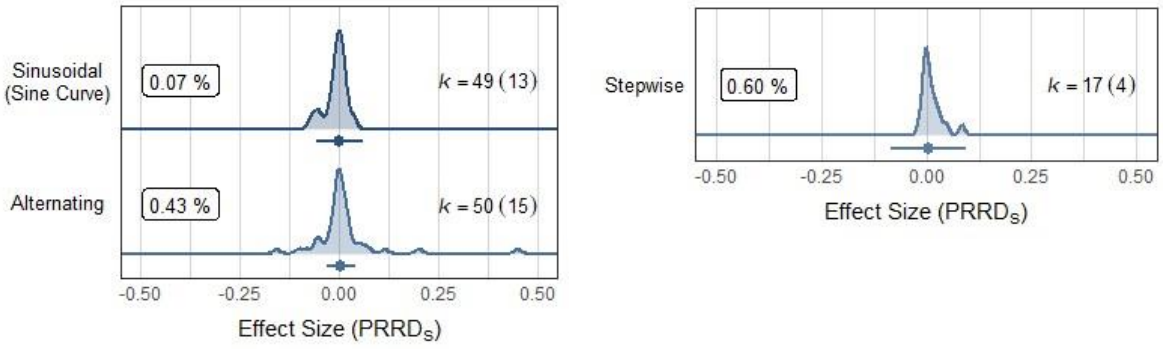


**Figure S22 Meta-regression results for the developmental subset with fluctuation type as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

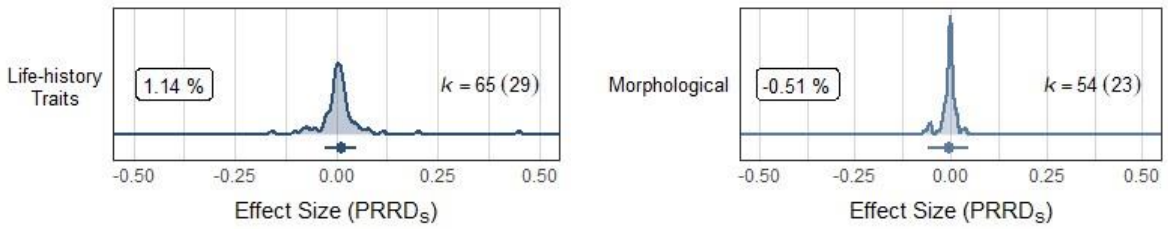


**Figure S23 Meta-regression results for the developmental subset with phenotypic trait category as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

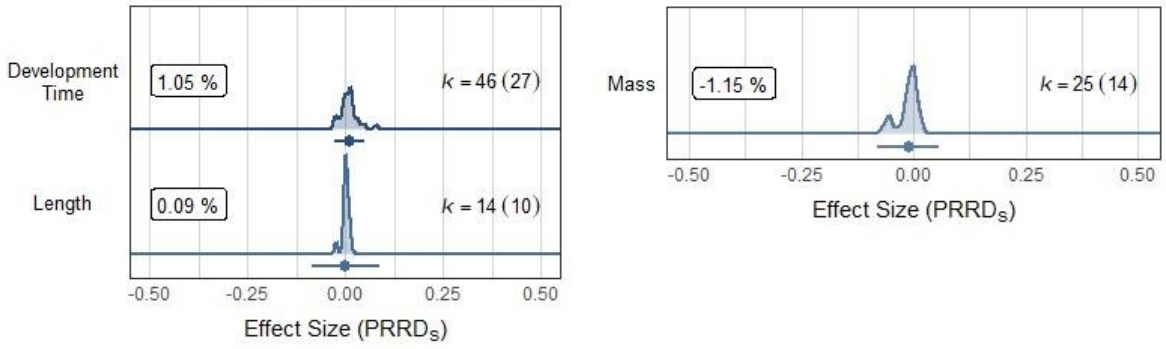


**Figure S24 Meta-regression results for the developmental subset with specific phenotypic traits as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. 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 species in brackets. X-axis limits are cropped for presentation.

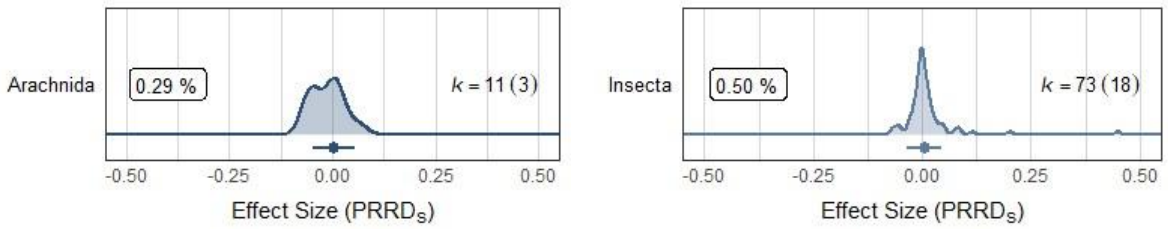


**Figure S25 Meta-regression results for the developmental subset with taxonomic class as the moderator.** There were no significant differences in the phenotypic plasticity between the constant and fluctuating temperature treatments (indicated by 95% CIs crossing zero). Mean effect size (plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as distributions of individual effect sizes. Percentage labels are the mean $PRRD_S$ estimates transformed to show a proportional difference between the fluctuating and stable temperature treatments. K = number of effect sizes with the number of species in brackets. X-axis limits are cropped for presentation.

# Chapter 4

## Quantifying population-level variation in phenotypic plasticity – a meta-analysis

# 1 | Abstract

Natural and anthropogenic climate changes can impose acute and prolonged environmental stresses. Phenotypic plasticity allows individuals to compensate for variable environments and can determine their resilience and persistence in the face of future climate changes. Our aim was to establish the current state-of-knowledge regarding variation in phenotypic plasticity among populations within ectothermic species. We conducted a quantitative synthesis of 52 studies (883 effect sizes from 279 population comparisons across 42 species) that had population-level comparisons of plastic responses (behavioural, biochemical assay, gene expression, life-history traits, morphological, and physiological). The data set permitted comparisons across different ecosystems (terrestrial and aquatic), environmental conditions (e.g., temperature and salinity), mechanisms of phenotypic plasticity (acclimation, developmental plasticity, and transgenerational effects), and taxonomic classes (e.g., Actinopteri, Gastropoda, and Insecta). Our analysis indicates that there are differences in the capacity for phenotypic plasticity among populations of the same species. The disparity between populations was particularly evident in physiological responses, and in the developmental plasticity of morphological traits. We suggest that intraspecific differences in plastic responses may reflect bet-hedging within species. Furthermore, predictions of the impacts of climate change and the resulting conservation strategies, need to be resolved at the population-level.

## 2 | Introduction

Ectotherms experience a range of acute and prolonged environmental stresses that arise from natural and anthropogenic climate changes (Arnell et al., 2019). The failure to compensate for unfavourable conditions can have detrimental consequences for performance and survival (Nunez et al., 2019; Pacifici et al., 2017; Warner et al., 2021). Organisms can shift their geographic range to align with more favourable conditions, or attempt to mitigate the impacts of environmental change (Bellard et al., 2012; Guisan and Zimmermann, 2000). Genetic adaptation can occur in response to persistent environmental signals over many generations (Chevin et al., 2010; Hoffmann and Sgro, 2011). However, rapid environmental changes with unpredictable directionality can render genetic adaptation inefficient (Alley et al., 2003; Kawecki, 2000).

Phenotypic plasticity remodels phenotypes independently of the genotype, and can at least partially buffer organisms from harmful conditions (Beaman et al., 2016; Loughland et al., 2021). The phenotypes of adults can be influenced by the environments experienced during early life history stages (developmental plasticity; Burggren, 2018; Le Roy et al., 2017; Warner et al., 2018). Adults and juveniles can also induce reversible and repeatable phenotypic modifications in response to relatively long-term intragenerational variation (acclimation; Guderley, 2004; Loughland and Seebacher, 2020). Both developmental plasticity and reversible acclimation can occur concurrently in response to environmental change (Beaman et al., 2016). Compensatory responses can also occur across generations (transgenerational effects) where (grand)parental environmental conditions determine offspring phenotypes (Le Roy et al., 2017). Each of these mechanisms can allow individuals to maintain relatively stable phenotypes across environmental gradients that mitigate the negative impacts of climate change (Lagerspetz, 2006; Schulte et al., 2011). Therefore, the

capacity to adjust phenotypes can dictate the resilience and persistence of ectotherms in variable environments (Botero et al., 2015; Kawecki, 2000).

Plastic responses to acute environmental variations are often characterised by non-linear performance curves (Huey and Kingsolver, 1989). Perfect phenotypic plasticity occurs when the performance curve shifts so that optimal performance coincides with the novel environmental conditions (Wilson and Franklin, 2002). It is rare to completely maintain optimal performance across environmental gradients (perfect plasticity) and differences in the capacity for phenotypic plasticity have been reported across phylogenetic lineages, habitats, and phenotypic traits (Seebacher et al., 2012; Seebacher et al., 2015). An understanding of differences in compensatory responses is essential as it can identify ectotherms at risk and where conservation efforts should be prioritised.

It is crucial to understand the extent to which populations differ in their capacity for phenotypic plasticity, because it could dictate the most effective application of conservation approaches (Hanski, 1998). Differences between populations and their capacities for phenotypic plasticity could result in a gradient of vulnerability within a species, that influences their environmental range and ecological success during variable climates (Kamo et al., 2011). Failure to account for intraspecific differences could induce inaccurate predictions regarding the impacts of environmental change (Pearman et al., 2010). However, empirical evidence of the divergence of plastic responses across populations is equivocal. It is possible that populations are genetically constrained and the capacity for phenotypic plasticity is fixed within a species (Levins, 1968). This would indicate that the resilience to environmental change is uniform across populations and that conservation approaches should be applied with a species-level resolution (Bellard et al., 2012; Tewksbury et al., 2008).

Understanding variation in plasticity is crucial to predict which species and populations are likely to persist in the face of future climate changes. Our aim was to establish the current state-of-knowledge regarding variation in phenotypic plasticity among populations within ectotherms. We conducted a systematic meta-analysis of the literature, that investigated whether the capacity for phenotypic plasticity differed between populations of the same ectothermic species. Through subset and meta-regression analyses the extent that populations differed in plastic responses was also investigated across different: (i) ecosystems (terrestrial and aquatic organisms), (ii) environmental conditions (e.g., temperature and salinity), (iii) mechanisms of phenotypic plasticity (acclimation, developmental plasticity, and transgenerational effects), (iv) phenotypic traits, and (v) taxonomic classes.

## 3 | Materials and Methods

We followed 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) in our study. Question formulation, literature searching and screening were performed in accordance with the guidelines set out in Foo et al., (2021).

### 3.1 | Systematic Literature Search and Screening

The PECO (Population, Exposure, Comparator, Outcome) framework was followed to establish focus questions and assist in the literature search and screening processes (Supplementary Materials 7.1; Morgan et al., 2018). A systematic literature search was conducted in the Scopus database on the 7th of February 2022. Scopus is a database commonly utilised in ecological meta-analyses that indexes from more than 27,000 peer-reviewed journals (Kelly et al., 2018; Zhu and Liu, 2020). To identify studies that investigated differences in phenotypic plasticity across populations of the same species, a

search string was implemented that included four key features. To narrow the list of studies to those that compared responses of at least two populations, the first two components of the search string were synonyms for 'population' and 'comparison' (Liao et al., 2016; Matesanz and Ramírez-Valiente, 2019). Synonyms for each mechanism of phenotypic plasticity (acclimation, developmental plasticity, and transgenerational effects) were then added to search explicitly for differences in the capacity for phenotypic plasticity (Noble et al., 2018; Pottier et al., 2021; Sánchez-Tójar et al., 2020a). The search string ended with exclusionary keywords and subject areas. The complete Scopus search string can be found in Supplementary Materials 7.2.

The initial search returned a total of 2,296 unique studies (Supplementary Materials 7.3), that underwent abstract screenings following the exclusion and inclusion criteria (Supplementary Materials 7.4). Briefly, the study had to be in English and experimentally compare the capacity for phenotypic plasticity across populations of the same ectothermic species. There were 417 studies that fulfilled these criteria, and we used these for full-text screening (Supplementary Materials 7.4).

To qualify for inclusion in the analysis, a study had to have: (a) identical housing and habituation periods across all treatments; (b) treatments that were a high and low state of the natural environment (e.g., diet, humidity, predator, salinity, temperature, or water level) and that matched one of the three mechanisms of phenotypic plasticity (acclimation, developmental plasticity, or transgenerational effects); (c) comparison of phenotypic plasticity across two or more wild populations in controlled-laboratory conditions; (d) a phenotypic response measured following each treatment (e.g., behavioural, biochemical assay, gene expression, life-history, morphological, physiological, or thermal tolerance traits). Transient phenotypic responses (e.g., development time), principal components and factors loading were excluded from the analysis (Tarka et al., 2018). In total, there were 52 studies

that adhered to both screening protocols and were included in the analysis (Supplementary Materials 7.5).

### 3.2 | Data Extraction

The effect size and sampling variance calculations required the sample sizes, means, and error metrics of phenotypic traits measured in each of four conditions: a high and low state of prolonged environmental exposure, measured in each of two populations of the same species. Acute test conditions of the phenotypic trait measurements had to match the corresponding environmental conditions (Seebacher et al., 2015). Measurements of thermal tolerance (e.g., critical thermal minimum and maximum) that involved dynamic acute test conditions, had to start at the corresponding treatment temperature. If there were more than two treatments conducted within a population (e.g., high, medium, and low), we selected the two experimental manipulations that coincided best with natural conditions experienced by the species (Seebacher et al., 2015). For studies that included more than two populations, we recorded the required information for every combination of binary population comparisons. Statistical information was manually extracted from texts, tables, supplementary materials, and figures (using the R package *metaDigtise* to extract data from figures; Pick et al., 2019). The sample sizes recorded were biological replicates, rather than technical replicates, to avoid non-independence (Wu and Seebacher, 2020).

Descriptive statistics of variables that had the potential to influence the difference in the capacity for phenotypic plasticity between populations were additionally recorded, for use in subset analyses and as moderators in meta-regression analyses. Taxonomic information and preferred ecosystem were recorded as plastic responses can differ across both phylogenetic lineages and habitat (Seebacher et al., 2012; Seebacher et al., 2015). Trait category was recorded because the capacity for phenotypic plasticity can differ across traits

(Le Roy et al., 2017). Lastly, information on the experimental design was recorded (environmental condition manipulated and mechanism of phenotypic plasticity).

### 3.3 | Data Processing and Transformations

Standard errors (SE; Equation 1) and 95% confidence internals (CI; Equation 2) were converted to standard deviations (SD), as required for effect size calculations (Quinn and Keough, 2002):

$$SD = SE \times \sqrt{n} \tag{1}$$

$$SD = \sqrt{n} \times \frac{CI_{High} - CI_{Low}}{3.92} \tag{2}$$

where n is sample size and $CI_{High}$ and $CI_{Low}$ represent the upper and lower 95% CIs respectively.

Data reported as percentages (e.g., survival) are bounded between 0 and 1. As such, they were transformed prior to effect size calculations using Equation 3 and 4, to obtain means and variances that were more likely to satisfy normal assumptions (Macartney et al., 2022):

$$\bar{x} = \arcsin\left(\sqrt{\bar{z}}\right) \tag{3}$$

$$SD_{\bar{x}} = \sqrt{\frac{SD_z^2}{4\bar{z}(1 - \bar{z})}} \quad (4)$$

where $\bar{x}$ and $SD_{\bar{x}}$ are the transformed means and SD, $\bar{z}$ and $SD_{\bar{z}}$ is the means and SD on the percentage scale. Different terminology for individual traits were consolidated to ensure consistency in terminology used across different studies (Supplementary Materials 7.6). Scientific names were adjusted to match the taxonomic information retrieved from the Open Tree of Life (Michonneau et al., 2016). Amphibians were considered aquatic organisms for the analysis.

### 3.4 | Effect Size Calculations

We calculated an interaction-based plasticity response ratio difference (PRRD) and its corresponding sampling variance to quantify the difference in phenotypic plasticity between two populations. The PRRD is derived by first calculating log response ratios for the change in phenotypic traits following either acclimation, developmental, or transgenerational exposures to different environments (lnRR; Equation 5; Hedges et al., 1999; Lajeunesse, 2011). Hence, the lnRR is indicative of the capacity for phenotypic plasticity. An effect size approaching zero suggests increasing plasticity as phenotypic trait values are remaining constant despite environmental differences. To capture the differences in phenotypic plasticity between populations we then subtracted the lnRR of one population from the other (Equation 6 for effect size; Equation 7 for sampling variance; Pottier et al., 2021).

$$\text{lnRR} = \ln\left(\frac{\bar{x}_H}{\bar{x}_L}\right) \quad (5)$$

$$\text{PRRD} = lnRR_{\text{P1}} - lnRR_{\text{P2}} \quad (6)$$

$$v_{\text{PRRD}} = \left(\frac{SD^2_{\text{P1H}}}{n_{\text{P1H}}\bar{x}^2_{\text{P1H}}} + \frac{SD^2_{\text{P1L}}}{n_{\text{P1L}}\bar{x}^2_{\text{P1L}}} + \frac{SD^2_{\text{P2H}}}{n_{\text{P2H}}\bar{x}^2_{\text{P2H}}} + \frac{SD^2_{\text{P2L}}}{n_{\text{P2L}}\bar{x}^2_{\text{P2L}}}\right) \quad (7)$$

To control for the fact that environmental gradients varied among effect sizes within and across studies, we standardised PRRD to a one degree or one ppt change in data subsets that investigated the effects of temperature and salinity, respectively (Equation 8 for effect size; Equation 9 for sampling variance; Noble et al., 2022; Pottier et al., 2021).

$$\text{PRRD}_{\text{S}} = \frac{lnRR_{\text{P1}} - lnRR_{\text{P2}}}{T_{\text{H}} - T_{\text{L}}} \quad (8)$$

$$v_{\text{PRRD}_{\text{S}}} = \left(\frac{1}{T_H - T_L}\right)^2 \left(\frac{SD^2_{\text{P1H}}}{n_{\text{P1H}}\bar{x}^2_{\text{P1H}}} + \frac{SD^2_{\text{P1L}}}{n_{\text{P1L}}\bar{x}^2_{\text{P1L}}} + \frac{SD^2_{\text{P2H}}}{n_{\text{P2H}}\bar{x}^2_{\text{P2H}}} + \frac{SD^2_{\text{P2L}}}{n_{\text{P2L}}\bar{x}^2_{\text{P2L}}}\right) \quad (9)$$

where $\bar{x}$ is the mean response, subscripts H and L denote the high and low environments, subscript P denotes population (first and second, respectively), subscripts S denotes a standardised PRRD, and T is the degrees or ppt for temperature or salinity treatments, respectively.

We ensured that the directionality in comparing environments within a population was consistent (e.g., high environments divided by lower environments for lnRR calculations). However, the directionality of the population comparisons (PRRD and PRRDs) is not important and requires the absolute magnitude of the effect sizes to be analysed (see 'analyse-then-transform' approach below). Therefore, a PRRD or PRRDs of zero would indicate that there is no variation in the capacity for phenotypic plasticity between populations of the same species. In contrast, effect size estimates greater than zero suggests a difference in the capacity for phenotypic plasticity.

### 3.5 | Statistical Analysis

All calculations, analyses, and figures were completed with R.4.2.2 in RStudio version 2022.12.0 (using R Package *ggplot2* for figures; Wickham, 2016). All data and code are available at https://github.com/ClaytonStocker/Pop_Plasticity_Meta (Supplementary Materials 7.7). Mode effect size estimates + 95% CIs are reported, along with a percentage difference in the capacity of phenotypic plasticity between populations of the same species (exponentially transformed; Pustejovsky, 2018).

### 3.6 | Meta-analyses and Non-independence

To estimate absolute mode effect size in plasticity across populations we used a Bayesian approach to fit multi-level meta-analytic models (MLMA) as they provide substantial flexibility when converting effect sizes to the absolute scale (see below; using *rstan* through the R Package *brms*; Bürkner, 2017; Bürkner, 2018; Morrissey, 2016b). Analysing raw absolute effect sizes can bias effect estimates (described in detail by Morrissey, 2016a). Therefore, we took an 'analyse-then-transform' approach (Morrissey, 2016a), which involved estimating the signed mode effect sizes and then transforming these modes to their corresponding absolute magnitudes by applying the folded normal distribution

(Morrissey, 2016a; Morrissey, 2016b). A Bayesian approach enabled us to easily propagate the uncertainty in mode estimates and allow for the comparison of different magnitudes across subsets and moderators through 95% CIs.

Bayesian models were run with four MCMC chains sampling 3000 posterior samples from each chain. The first 2000 iterations were discarded as burning, and a thinning rate of 5 was applied, meaning that 8000 posterior samples were retained across all MCMC chains. Models were fitted with an adapt delta of 0.99 and a maximum tree depth of 15. A half student-t prior with 3 degrees of freedom was used for the intercept (0, 25) and variance (0, 20), and a flat uniform prior was used for the fixed effects. MCMC chain mixing, and convergence were assessed by visually inspecting diagnostic plots and ensuring a Rhat < 1.01 for all parameters (Supplementary Materials 7.8).

Our Bayesian multi-level meta-analytic models dealt with a number of sources of non-independence (Noble et al., 2017). The same populations and treatments were used to derive multiple effect sizes from studies, introducing shared sampling covariance among effect sizes (Noble et al., 2017). To control for this, we divided the sample sizes for each treatment group of a population by the number of times a treatment and/or population was repeated across effect sizes (Sánchez-Tójar et al., 2020a). The sampling variances associated with the PRRD and $PRRD_S$ were subsequently calculated based on these adjusted sample sizes.

Random effects were added to our models to control for the remaining dependencies (Nakagawa et al., 2017; Sánchez-Tójar et al., 2020b). To account for several effect sizes coming from the same study, a Study ID random effect was included. A Shared Animal random effect was added to account for multiple responses measured in the same individuals. To control for phylogenetic relatedness, we derived a phylogenetic tree (Supplementary Materials 7.9) using the Open Tree of Life database (Michonneau et al., 2016). The *ape* R Package (Paradis and Schliep, 2019) was used to resolve polytomies and calculate branch lengths using the Grafen's method (Power = 1; Grafen, 1989). We then converted the phylogenetic tree to a phylogenetic correlation/covariance matrix and included this matrix when estimating the phylogenetic variance. In addition, we included a species level random effect (Species ID) to account for the repeated use of the same species across studies, which captures additional species-specific effects not captured by our phylogenetic random effect (Cinar et al., 2022). A trait-level random effect was included to control for the different frequencies that phenotypic traits were measured across the literature. Lastly, all models included an observational-level random effect to estimate 'residual' variation in effect sizes (Nakagawa and Santos, 2012).

The random effect structure for our models always included phylogenetic relatedness, study ID and observational-level ('within-study') random effects. To identify the most parsimonious model and the corresponding random effect structure, we compared all possible combinations of the remaining random effects using the Akaike Information Criterion small-sample size equivalent (AICc; Slein et al., 2023). For models using PRRDs a random effect structure that included phylogeny, study, and observation-level random effects was most parsimonious. The same was true if effects were standardised for temperature or salinity differences. Models using PRRD included a trait-level random effect (Supplementary Materials 8.10).

### 3.7 | Multi-level Meta-analytic Models

A MLMA model was first implemented to estimate the overall meta-analytic mode (intercept only) while accounting for random effects. We then conducted subset analyses to estimate the meta-analytic modes for moderators, which included terrestrial and aquatic organisms, studies that manipulated temperature as the environmental condition, terrestrial organisms exposed to temperature as the environmental condition, and aquatic organisms exposed to temperature or salinity as the environmental conditions (Figure 1, Table 1). For each subset model the total heterogeneity ($I^2_{Total}$) along with heterogeneity for each relevant random effect ($I^2_{Obs}$, $I^2_{Phylo}$, $I^2_{Study}$, and $I^2_{Trait}$,) was calculated to quantify the proportion of effect size heterogeneity after removing known sampling variance (high $I^2$ = 75%, medium $I^2$ = 50%, small $I^2$ = 25%; Borenstein et al., 2021; Higgins et al., 2003; Nakagawa and Santos, 2012). It is common in multi-species meta-analyses to achieve an $I^2_{Total}$ of greater than 90% (Senior et al., 2016). Heterogeneity statistics for each subset analysis are given in Supplementary Materials 7.11.

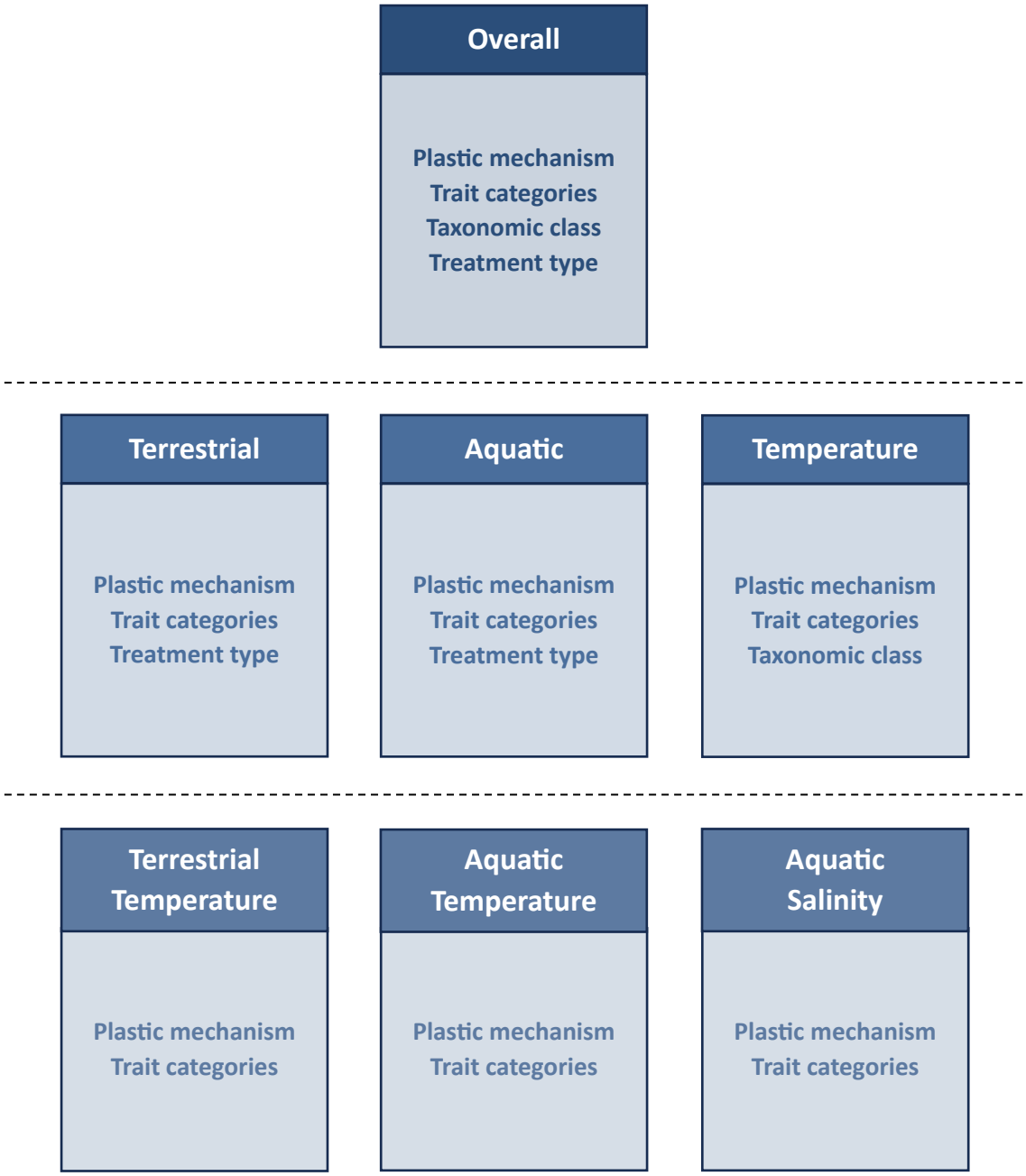


**Figure 1 The order of subset MLMA analyses and the corresponding meta-regression analyses.** White text = MLMA model. Blue text = meta-regression model. “Plastic mechanisms” distinguishes between plasticity treatments including acclimation, developmental plasticity, and transgenerational effects. “Trait categories” refer to the categorised groups of phenotypic traits measured following treatments. “Taxonomic class” is the class of each experimental species. "Treatment type” refers to the environmental variable

used in treatments. Subset analyses and meta-regressions were included when the number of effect sizes > 10. Definitions of MLMA and meta-regression terms are in Table 1.

**Table 1 Definitions of included terms in each subset.** Inclusion criteria for each subset term.

| Subset Terms | Definitions |
|---|---|
| **Terrestrial Organisms** | Organisms that spend the predominant amount of their lifecycle in terrestrial ecosystems. |
| **Aquatic Organisms** | Organisms that spend the predominant amount of their lifecycle in aquatic ecosystems, including amphibians. |
| **Temperature Manipulations** | The four natural environmental treatments required for the effect size calculations were temperature manipulations. |
| **Salinity Manipulations** | The four natural environmental treatments required for the effect size calculations were salinity manipulations. |

### 3.8 | Meta-regression Models

For each data subset we conducted separate meta-regression models to compare moderators that had the potential to influence the observed mode effect size estimate (Figure 1). The moderators that we explored included mechanisms of phenotypic plasticity (acclimation, developmental plasticity, and transgenerational effects), taxonomic classes (Actinopteri, Amphibia, Branchiopoda, Gastropoda, and Insecta), phenotypic trait categories (behavioural, biochemical assay, gene expression, life-history traits, morphological, physiological, and tolerance), and the type of treatment manipulation (diet, humidity, predator, salinity, temperature, and water level). Moderators were analysed only when there were > 10 effect sizes (O'Dea et al., 2021).

### 3.9 | Publication Bias and Sensitivity Analysis

We explored evidence of publication bias by visually inspecting funnel plots of meta-analytic residuals from Bayesian models, and including both the z-transformed publication year and precision (inverse of variance) as moderators in frequentist models (Nakagawa et al., 2022; Sterne et al., 2005). There was no evidence of publication bias found in models using PRRD and $PRRD_S$ (data standardised for temperature and salinity differences) as the effect size (Supplementary Materials 7.11).

We conducted a sensitivity analysis by estimating the influence of each data point on frequentist models (using the function *cooks.distance* in the R Package *metafor*; Viechtbauer, 2010) to test the 'robustness' of our results. Bayesian models were also compared to Bayesian models fitted with untransformed data (effect sizes calculated prior to percentage and sample size transformations; Knol et al., 2011; Macartney et al., 2022). The outcome of these two analyses suggests that models using PRRD and $PRRD_S$ (standardised for temperature and salinity differences) as the effect size, are both robust and free from significantly influential outliers (Supplementary materials 7.11).

## 4 | Results

The overall data set included 279 population comparisons resulting in 883 effect sizes from 52 studies (Supplementary Materials 7.5) across 42 species (Supplementary Materials 7.9). In the overall MLMA analysis and the subsequent subset analyses (Supplementary Materials 7.12), the capacity for phenotypic plasticity differed between populations of the same species (Figure 2). There was a high heterogeneity ($I^2_{Total}$ = 83.55%; 2.d.p), which is not surprising due to the diversity of species, populations, treatments, and phenotypic trait measurements (Supplementary Materials 7.13). Most variation was derived from the observation-level random effect ($I^2_{Obs}$ = 43.35%) and the specific phenotypic trait measured

($I^2_{Trait}$ = 23.49%). Relatively little variation stemmed from study ($I^2_{Study}$ = 10.46%) and phylogenetic relatedness ($I^2_{Phylo}$ = 6.24%).

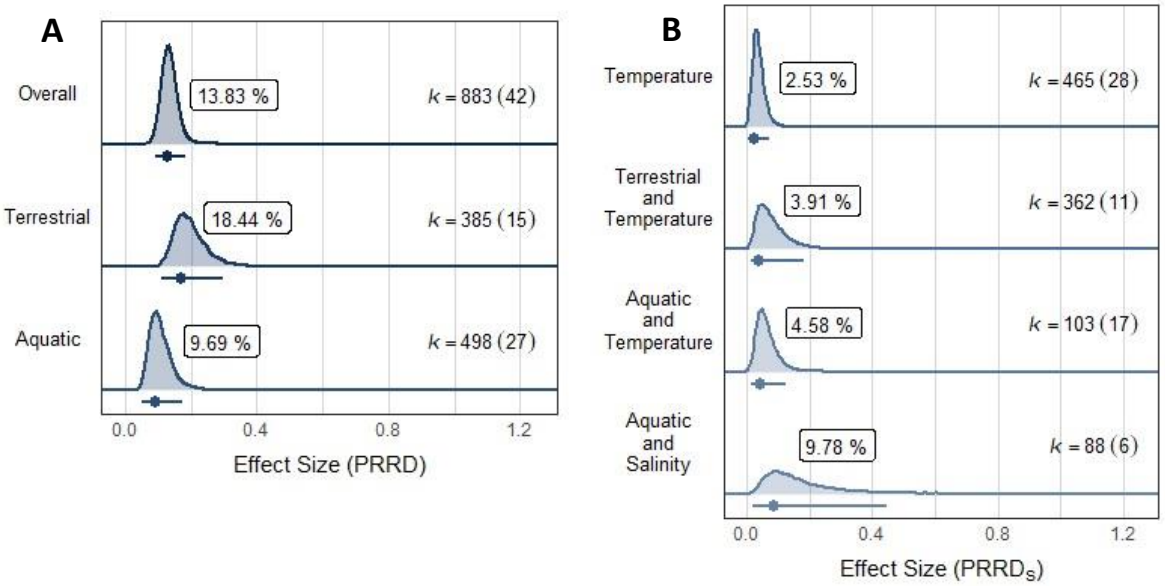


**Figure 2 Multi-level meta-analytic results for the overall data set and subset analyses.** All MLMA models showed that the capacity for phenotypic plasticity differed between populations of the same species (range = 2.53% - 18.44%). Mode effect size (A = folded normal plasticity response ratio difference, PRRD; B = folded normal standardised plasticity response ratio difference, PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode effect size estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

There were differences in the capacity for phenotypic plasticity between populations found in all meta-regression analyses of the overall and subset data sets (Supplementary Materials 7.12 and 8.14). Differences in the capacity for developmental plasticity between populations were statistically larger than acclimation (6.21% larger, p = 0.0023) and transgenerational effects (7.56% larger, p = 0.0055; Figure 3).

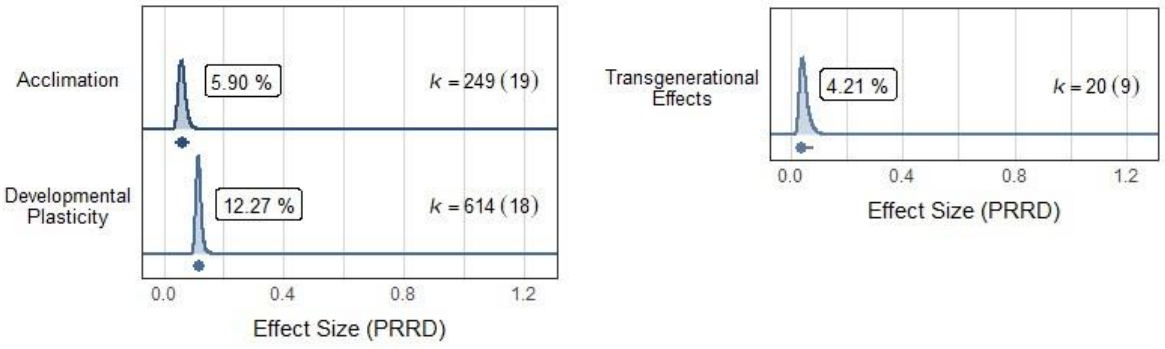


**Figure 3 Meta-regression results for the overall data set with the mechanism of plasticity as the moderator.** The capacity for acclimation, developmental plasticity and transgenerational effects all differed between populations of the same species (range = 4.21% - 12.27%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

Populations of gastropods had statistically larger differences in the capacity for phenotypic plasticity between their populations than all other taxonomic classes, including Actinopteri (46.24% larger, p = 0.0003), Amphibia (45.31% larger, p = 0.0005), Branchiopoda (36.62% larger, p = 0.0000), and Insecta (40.32% larger, 0.0000; Figure 4).

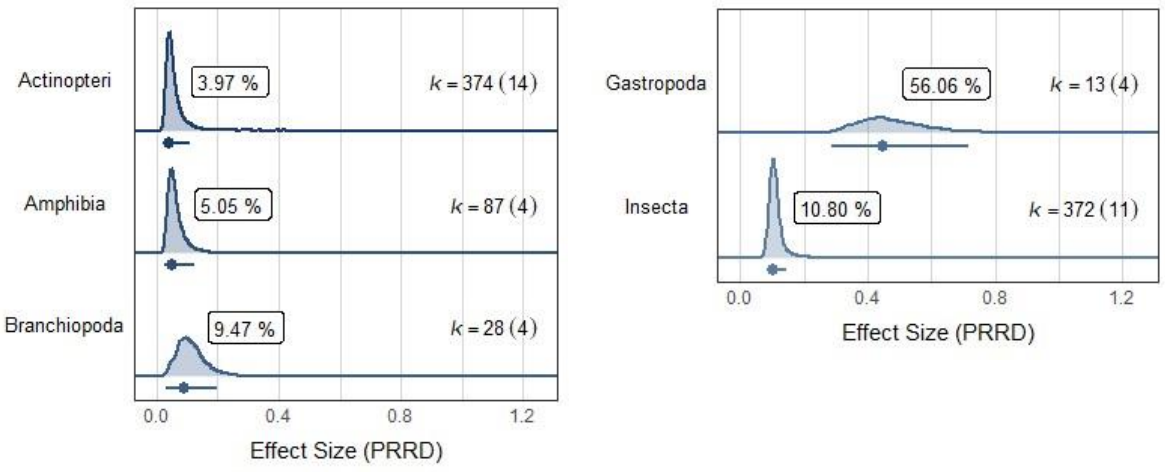


**Figure 4 Meta-regression results for the overall data set with taxonomic class as the moderator.** All taxonomic classes showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 3.97% - 56.06%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

There was a statistically increased difference between populations in the capacity for morphological plastic responses when compared to biochemical (6.53% increase, p = 0.0063) and thermal tolerance measurements (7.33% increase, p = 0.0100; Figure 5). The capacity for physiological plastic responses had statistically larger differences between populations than behavioural (79.26% larger, p = 0.0013), biochemical (88.90% larger, p = 0.0000), gene expression (83.49% larger, p = 0.0013), life-history (72.16% larger, p = 0.0020), morphological (75.14% larger, p = 0.0008), and thermal tolerance traits (80.91% larger, p = 0.0000; Figure 5), and this was particularly evident in aquatic environments (Supplementary Materials 7.14).

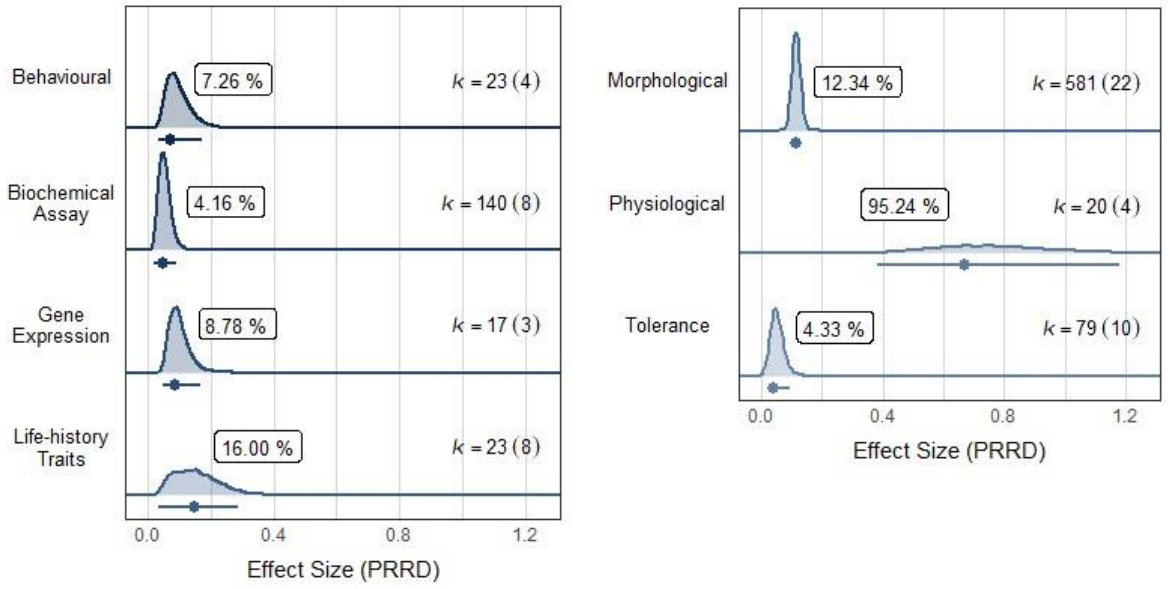


**Figure 5 Meta-regression results for the overall data set with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 4.16% - 95.24%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

Plastic responses to changes in humidity had a statistically greater difference between populations than responses to variation in diet (34.8% greater, p = 0.0003), predators (38.84% greater, p = 0.0000), salinity (22.88% greater, p = 0.0045), temperature (33.30% greater, 0.0000), and water level (37.98% greater, 0.0000; Figure 6), and this was particularly evident in terrestrial environments (Supplementary Materials 7.14).

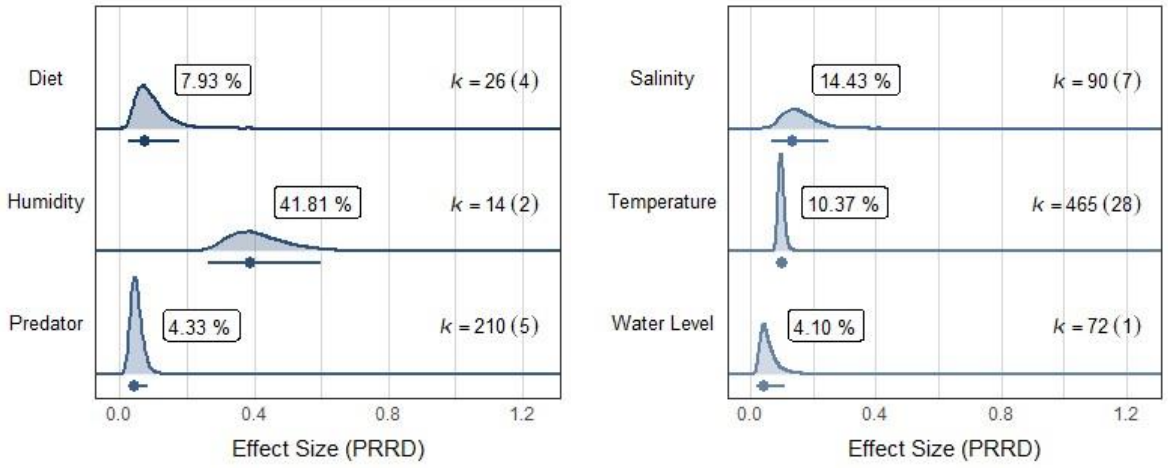


**Figure 6 Meta-regression results for the overall data set with the type of treatment as the moderator.** All types of treatments showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 4.10% to 41.81%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

## 5 | Discussion

Our meta-analysis of the overall data set showed that there are differences in the capacity for phenotypic plasticity among populations of the same ectothermic species. The data set included comparisons of phenotypic plasticity across various ecosystems, environmental conditions, plasticity mechanisms, traits, and taxonomic classes. Analyses of these factors consistently indicated differential plastic responses between populations. However, there were specific scenarios where the disparity between populations was particularly evident, and we discuss these below. Our analyses provide evidence that

compensatory responses are not uniform across a species and, instead, populations within species may have different levels of resilience to variable environments.

Perfect phenotypic plasticity would manifest as a shift in the thermal performance curve so that optimal performance aligns exactly with the current conditions (Murren et al., 2015; Wilson and Franklin, 2002). Our findings indicate that perfect compensation does not always occur and instead there are population-level differences in the capacity for phenotypic plasticity. The diversity of plastic responses across a species suggests that the benefit of phenotypic plasticity is limited and that some environments may instead favour relatively fixed phenotypes (DeWitt et al., 1998; Seebacher et al., 2015). Neither plastic nor fixed phenotypes are beneficial across all environments (e.g., fixed phenotypes can have greater performance at high temperatures; Loughland and Seebacher, 2020). Circumstances where a species contains populations with compensatory responses that range from a high capacity for phenotypic plasticity (full compensation for environmental change) to completely fixed phenotypes (no compensation at all), could therefore resemble a bet-hedging scenario (Seebacher and Little, 2021). In theory, a bet-hedging strategy would render a species more resilient to climate change as it increases the likelihood that at least some populations have phenotypes that function well in the novel environment (Haaland et al., 2020; Schindler et al., 2015).

The extent to which populations differ in their capacity for phenotypic plasticity could be determined by the endocrine and epigenetic mechanisms that regulate plastic responses (Ashe et al., 2021; Cedar and Bergman, 2009). Epigenetic processes including DNA methylation and histone modifications (acetylation and methylation), can result in relatively permanent phenotypic changes in response to (grand)parental and developmental environments (Ho and Burggren, 2010; Loughland et al., 2021). Reversible acclimation to relatively long-term intragenerational environmental change can be mediated by hormones

(such as thyroid and glucocorticoid) that also play a role in recruiting and regulating epigenetic mechanisms (Bartlett et al., 2019; Little et al., 2013). The genetic bases of these mechanisms suggests that sources of genetic diversity could influence the efficacy of plastic responses (Laskowski et al., 2021). Many species consist of genetically distinct populations (e.g., resulting from local adaptation or genetic drift; Blanquart et al., 2013; Gárate-Escamilla et al., 2019), and it may be that genetic and demographic processes interact with the mechanisms that regulate phenotypic plasticity to mediate the observed population-level differences (Seebacher et al., 2012).

Relatively large differences between populations were found in the capacity for developmental plasticity compared to acclimation and transgenerational effects. An organism can experience costs associated with acclimation capacity, including fitness-related costs that result from a phenotype-environment mismatch, or energetic costs from the production of the compensatory responses (Auld et al., 2010; Lande, 2014; Murren et al., 2015). Recent literature has suggested that developmental modifiers are mechanistically linked to acclimation capacity to reduce the costs involved (Beaman et al., 2016). Environmental history experienced during development can also influence the extent of transgenerational effects in guppies (*Poecilia reticulata*; Seebacher et al., 2023). The importance of the developmental environment to the overall phenotypic plasticity of an organism could explain the particularly large population-level differences in developmental plasticity. Populations with effective developmental plastic responses could reduce the need for equally varied acclimatory and transgenerational responses within a species. There were relatively large differences in the capacity for morphological responses that were investigated mostly following developmental treatments. Morphologies can be dependent on developmental temperatures and the specific habitat within species despite gene flow between the populations (Seebacher et al., 2016; Warner et al., 2020), which could extend to their plastic

capacities. The diverse habitats and developmental conditions experienced by different populations could explain the particularly large differences in the capacity for developmental plasticity of morphological responses.

Anthropogenic and natural environmental changes do not occur uniformly across a species' geographical range. Instead, populations within a species can encounter variable environments at different temporal- and spatial scales that impose unique limitations on the availability of resources (Arnell et al., 2019). The capacity for phenotypic plasticity of physiological responses can be constrained by the inadequate supply of energy and nutrients (Raubenheimer et al., 2012). The reduction in mitochondrial efficiency in the production of chemical energy (ATP) can also be a limiting factor (Brand et al., 2004). Our analyses found that differences between populations in the capacity for phenotypic plasticity of physiological responses were larger than all other trait categories. The extent of intraspecific differences could be characteristic of the unique history of resource limitations experienced by the populations.

The capacity for phenotypic plasticity can vary across phylogenetic lineages, habitats, and phenotypic traits (Seebacher et al., 2012; Seebacher et al., 2015). Populations of gastropods had a particularly large difference in plastic responses when compared to other taxonomic classes. Similarly, differences between populations in the plastic responses to changes in humidity were relatively larger than other environmental conditions. Although there were considerable overlaps in the effect sizes for these variables, they together suggest that there might be a taxonomic and environmental dependency to the extent in which populations differ in their capacity for phenotypic plasticity. There was considerable variation in the number of studies across the different taxonomic classes and environmental conditions, with relatively few investigating these potential sources for variation. However, the identification of potential sources of variation in the population-level differences of plastic

responses, emphasises that a universal approach to conservation does not exist, and instead strategies need be specific to the unique situations of the ectotherms at risk.

An organism's capacity to adjust phenotypes is indicative of their resilience and persistence in the face of anthropogenic and natural changes to the environment (Botero et al., 2015; Kawecki, 2000). Therefore, accurate predictions of the impacts of future climate changes require an understanding of the different compensatory responses across animals. The current state-of-knowledge we establish is that populations within species can have substantial variation in their capacity for phenotypic plasticity. These findings suggest that species could have a gradient of vulnerability consisting of populations with different environmental ranges and ecological successes during variable climates (Kamo et al., 2011). In a conservation sense, our quantitative synthesis of the literature emphasises the need for predictive models and conservation approaches to be developed at the population-level, rather than a species-level resolution. Our analyses have identified potential scenarios where the extent of population-level differences is exacerbated, including different taxonomic classes, environmental conditions, and trait responses. The current data coverage across species, habitats, and phenotypic traits is relatively sparce and future research into these sources of variation could prove crucial in identifying ectotherms at risk and where conservation efforts should be prioritised.

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## 7 | Supplementary Materials

### 7.1 | PECO Framework

**Table S1 PECO Framework table.** Populations, Exposures, Comparator and Outcomes that were the foundation for the focus question of the meta-analysis, the database search strings (Supplementary Materials 7.2) and the decision tree (Supplementary Materials 7.4).

| PECO | Description |
|---|---|
| **Population** | Two ectothermic metazoan populations of the same species. The two populations had to have restricted gene flow between them. |
| **Exposure** | A treatment replicating a natural environment. This treatment was either higher than (i.e., salinity or temperature) or present (i.e., diet or predator), when compared to the comparator. |
| **Comparator** | A treatment replicating a natural environment. This treatment was either lower than (i.e., salinity or temperature) or absent (i.e., diet or predator), when compared to the exposure. |
| **Outcome** | Any phenotypic trait that:<br>- was measured in the same environment as the corresponding treatment (comparator or exposure);<br>- was measured following an uninterrupted treatment condition;<br>- was not a transient response measured during the treatment exposure. |

### 7.2 | Search Strings and Protocols

Scopus

TITLE-ABS-KEY((population OR intraspecific OR intra-specific OR conspecific OR "within species") AND (compar* OR diff* OR var* OR diverge* OR capacit* OR homogen* OR heterogen*) AND (plasticity OR "plastic response" OR acclimat* OR "develop* effect" OR epigenetic* OR transgeneration* OR trans-generation* OR intergeneration* OR inter-generation* OR "cross generation*" OR "paternal effect" OR "maternal effect" OR "parent* effect" OR "grandparent* effect" OR "grandmater* effect" OR "grandpater* effect" OR "silver-spoon effect" OR "anticipatory effect" OR "carry-over effect" OR "carry over effect")) AND NOT (endotherm* OR bacter* OR fung* OR microorganism OR micro-organism OR plant* OR bird OR mammal OR rat OR mouse OR cattle OR human OR woman OR man OR "parental care" OR child* OR infant OR educat* OR industr* OR commerc* OR domest* OR cancer) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (SUBJAREA,"AGRI") OR LIMIT-TO (SUBJAREA,"ENVI") OR LIMIT-TO (SUBJAREA,"BIOC")) AND (LIMIT-TO (LANGUAGE,"English"))

### 7.3 | PRISMA Flow Diagram

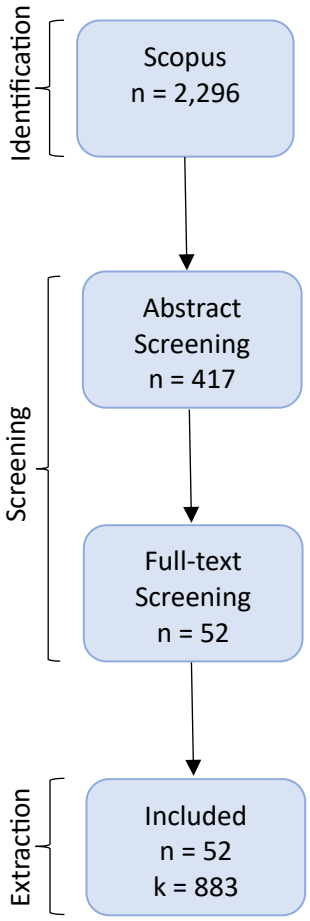


**Figure S1 PRISMA flow diagram for the systematic search and screening processes.** n = number of studies remaining after that respective stage. k = number of effect sizes.

### 7.4 | Decision Tree

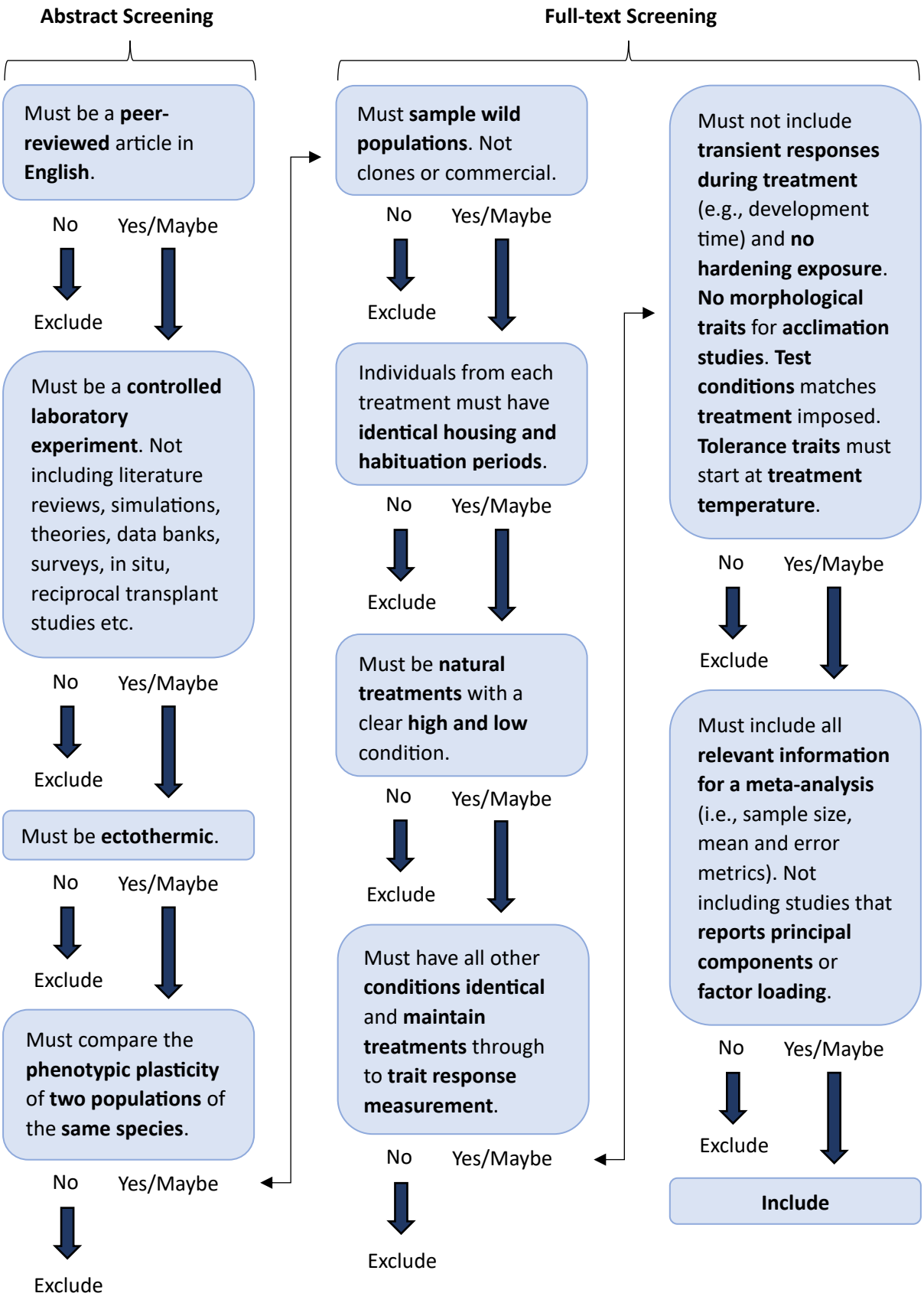


**Figure S2 Decision tree and inclusion criteria.**

## 7.5 | List of Included Studies


Aalberg Haugen, I. M., Berger, D. and Gotthard, K. (2012). The evolution of alternative developmental pathways: Footprints of selection on life-history traits in a butterfly. *Journal of Evolutionary Biology* **25**(7), 1377-1388. doi: 10.1111/j.1420-9101.2012.02525.x

Abayarathna, T., Murray, B. R. and Webb, J. K. (2019). Higher incubation temperatures produce long-lasting upward shifts in cold tolerance, but not heat tolerance, of hatchling geckos. *Biology Open* **8**(4). doi: 10.1242/bio.042564

Bochdanovits, Z., Van Der Klis, H. and De Jong, G. (2003). Covariation of larval gene expression and adult body size in natural populations of *Drosophila melanogaster*. *Molecular Biology and Evolution* **20**(11), 1760-1766. doi: 10.1093/molbev/msg179

Brennan, R. S., Galvez, F. and Whitehead, A. (2015). Reciprocal osmotic challenges reveal mechanisms of divergence in phenotypic plasticity in the killifish *Fundulus heteroclitus*. *Journal of Experimental Biology* **218**(8), 1212-1222. doi: 10.1242/jeb.110445

Brennan, R. S., Hwang, R., Tse, M., Fangue, N. A. and Whitehead, A. (2016). Local adaptation to osmotic environment in killifish, *Fundulus heteroclitus*, is supported by divergence in swimming performance but not by differences in excess post-exercise oxygen consumption or aerobic scope. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* **196**, 11-19. doi: 10.1016/j.cbpa.2016.02.006

Bubliy, O. A. and Loeschcke, V. (2005). Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *Drosophila simulans* collected along an altitudinal gradient from a Canary island. *Biological Journal of the Linnean Society* **84**(1), 119-136. doi: 10.1111/j.1095-8312.2005.00419.x

Castañeda, L. E., Balanyà, J., Rezende, E. L. and Santos, M. (2013). Vanishing chromosomal inversion clines in *Drosophila subobscura* from Chile: Is behavioral thermoregulation to blame? *The American Naturalist* **182**(2), 249-259. doi: 10.1086/671057

Chopelet, J., Blier, P. U. and Dufresne, F. (2008). Plasticity of growth rate and metabolism in *Daphnia magna* populations from different thermal habitats. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* **309A**(9), 553-562. doi: 10.1002/jez.488

Christensen, E. A. F., Grosell, M. and Steffensen, J. F. (2019). Maximum salinity tolerance and osmoregulatory capabilities of European perch *Perca fluviatilis* populations originating from different salinity habitats. *Conservation Physiology* **7**(1), coz004. doi: 10.1093/conphys/coz004

Collin, R. and Salazar, M. Z. (2010). Temperature-mediated plasticity and genetic differentiation in egg size and hatching size among populations of *Crepidula* (Gastropoda: Calyptraeidae). *Biological Journal of the Linnean Society* **99**(3), 489-499. doi: 10.1111/j.1095-8312.2009.01388.x

Cooper, B. S., Hammad, L. A. and Montooth, K. L. (2014). Thermal adaptation of cellular membranes in natural populations of *Drosophila melanogaster*. *Functional Ecology* **28**(4), 886-894. doi: 10.1111/1365-2435.12264

Dalesman, S. and Rundle, S. D. (2010). Influence of rearing and experimental temperatures on predator avoidance behaviour in a freshwater pulmonate snail. *Freshwater Biology* **55**(10), 2107-2113. doi: 10.1111/j.1365-2427.2010.02470.x

Delpuech, J. M., Moreteau, B., Chiche, J., Pla, E., Vouidibio, J. and David, J. R. (1995). Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster*: Ovarian size and developmental temperature. *Evolution* **49**(4), 670-675. doi: 10.1111/j.1558-5646.1995.tb02303.x

Elliott, J. M., Elliott, J. A. and Allonby, J. D. (1994). The critical thermal limits for the stone loach, *Noemacheilus barbatulus*, from three populations in north-west England. *Freshwater Biology* **32**(3), 593-601. doi: 10.1111/j.1365-2427.1994.tb01150.x

Elliott, J. M. and Elliott, J. A. (1995). The critical thermal limits for the bullhead, *Cottus gobio*, from three populations in north-west England. *Freshwater Biology* **33**(3), 411-418. doi: 10.1111/j.1365-2427.1995.tb00403.x

Fangue, N. A., Podrabsky, J. E., Crawshaw, L. I. and Schulte, P. M. (2009). Countergradient variation in temperature preference in populations of killifish *Fundulus heteroclitus*. *Physiological and Biochemical Zoology* **82**(6), 776-786. doi: 10.1086/606030

Fernando, A. V., Lochmann, S. E. and Haukenes, A. H. (2016). Critical thermal maxima of juvenile alligator gar (*Atractosteus spatula*, Lacépède, 1803) from three Mississippi-drainage populations acclimated to three temperatures. *Journal of Applied Ichthyology* **32**(4), 701-705. doi: 10.1111/jai.13047

Gibbons, T. C., Metzger, D. C. H., Healy, T. M. and Schulte, P. M. (2017). Gene expression plasticity in response to salinity acclimation in threespine stickleback ecotypes from different salinity habitats. *Molecular Ecology* **26**(10), 2711-2725. doi: 10.1111/mec.14065

Gillis, M. K. and Walsh, M. R. (2019). Individual variation in plasticity dulls transgenerational responses to stress. *Functional Ecology* **33**(10), 1993-2002. doi: 10.1111/1365-2435.13409

Howard, J. H., Wallace, R. L. and Stauffer Jr, J. R. (1983). Critical thermal maxima in populations of *Ambystoma macrodactylum* from different elevations. *Journal of Herpetology* **17**(4), 400-402. doi: 10.2307/1563592

Kearney, B. D., Byrne, P. G. and Reina, R. D. (2015). Anuran developmental plasticity loss: The cost of constant salinity stress. *Australian Journal of Zoology* **63**(5), 331-337. doi: 10.1071/ZO15017

Kelley, A. L., de Rivera, C. E. and Buckley, B. A. (2011). Intraspecific variation in thermotolerance and morphology of the invasive European green crab, *Carcinus maenas*, on the west coast of North America. *Journal of Experimental Marine Biology and Ecology* **409**(1-2), 70-78. doi: 10.1016/j.jembe.2011.08.005

Kolbe, J. J., Ehrenberger, J. C., Moniz, H. A. and Angilletta Jr, M. J. (2014). Physiological variation among invasive populations of the brown anole (*Anolis sagrei*). *Physiological and Biochemical Zoology* **87**(1), 92-104. doi: 10.1086/672157

Larsen, P. F., Nielsen, E. E., Koed, A., Thomsen, D. S., Olsvik, P. A. and Loeschcke, V. (2008). Interpopulation differences in expression of candidate genes for salinity tolerance in winter migrating anadromous brown trout (*Salmo trutta* L.). *BMC Genetics* **9**(12). doi: 10.1186/1471-2156-9-12

Lin, T. E., Chen, T. Y., Wei, H. L., Richard, R. and Huang, S. P. (2019). Low cold tolerance of the invasive lizard *Eutropis multifasciata* constrains its potential elevation distribution in Taiwan. *Journal of Thermal Biology* **82**, 115-122. doi: 10.1016/j.jtherbio.2019.03.015

Lucas, P. and Hunter, F. F. (1999). Phenotypic plasticity in the labral fan of simuliid larvae (Diptera): Effect of seston load on primary-ray number. *Canadian Journal of Zoology* **77**(12), 1843-1849. doi: 10.1139/z99-173

May, C., Hillerbrand, N., Thompson, L. M., Faske, T. M., Martinez, E., Parry, D., Agosta, S. J. and Grayson, K. L. (2018). Geographic variation in larval metabolic rate between northern and southern populations of the invasive gypsy moth. *Journal of Insect Science* **18**(4). doi: 10.1093/jisesa/iey068

McDermid, J. L., Wilson, C. C., Sloan, W. N. and Shuter, B. J. (2013). Intraspecific differences in thermal biology among inland lake trout populations. *Transactions of the American Fisheries Society* **142**(3), 756-766. doi: 10.1080/00028487.2013.768548

Michimae, H. and Emura, T. (2012). Correlated evolution of phenotypic plasticity in metamorphic timing. *Journal of Evolutionary Biology* **25**(7), 1331-1339. doi: 10.1111/j.1420-9101.2012.02523.x

Miller, S. E., Metcalf, D. and Schluter, D. (2015). Intraguild predation leads to genetically based character shifts in the threespine stickleback. *Evolution* **69**(12), 3194-3203. doi: 10.1111/evo.12811

Parkash, R. and Munjal, A. K. (1999). Phenotypic variability of thoracic pigmentation in indian populations of *Drosophila melanogaster*. *Journal of Zoological Systematics and Evolutionary Research* **37**(3), 133-140. doi: 10.1111/j.1439-0469.1999.tb00975.x

Petino Zappala, M. A., Ortiz, V. E. and Fanara, J. J. (2018). Study of natural genetic variation in early fitness traits reveals decoupling between larval and pupal developmental time in *Drosophila melanogaster*. *Evolutionary Biology* **45**(4), 437-448. doi: 10.1007/s11692-018-9461-z

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Pulgar, J. M., Bozinovic, F. and Ojeda, F. P. (2007). Inter-population thermal variability and physiological response in the intertidal fish *Scartichthys viridis* (Blenniidae). *Revista Chilena de Historia Natural* **80**(4), 439-446. doi: 10.4067/S0716-078X2007000400005

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Rabus, M., Waterkeyn, A., Van Pottelbergh, N., Brendonck, L. and Laforsch, C. (2012). Interclonal variation, effectiveness and long-term implications of Triops-induced morphological defences in *Daphnia magna* Strauss. *Journal of Plankton Research* **34**(2), 152-160. doi: 10.1093/plankt/fbr092

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Simões, P., Santos, M. A., Carromeu-Santos, A., Quina, A. S., Santos, M. and Matos, M. (2020). Beneficial developmental acclimation in reproductive performance under cold but not heat stress. *Journal of Thermal Biology* **90**, 102580. doi: 10.1016/j.jtherbio.2020.102580

Stucki, D., Freitak, D. and Sundström, L. (2017). Survival and gene expression under different temperature and humidity regimes in ants. *PloS One* **12**(7), e0181137. doi: 10.1371/journal.pone.0181137

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Underwood, Z. E., Myrick, C. A. and Rogers, K. B. (2012). Effect of acclimation temperature on the upper thermal tolerance of Colorado River cutthroat trout *Oncorhynchus clarkii pleuriticus*: Thermal limits of a North American salmonid. *Journal of Fish Biology* **80**(7), 2420-2433. doi: 10.1111/j.1095-8649.2012.03287.x

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### 7.6 | Phenotypic Trait Measurements Adjustments and Categorisation

**Table S2 Behavioural traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Behavioural traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Behavioural Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Crawl Out | Crawl Out | 1 | 1 | 1 |
| Thermal Preference | Thermal Preference | 3 | 3 | 22 |
| | **Category Totals** | **4** | **4** | **23** |

**Table S3 Biochemical traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Biochemical Assay traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Biochemical Assay | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Axial Muscle Alanine | Axial Muscle Alanine | 1 | 1 | 6 |
| Axial Muscle Asparagine | Axial Muscle Asparagine | 1 | 1 | 6 |
| Axial Muscle Glutamine | Axial Muscle Glutamine | 1 | 1 | 6 |
| Axial Muscle Glycine | Axial Muscle Glycine | 1 | 1 | 6 |
| Axial Muscle Proline | Axial Muscle Proline | 1 | 1 | 6 |
| Axial Muscle Taurine | Axial Muscle Taurine | 1 | 1 | 6 |
| Fructose Content | Fructose Content | 1 | 1 | 1 |
| Glucose Content | Glucose Content | 1 | 1 | 1 |
| Glycerol Content | Glycerol Content | 1 | 1 | 1 |
| Lipids | Lipids | 1 | 1 | 2 |
| Liver Muscle Alanine | Liver Muscle Alanine | 1 | 1 | 6 |
| Liver Muscle Asparagine | Liver Muscle Asparagine | 1 | 1 | 6 |
| Liver Muscle Glutamine | Liver Muscle Glutamine | 1 | 1 | 6 |
| Liver Muscle Glycine | Liver Muscle Glycine | 1 | 1 | 6 |
| Liver Muscle Proline | Liver Muscle Proline | 1 | 1 | 6 |
| Liver Taurine | Liver Taurine | 1 | 1 | 6 |
| Liver Water Content | Liver Water Content | 1 | 1 | 1 |
| Mannitol Content | Mannitol Content | 1 | 1 | 1 |
| Muscle Water Content | Muscle Water Content | 1 | 1 | 1 |

| | | | | |
|---|---|---|---|---|
| Myo-inositol Content | Myo-inositol Content | 1 | 1 | 1 |
| NKA | NKA | 2 | 2 | 9 |
| PE/PC | PE/PC | 1 | 1 | 3 |
| Pectoral Fin Muscle Alanine | Pectoral Fin Muscle Alanine | 1 | 1 | 6 |
| Pectoral Fin Muscle Asparagine | Pectoral Fin Muscle Asparagine | 1 | 1 | 6 |
| Pectoral Fin Muscle Glutamine | Pectoral Fin Muscle Glutamine | 1 | 1 | 6 |
| Pectoral Fin Muscle Glycine | Pectoral Fin Muscle Glycine | 1 | 1 | 6 |
| Pectoral Fin Muscle Proline | Pectoral Fin Muscle Proline | 1 | 1 | 6 |
| Pectoral Fin Muscle Taurine | Pectoral Fin Muscle Taurine | 1 | 1 | 6 |
| Plasma Chloride Concentration | Plasma Chloride Concentration | 1 | 1 | 1 |
| Plasma Osmolality | Plasma Osmolality | 2 | 2 | 4 |
| Plasma Sodium Concentration | Plasma Sodium Concentration | 1 | 1 | 1 |
| Polyunsaturated GPLs | Polyunsaturated GPLs | 1 | 1 | 3 |
| Sorbitol Content | Sorbitol Content | 1 | 1 | 1 |
| Trehalose Content | Trehalose Content | 1 | 1 | 1 |
| | **Category Totals** | **8** | **8** | **140** |

**Table S4 Gene expression traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Gene Expression traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Gene Expression Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Arylphorin | Arylphorin | 1 | 1 | 2 |
| atp1a1a.5 | atp1a1a.5 | 2 | 2 | 2 |
| hsp70 | hsp70 | 1 | 1 | 1 |
| HSP75 | HSP75 | 1 | 1 | 2 |
| IR3 | IR3 | 1 | 1 | 2 |
| Pro-Phenoloxidase | Pro-Phenoloxidase | 1 | 1 | 2 |
| slc9a3.2 | slc9a3.2 | 1 | 1 | 1 |
| Toll-receptor | Toll-receptor | 1 | 1 | 2 |
| trpv6 | trpv6 | 1 | 1 | 1 |
| β-1,3-glucan-binding protein | β-1,3-glucan-binding protein | 1 | 1 | 2 |
| | **Category Totals** | **3** | **3** | **17** |

**Table S5 Life-history traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Life-history traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Life-history Traits Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Brood Size | Brood Size | 1 | 1 | 1 |
| Development Time | Age at First Reproduction | 2 | 1 | 2 |
| | Pupal Development Time | 1 | 1 | 1 |
| | **Adjusted Record Totals** | **3** | **2** | **3** |
| Fecundity | Fecundity | 5 | 4 | 13 |
| First Clutch Size | First Clutch Size | 1 | 1 | 1 |
| Individual Growth Rate | Growth Rate | 1 | 1 | 1 |
| Interbrood Interval | Interbrood Interval | 1 | 1 | 1 |
| Interclutch Interval | Interclutch Interval | 1 | 1 | 2 |
| Superfetation | Superfetation | 1 | 1 | 1 |
| | **Category Totals** | **9** | **8** | **23** |

**Table S6 Morphological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Morphological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Morphological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Aperture Length | Aperture Length | 1 | 1 | 2 |
| Aperture Width | Aperture Width | 1 | 1 | 2 |
| Defense | Defense | 1 | 1 | 6 |
| Egg Diameter | Diameter | 1 | 2 | 2 |
| First Dorsal Spine Length | First Dorsal Spine length | 1 | 1 | 28 |
| Gape Width | Gape Width | 1 | 1 | 28 |
| Head-fan Rays | Head-fan Rays | 1 | 1 | 3 |
| Height | Height | 1 | 1 | 1 |
| Lateral Plate Number | Lateral Plate Number | 1 | 1 | 28 |
| Length | Body Length | 1 | 1 | 1 |
| | Length at Maturity | 1 | 1 | 2 |
| | Size at First Paturition | 1 | 1 | 1 |
| | Size at Maturation | 2 | 2 | 8 |
| | Size at Primipara | 1 | 1 | 1 |
| | Snout Vent Length | 2 | 2 | 4 |
| | Standard Length | 2 | 3 | 30 |
| | Total Length | 1 | 1 | 36 |
| | **Adjusted Record Totals** | **11** | **12** | **83** |
| Lorica Length | Lorica Length | 1 | 1 | 1 |

| | | | | |
|---|---|---|---|---|
| Lorica Width | Lorica Width | 1 | 1 | 1 |
| Ovariole | Ovariole | 1 | 1 | 3 |
| Pelvic Girdle Length | Pelvic Girdle Length | 1 | 1 | 28 |
| Pelvic Length | Pelvic length | 1 | 1 | 28 |
| Posterior Spine Length | Posterior Spine Length | 1 | 1 | 1 |
| Second Dorsal Spine Length | Second Dorsal Spine length | 1 | 1 | 28 |
| Shell Length | Shell Length | 1 | 1 | 2 |
| Shell Width | Shell Width | 1 | 1 | 2 |
| Tail Length | Tail Length | 1 | 1 | 1 |
| Thorax Length | Thorax Length | 1 | 2 | 20 |
| Trident Pigmentation | Trident Pigmentation | 1 | 1 | 182 |
| Weight | Weight | 6 | 5 | 48 |
| | Weight at Metamorphosis | 2 | 2 | 9 |
| | **Adjusted Record Totals** | **8** | **7** | **57** |
| Wing Centroid Size | Wing Centroid Size | 1 | 1 | 1 |
| Wing Length | Wing Length | 2 | 3 | 23 |
| Wing Width | Wing Width | 1 | 2 | 20 |
| | **Category Totals** | **24** | **22** | **581** |

**Table S7 Physiological traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Physiological traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Physiological Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Aerobic Scope | Aerobic Scope | 1 | 1 | 1 |
| Citrate Synthase | Citrate Synthase | 1 | 1 | 6 |
| Creatine Kinase | Creatine Kinase | 1 | 1 | 6 |
| Distance to Exhaustion | Distance to Exhaustion | 1 | 1 | 1 |
| EPOC | EPOC | 1 | 1 | 1 |
| Metabolic Rate | Metabolic Rate | 2 | 2 | 4 |
| Time to Exhaustion | Time to Exhaustion | 1 | 1 | 1 |
| | **Category Totals** | **4** | **4** | **20** |

**Table S8 Tolerance traits.** The consistency adjustments (if any) for the phenotypic trait measurements that were categorised as Tolerance traits. These records were used in all MLMAs and meta-regressions as either a part of the trait random effect, or the phenotypic trait category moderator. Number of studies, species and effects sizes are total from the overall data set.

| Tolerance Traits Category | | | | |
|---|---|---|---|---|
| **Adjusted Records** | **Original Records** | **Studies** | **Species** | **Effect Sizes** |
| Chill Coma Recovery Time | Chill Coma Recovery Time | 1 | 1 | 28 |
| Critical Thermal Maximum | Critical Thermal Maximum | 7 | 7 | 15 |
| Critical Thermal Minimum | Critical Thermal Minimum | 4 | 4 | 8 |
| Heat Knockdown | Heat Knockdown | 1 | 1 | 28 |
| | **Category Totals** | **10** | **10** | **79** |

### 7.7 | Codebook and Metadata

**Table S9 Codebook and Metadata for the Raw Data Set.** Raw data descriptors for the meta-analysis.

| Column Name | Description |
|---|---|
| Effect_Size_ID | Unique Identifiers for individual effect sizes. |
| Study_ID | Unique identifiers for each paper. |
| Species_ID | Within study identifier for each species. |
| Study_Pop_ID_(1) | Within study identifier for the first population compared in this effect size. |
| Study_Pop_ID_(2) | Within study identifier for the second population compared in this effect size. |
| Treatment_ID | Within study identifier for the treatment imposed. |
| Trait_ID | Within study identifier for the phenotypic trait measured. |
| First_Author | Initials and surname for the first author of the study. |
| Title | Title of the study. |
| Publication_Year | Year of publication. |
| Publication_Year_Z | Z-transformed year of publication. |
| Journal | Name of the journal the study was published. |
| Impact_Factor | Journal impact factor as of 2020 (most recent records at time of data collection). |
| Kingdom | Kingdom of each species that a study investigates. |
| Phylum | Phylum for each species that a study investigates. |
| Class | Class for each species that a study investigates. |
| Order | Order for each species that a study investigates. |
| Family | Family for each species that a study investigates. |
| Scientific_Name | Binomial nomenclature for species. |
| Ecosystem | Ecosystem the species is naturally observed (Aquatic and Terrestrial). Amphibians considered aquatic. |
| First_Population | Name that the first population is referred to in the study. |
| Second_Population | Name that the second population is referred to in the study. |
| Plasticity_Category | Mechanism of plasticity the treatments were exposed to to (Acclimation, Developmental Plasticity, or Transgenerational Effects). |

| | |
|---|---|
| Type | Type of treatment that replicates the natural environment (e.g., temperature and salinity). |
| T1 | Details of the first treatment imposed (temperature, salinity, high/low, present/absent). |
| T1_Number | Separation of the temperature and salinity details for subset analyses. |
| T2 | Details of the second treatment imposed (temperature, salinity, high/low, present/absent). |
| T2_Number | Separation of the temperature and salinity details for subset analyses. |
| Treatment_Unit | Original unit of the treatment imposed prior to categorisation to high/low or present/absent). |
| high | Identifier for the treatment that involved the higher exposure (higher temperature or salinity, high, or present), eight T1 or T2. |
| Category | Categorisation of trait measurements. |
| Measurement | Phenotypic traits measured following treatment exposure. |
| Trait_Unit | Units for measurements. |
| Total_Shared_Control | Count of how many times the treatments within an effect size were shared across multiple effects. |
| Shared_Animal_Number | Unique identifier for effect sizes that measured phenotypic traits in the same animals. |
| n_(P1T1) | Sample size for the first treatment in the first population. |
| Mean_(P1T1) | Mean response from the first treatment in the first population. |
| SD_(P1T1) | Standard deviation from the first treatment in the first population. |
| n_(P1T2) | Sample size for the second treatment in the first population. |
| Mean_(P1T2) | Mean response from the second treatment in the first population. |
| SD_(P1T2) | Standard deviation from the second treatment in the first population. |
| n_(P2T1) | Sample size for the first treatment in the second population. |
| Mean_(P2T1) | Mean response from first treatment in the second population. |
| SD_(P2T1) | Standard deviation from the first treatment in the second population. |
| n_(P2T2) | Sample size for the second treatment in the second population. |

| | |
|---|---|
| Mean_(P2T2) | Mean response from the second treatment in the second population. |
| SD_(P2T2) | Standard deviation from the second treatment in the second population. |
| Effect_Size | Plasticity response ratio difference. Represents the difference in plastic responses between the two populations. |
| Variance | Plasticity response ratio difference sampling variance. |
| Effect_Size_Type | Plasticity response ratio difference standardised to one-degree or ppt change for temperature and salinity data, respectively. |
| Variance_Type | Standardised plasticity response ratio difference sampling variance. |
| Percentage_Transformation | Whether the recorded means were recorded as a percentage (Yes, No). |
| Proportion_Transformation | Whether the recorded means were recorded as a proportion (Yes, No). |
| n_(P1T1)_Adjusted | Sample size for the first treatment in the first population, adjusted for the Total Shared Control. |
| Mean_(P1T1)_Adjusted | Mean response from the first treatment in the first population, with percentage and proportion data transformed. |
| SD_(P1T1)_Adjusted | Standard deviation from the first treatment in the first population, with percentage and proportion data transformed. |
| n_(P1T2)_Adjusted | Sample size for the second treatment in the first population, adjusted for the Total Shared Control. |
| Mean_(P1T2)_Adjusted | Mean response from the second treatment in the first population, with percentage and proportion data transformed. |
| SD_(P1T2)_Adjusted | Standard deviation from the second treatment in the first population, with percentage and proportion data transformed. |
| n_(P2T1)_Adjusted | Sample size for the first treatment in the second population, adjusted for the Total Shared Control. |
| Mean_(P2T1)_Adjusted | Mean response from the first treatment in the second population, with percentage and proportion data transformed. |
| SD_(P2T1)_Adjusted | Standard deviation from the first treatment in the second population, with percentage and proportion data transformed. |

| | |
|---|---|
| n_(P2T2)_Adjusted | Sample size for the second treatment in the second population, adjusted for the Total Shared Control. |
| Mean_(P2T2)_Adjusted | Mean response from the second treatment in the second population, with percentage and proportion data transformed. |
| SD_(P2T2)_Adjusted | Standard deviation from the second treatment in the second population, with percentage and proportion data transformed. |
| Effect_Size_Adjusted | Plasticity response ratio difference, calculated with adjusted data. Represents the difference in plastic responses between the two populations. |
| Variance_Adjusted | Plasticity response ratio difference sampling variance, calculated with adjusted data. |
| Precision | Z-transformed inverse of Effect Size Adjusted. |
| Effect_Size_Type_Adjusted | Plasticity response ratio difference standardised to one-degree or ppt change for temperature and salinity data, respectively. Calculated with adjusted data. |
| Variance_Type_Adjusted | Standardised plasticity response ratio difference sampling variance, calculated with adjusted data. |
| Precision_Type | Z-transformed inverse of Effect Size Type Adjusted. |

### 7.8 | MCMC Chain Diagnostics

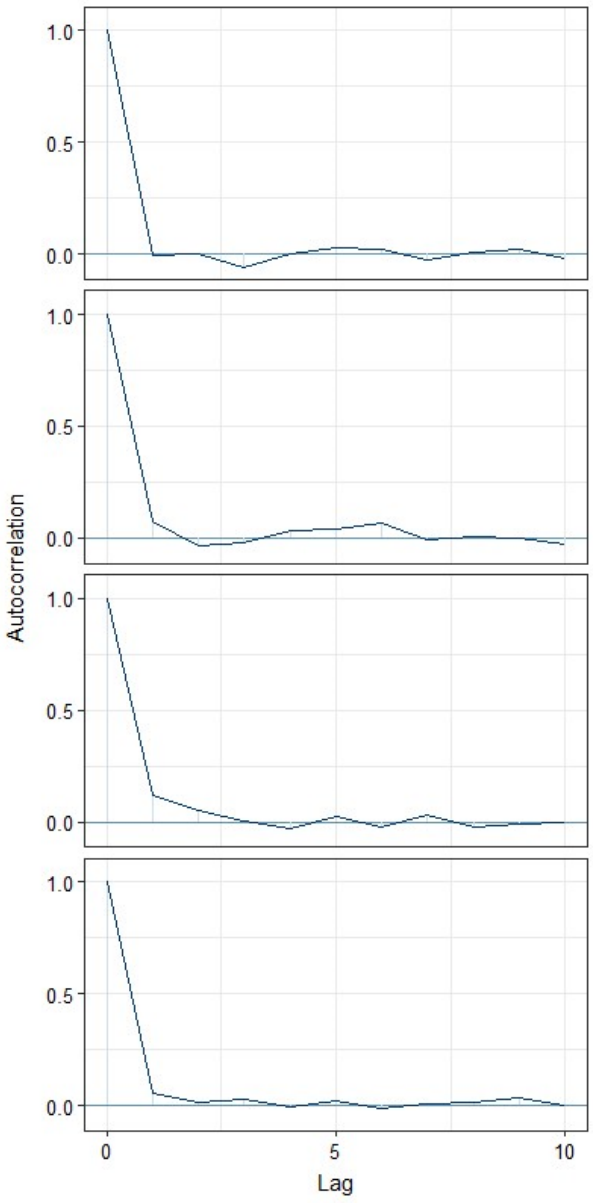


**Figure S3 Overall MCMC Chain Diagnostics.** Graph showing the autocorrelation of the MCMC chains for the overall data model.

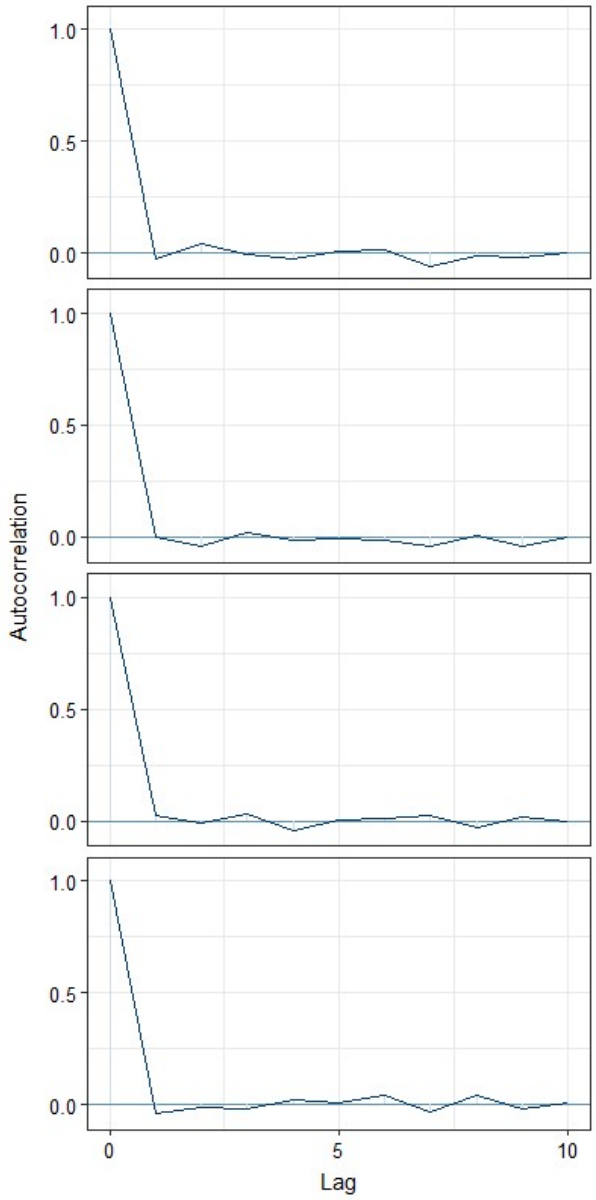


**Figure S4 Temperature MCMC Chain Diagnostics.** Graph showing the autocorrelation of the MCMC chains for the model fitted with PRRDs standardised for differences in temperature as the effect size.

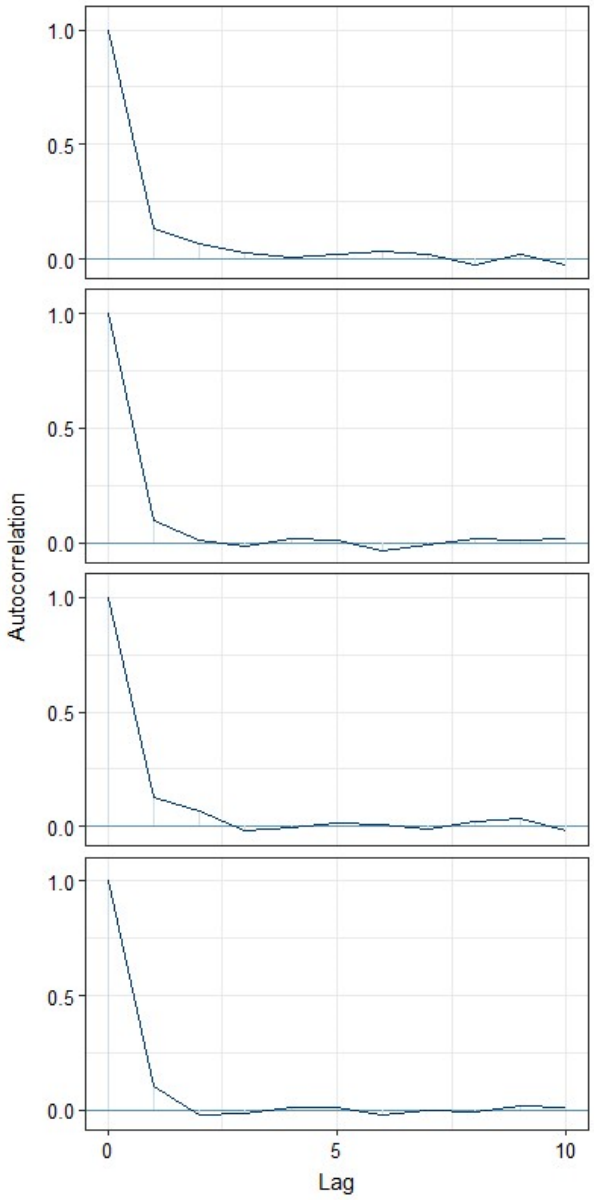


**Figure S5 Salinity MCMC Chain Diagnostics.** Graph showing the autocorrelation of the MCMC chains for the model fitted with PRRDs standardised for differences in salinity as the effect size.

## 7.9 | Phylogenetic Tree

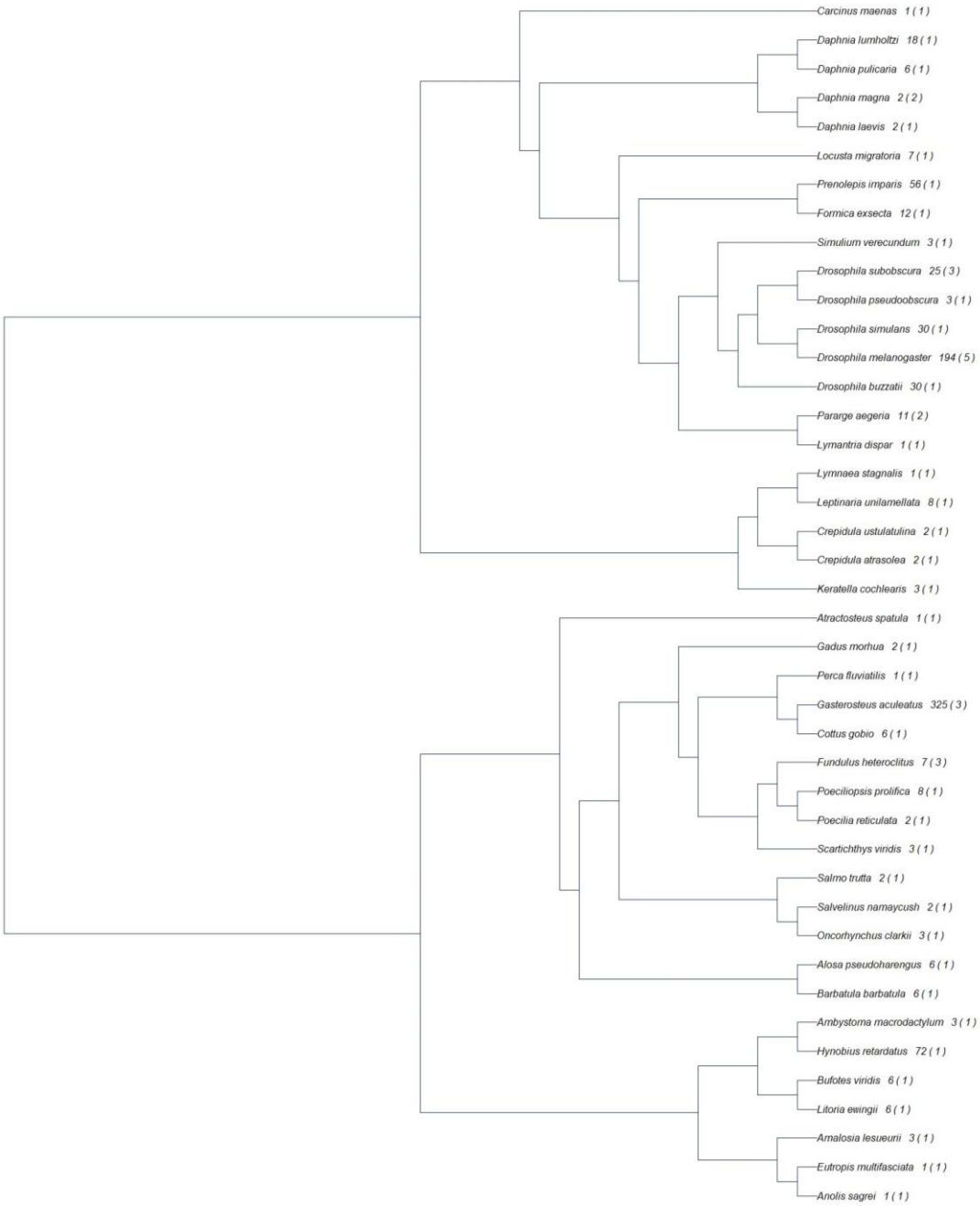


**Figure S6 Phylogenetic tree of species present in the meta-analysis.** Node labels are scientific names followed by the number of effect sizes with the number of studies in brackets.

### 7.10 | AICc Results

**Table S10 A comparison of the overall AICc values and heterogeneity across different random effect structures.** MLMA models fitted with PRRD effect sizes. $I^2$ values reported as percentages. Bold AICc = lowest value retained and therefore, the random effect structure chosen for the analyses.

| Random Effect Structure (Overall Data) | AICc | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|---|
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = n/a | 112.1657 | 2.30 | 44.12 | 0.00 | 0.00 | 8.41 | 26.86 | 81.69 |
| *Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal* | 110.5124 | n/a | 46.51 | 0.00 | 0.00 | 9.57 | 25.47 | 81.55 |
| *Shared Animal; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Trait* | 112.9825 | 1.17 | 62.93 | 0.00 | 0.00 | 13.35 | n/a | 77.44 |
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Species* | 110.1336 | 2.30 | 44.12 | 0.00 | n/a | 8.41 | 26.86 | 81.69 |
| *Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal; Trait* | 111.0102 | n/a | 63.64 | 0.00 | 0.00 | 13.87 | n/a | 77.51 |
| *Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Shared Animal; Species* | **108.4849** | n/a | 46.51 | 0.00 | n/a | 9.57 | 25.47 | 81.55 |
| *Shared Animal; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Trait; Species* | 110.9550 | 1.17 | 62.93 | 0.00 | n/a | 13.35 | n/a | 77.44 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Obs; Phylogenetic Relatedness; Study ID* **Removed =** *Shared Animal; Trait; Species* | 108.9874 | n/a | 63.64 | 0.00 | n/a | 13.87 | n/a | 77.51 |

**Table S11 A comparison of the temperature AICc values and heterogeneity across different random effect structures**. MLMA models fitted with PRRDs standardised for differences in temperature as the effect sizes. $I^2$ values reported as percentages. Bold AICc = lowest value retained and therefore, the random effect structure chosen for the analyses.

| Random Effect Structure (Temperature Data) | AICc | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|---|
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = n/a | 175.7246 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal* | 173.6629 | n/a | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Shared Animal; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Trait* | 173.6629 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | n/a | 0.00 |
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Species* | 173.6629 | 0.00 | 0.00 | 0.00 | n/a | 0.00 | 0.00 | 0.00 |
| *Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal; Trait* | 171.6102 | n/a | 0.00 | 0.00 | 0.00 | 0.00 | n/a | 0.00 |
| *Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Shared Animal; Species* | 171.6102 | n/a | 0.00 | 0.00 | n/a | 0.00 | 0.00 | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Shared Animal; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed =** *Trait; Species* | 171.6102 | 0.00 | 0.00 | 0.00 | n/a | 0.00 | n/a | 0.00 |
| *Obs; Phylogenetic Relatedness; Study ID*<br>**Removed =** *Shared Animal; Trait; Species* | **169.5665** | n/a | 0.00 | 0.00 | n/a | 0.00 | n/a | 0.00 |

**Table S12 A comparison of the salinity AICc values and heterogeneity across different random effect structures**. MLMA models fitted with PRRDs standardised for differences in salinity as the effect sizes. $I^2$ values reported as percentages. Bold AICc = lowest value retained and therefore, the random effect structure chosen for the analyses.

| Random Effect Structure (Salinity Data) | AICc | $I^2_{Animal}$ | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Species}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|---|---|
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = n/a | -1.2794 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Trait; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal* | -3.6423 | n/a | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Shared Animal; Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Trait* | -3.6423 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | n/a | 0.00 |
| *Shared Animal; Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Species* | -3.6423 | 0.00 | 0.00 | 0.00 | n/a | 0.00 | 0.00 | 0.00 |
| *Obs; Phylogenetic Relatedness; Species; Study ID*<br>**Removed** = *Shared Animal; Trait* | -5.9477 | n/a | 0.00 | 0.00 | 0.00 | 0.00 | n/a | 0.00 |
| *Trait; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Shared Animal; Species* | -5.9477 | n/a | 0.00 | 0.00 | n/a | 0.00 | 0.00 | 0.00 |
| *Shared Animal; Obs; Phylogenetic Relatedness; Study ID*<br>**Removed** = *Trait; Species* | -5.9477 | 0.00 | 0.00 | 0.00 | n/a | 0.00 | n/a | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Obs; Phylogenetic Relatedness; Study ID* **Removed =** *Shared Animal; Trait; Species* | **-8.1974** | n/a | 0.00 | 0.00 | n/a | 0.00 | n/a | 0.00 |

### 7.11 | Publication Bias and Sensitivity Analysis

*PRRD*

There was no indication of the presence of asymmetry in the funnel plot of the meta-analytic residuals of the overall Bayesian model (Figure S7). A frequentist model of the overall data fitted with z-transformed publication year (effect size estimate = -0.0039; 95% CIs = [-0.0250, 0.0171]; p = 0.7080) and precision (effect size estimate = -0.0011; 95% CIs = [-0.0058, 0.0036]; p = 0.6465) as moderators, found no significant relationships with PRRD (absolute value; Figure S8). These results together indicate no evidence of funnel plot asymmetry or time-lag bias in our overall data set.

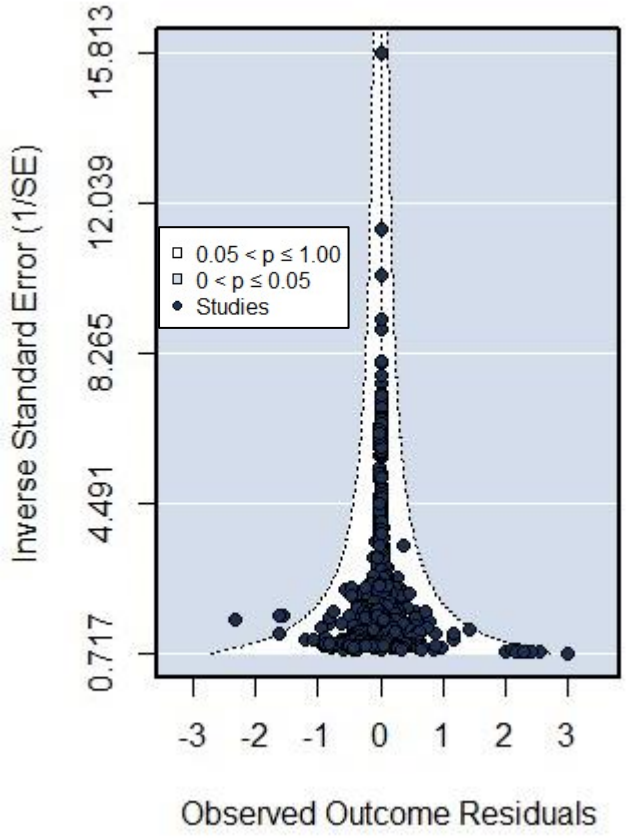


**Figure S7 Overall Funnel plot.** Showing the observed outcome residuals from the overall Bayesian model against the inverse standard error (precision). Effect Size = PRRD. Dotted lines = 95% pseudo confidence intervals.

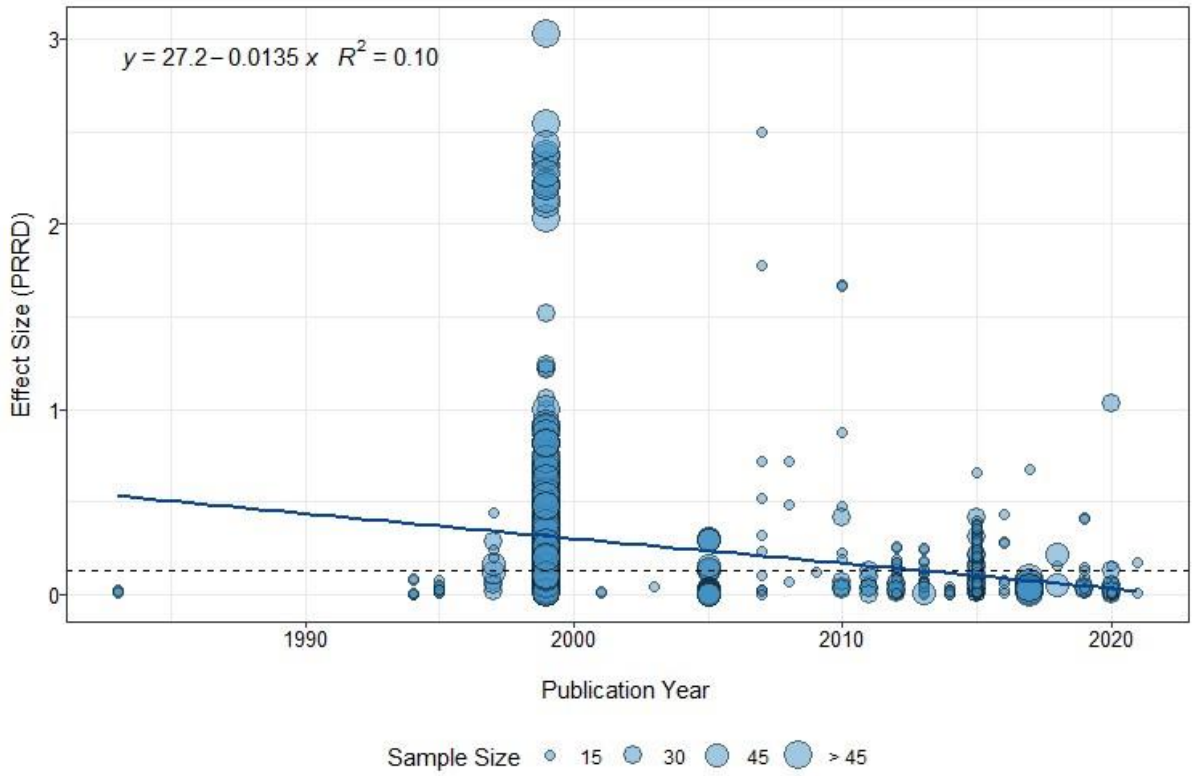


**Figure S8 The relationship between the absolute values of effect sizes (PRRD) from the overall data set, and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = mode PRRD estimate from the overall Bayesian model. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

The observed outcome of residuals from the Cook's distance analysis never exceeded 1, suggesting that there were no implausible data points identified in the overall data set. A Bayesian model fitted with untransformed effect sizes (original data prior to percentage and sample size transformations) were found to be quantitatively similar to the overall Bayesian model (Table S13). These two results combined suggest that our data is both robust and free from significantly influential outliers.

**Table S13 Overall MLMA outputs.** Comparison of the overall Bayesian model with a Bayesian model fitted with untransformed data. Untransformed data includes effect sizes calculated prior to percentage and sample size transformations. Effect size = PRRD. Estimates reported as mode. $I^2$ values reported as percentages.

| Model (Overall Data) | Estimate | CI Low | CI High | $I^2_{Total}$ |
|---|---|---|---|---|
| **MLMA (PRRD)** | 0.1295 | 0.0894 | 0.1806 | 83.55 |
| **Untransformed Data** | 0.1937 | 0.1488 | 0.2626 | 96.71 |

*PRRDs Standardised for Differences in Temperature*

The funnels plots of the meta-analytic residuals for the Bayesian model fitted with PRRDs, standardised for differences in temperature, found no indication of the presence of asymmetry (Figure S9). There were no significant relationships with PRRDs (absolute value standardised for differences in temperature; Figure S10) found in a frequentist model fitted with z-transformed publication year (effect size estimate = -0.0018; 95% CIs = [-0.0237, 0.0201]; p = 0.8700) and precision (effect size estimate = 0.0001; 95% CIs = [-0.0069, 0.0071]; p = 0.9731). These results together indicate no evidence of funnel plot asymmetry or time-lag bias in our temperature data.

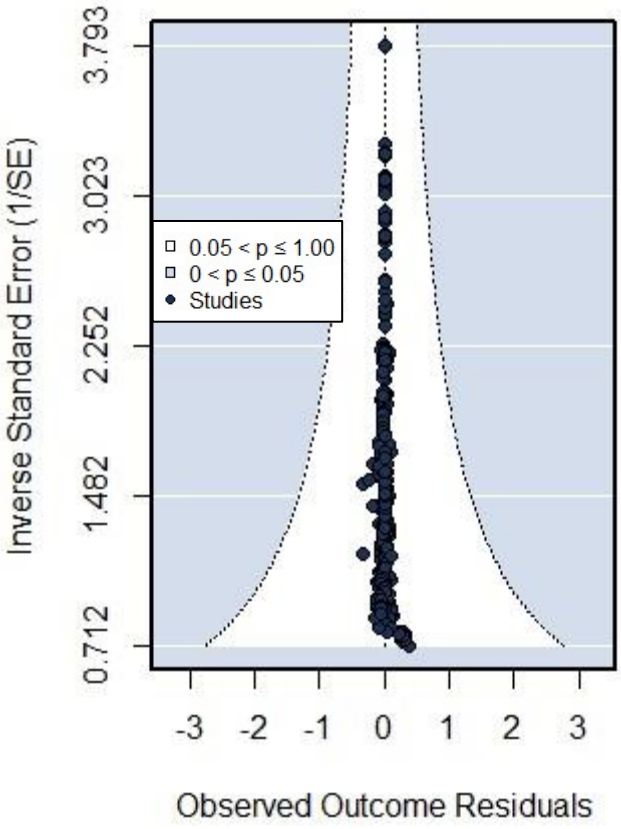


**Figure S9 Temperature Funnel plot.** Showing the observed outcome residuals from the Bayesian model fitted with PRRDs standardised for difference in temperature, against the inverse standard error (precision). Dotted lines = 95% pseudo confidence intervals.

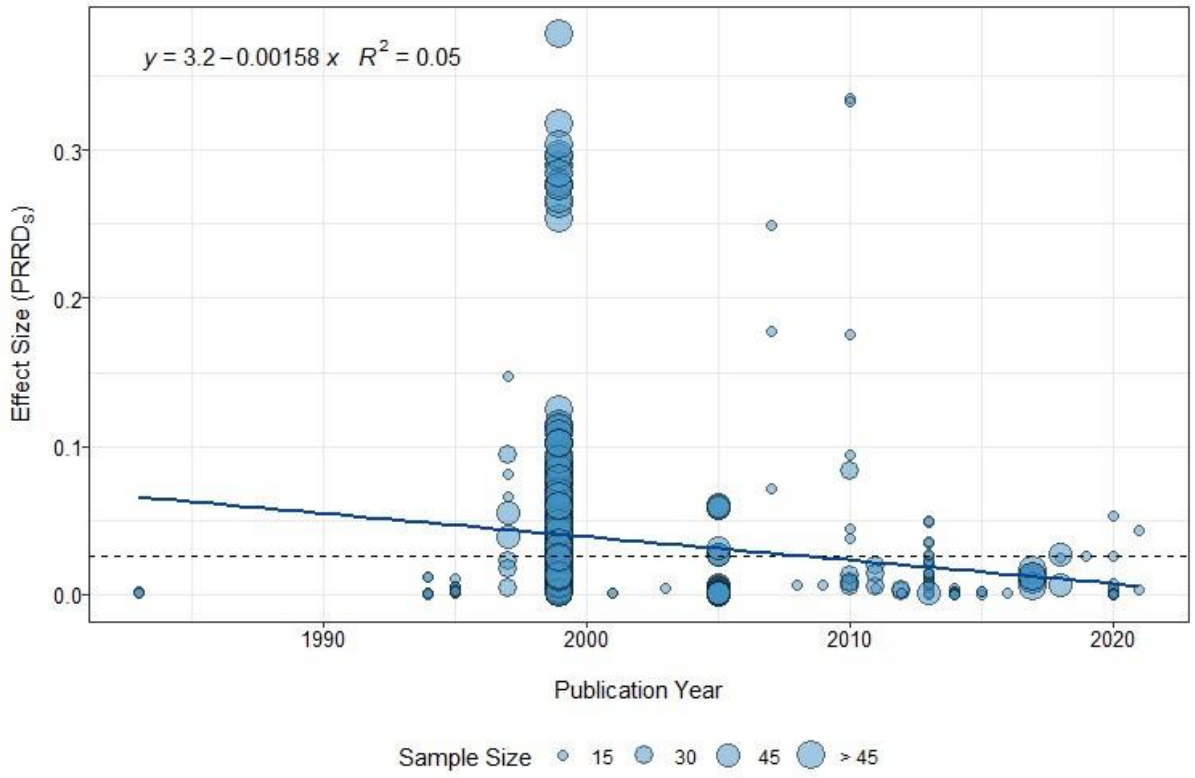


**Figure S10 The relationship between the absolute values of effect sizes (PRRDs) from the temperature data set, and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = mode estimate from the Bayesian model fitted with PRRDs standardised for difference in temperature. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

The Cook's distance analysis of the temperature data found that the observed outcome of the residuals never exceeded 1, suggesting that there were no implausible data points. Both the Bayesian model fitted with PRRDs standardised for differences in temperature, and the Bayesian model fitted with untransformed effect sizes (PRRDs standardised for difference in temperature, calculated prior to percentage and sample size transformations) were found to be quantitatively similar (Table S15). These two results combined suggest that our temperature data is both robust and free from significantly influential outliers.

**Table S14 Temperature MLMA outputs.** Comparison of the Bayesian model fitted with PRRDs standardised for differences in temperature with a Bayesian model fitted with untransformed data. Untransformed data included effect sizes (PRRDs) calculated prior to percentage and sample size transformations. Estimates reported as mode. $I^2$ values reported as percentages.

| Model (Temperature Data) | Estimate | CI Low | CI High | $I^2_{Total}$ |
|---|---|---|---|---|
| **MLMA (PRRDs)** | 0.0257 | 0.0069 | 0.0722 | 1.47 |
| **Untransformed Data** | 0.0228 | 0.0063 | 0.0617 | 1.77 |

*PRRDs Standardised for Differences in Salinity*

Visual inspections of funnel plots of the meta-analytic residuals for the Bayesian model fitted with PRRDs standardised for differences in salinity, indicated no presence of asymmetry (Figure S11). The frequentist model fitted with z-transformed publication year (effect size estimate = 0.0058; 95% CIs = [-0.0741, 0.0858]; p = 0.8640) and precision (effect size estimate = -0.0495; 95% CIs = [-0.3924, 0.2933]; p = 0.7747), found no relationship with PRRDs (absolute standardised for differences in salinity; Figure S12). These results together indicate no evidence of funnel plot asymmetry or time-lag bias in our salinity data.

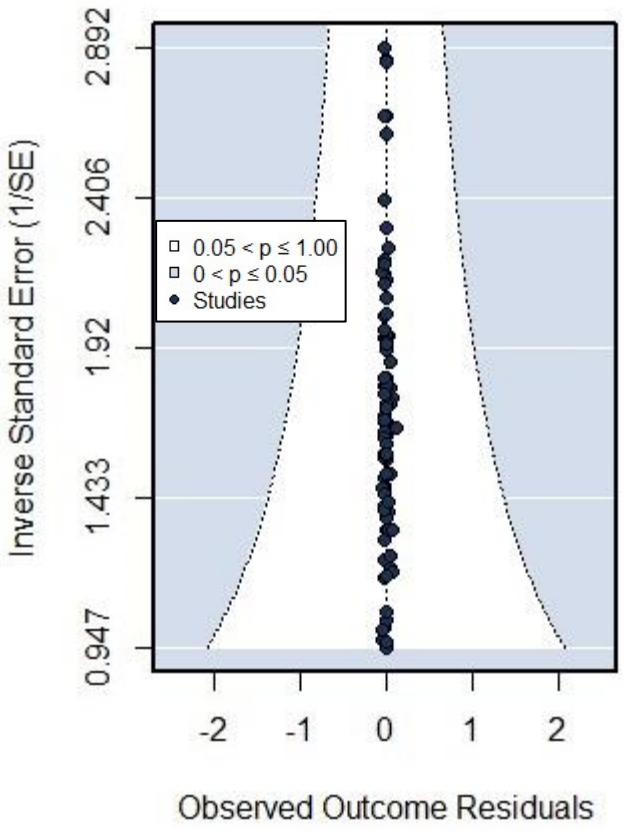


**Figure S11 Salinity Funnel plot.** Showing the observed outcome residuals from the Bayesian model fitted with PRRDs standardised for difference in salinity, against the inverse standard error (precision). Dotted lines = 95% pseudo confidence intervals.

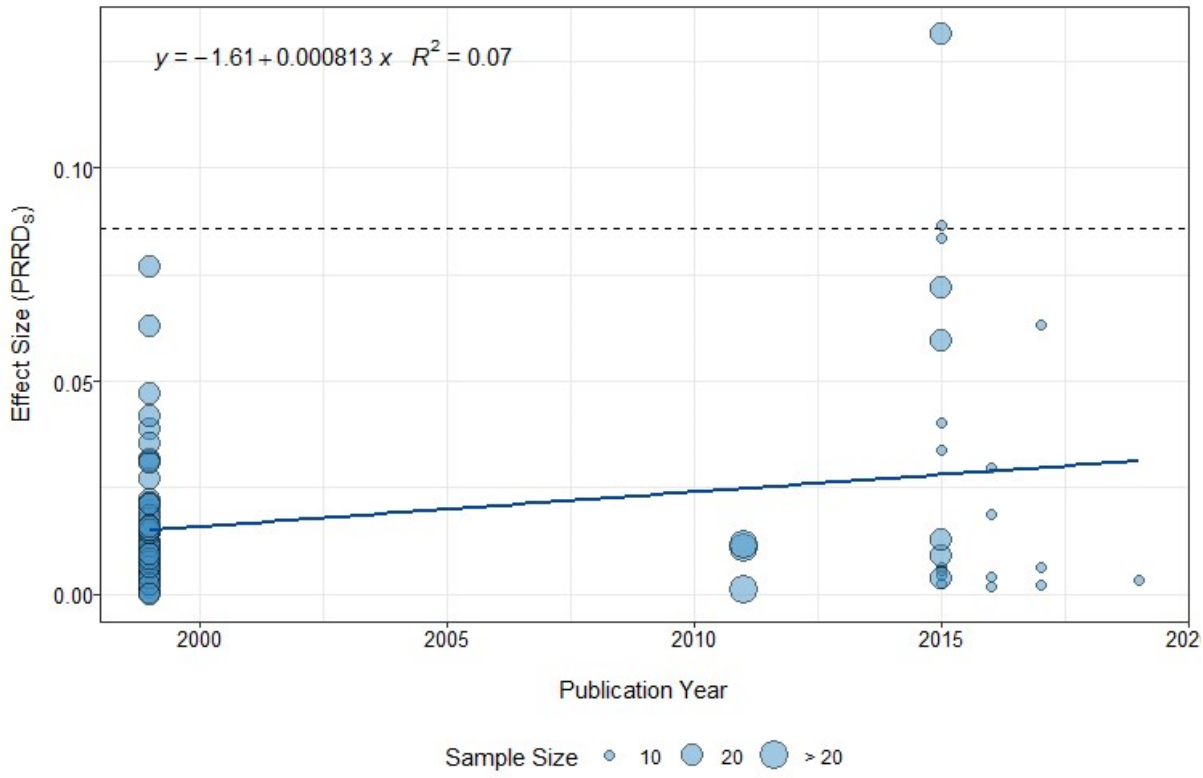


**Figure S12 The relationship between the absolute values of effect sizes (PRRDs) from the temperature data set, and the studies' year of publication to identify evidence of time-lag bias.** Dashed line = mode estimate from the Bayesian model fitted with PRRDs standardised for difference in salinity. Solid line = model prediction. Sample sizes are those used to calculate each individual effect size.

There were no implausible data points found in the Cook's distance analysis of salinity data, with the observed outcome of the residuals never exceeding 1. The comparison of the Bayesian model fitted with PRRDs standardised for difference in salinity and the Bayesian model fitted with untransformed effect sizes (PRRDs standardised for differences in salinity, calculated prior to percentage and sample size transformations), found them both to be quantitatively similar (Table S15). These two results combined suggest that our salinity data is both robust and free from significantly influential outliers.

**Table S15 Salinity MLMA outputs.** Comparison of the Bayesian model fitted with PRRDs standardised for differences in salinity with a Bayesian model fitted with untransformed data. Untransformed data included effect sizes (PRRDs) calculated prior to percentage and sample size transformations. Estimates reported as mode. $I^2$ values reported as percentages.

| Model (Salinity Data) | Estimate | CI Low | CI High | $I^2_{Total}$ |
|---|---|---|---|---|
| **MLMA (PRRDs)** | 0.0857 | 0.0144 | 0.4211 | 19.48 |
| **Untransformed Data** | 0.0903 | 0.0139 | 0.4174 | 26.42 |

### 7.12 | Raw MLMA and Meta-regression Outputs

**Table S16 Results of the overall MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Overall Data (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 52 | 42 | 883 | 0.1295 | 0.0894 | 0.1806 |

**Table S17 Results of the meta-regression of the overall data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Overall Data (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 22 | 19 | 249 | 0.0623 | 0.0395 | 0.0875 |
| **Developmental Plasticity** | 23 | 18 | 614 | 0.1159 | 0.1005 | 0.1343 |
| **Transgenerational Effects** | 9 | 9 | 20 | 0.0413 | 0.0239 | 0.0836 |

**Table S18 Results of the meta-regression of the overall data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Overall Data (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Behavioural** | 4 | 4 | 23 | 0.0701 | 0.0330 | 0.1694 |
| **Biochemical Assay** | 8 | 8 | 140 | 0.0460 | 0.0193 | 0.0888 |
| **Gene Expression** | 3 | 3 | 17 | 0.0838 | 0.0479 | 0.1646 |
| **Life-history Traits** | 9 | 8 | 23 | 0.1492 | 0.0338 | 0.2861 |
| **Morphological** | 24 | 22 | 581 | 0.1163 | 0.0990 | 0.1328 |
| **Physiological** | 4 | 4 | 20 | 0.6712 | 0.3801 | 1.1784 |
| **Tolerance** | 10 | 10 | 79 | 0.0423 | 0.0193 | 0.0926 |

**Table S19 Results of the meta-regression of the overall data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Overall Data (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Actinopteri** | 18 | 14 | 374 | 0.0387 | 0.0228 | 0.1112 |
| **Amphibia** | 4 | 4 | 87 | 0.0492 | 0.0242 | 0.1238 |
| **Branchiopoda** | 5 | 4 | 28 | 0.0910 | 0.0325 | 0.1971 |
| **Gastropoda** | 3 | 4 | 13 | 0.4451 | 0.2844 | 0.7147 |
| **Insecta** | 17 | 11 | 372 | 0.1025 | 0.0837 | 0.1454 |

**Table S20 Results of the meta-regression of the overall data set with the treatment type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Overall Data (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Diet** | 4 | 4 | 26 | 0.0761 | 0.0254 | 0.1766 |
| **Humidity** | 2 | 2 | 14 | 0.3874 | 0.2615 | 0.6006 |
| **Predator** | 5 | 5 | 210 | 0.0424 | 0.0235 | 0.0829 |
| **Salinity** | 9 | 7 | 90 | 0.1351 | 0.0670 | 0.2506 |
| **Temperature** | 32 | 28 | 465 | 0.0986 | 0.0817 | 0.1172 |
| **Water Level** | 1 | 1 | 72 | 0.0399 | 0.0168 | 0.1111 |

**Table S21 Results of the terrestrial subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Terrestrial Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 21 | 15 | 385 | 0.1698 | 0.1110 | 0.2953 |

**Table S22 Results of the meta-regression of the terrestrial data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Terrestrial Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 5 | 5 | 77 | 0.1965 | 0.1219 | 0.3557 |
| **Developmental Plasticity** | 14 | 9 | 300 | 0.1906 | 0.1454 | 0.3495 |

**Table S23 Results of the meta-regression of the terrestrial data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Terrestrial Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Behavioural** | 1 | 1 | 20 | 0.1082 | 0.0387 | 0.2533 |
| **Biochemical Assay** | 2 | 2 | 13 | 0.0721 | 0.0229 | 0.2417 |
| **Gene Expression** | 1 | 1 | 12 | 0.0913 | 0.0457 | 0.2370 |
| **Morphological** | 12 | 9 | 273 | 0.1742 | 0.1361 | 0.2600 |
| **Tolerance** | 3 | 3 | 58 | 0.0995 | 0.0424 | 0.2451 |

**Table S24 Results of the meta-regression of the terrestrial data set with the treatment type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Terrestrial Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Humidity** | 2 | 2 | 14 | 0.3639 | 0.2633 | 0.6090 |
| **Temperature** | 16 | 11 | 362 | 0.1079 | 0.0852 | 0.1973 |

**Table S25 Results of the aquatic subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Aquatic Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 31 | 27 | 498 | 0.0917 | 0.0522 | 0.1737 |

**Table S26 Results of the meta-regression of the aquatic data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Aquatic Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 17 | 14 | 172 | 0.0688 | 0.0396 | 0.1405 |
| **Developmental Plasticity** | 9 | 9 | 314 | 0.0642 | 0.0372 | 0.1249 |
| **Transgenerational Effects** | 6 | 6 | 12 | 0.0721 | 0.0370 | 0.1428 |

**Table S27 Results of the meta-regression of the aquatic data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Aquatic Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Biochemical Assay** | 6 | 6 | 127 | 0.0624 | 0.0271 | 0.1942 |
| **Life-history Traits** | 5 | 5 | 15 | 0.1845 | 0.0565 | 0.3955 |
| **Morphological** | 12 | 13 | 308 | 0.0444 | 0.0269 | 0.0793 |
| **Physiological** | 3 | 3 | 19 | 0.7458 | 0.4223 | 1.2554 |
| **Tolerance** | 7 | 7 | 21 | 0.0418 | 0.0230 | 0.0871 |

**Table S28 Results of the meta-regression of the aquatic data set with the treatment type as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRD.

| Aquatic Organisms (PRRD) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Diet** | 3 | 3 | 23 | 0.1157 | 0.0432 | 0.2488 |
| **Predatory** | 5 | 5 | 210 | 0.0582 | 0.0273 | 0.1201 |
| **Salinity** | 9 | 7 | 90 | 0.1409 | 0.0738 | 0.2445 |
| **Temperature** | 16 | 17 | 103 | 0.0545 | 0.0271 | 0.1178 |
| **Water Level** | 1 | 1 | 72 | 0.0612 | 0.0258 | 0.1601 |

**Table S29 Results of the temperature subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 32 | 28 | 465 | 0.0252 | 0.0071 | 0.0722 |

**Table S30 Results of the meta-regression of the temperature data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 15 | 15 | 161 | 0.0319 | 0.0094 | 0.0759 |
| **Developmental Plasticity** | 11 | 7 | 291 | 0.0362 | 0.0094 | 0.0791 |
| **Transgenerational Effects** | 6 | 7 | 13 | 0.0534 | 0.0106 | 0.1391 |

**Table S31 Results of the meta-regression of the temperature data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Behavioural** | 4 | 4 | 23 | 0.0520 | 0.0153 | 0.1414 |
| **Biochemical Assay** | 4 | 4 | 72 | 0.0432 | 0.0128 | 0.1021 |
| **Morphological** | 11 | 10 | 267 | 0.0340 | 0.0096 | 0.0819 |
| **Tolerance** | 9 | 9 | 78 | 0.0338 | 0.0083 | 0.0849 |

**Table S32 Results of the meta-regression of the temperature data set with taxonomic class as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Actinopteri** | 9 | 9 | 87 | 0.0401 | 0.0106 | 0.1274 |
| **Insecta** | 15 | 10 | 361 | 0.0344 | 0.0087 | 0.1214 |

**Table S33 Results of the terrestrial and temperature subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Terrestrial Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 16 | 11 | 362 | 0.0388 | 0.0112 | 0.1824 |

**Table S34 Results of the meta-regression of the terrestrial and temperature data set with the mechanism of plasticity as the moderator.**

Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Terrestrial Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 4 | 4 | 70 | 0.0796 | 0.0156 | 0.2288 |
| **Developmental Plasticity** | 10 | 6 | 288 | 0.0474 | 0.0083 | 0.2288 |

**Table S35 Results of the meta-regression of the terrestrial and temperature data set with phenotypic trait category as the moderator.**

Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Terrestrial Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Behavioural** | 1 | 1 | 20 | 0.0864 | 0.0216 | 0.3136 |
| **Biochemical Assay** | 2 | 2 | 13 | 0.0994 | 0.0202 | 0.3046 |
| **Morphological** | 8 | 6 | 257 | 0.0450 | 0.0112 | 0.2774 |
| **Tolerance** | 2 | 2 | 57 | 0.0898 | 0.0173 | 0.2916 |

**Table S36 Results of the aquatic and temperature subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 16 | 17 | 103 | 0.0443 | 0.0111 | 0.1243 |

**Table S37 Results of the meta-regression of the aquatic and temperature data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 11 | 11 | 91 | 0.0440 | 0.0115 | 0.1602 |

**Table S38 Results of the meta-regression of the aquatic and temperature data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Temperature Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Biochemical Assay** | 2 | 2 | 59 | 0.0783 | 0.0149 | 0.2017 |
| **Tolerance** | 7 | 7 | 21 | 0.0581 | 0.0142 | 0.1802 |

**Table S39 Results of the aquatic and salinity subset MLMA model.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Salinity Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **MLMA** | 8 | 6 | 88 | 0.0874 | 0.0181 | 0.4443 |

**Table S40 Results of the meta-regression of the aquatic and salinity data set with the mechanism of plasticity as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Salinity Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Acclimation** | 6 | 4 | 79 | 0.1194 | 0.0118 | 0.8724 |

**Table S41 Results of the meta-regression of the aquatic and salinity data set with phenotypic trait category as the moderator.** Number of studies, species and effect sizes are totals from the overall data set. Effect size = PRRDs.

| Aquatic Organisms in Salinity Treatments (PRRDs) | Studies | Species | Effect Sizes | Estimate | CI Low | CI High |
|---|---|---|---|---|---|---|
| **Biochemical Assay** | 4 | 4 | 66 | 0.1830 | 0.0180 | 1.8522 |

### 7.13 | Heterogeneity Results

**Table S42 Heterogeneity results for the MLMA models.** PRRD = population response ratio difference; PRRDs = standardised population response ratio difference. $I^2$ values reported as percentages.

| MLMA Models | Effect Size | $I^2_{Obs}$ | $I^2_{Phylo}$ | $I^2_{Study}$ | $I^2_{Trait}$ | $I^2_{Total}$ |
|---|---|---|---|---|---|---|
| **Overall** | **PRRD** | 43.35 | 6.24 | 10.46 | 23.49 | 83.55 |
| **Terrestrial** | **PRRD** | 44.39 | 33.35 | 4.34 | 11.11 | 93.20 |
| **Aquatic** | **PRRD** | 1.62 | 9.82 | 28.39 | 25.56 | 65.39 |
| **Temperature** | **PRRD$_S$** | 0.33 | 0.68 | 0.40 | n/a | 1.41 |
| **Terrestrial and Temperature** | **PRRD$_S$** | 0.75 | 3.45 | 1.11 | n/a | 5.30 |
| **Aquatic and Temperature** | **PRRD$_S$** | 1.55 | 3.08 | 1.86 | n/a | 6.49 |
| **Aquatic and Salinity** | **PRRD$_S$** | 2.70 | 12.78 | 4.85 | n/a | 20.33 |

## 7.14 | Graphical Representation of the MLMA and Meta-regression Results

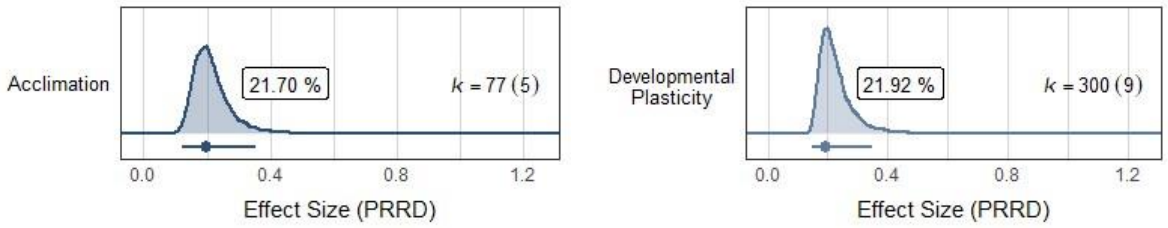


**Figure S13 Meta-regression results for the terrestrial subset with the mechanism of plasticity as the moderator.** The capacity for acclimation and developmental plasticity both differed between populations of the same species (range = 21.70% - 21.92%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

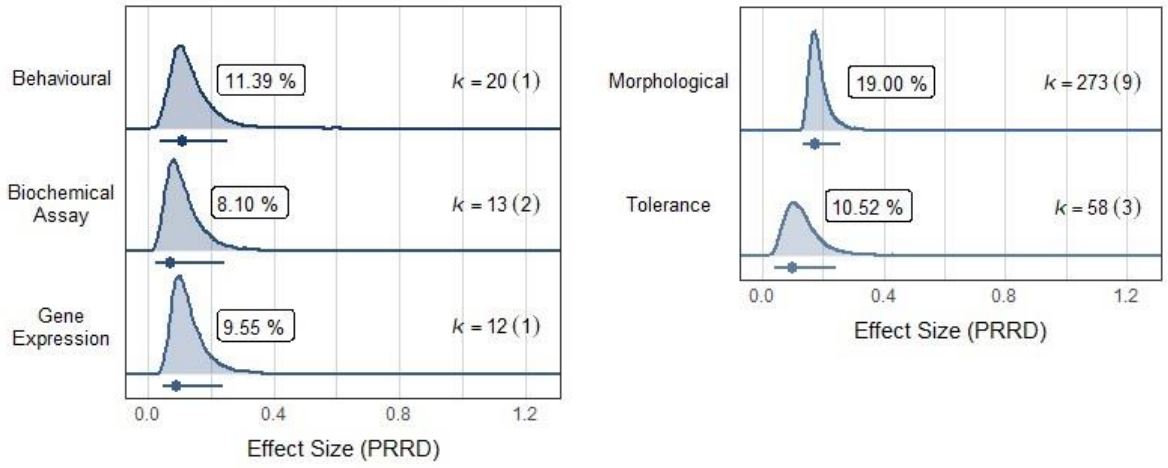


**Figure S14 Meta-regression results for the terrestrial subset with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 8.10% - 19.00%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

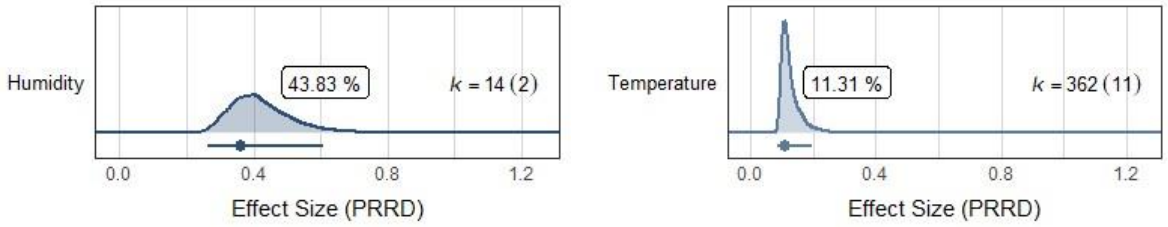


**Figure S15 Meta-regression results for the terrestrial subset with the type of treatment as the moderator.** All types of treatments showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 11.31% - 43.83%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

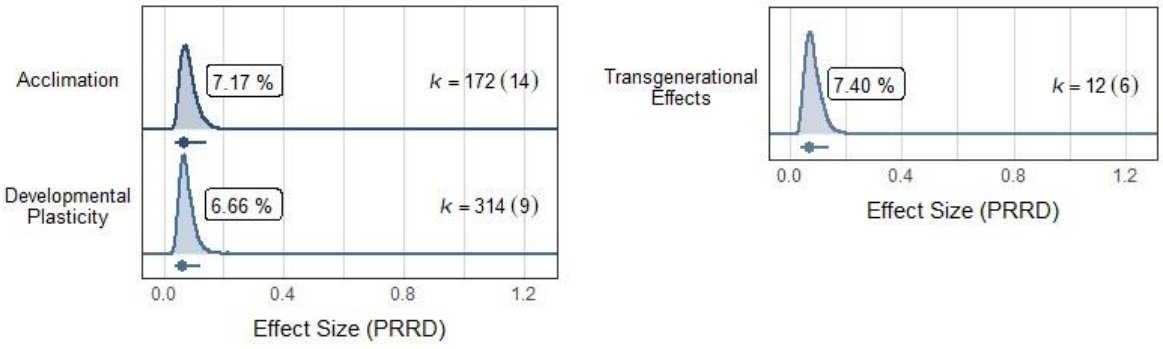


**Figure S16 Meta-regression results for the aquatic subset with the mechanism of plasticity as the moderator.** The capacity for acclimation, developmental plasticity, and transgenerational effects all differed between populations of the same species (range = 6.66% - 7.40%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

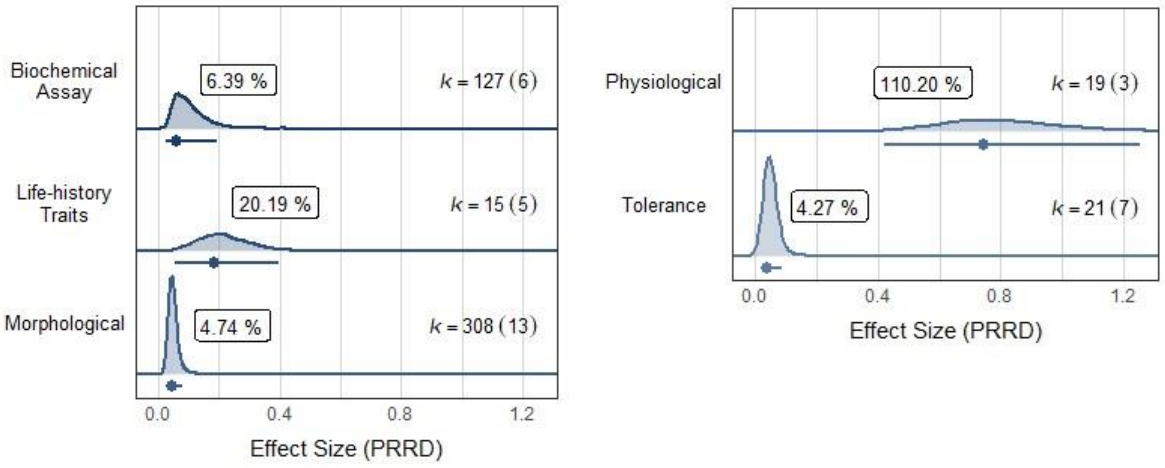


**Figure S17 Meta-regression results for the aquatic subset with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 4.27% - 110.20%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

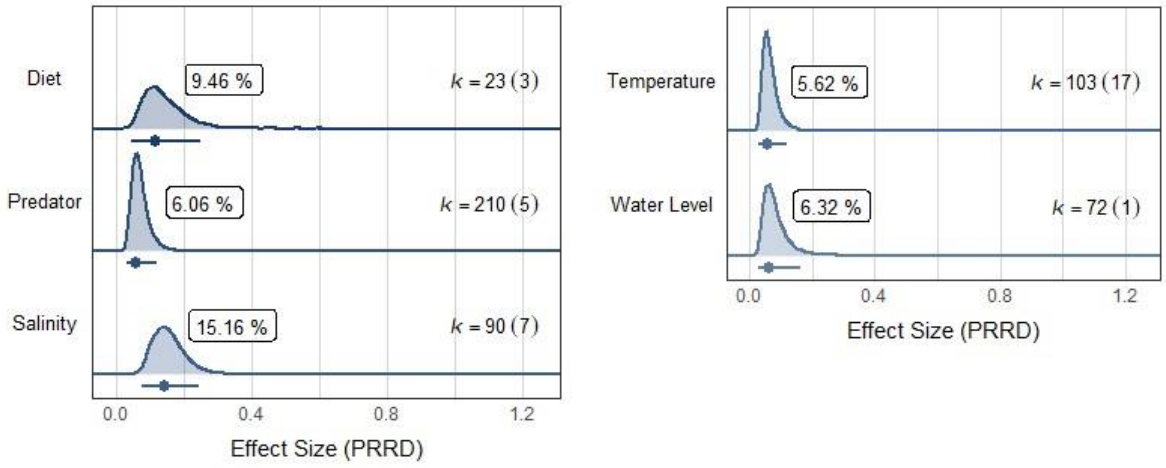


**Figure S18 Meta-regression results for the aquatic subset with the type of treatment as the moderator.** All types of treatments showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 5.62% - 15.16%). Mode effect size (folded normal plasticity response ratio difference; PRRD) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRD estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

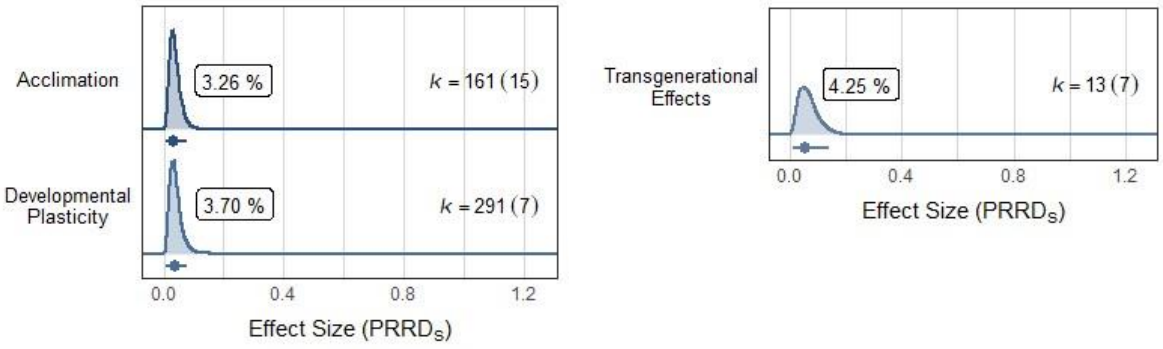


**Figure S19 Meta-regression results for the temperature subset with the mechanism of plasticity as the moderator.** The capacity for acclimation, developmental plasticity, and transgenerational effects all differed between populations of the same species (range = 3.26% - 4.25%). Mode effect size (folded normal standardised plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode $PRRD_S$ estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

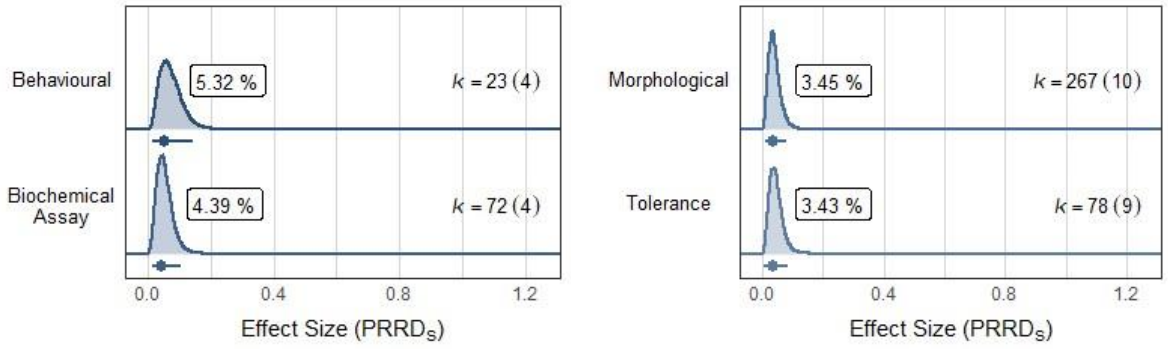


**Figure S20 Meta-regression results for the temperature subset with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 3.43% - 5.32%). Mode effect size (folded normal standardised plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode $PRRD_S$ estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

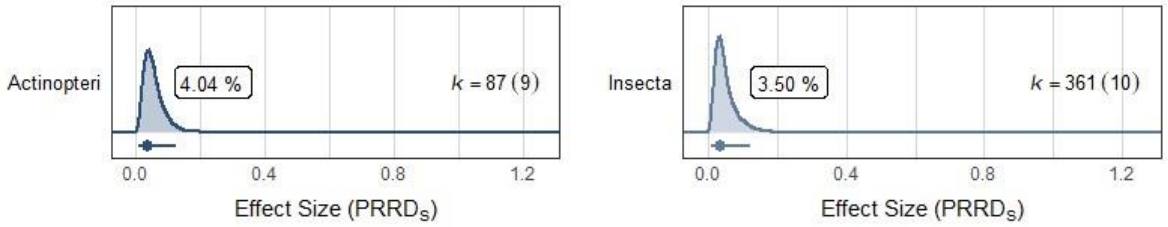


**Figure S21 Meta-regression results for the temperature subset with taxonomic class as the moderator.** All taxonomic classes showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 3.50% - 4.04%). Mode effect size (folded normal standardised plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode $PRRD_S$ estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

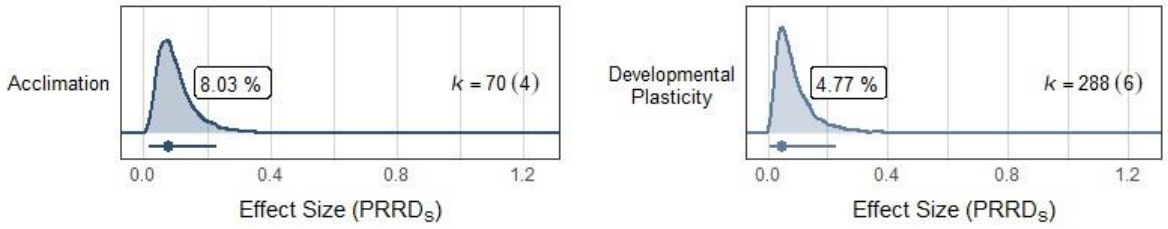


**Figure S22 Meta-regression results for the terrestrial and temperature subset with the mechanism of plasticity as the moderator.** The capacity for acclimation and developmental plasticity both differed between populations of the same species (range = 4.77% - 8.03%). Mode effect size (folded normal standardised plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode $PRRD_S$ estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

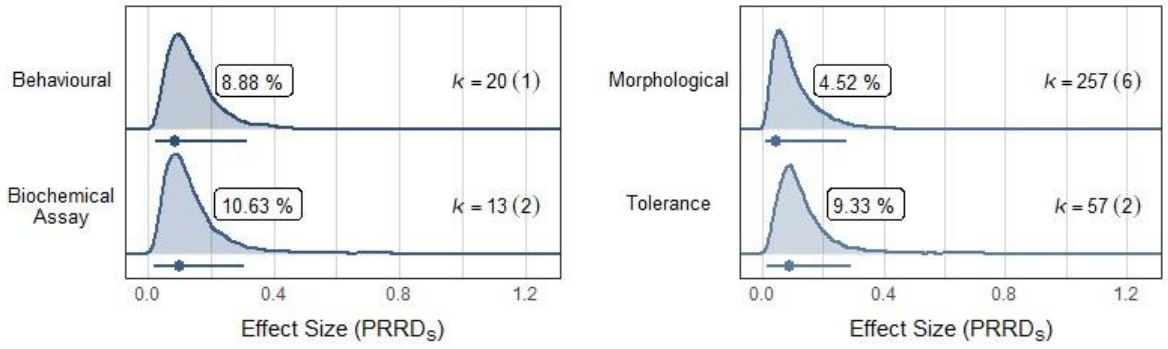


**Figure S23 Meta-regression results for the terrestrial and temperature subset with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 4.52% - 10.63%). Mode effect size (folded normal standardised plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRDs estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

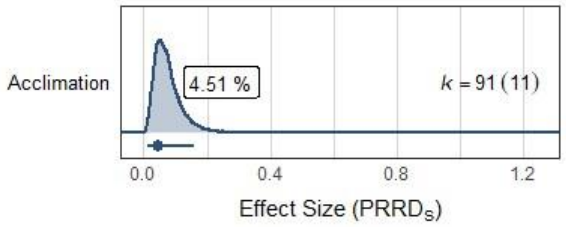


**Figure S24 Meta-regression results for the aquatic and temperature subset with the mechanism of plasticity as the moderator.** The capacity for acclimation differed between populations of the same species (4.51%). Mode effect size (folded normal standardised plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRDs estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

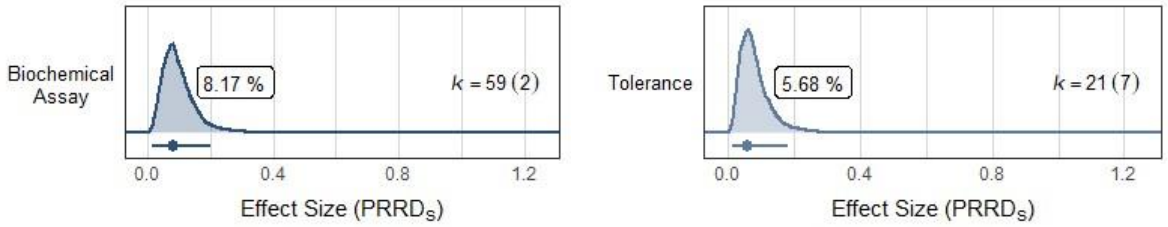


**Figure S25 Meta-regression results for the aquatic and temperature subset with phenotypic trait category as the moderator.** All phenotypic trait categories showed a difference in the capacity for phenotypic plasticity between populations of the same species (range = 5.68% - 8.17%). Mode effect size (folded normal standardised plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRDs estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

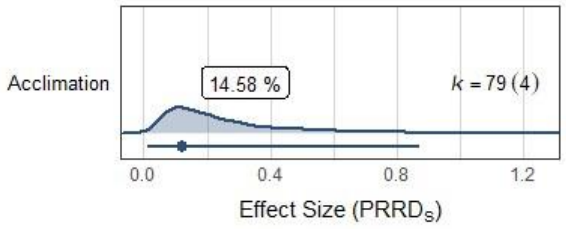


**Figure S26 Meta-regression results for the aquatic and salinity subset with the mechanism of plasticity as the moderator.** The capacity for acclimation differed between populations of the same species (14.58%). Mode effect size (folded normal standardised plasticity response ratio difference; PRRDs) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode PRRDs estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

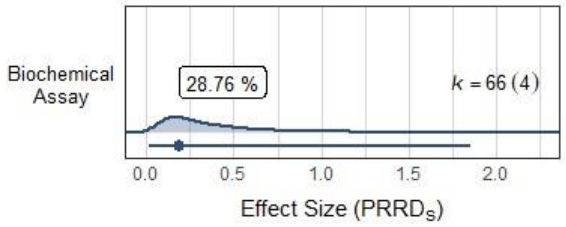


**Figure S27 Meta-regression results for the aquatic and salinity subset with phenotypic trait category as the moderator.** Biochemical assay traits showed a difference in the capacity for phenotypic plasticity between populations of the same species (28.76%). Mode effect size (folded normal standardised plasticity response ratio difference; $PRRD_S$) estimates ± 95% CIs (solid circles and horizontal bars, respectively) are shown, as well as the Bayesian posterior distributions. Percentage labels are the mode $PRRD_S$ estimates transformed to show a proportional difference between two populations. K = number of effect sizes with the number of species in brackets.

# General Discussion

Anthropogenic changes are expected to rapidly impose novel environmental signals that can impact the biological and ecological functions of ectotherms. Currently, the most critical anthropogenic drivers to the natural environment are chemical pollution, artificial light-at-night (ALAN), and climate change (Borrelle et al., 2020; IPCC, 2022; Sanders et al., 2021). In this thesis, I have experimentally demonstrated the absence of an interactive effect between chemical and light pollution on the physiological performance of zebrafish (*Danio rerio*). I have also conducted several meta-analyses that have established the current state-of-knowledge regarding the differences in phenotypic traits and their plastic responses between constant and fluctuating thermal environments, and the extent to which populations vary in their capacity for phenotypic plasticity.

Urbanisation is a primary cause for the rapid increase in chemical and light pollution experienced in nature (Falchi et al., 2016; Landrigan et al., 2023). Plastic manufacturing and pollution have released endocrine disrupting chemicals (EDCs) into the environment, the most prominent of which is bisphenol A (BPA; Hahladakis et al., 2018; Huang et al., 2017). To counteract the growing conservation and health concerns surrounding BPA, alternatives such as bisphenol S (BPS) have been increasingly utilised (Flint et al., 2012). Light pollution from artificial light sources has fundamentally changed natural photoperiods and increased the occurrence of ALAN (Kyba et al., 2017). Increased light levels can extend to remote areas through skyglow and can interfere with the natural circadian rhythms of organisms (Gaston, 2018; Russart and Nelson, 2018). Unexpectedly, photoperiods simulating ALAN (extended and continuous light regimes) and their interaction with BPS had no effect on the physiological responses of *D. rerio* (Chapter 1). Biological outcomes resulting from multiple anthropogenic drivers are not always additive and often differ unpredictably from the

individual effects (Caporale et al., 2022; Orr et al., 2020). The impact of EDCs on physiological responses has been found to be temperature dependent (Wu and Seebacher, 2021), but the findings of this thesis suggest that there is no interactive effect between BPS exposure and photoperiod (Chapter 1). Therefore, the impact that EDCs have on the phenotypic response of organisms is dependent on the environmental condition that has changed.

Exposure to BPS, on the other hand, was found to negatively impact the metabolism and swimming performance of *D. rerio* (Chapter 1) which aligns with recent studies that have questioned the utilisation of BPS in plastic manufacturing (Pelaia et al., 2023; Wu and Seebacher, 2021). The current consensus in the literature is that the physiological decline following BPS exposure could be mechanistically explained by the disruption potential of EDCs on hormone signalling pathways (Caporale et al., 2022; Toporova and Balaguer, 2020). For example, bisphenols can bind to thyroid hormone receptors and reduce or eliminate signals to inhibit the physiological performance of organisms (Gentilcore et al., 2013; Zhang et al., 2017). The negative implications of BPS exposure to the metabolic and locomotor functions of organisms can affect their ecological and evolutionary processes (Lailvaux and Husak, 2014; Norin and Metcalfe, 2019). Therefore, the findings of this thesis suggest the reconsideration of BPS use in plastic manufacturing and emphasise the need for additional alternatives to BPA.

In addition to chemical and light pollution, this thesis has synthesised our understanding of how ectotherms respond to changing climates. Current models predict that global climate change will manifest as the increase in mean temperatures, and the amplitude of thermal fluctuations (IPCC, 2021; Vasseur et al., 2014). This thesis establishes that ectotherms exhibit the same phenotypic traits (mean or variance; Chapter 2) and capacities for phenotypic plasticity (Chapter 3), following both fluctuating and constant thermal

environments. Therefore, an organism's performance, and its ability to maintain relatively constant phenotypes across environmental gradients, are not influenced by short periods of exposure outside of the thermal mean. These findings could be evidence of an advantageous mechanism that ectotherms have to reduce the costs incurred during phenotypic regulation. Continual phenotypic responses to regular, short-term thermal fluctuations, such as daily variation, could increase the occurrence of phenotype-environment mismatches and exacerbate the energetic costs to the organism (Angilletta, 2009; Chevin and Lande, 2015). Instead, the ability to filter out thermal fluctuations could resemble a 'band-pass filter' that increases the efficacy of plastic responses. A 'band-pass filter' is when regular thermal noise around the mean is filtered out and does not contribute to the capacity for phenotypic plasticity (Lattin and Kelly, 2020; Pérez et al., 2020). The effective compensation for long-term changes in mean temperatures that reduces the overall vulnerability of an organism to climate changes, could be the primary benefit of phenotypic plasticity (Leung et al., 2020).

Previous meta-analyses on the impacts of thermal fluctuations during acclimation (Slein et al., 2023) and reptilian developmental periods (Raynal et al., 2022), have demonstrated the detrimental effects of extreme variability. In this thesis, we also show that there are special cases where fluctuating temperatures do make a difference to the phenotypic outcomes of ectotherms. Importantly, increasing amplitudes of fluctuations decreased mean phenotypic trait values relative to the constant thermal environment (Chapter 2). Extreme changes to thermal regimes could frequently expose ectotherms to internal body temperatures that are close to their critical limits and explain the decrease in performance (Amarasekare and Savage, 2012; Schulte et al., 2011). As the occurrence of extreme climatic events are expected to increase due to climate change, it is important to consider that the impacts of thermal change are mediated by the intensity of the fluctuation experienced (IPCC, 2021; Kellermann et al., 2019). The negative consequences of extreme climatic events did not

extend to plastic responses, with increases to fluctuation amplitudes not impacting the capacity for phenotypic plasticity and unlikely to affect the resilience and persistence of ectotherms to future environmental change (Chapter 3).

Although plastic responses were consistent across constant and fluctuating thermal environments (Chapter 3), it is rare to completely maintain optimal performance across environmental gradients (perfect phenotypic plasticity; Seebacher et al., 2015; Warner et al., 2018; Wilson and Franklin, 2002). A quantitative synthesis in this thesis showed that there were population-level differences in the capacity for phenotypic plasticity within ectothermic species (Chapter 4). As the capacity to adjust phenotypes can be indicative of an ectotherm's resilience to environmental change (Botero et al., 2015; Kawecki, 2000), populations with diverse compensatory responses could represent a gradient of vulnerability within a species (Kamo et al., 2011). However, neither plastic or fixed phenotypes are completely beneficial across all environments and, instead, a range of plastic responses could resemble a bet-hedging scenario (Loughland and Seebacher, 2020; Seebacher and Little, 2021). A bet-hedging strategy would always render some populations at risk during environmental changes, but in return would increase the likelihood that some populations would have phenotypes that align with the novel environment (Haaland et al., 2020). In theory, this would increase the overall resilience of a species to variable environments (Schindler et al., 2015).

The capacity for phenotypic plasticity has been previously reported to differ across taxonomic lineages, habitats, and phenotypic traits (Seebacher et al., 2012; Seebacher et al., 2015). These specific scenarios were found in this thesis to also influence the extent to which populations differ in their capacity for phenotypic plasticity (Chapter 4). The disparity between populations was found to be particularly evident during developmental plasticity in populations of gastropods, following changes in humidity, and in physiological and morphological responses. The sources for variation that have been identified in this thesis,

emphasise that a universal conservation approach does not exist. Vulnerabilities to climate change and the diversification of plastic responses within a species can be dependent on the unique situations experienced at each level of organisation.

The work presented in this thesis improves our understanding of the impacts of anthropogenic changes and the factors that undermine the compensatory responses of ectotherms. Crucial insights into the temporal relationship between fluctuating temperatures and phenotypic responses (trait values and plastic responses) are provided, with the potential to inform conservation efforts. Overall, the outcomes of this thesis indicate that effective conservation policies and strategies should consider mean temperatures, and extreme events such as heatwaves (Bauerfeind and Fischer, 2014; Breitenbach et al., 2020), but daily temperature fluctuations are of lesser importance (Chapter 2). Furthermore, the quantitative syntheses of the thesis emphasise that the vulnerability of ectotherms is not determined by thermal fluctuations (Chapter 3), and that a population-level resolution is required for accurate predictive models and conservation approaches (Chapter 4). The impacts that EDCs have on the phenotypic response of organisms to climate change (or other anthropogenic drivers) can vary depending on the environment being altered. Future research should focus on the complex relationships between the chapters presented in this thesis, to determine whether exposure to multiple anthropogenic drivers (including EDCs) will impact the relationship between thermal fluctuations and plastic responses (Chapter 2 and 3), and the extent that populations differ in their capacity for phenotypic plasticity (Chapter 4).

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