# Methods

### *Effect size*

To quantify the impacts of developmental stress on mitochondrial function we calculated the standardized mean difference, assuming heteroscedastic population variances () (Bonett, 2008, 2009) along with its associated sampling error. We used the *escalc* function of the *metafor* package (vers. 4.6.0) (Viechtbauer, 2010) in R (vers. 4.3.1) which implements a correction to account for possible bias resulting from effect sizes calculated with small sample sizes (Borenstein & Hedges, 2009). We choose to use as our effect size because our data: 1) contained substantial amounts of ratio data (e.g., respiratory control ratios, RCR, relative gene expression) which makes interpretation with alternative effect size measures, such as log response ratio, challenging; 2) percentages and zero measurement variables and 3) skewed measurement variables. is more robust to these types of measurement variables. We calculated as the mean difference between control and treatment groups divided by the pooled standard deviation. As such, positive effect sizes represent situations where the mean of the experimental group was larger than the control group. Given the diversity of measurement variables we paid particular attention to effect size direction to ensure that positive increase of means all represent increased in mitochondrial function or efficiency. Any measurement type with teh opposite meaning was ‘coined’ (multiplied by -1) to ensure the meaning was consistent. For example, positive mean increases in rates of oxidative phosphorylation (OXPHOS) indicate higher mitochondrial function whereas negative RCRs calculated as XXX, indicate greater mitochondrial efficiency and thus better mitochondrial function.

### *Meta-Analysis*

We analysed our data using multilevel meta-analytic (MLMA) and meta-regression (MLMR) models in R using *metafor* (vers. 4.6.0 Viechtbauer, 2010). Meta-analytic mean estimates and meta-regression models were plotted using orchard plots which convey overall meta-analytic means, alongside raw effect sizes and prediction intervals, which describe effect heterogeneity (vers. 2.0, Nakagawa *et al.*, 2021, 2023).

#### *Multi-level Meta-analysis (MLMA) Models*

Using data subsets for each of our major stressor categories, we first fit multi-level meta-analysis (MLMA) models (i.e., intercept-only models) with , that included study, species, tissue type, and phylogeny as random effects to account for non-independence and identify sources of variability. Given *metafor* does not estimate a residual variance by default we also included an observation-level random effect in our models. Our MLMA models allowed us to partition variation in among these key sources while accounting for total sampling variance. This allowed us to calculate the proportion of total heterogeneity [i.e., ; *sensu* Nakagawa & Santos (2012); Noble *et al.* (2022)] along with various metrics describing the proportion of variance explained by each random effect level (Nakagawa & Santos, 2012). As a second measure of heterogeneity we also present 95% prediction intervals which describe the expected distribution of effects that are expected from future studies (Nakagawa *et al.*, 2021; Noble *et al.*, 2022).

A phylogeny was derived by first cross-checking taxa names using the Open Tree of Life (OTL) with the *rotl* package in R (vers. 3.1.0) (Michonneau, Brown, & Winter, 2016), and plotting taxa using *ggtree* to visualize the tree (vers. 3.9.0) (Yu *et al.*, 2017). We resolved any taxa names that were outdated or changed. Once names in the dataset and *rotl* database matched we exported species names and built a time calibrated phylogeny for the species in our dataset using TimeTree [timetree.org; Kumar *et al.* (2022)]. We used the R packages *ape* (vers. 5.7.1) (Paradis & Schliep, 2019) and *phytools* (vers. 1.9.16) (Revell, 2012) to prune the tree for individual analyses and calculate phylogenetic correlation matrices used in meta-analytic models.

#### *Multi-level Meta-Regression (MLMR) Models*

After quantifying levels of heterogeneity in each of the different stressor datasets, we fit a series of multi-level meta-regression (MLMR) models to test our key questions. In all models, we included the same random effects as we used in our MLMA models, however, MLMR models differed in the moderators included because each stressor type had unique sources of heterogeneity that we expected would explain effect variance.

# Results

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| Table 1- Heterogeneity estimates for developmental stressor datasets   |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Corticosterone | 184 | 9 | 11 | 91 | 2.8 | 0.00 | 0 | 80.53 | 8 | | Social Deprevation | 175 | 2 | 11 | 85 | 11.4 | 0.00 | 0 | 0.00 | 74 | | Disturbance | 192 | 6 | 15 | 89 | 35.1 | 1.15 | 29 | 5.29 | 19 | | Nutrition | 745 | 16 | 53 | 82 | 21.1 | 0.72 | 0 | 0.56 | 59 | | Temperature | 438 | 25 | 34 | 93 | 21.0 | 1.89 | 0 | 0.24 | 70 | |

## Corticosterone developmental impacts on mitochondrial function

Overall, exposure to corticosterone during development had a positive, but non-significant, impact on mitochondrial function ( = 0.18, 95% CI = -0.88 to 1.23, = 0.7; [Figure 1](#fig-orchard_int)a). However, effect heterogeneity was high (95% Prediction Intervals: -3.13 to 3.48; [Table 1](#tbl-heterogeneity)), with 80.53% of variation being driven by the type of tissue measured (; [Table 1](#tbl-heterogeneity)) with little to no variation explained by species/phylogeny and very small amount of between study variation ([Table 1](#tbl-heterogeneity)).

## Social deprivation developmental impacts on mitochondrial function

Social deprivation during development had a negative impact on mitochondrial function ( = -0.47, 95% CI = -3.24 to 2.3, = 0.28; [Figure 1](#fig-orchard_int)b). Effect heterogeneity was again high (95% Prediction Intervals: -19.2 to 18.26; [Table 1](#tbl-heterogeneity)), with only a small amount of variation across studies ( = 11.41%) and little to no variation explained by species/phylogeny ([Table 1](#tbl-heterogeneity)).

## Disturbance developmental impacts on mitochondrial function

Disturbance during development had a positive impact on mitochondrial function ( = 0.57, 95% CI = -0.91 to 2.06, = 0.37; [Figure 1](#fig-orchard_int)c). Effect heterogeneity was high (95% Prediction Intervals: -3.83 to 4.98; [Table 1](#tbl-heterogeneity)), with a moderate amount of variation being driven by differences between studies ( = 35.07%) and species ( = 28.63%) ([Table 1](#tbl-heterogeneity)).

## Nutrition developmental impacts on mitochondrial function

Nutritional stress during development also had limited impact on mitochondrial function overall ( = -0.05, 95% CI = -0.36 to 0.26, = 0.74; [Figure 1](#fig-orchard_int)d), but as expected, effect heterogeneity was high (95% Prediction Intervals: -2.78 to 2.68; [Table 1](#tbl-heterogeneity)), with a moderate amount of variation being driven by differences between studies ( = 21.14%; [Table 1](#tbl-heterogeneity)).

## Temperature developmental impacts on mitochondrial function

Temperature stress had a positive impact on mitochondrial function ( = 0.37, 95% CI = -0.14 to 0.88, = 0.14; [Figure 1](#fig-orchard_int)e) with effect heterogeneity being around 92.66% (95% Prediction Intervals: -3.59 to 4.33; [Table 1](#tbl-heterogeneity)), with a moderate amount of variation being driven by differences between studies ( = 20.97%)([Table 1](#tbl-heterogeneity)).

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| Figure 1- Orchard plots showing the overall meta-analytic mean effect size and 95% prediction intervals for each stressor category. |

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