Daniel W.A. Noble, Fonti Kar, Frank Seebacher, Alex Bush, & Shinichi Nakagawa

2022-11-20

## Materials and Methods

### *Literature collection*

We compiled literature on ectothermic animals that measured physiological rates (e.g., metabolic rate) at two or more temperatures after having been acclimated (or acclimatized) at these temperatures. We used data from a previous meta-analysis (Seebacher *et al.* 2015) and updated Seebacher *et al.* (2015)’s data by extracting data from suitable studies from our own searches that followed the same search protocol. More specifically, we performed a literature search on the 28th of June 2017 using the Web of Science database. We limited our search to articles or proceedings papers published in English from 2013 to 2017 (the date after Seebacher *et al.* 2015 searches were conducted) using the following topic search string: *“(acclimat* AND (therm\* OR temp*) NOT (plant* OR tree\* OR forest\* OR fung\* OR mammal\* OR marsup\* OR bird\* OR human OR exercis\* OR train\* OR hypoxi*))“*. We further limited to the following research areas: Anatomy Morphology; Biodiversity Conservation; Biology; Ecology; Endocrinology Metabolism; Entomology; Evolutionary Biology; Marine Freshwater Biology; Physiology; Respiratory System, Reproductive Biology, Zoology.

Our search resulted in 1,321 papers for screening in Rayyan (Ouzzani *et al.* 2016). We also cross-checked papers we found in our searches with a recent paper by Havird *et al.* (2020), which also updates Seebacher *et al.* (2015)’s dataset. We included any papers that were missed between our searches and those of Havird *et al.* (2020) from the dates 2013-2017. Havird *et al.* (2020) added 7 new studies between 2013-2017 (mainly because they were focused on metabolic rates), and our searches differed from theirs by only a single paper (i.e., Bulgarella *et al.* 2015). Given the physiological traits we included were broader, we had a substantial increase in additional papers that we added to Seebacher *et al.* (2015)’s dataset. More specifically, in addition to the 191 papers we included from the Seebacher *et al.* (2015) dataset, we extracted data from an extra 65 papers (with a total of 238 effects) that were published between 2013 - 2017 (a 34.03% increase in the number of published articles). Note that Seebacher *et al.* (2015) included a total of 205 publications, however, not all these contained the necessary statistics we needed to derive effect sizes and associated sampling variances (see below). While we may have missed papers, our goal was to obtain a large representative (and unbiased) sample of acclimation research rather than a comprehensive dataset. As such, our database represents the most up-to-date dataset used by Seebacher *et al.* (2015) to answer questions on acclimation across ectotherms.

We split the screening of titles and abstracts for the 1,321 papers found in our search among all authors evenly. To ensure consistency among authors in title and abstracts that should be included, prior to screening all authors went through a randomly selected set of papers together - agreeing on those that were relevant and those that were not based on our inclusion criteria (see below). Where any authors were uncertain about whether to include a paper in the sub-sample they screened, we conservatively included the paper for full text screening and discussed uncertain papers among authors to come to a decision on whether to include the paper. After title and abstract screening, we were left with a total of 149 papers for full text screening. Papers were included only if they: 1) measured a physiological rate acutely at two temperatures on a sample of animals chronically exposed to the same two temperatures for at least 1 week; and 2) where physiological rates measured were burst and sustained locomotion, metabolic rates (standard, resting, routine and maximal), heart rates, and/or enzyme activities.

### *Data Compilation*

We extracted means, standard deviations and sample sizes for physiological rates at the two test temperatures. If there were more than two test temperatures, we choose only the test temperatures that fell within the most likely natural range of temperatures experienced by the species in question. We extracted these data from text, tables or figures of a given paper. Data were extracted from figures using the R package *metaDigitise* (Pick *et al.* 2019). We also recorded the phylum, class, order, genus and species under study and the latitude and longitude of the population that was being studied. For studies that did not provide latitude and longitude for the population, we searched for similar studies by the lab group to identify where the population was likely to have been sourced or derived from when needed. If the population was derived from the wild, we recorded the nearest latitude and longitude of the population to the field collection site. If the animals had been sourced from a commercial supplier, we took the latitude and longitude of the supplier that the paper identified the animals to have originated from. When it was not possible to find latitude and longitude using these methods, we looked up the distribution of the species in question and took the latitude and longitude of the centroid of the species’ distributional range.

### *Based Effect Sizes and Sampling Variances for Means and Variances*

Following Noble *et al.* (2022) we calculated a series of temperature corrected effect sizes that compared mean physiological rates () as well as the variability in physiological rates ( and ). These effect sizes are essentially similar to the traditional temperature coefficient (), but with formal analytical approximations for their sampling variances. Sampling variances for effect sizes allowed us to make use of traditional meta-analytic modelling approaches.

#### *Comparing changes in mean physiological rates*

To compare mean physiological rates we calculated the log response ratio, (Noble *et al.* 2022) as follows:

Where, and are mean physiological rates and and are the temperatures that these rates are measured. Log transformation of this ratio makes the effect size normally distributed. Equation (1) is essentially a temperature corrected equivalent to the log response ratio (lnRR) (Hedges *et al.* 1999; Lajeunesse 2011) when the numerator and denominator are measured at different temperatures. This allows one to compare the mean of two temperature treatments directly regardless of the temperatures that these groups have been measured. The sampling variance for equation (1) can be computed as follows (as described in Noble *et al.* (2022)):

Here, and are the standard deviations and and are the sample sizes in group 1 and 2, respectively.

#### *Comparing variance physiological rates*

Nakagawa *et al.* (2015) recently proposed analogous effect size estimates to *lnRR* that allow for comparisons of changes in variance between two groups, the log variance ratio (*lnVR*) and the log coefficient of variation (*lnCVR*). *lnVR* and *lnCVR* are ratios that describe the relative difference in trait variability between two groups. We refer readers to Nakagawa *et al.* (2015) for the equations describing *lnVR* and *lnCVR*, but these can easily be extended to their analogues (and associated sampling variance) as follows:

Equations (3) and (4) describe the change in physiological rate variance (eqn (3)) across a 10°C temperature change along with its sampling variance (eqn (4)). While this is a useful metric, as discussed by Nakagawa *et al.* (2015) there is often a strong mean-variance relationship that needs to be accounted for in analysing changes in variance. As such, we calculated the coefficient of variation, which standardizes changes in variance for changes in means as follows:

where is the coefficient of variation defined as .

#### *Calculating acute and acclimation , and estimates*

Using the mean, standard deviation and sample size for all acute and acclimation treatments of studies in our databases we derived acute and acclimation , and estimates. For all effect sizes the higher acute or acclimation temperature was in the numerator and the lower of the two temperatures in the denominator. As such, positive effect sizes suggest that the mean or variance is larger at the higher of the two temperatures, standardized to 10°C.

### *Moderator Variables*

We recorded or derived a series of moderator variables from each study that are expected to have an impact on our effect size estimates. These included the duration of acclimation in days and acclimation type (“acclimation” or “acclimatization”) given that acclimation responses are expected to depend both on how long chronic temperature exposure occurs (longer exposure = better acclimation response) and whether exposure took place in the wild (“acclimatized”) or lab (“acclimated”) (Seebacher *et al.* 2015). We also recorded if the sample of animals were derived from captive or wild stocks, the life-history stage of the animals used (“adult” or “juvenile”) and the habitat type (“freshwater”, “marine” or “terrestrial”) given that Seebacher *et al.* (2015) show that these factors can impact estimates. Physiological rate measures varied widely across the studies but could generally be grouped into discrete trait categories (Seebacher *et al.* 2015). As such, using the detailed information on the trait type and its associated units from a given study we categorized each effect size into one of 12 trait categories. These categories included measures of whole organism performance measures including cardiac (i.e., ‘cardiac’) and muscle (‘muscle’) function, sprint speed (‘sprint’) and endurance (‘endurance’) and metabolic rates (i.e., maximal and resting metabolic rate; max MR’, ‘rest MR’, respectively). Studies also quantified various enzymatic reaction rates, including enzymes involved in general metabolic responses (categorized as ‘metabolic enzyme’), various parts of the electron transport chain, including ATPase activity (‘ATPase’), mitochondrial leak (‘mito\_leak’) and oxidation (‘mito\_oxidation’) as well as antioxidant enzymes (‘antiox’). All other traits not falling within these categories were placed into ‘other’.

### *Climate Data*

To understand how climate has impacted species’ physiological acclimation abilities we used the coordinates reported by each study to extract temperature data from terrestrial and aquatic environments. Only studies on wild populations were used for climate analyses because temperature data at locations was likely reflective of past climate history. Temperature data was extracted using the monthly averages provided by the ERA5 climate model, available from the Copernicus climate data store (Hersbach *et al.* 2020). For each population and species in the dataset we extracted a 30-year period (1958-2022) of either surface temperature at 2 meters for terrestrial and freshwater taxa, or sea surface temperature for the marine taxa. We chose a 2 meters resolution because we believed that it more likely to reflects the micro-thermal environment experienced by terrestrial and freshwater ectotherms.

Using thermal time-series data for each location we summarised various metrics of thermal variability across months and years. We calculated the coefficient of variation (, where SD = standard deviation in temperature and M = the mean temperature for each year).

### *Meta-Analysis*

We analysed our data using Bayesian multilevel meta-analytic (MLMA) and meta-regression (MLMR) models in R (vers. 4.2.1) using *brms* (Bürkner 2017; vers. 2.18.0 Bürkner 2018; “Stan development team. RStan” 2021). We included study, species and phylogeny as random effects to account for non-independence and also explore drivers of variation among estimates.

Yu *et al.* (2017)

### *Sensitivity Analyses*

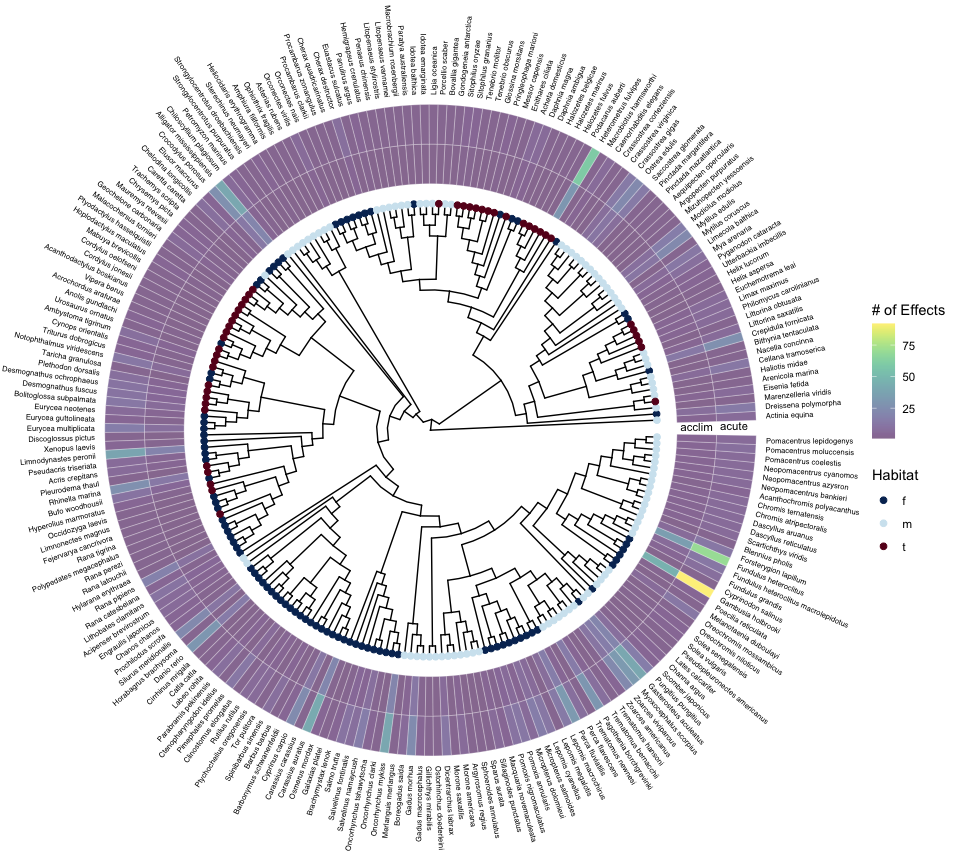
### *Publication Bias*

## *Results*

### *Do terrestrial and aquatic ectotherms differ in their capacity to acclimate?*

### *Does climate variability predict acclimation capacity among aquatic and terrestrial ectotherms?*

### *Does between-individual variation in acclimation capacities differ across terrestrial and aquatic ectotherms?*



**Figure. 1.** Phylogenetic tree

## References

Bulgarella, M., Trewick, S.A., Godfrey, A.J.R., Sinclair, B.J. & Morgan-Richards, M. (2015). Elevational variation in adult body size and growth rate but not in metabolic rate in the tree weta hemideina crassidens. *Journal of Insect physiology*, 75, 30–38.

Bürkner, P.-C. (2017). Brms: An r package for bayesian multilevel models using stan. *Journal of Statistical Software*, 80, 1–28., doi:10.18637/jss.v080.i01.

Bürkner, P.-C. (2018). Advanced bayesian multilevel modeling with the r package brms. *The R Journal*, 10, 395–411.

Havird, J.C., Neuwald, J.L., Shah, A.A., Mauro, A., Marshall, C.A. & Ghalambor, C.K. (2020). Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q10 effects: Why methodology matters. *Functional Ecology*, 0, 1–14.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.

Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz-Sabater, J., *et al.* (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal Meteorological Society*, 146, 1999–2049.

Lajeunesse, M.J. (2011). On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology*, 92, 2049–2055.

Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., *et al.* (2015). Meta-analysis of variation: Ecological and evolutionary applications and beyond. *Methods in Ecology and Evolution*, 6, 143–152.

Noble, D.W.A., Pottier, P., Lagisz, M., Burke, S., Drobniak, S.M., O’Dea, R.E., *et al.* (2022). Meta-analytic approaches and effect sizes to account for “nuisance heterogeneity” in comparative physiology. *Journal of Experimental Biology*, 225, jeb243225.

Ouzzani, M., Hammady, H., Fedorowicz, Z. & Elmagarmid, A. (2016). Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews*, 5, 210–220.

Pick, J.L., Nakagawa, S. & Noble, D.W.A. (2019). Reproducible, flexible and high throughput data extraction from primary literature: The metaDigitise r package. *Methods in Ecology and Evolution*, 10, 426–431.

Seebacher, F., White, C.R. & Franklin, C.E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5, 61–66.

Stan development team. RStan: The r interface to stan. (2021). *R package version 2.21.3. https://mc-stan.org/.*

Yu, G., Smith, D., Zhu, H., Guan, Y. & Lam, T.T.-Y. (2017). Ggtree: An r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, 8, 28–36, doi:10.1111/2041–210X.12628.