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

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**Supplementary Materials**

**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 and the decision tree.

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

**Table S2 Directionality of the PRRD effect size based on the response of traits to temperature.** Hypothetical scenarios of trait responses to temperature depending on how traits respond to temperature (i.e., direction of reaction norm). In our analyses, all trait responses were standardised such that negative values indicate situations when the slope in the fluctuating treatments were shallower compared to constant conditions. The opposite was true of positive signed PRRD values. For situations where reaction norms were opposite, we xx. Note that values are not temperature corrected for simplicity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Constant** |  | **Flutuating** |  |  |
|  | **Low Temp** | **High Temp** | **Low Temp** | **High Temp** | **PRRD** |
| **Scenario** |  |  |  |  |  |
| *Positive slopes, steeper in C* | 0.70 | 1.00 | 0.90 | 1.00 |  |
| lnRR/PRRD |  | 0.15 |  | 0.05 | -0.11 |
|  |  |  |  |  |  |
| *Negative slopes, steeper in C* | 1.00 | 0.70 | 1.00 | 0.90 |  |
| lnRR/PRRD |  | -0.15 |  | -0.05 | 0.11 |
|  |  |  |  |  |  |
| *Positive slopes, steeper in F* | 0.90 | 1.00 | 0.70 | 1.00 |  |
| lnRR/PRRD |  | 0.05 |  | 0.15 | 0.11 |
|  |  |  |  |  |  |
| *Negative slopes, steeper in F* | 1.00 | 0.90 | 1.00 | 0.70 |  |
| lnRR/PRRD |  | -0.05 |  | -0.15 | -0.11 |
|  |  |  |  |  |  |
| *Same slope, opposite direction* | 1.00 | 0.90 | 0.90 | 1.00 |  |
| lnRR/PRRD |  | -0.05 |  | 0.05 | 0.09 |
|  |  |  |  |  |  |
| *Same slope, opposite direction* | 0.90 | 1.00 | 1.00 | 0.90 |  |
| lnRR/PRRD |  | 0.05 |  | -0.05 | -0.09 |
|  |  |  |  |  |  |

**Search Strings**

*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*

Note that the search engine accepts a maximum 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)

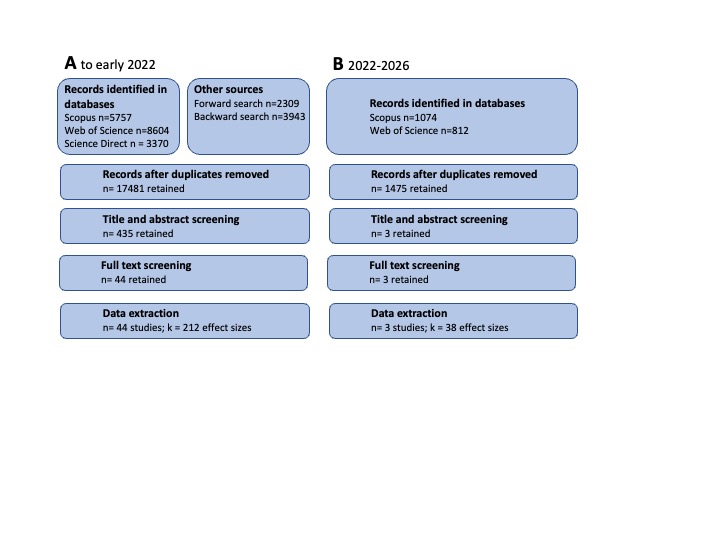
**7.** (“development effect" OR "developmental effect" OR "develop effect”) AND (thermal OR temperature) AND (vary OR change OR irregular) NOT (endotherm)

**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

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

**Abstract Screening**

**Full-text Screening**

Must not include **metrics of thermal performance** (critical thermal maximum, critical thermal minimum, or thermal preference) and **not experience hardening** prior to trait measurement. **Acclimation** studies must **not include morphological** traits.

No

Yes/Maybe

Exclude

Treatments must have: all other **conditions identical**, the **mean** of **fluctuations** **match** the **constant temperatures**,and treatments **maintained through to phenotypic trait measurement**.

Treatments must be imposed **within the lifespan** of an individual. Not including **transgenerational studies**.

Individuals from each treatment must have **identical housing and habituation periods**.

Must be a **peer-reviewed** article in **English**.

Yes/Maybe

No

Exclude

Must be a **controlled laboratory experiment**. Not including literature reviews, simulations, theories, data banks, surveys, in situ, reciprocal transplant studies etc.

Must include all **relevant information for a meta-analysis** (i.e., sample size, mean and error metrics). Not including studies that **reports principal components** or **factor loading**.

Exclude

No

Yes/Maybe

Exclude

No

Yes/Maybe

**Include**

Exclude

No

Yes/Maybe

Exclude

No

Yes/Maybe

Must be **ectothermic**.

Exclude

No

Exclude

No

Yes/Maybe

Yes/Maybe

Must compare **fluctuating** and **constant phenotypic plasticity.**

Yes/Maybe

Exclude

No

**Figure S2**

**Decision tree and inclusion criteria.**

**Studies included in the analysis**

Arrighi, J. M., Lencer, E. S., Jukar, A., Park, D., Phillips, P. C. and Kaplan, R. H. (2013). Daily temperature fluctuations unpredictably influence developmental rate and morphology at a critical early larval stage in a frog. *BMC Ecology* **13**, 18. doi: 10.1186/1472-6785-13-18

Bahar, H. M., Soroka, J. J. and Dosdall, L. M. (2012). Constant versus fluctuating temperatures in the interactions between *Plutella xylostella* (Lepidoptera: Plutellidae) and its larval parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Environmental Entomology* **41**(6), 1653-1661. doi: 10.1603/EN12156

Bayu, M. S. Y. I., Ullah, M. S., Takano, Y. and Gotoh, T. (2017). Impact of constant versus fluctuating temperatures on the development and life history parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology* **72**, 205-227. doi: 10.1007/s10493-017-0151-9

Boher, F., Trefault, N., Estay, S. A. and Bozinovic, F. (2016). Ectotherms in variable thermal landscapes: A physiological evaluation of the invasive potential of fruit flies species. *Frontiers in Physiology* **7**, 302. doi: 10.3389/fphys.2016.00302

Brakefield, P. M. and Kesbeke, F. (1997). Genotype-environment interactions for insect growth in constant and fluctuating temperature regimes. *Proceedings of the Royal Society B: Biological Sciences* **264**(1382), 717-723. doi: 10.1098/rspb.1997.0102

Breitenbach, A. T., Bowden, R. M., and Paitz, R. T. (2022). Effects of constant and fluctuating temperatures on gene expression during gonadal development. *Integrative and Comparative Biology* **62**, 21-29.

Bryant, S. R., Bale, J. S. and Thomas, C. D. (1999). Comparison of development and growth of nettle-feeding larvae of Nymphalidae (Lepidoptera) under constant and alternating temperature regimes. *European Journal of Entomology* **96**, 143-148.

Carrington, L. B., Armijos, M. V., Lambrechts, L., Barker, C. M. and Scott, T. W. (2013). Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. *PloS One* **8**(3), e58824. doi: 10.1371/journal.pone.0058824

Cavieres, G., Bogdanovich, J. M. and Bozinovic, F. (2016). Ontogenetic thermal tolerance and performance of ectotherms at variable temperatures. *Journal of Evolutionary Biology* **29**(7), 1462-1468. doi: 10.1111/jeb.12886

Chen, C. Y., Chiu, M. C. and Kuo, M. H. (2013). Effect of warming with temperature oscillations on a low-latitude aphid, *Aphis craccivora*. *Bulletin of Entomological Research* **103**(4), 406-413. doi: 10.1017/S0007485312000867

Chen, W., Yang, L., Ren, L., Shang, Y., Wang, S. and Guo, Y. (2019). Impact of constant versus fluctuating temperatures on the development and life history parameters of *Aldrichina grahami* (Diptera: Calliphoridae). *Insects* **10**(7), 184. doi: 10.3390/insects10070184

Chown, S. L., Haupt, T. M. and Sinclair, B. J. (2016). Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth. *Journal of Insect Physiology* **85**, 10-16. doi: 10.1016/j.jinsphys.2015.11.010

Czarnoleski, M., Cooper, B. S., Kierat, J. and Angilletta Jr, M. J. (2013). Flies developed small bodies and small cells in warm and in thermally fluctuating environments. *Journal of Experimental Biology* **216**(15), 2896-2901. doi: 10.1242/jeb.083535

de Majo, M. S., Zanotti, G., Campos, R. E. and Fischer, S. (2019). Effects of constant and fluctuating low temperatures on the development of *Aedes aegypti* (Diptera: Culicidae) from a temperate region. *Journal of Medical Entomology* **56**(6), 1661-1668. doi: 10.1093/jme/tjz087

Dhaliwal, N. K. and Aggarwal, N. (2021). Development and survival of brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee (Crambidae: Lepidoptera) at constant and alternating temperatures. *International Journal of Tropical Insect Science* **41**(2), 1717-1728. doi: 10.1007/s42690-020-00376-5

Dhillon, R. S. and Fox, M. G. (2007). Growth-independent effects of a fluctuating thermal regime on the life-history traits of the Japanese medaka (*Oryzias latipes*). *Ecology of Freshwater Fish* **16**(3), 425-431. doi: 10.1111/j.1600-0633.2007.00240.x

Dong, Y. and Dong, S. (2006). Growth and oxygen consumption of the juvenile sea cucumber *Apostichopus japonicus* (Selenka) at constant and fluctuating water temperatures. *Aquaculture Research* **37**(13), 1327-1333. doi: 10.1111/j.1365-2109.2006.01570.x

Dong, Y., Dong, S., Tian, X., Wang, F. and Zhang, M. (2006). Effects of diel temperature fluctuations on growth, oxygen consumption and proximate body composition in the sea cucumber *Apostichopus japonicus* Selenka. *Aquaculture* **255**(1-4), 514-521. doi: 10.1016/j.aquaculture.2005.12.013

Du, W. G. and Feng, J. H. (2008). Phenotypic effects of thermal mean and fluctuations on embryonic development and hatchling traits in a lacertid lizard, *Takydromus septentrionalis*. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* **309A**(3), 138-146. doi: 10.1002/jez.442

Fischer, K., Kölzow, N., Höltje, H. and Karl, I. (2011). Assay conditions in laboratory experiments: Is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* **166**, 23-33. doi: 10.1007/s00442-011-1917-0

Foray, V., Desouhant, E. and Gibert, P. (2014). The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps. *Functional Ecology* **28**(2), 411-423. doi: 10.1111/1365-2435.12171

Hagstrum, D. W. and Milliken, G. A. (1991). Modeling differences in insect developmental times between constant and fluctuating temperatures. *Annals of the Entomological Society of America* **84**(4), 369-379. doi: 10.1093/aesa/84.4.369

Hall, J. M. and Warner, D. A. (2020). Ecologically relevant thermal fluctuations enhance offspring fitness: Biological and methodological implications for studies of thermal developmental plasticity. *Journal of Experimental Biology* **223**(19), jeb231902. doi: 10.1242/jeb.231902

Jiang, L., Sun, Y. F., Zhang, Y. Y., Zhou, G. W., Li, X. B., McCook, L. J., Lian, J. S., Lei, X. M., Liu, S., Cai, L., Qian, P. Y. and Huang, H. (2017). Impact of diurnal temperature fluctuations on larval settlement and growth of the reef coral *Pocillopora damicornis*. *Biogeosciences* **14**(24), 5741-5752. doi: 10.5194/bg-14-5741-2017

Joshi, D. S. (1996). Effect of fluctuating and constant temperatures on development, adult longevity and fecundity in the mosquito *Aedes krombeini*. *Journal of Thermal Biology* **21**(3), 151-154. doi: 10.1016/0306-4565(95)00035-6

Ketola, T., Kellermann, V., Kristensen, T. N. and Loeschcke, V. (2012). Constant, cycling, hot and cold thermal environments: Strong effects on mean viability but not on genetic estimates. *Journal of Evolutionary Biology* **25**(6), 1209-1215. doi: 10.1111/j.1420-9101.2012.02513.x

Kingsolver, J. G., Higgins, J. K. and Augustine, K. E. (2015). Fluctuating temperatures and ectotherm growth: Distinguishing non-linear and time-dependent effects. *Journal of Experimental Biology* **218**(14), 2218-2225. doi: 10.1242/jeb.120733

Kingsolver, J. G., Moore, M. E., Hill, C. A. and Augustine, K. E. (2020). Growth, stress, and acclimation responses to fluctuating temperatures in field and domesticated populations of *Manduca sexta*. *Ecology and Evolution* **10**(24), 13980-13989. doi: 10.1002/ece3.6991

Les, H. L., Paitz, R. T. and Bowden, R. M. (2009). Living at extremes: Development at the edges of viable temperature under constant and fluctuating conditions. *Physiological and Biochemical Zoology* **82**(2), 105-112. doi: 10.1086/590263

Malek, D., Drobniak, S., Gozdek, A., Pawlik, K. and Kramarz, P. (2015). Response of body size and developmental time of *Tribolium castaneum* to constant versus fluctuating thermal conditions. *Journal of Thermal Biology* **51**, 110-118. doi: 10.1016/j.jtherbio.2015.04.002

Meyers, D. G. (1984). Egg development of a chydorid cladoceran, *Chydorus sphaericus*, exposed to constant and alternating temperatures: Significance to secondary productivity in fresh waters. *Ecology* **65**(1), 309-320. doi: 10.2307/1939483

Moore, M. E., Hill, C. A. and Kingsolver, J. G. (2021). Differing thermal sensitivities in a host–parasitoid interaction: High, fluctuating developmental temperatures produce dead wasps and giant caterpillars. *Functional Ecology* **35**(3), 675-685. doi: 10.1111/1365-2435.13748

Orcutt Jr., J. D. and Porter, K. G. (1983). Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of *Daphnia*. *Limnology and Oceanography* **28**(4), 720-730. doi: 10.4319/lo.1983.28.4.0720

Paaijmans, K. P., Blanford, S., Bell, A. S., Blanford, J. I., Read, A. F. and Thomas, M. B. (2010). Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Sciences of the United States of America* **107**(34), 15135-15139. doi: 10.1073/pnas.1006422107

Patterson, L. D. and Blouin-Demers, G. (2008). The effect of constant and fluctuating incubation temperatures on the phenotype of black ratsnakes (*Elaphe obsoleta*). *Canadian Journal of Zoology* **86**(8), 882-889. doi: 10.1139/Z08-067

Salo, T., Kropf, T., Burdon, F. J. and Seppälä, O. (2019). Diurnal variation around an optimum and near-critically high temperature does not alter the performance of an ectothermic aquatic grazer. *Ecology and Evolution* **9**(20), 11695-11706. doi: 10.1002/ece3.5666

Schweiterman, G. D., Hardison, E. A., Eliason, E.J. (2022). Effect of thermal variation on the cardiac thermal limits of a eurythermal marine teleost (*Girella nigricans*). *Current Research in Physiology* **5**, 109-117.

Spanoudis, C. G., Andreadis, S. S., Tsaknis, N. K., Petrou, A. P., Gkeka, C. D. and Savopoulou–Soultani, M. (2019). Effect of temperature on biological parameters of the west Nile virus vector *Culex pipiens* form ‘molestus’ (Diptera: Culicidae) in Greece: Constant vs fluctuating temperatures. *Journal of Medical Entomology* **56**(3), 641-650. doi: 10.1093/jme/tjy224

Steele, A. L. and Warner, D. A. (2020). Sex-specific effects of developmental temperature on morphology, growth and survival of offspring in a lizard with temperature-dependent sex determination. *Biological Journal of the Linnean Society* **130**(2), 320-335. doi: 10.1093/biolinnean/blaa038

Theys, C., Verheyen, J., Tüzün, N. and Stoks, R. (2021). Higher mean and fluctuating temperatures jointly determine the impact of the pesticide chlorpyrifos on the growth rate and leaf consumption of a freshwater isopod. *Chemosphere* **273**, 128528. doi: 10.1016/j.chemosphere.2020.128528

Tian, X. and Dong, S. (2006). The effects of thermal amplitude on the growth of Chinese shrimp *Fenneropenaeus chinensis* (Osbeck, 1765). *Aquaculture* **251**(2-4), 516-524. doi: 10.1016/j.aquaculture.2005.05.031

Tian, X., Dong, S., Wang, F. and Wu, L. (2006). The growth of juvenile Chinese shrimp, *Fenneropenaeus chinensis* Osbeck, at constant and diel fluctuating temperatures. *Journal of Shellfish Research* **25**(3), 1007-1011. doi: 10.2983/0730-8000(2006)25[1007:TGOJCS]2.0.CO;2

Uvarov, A. V. and Scheu, S. (2004). Effects of temperature regime on the respiratory activity of developmental stages of *Lumbricus rubellus* (Lumbricidae). *Pedobiologia* **48**(4), 365-371. doi: 10.1016/j.pedobi.2004.05.002

Vangansbeke, D., Nguyen, D. T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. and De Clercq, P. (2015). Prey consumption by phytoseiid spider mite predators as affected by diurnal temperature variations. *BioControl* **60**(5), 595-603. doi: 10.1007/s10526-015-9677-0

Verheyen, J. and Stoks, R. (2019). Temperature variation makes an ectotherm more sensitive to global warming unless thermal evolution occurs. *Journal of Animal Ecology* **88**(4), 624-636. doi: 10.1111/1365-2656.12946

Verheyen, J. and Stoks, R. (2020). Negative bioenergetic responses to pesticides in damselfly larvae are more likely when it is hotter and when temperatures fluctuate. *Chemosphere* **243**. doi: 10.1016/j.chemosphere.2019.125369

Xu, W., Chang, M., Li, J., Li, M., Stoks, R. and Zhang, C. (2024). Local thermal adaptation mediates the sensitivity of Daphnia magna to nanoplastics under global arming scenarios. Journal of Hazardous Materials 476, 134921. doi: 10.1016/j.jhazmat.2024.134921

**Table S3 Metadata for the Raw Data Set.** Column names and descriptions used in the Excel sheet with raw data.

|  |  |
| --- | --- |
| **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. |

![A screen shot of a computer

AI-generated content may be incorrect.](data:application/pdf;base64,)

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

**Publication bias and sensitivity analysis**

The overall MLMA model that included all random effects, showed no evidence for small-study effects (inverse effective sample size estimate = 0.0046; 95% CIs = [-0.0194, 0.0286]; p = 0.7073) or time lag bias in PRRDS (estimate = -0.0026, 95% CI = -0.0080, 0.0027], p = 0.3290). No implausible data points were identified in the sensitivity analysis, with the Cook’s distant never exceeding 1.

**Table S4 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** | 46 | 41 | 241 | 0.004 | -0.002 | 0.01 | 240 | 0.188 |

**Table S5 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. Italics indicates that mean PRRDS p < 0.1 but > 0.05.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Overall Data (PRRDS)** | **Studies** | **Species** | **Effect Sizes** | **Estimate** | **CI Low** | **CI High** | **df** | **p-value** |
| **Intercept** | 46 | 41 | 241 | -0.005 | -0.018 | 0.007 | 239 | 0.407 |
| **Fluctuation Amplitude (slope)** |  |  |  | *0.001* | *0* | *0.002* | *239* | *0.056* |

**Table S6 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** |
| **Alternating** | 15 | 17 | 56 | 0.009 | -0.002 | 0.021 | 211 | 0.113 |
| **Sinusoidal (Sine Curve)** | 20 | 17 | 110 | 0.003 | -0.008 | 0.013 | 211 | 0.623 |
| **Stepwise** | 7 | 6 | 48 | -0.001 | -0.014 | 0.013 | 211 | 0.904 |

**Table S7 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** | 6 | 6 | 52 | 0.001 | -0.015 | 0.016 | 224 | 0.946 |
| **Life-History Traits** | 28 | 30 | 66 | 0.002 | -0.012 | 0.016 | 224 | 0.771 |
| **Morphology** | 20 | 23 | 54 | 0.004 | -0.008 | 0.016 | 224 | 0.489 |
| **Physiological** | 16 | 14 | 56 | 0.003 | -0.009 | 0.015 | 224 | 0.641 |

**Table S8 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. Bold indicates significant mean PRRDS and italics p < 0.1 but > 0.05.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Overall Data (PRRDS)** | **Studies** | **Species** | **Effect Sizes** | **Estimate** | **CI Low** | **CI High** | **df** | **p-value** |
| **Development Time** | **26** | **27** | **46** | **-0.01** | **-0.016** | **-0.004** | **93** | **0.002** |
| **Length** | 9 | 10 | 14 | 0 | -0.011 | 0.011 | 93 | 0.951 |
| **Mass** | 12 | 14 | 25 | 0.004 | -0.005 | 0.012 | 93 | 0.401 |
| **Metabolic Rate** | *6* | *5* | *12* | *0.021* | *-0.001* | *0.043* | *93* | *0.061* |

**Table S8 Results of the meta-regression of the overall data set with taxonomic group (invertebrates vs vertebrates) 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** |
| **Invertebrates** | 37 | 32 | 182 | 0.002 | -0.004 | 0.009 | 239 | 0.488 |
| **Vertebrates** | 9 | 9 | 59 | 0.009 | -0.001 | 0.019 | 239 | 0.089 |

**Table S9 Results of the meta-regression of the overall data set with taxonomic habitat type (aquatic vs terrestrial) 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** |
| **Aquatic** | 15 | 13 | 90 | 0.001 | -0.01 | 0.011 | 239 | 0.874 |
| **Terrestrial** | 31 | 28 | 151 | 0.006 | -0.002 | 0.014 | 239 | 0.144 |

**Table S10 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** | 45 | 41 | 234 | 0.004 | -0.002 | 0.011 | 233 | 0.205 |

**Table S11 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** |
| **Intercept** | 45 | 41 | 234 | -0.005 | -0.017 | 0.008 | 232 | 0.467 |
| **Fluctuation Amplitude (slope)** |  |  |  | 0.001 | 0 | 0.002 | 232 | 0.079 |

**Table S12 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** |
| **Alternating** | 14 | 17 | 55 | 0.009 | -0.003 | 0.02 | 204 | 0.138 |
| **Sinusoidal (Sine Curve)** | 20 | 17 | 105 | 0.002 | -0.008 | 0.012 | 204 | 0.647 |
| **Stepwise** | 7 | 6 | 47 | 0 | -0.014 | 0.013 | 204 | 0.966 |

**Table S13 Results of the meta-regression of the individual-level traits data set with group (invertebrates vs vertebrates) 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** |
| **Invertebrate** | 36 | 32 | 177 | 0.003 | -0.004 | 0.01 | 232 | 0.459 |
| **Vertebrate** | 9 | 9 | 57 | 0.008 | -0.003 | 0.019 | 232 | 0.136 |

**Table S14 Results of the meta-regression of the individual-level traits data set with habitat type (terrestrial & aquatic) 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** |
| **Aquatic** | 15 | 13 | 86 | 0.001 | -0.01 | 0.011 | 232 | 0.861 |
| **Terrestrial** | 30 | 28 | 148 | 0.006 | -0.002 | 0.014 | 232 | 0.144 |



**Table S15 Heterogeneity statistics based on I2, proportion of variance explained relative to total, along with two magnitude measures of heterogeneity, CVH2, mean-standardised measure of heterogenity and, M2, variance and mean standardised heterogeneity.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Total** | **Phylogeny** | **Study** | **Obs** | **Species** | **Shared Animal ID** | **Measurement** |
| **Proportion of Variances (%) - I2** | 99.25 | 0 | 1.51 | 90.29 | 0 | 0 | 7.46 |
| **CVH2** | 50.92 | 0 | 0.77 | 46.32 | 0 | 0 | 3.83 |
| **M2** | 0.98 | 0 | 0.01 | 0.89 | 0 | 0 | 0.07 |