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Developmental environments do not affect thermal physiological traits in reptiles: An experimental test and meta-analysis

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Does your article include research that required ethical approval or permits?:
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Data

*It is a condition of publication that data, code and materials supporting your paper are made publicly
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Yes

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Conflict of interest

I/We declare we have no competing interests

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1 **Developmental environments do not affect thermal physiological traits in reptiles: An**
2 **experimental test and meta-analysis**
3
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16 **Abstract**:
17 On a global scale, organisms face significant challenges due to climate change and
18 anthropogenic disturbance. In many ectotherms, developmental and physiological processes are
19 sensitive to changes in temperature and resources. Developmental plasticity in thermal
20 physiology may provide adaptive advantages to environmental extremes if early environmental
21 conditions are predictive of late-life environments. Here, we conducted a laboratory experiment
22 to test how developmental temperature and maternal resource investment influence thermal
23 physiological traits (critical thermal maximum: CT_{max} & thermal preference: T_{pref}) in a common
24 skink (*Lampropholis delicata*). We then compared our experimental findings more broadly
25 across reptiles (snakes, lizards, turtles) using meta-analysis. In both our experimental study and
26 meta-analysis, we did not find evidence that developmental environments influence CT_{max} or
27 T_{pref} . Furthermore, the effects of developmental environments on thermal physiology did not
28 vary by age, taxon, or climate zone (temperate/tropical). Overall, the magnitude of
29 developmental plasticity on thermal physiology appears to be limited across reptile taxa
30 suggesting that behavioural or evolutionary processes may be more important. However, there is
31 a paucity of information across most reptile taxa, and a broader focus on thermal performance
32 curves themselves will be critical in understanding the impacts of changing thermal conditions
33 on reptiles in the future.

Introduction

34 **Introduction**
35 Climate warming and anthropogenic stressors pose significant challenges to organisms on a
36 global scale [1,2]. Rapidly increasing temperatures are a particularly significant threat for
37 ectothermic species. Indeed, increasing temperatures can drive fitness declines due to
38 physiological intolerance [3], and alter the distribution of species [4]. Inevitably, these impacts are
39 primarily mediated by how organisms change their behaviour and physiology through
40 development and evolutionary time in response to shifting environments. Phenotypic changes
41 that occur during an animal's lifetime in response to changing environments (i.e., phenotypic
42 plasticity), are important mechanisms by which ectotherms can cope with climate change over
43 short time scales [5]. However, the magnitude of plastic responses is widely trait- and species-
44 specific [5–7]
45   Temperature can also have transgenerational effects by impacting parental generations
46 [8,9]. For instance, recent evidence indicates that some ectotherms can tolerate heat events for
47 long periods [5,10]. Thermal ecology of ectotherms can also be shaped by other factors, such as
48 diet or maternal investment, which can influence physiological traits that are temperature
49 dependent [11–13]. For example, a diet high in nutrients (carbohydrate or protein) leads to higher
50 metabolic rates and CT_{max}, while a diet low in these nutrients can result in lower physiological
51 estimates [14–16]. Additionally, the resources a mother invests in her offspring (i.e., the energetic
52 provisioning of eggs) can influence metabolic processes like growth and development [17].
53 Determining how thermal and resource environments during development affect key thermal
54 physiological traits in various taxa may provide an understanding of how species are likely to
55 cope with changing environments.
56   While phenotypic plasticity can adjust phenotypes throughout life, developmental
57 plasticity – plasticity occurring during early embryonic development – can have organisational
58 effects on phenotypes that can affect responses later in life[6]. For vertebrates in particular, such
59 effects may be adaptive or maladaptive depending on whether early-life environments are
60 predictive of late-life environments. While temperature and early resource provisioning can
61 influence thermal traits in ectotherms [18], most research effort has focused on temperature, which
62 is known to have a profound effect on fitness [19,20]. In reptiles, temperatures during embryonic
63 development are known to affect phenotypes throughout ontogeny [7]. For example, incubation
64 conditions of developing reptile embryos can impact a variety of traits including sex, growth
65 rate, morphology, behaviour, and cognition[7,20,21]. However, there is a dearth of evidence linking
66 developmental factors more generally to thermal traits, and whether these differences persist
67 through various stages of ontogeny in reptiles[22,23].
68   Here, we aim to determine how early developmental environments affect thermal
69 physiology (critical thermal maximum: CT_{max} & thermal preference: T_{pref}) in reptiles. CT_{max} &
70 T_{pref} are two common thermal indices used as proxies for how the environment influences
71 individual fitness and are used to predict how species distributions are predicted to shift with
72 climate change [3,24,25]. We first conduct a laboratory experiment to test how maternal investment
73 and developmental temperature both influence CT_{max} & T_{pref} in a common skink (*Lampropholis*
74 *delicata*). We then compare our experimental findings with quantitative results testing this same
75 question more broadly in reptiles using a meta-analysis.
76 **Method and materials**
77 *(a) Consequences of incubation temperature and resource allocation on thermal physiology: an*
78 *experimental manipulation*

79   We collected gravid *Lampropholis delicata* (common garden skink, n = 100) from
80 populations in Sydney (Australia) and transported them back to the Australian National
81 University, where females were housed until eggs (n =40) were laid. We then pseudo-randomly
82 (to ensure equal sample sizes) assigned eggs (n = 20) to both a resource allocation treatment ('R'
83 - yolk removal or 'C' – control) and an incubation temperature (23°C or 28°C SD $\pm$ 3.0)
84 treatment (*See Supplementary materials for details on husbandry of hatchlings*). Egg incubation
85 temperatures were chosen to mimic conditions experienced at extremes of natural nest
86 temperatures in nature while also showing natural thermal fluctuations throughout the day [26].
87 Yolk removal treatments followed Sinervo[16], with 15-20% of the total egg mass being removed
88 via a sterilised syringe. Control treatments were punctured with the syringe without any yolk
89 removal. For further description of husbandry conditions of adults and incubation details, *see*
90 *Kar et al.*[28].
91   Hatchlings from their respective treatment were housed in mixed treatment groups of 5-6
92 within 20 L [40 cm (l) x 29.5 cm (w) x 20.5 cm (h)] plastic enclosures, with UVA/UVB lighting
93 and a 20W heat lamp in each enclosure. Water was provided *ad libitum*, with enclosures misted
94 daily. Lizards were fed calcium and vitamin-dusted crickets (*Acheta domesticus*) every second
95 day. At eight to eleven months post-hatching, lizards were selected at random, and thermal traits
96 (CT_{max} and T_{pref}) measured. Briefly, after undergoing a 24-hour fasting period, animals were
97 transferred into individual lanes of a thermal gradient (5°C to 55°C) to measure T_{pref}. A FLIR
98 T640 thermal camera was used to take thermal images of all lanes every 15-minutes over an
99 eight-hour observation period. T_{pref} was defined as the mean skin surface temperature (on the
100 neck) over the eight-hour observation period. Given the small size of lizards (i.e., 1.3 g) we
101 assumed skin surface temperature reflected body temperature, which has been shown for many
102 small lizards [29]. For CT_{max} we followed the same fasting period used for T_{pref} experiments. Here,
103 lizards were placed in falcon tubes in a water bath for 5 min at a temperature of 30°C. The water
104 temperature was increased to 38° C at a rate of 1° C/min. We used a control falcon tub with a
105 thermal couple attached to the bottom of the tub where lizards were positioned to record the
106 temperature of the tube surface, which we took to be the temperature experienced by the lizards.
107 This approach was needed because it was not possible to have a thermal couple in each lizards
108 Falcon tube when measuring righting responses in the CT_{max} procedure [30]. CT_{max} was defined
109 as the temperature at which an individual lost their righting reflex (for further details in
110 collection methods, *see Supp.*).
111   All statistical analyses were conducted using the R environment, ver. 4.1.0 (www.r.-
112 project.org). We used linear mixed-effects models to analyse thermal traits (T_{pref} and CT_{max}). We
113 constructed models that contained the main effects of body mass, sex, incubation temperature
114 and resource treatment. We also tested for the interaction between incubation temperature and
115 resource treatment (*see Supp. for more details*). If the interaction was not significant, we
116 removed it and presented the full main effects model.
117
118 *(b) Meta-analysis of early thermal effects on thermal physiology in reptiles*
119 To understand more broadly the impact of developmental environments on thermal physiology,
120 we systematically searched for studies manipulating early developmental environments and
121 subsequently measuring thermal physiological traits. Unfortunately, few studies manipulated egg
122 resource investment and measured thermal tolerance. As such, it was only possible to focus on
123 developmental temperature manipulations. Our meta-analysis collected data on offspring's

124 thermal preference (T_{pref}) and critical thermal maximum (CT_{max}) in lizards, snakes, tortoises,
125 turtles, and tuatara. Our search string included cold tolerance (i.e., critical thermal minimum,
126 CT_{min}), but there were too few studies that manipulated developmental environments and
127 measured this trait to conduct a formal meta-analysis. As such, we focus on T_{pref} and CT_{max}.
128 In brief, we conducted a systematic literature search in Scopus, ISI Web of Science (core
129 collection), and ProQuest (dissertations and thesis) and did not apply a timespan limit. We
130 followed the PRISMA-EcoEvo (Preferred Reporting Items for Systematic Reviews & Meta-
131 Analyses in Ecology and Evolutionary biology) guidelines for reporting[31]. Full search strings,
132 search methods, and selection criteria are described in detail in supporting information (Figs.
133 S1&2). We obtained 485 original records, and 15 articles satisfied our selection criteria.
134 Multilevel meta-analytic (MLMA) models were constructed using the *rma.mv* function in
135 the *metafor* package (version 3.8)[32]. To determine the ability of an organism to acclimate to
136 changes in the environment, we used the acclimation response ratio (ARR) as our effect size [33].
137 Sampling variance for the ARR was derived in Pottier et al.,[34]. Study, phylogeny, and study
138 species were designated as random effects and we included an observation-random effect (effect
139 size ID). A model that included only study, species and effect size ID was best supported over
140 one with phylogeny, so we present meta-analytic results from a model without phylogeny.
141 Studies often had more than two temperature treatments. As such, we derived all pairwise effect
142 size comparisons. This, however, does induce a correlation between effect size sampling errors ,
143 which we controlled for through the inclusion of a sampling (co)variance matrix derived by
144 assuming effect sizes are correlated by r = 0.5[35]. Thermal trait (T_{pref} or CT_{max}), life stage at
145 measurement (hatchling, juvenile or adult), climate zone (temperate or tropical), and major
146 taxonomic group (lizard, snake, tuatara or turtle) were included as fixed factors in separate multi-
147 level meta-regression (MLMR) models. We also tested for publication bias using a MLMR
148 model with sampling variance and standard error as predictors [36] and was visually inspected
149 using a funnel plot (*see Supp. for more details*). We present effect size heterogeneity by
150 constructing prediction intervals [37] and presenting I^2 using the *orchaRd* package (version
151 2.0)[38].
152 **Results**
153 *a)Incubation temperature and resource allocation consequences on thermal preference and*
154 *critical thermal maximum*
155 Mean T_{pref} was 31°C ±0.47 (mean ±SE) and ranged from 20.99–34.26°C. Mean CT_{max} was
156 43.04°C ±0.23 and ranged from 38.6–45.2 °C. We did not detect any effect of incubation
157 temperature, yolk treatment, sex, or body mass on T_{pref} or CT_{max} (Figure 1A|B; Table 1).
158
159 *(b) Meta-analysis of early thermal effects on thermal physiology in reptiles*
160 Across reptiles, developmental temperatures did not influence thermal traits (T_{pref} or CT_{max}), but
161 heterogeneity was high (ARR =0.05, 95% CI:-0.28-0.37; I^2_{Total} = 99.53%, Prediction Interval: -
162 1.23-1.32; Fig. 2A, n = 69 effects from 14 species). Overall, we found no evidence for
163 publication biases (β=-0.81, 95%CI=-1.92-0.3, *p=0.15*; Fig S3; for further details see electronic
164 supplementary materials). Species effects ($I^2_{Species}$ = 70.57%) drove most of the heterogeneity in
165 ARR, but thermal traits were not influenced by life stage, climate zone, or major taxonomic
166 group (i.e., snakes, turtles, lizards) (Fig. 2B|C). While there was a significant increase in thermal
167 traits in snakes (Fig 2D), this was driven by a single species (*Nerodia sipdedon*), and given the
168 small sample sizes, we need to caution whether any true differences between snakes and other
169 groups exists.

Discussion

170 Genetic adaptation and phenotypic plasticity are two hypotheses for how ectotherms can cope
171 with warming temperatures associated with anthropogenic climate change [3,39–41]. Plastic
172 responses occurring early in development can have long-lasting effects on organisms, with
173 significant implications for how they cope with environmental stressors.
174
175   We show that early developmental environments do little to modify thermal
176 physiological traits (CT_{max} & T_{pref}) in most reptile taxa. Both our experimental and meta-analytic
177 approaches suggest that the magnitude of developmental plasticity on thermal indices appears to
178 be canalised across reptile taxa. For example, our meta-analysis indicated that for every 1°C
179 change in developmental temperature, we only expect a 0.05°C change in thermal physiology.
180 Our findings are consistent with those of other ectotherm systems, which show that
181 developmental plasticity has little impact on adult heat tolerance [6,42–44]. Nonetheless, we
182 detected significant species-specific heterogeneity ($I^2_{Species}$= 70.57%), suggesting substantial
183 differences across species that cannot be ignored. Such variability may be driven by species
184 differences in micro-habitat selection of nests or nesting phenology in the wild and whether
185 developmental conditions in the field corroborate with conditions chosen for laboratory
186 experiments. It has been indicated in other studies[45–48] that differences in nest depth, nest
187 location, clutch density or maternal condition may select for developmentally plastic responses
188 in offspring. Together, these data highlight that further ecological data on developmental
189 environments in nature is needed to test if static manipulations in the lab provide a functional
190 link to how species can cope with environmental change.
191   While there are still limited empirical studies, across reptile taxa, plasticity in thermal
192 physiology did not differ by age, taxon or climate zone. We expected that the earlier age at
193 which thermal traits were measured would be more likely to detect effects of early environments.
194 In addition, tropical species are expected to maintain body temperatures near their thermal limits,
195 and an increase in temperature can push these species to physiological extremes compared to
196 temperate species [3,41,49]. Greater thermal variability in temperate regions should select for
197 greater plasticity. However, our meta-analysis does not support these hypotheses. Instead, the
198 microthermal environments and behavioural flexibility may be a more important driving
199 mechanism as to whether species respond plastically to developmental environments or not [3,50].
200 Future studies looking at the autocorrelation between early and late developmental environments
201 would be fruitful in helping elucidate species-specific responses to thermal environments.
202 Overall, our results suggest that most reptiles may have limited developmental plasticity in
203 thermal traits, relying instead on energetically expensive behaviours (i.e., thermoregulation) [3,51]
204 or responses that operate on slower time scales (i.e., local adaptation) [40,52]. Given the small
205 effect sizes we observed, statistical power is likely an issue in ours and others' empirical work.
206 However, ethical constraints in measuring thermal limits in large numbers of animals will mean
207 such studies are likely to be common. As such, we will need to rely on meta-analysis to help
208 circumvent power limitations in individual studies (as we have done here)[53]. We have also
209 identified clear gaps in the literature that should help pave the way for future research. First, we
210 encourage measuring thermal physiology under different developmental manipulations across a
211 greater diversity of reptile taxa. Greater taxonomic diversity will clarify when developmental
212 environments matter and allow us to explore reasons for this heterogeneity. Second, we
213 encourage measuring CT_{min}, in addition to other thermal physiological traits (i.e., CT_{max}, T_{Pref},
214 etc) as it is often more environmentally flexible than upper thermal limits. Despite these gaps,

215   our results provide valuable insights into possible responses that are plausible under changing
216   thermal conditions.

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217

218

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396   **Tables & Figures**

397   Table 1. Model outputs coefficients for testing wither sex, body mass, incubation temperature,
398   resource, or the interaction between resource and temperature had an effect on T_{Pref} or CT_{Max} in
399   hatchling *Lampropholis delicata*. Est. value describes the estimated coefficient value and 95% CI
400   describes the lower and upper bound of the 95% credible interval for each coefficient value.
401   Intercept is the estimated mean of each thermal trait from the null model.

| Thermal Index	Covariate	Estimate	l-95% CI	u-95% CI	p value
Tpref	**(Intercept)**	**30.94**	**28.67**	**33.20**	**0.00**
	Body Mass	0.44	-0.97	1.86	0.53
	Sex	0.30	-2.50	3.09	0.83
	Incubation Temperature	-0.35	-2.36	1.66	0.72
	Resource	0.19	-1.83	2.20	0.85
	Incubation Temperature*Resource	-0.22	-4.31	3.87	0.91
CTmax	**(Intercept)**	**43.27**	**42.17**	**44.37**	**0.00**
	Body Mass	-0.41	-1.08	0.25	0.21
	Sex	-0.03	-1.35	1.28	0.96
	Incubation Temperature	-0.18	-1.14	0.78	0.70
	Resource	-0.24	-1.20	0.71	0.61
	Incubation Temperature*Resource	-0.52	-2.47	1.44	0.59

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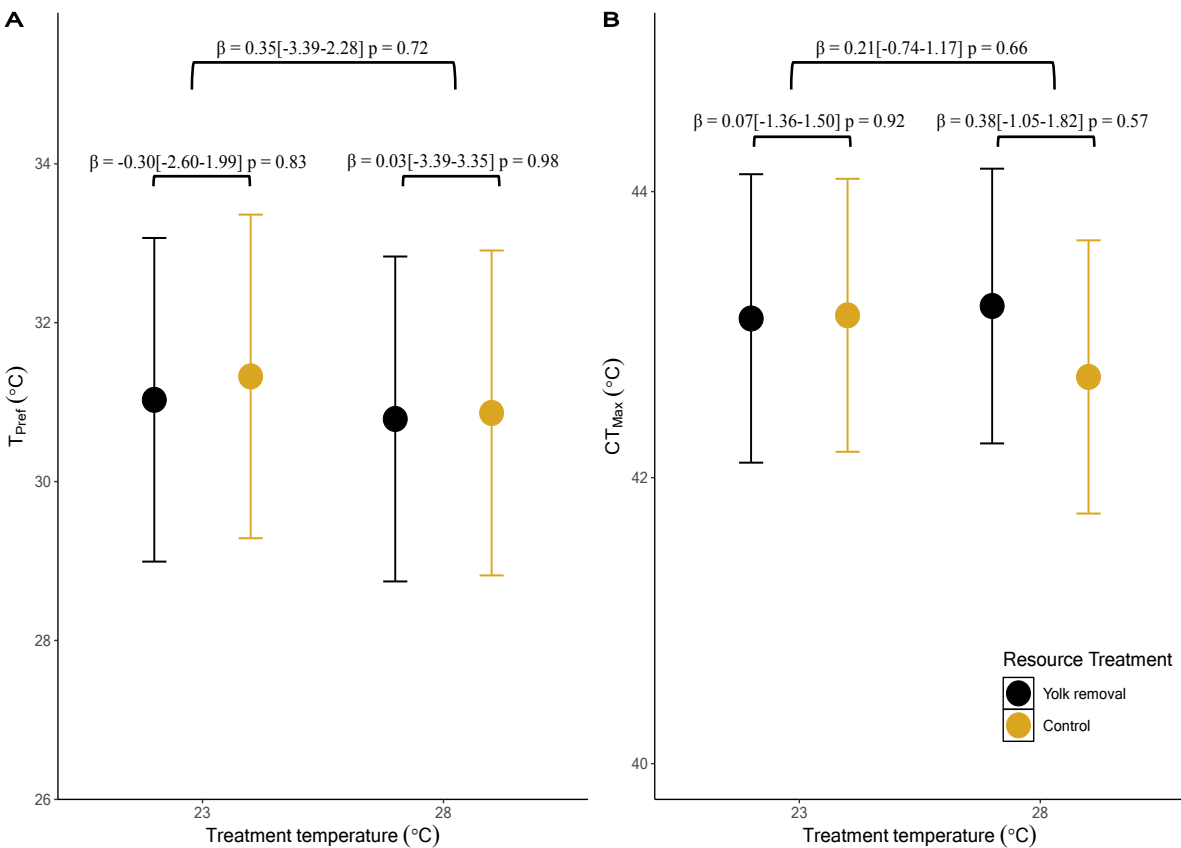
405 Figure 1. Thermal indices across different incubation temperatures and resource treatments for
406 hatchling *Lampropholis delicata* (n=10 per temperature and treatment). (A) Thermal preference
407 (T$_{pref}$) in lizards incubated at 23 & 28°C for each resource treatment (yolk ablation & control).
408 (B) Critical thermal maximum (CT$_{max}$) in lizards incubated at 23 & 28°C for each resource
409 treatment. Bars above plots indicate pairwise comparisons of thermal indices between treatment
410 temperature and the interaction between treatment temperature and resource treatment. Means
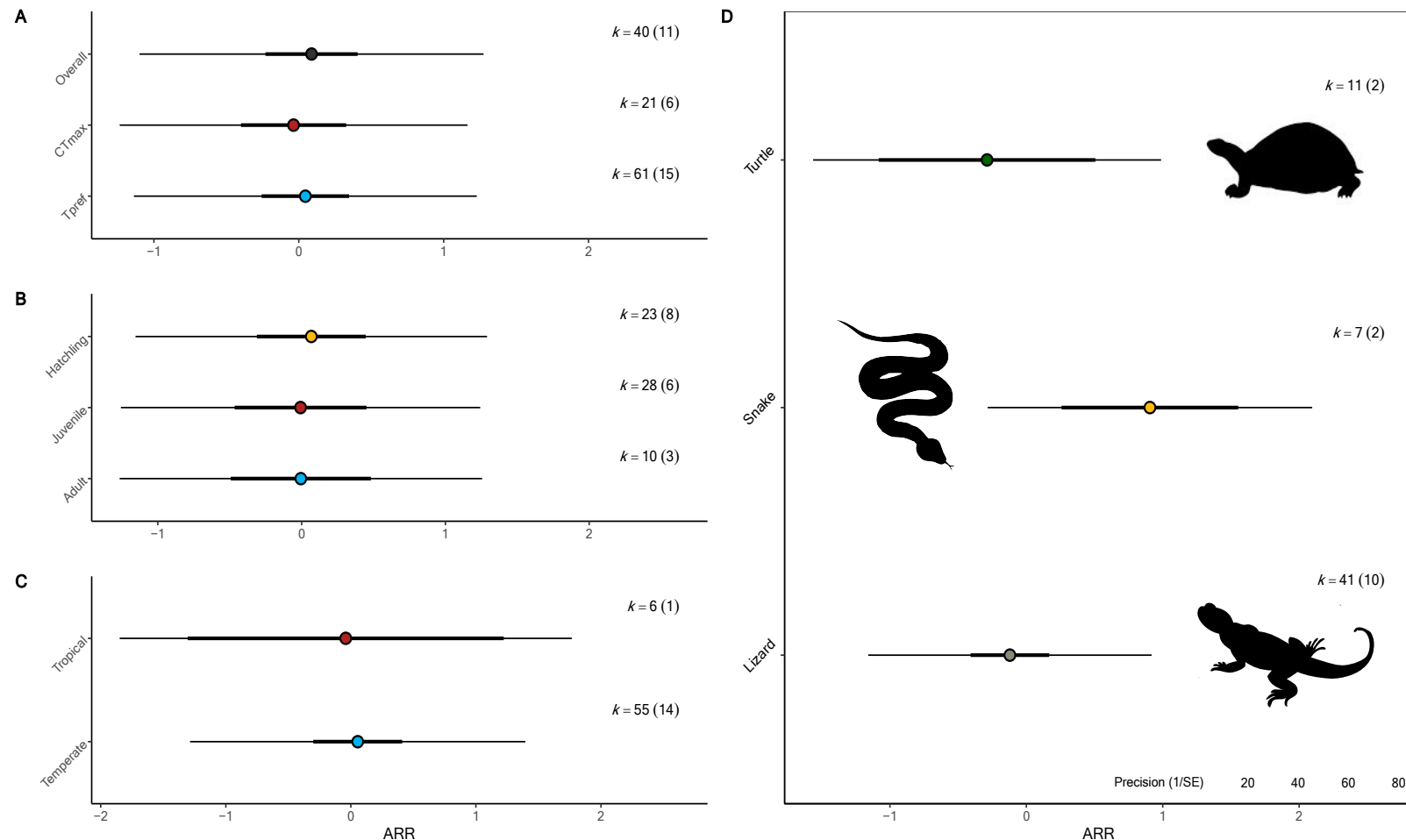411 and 95% confidence intervals are provided along with the *p*-value for each contrast.
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414 Figure 2. The magnitude of the effect of developmental temperature on thermal indices (T_{pref} & CT_{max}) in reptiles (A) concerning
415 age class of thermal physiological measurement (B), climate zone (C), and taxon (D). Mean meta-analytic ARR estimates (circles)
416 with their 95% confidence intervals (thicker error bars) and prediction intervals (thinner error bars). Data points from each study from
417 the meta-analysis are scaled by precision (inverse of standard error), and k is the number of effect sizes with the number of species in
418 brackets. ARR is the acclimation response ratio. 95% confidence intervals not overlapping 0 are statistically significant. Graphs were
419 constructed using the *orchaRd* package[54]. Tuatara was removed for visual purposes due to the small number of effect sizes (n=3)

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