Running head: Incubation of TSD Species

Title: Artificial incubation in species with temperature-dependent sex determination. How hot is too hot?

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Abstract

Reptiles are declining globally, and turtles in particular are among the most imperiled group of vertebrates on earth. Common turtle conservation strategies include artificial incubation and 'head-starting' to rear healthy juvenile turtles for release into wild populations. Temperature-dependent sex determination (TSD) in turtles and other reptiles allows conservation managers to bias sex ratios towards females by using high incubation temperatures, allowing the introduction of more egg-bearing individuals into populations. However, theory and data suggest high incubation temperature may potentially degrade phenotypic quality, and therefore investigation into incubation temperature and fitness outcomes is warranted for species with TSD. We performed a meta-analysis to explore the relationship between hatchling mortality, constant temperature incubation, and pivotal temperature (TPiv, the temperature where the sex ratio is 1:1). We found models incorporating species-specific TPivs better explained hatchling mortality data than models without species-specific information, suggesting TPivs exhibit a correlated evolution with embryonic thermal tolerance. Moreover, we found embryo mortality followed a parabolic distribution centered near the pivotal temperature, and critically, depreciation of fitness was relatively rapid above vs below the pivotal temperature. These results suggest artificial incubation regimes may benefit from avoiding high-temperature incubation. We suggest that fluctuating temperature regimes are easily implemented and are representative of the environmental context in which turtles evolved over millions of years. Further, recent models allow the prediction of sex ratio under fluctuating regimes, thereby allowing managers to maintain sex ratio bias of wild releases without compromising phenotypic quality.

Key words: pivotal temperature, reptiles, mortality, hatchling, population growth, conservation

Introduction

Despite recent and widespread interest in reptile conservation (Roll et al. 2017), reptile populations are declining globally (Todd et al. 2010). Turtles in particular are among the most imperiled group of vertebrates in the world (Rhodin et al. 2018; Gibbons & Lovich 2019), and are naturally susceptible to declines due to low genetic diversity (Romiguier et al. 2014) coupled with high juvenile mortality and slow life histories (e.g., Kondo, Morimoto, Sato, & Suganuma, 2017). Elevated adult mortality of turtles arising from road collisions (Steen & Gibbs 2004), fishing gear entrapment (Lewison et al. 2004; Bolten et al. 2011), predation (Bolten et al. 2011), and direct consumption as a food source (Conway-Gómez 2007; Hancock et al. 2017), has therefore resulted in dramatic population declines, as natural rates of replacement and population growth are low, and populations are unable to adapt to environmental change on the time scale of anthropogenic impacts (Hawkes et al. 2009). At present, 56.3% of data sufficient species are considered critically endangered, endangered, or vulnerable by the International Union for Conservation of Nature (Rhodin et al. 2018).

There is now widespread interest in turtle conservation (Gibbons & Lovich 2019), and common initiatives include protecting nesting turtles from natural predation and poachers (Eckert et al. 1999), rehabilitation programs at trauma centers (Feck & Hamann 2013), and education initiatives (Hassan et al. 2017). Another common initiative undertaken by zoos, parks, conservation authorities, and even private individuals, involves incubation of turtle eggs in an ex-situ setting followed by hatchling or juvenile release, ultimately protecting eggs from predation, poaching, and climatic uncertainty (Eckert et al. 1999). This practice, which is called “artificial incubation”, typically involves removing eggs from natural nests, placing the eggs in incubators, and allowing eggs to hatch under a specified incubation regime (Páez et al.

2015). After hatching, juveniles are either released immediately, or are ‘head-started’, which involves raising individuals to larger body sizes before release to increase survival in the wild (Kingsolver & Pfennig 2004; Kingsolver 2009). While some studies suggest head-starting can be beneficial (Cunnington & Brooks 1996; Crowder & Heppell 2011; Mullin 2019), the long-term benefits of head starting or often unclear or negligible (Páez et al., 2015; Spencer, Van Dyke, & Thompson, 2017).

The head-starting process assumes protecting eggs during incubation and rearing juveniles to a larger size will give juveniles a survival advantage compared to their non-reared counterparts (Mullin 2019). Although head-starting programs vary in the size and age of juveniles at release, it is notable that survival of wild hatchlings and juveniles is very low (Heppell 1998). Therefore, a very large effort may be needed to release a quantity of juveniles sufficient to impact recruitment rates. Any practice that increases survival of individuals released into the wild would likely increase the long-term benefits of head starting, and would therefore be desirable.

Incubation temperatures experienced by embryos may represent an important axis of variation with respect to performance of wild-released hatchlings or juveniles. This is because incubation temperature has profoundly impacts many aspects of hatchling phenotypes (Noble et al. 2018b), as well as life-history traits such as survival (Bobyn & Brooks 1994a, 1994b). There is, however, essentially no primary literature how artificial incubation is performed as a component of wild-release and head-starting practices, suggesting that artificial incubation regimes are unstandardized and may not resemble natural incubation regimes. For instance, by introducing embryos to evolutionarily novel environments, such as constant temperatures throughout incubation, it is possible artificial incubation regimes inadvertently degrade the

quality of juvenile phenotypes released into the wild. If this is the case, then juvenile mortality would likely be exacerbated after release, relative to a more naturalized incubation regime (Páez et al. 2015; Mullin 2019; Gibbons & Lovich 2019).

If artificial incubation programs are geared toward conservation, then presumably artificial incubation regimes fall within a normal range of incubation temperatures experienced by the focal population. Under a constant temperature incubation, for example, intermediate temperature leads to a relatively high performance and survival compared to high temperature incubation (Fisher et al. 2014; Noble et al. 2018b). Yet, despite the benefits of intermediate incubation temperatures, there may be strong incentive to incubate embryos at relatively elevated temperatures, perhaps to the detriment of embryos. This is because a major goal of conservation programs is ultimately to increase the number of wild births in the population, and births are more strongly related to female than male abundance (Girondot et al. 2008). Most turtles exhibit temperature-dependent sex determination (TSD), where relatively warm temperatures result in an over-production of female hatchlings (Ewert et al. 1994). Additionally, raising incubation temperature results in shorter incubation time, potentially reducing the expense associated with husbandry (Du et al. 2006). Yet, conservation managers are not able to weigh the costs and benefits of implementing high incubation temperatures, primarily because no study has systematically examined the relationship between hatchling phenotypes and incubation temperature in the context of female production and TSD. Our study therefore assesses how incubation environments affect offspring quality across many species.

In the present study, we explore the fitness consequences of artificial incubation regimes in turtles, with the goal of informing best practices for artificial incubation programs geared towards conservation. We explicitly examine the relationship between embryonic mortality with

respect to species-specific or population-specific pivotal temperatures (TPiv), which is the species- or population-specific constant temperature at which the sex ratio becomes 1:1 (Bull 1980; Ewert et al. 1994). We hypothesize that embryo physiology and thermal tolerance exhibit a correlated evolution with TPiv. Therefore, we predict that (1) mortality will be better explained by the difference between incubation temperature and TPiv compared to raw incubation temperature (i.e., the actual incubation temperature), and (2) mortality will be positively associated with the difference between incubation temperature and species-specific (or population specific) TPiv. Finally, recent theory suggests TPivs evolve to be closer to the upper critical thermal maximum than the lower critical thermal maximum. We therefore predict that (3) mortality will increase relatively quickly above vs below TPiv. For conservation managers, the present study emphasizes that the TPiv should act as a thermal reference point for phenotypic outcomes under constant temperature incubation. We use mortality as a proxy for negative phenotypic outcomes, as mortality is a commonly measured trait and it represents the maximum fitness loss that can be associated with a stressor.

Methods

Data collection

Data was collected from previously published studies collated in the Reptile Development Database (Noble et al. 2018a). Studies from this database were included in the present study if they featured (1) a TSD turtle species (Sabath et al. 2016), (2) artificial incubation under constant temperature, (3) embryonic mortality (or survival) specific to a temperature treatment, and (4) the number of embryos comprising the sample. Data collected from studies fitting inclusion criteria comprised of species, incubation temperature, pivotal temperature, percent mortality per incubation regime, ~~sample size (~~number of eggs incubated),

and location of egg collection. Exclusion factors included genetic sex determined species (GSD), In situ incubation, or fluctuating temperature trials. In studies including both TSD and GSD species, and studies including both constant and fluctuating incubation trials, only TSD and constant trial results were extracted. Trials involving temperature shifts were also excluded. Several studies report mortality results in figures and do not specify results textually; in these cases we extracted data from figures using WebPlotDigitzer (Rohatgi 2019). We also extracted all available information on substrate moisture; however, moisture was only measured directly in ten studies. Notably, all studies either reported moisture values or maintained moisture at approximately constant levels across the incubation regime; further, the few studies that manipulated moisture and temperature did so in a factorial manner. It is therefore unlikely that moisture was confounded with temperature in the present study.

Ultimately, 29 studies in the Reptile Development Database fit inclusion criteria for the current study (Table A1). In total, 202 data points were collected on a total of 15 different species in 6 different families of turtle, representing 43% of known taxonomic families.

Pivotal temperatures

In cases where species exhibit female-male-female (Type II TSD) sex determination the upper pivotal temperature was used for analyses, since high temperature incubation is the focus of this study. Papers which did not explicitly state the species-specific or population-specific pivotal temperature were supplemented with pivotal temperatures from geographically similar populations of the same species, as pivotal temperature can be both species dependent and geographically dependent (Bull 1980; Ewert et al. 1994). Literature searches were performed to find pivotal temperatures of species using the species name, ‘pivotal temperature’, and ‘sex ratios’. Species-specific pivotal temperatures were found, and the closest geographically

available population pivotal temperature found was used as the pivotal temperature. In total, thirteen studies required supplementation of pivotal temperatures from previously published literature (Table A1).

Data Analysis

To test Prediction 1 (i.e., mortality will be better explained by the difference between incubation temperature and TPiv compared to raw incubation temperature) and Prediction 2 (i.e., mortality will increase with increasing deviation of incubation temperature from species-specific pivotal temperatures), we generated plausible hypotheses for how incubation temperature may affect mortality and expressed these hypotheses as statistical models. First, mortality may simply be a function of absolute incubation temperature. Second, if TPiv evolves to match local incubation regimes, then mortality may be better explained by the difference between TPiv and the incubation temperature, rather than absolute incubation temperature. Finally, regardless of which of the above hypotheses is correct, temperature may be linearly or non-linearly related to temperature. In sum, we compared four models: two hypotheses regarding which temperatures affect mortality crossed with two hypotheses regarding the form of the relationship between temperature and mortality. We fit four mixed effect models using maximum likelihood estimation, where mortality values were weighted by sample size of embryos in each treatment. Models took the form:

1.1) $Y_{ij} = \beta_0 + \beta_1 \text{Raw.Inc}_{ij} + \gamma_j + \varepsilon_{ij}$

1.2) $Y_{ij} = \beta_0 + \beta_1 \text{Raw.Inc}_{ij} + \beta_2 \text{Raw.Inc}_{ij}^2 + \gamma_j + \varepsilon_{ij}$

1.3) $Y_{ij} = \beta_0 + \beta_1 \text{Dev.TPiv}_{ij} + \gamma_j + \varepsilon_{ij}$

1.4) $Y_{ij} = \beta_0 + \beta_1 \text{Dev.TPiv}_{ij} + \beta_2 \text{Dev.TPiv}_{ij}^2 + \gamma_j + \varepsilon_{ij}$

Where *Raw.Inc* represents the fixed effect of absolute incubation temperature, *Dev.TPiv* is the difference between the incubation temperature and the pivotal temperature, *Y* represents logit-transformed mortality, ε_{ij} represents error, γ_j represents the random intercept of Study ID, β represents parameters to be estimated. Preliminary modelling revealed the random intercept of *Species* was associated with very little variance, so *Species* was not modelled (and results are qualitatively the same if *Species* is included). Analysis was performed in R using the function 'lmer' from the package 'lme4' in R (Bates et al. 2015). The four models were compared using second order Akaike Information Criterion (AIC_c) and Akaike weights (w_i) within the package 'AICmodavg' in R (Fabozzi et al. 2014; Mazzerole 2019). For visualization only, a generalized additive model (GAM) was fit to these data, using the 'gamm' function from the R package 'mgcv' (Wood 2004). Values were predicted using 'predict.gam' function from the package 'mgcv' and then back transformed from logit data (Wood, 2000; Wood, 2003)

To test Prediction 3 (i.e., mortality will therefore increase relatively quickly above vs below TPiv), we used maximum likelihood estimation to compare three models exploring whether the rate at which mortality accrued is different above versus below TPiv. Instead of assigning a sign (i.e., -ve vs +ve) to *Dev.TPiv*, we took the absolute value of *Dev.TPiv* and created a new categorical factor with two levels, "Above" or "Below", which identified whether the datapoint was from above or below the TPiv. Mortality values were weighted by sample size of embryos in each treatment, and models took the form,

2.1) $Y_{ij} = \beta_0 + \beta_1 \text{Abs.Dev.TPiv}_{ij} + \beta_2 \text{Abs.Dev.TPiv}_{ij}^2 + \gamma_j + \varepsilon_{ij}$

2.2) $Y_{ij} = \beta_0 + \beta_1 \text{Abs.Dev.TPiv}_{ij} + \beta_2 \text{Abs.Dev.TPiv}_{ij}^2 + \beta_3 \text{Direction}_{ij} + \gamma_j + \varepsilon_{ij}$

2.3) $Y_{ij} = \beta_0 + \beta_1 \text{Abs.Dev.TPiv}_{ij} + \beta_2 \text{Abs.Dev.TPiv}_{ij}^2 + \beta_3 \text{Direction}_{ij} + \beta_4(\text{Direction x Abs.Dev.TPiv})_{ij} + \gamma_j + \varepsilon_{ij}$

Where *Abs.Dev.TPiv* is the absolute value of *Dev.TPiv*, and *Direction* is a two-level categorical variable identifying whether *Abs.Dev.TPiv* is from above or below the TPiv. Models were compared using w_i and AICc, as above.

Results

We tested the prediction that (1) mortality will be better explained by the difference between incubation temperature and TPiv (Dev.TPiv) compared to raw incubation temperature (Raw.Inc), and (2) mortality will increase with increasing deviation of incubation temperature from species-specific (or population specific) pivotal temperatures in a U-shaped fashion. We found overwhelming support for model 1.4, supporting both predictions (w_i = 1.0, Tables 1, 2). Visualization of the relationship between mortality and Dev.TPiv, using a generalized additive model, shows a concave-up relationship centered near the pivotal temperature (Figure 1). Next, we tested the prediction that mortality will accrue relatively rapidly above vs below TPiv. We found strong support for model 2.3 (w_i = 0.99), the only model that allows the slope of mortality vs Abs.Dev.TPiv to differ above vs below TPiv; the direction of the relationship supports the prediction that mortality accrues relatively rapidly above TPiv (Figure 2). Of note, is that a temperature of 3.2 – 3.5°C above the pivotal temperature is associated with 50% clutch mortality (depending on the model and analysis, Figures 1, 2), while 50% mortality occurs 5.2 – 5.5°C below the pivotal temperature.

Discussion

The present study communicates three major findings. First, we show embryonic mortality is positively related to the difference between incubation temperature and species-

specific TPiv, and that raw incubation temperature values explain mortality patterns relatively poorly. Second, and perhaps most importantly, we demonstrate negative fitness outcomes (i.e., mortality) accrue relatively rapidly above the TPiv, compared to below the TPiv, which has broad ecological and evolutionary implications. Finally, we also provide the first quantitative, multi-species analysis supporting the notion that embryonic thermal tolerance experiences a correlated evolution with TPiv. Below we explore these results and emphasize their significance in ecology, evolution, and conservation.

The overarching message communicated by the present study is that a constant temperature used to produce females is likely associated with thermal stress. Embryonic mortality was lowest within 1-2 °C of TPiv, but then began to increase with increasing temperature difference from TPiv; this occurred much more rapidly above TPiv than below TPiv. Importantly, stress can be defined as a condition disrupting normal functions or decreasing fitness, and it is critical to note the current study's measure of stress (mortality) represents the most extreme outcome possible with respect to fitness, and is thus a conservative measure of stress. Indeed, mortality suggests the inability to persist, let alone reproduce (Kingsolver 2009). Thermal stress can be induced at lower temperatures which do not necessarily induce mortality but may induce phenotypic morbidity (Refsnider et al. 2015; Cavallo et al. 2015; Kingsolver & Woods 2016). For example, previous studies suggest warm temperatures lead to developmental instability (Noble et al. 2018b), and indeed, several studies included in this study report abnormalities in hatchlings reared in high temperature incubation trials (Packard et al. 1987; Zhu et al. 2006). We suggest that the increase in mortality associated with extreme incubation conditions are associated with phenotypic degradation among the surviving hatchlings, and as such represent sub-optimal incubation environments.

The relatively rapid accumulation of negative phenotypic outcomes above the TPiv has interesting ecological and evolutionary implications. Mechanistically, fitness may depreciate rapidly above the TPiv because TPiv evolves toward the optimal incubation temperature, and reaction rates for most or all processes changes more rapidly above than below the optimum temperature (Sharpe & DeMichele 1977; Kingsolver 2009). Indeed, the lowest level of mortality observed in the present study was just below TPiv, suggesting TPiv often falls near (or just above) a narrow and intermediate thermal range that is optimal for development (Stubbs & Mitchell 2018). One interpretation of our results, therefore, is that female-producing temperatures coincide with a departure from optimal incubation temperatures, which has a strong bearing on the evolution of TSD. Indeed, despite decades of theory and experiments, there is no single explanation that can account for the evolution and maintenance of TSD in reptiles (Janzen and Philips 2006). A minority of previous work suggests the evolution and maintenance of TSD is related to the relative quality of male-producing vs female-producing environments (e.g., Ewert et al., 1994). Indeed, there are theoretical grounds to suspect that males produced in relatively high-quality environments, such as those with low thermal stress, provide greater fitness returns than females produced in the same environment (Trivers & Willard 1973). The present study is therefore consistent with an explanation in which temperatures that are slightly below the TPiv provide relatively high-quality environments, and hence high-quality phenotypes that are male. Thus, our work invigorates an exploration of how classical models in evolutionary ecology (e.g., Trivers – Willard) can be incorporated into theories of TSD.

Whether the negative phenotypic effects of high temperature are persistent was not addressed herein, but is crucial to the thesis we present. Although mortality is used as the response variable in the current study, several studies used in the present work included

measurements of post-hatching fitness and mortality. Fitness measures included both growth and performance metrics. Within the studies included in the current study, thirteen included a measure of post-hatching phenotype, encompassing a total of twenty-three discrete measurements (Table 3). Fifty-two percent of the measurements indicated a significant temperature effect, and the direction of the effect between studies was variable (Table 3). Due to the heterogeneity of the papers and fitness metrics, and the multi-level comparisons within some studies, a formal meta-analysis was not possible. Although long-term studies of lab-reared hatchling turtles are uncommon, our inclusion criteria for the present study likely resulted in the exclusion of many studies examining post-hatching growth or performance. It may prove fruitful for future research to formally examine post-hatching growth and performance under different incubation regimes, as impacts of temperature stress may be observed at a variety of life stages (Jonsson et al. 2014).

More broadly, the results of our study suggest incubating near the pivotal temperature may result in the highest hatching success and perhaps less thermal stress, suggesting this will result in better phenotypes for release into wild populations. If head-starting is to be used, the results of this study indicate incubation methods for conservation must be reviewed to prevent the development of low-quality phenotypes which may in turn have a negative effect on population growth rate. While constant temperature incubation is common for both conservation and research, further research should investigate the use of fluctuating incubation regimes, which is more similar to environments in natural nests. When constant and extreme incubation temperatures are used, such as high temperatures relative to TPiv, cellular processes can become impaired (Niehaus et al. 2012). If fluctuating temperatures are used, optimal temperatures for cellular processes will be experienced during fluctuation periods, with short incursions to

suboptimal temperatures likely inflicting less damage than constant chronic incubation at suboptimal temperatures (Niehaus et al. 2012). Recent advances in technology, such as low-cost methods of controlled temperature fluctuation (Greenspan et al. 2016), facilitate the study and implementation of incubation environments that more closely resemble natural environments (Bowden et al. 2014). Further, recent advances allow the sex ratio of a clutch to be accurately predicted under fluctuating temperatures (Carter et al. 2018; Massey et al. 2019), such that conservation managers can impose fluctuating regimes both easily and without compromising desired (female-biased) sex ratios.

Meta-analyses are able to identify large-scale patterns, and ultimately help strengthen evidence-based decision-making (Stewart 2010). Indeed, it is important for conservation managers to periodically review methodologies used for species recovery, and with the substantial increase in reptile conservation organizations since the 1990s, there are likely many different initiatives that leverage artificial incubation (Gibbons & Lovich 2019). The present study implies that the method of artificial egg incubation may be an important source of variation in phenotypic quality. More broadly, however, there is little published literature on formal methods of artificial egg incubation, and as such there is little to no formal discussion or exchange of information with respect to incubation methodologies. We suggest that head-starting programs may benefit from avoiding high temperature incubation environments, and that the same outcomes with respect to sex can be achieved under fluctuating temperature regimes.

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Tables

Table 1 Model selection results for four models comparing the relationship between mortality and incubation temperature.

Models	*K*	$\Delta AICc$	w_i	*LL*
Mortality ~ (deviation from pivotal temperature)^2	*5*	*0.00*	*1*	*-340.8*
Mortality ~ (incubation temperature)^2	*5*	*50.63*	*0*	*-366.1*
Mortality ~ incubation temperature	*4*	*91.16*	*0*	*-387.5*
Mortality ~ deviation from pivotal temperature	*4*	*91.16*	*0*	*-387.5*

*Study is included as a random effect in all models

Table 2 Summary statistics of model 1.4 (refit with REML), the top-ranked model.

Parameters	Effect type	Estimate[a]	SE[b]
Study	Random	1.36	-
Residual	Random	25.8	-
Intercept	Fixed	-1.392	0.238
Deviation from TPiv	Fixed	0.174	0.031
(Deviation from TPiv)^2	Fixed	0.081	0.007

[a]Estimates for random effects are variance estimates

[b]Standard error of the estimate

Table 3 Post-hatching performance and growth temperature effect for papers included in the current study.

Species	Trait	Effect	Is cooler better?	Study
Chinemys reevesii	Condition - body condition	No	-	Du et al. (2009)
Chinemys reevesii	Condition - body shape	No	-	Du et al. (2009)
Maureymys mutica	Condition - size	No	-	Zhao et al. (2015)
Maureymys mutica	Condition - size	Yes	No	Zhao et al. (2015)
Malaclemys terrapin	Growth	Yes/No *	-	Roosenburg and Kelley (1996)
Chrysemys picta	Growth	No	-	Les et al. (2009)
Trachemys scripta	Growth	No	-	Les et al. (2009)
Emys marmorata	Growth	No	-	Geist et al. (2015)
Ocadia sinensis	Growth	No	-	Du et al. (2010)
Chinemys reevesii	Growth	Yes**	-	Du et al. (2009)
Chinemys reevesii	Growth	Yes**	-	Du et al. (2007)
Gopherus agassizii	Growth	Yes	Yes	Spotila et al.(1994)
Mauremys mutica	Growth	Yes	Yes	Du et al. (2010)
Chelonia mydas	Growth	Yes	Yes	Booth et al. (2004)
Gopherus Polyphemus	Growth	Yes***	-	Demuth (2004)
Chelydra serptenina	Growth - mass	Yes	Yes/No****	Bobyn and Brooks (1994)
Podocnemis lewyana	Growth - straight line carapace	Yes	Yes	Paez et al. (2009)
Gopherus Polyphemus	Performance - crawl speed	No	-	Demuth (2001)
Maureymys mutica	Performance - righting response	No	-	Zhao et al. (2015)
Mauerymys mutica	Performance - righting response	Yes	No	Zhao et al. (2015)
Caretta caretta	Performance - righting response,	Yes	No	Fisher et al.

	crawl test, swim test			(2014)
Chinemys reevesii	Performance - swimming ability	No	-	Du et al. (2009)
Chelonia mydas	Performance - swimming trials	Yes	No	Booth et al. (2004)

*Not able to determine if due to temperature or due to sex

** Temperature effect gone after three months

**Temperature effect gone after 271 days

****May be due to sexual dimorphism, intermediate (male temperature) led to larger

Figures

Figure 1: GAM representation of model 1.4, the top ranked model predicted values in black with 95% confidence intervals in shaded grey. Points represent raw mortality data collected from previously published literature (Table A1) as a function of the difference between species TPiv and incubation temperature.

Figure 2 Relationship between logit clutch mortality and absolute incubation temperature deviation above vs below species-specific pivotal temperatures. A value of 0 on the y-axis represents 50% mortality. Fits are from model 2.3, refit with REML estimation.

Figures

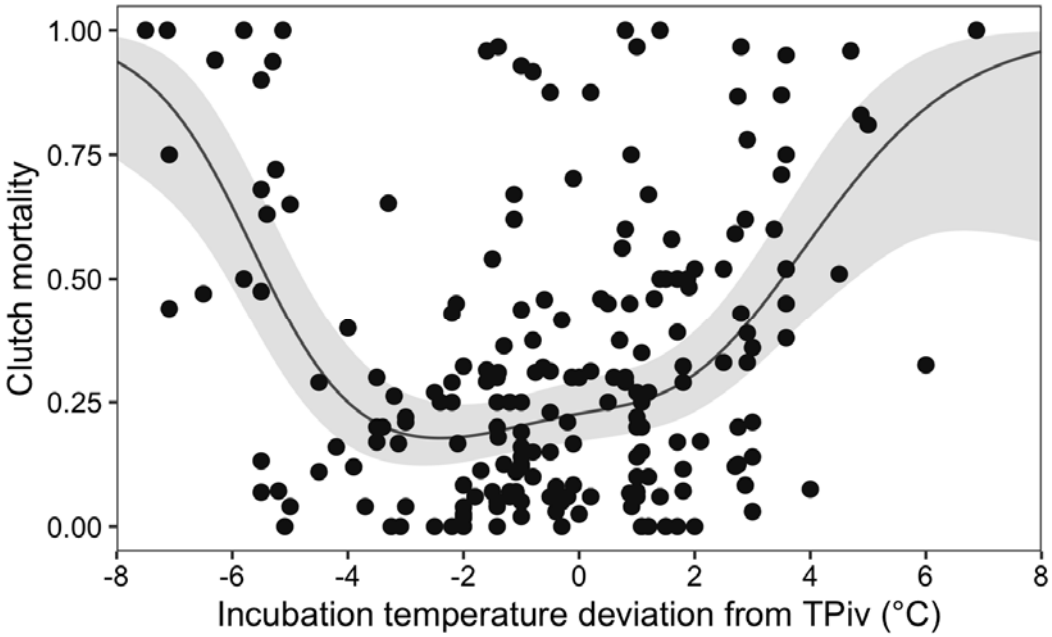

Figure 1

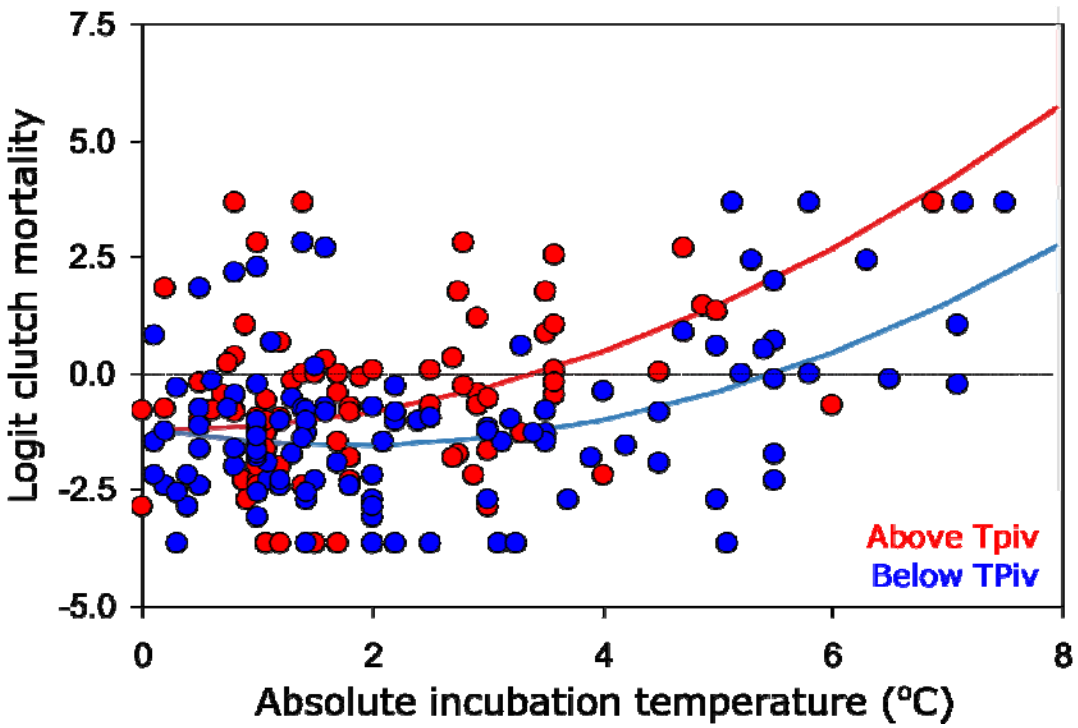
7.5
5.0
2.5
0.0
-2.5
-5.0
Logit clutch mortality
0
2
4
6
8
Absolute incubation temperature (°C)
Above Tpiv
Below TPiv

Figure 2

Appendix

Table A1 Study name, species, and source of pivotal temperature for studies included in the meta-analysis of the current study.

Study #	Study	Study species	Pivotal temperature source
1	(Yntema & Mrosovsky 1980)	*Caretta caretta*	Average of (Marcovaldi et al. 1997; Mrosovsky et al. 2002; Öz et al. 2004)
2	(Yntema 1976)	*Chelydra serpentina*	(Ewert et al. 2005)
3	(Wibbels et al. 1998)	*Lepidochelys olivacea*	Own paper
4	(Demuth 2001)	*Gopherus polyphemus*	Own paper
5	(Gómez-Saldarriaga et al. 2016)	*Podocnemis lewyana*	Own paper
6	(Marcovaldi et al. 1997)	*Caretta caretta*	Own paper
7	(Schwarzkopf & Brooks 1985)	*Chrysemys picta*	Own paper
8	(Bobyn & Brooks 1994b)	*Chelydra serpentina*	(Passmore & Brooks n.d.; Massey et al. In press)
9	(Páez et al. 2009)	*Podocnemis lewyana*	Own paper
10	(Du et al. 2009)	*Chinemys reevevsii*	(Du et al. 2007)
11	(Lewis-Winokur & Winokur 1995)	*Gopherus agassizii*	Own paper
12	(Les et al. 2009)	*Chrysemys picta and Trachemys scripta*	(Dodd et al. 2006; Refsnider et al. 2014)
13	(Zhu et al. 2006)	*Mauremys mutica*	(Du et al. 2010)
14	(Reid et al. 2009)	*Caretta caretta*	Own paper
15	(Roosenburg & Kelley 1996)	*Malaclemys terrpain*	(Burke & Calichio 2014)
16	(Spotila et al. 1994)	*Gopherus agassizii*	Own paper
17	(Valenzuela 2001)	*Podocnemis expansa*	Own paper
18	(Packard et al. 1987)	*Chelydra serpentina*	(Ewert et al. 2005)
19	(Du et al. 2007)	*Chinemys reevevsii*	Own paper
20	(Godfrey & Mrosovsky 2006)	*Chelonia mydas*	Own paper
21	(Mrosovsky et al. 2002)	*Caretta caretta*	Own paper

22	(Rafferty & Reina 2014)	*Chelonia mydas*	(Jensen et al. 2018)
23	(Steyermark & Spotila 2001)	*Chelydra serpentina*	(Ewert et al. 2005)
24	(Fisher et al. 2014)	*Caretta caretta*	Average of (Marcovaldi et al. 1997; Mrosovsky et al. 2002; Öz et al. 2004)
25	(Geist et al. 2015)	*Emys marmorata*	Own paper
26	(Zhao et al. 2015)	*Mauremys mutica*	(Du et al. 2010)
27	(Lubiana & Ferreira Júnior 2009)	*Podocnemis expansa*	Own paper
28	(Du et al. 2010)	*Ocadia sinensis and Mauremys mutica*	Own paper
29	(Booth et al. 2004)	*Chelonia mydas*	(Stubbs & Mitchell 2018)
30	(Stubbs & Mitchell 2018)	*Chelonia mydas*	Own paper

[*] Own paper indicates the paper itself, listed under Study, includes the pivotal temperature.