

Rate of egg maturation in marine turtles exhibits 'universal temperature dependence'

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Summary

1. The metabolic theory of ecology (MTE) predicts that, after correcting for body mass variation among organisms, the rates of most biological processes will vary as a universal function of temperature. However, empirical support for 'universal temperature dependence' (UTD) is currently equivocal and based on studies of a limited number of traits.

2. In many ectothermic animals, the rate at which females produce mature eggs is temperature dependent and may be an important factor in determining the costs of reproduction.

3. We tested whether the rate of egg maturation in marine turtles varies with environmental temperature as predicted by MTE, using the time separating successive clutches of individual females to estimate the rate at which eggs are formed. We also assessed the phenotypic contribution to this rate, by using radio telemetry to make repeated measurements of interclutch intervals for individual green turtles (*Chelonia mydas*).

4. Rates of egg maturation increased with seasonally increasing water temperatures in radio-tracked green turtles, but were not repeatable for individual females, and did not vary according to maternal body size or reproductive investment (number and size of eggs produced).

5. Using a collated data set from several different populations and species of marine turtles, we then show that a single relationship with water temperature explains most of the variation in egg maturation rates, with a slope that is statistically indistinguishable from the UTD predicted by MTE. However, several alternative statistical models also described the relationship between temperature and egg maturation rates equally parsimoniously.

6. Our results offer novel support for the MTE's predicted UTD of biological rates, although the underlying mechanisms require further study. The strong temperature dependence of egg maturation combined with the apparently weak phenotypic contribution to this rate has interesting behavioural implications in ectothermic animals. We suggest that maternal thermoregulatory behaviour in marine turtles, and many other reptiles, is consistent with a strategy of adaptively increasing body temperatures to accelerate egg maturation.

Key-words: behavioural thermoregulation, interesting interval, radio telemetry, sea surface temperature, reptile

Introduction

Virtually all biological rates are affected by temperature. This is particularly apparent in ectotherms where body temperatures are more sensitive to fluctuations in ambient conditions. Development times (Gillooly *et al.* 2002), growth rates (Lopez-Urrutia 2008), fecundity (Berger, Walters & Gotthard 2008), and longevity (Munch & Salinas 2009) of ectothermic animals have been shown to be temperature

dependent, which may in turn favour thermoregulatory behaviour that optimises such rates (Lillywhite, Licht & Chelgren 1973; Autumn & De Nardo 1995). Although ubiquitous in nature, the mechanisms underpinning biological rate–temperature relationships remain controversial. According to the metabolic theory of ecology (MTE), biological rates at all levels of organisation are rooted in the thermodynamics of enzyme-catalysed reactions and will therefore share a common dependence on temperature (Brown *et al.* 2004). MTE is founded on the premise that by limiting the flux of energy and resources in organisms,

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metabolic rate sets the pace of most higher-order ecological processes, ranging from whole-organism physiology to ecosystem dynamics (Brown *et al.* 2004). Proponents of MTE further suggest that whole-organism metabolism is governed primarily by body size and the effects of temperature on biochemical kinetics (Gillooly *et al.* 2001). Thus, after correcting for body mass variation, the majority of biological rates (R) are predicted to vary as a function of temperature according to a Boltzmann-Arrhenius relationship of the following form (Gillooly *et al.* 2001; Brown *et al.* 2004):

$$R \sim b_0 e^{-E/kT} \quad \text{eqn 1}$$

where b_0 is a normalisation constant, T is the absolute temperature (in °K), k is Boltzmann's constant (8.62×10^{-5} eV/K), and E is the activation energy of rate-limiting metabolic reactions (in electron-volts, or eV). Moreover, MTE makes the more specific prediction that E will consistently fall in the range 0.6–0.7 eV for aerobic animals, reflecting the average activation energy of the respiratory complex (Gillooly *et al.* 2001; Brown *et al.* 2004). This has been termed 'universal temperature dependence' (UTD; Gillooly *et al.* 2001) by proponents of MTE and supported with empirical data for a number of different biological rates (e.g. Gillooly *et al.* 2001, 2002; Brown *et al.* 2004; Savage *et al.* 2004; Allen *et al.* 2006).

While appealing in its generality, UTD has provoked a great deal of criticism (reviewed in O'Connor *et al.* 2007a). Points of contention include the validity of extrapolating Boltzmann kinetics to describe complex biological pathways (Clarke 2006; O'Connor *et al.* 2007a); the failure of UTD to account for acclimation, phenotypic variation or evolutionary adaptation in the temperature responses of biological rates (Clarke 2004; O'Connor *et al.* 2007a); and the difficulty with constructing a test of the theory itself, given that its quantitative predictions do not necessarily rely on the proposed mechanisms (O'Connor *et al.* 2007a). On these grounds, critics have argued that UTD should be considered a statistical approximation rather than a mechanistic model based on first principles (Clarke 2004, 2006; O'Connor *et al.* 2007a; but see Gillooly *et al.* 2006; Allen & Gillooly 2007 for a rebuttal). Nonetheless, UTD is at the very least an intriguing phenomenological relationship that warrants further investigation. For example, mass-corrected metabolic rate (Gillooly *et al.* 2001; Meehan 2006), development times (Gillooly *et al.* 2002; O'Connor *et al.* 2007b), individual and population growth rates (Savage *et al.* 2004; Lopez-Urrutia 2008), longevity (Munch & Salinas 2009) and rates of DNA evolution and speciation (Allen *et al.* 2006) have all been shown to exhibit Arrhenius temperature dependence that approximates the predicted range of $E = 0.6$ – 0.7 eV. However, other studies have reported significant deviations from UTD for many of the same rates (Frazier, Huey & Berrigan 2006; Algar, Kerr & Currie 2007; de Castro & Gaedke 2008). Empirical tests drawing on a wider range of biological rate processes are therefore an important step in evaluating the predictions of UTD.

In many ectothermic animals, the rate at which females produce mature eggs is temperature dependent (Carroll & Quiring 1993; Calbet & Agusti 1999; Hays *et al.* 2002a; Berger, Walters & Gotthard 2008; Lourdaïs, Heulin & DeNardo 2008). This rate is likely to affect important fitness outcomes, such as fecundity (Carroll & Quiring 1993; Berger, Walters & Gotthard 2008), or the time invested in reproduction and the associated physiological and/or survival costs for females (see Shine 1980 and Williams 2005 for reviews). However, studying the factors that influence egg maturation rates is difficult in practice. In this study, we use marine turtles as models to test whether rates of egg maturation vary with temperature in accordance with UTD. Female marine turtles lay multiple clutches within a season (Miller 1997), and their reproductive physiology is such that the interval separating successive clutches ('internesting intervals') can be used to estimate the time required to produce mature eggs. In particular, ovulation immediately follows the laying of a previous clutch (Owens 1980), and embryonic development arrests at a very early stage prior to oviposition (mid gastrulation; Miller 1985), meaning ontogenetic growth is expected to contribute relatively little to overall rates of egg maturation. As yolk formation is complete prior to the arrival at the nesting grounds in marine turtles (Rostal *et al.* 1997), we define 'egg maturation' as the post-ovulatory phase of egg production, including fertilisation, albumen deposition, shell membrane development and calcification (Owens 1980). Although Ridley turtles (*Lepidocelys* spp.) often retain mature eggs in the oviducts to synchronise with mass nesting events (Owens & Morris 1985), egg retention has not been reported in other species of marine turtles when suitable nesting conditions are available. We therefore expect internesting intervals to be a reliable (inverse) estimate of the rate at which eggs mature in non-Ridley species.

Previous studies of green (*Chelonia mydas*) and loggerhead turtles (*Carretta carretta*) have shown that the lengths of internesting intervals decrease as a function of increasing water temperature (Sato *et al.* 1998; Hays *et al.* 2002a), in a manner that is consistent among different species and populations (Hays *et al.* 2002a). However, these studies have also revealed considerable residual variation not explained by temperature, which might indicate an additional phenotypic component to the rate of egg maturation (Sato *et al.* 1998; Hays *et al.* 2002a). The objectives of this study were therefore twofold. First, we assess the relative contributions of temperature and maternal phenotypes to rates of egg maturation in green turtles, using radio-telemetry to make repeated measurements of internesting interval lengths for individual females across a seasonal water temperature gradient. We also relate internesting intervals to specific maternal phenotypes which might be expected to influence egg maturation rates, such as body size (as per the MTE; Brown *et al.* 2004) and reproductive investment (size and number of eggs produced). Second, we examine whether the temperature-dependence of egg maturation supports the quantitative predictions of MTE, i.e. that variation in rate with temperature is described by an Arrhenius relationship with a slope in the range of $E = 0.6$ – 0.7 eV.

Materials and methods

STUDY SITE AND FIELD PROCEDURES

Data were collected for green turtles (*Chelonia mydas*) nesting at Ascension Island, South Atlantic Ocean (14°20'W, 7°55'S) during the 2007 breeding season. The study was conducted on Long Beach, which supports the highest density and numbers of nesting turtles on the island (Godley, Broderick & Hays 2001). To assess the contribution of maternal phenotype to the rate of egg production, radio-telemetry was used to monitor the nesting activity of individual females and make repeated measures of nesting interval length (days from laying until subsequent nesting activity; Sato *et al.* 1998; Alvarado & Murphy 1999; Hays *et al.* 2002a) as they returned to lay successive clutches. VHF radio transmitters were attached to the carapaces of a randomly selected sample of females ($n = 20$) nesting between the 2nd and 12th of January, using a two-part epoxy resin (Power-Fast+; Powers Fasteners Inc., Brewster, NY, USA). Females were also fitted with a Passive Integrated Transponder tag (PIT, Identichip; Animalcare Ltd., York, UK) implanted into the triceps muscle of the right fore-flipper to assist identification (Godley, Broderick & Moraghan 1999). Over 95% of nesting activity at Ascension Island occurs between January and May (Godley, Broderick & Hays 2001), so there is a high probability that tagged females were encountered while depositing their first clutch. Nesting females were subsequently re-located using a scanning AR-8200 VHF receiver (AOR, Tokyo, Japan) and YAGI antenna (Biotrack, Dorset, UK) during nightly patrols of the nesting beach (20.00–05.00) conducted at 1-h intervals until 1st April when tracking was terminated. Females were observed from a distance to allow nest excavation and then approached to confirm whether eggs were present. Nesting interval length was then calculated as the number of days elapsed between laying and the subsequent nesting attempt (following Sato *et al.* 1998; Alvarado & Murphy 1999; Hays *et al.* 2002a).

Body mass is a significant determinant of metabolic rate and the rates of other biological processes (Gillooly *et al.* 2001), but is logistically difficult to measure in adult marine turtles (e.g. Hays *et al.* 2002b). Curved carapace length (CCL; ± 1 cm) was therefore used as an estimator of body mass for study females, as CCL and mass are highly correlated in the study population ($r^2 = 0.69$; Hays *et al.* 2002b). As the size or number of eggs produced may also contribute to the time necessary to produce a clutch, mean egg mass (± 0.2 g) for each clutch was calculated from a sample of 3 eggs collected at approximately the 10th, 50th and ca. 10th from last positions in the laying sequence (to capture the full range of intra-clutch egg size variation; Hays, Adams & Speakman 1993). The locations of all clutches were marked and clutch size estimated post-hatching from the number of hatched and unhatched eggs (see Broderick *et al.* 2003). Total clutch mass was calculated as clutch size \times mean egg mass.

WATER TEMPERATURE MEASUREMENTS

Mean water temperature for the duration of each observed nesting interval was estimated from night-time MODIS-Aqua sea surface temperature (SST) images (4 km resolution; Goddard Space Flight Centre). Daily mean SST for a box of 1° latitude \times 1° longitude centred on Ascension Island (14°20'W, 7°55'S) was extracted using MATLAB v7 (MathWorks, Natick, MA, USA), and these data were used to calculate mean SST between the start and end date of each nesting interval.

The reliability of SST as a proxy for water temperatures experienced by internesting green turtles was validated using data from ani-

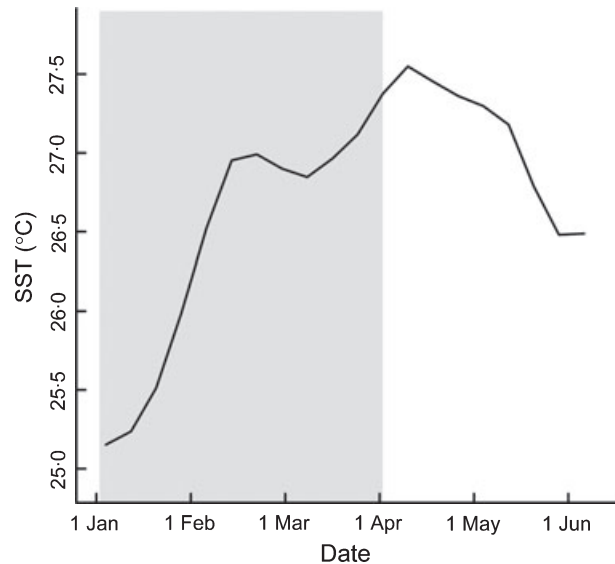


Fig. 1. Sea surface temperature at Ascension Island during the 2007 green turtle nesting season (from night-time MODIS-Aqua satellite data). The shaded area shows the period during which the present study was conducted.

mal mounted time-depth recorders (TDRs) collected as part of a previous study at Ascension Island ($n = 6$ females; Hays, Metcalfe & Walne 2004). Mean SST during the period for which each TDR was deployed was calculated as described earlier. Mean TDR temperatures were slightly higher than mean SST measured across the same inter-nesting period (TDR range = 27.60–27.75 °C; SST range = 27.53–27.59 °C; Generalised linear mixed models (GLMM) with measurement method as a fixed factor and internesting period as a random effect, $\chi^2_1 = 5.1$, $P = 0.004$), but the difference between methods was marginal (estimate \pm SE = 0.12 \pm 0.02 °C) when compared with the 2.5 °C variation in SST over the course of the present study (range = 25.2–27.7 °C; Fig. 1). A close correspondence between SST and water temperatures experienced by turtles is expected at an oceanic nesting site such as Ascension Island, where substantial surface mixing dispels any thermal gradients over the normal range of dive depths (e.g. Hays *et al.* 2000).

STATISTICAL ANALYSIS

Generalised linear mixed models with female identity included as a random factor were used to evaluate the effects of SST, clutch size, egg size and total clutch mass (clutch size multiplied by mean egg mass) on internesting interval length. Clutch characteristics could not be obtained for all nesting intervals as females were occasionally encountered during the post-laying, cover-up phase of nesting and a number of clutches were destroyed by tidal inundation or excavation by other turtles before clutch size could be determined. Analyses including measures of maternal reproductive investment were therefore performed using a subset of intervals for which clutch data were available (see Results for sample sizes). The significance of fixed effects in GLMMs (SST and clutch characteristics) was assessed using likelihood ratio tests, and models were simplified by stepwise deletion of non-significant predictors ($\alpha = 0.05$), starting with the effect with the highest P -value (Crawley 2007).

To estimate the overall proportion of variation in nesting interval length explained by phenotypic differences among females, we

calculated the intra-class correlation coefficient, or repeatability score (R) using the equation $R = S_B^2 / [S_B^2 + S_E^2]$, where S_B^2 is the among-female variance component and S_E^2 is the within-female (or residual) variance component from a GLMM fit using restricted maximum likelihood (Nakagawa & Cuthill 2007; Zuur *et al.* 2009). We limited the analysis to only those females for which two or more interval length measurements were made during the study. Significance of the among-female variance component was assessed by comparing a GLMM containing the random effect to a linear model fit by generalised least squares using a likelihood ratio test (as recommended by Zuur *et al.* 2009).

Details of further statistical tests are presented in Results. All analyses were performed using R v. 2.5.1 (R Development Core Team 2007).

Results

DETERMINANTS OF INTERNESTING INTERVAL LENGTH IN GREEN TURTLES

A total of 46 internesting intervals from 18 individual green turtles was recorded over the course of the study (two tagged females were never relocated). Females that were relocated laid a minimum average \pm SD of 3.9 ± 1.1 clutches (range = 2–5) over the study period, separated by an average interval of 13.3 ± 1.6 days (range = 11–17 days).

As a result of climatic trends at Ascension Island, SST increased across the study period (Fig. 1), and this was associated with a significant reduction in the length of internesting intervals of study females (likelihood ratio test, $\chi_1^2 = 33.5$, $P < 0.001$, $n = 46$ intervals from 18 females; Fig. 2a). To account for the possibility that seasonally changing factors other than temperature contributed to this trend (e.g. increased efficiency of egg production, reduced harassment by males), we constructed a model containing both SST and the midpoint date of each internesting interval (days since 1st January 2007) as explanatory variables. Date did not explain significant variation in interval length after controlling for SST (likelihood ratio test, $\chi_1^2 = 1.2$, $P = 0.27$), suggesting that temperature drove the observed seasonal decrease in interval lengths.

In contrast to the effects of temperature, internesting interval length was largely independent of maternal phenotype.

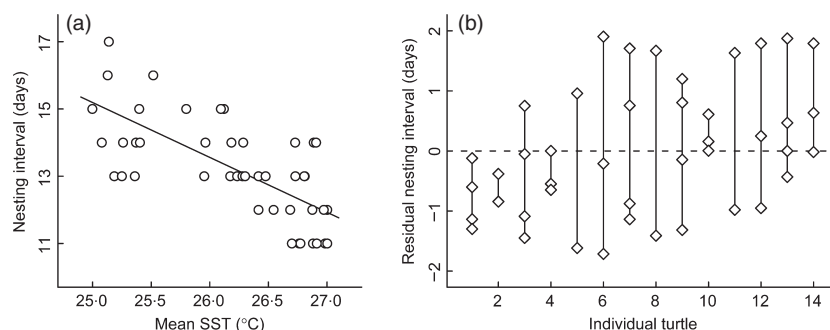


Fig. 2. Effects of temperature and maternal phenotype on nesting interval length for green turtles nesting at Ascension Island. (a) Relationship between mean sea surface temperature (SST) during the nesting interval and interval length. (b) Repeatability of nesting interval lengths for individual turtles measured two or more times during the season ($n = 14$) after controlling for the effects of SST. Positive values indicate longer nesting interval lengths than predicted by the relationship with water temperature.

For females where two or more measurements were made ($n = 14$ females and 42 intervals), individual repeatability of nesting interval length was low and not statistically significant ($R = 0.03$, $\chi_1^2 = 0.013$, $P = 0.91$; Fig. 2b). Similarly, after controlling for SST there were no significant relationships between the length of the interval preceding a clutch and the number, size or total mass of eggs produced (likelihood ratio test, clutch size: $\chi_1^2 = 0.13$, $P = 0.71$; egg mass: $\chi_1^2 = 0.017$, $P = 0.90$; total clutch mass: $\chi_1^2 = 0.07$, $P = 0.79$; $n = 36$ clutches from 15 females; Fig. 3). Female size, measured as CCL, was also unrelated to interval length (multiple regression with SST as a covariate; $F_{1,44} = 0.04$, $P = 0.85$). Thus, of the variables measured, SST was the only significant predictor of inter-nesting interval lengths in green turtles.

DOES EGG MATURATION EXHIBIT UTD?

To test whether the temperature dependence of egg maturation supports the quantitative predictions of UTD, we compiled a data set of 82 internesting interval lengths and water temperatures for green and loggerhead turtles nesting in Japan ($n = 24$; Sato *et al.* 1998), Cyprus ($n = 10$; Hays *et al.* 2002a) and Ascension Island ($n = 46$, this study; $n = 2$, Hays *et al.* 2002a). Water temperature was measured directly using animal-borne data loggers in previous studies (Sato *et al.* 1998; Hays *et al.* 2002a) and estimated from nocturnal SST in the present study. Egg maturation rate (R) was calculated as the reciprocal of internesting interval length (in days⁻¹) and modelled as a function of water temperature according to an Arrhenius relationship of the form proposed by MTE (eqn 1). Maternal body size is not a significant determinant of interval length in either species represented in the combined data set (this study; Sato *et al.* 1998), so R was not mass-corrected. Rearranging eqn (1) generates a linear model of the following form:

$$\ln(R) = \ln(b_0) - E(1/kT) \quad \text{eqn 2}$$

Thus, according to MTE, an Arrhenius plot of log-transformed rate against inverse absolute temperature ($1/kT$)

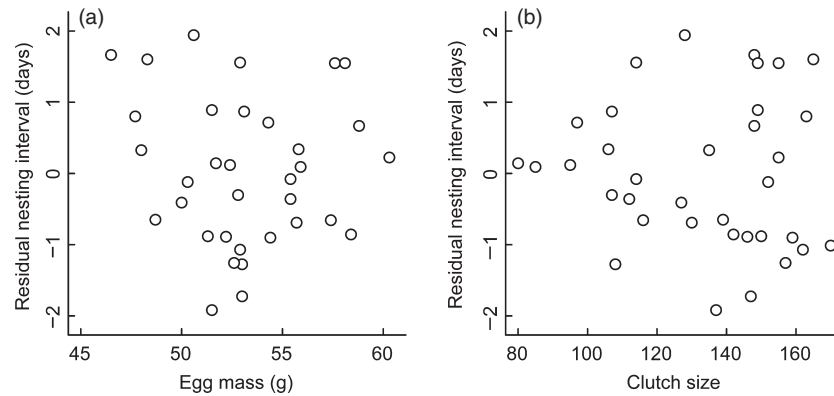


Fig. 3. Effect of maternal reproductive investment on nesting interval length for green turtles at Ascension Island. Relationships between (a) egg size and (b) clutch size and nesting interval length after controlling for the effects of sea surface temperature.

should generate a linear relationship with a slope of E that is consistently in the range of 0.6–0.7 eV (i.e. UTD). This prediction was supported by our data set. A linear regression of R on $1/kT$ explained a majority of variation in egg maturation rates ($F_{1,80} = 226.7$, $P < 0.001$, $r^2 = 0.74$; Fig. 4), with an estimated slope of $E = 0.78$ eV (95% CI: 0.68–0.89) that was not significantly different from $E = 0.7$ eV at the upper bound of the MTE predicted range (F -test, $F_{1,80} = 2.486$, $P = 0.12$). Residual deviances from the fitted model were not significantly different for data taken from different sources (one-way ANOVA, $F_{2,79} = 0.035$, $P = 0.97$) or species ($F_{1,80} = 0.09$, $P = 0.76$), indicating that the single model was sufficient to describe the temperature dependence of egg maturation in all cases.

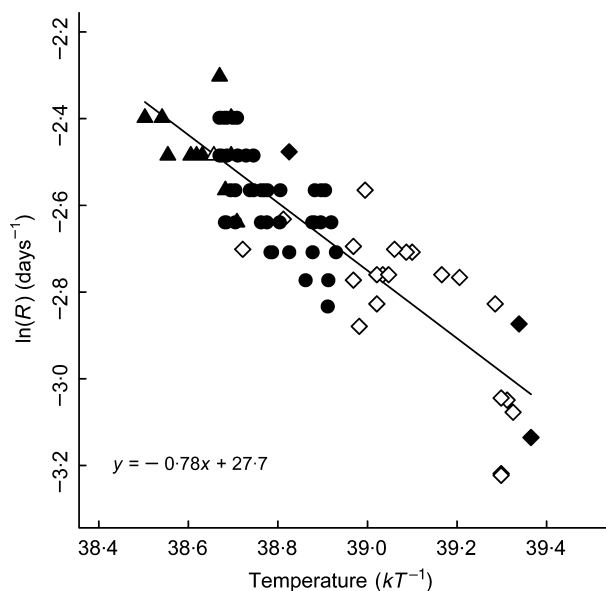


Fig. 4. Arrhenius plot of the relationship between inverse water temperature ($1/kT$) and rate of egg maturation (R , days^{-1}) for green turtles (solid symbols) and loggerhead turtles (open symbols) nesting in Cyprus (triangles), Ascension Island (circles) and Japan (diamonds). Data are plotted according to the Boltzmann-Arrhenius factor (eqn 2), where k is Boltzmann's constant (8.62×10^{-5} eV/K) and T is absolute water temperature (in °K).

Others have noted that the Arrhenius thermal scaling relationship favoured by MTE is only one of several statistical models that can be used to describe the temperature dependence of biological rates (Clarke 2004, 2006; O'Connor *et al.* 2007a). We therefore compared the relationship between egg maturation rates and water temperature using several different models including exponential, double logarithmic, and quadratic. All of the models fit the data equally parsimoniously when evaluated using the sample size-corrected Akaike Information Criterion (i.e. all $\Delta\text{AIC}_c < 2$; Burnham & Anderson 2002).

Discussion

This study has shown that egg maturation rates in marine turtles vary with environmental temperature in accordance with the predictions of the MTE, but are largely independent of maternal phenotype. An Arrhenius thermal scaling relationship of the form proposed by MTE (eqn 1) explained 75% of the variation in egg maturation rates, with a slope that was statistically indistinguishable from the predicted range of $E = 0.6$ –0.7 eV. This range has been described as the UTD of biological rates (Gillooly *et al.* 2001). Interestingly, while the estimated value of $E = 0.78$ eV for egg maturation rates in marine turtles is marginally outside the MTE predicted range (albeit not significantly so), it is remarkably consistent with the temperature dependence of basal metabolic rate estimated for reptiles in general ($E = 0.76$ eV, extracted from figure 1F in Gillooly *et al.* 2001).

Such close correspondence in the temperature dependence of metabolic rate and higher-order physiological processes is intriguing; however, the mechanistic implications are far from clear (Clarke 2006; O'Connor *et al.* 2007a). Proponents of MTE argue that because UTD is derived from first principles, empirical support for its predictions helps to validate the underlying mechanism, i.e. that the thermodynamics of enzyme-catalysed metabolic reactions underpins many other biological rates (Brown *et al.* 2004). However, critics have questioned the mechanistic provenance of UTD and suggested it should (at best) be considered a statistical approximation with as yet unknown causes (Clarke 2004, 2006). We

should note that our purpose here was to evaluate the quantitative predictions of UTD, not the underlying mechanisms. However, several points are worth making. First, the time taken for females to produce mature eggs is likely to reflect a complex integration of physiological rates (e.g. albumen biosynthesis, active transport of Ca^{2+} into the oviducts), many of which are dependent on cellular energy production (e.g. Thompson *et al.* 2007). It therefore seems intuitive that maternal metabolism might limit egg maturation rates, and that we might expect similar temperature dependences for these rates. However, it is also clear that statistical similarities in the fit of empirical data by an Arrhenius relationship do not alone support a particular causal mechanism (O'Connor *et al.* 2007a). Indeed, consistent with other recent studies (Clarke 2004; O'Connor *et al.* 2007a; Munch & Salinas 2009), we found that alternative statistical models described the relationship between temperature and egg maturation rates equally parsimoniously. While this does not invalidate our main finding that the temperature dependence of egg maturation in marine turtles is quantitatively similar to that of many other ecological rates (e.g. Gillooly *et al.* 2001, 2002; Brown *et al.* 2004; Allen *et al.* 2006; O'Connor *et al.* 2007b), it highlights a clear need for robust tests of underlying mechanisms.

Despite agreement with UTD, there was nonetheless significant residual variation in egg maturation rates that could not be explained by either temperature or phenotypic differences among females. Contrary to the allometric scaling predictions of MTE, egg maturation rates in marine turtles were independent of maternal body size (a strong correlate of mass; see also Sato *et al.* 1998). This is consistent with several recent studies suggesting that body size is a weak predictor of biological rates at the intra-specific level (Tilman *et al.* 2004; Munch & Salinas 2009), while temperature continues to explain substantial variation (Munch & Salinas 2009). Egg maturation rates were also independent of either the size or number of eggs produced in a clutch. This might be expected in marine turtles, given that all of the eggs in a clutch mature simultaneously (Owens 1980) and albumen deposition, which accounts for much of the variation in egg mass (Wallace *et al.* 2006), is completed soon after ovulation and unlikely to be rate limiting (Owens 1980). Sato *et al.* (1998) hypothesised that physiological differences among females (e.g. hormonal state or fat reserves) might account for much of the residual variation in internesting interval lengths not explained by temperature. However, the low intra-individual repeatability of interval length in the present study suggests that such endogenous factors may be of minor importance.

Given the small amount of variance explained by maternal phenotypes, it seems possible that much of the residual variation in internesting interval lengths may arise from stochastic events that interfere with nesting patterns, e.g. rough sea states, human disturbance, level of female harassment by males, and timing of arrival at beaches relative to tidal cycles. The integer scale on which nesting interval length is typically measured may also introduce error, as the nocturnal nesting habits of most marine turtle species

mean that interval lengths cannot be measured on a truly continuous axis. However, while many unknown factors could plausibly influence nesting periodicity (and therefore our estimates of egg maturation rates), it is worth noting that the mean (\pm SD) residual deviation in internesting-interval length from the fitted relationship with water temperature was just 1.0 ± 0.1 days (back transformed from Fig. 4). Thus, females typically nested within 24 h of the exact time predicted by temperature.

The strong temperature dependence of egg maturation, combined with the relatively weak phenotypic contribution to this rate, has potentially important behavioural implications in ectothermic animals. Reproduction is costly for females: in addition to the direct energetic investment in eggs, mothers also incur costs through the physiological and behavioural changes that accompany vitellogenesis, e.g. altered hormonal states and reduced foraging opportunities (Shine 1980; Williams 2005). Many of these indirect costs are likely to be proportional to the time invested in reproduction, and may therefore favour thermoregulatory strategies that increase maternal body temperature in order to accelerate egg maturation.

Indeed, descriptions of habitat use by breeding marine turtles are broadly consistent with this hypothesis. For example, studies of loggerhead turtles suggest that females actively seek out warm water microhabitats during the internesting period (Naito *et al.* 1990; Sakamoto *et al.* 1993; Schofield *et al.* 2009). Female leatherback turtles (*Dermocelys coriacea*) have similarly been shown to restrict their movements to the warmest habitat patches at some nesting sites (Fossette *et al.* 2009; but not others, Wallace *et al.* 2005). Thermal stratification of the water column means that thermoregulatory opportunities also exist through selection of diving depths in marine turtles. For example, the contrasting dive profiles of gravid green turtles in the Mediterranean (shallow dives, stratified water column; Hays *et al.* 2002a,c; Schofield *et al.* 2009; Fuller *et al.* 2009) and Ascension Island (deep dives, mixed water column; Hays *et al.* 2000, 2002c) are consistent with a strategy of remaining in the warm surface mixed layer while maximising more energy-efficient deep dives (see Hays *et al.* 2000). Dive profiles also suggest that female sea turtles may alter their depth selection diurnally and seasonally to track variations in water column stratification, e.g. diving shallower in summer or at midday when warm surface layers form (Hays *et al.* 2000, 2002c; Yasuda *et al.* 2008). Maternal thermoregulatory strategies that maximise body temperature are perhaps not surprising in sea turtles, given the prevalence of such behaviour in other reptiles. In many species of lizards and snakes, gravid females maintain higher body temperatures than conspecifics through behavioural thermoregulation (reviewed in Shine 2006), which reduces the time for which eggs must be carried (Lourdais, Heulin & DeNardo 2008). However, the adaptive significance of 'maternal thermophily' is ambiguous in these taxa. Lizards and snakes are unusual among reptiles in having significant embryonic develop-

ment prior to oviposition (Andrews 2004), meaning that thermoregulatory behaviour may serve to optimise the developmental conditions for offspring as well as accelerating egg maturation (Wapstra 2000; Shine 2006). In contrast, marine turtles (like other chelonids) lay their eggs at a very early stage in development (Miller 1985), suggesting that maternal thermophily is more likely to reflect a strategy of increasing egg maturation rates in order to reduce the time invested in reproduction.

In conclusion, we have shown that egg maturation rates in marine turtles vary with temperature in accordance with the UTD predicted by MTE, but are largely independent of maternal phenotypes. This may have significant behavioural implications for in ectothermic animals, favouring a strategy of 'maternal thermophily' to accelerate egg maturation and reduce the time invested in reproduction. Empirical tests of UTD have so far yielded mixed results, with some studies supporting its quantitative predictions (this study, Gillooly *et al.* 2001, 2002; Allen *et al.* 2006; Meehan 2006; O'Connor *et al.* 2007b; Lopez-Urrutia 2008), and others rejecting them (Frazier, Huey & Berrigan 2006; Algar, Kerr & Currie 2007; de Castro & Gaedke 2008; Irlich *et al.* 2009). This has prompted suggestions that UTD at best describes a central tendency in ecology, with much of the interesting biology lying in deviations from the general model (Clarke 2006; Irlich *et al.* 2009). Rigorous tests of the mechanisms underlying UTD are clearly needed to resolve these issues. However, we agree with Clarke (2006) that, at the very least, UTD represents an intriguing phenomenological description of ecological rate-temperature relationships. It is striking that processes as apparently unrelated as egg maturation in sea turtles, larval development in fish (O'Connor *et al.* 2007b) and speciation rates in foraminifera (Allen *et al.* 2006) converge on a similar dependence on temperature. Explaining why this is so (and indeed why some rate-temperature relationships differ) remains an important challenge for the future.

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References

Algar, A.C., Kerr, J.T. & Currie, D.J. (2007) A test of metabolic theory as the mechanism underlying broad-scale species-richness gradients. *Global Ecology and Biogeography*, **16**, 170–178.

Allen, A.P. & Gillooly, J.F. (2007) The mechanistic basis of the metabolic theory of ecology. *Oikos*, **116**, 1073–1077.

Allen, A.P., Gillooly, J.F., Savage, V.M. & Brown, J.H. (2006) Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences*, **103**, 9130–9135.

Alvarado, J. & Murphy, T.M. (1999) Nesting periodicity and interesting behaviour. *Research and Management Techniques for the Conservation of Sea Turtles* (eds K.L. Eckert, K.A. Bjorndal, F.A. Abreu-Grobois & M. Donnelly), pp. 115–118. IUCN/SSC Marine Turtle Specialist Group, Washington D.C.

Andrews, R.M. (2004) Patterns of embryonic development. *Reptilian Incubation: Environment, Evolution and Behaviour* (ed D.C. Deeming), pp. 75–102. Nottingham University Press, Nottingham, UK.

Autumn, K. & De Nardo, D.F. (1995) Behavioral thermoregulation increases growth rate in a nocturnal lizard. *Journal of Herpetology*, **29**, 157–162.

Berger, D., Walters, R. & Gotthard, K. (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly *Functional Ecology*, **22**, 523–529.

Broderick, A.C., Glen, F., Godley, B.J. & Hays, G.C. (2003) Variation in reproductive output of marine turtles. *Journal Experimental Marine Biology and Ecology*, **288**, 95–109.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical-Theoretic Approach*. Springer, New York.

Calbet, A. & Agustí, S. (1999) Latitudinal changes of copepod egg production rates in Atlantic water: temperature and food availability as the main driving factors. *Marine Ecology Progress Series*, **181**, 155–162.

Carroll, A.L. & Quiring, D.T. (1993) Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis*. *Oecologia*, **93**, 233–241.

de Castro, F. & Gaedke, U. (2008) The metabolism of lake plankton does not support the metabolic theory of ecology. *Oikos*, **117**, 1218–1226.

Clarke, A. (2004) Is there a universal temperature dependence of metabolism? *Functional Ecology*, **18**, 252–256.

Clarke, A. (2006) Temperature and the metabolic theory of ecology. *Functional Ecology*, **20**, 405–412.

Crawley, M.J. (2007) *The R Book*. John Wiley & Sons Ltd., Chichester, UK.

Fossette, S., Girard, C., Bastian, T., Calmettes, B., Ferraroli, S., Vendeville, P., Blanchard, F. & Georges, J.-Y. (2009) Thermal and trophic habitats of the leatherback turtle during the nesting season in French Guiana. *Journal of Experimental Marine Biology and Ecology*, **378**, 8–14.

Frazier, M.R., Huey, R.B. & Berrigan, D. (2006) Thermodynamics constrains the evolution of insect population growth rates: "warmer is better". *American Naturalist*, **168**, 512–520.

Fuller, W.J., Broderick, A.C., Hooker, S.K., Witt, M.J. & Godley, B.J. (2009) Insights into habitat utilization by green turtles (*Chelonia mydas*) during the inter-nesting period using animal-borne digital cameras. *Mar Technol Soc J*, **43**, 51–59.

Gillooly, J.F., Allen, A.P., Savage, V.M., Charnov, E.L., West, G.B. & Brown, J.H. (2006) Response to Clarke and Fraser: effects of temperature on metabolic rate. *Functional Ecology*, **20**, 400–404.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001) Effects of size and temperature on metabolic rate. *Science*, **293**, 2248–2251.

Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M. & Brown, J.H. (2002) Effects of size and temperature on developmental time. *Nature*, **417**, 70–73.

Godley, B.J., Broderick, A.C. & Hays, G.C. (2001) Nesting of green turtles (*Chelonia mydas*) at Ascension Island, South Atlantic. *Biological Conservation*, **97**, 151–158.

Godley, B.J., Broderick, A.C. & Moraghan, S. (1999) Short term effectiveness of Passive Integrated Transponders (PITs) used in the study of Mediterranean marine turtles. *Chelonian Conservation and Biology*, **3**, 477–479.

Hays, G.C., Adams, C.R. & Speakman, J.R. (1993) Reproductive investment by green turtles nesting on Ascension Island. *Canadian Journal of Zoology*, **71**, 1098–1103.

Hays, G.C., Metcalfe, J.D. & Walne, A.W. (2004) The implications of lung-regulated buoyancy control for dive depth and duration. *Ecology*, **85**, 1137–1145.

Hays, G.C., Adams, C.R., Broderick, A.C., Godley, B.J., Lucas, D.J., Metcalfe, J.D. & Prior, A.A. (2000) The diving behaviour of green turtles at Ascension Island. *Animal Behaviour*, **59**, 577–586.

Hays, G.C., Broderick, A.C., Glen, F., Godley, B.J., Houghton, J.D.R. & Metcalfe, J.D. (2002a) Water temperature and internesting intervals for loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. *Journal of Thermal Biology*, **27**, 429–432.

Hays, G.C., Broderick, A.C., Glen, F. & Godley, B.J. (2002b) Change in body mass associated with long-term fasting in a marine reptile: the case of green turtles (*Chelonia mydas*) at Ascension Island. *Canadian Journal of Zoology*, **80**, 1299–1302.

- Hays, G.C., Glen, F., Broderick, A.C., Godley, B.J. & Metcalfe, J. (2002c) Behavioural plasticity in a large marine herbivore: contrasting patterns of depth utilisation between two green turtle (*Chelonia mydas*) populations. *Marine Biology*, **141**, 985–990.
- Irlich, U.M., Terblanche, J.S., Blackburn, T.M. & Chown, S.L. (2009) Insect rate-temperature relationships: environmental variation and the metabolic theory of ecology. *American Naturalist*, **174**, 819–835.
- Lillywhite, H.B., Licht, P. & Chelgren, P. (1973) The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology*, **54**, 375–383.
- Lopez-Urrutia, A. (2008) The metabolic theory of ecology and algal bloom formation. *Limnology and Oceanography*, **53**, 2046–2047.
- Lourdais, O., Heulin, B. & DeNardo, D.F. (2008) Thermoregulation during gravidity in the children's python (*Antaresia childreni*): a test of the preadaptation hypothesis for maternal thermophily in snakes. *Biological Journal of the Linnean Society*, **93**, 499–508.
- Meehan, T.D. (2006) Mass and temperature dependence of metabolic rate in litter and soil invertebrates. *Physiological and Biochemical Zoology*, **79**, 878–884.
- Miller, J.D. (1985) Embryology of marine turtles. *Biology of the Reptilia*, vol. 14A (eds C. Gans, F. Billett & P.F.A. Maderson), pp. 14. Wiley-Interscience, New York.
- Miller, J.D. (1997) Reproduction in sea turtles. *The Biology of Sea Turtles* (eds P.L. Lutz & J.A. Musick), pp. 51–81. CRC Press, Boca Raton.
- Munch, S.B. & Salinas, S. (2009) Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. *Proceedings of the National Academy of Science*, **106**, 13860–13864.
- Naito, Y., Sakamoto, W., Uchida, I., Kureha, K. & Ebisawa, T. (1990) Estimation of migration route of the loggerhead turtle *Caretta caretta* around the nesting ground. *Nippon Suisan Gakkaishi*, **56**, 255–262.
- Nakagawa, S. & Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, **82**, 591–605.
- O'Connor, M.P., Kemp, S.J., Agosta, S.J., Hansen, F., Sieg, A.E., Wallace, B.P., McNair, J.N. & Dunham, A.E. (2007a) Reconsidering the mechanistic basis of the metabolic theory of ecology. *Oikos*, **116**, 1058–1072.
- O'Connor, M.I., Bruno, J.F., Gaines, S.D., Halpern, B.S., Lester, S.E., Kinlan, B.P. & Weiss, J.M. (2007b) Temperature control of larval dispersal and the implications for marine ecology, evolution and conservation. *Proceedings of the National Academy of Science*, **104**, 1266–1271.
- Owens, D.W. (1980) The comparative reproductive physiology of sea turtles. *American Zoologist*, **20**, 549–563.
- Owens, D.W. & Morris, Y.A. (1985) The comparative endocrinology of sea turtles. *Copeia*, **1985**, 723–735.
- R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rostal, D.C., Grumbles, J.S., Byles, R.A., R., M.-M. & Owens, D.W. (1997) Nesting physiology of Kemp's Ridley sea turtles, *Lepidochelys kempi*, at Ranco Nuevo, Tampaulipas, with observations on population estimates. *Chelonian Conservation and Biology*, **2**, 538–547.
- Sakamoto, W., Sato, K., Tanaka, H. & Naito, Y. (1993) Diving patterns and swimming environment of two loggerhead turtles during internesting. *Nippon Suisan Gakkaishi*, **59**, 1129–1137.
- Sato, K., Matsuzawa, Y., Tanaka, H., Bando, T., Minamikawa, S., Sakamoto, W. & Naito, Y. (1998) Interesting intervals for loggerhead turtles, *Caretta caretta*, and green turtles, *Chelonia mydas*, are affected by temperature. *Canadian Journal of Zoology*, **76**, 1651–1662.
- Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B. & Charnov, E.L. (2004) Effects of body size and temperature on population growth. *American Naturalist*, **163**, 429–441.
- Schofield, G., Bishop, C.M., Katselidis, K.A., Dimopoulos, P., Pantis, J.D. & Hays, G.C. (2009) Microhabitat selection by sea turtles in a dynamic thermal marine environment. *Journal of Animal Ecology*, **78**, 14–21.
- Shine, R. (1980) "Costs" of Reproduction in Reptiles. *Oecologia*, **46**, 92–100.
- Shine, R. (2006) Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *Journal of Experimental Biology*, **305A**, 524–535.
- Thompson, M.B., Lindsay, L.A., Herbert, J.F. & Murphy, C.R. (2007) Calcium ATPase expression in the oviducts of the skink, *Lampropholis guichenoti*. *Comparative Biochemistry and Physiology A*, **147**, 1090–1094.
- Tilman, D., HilleRisLambers, J., Harpole, S., Dyzinski, R., Fargione, J., Clark, C. & Lehman, C. (2004) Does metabolic theory apply to community ecology? It's a matter of scale. *Ecology*, **85**, 1797–1799.
- Wallace, B.P., Williams, C.L., Paladino, F.V., Morreale, S.J., Lindstrom, R.T. & Spotila, J.R. (2005) Bioenergetics and diving activity of interinteresting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marino Las Baulas, Costa Rica. *Journal of Experimental Biology*, **208**, 3873–3884.
- Wallace, B.P., Sotherland, P.R., Tomillo, P.S., Bouchard, S.S., Reina, R.D., Spotila, J.R. & Paladino, F.V. (2006) Egg components, egg size and hatching size in leatherback turtles. *Comparative Biochemistry and Physiology A*, **145**, 524–532.
- Wapstra, E. (2000) Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology*, **14**, 345–352.
- Williams, T.D. (2005) Mechanisms underlying the cost of egg production. *BioScience*, **55**, 39–48.
- Yasuda, T., Kittiwattanawong, K., Klom-in, W. & Arai, N. (2008) Vertical distribution of water temperature around female green turtles during the interinteresting period. *Proceedings of the 3rd International Symposium on SEASTAR2000 and Asian Bio-logging Science*, pp. 27–30.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer Science and Business Media, New York.

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