

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

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**Running headline:** Egg production conforms to metabolic theory

## SUMMARY

1. The metabolic theory of ecology (MTE) predicts that, after accounting for variation in body mass amongst organisms, the rates of many ecological processes should vary as a universal function of temperature, governed by the thermodynamics of enzyme-catalysed metabolic reactions.
2. In ectothermic animals, the rate at which females can produce mature eggs is profoundly influenced by environmental temperature, and may be an important factor in determining the costs of reproduction. However, whether the temperature-dependence of this rate conforms to the predictions of MTE is not known.
3. We tested whether the rate of egg production in marine turtles varies with ambient temperature as predicted by MTE, using the time separating successive clutches of individual females as an estimate of the rate at which eggs are formed. We also examined the contribution of maternal phenotype to this rate by making repeated measurements of inter-clutch intervals for individual, radio-tracked green turtles (*Chelonia mydas*).
4. Rates of egg production in green turtles increased with seasonally increasing water temperatures, but were not significantly repeatable for individual females, and did not vary according to maternal reproductive investment (number and size of eggs produced).
5. Using a collated dataset from several different populations and species of marine turtles, we then show that a single thermodynamic function explains most of the variation in egg production rates, with an activation energy (or temperature dependence) that is consistent with the universal value predicted by MTE.

6. Since the effect of temperature on metabolic rate is the principle determinant of egg production rates, gravid females might be expected to adaptively increase their operating temperatures through behavioural thermoregulation in order to minimise the time invested in reproduction. Indeed, based on a review of studies describing habitat use by breeding marine turtles, we suggest that ‘maternal thermophily’ may be a widespread strategy for accelerating egg production in these species.

KEYWORDS: behavioural thermoregulation | interesting interval | metabolic rate | sea surface temperature | reptile

# 1 INTRODUCTION

2

3 Variation in environmental temperature has a profound effect on the ecology of  
4 ectothermic animals. Many key life history traits including development times,  
5 growth rates, fecundity and longevity vary as direct a function of temperature  
6 (Gillooly et al. 2002; Angilletta 2009; Munch & Salinas 2009), which may in turn  
7 favour thermoregulatory behaviour that optimises such rates (Lillywhite, Licht &  
8 Chelgren 1973; Autumn & De Nardo 1995). Recently, the metabolic theory of  
9 ecology (MTE) has proposed a unifying theoretical framework that relates  
10 temperature to ecological processes in terms of first principals of thermodynamics  
11 (Brown et al. 2004). According to MTE, metabolic rate varies among organisms  
12 largely as a result of body temperature, through its effects on biochemical kinetics,  
13 and body mass (Gillooly et al. 2001; Brown et al. 2004). Thus, for a given body mass,  
14 metabolic rate in all aerobic organisms is predicted to increase as a single, universal  
15 function of temperature (universal temperature dependence; UTD), with a slope  
16 defined by the activation energy of enzyme-catalysed reactions (Gillooly et al. 2001).  
17 Moreover, because metabolism sets the rate of higher-order ecological processes such  
18 as growth and reproduction, many processes ranging from life history traits to  
19 ecosystem dynamics are expected to share a similar UTD (Brown et al. 2004). Whilst  
20 MTE is controversial (e.g. Clarke 2004), these predictions have received considerable  
21 empirical support in both marine and terrestrial ecosystems (Duarte 2007; Lopez-  
22 Urrutia 2008; Munch & Salinas 2009).

23

24 In ectothermic animals, the time required for females to produce mature eggs is highly  
25 dependent on ambient temperature (Carroll & Quiring 1993; Calbet & Augusti 1999;

1 Hays et al. 2002a; Berger, Walters & Gotthard 2008; Lourdais, Heulin & DeNardo  
2 2008). Given the physiologically integrated nature of egg production, such effects are  
3 probably largely driven by changes in metabolic rate; however, whether the rate of  
4 egg production conforms to the UTD predicted by metabolic theory has not yet been  
5 established for any species. This rate may determine important fitness outcomes, such  
6 as fecundity (e.g. Carroll & Quiring 1993; Berger, Walters & Gotthard 2008) or the  
7 time invested in reproduction and its associated costs to females e.g. increased  
8 predation risks, reduced foraging opportunities, and altered hormonal states and  
9 haematology (Shine 1980; Williams 2005). If rapid egg production confers a selective  
10 advantage, reproductive females may be expected to adaptively increase their  
11 operating temperatures through behavioural modifications to optimise their laying  
12 rate. Such ‘maternal thermophily’ is known to occur in some species of terrestrial,  
13 squamate reptiles (i.e. lizards and snakes; Shine 2006; Lourdais, Heulin & DeNardo  
14 2008); however, the squamata are unusual amongst reptiles in having significant  
15 embryonic development prior to oviposition (Andrews 2004), meaning thermophilic  
16 behaviour in these taxa may serve to optimise offspring developmental conditions *in*  
17 *utero* (Wapstra 2000; Shine 2006), rather than to promote egg production. However, a  
18 recent study has suggested that gravid marine turtles may also actively seek out warm  
19 micro-habitats (Schofield et al. 2009). Unlike squamate reptiles, chelonids lay their  
20 eggs at a very early stage in development (gastrulation; Miller 1985) suggesting that  
21 maintenance of optimal conditions for embryogenesis is unlikely to drive such  
22 behaviour and accelerating egg production may be a proximate explanation.

23

24 All species of marine turtle lay multiple clutches within a single nesting season  
25 (Miller 1997), and it is known that the interval separating successive clutches declines

1 as a function of increasing ambient water temperature (Sato et al. 1998; Hays et al.  
2 2002a). Since ovulation of the follicles for each clutch immediately follows the  
3 previous nesting attempt in sea turtles (Owens 1980; Licht 1982), and eggs are laid at  
4 a fixed developmental stage (Miller 1985), such ‘nesting interval lengths’ constitute a  
5 robust measure of the time required to produce mature eggs. Comparative analyses  
6 have suggested that the relationship between nesting interval length and ambient  
7 temperature may be similar among species and populations of marine turtle (Hays et  
8 al. 2002a), as would be predicted if there is a common metabolic mechanism.  
9 However, there is also considerable unexplained variation (Hays et al. 2002), which  
10 might suggest a phenotypic component to the rate of egg production.

11

12 The aims of the present study are threefold. Firstly, by making repeated measures of  
13 nesting interval length for individual green turtles (*Chelonia mydas*) across a seasonal  
14 water temperature gradient, we assess the contributions of ambient temperature and  
15 maternal phenotypes to the rate of egg production in marine turtles. Secondly, using a  
16 collated dataset of water temperatures and nesting interval lengths taken from  
17 different species and populations of marine turtles, we test the hypothesis that the  
18 temperature dependence of egg production conforms to the predictions of MTE.  
19 Thirdly, since rapid egg production may help to reduce the costs of reproduction, we  
20 review evidence that female marine turtles actively adjust their operating temperatures  
21 to maximise their laying rate.

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# 1 **Materials and Methods**

2

## 3 **Study site and field procedures**

4 Data were collected for green turtles (*Chelonia mydas*) nesting at Ascension Island,  
5 South Atlantic Ocean (14°20'W, 7°55'S) during the 2007 breeding season. The study  
6 was conducted on Long Beach, which supports the highest density and numbers of  
7 nesting turtles on the island (Godley, Broderick & Hays 2001). To assess the  
8 contribution of maternal phenotype to the rate of egg production, radio-telemetry was  
9 used to monitor the nesting activity of individual females and make repeated measures  
10 of nesting interval length (days from laying until subsequent nesting activity; Sato et  
11 al. 1998; Alvarado & Murphy 1999; Hays et al. 2002a) as they returned to lay  
12 successive clutches. VHF radio transmitters were attached to the carapaces of a  
13 randomly selected sample of females ( $N = 20$ ) nesting between the 2<sup>nd</sup> and 12<sup>th</sup> of  
14 January, using a two-part epoxy resin (Power-Fast+, Powers Fasteners Inc., NY).  
15 Females were also fitted with a PIT tag (Passive Integrated Transponder; Identichip,  
16 Animalcare Ltd., UK) implanted into the triceps muscle of the right fore-flipper to  
17 assist identification (Godley et al 1999). Over 95% of nesting activity at Ascension  
18 Island occurs between January and May (Godley, Broderick & Hays 2001), so there is  
19 a high probability that tagged females were encountered whilst depositing their first  
20 clutch. Nesting females were subsequently re-located using a scanning AR-8200 VHF  
21 receiver (AOR, Derbyshire, UK) and YAGI antenna (Biotrack, Dorset, UK.) during  
22 nightly patrols of the nesting beach (20:00 – 05:00) conducted at 1 h intervals until 1<sup>st</sup>  
23 April when tracking was terminated. Females were observed from a distance to allow  
24 nest excavation and then approached to confirm whether eggs were present. Nesting  
25 interval length was then calculated as the number of days elapsed between laying and

1 the subsequent nesting attempt (following Sato et al. 1998; Alvarado & Murphy 1999;  
2 Hays et al. 2002a).

3  
4 Body mass has important effects on metabolic rate and thus on the rate of biological  
5 processes (Gillooly et al. 2001), but is logistically difficult to measure in adult marine  
6 turtles (e.g. Hays et al. 2002b). Thus, curved carapace length (CCL;  $\pm 1$  cm) was used  
7 as an estimator of body size for study females, as it is highly correlated with body  
8 mass in this population (Hays et al. 2002b). Since the size or number of eggs  
9 produced may also contribute to the time necessary to produce a clutch, mean egg  
10 mass ( $\pm 0.2$  g) for each clutch laid by study females was calculated for a sample of 3  
11 eggs collected from the 10<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> positions in the laying sequence (to account  
12 for within-clutch variation in egg size; Hays, Adams & Speakman 1993). The  
13 locations of all clutches were then marked and clutch size estimated post-hatching  
14 from the number of hatched and unhatched eggs (see Broderick et al. 2003)

15

16 **Measuring water temperature**

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

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## 1 **Statistical analysis**

2 Generalised linear mixed models (GLMM) with female identity included as a random  
3 factor were used to test the effects of SST, clutch size, egg size and total clutch mass  
4 (clutch size multiplied by egg mass) on nesting interval length. Clutch characteristics  
5 could not be obtained for all nesting intervals as females were occasionally  
6 encountered during the post-laying, cover-up phase of nesting and a number of  
7 clutches were destroyed by tidal inundation or excavation by other turtles before  
8 clutch size could be determined. Analyses including measures of maternal  
9 reproductive investment were therefore performed using a subset of intervals for  
10 which clutch data were available (see Results). The significance of fixed effects in  
11 GLMMs (SST and clutch characteristics) was assessed using likelihood ratio tests to  
12 compare the full model with a reduced model minus the effect of interest (Crawley  
13 2007). Models were simplified by stepwise deletion of non-significant predictors ( $\alpha =$   
14 0.05), starting with the effect with the highest  $p$ -value (Crawley 2007)

15  
16 In order to estimate the overall proportion of variation in nesting interval length  
17 explained by phenotypic differences among females, we restricted the dataset to those  
18 individuals for which two or more measurements were made during the study and  
19 calculated the intra-class correlation coefficient, or repeatability score ( $R$ ) using the  
20 equation  $R = S^2_B / [S^2_B + S^2_E]$ ; where  $S^2_B$  is the among-female variance component  
21 and  $S^2_E$  is the within-female (or residual) variance component from a GLMM fit using  
22 restricted maximum likelihood (Nakagawa & Cuthill 2007; Zuur et al. 2009). SST  
23 was included in the model as a fixed effect. The significance of the among-female  
24 variance component was assessed by comparing a GLMM containing the random  
25 effect to a simple linear model fit by generalized least squares using a likelihood ratio

1 test (as recommended by Zuur et al. 2009). Details of further statistical analyses are  
2 provided in the text of the results. All statistical analyses were performed using R v.  
3 2.5.1 (R Development Core Team 2009).

4

## 5 **Results**

### 6 **Determinants of nesting interval length.**

7 A total of 46 inter-nesting intervals from 18 individual turtles were recorded over the  
8 course of the study (2 tagged females were never relocated). Females that were  
9 relocated were recorded as laying a minimum average  $\pm$  SD of  $3.9 \pm 1.1$  clutches  
10 (range = 2 – 5) over the course of the study period separated by an average interval of  
11  $13.3 \pm 1.6$  days (range = 7 – 11). Due to seasonal climatic fluctuations at Ascension  
12 Island, sea surface temperature (SST) increased during the study period (Fig. 1) and  
13 this had a profound effect on the length of the interval separating successive clutches  
14 of individual turtles: nesting interval length declined significantly as water  
15 temperature increased (likelihood ratio test;  $\chi_1^2 = 33.5$ ,  $p < 0.001$ ,  $N = 46$  intervals  
16 from 18 females; Fig. 2A). In contrast, maternal phenotype and reproductive  
17 investment explained little of the observed variation in interval length. For turtles  
18 where two or more measurements were made ( $N = 14$  females and 42 intervals),  
19 individual repeatability of nesting interval length was low and not statistically  
20 significant ( $R = 0.03$ ,  $\chi_1^2 = 0.013$ ,  $p = 0.91$ ; Fig. 2B). Consistent with the low intra-  
21 individual repeatability of nesting interval length, after controlling for SST there were  
22 no significant relationships between the length of the interval preceding a clutch and  
23 the number, size or total mass of eggs produced (likelihood ratio test, clutch size:  
24  $\chi_1^2 = 0.13$ ,  $p = 0.71$ ; egg mass:  $\chi_1^2 = 0.017$ ,  $p = 0.90$ ; total clutch mass:  $\chi_1^2 = 0.07$ ,  $p =$   
25  $0.79$ ;  $N = 36$  clutches from 15 females; Fig. 3). Female size, measured as curved

1 carapace length (CCL), was also unrelated to nesting interval length (multiple  
2 regression with SST as a covariate;  $F_{1,44} = 0.04, p = 0.85$ ).

3

4 Thus, SST was the only significant determinant of nesting interval length for green  
5 turtles at Ascension Island. Previous studies using animal-mounted temperature  
6 loggers have directly estimated the relationship between water temperature and  
7 nesting interval length in sea turtles to fit the linear function  $\log(\text{length}) = 2.25 -$   
8  $0.043 \times \text{temperature}$  (Hays et al. 2002a). For comparison, the regression equation  
9 linking log-transformed nesting interval length and remotely sensed SST in the  
10 present study was  $\log(\text{length}) = 2.29 - 0.045 \times \text{SST}$  ( $r^2 = 0.50$ ), which did not differ  
11 significantly from the slope and intercept estimated by Hays et al. ( $F$ -test,  $F_{2,44} = 1.2,$   
12  $p = 0.30$ ). This not only suggests that SST was a reliable measure of the actual water  
13 temperature experienced by females, but also implies that a single thermodynamic  
14 relationship may relate temperature and nesting interval length across different  
15 populations and species of marine turtles.

16

### 17 **Metabolic rate and egg production**

18 The metabolic theory of ecology (MTE; Brown et al. 2004) predicts that metabolic  
19 rate, and thus many other biological rates, will vary with body mass and temperature  
20 according to the fundamental equation:

21

$$22 \text{ rate} = b_0 M^{3/4} e^{-E/kT} \quad [1]$$

23

24 Where  $b_0$  is a normalisation constant,  $e^{-E/kT}$  is the Boltzmann-Arrhenius factor  
25 describing the temperature dependence of the rate (see below), and  $M^{3/4}$  is an

1 allometric scaling of body mass to account for the fractal nature of resource  
2 distribution networks in animals, and the effects this has on metabolic rate over orders  
3 of magnitude of body size (Gillooly et al. 2001). This latter parameter is often less  
4 important in intraspecific comparisons as the range in body size tends to be relatively  
5 small (e.g. Tilman et al. 2004; Munch & Salinas 2009). In our study population  
6 female CCL ranged from 107 – 123 cm (equivalent to a range in mass of ~140 – 200  
7 kg using the equation given by Hays et al. 2002b) and was unrelated to nesting  
8 interval length (see above). A previous study similarly found no correlation between  
9 body mass and nesting interval length in marine turtles (Sato et al. 1998). Thus, the  
10 temperature-dependence of nesting interval length does not appear to be conditional  
11 on maternal body mass. Rearranging equation [1] to include only temperature effects  
12 gives the following:

13

$$14 \ln(\text{rate}) = \ln(b_0) - E(1/kT) \quad [2]$$

15 Thus, according to MTE, log-transformed rate should vary as a linear function of  
16  $1/kT$ , where  $k$  is the Boltzmann constant and  $T$  is absolute temperature (°K), with a  
17 slope of  $E$  which is the activation energy of the process. Moreover, for biological rates  
18  $E$  is predicted to fall in the range of activation energies for enzyme catalysed  
19 metabolic reactions (i.e.  $E = 0.6 - 0.7$  eV; Gillooly et al. 2001; Brown et al. 2004).

20

21 To test whether this prediction is upheld for the rate of egg production in sea turtles  
22 we compiled a dataset of published nesting interval lengths and water temperatures  
23 for green and loggerhead turtles nesting in Japan ( $N = 24$ ; Sato et al. 1998), Cyprus ( $N$   
24  $= 10$ ; Hays et al. 2002a) and Ascension Island ( $N = 46$ , this study;  $N = 2$ , Hays et al.  
25 2002a). Nesting interval length was converted to a rate of egg production by taking

1 the reciprocal ( $R = \text{length in days}^{-1}$ ) and modelled as a linear function of  $1/kT$   
2 following equation [2]. The model explained a majority of the variation in the rate of  
3 egg production across populations (linear regression,  $F_{1,80} = 226.7$ ,  $p < 0.001$ ,  $r^2 =$   
4  $0.75$ ; Fig. 4) and was described by the linear function  $\ln(R) = 27.7 - 0.78(1/kT)$ .  
5 Residual deviances from the fitted model were not significantly different for nesting  
6 interval data taken from different sources (one-way ANOVA,  $F_{2,79} = 0.035$ ,  $p = 0.97$ ),  
7 indicating that the single model fits all nesting interval length data equally.

8

9 The slope of the fitted model estimates the activation energy ( $E$ ), or temperature  
10 dependence, for the rate of egg production in sea turtles as  $E = 0.78$  eV. This is  
11 slightly outside the range of activation energies of  $0.6 - 0.7$  eV predicted for  
12 biological rates by MTE (Gillooly et al. 2001; Brown et al. 2004), although was not  
13 significantly different than a slope of  $E = 0.7$  eV at the upper limit of this range ( $F$ -  
14 test,  $F_{1,80} = 2.486$ ,  $p = 0.12$ ). Moreover, whilst empirical estimates of  $E$  for metabolic  
15 rate have been shown to centre on the MTE predicted range, there are subtle  
16 variations among different taxonomic groups ( $E = 0.43 - 0.78$  eV; Gillooly et al.  
17 2001). Taxon-specific estimates of  $E$  may therefore be more appropriate for relating  
18 the temperature-dependence of higher order processes to metabolic rate. Indeed, the  
19 activation energy for mass-normalised metabolic rate in reptiles specifically has been  
20 estimated as  $E = 0.76$  eV (extracted from Figure 1F in Gillooly et al. 2001), which is  
21 remarkably consistent with  $E = 0.78$ eV estimated herein for the rate of egg production  
22 in marine turtles ( $F$ -test,  $F_{1,80} = 0.178$ ,  $p = 0.67$ ; Fig. 4).

23

24

25

# 1 **Discussion**

## 2 **Metabolic rate and egg production**

3 The factors which determine how quickly females can produce eggs have been little  
4 studied in oviparous vertebrates, possibly reflecting technical difficulties associated  
5 with the measurement of this rate. In this respect marine turtles offer a rare  
6 opportunity, as the length of the interval separating successive clutches of individual  
7 females can be used as an inverse measure of the rate at which eggs mature. In this  
8 study we have shown that the rate of egg production in marine turtles varies as a  
9 function of ambient temperature in a manner that is broadly consistent with the  
10 universal temperature dependence (UTD) predicted by the metabolic theory of  
11 ecology (MTE; Gillooly et al. 2001; Brown et al. 2004), but is largely independent of  
12 maternal phenotype and reproductive investment.

13

14 According to the MTE, the Arrhenius temperature-dependence of biological processes  
15 should consistently fall in the range  $E = 0.6 - 0.7$  eV which encompasses the average  
16 activation energies of enzyme-catalysed metabolic reactions (Gillooly et al. 2001;  
17 Brown et al. 2004). The slope of 0.78 estimated herein for the rate of egg production  
18 was not significantly different from the upper bound of the MTE predicted range, and  
19 closely approximated the slope of 0.76 estimated for metabolic rate across a wide  
20 range of reptiles (Gillooly et al. 2001; Fig. 4). This clearly implies that the  
21 thermodynamics of metabolism is rate-limiting for egg production. The observation  
22 that temperature affects the rate of egg maturation in oviparous animals and that this  
23 relationship probably has a metabolic basis is by no means new (Carroll & Quiring  
24 1993; Calbet & Agusti 1999; Hays et al. 2002b), however the results of this study

1 integrate egg production into the wider context of metabolic theory and provide  
2 further support for a UTD for biological rates.

3

4 Despite broad agreement with the temperature-dependence predicted by MTE, there  
5 was nonetheless considerable residual variation in egg production rates that could not  
6 be explained by either water temperature or phenotypic differences among females.

7 Nesting interval lengths were not dependent on the size, number or total mass of eggs  
8 produced in a clutch (Fig. 3; see also Sato et al. 1998) and had low overall  
9 repeatability for individual females measured multiple times over the course of the

10 nesting season (Fig. 2B). Female body size was also unrelated to nesting interval  
11 length, which supports the finding that body mass generally has low predictive power  
12 for metabolic processes in intraspecific comparisons (Tilman et al. 2004; Munch &

13 Salinas 2009). Given the small amount of variance explained by maternal phenotypes,  
14 it is possible that much of the residual variation in nesting interval lengths may be due  
15 to stochastic events that interfere with nesting patterns (e.g. rough seas, human

16 disturbance), or an artefact of the integer scale on which nesting interval length is  
17 typically measured. Because most species of sea turtle nest nocturnally, nesting can  
18 only occur on one night or the next, and therefore intervals cannot be measured on a

19 truly continuous timescale. Indeed, for the combined dataset the mean ( $\pm$  SE) residual  
20 deviation in interval length from the fitted relationship with water temperature was  $1.0$   
21  $\pm 0.1$  days (Fig. 4), indicating that females typically nested on the night before or

22 night after the exact time predicted by body temperature.

23

24 Given the physiologically integrated nature of egg production, it is perhaps  
25 unsurprising that the rate at which eggs are formed is dependent on metabolic

1 processes. However, based on current understanding of the reproductive cycle of  
2 marine turtles it may be possible to elaborate on the rate-limiting step(s). According to  
3 Owens (1980) and Licht (1982), ovulation of the full complement of yolky follicles  
4 for a clutch occurs soon after the previous clutch is laid, and is followed by an  
5 extremely rapid phase of albumen deposition in the oviducts that is typically complete  
6 within 72 hours. Thus, formation of the major constituent parts of eggs is unlikely to  
7 limit the rate of egg production, which is consistent with the finding that neither the  
8 size nor number of eggs in a clutch contributes to nesting interval length. In contrast,  
9 shell membrane formation and calcification in the lower oviduct occurs more slowly  
10 and may therefore limit how quickly eggs can be produced (Owens 1980; Hamann et  
11 al. 2003). Egg shell mineralisation is an active process involving ATP-dependent  
12 transport of calcium ions across the uterine epithelium (Thompson et al. 2007) and  
13 might therefore be limited by maternal metabolic rate. In other reptilian species,  
14 including many snakes and lizards, embryonic development within the oviducts may  
15 limit the rate at which mature eggs can be produced (e.g. Lourdais et al. 2008). This  
16 seems unlikely in sea turtles as embryonic development is arrested at a very early  
17 stage until shortly after oviposition (Miller 1985). Nonetheless, developmental rate is  
18 known to vary with temperature according to the predictions of MTE in various  
19 species (Gillooly et al. 2002) and may potentially contribute to the overall rate of egg  
20 production.

21

## 22 **Implications for maternal behaviour**

23 Because operating temperature and its effects on metabolism apparently underpin  
24 much of the variation in fitness-related biological rates, there is considerable scope for  
25 selection to act on this property of organisms and shape it adaptively (Brown et al.



1 2004). Egg production is costly for females, diverting time and resources away from  
2 other life-history processes. In addition to the direct energetic and nutritional  
3 investment in eggs, females may also incur costs through the physiological and  
4 behavioural changes that accompany reproduction e.g. altered hormonal states and  
5 haematology, reduced foraging opportunities and food intake, and increased predation  
6 risks associated with carrying eggs (Shine 1980; Lourdais, Bonnet & Doughty 2002;  
7 Williams 2005). Many of these latter costs are likely to be conditional on the duration  
8 of the reproductive period, and may therefore confer a selective advantage to females  
9 that can produce eggs quickly. Given that the rate of egg production in marine turtles  
10 is largely determined by ambient temperature and is influenced little by maternal  
11 phenotypes, if rapid egg production is adaptive we may expect breeding females to  
12 facultatively increase their operating temperatures through behavioural  
13 thermoregulation.

14

15 A recent study by Schofield et al. (2009) has provided the first explicit evidence that  
16 gravid loggerhead turtles (*Caretta caretta*) maintain higher body temperatures than  
17 would be expected from ambient conditions by actively tracking warm water  
18 microhabitats. Schofield et al. (2009) suggest that this behaviour may serve to  
19 accelerate egg production in a population at the latitudinal extreme of the species'  
20 range, where the window of suitable climatic conditions for egg incubation is  
21 correspondingly short. However, the fact that thermophilic behaviour has only  
22 recently been recognised in breeding marine turtles may reflect the technical  
23 difficulties associated with monitoring behaviour in these species, rather than it being  
24 a localised phenomenon. Indeed, evidence from globally distributed populations  
25 suggests that the need to maximise operating temperatures may have a profound effect

1 on female habitat utilisation during egg production. For example, loggerhead turtles  
2 nesting in Japan have been shown to travel considerable distances during the inter-  
3 nesting period, actively seeking out warm water currents (Naito et al 1990; Sakamoto  
4 et al. 1993), which suggests that thermoregulatory requirements may sometimes  
5 outweigh the energetic costs associated with such migrations. Leatherback turtles  
6 (*Dermochelys coriacea*) nesting in French Guiana also range widely between nesting  
7 events, yet similarly restrict their movements to the warmest areas of the inter-nesting  
8 habitat (Fossette et al. 2009). In contrast, Wallace et al. (2005) have suggested that  
9 leatherbacks nesting in tropical regions may actually seek out cooler water to avoid  
10 overheating. Uniquely for a marine turtle, leatherbacks have physiological adaptations  
11 that enable body temperatures to be maintained significantly higher than ambient,  
12 which probably explains the consistently shorter inter-clutch intervals in this species  
13 compared to other marine turtles (Alvarado & Murphy 1999). Variations in ambient  
14 water temperature and thermoregulatory behaviour might therefore be less important  
15 in determining egg production rates in this species.

16

17 With the exception of leatherbacks, wide-ranging movements are relatively rare in  
18 gravid marine turtles, with females at most breeding sites preferring to rest in shallow  
19 coastal waters during the inter-nesting period in order to conserve energy (e.g. Hays et  
20 al. 2000; Houghton et al. 2002). Nonetheless, significant opportunities for  
21 thermoregulation may still arise from selection of vertical position in the water  
22 column. Water temperatures decrease from the ocean surface to greater depths,  
23 however the degree of thermal stratification varies among breeding sites and depth  
24 selection by gravid females appears to track this variation. For example, in  
25 Mediterranean rookeries such as Cyprus and Zakynthos, where sheltered conditions

1 and calm waters lead to the formation of a relatively shallow surface mixed layer  
2 (with mean water temperature dropping by 1-2 °C between the surface and a depth of  
3 5-10 m; Hays et al. 2002a; Schofield et al 2009), female green and loggerhead turtles  
4 almost exclusively select resting depths shallower than 4-6 m (Hays et al. 2002c;  
5 Schofield et al. 2009; Fuller et al. 2009). By contrast at exposed oceanic nesting sites  
6 such as Ascension Is., where the warm surface mixed layer extends deeper, with little  
7 variation in mean water temperature from the surface to 20 m (Hays et al. 2002a),  
8 female green turtles select resting depths between 12 - 18 m (Hays et al. 2000; Hays et  
9 al. 2002c).

10

11 Deeper resting depths are thought to be advantageous for gravid turtles, allowing them  
12 to maintain neutral buoyancy whilst holding greater amounts of air in the lungs, thus  
13 minimising the energy expended on resurfacing to breathe (Hays et al. 2000).  
14 However, the available evidence suggests that females must balance this against the  
15 need to remain in the warm surface layer and maximise body temperature. Indeed,  
16 whilst females nesting at Ascension Is. generally select deeper resting depths than  
17 those in the Mediterranean, behaviour at this rookery varies diurnally, with females  
18 spending less time resting and moving to depths shallower than 5 m in the middle of  
19 the day when transient warm surface layers may form (Hays et al 2000; Hays et al  
20 2002c), consistent with active thermoregulation. In some tropical breeding grounds  
21 where nesting occurs year round, females also appear to alter their depth selection in  
22 response to seasonal changes in water column stratification in order to stay within the  
23 warm surface mixed layer (Yasuda et al. 2008). At a small number of nesting sites  
24 females may even leave the water column altogether and bask on land (summarised in  
25 Whittow & Balazs 1982). This behaviour is relatively rare and its functional

1 significance is not well understood, however terrestrial basking significantly elevates  
2 maternal body temperatures and it has been suggested that it may serve to accelerate  
3 egg production (Whittow & Balazs 1982).

4

5 Overall, while explicit behavioural studies are currently lacking, there is compelling  
6 evidence to suggest that gravid marine turtles actively increase their body  
7 temperatures through behavioural thermoregulation and microhabitat selection. This  
8 finding may be unsurprising given the prevalence of ‘maternal thermophily’ in  
9 terrestrial reptiles, particularly amongst the squamata (lizards and snakes). Gravid  
10 lizards and snakes are frequently observed to bask more and maintain higher body  
11 temperatures compared to conspecifics (e.g. Graves & Duvall 1993; Blazquez 1995;  
12 Blouin-Demers & Weatherhead 2001; Lourdais, Heulin & DeNardo 2008; reviewed  
13 in Shine 2006). However the adaptive significance of maternal thermophily in  
14 squamates is unclear. While such behaviour can significantly increase the rate at  
15 which eggs mature, and so reduce length of time they must be carried by females prior  
16 to oviposition (Lourdais et al. 2008), squamates also have prolonged retention of eggs  
17 *in utero* (with as much as one-third of embryonic development occurring prior to  
18 oviposition; Andrews 2004) meaning this behaviour may equally serve to optimise the  
19 developmental environment for offspring (Wapstra 2000; Shine 2006). In contrast,  
20 chelonids lay their eggs at a very early stage in development (when the embryo is not  
21 yet visible; Miller 1985) suggesting there is little scope for maternal thermoregulatory  
22 behaviour to influence offspring phenotypes. Thus, maternal thermophily in marine  
23 turtles provides less ambiguous evidence that females may adjust their body  
24 temperature in order to increase metabolic rate and accelerate egg development. The  
25 benefits of such behaviour may be profound. Based on the relationship estimated in

1 this study (Fig. 4), a female that lays 4 clutches within a nesting season and  
2 experiences an ambient water temperature of 25 °C, can reduce the total time invested  
3 in egg production by 11 days (or c. 20 %) by maintaining a body temperature 2 °C  
4 higher than ambient through behavioural thermoregulation. At breeding sites with a  
5 short reproductive season, increased rates of egg production may also allow females  
6 to lay additional clutches and thereby increase overall fecundity (Schofield et al.  
7 2009).

8

9 In summary, this study has shown that the rate of egg production in marine turtles is  
10 predominantly determined by water temperature through its affect on metabolic rate,  
11 and provides further support for the universal temperature dependence of biological  
12 rates predicted by MTE (Gillooly et al. 2001; Brown et al. 2004). An interesting  
13 implication of this work is that females may only be able to modify rates of egg  
14 production through careful behavioural regulation. Indeed, the evidence reviewed here  
15 suggests that female marine turtles may actively seek out warm water habitats during  
16 the inter-nesting period, potentially reflecting an adaptive strategy to accelerate egg  
17 production and thus reduce the costs associated with reproduction.

18

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## Figure Legends.

**Figure 1.** Sea surface temperature (SST) 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.

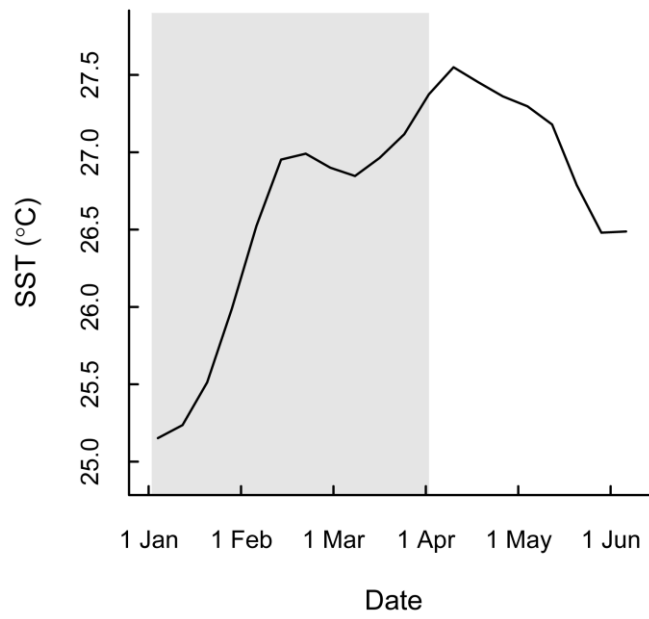
**Figure 2.** Effects of temperature and maternal phenotype on nesting interval length for green turtles nesting at Ascension Island. (A) Relationship between mean sea surface temperature (SST) during the nesting interval and interval length. (B) Repeatability of nesting interval lengths for individual turtles measured 2 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.

**Figure 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 SST.

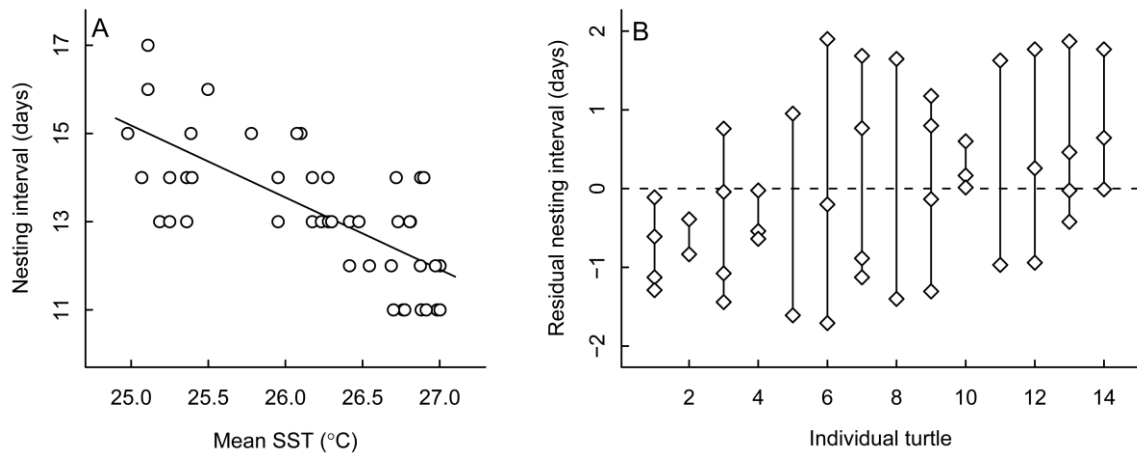
**Figure 4.** The relationship between ambient water temperature ( $1/kT$ ) and the rate of egg production ( $R = \text{nesting interval length}^{-1}$ ) for green turtles (solid symbols) and loggerhead turtles (open symbols) nesting in Cyprus (triangles), Ascension Island (circles) and Japan (diamonds). Data are plotted according to the Boltzmann-Arrhenius relationship (see equation [2]) where  $k$  is Boltzmann's constant and  $T$  is water temperature in °K. The slope of the regression line fitted to these data (solid line,  $y = 27.7 - 0.78x$ ) estimates the activation energy ( $E$ ), or temperature dependence,

for the rate of egg production as  $E = 0.78$  eV. For comparison, the broken line shows a linear regression fit to these same data with slope constrained to  $E = 0.76$  eV, which is the estimated temperature dependence for mass-normalised metabolic rate in reptiles (Gillooly et al. 2001).

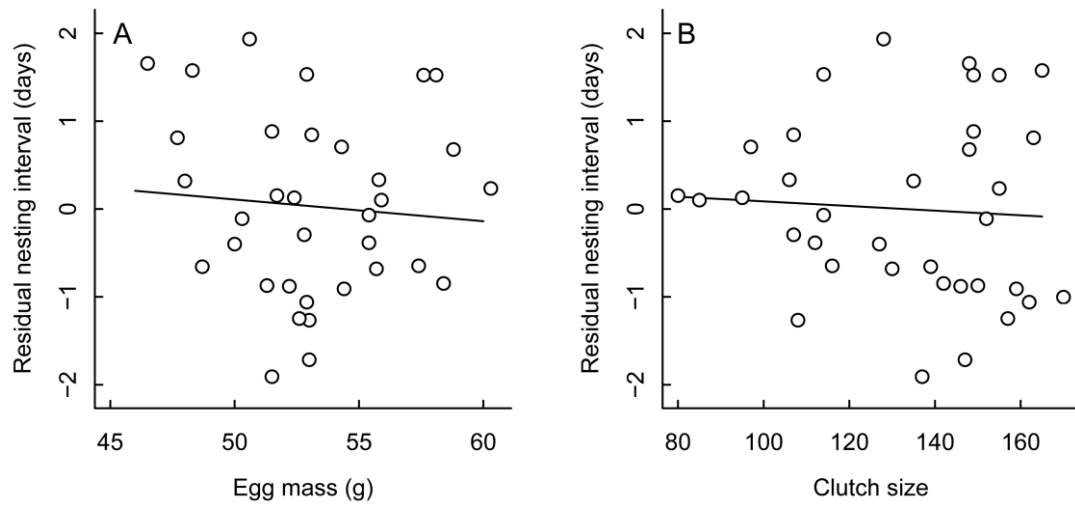
**Figure 1.**



**Figure 2.**



**Figure 3.**



**Figure 4.**

