**Questions that need to be addressed:**

In silico DEB experiment:

1. How do we get the temperature size rule we observe using empirical data from control animals at different incubation temperatures (23C & 28C)?
   1. Can maintenance costs be predicted based on developmental temperature in relation to predictions of how these costs would change across temperature gradients?
      1. Here, we test if decreasing structural costs (EG) for cold incubated individuals and increasing structural costs for hot incubated individuals accurately predict the developmental time and offspring body mass
2. Can DEB model capture the developmental effects of yolk ablation experiments at different incubation temperatures (23C & 28C)?

Converting proton gradient and protein function at higher temperatures. How can we capture the inefficiency of aerobic respiration at higher temperatures.

**Code links:**

Insilco DEB experiment code - for Mike

* [Analysis](https://www.dropbox.com/scl/fi/4lu1409avll291h2grsdn/In_silco_DEB_experiment.R?rlkey=uqpub4si6iky7o9edurwp9qh0&dl=0):
* [Main functions](https://www.dropbox.com/scl/fi/32w8l2jh57naueygjg6x5/simulations.R?rlkey=egh9g26b3acd6lrnn07d2ud07&dl=0):
* [DEB egg simulation function](https://www.dropbox.com/scl/fi/sto5hkj4oagmmb13zgan6/run.DEB.sim.R?rlkey=5d3ie16bmh5dzj51pjypk3a6c&dl=0):
* [Matlab parameters used in run.DEB.sim:](https://www.dropbox.com/scl/fi/zaomxblvd5s7h2euowyw1/parameters_matlab.R?rlkey=i55cfh1u8ap2pcwhu9tlefwem&dl=0)

**1| Introduction**



Figure 1: Theoretical expectations on the relationship of ROS, ATP, and respiration relate across temperature, contributing to higher maintenance costs at higher temperatures. Panel A shows two expectations for repair as temperatures get warmer. The null hypothesis is that there is no relationship between repair and temperature, and the alternative hypothesis suggests that repair increases linearly with ROS until a temperature point where it rapidly declines. Here, individual damage costs from ROS would outstrip repairs. Panel B shows the relationship between respiration (O2) and ATP and how these traits vary across temperatures.

**2| Methods**

*2.1 Empirical data and parameter estimation*

*2.2. Egg simulations within a DEB framework*

Dynamic energy budget (DEB) theory (Koojiman, 1993) was used to help predict how maintenance costs drive embryonic and adult life history traits. In brief, the Dynamic Energy Budget theory offers a unified quantitative framework for dynamically explaining how metabolism (both energy and mass budgets) operates across all living organisms at the individual level. This theory is grounded in specific assumptions regarding how biomass is partitioned into three types: reserve, structure and reproduction buffer. During embryonic stages embryos do not feed or allocate energy to reproduction buffer, but rather use high initial reserves for growth and maintenance until they are hatched. Here we tested..

**3| Results**

*3.1 Empirical data*

Between 2019-2021 adult gravid *Lampropholis delicata* were housed until eggs were laid. Eggs were then pseudo-randomly (to ensure equal sample sizes) assigned to both incubation treatments ‘cold’ (23°C; n = 205) or ‘hot’ (28°C s.d. ± 3.0). Egg incubation temperatures were chosen to mimic conditions experienced at extremes of natural nest temperatures in nature while also showing natural thermal fluctuations throughout the day. To capture variation in egg mass yolk was removed on a subset of eggs (yolk removal: n = 218; control: n = 186). Yolk removal treatments followed Sinervo et al., with 15–20% of the total egg mass being removed via a sterilized syringe. Control treatments were punctured with the syringe without any yolk removal. As expected, developmental time was slower in ‘cold’ incubation treatments (46 d) in comparison to shorter developmental times in ‘hot’ incubation treatments (29.3 d; Table 1). Mass (g) was higher (0.116 g) in cold incubation temperatures in comparison mass (0.103 g) in hot incubation temperatures (Table 1).

Table 1. Empirical data of mean mass and developmental times by incubation temperature treatment for each species. Values in brackets are SD of mean values.



3.2 *Null model: Can DEB model predictions accurately predict developmental time and offspring body mass under different environmental scenarios?*

Under null expectations, where structural costs (EG) were unchanged (7837.66 J/cm3), incubation in ‘cold’ incubation treatment (23°C) accurately predicted developmental time 46d when comparing it to the empirical observations (Table 1), but simulated mass under these developmental environments were below 0.104g observed values. When running the null simulation for ‘hot’ incubation treatment (28°C), simulated developmental times were higher 32d than empirical observations and simulated mass was above 0.140g observed values (Table 1).

3.3 *Structure costs model under varying temperature conditions: Does decreasing structural costs (EG) for cold incubated individuals and increasing structural costs for hot incubated individuals accurately predict developmental time and offspring body mass?*

Simulations were run where structural costs (EG) from our null model were changed (10%, 20%, 30%*)* depending on the incubation temperature treatment (23°C or 28°C) to test theoretical expectations (Figure 1). In this simulation, cold incubation treatments decreased, whereas hot incubation treatments increased.Reducing the EG by 10% for the ‘cold’ incubation treatment caused the incubation time to become 48d and the wet mass of hatching to become 0.118g, whereas increasing EG by 10% in the hot incubation treatment caused the incubation time to become 31d and the wet mass of hatching to become 0.124g (Figure 2A). When decreasing EG by 20% for the ‘cold’ incubation treatment caused the incubation time to become 49d and the wet mass of hatching to become 0.116g, whereas increasing EG by 20% in the hot incubation treatment caused the incubation time to become 30d and the wet mass of hatching to become 0.109g (Figure 2B). These values were remarkably close to empirical data (Table 2). Finally decreasing EG by 30% for ‘cold’ incubation treatment caused incubation time to become 51d and wet mass of hatching to become 0.131g, whereas increasing EG by 30% in the hot incubation treatment caused incubation time to become 29d and wet mass of hatching to become 0.105g (Figure 2C).

Table 2. Comparison of mass and developmental time vary between empirical data and simulation data where cost of structure (EG) is changed. Null simulation is where the cost of structure was unchanged (7837.66 J/cm3). For each “EG” simulation the precent that follows indicates the percent change in EG depending on incubation temperature, decrease in cold incubation temperatures and increase in hot temperatures. EG percent change simulation estimations correspond with figure 1 (A-C).



*3.4 Changing the ‘zoom factor’ under varying developmental temperatures predict hatchling mass and developmental times?*

Here we ran simulations where the scaling factor "z\_mult" was manipulated for the following parameters: E\_Hb - Maturity at birth (J), E\_Ho - Maturity of embryo at oviposition (J), E\_Hp - Maturity at puberty (J), h\_a (Weibull ageing acceleration (1/h2). The scaling for these parameters have been changed while not changing the scaling factor of the energy content of the egg (EO).





Figure 2. Comparison of mass (g) and developmental time (d) vary when the cost of structure (EG) is changed during embryonic development. Grey lines indicate reserve, green lines indicate structure and brown lines indicate food in the gut during development. Vertical dashed grey lines indicate the hatch time for the simulated animals. The initial EG (7837.66 J/cm3) was estimated from our Null simulation. For each simulation, the per cent change in EG (A - 10%; B - 20%; C - 30%) is indicated following the incubation temperature of the simulation (23°C top rows and 28°C bottom rows). Depending on the incubation temperature of the simulation, there was a decrease in EG in cold incubation temperatures and an increase in EG in warmer temperatures.

3.4 *How does decreasing energy content of the egg (EO) effect developmental time and offspring body mass?*

During these simulations initial energy content of egg (EO) was decreased by 10% until 50% was reached for each incubation temperature treatment (23°C or 28°C). The energy content of egg was (1145.0 J). During each simulation, cost of structure (EG) was adjusted according to the incubation treatment (23°C or 28°C) that best fit the empirical data (-20% ‘cold’ & +20% ‘hot’). Regardless of the incubation treatment there was a decrease in mass when EO was decreased. The overall mass change was greater in the cold incubation temperatures, but lower overall mass values were detected in hot temperatures.

