Egg hormone methods and results

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# Methods

## Egg collection and dissection

I collected clutches of *L. delicata* and *L. guichenoti* from respective lizard enclosure egg boxes on Monday and Wednesday each week for 9 weeks over November 2022 to February 2023. I collected a total of 21 clutches each for *L. delicata* and *L. guichenoti*, equating to 146 eggs (79 for *L. delicata* and 67 for *L. guichenoti*).

During extraction, 19 eggs were lost due to not running in the silica-bonded column (0 for *L. delicata* and 19 for *L. guichenoti*).

21 clutches were tested for CORT for each species, and 16 clutches for T4 for each species, as I decided to measure T4 concentration after previously assaying 5 clutches.

## Statistical analyses

I performed all statistical analyses in R studio using R version 4.2.2. Upon initial analysis, 69.75% of total variation in CORT and 24.59% of total variation in T4 was due to differences between EIA plates, confounding clutch variation determination. As this variation is not biologically relevant, I used a conditional repeatability approach, and included EIA plate variation as a fixed factor in the repeatability models. Between and within clutch variation is calculated from variation excluding plate variation.

### Between clutch variation

I estimated between clutch variation for CORT and T4 for each of *L. delicata* and *L. guichenoti* by estimating between plate (), between clutch () and within clutch () variance parameters. I then calculated between clutch *ICC* () using the following formula with the rptR package in R as described by Nakagawa and Schielzeth (2010):

Plate variance was included as a fixed effect in the model.

where is the between clutch variance estimate and is the within-clutch variance estimate.

### Within clutch variation

Between plate (), between clutch () and within clutch () variance parameters estimates from between clutch variation from CORT/T4 and *L. delicata*/*L. guichenoti* were used to calculate within clutch *ICC* () using the following formula with the rptR package in R as described by Nakagawa and Schielzeth (2010):

Plate variance was included as a fixed effect in the model.

### Species comparison

*L. delicata* and *L. guichenoti* concentrations of CORT and T4 were compared using a linear model, accounting for egg mass as a fixed effect.

### CORT and T4 relationship

A linear regression was used to compare CORT and T4 concentrations for each egg. A linear model was used to determine scaling coefficient and significance.

# Results

## *Lampropholis delicata*

I obtained an average of 3.76 eggs per clutch (21 clutches in total) for *L. delicata* (SD = 0.7) with a minimum of 2 and maximum of 5 eggs for each clutch. The average log CORT concentration was 0.68 (SD = 1.08) with a minimum of -1.51 and maximum of 3.38 (Table 1). Overall, there was evidence of differences between clutch repeatability in CORT concentrations (R = 0.36, 95% CI: 0.09 to 0.61; [Figure 1](#fig-fig1)), suggesting approximately 35.64% of the variation was the result of differences between mothers. Additionally, there was evidence of repeatability difference within clutches in CORT concentrations (R = 0.64, 95% CI: 0.39 to 0.91; [Figure 1](#fig-fig1)), suggesting approximately 64.36% of the variation was due to egg hormone deposition differences in respective clutches.

The average log T4 concentration for *L. delicata* was -1 (SD = 0.51) with a minimum of -1.85 and maximum of 0.53 (Table 2). For T4 concentration, there was no strong evidence for between clutch repeatability difference (R = 0.14, 95% CI: 0 to 0.42); [Figure 2](#fig-fig2)). This suggests approximately 14.34% of T4 variation was due to differences between mothers. Additionally, there was evidence of repeatability difference within clutches in T4 concentrations (R = 0.86, 95% CI: 0.58 to 1; [Figure 2](#fig-fig2)), suggesting approximately 85.66% of the variation was due to egg hormone deposition differences in respective clutches.

## *Lampropholis guichenoti*

I obtained an average of 3.33 eggs per clutch (21 clutches in total) for *L. guichenoti* (SD = 0.58) with a minimum of 3 and maximum of 5 eggs for each clutch. The average log CORT concentration was 0.69 (SD = 0.93) with a minimum of -1.24 and maximum of 2.53 (Table 1). Overall, there was evidence of between clutch repeatability differences in CORT concentrations (R = 0.43, 95% CI: 6.84^{-19} to 0.7; [Figure 1](#fig-fig1)), suggesting approximately 42.51% of the variation was the result of differences between mothers. Additionally, there was evidence of repeatability difference within clutches in CORT concentrations (R = 0.57, 95% CI: 0.3 to 1; [Figure 1](#fig-fig1)), suggesting approximately 57.49% of the variation was due to egg hormone deposition differences in respective clutches.

The average log T4 concentration for *L. guichenoti* was -1.19 (SD = 0.53) with a minimum of -2.5 and maximum of 0.01 (Table 2). For T4 concentration, there was no strong evidence for differences between clutch repeatability (R = 0.34, 95% CI: 0 to 0.73); [Figure 2](#fig-fig2)). This suggests approximately 33.82% of T4 variation was due to differences between mothers. Additionally, there was evidence of repeatability differences within clutches in T4 concentrations (R = 0.66, 95% CI: 0.27 to 1; [Figure 2](#fig-fig2)), suggesting approximately 66.18% of the variation was due to egg hormone deposition differences in respective clutches.

## Species comparisons

*L. delicata* had higher CORT concentrations, however there were no significant differences in CORT concentrations between species (mean difference = -0.13, 95% CI: -0.21 to 0.11, p = 0.45) controlling for egg mass. *L. delicata* had higher T4 concentrations accounting for egg mass, however this difference in T4 concentration between species was not significant (mean difference = -0.14, 95% CI: -0.18 to 0.09, p = 0.32).

## CORT and T4 relationship

There was no significant relationship of CORT and T4 (scaling coefficient = 0.06, SE = 0.21, p = 0.77; [Figure 3](#fig-fig3)), suggesting no correlation of these hormones.

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| Figure 1- Log CORT concentration (pg/mg yolk) in egg yolks for clutches of *L. delicata* (n = 21) and *L. guichenoti* (n = 21). Each box represents a unique clutch, where *L. delicata* clutches are shown in shades of warmer colours, and *L. guichenoti* clutches are shown in shades of cooler colours. |

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| Figure 2- Log T4 concentration (pg/mg yolk) in egg yolks for clutches of *L. delicata* (n = 16) and *L. guichenoti* (n = 16). Each box represents a unique clutch, where *L. delicata* clutches are shown in shades of pink, and *L. guichenoti* clutches are shown in shades of green. |

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| Figure 3- Relationship of log CORT concentration (pg/mg yolk) and log T4 concentration (pg/mg yolk) in egg yolks. Raw data is shown in black (n = 96), the linear regression line is represented in purple, and 95% confidence interval is shaded in grey. The scaling coefficient is 0.06 with a p-value of 0.77. |