Temporal effects of heat waves on sex ratios and gene expression in a freshwater turtle

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Climate Change and Heat Waves
Predicted changes in heat wave frequency (with respect to data from 1961-1990)

Fischer and Schär 2010
Predicted changes in heat wave amplitude (with respect to data from 1961-1990)

Fischer and Schär 2010
GENOTYPIC SEX DETERMINATION

Fertilization

Gonad Determining Genes → Gonad Formation → Hormones → Sexual Differentiation of Phenotype

TEMPERATURE-DEPENDENT SEX DETERMINATION

Fertilization

Temperature

Enzymes Hormones Receptors → Gonad Determining Genes → Gonad Formation → Hormones → Sexual Differentiation of Phenotype
Modified from Lance and Valenzuela 2004
Cool temps. (26°C)

Warm temps. (31°C)

Trachemys scripta

Modified from Lance and Valenzuela 2004
Oviposition

**SEX HAS NOT BEEN DETERMINED**

Bipotential gonads

**SEX IS BEING DETERMINED**

Warm temps.

Cool temps.

**SEX HAS BEEN DETERMINED**

Ovarian development

Testicular development

Female

Male

Thermosensitive Period (TSP) (middle third)

Hatching
Temperature affects TSP length and timing

Matsumoto and Crews 2012
Male producing temperature

- Dmrt1 (transcription factor)
- Sox9 (transcription factor)
- SF1 (nuclear receptor/mRNA splicing factor)
- AMH
- P450_{scc}, P450_{17a}, 3βHSD

Female producing temperature

- Foxl2 (transcription factor)
- Rspo1 (growth factor)
- Aromatase (estrogen synthase)

Testicular development

1. Cholesterol
2. Testosterone
3. Dihydrotestosterone

Ovarian development

- Estradiol

Testicular development

Change in temperature influences gene expression:
- Dmrt1
- Sox9
- SF1

Aromatase (estrogen synthase)

Modified from Matsumoto and Crews 2012
Male producing temperature

Dmrt1 (transcription factor)

Sox9 (transcription factor)

SF1 (nuclear receptor/mRNA splicing factor)

AMH

P450<sub>sc</sub>, P450<sub>17α</sub>, 3βHSD

Testicular development

Female producing temperature

Foxl2 (transcription factor)

Rspo1 (growth factor)

Aromatase (estrogen synthase)

Modified from Matsumoto and Crews 2012
A. FoxL2

B. Rspo1

C. Aromatase

D. Dmrt1

E. Sox9

Matsumoto and Crews 2012
Male-producing temperature cue

![Chemical diagram]

**Testicular development**
Female-producing temperature cue

Testosterone \rightarrow \text{aromatase} \rightarrow \text{estradiol} \downarrow \text{Ovarian development}

\[
\begin{align*}
\text{CH}_3 & \quad \text{O} & \quad \text{H} \\
\text{CH}_3 & \quad \text{C} & \quad \text{D} \\
\text{CH}_3 & \quad \text{A} & \quad \text{B} \\
\text{C} & \quad \text{B} & \quad \text{A} \\
\text{D} & \quad \text{C} & \quad \text{B} \\
\end{align*}
\]
Steroid hormones

Crews et al. 1994; Matsumoto and Crews 2012
Field versus Laboratory
Constant versus Fluctuating

- Daily/seasonal fluctuations

*Paitz et al. 2010*
Average temperatures are poor predictors of sex ratios

Les et al. 2007
Heat waves affect sex ratios

Carter et al. 2018
Current Understanding
Natural Fluctuations

Chrysemys picta:
Biased Sex Ratios

- Mrosovsky and Provancha 1989
- Wibbels et al. 1991
- Mrosovsky and Provancha 1992
- Marcovaldi et al. 1997
- Hanson et al. 1998
- Godley et al. 2001
- Öz et al. 2004

*Caretta caretta*
(Loggerhead Sea Turtle)

Female biased nests, juvenile and sub-adult
89-99% females
Potential Responses

• Behavioral Plasticity
  • **Nesting phenology***
    • *Chrysemys picta* nesting 27 days earlier on average over 13 year period (*Schwanz and Janzen 2008*)
  • Spatial changes in nesting
    • Lacks plasticity (*Refsnider and Janzen 2012; Refsnider 2013*)

• Evolve a sensitivity to a different pivotal temperature

• Migrate to cooler climate to balance sex ratios
  • Depends on water body distributions
Climate change, in the form of heat waves, will influence the physiological and endocrinological underpinnings of TSD in turtles.

1) How does TSD operate in the field under natural conditions?
2) How will climate change affect sex ratios in reptiles with TSD?
Model Species- *Trachemys scripta*
Hypothesis 1

Heat wave timing will influence the physiological and endocrinological underpinnings of TSD.

**Prediction 1**: A heat wave that occurs within the TSP will produce more female-biased sex ratios.

**Prediction 2**: A heat wave applied during the TSP will trigger relatively higher levels of aromatase expression and relatively lower levels of Dmrt1 expression.

a. Using fluctuating incubation temperatures, when does the TSP occur?

b. How does aromatase and Dmrt1 expression respond to a simulated heat wave during the predicted TSP?
Hypothesis 1 - Methods

• Male producing condition: $25 \pm 3^\circ C$
  • “Baseline”

• Female producing condition: $29.5 \pm 3^\circ C$
  • “Heat wave”

• 15-day heat wave varied temporally

• Eggs: gravid female (oxytocin)/nest
• Group eggs to avoid clutch/box/incubator effects

• 20 eggs per treatment (sex ratio) 15 extra eggs in control and 24-38 (qPCR)

Illinois Climate Network 2015; Carter et al. 2017
Hypothesis 1 - Methods (continued)

- Macroscopic gonad examination after euthasol injection
Hypothesis I - qPCR

- Sample embryonic tissues on days 38 and 43 in the control and day 24-38 groups
  - *Gapdh* as housekeeping gene

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward <em>Gapdh</em></td>
<td>GGCTTT CGTGT TCC AAC TC</td>
<td>Ge et al. 2017</td>
</tr>
<tr>
<td>Reverse <em>Gapdh</em></td>
<td>GAC AAC CTG GTC CTC CGT GTA TC</td>
<td>Ge et al. 2017</td>
</tr>
<tr>
<td>Forward <em>Aromatase</em></td>
<td>CGA CAT GGA CTT TGC ATC ACA</td>
<td>Ramsey et al. 2007</td>
</tr>
<tr>
<td>Reverse <em>Aromatase</em></td>
<td>GAA CCA TCA TCT CCA ACA CAC ACT GGT TC</td>
<td>Ramsey et al. 2007</td>
</tr>
<tr>
<td>Forward <em>Dmrt1</em></td>
<td>CAA CTA CTC CCA ATA CCA GAT GGC</td>
<td>Shoemaker et al. 2007</td>
</tr>
<tr>
<td>Reverse <em>Dmrt1</em></td>
<td>GGCTTC GCA GGCTGT TTT TC</td>
<td>Shoemaker et al. 2007</td>
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</tbody>
</table>
Sex ratio results:

Heatwave treatment timing: $\chi^2 = 29.324$, df = 4, p < 0.001
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. Middle Third</th>
<th>% Female</th>
<th>Stat. Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>≈28-56</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Heat wave 10-24</td>
<td>≈24-48</td>
<td>12%</td>
<td>a</td>
</tr>
<tr>
<td>Heat wave 17-31</td>
<td>≈24-48</td>
<td>72%</td>
<td>b</td>
</tr>
<tr>
<td>Heat wave 24-38</td>
<td>≈24-48</td>
<td>89%</td>
<td>b</td>
</tr>
<tr>
<td>Heat wave 31-45</td>
<td>≈25-50</td>
<td>84%</td>
<td>b</td>
</tr>
<tr>
<td>Heat wave 38-52</td>
<td>≈25-50</td>
<td>6%</td>
<td>a</td>
</tr>
</tbody>
</table>

Middle third?
Control
Heatwave

Relative Aromatase expression
$(2^{\Delta \text{dCT}})$

<table>
<thead>
<tr>
<th>Aromatase</th>
<th>df</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>11.32</td>
<td><strong>p = 0.003</strong></td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>15.16</td>
<td><em><strong>p &lt; 0.001</strong></em></td>
</tr>
<tr>
<td>Treatment*Day</td>
<td>1</td>
<td>11.37</td>
<td><strong>p = 0.003</strong></td>
</tr>
</tbody>
</table>

Day

- 15
- 20

Treatment

- Control
- Heatwave
**Relative Dmrt1 expression**

(2^−dCT)

<table>
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<th>df</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1</td>
<td>30.11</td>
<td>p &lt; 0.001 ***</td>
</tr>
<tr>
<td></td>
<td>Heatwave</td>
<td>1</td>
<td>15.42</td>
<td>p &lt; 0.001 ***</td>
</tr>
<tr>
<td></td>
<td>Treatment*Day</td>
<td>1</td>
<td>6.53</td>
<td>p = .0193 *</td>
</tr>
</tbody>
</table>
Control

Day 10-24

Day 17-31

Day 24-38

Day 31-45

Day 38-52

Predicted TSP

25.0 ± 3°C shifted to 29.5 ± 3°C
Hypothesis 1 Conclusions

- TSP is likely around days 20-40 using these temperature parameters
- Middle three heat waves have similar potency to produce females
- Heat waves induce *aromatase* expression
- MPTs induce *Dmrt1* expression

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Next Steps: Natural Sex Ratios

• Multi-year *T. scripta* field incubations
  • Eggs: gravid female (oxytocin)/nest
  • Individual nests dug for each clutch in nesting area
  • iButton data loggers
  • Macroscopic gonad examination

a. What sex ratios are being produced in nature?
Next Steps: Hypothesis 2

Heat wave continuity will influence the physiological and endocrinological underpinnings of TSD in turtles.

**Prediction 1:** With hot days held constant, more continuous heat waves will produce more female-biased sex ratios.

a. How does heat wave continuity affect sex determination?
Hypothesis 2 - Methods

<table>
<thead>
<tr>
<th>Heat Wave Length (Days)</th>
<th>Proportion Females</th>
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</thead>
<tbody>
<tr>
<td>8</td>
<td>24%</td>
</tr>
<tr>
<td>11</td>
<td>74%</td>
</tr>
<tr>
<td>14</td>
<td>94%</td>
</tr>
<tr>
<td>17</td>
<td>89%</td>
</tr>
<tr>
<td>20</td>
<td>90%</td>
</tr>
<tr>
<td>23</td>
<td>94%</td>
</tr>
<tr>
<td>26</td>
<td>90%</td>
</tr>
<tr>
<td>29</td>
<td>100%</td>
</tr>
<tr>
<td>32</td>
<td>100%</td>
</tr>
<tr>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
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Carter et al. unpublished
Next Steps: Hypothesis 3

**Aromatase and Dmrt1** expression will respond differentially to heat waves of varying lengths.

**Prediction 1**: Longer heat waves will produce higher levels of aromatase expression.

**Prediction 2**: Heat waves will down-regulate Dmrt1 expression.

**a.** How does aromatase expression respond to a heat waves of varying lengths?

**b.** How does Dmrt1 expression respond to a heat waves of varying lengths?

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</tr>
<tr>
<td>35</td>
<td>100%</td>
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Carter et al. unpublished
Hypothesis 3 - Methods

- Sample on days 30, 33, & 36
- Gapdh as housekeeping gene
Acknowledgements

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