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

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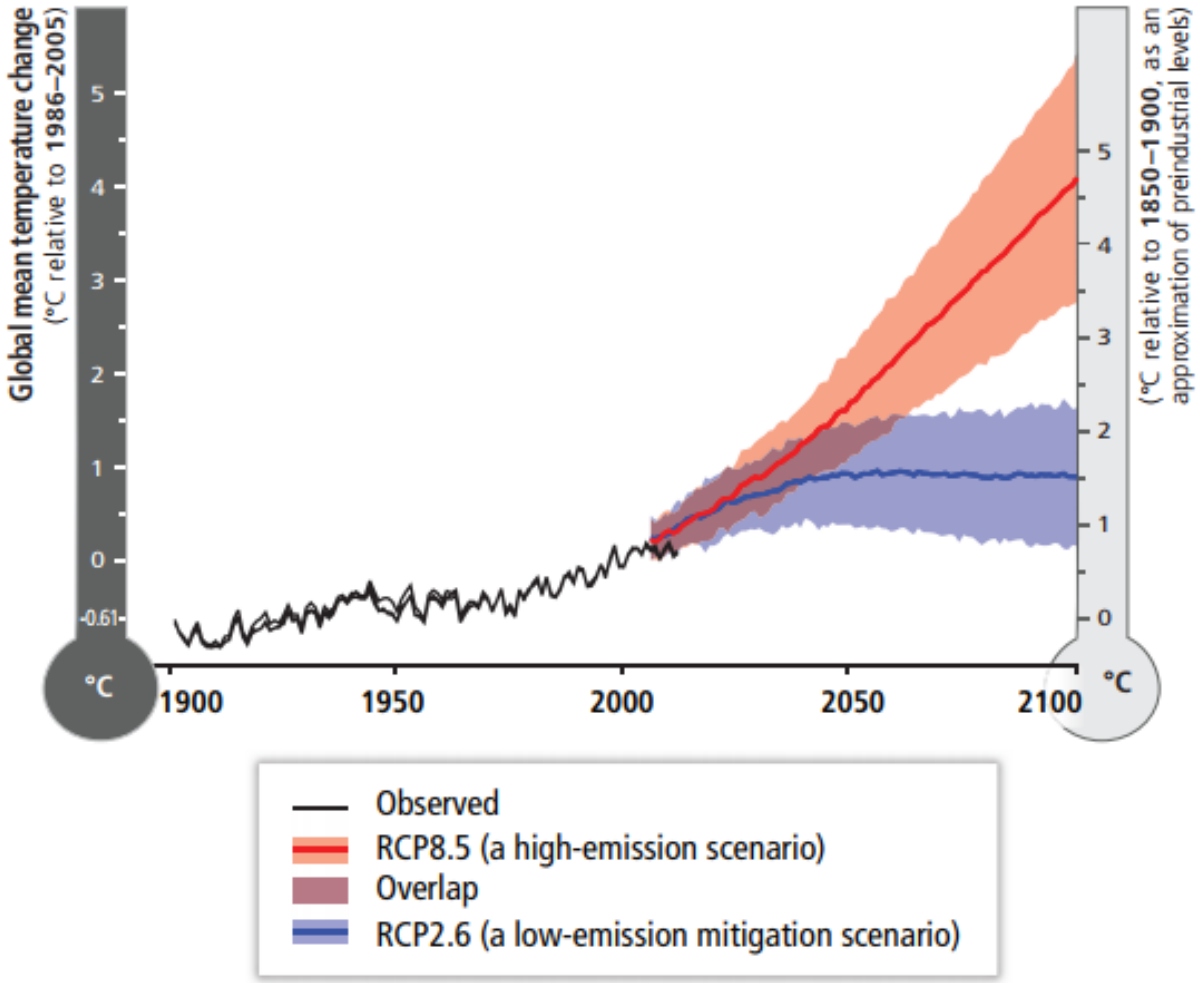
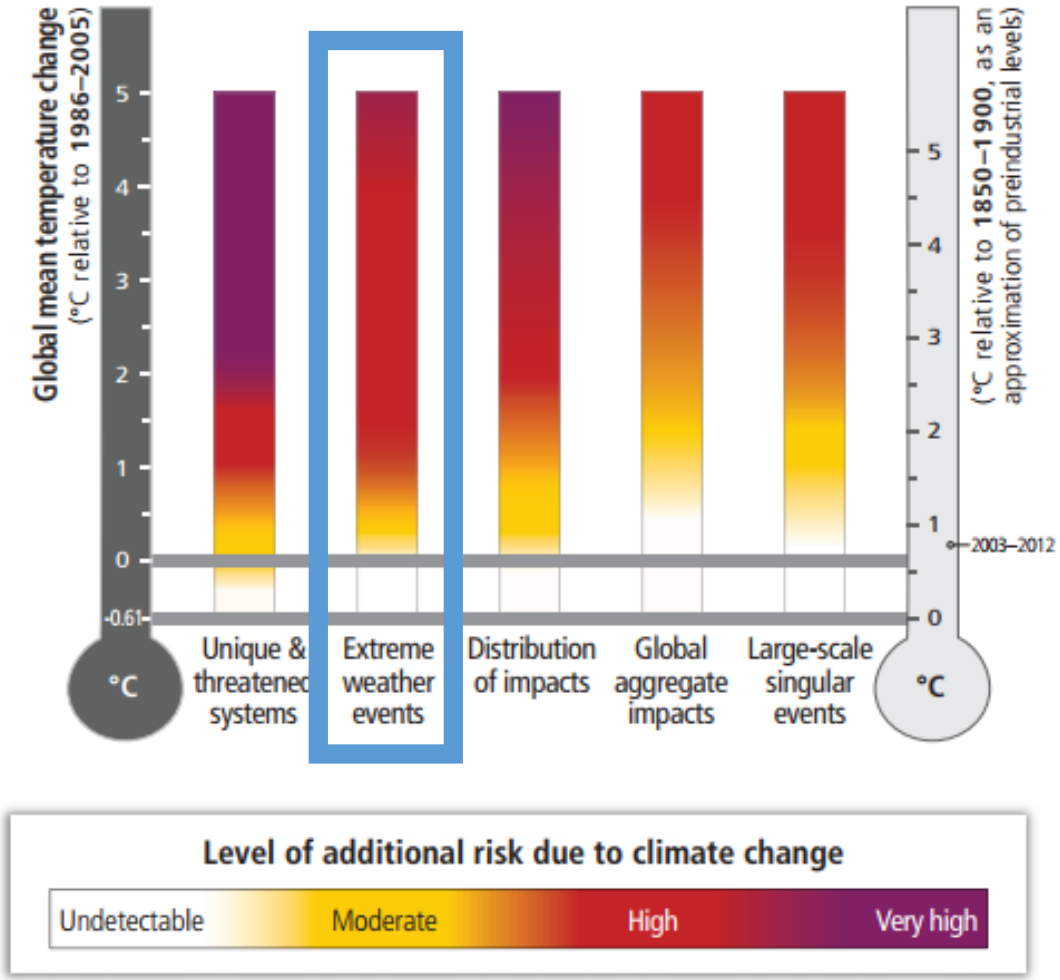
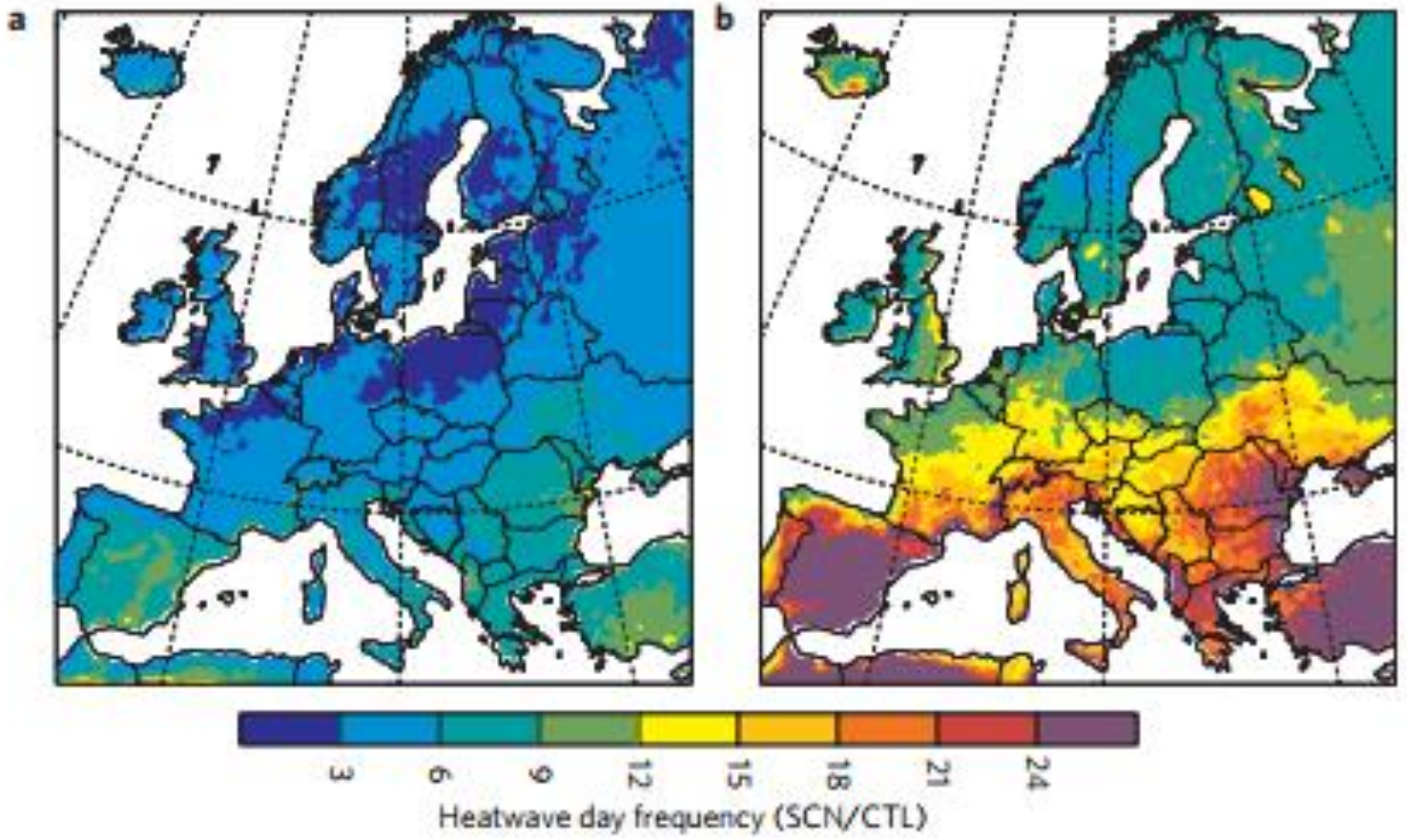
Climate Change and Heat Waves


Global mean temperature change
(°C relative to 1986–2005)
5
4
3
2
1
0
-0.61
°C
1900
1950
2000
2050
2100
(°C relative to 1850–1900, as an approximation of preindustrial levels)
°C
Observed
RCP8.5 (a high-emission scenario)
Overlap
RCP2.6 (a low-emission mitigation scenario)


Global mean temperature change
(°C relative to 1986–2005)
5
4
3
2
1
0
-0.61
°C
Unique & threatened systems
Extreme weather events
Distribution of impacts
Global aggregate impacts
Large-scale singular events
(°C relative to 1850–1900, as an approximation of preindustrial levels)
2003–2012
°C
Level of additional risk due to climate change
Undetectable
Moderate
High
Very high

IPCC 2014

Predicted changes in heat wave frequency (with respect to data from 1961-1990)

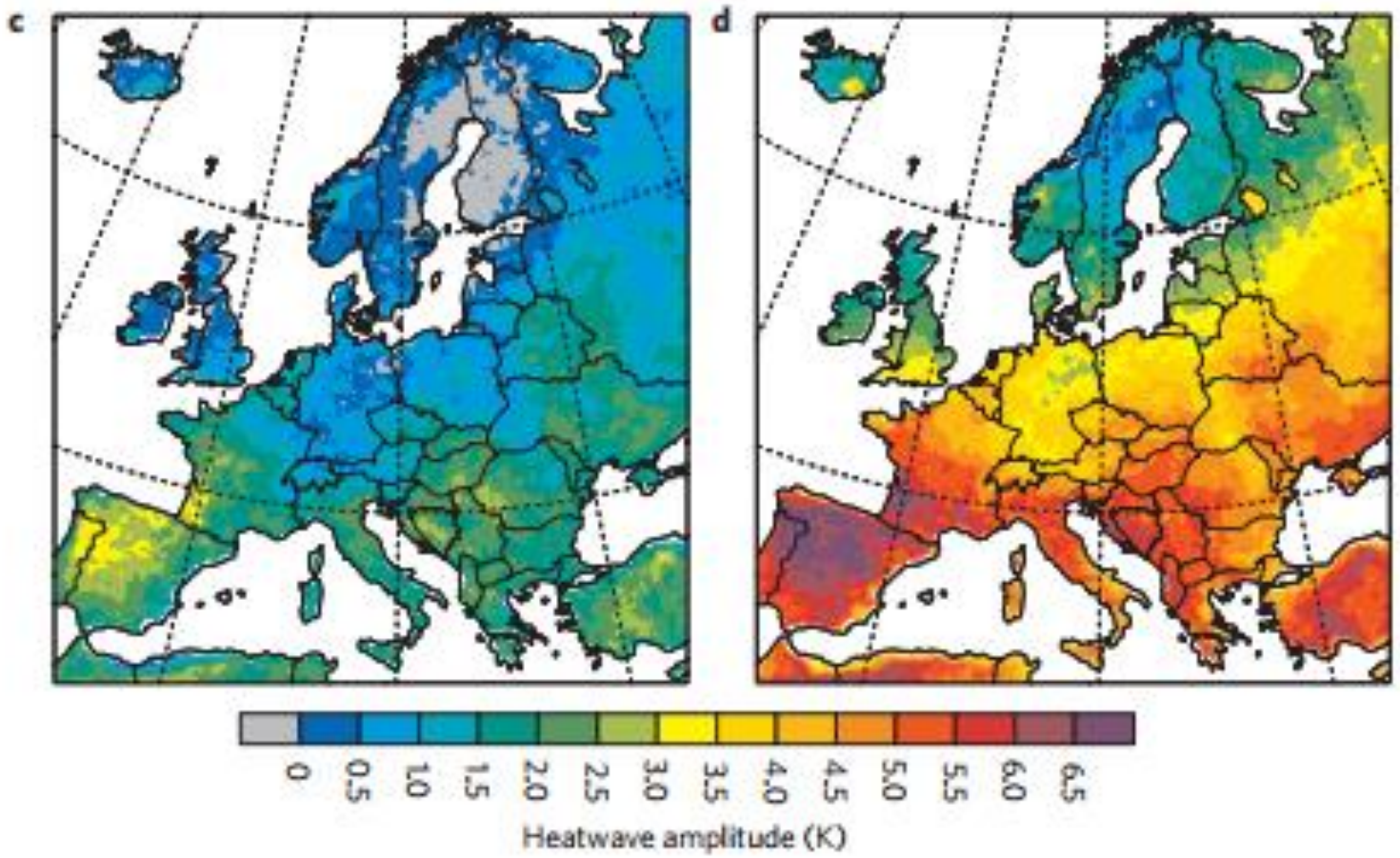


2021-2050

2071-2100

Fischer and Schär 2010

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



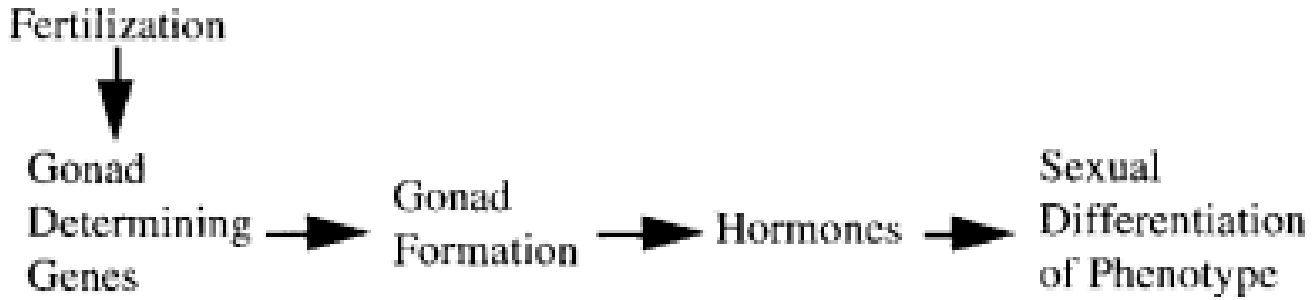
2021-2050

2071-2100

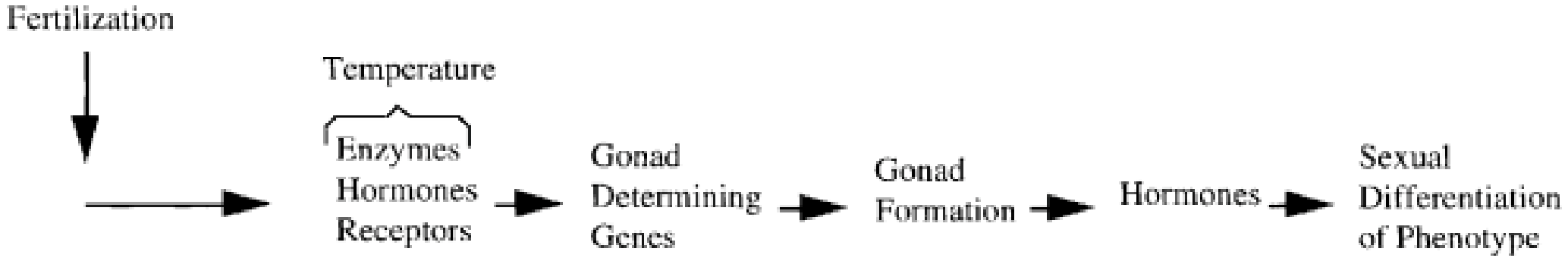
Fischer and Schär 2010

Reptiles with TSD

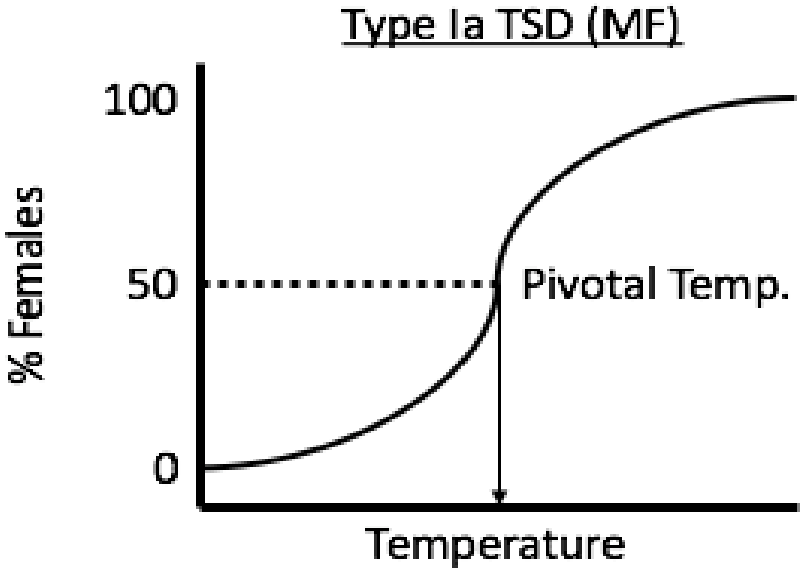
GENOTYPIC SEX DETERMINATION

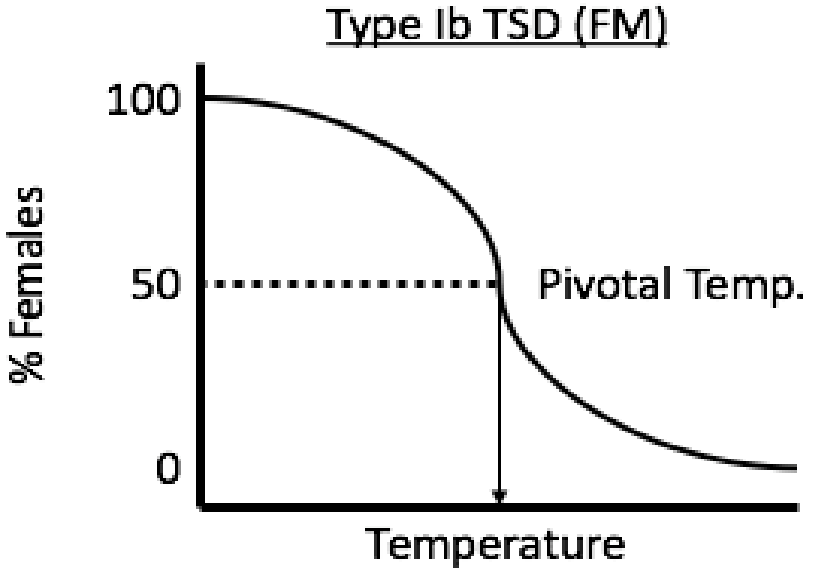


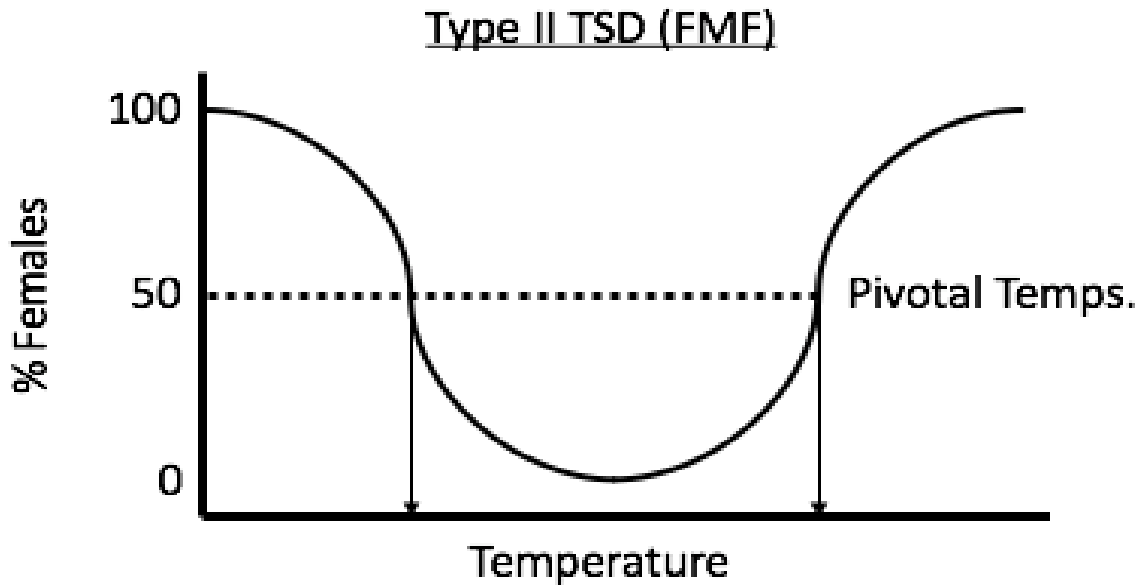
TEMPERATURE-DEPENDENT SEX DETERMINATION



Crews et al. 1994

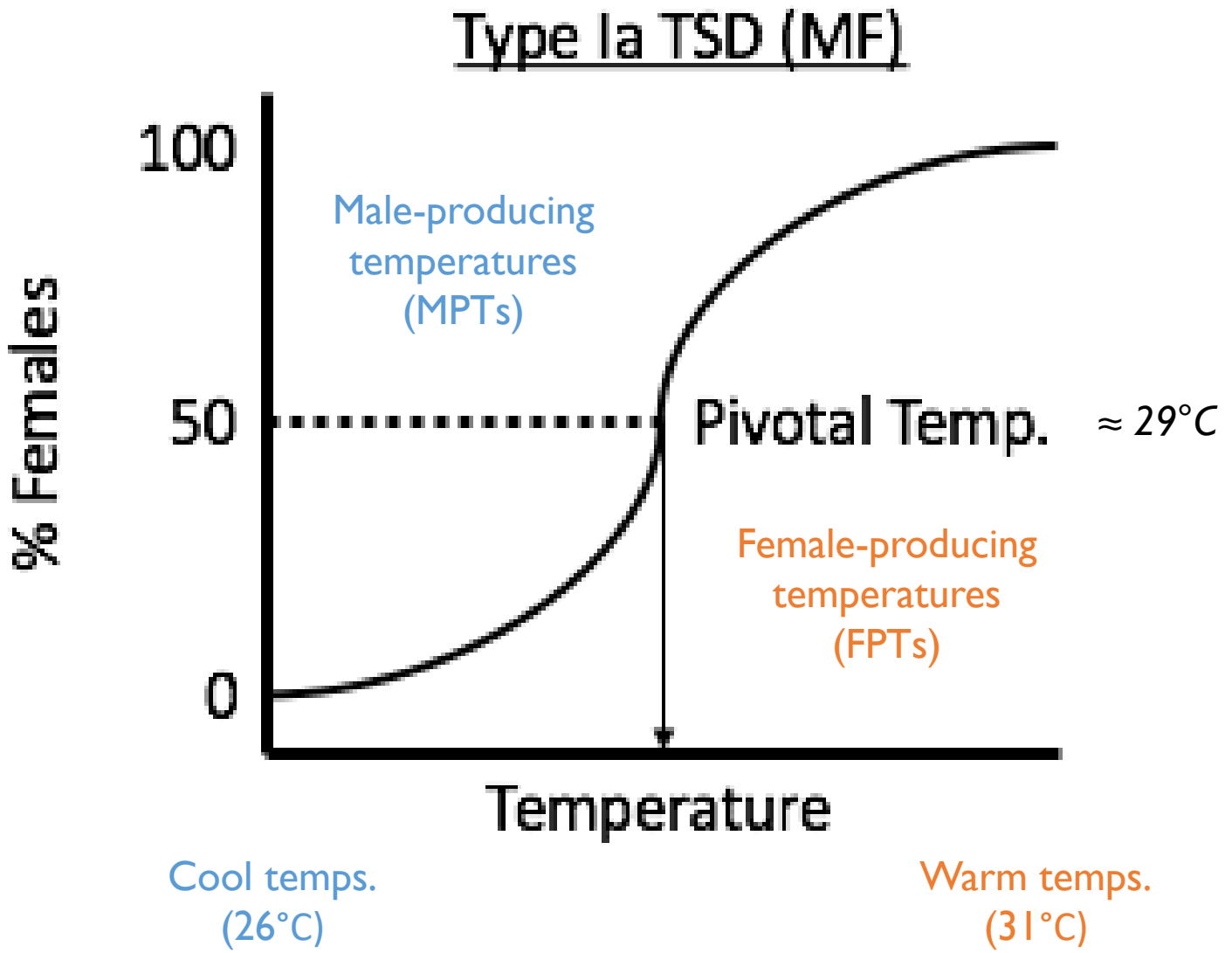






Modified from Lance and Valenzuela 2004

Trachemys scripta



Modified from Lance and Valenzuela 2004

SEX HAS NOT BEEN DETERMINED

SEX IS BEING DETERMINED

SEX HAS BEEN DETERMINED

Bipotential gonads

Warm temps.

Ovarian development

Female ♀

Cool temps.

Testicular development

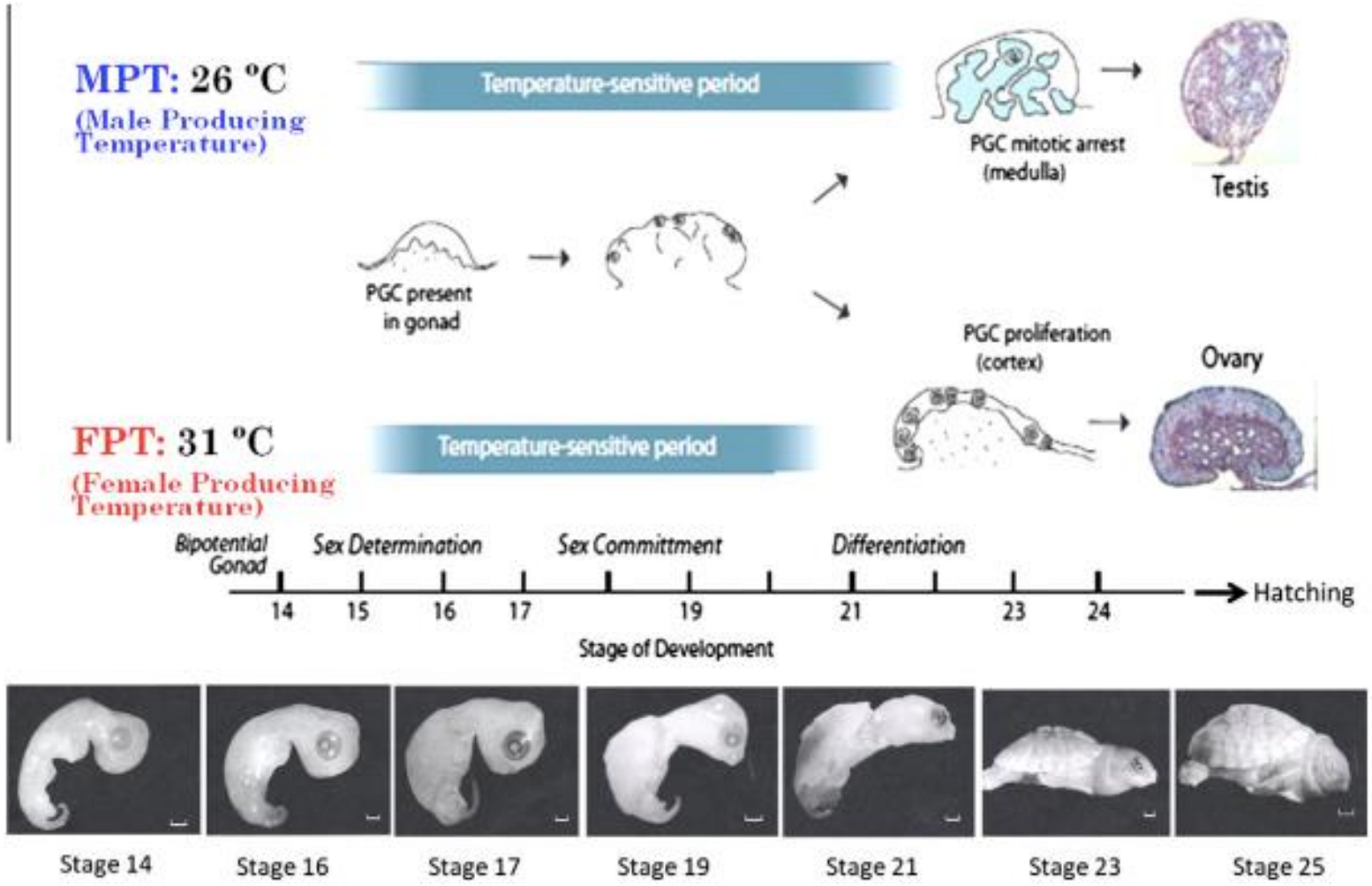
Male ♂

Oviposition

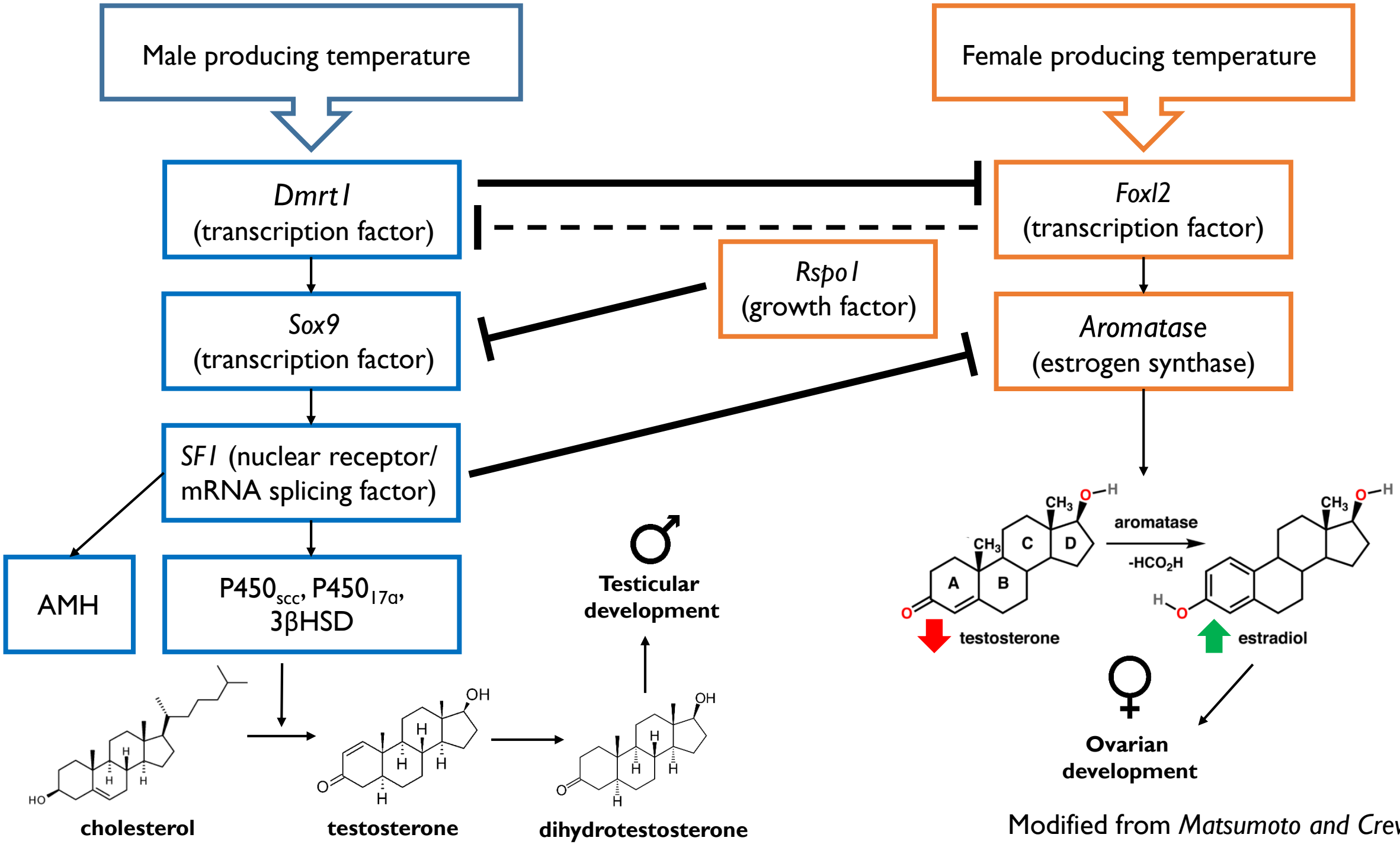
Thermosensitive Period (TSP)
(middle third)

Hatching

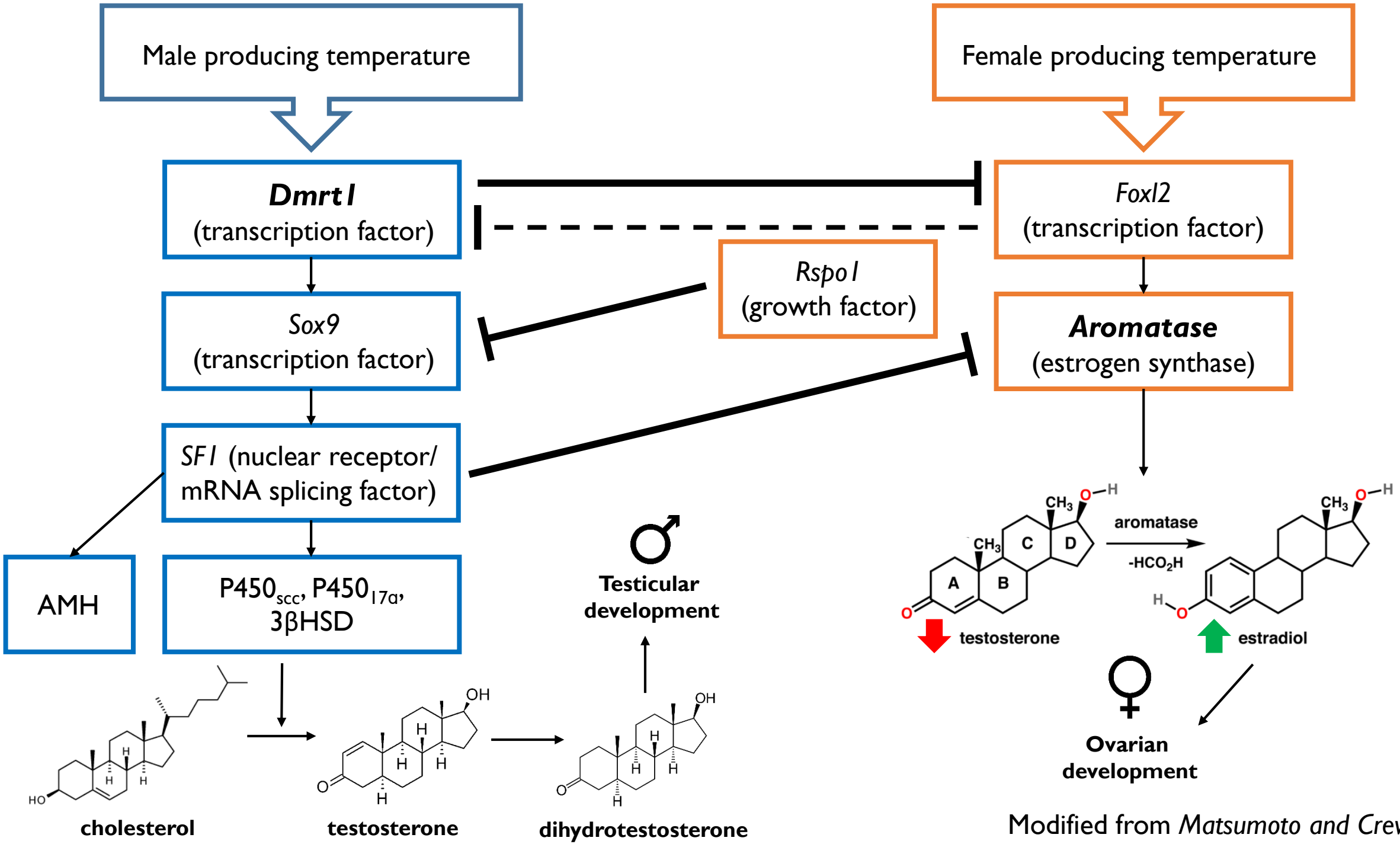
Temperature affects TSP length and timing



Matsumoto and Crews 2012



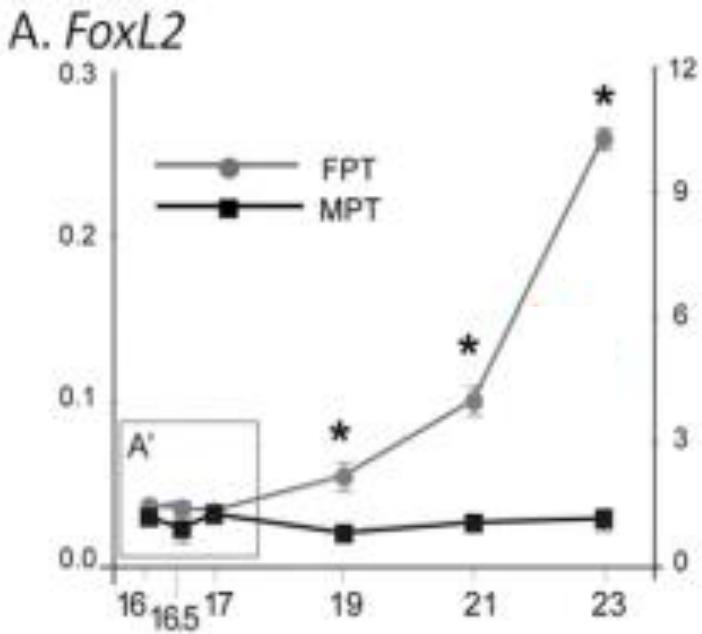
Modified from *Matsumoto and Crews 2012*

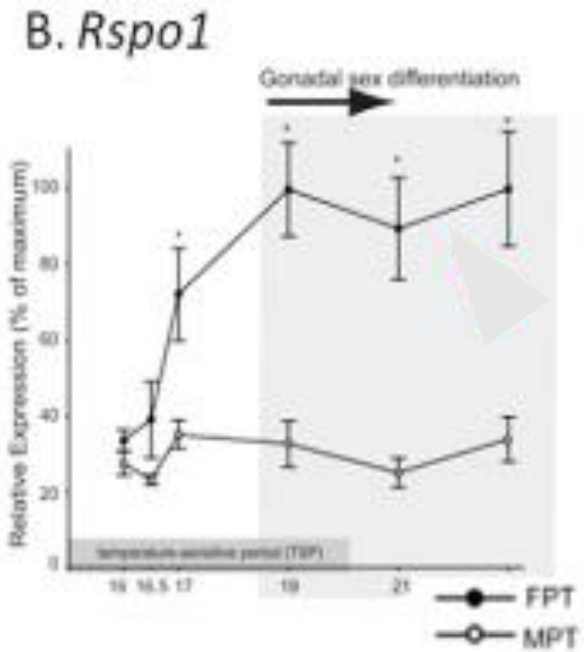


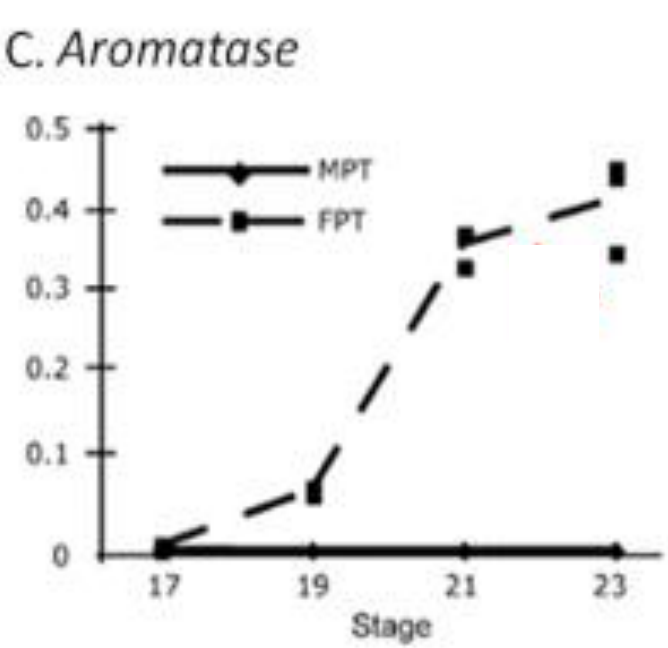
Modified from *Matsumoto and Crews 2012*

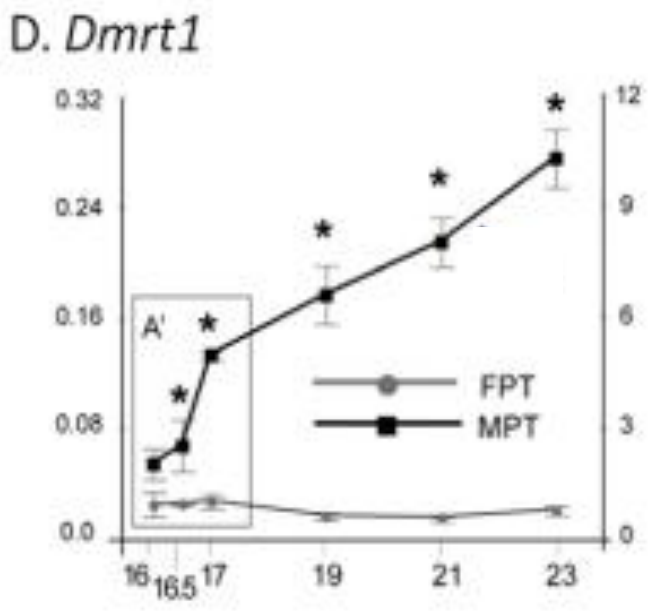
♀

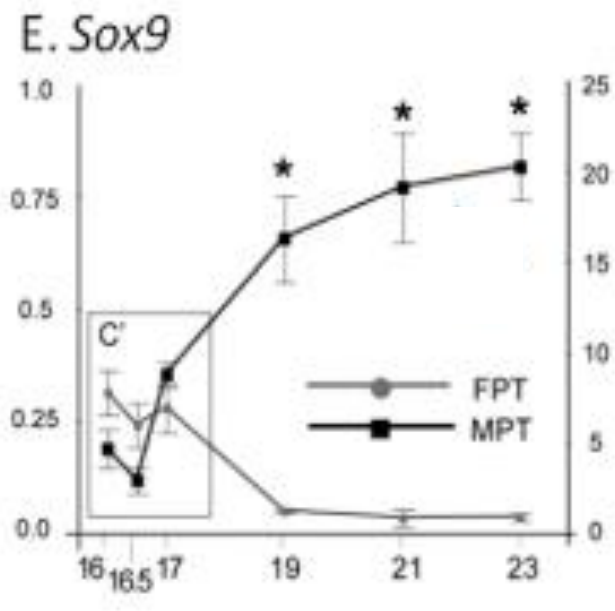
♂





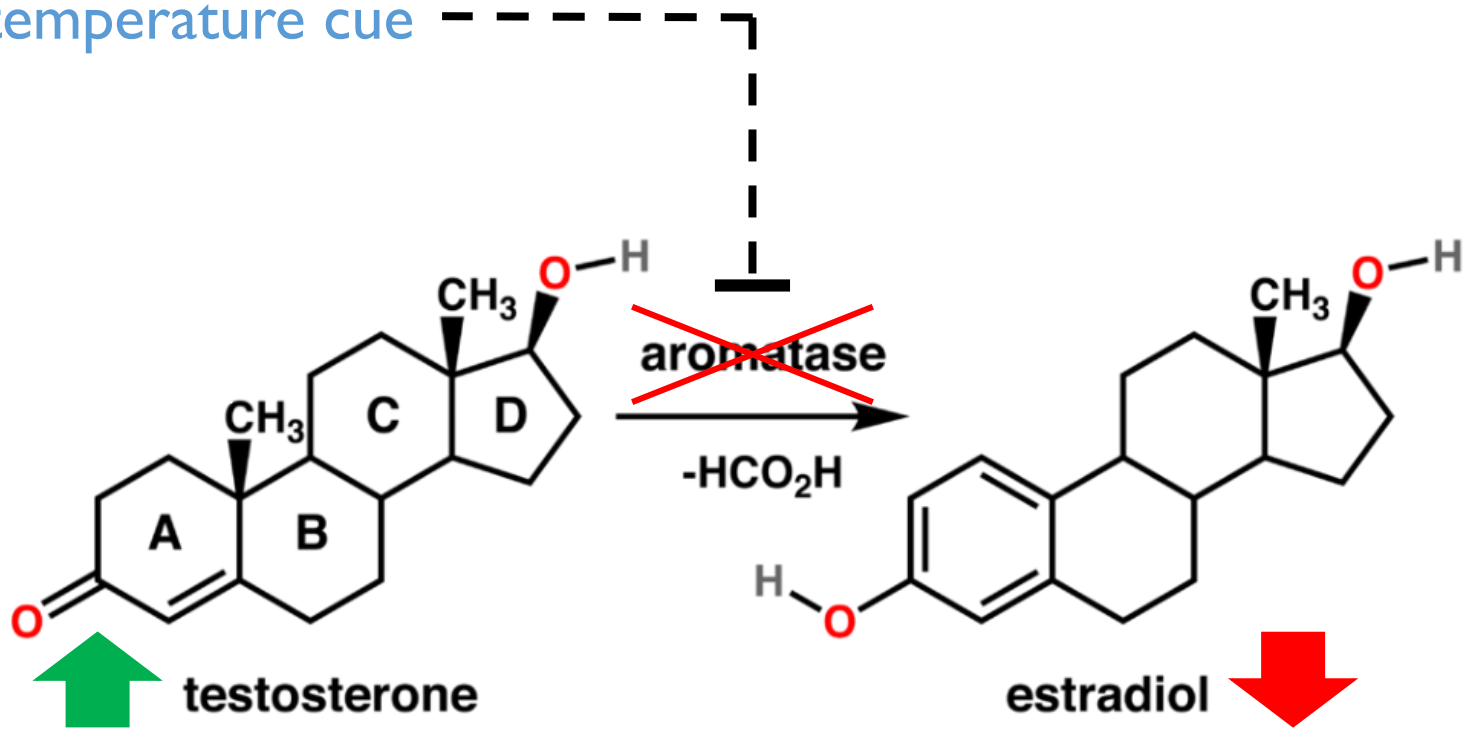




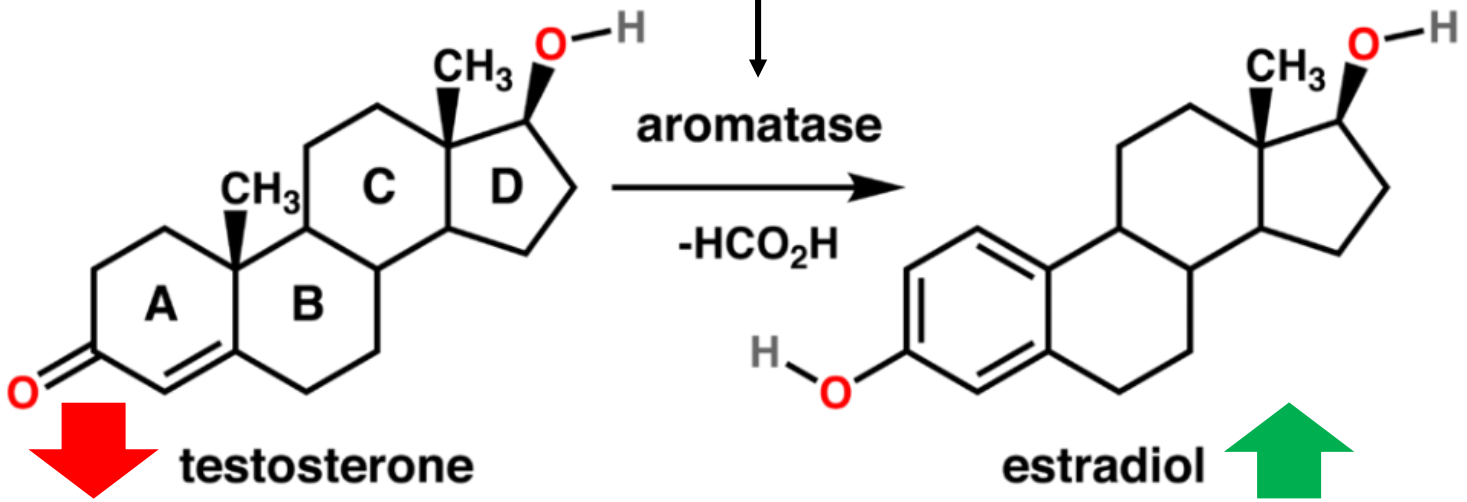


Matsumoto and Crews 2012

Male-producing temperature cue



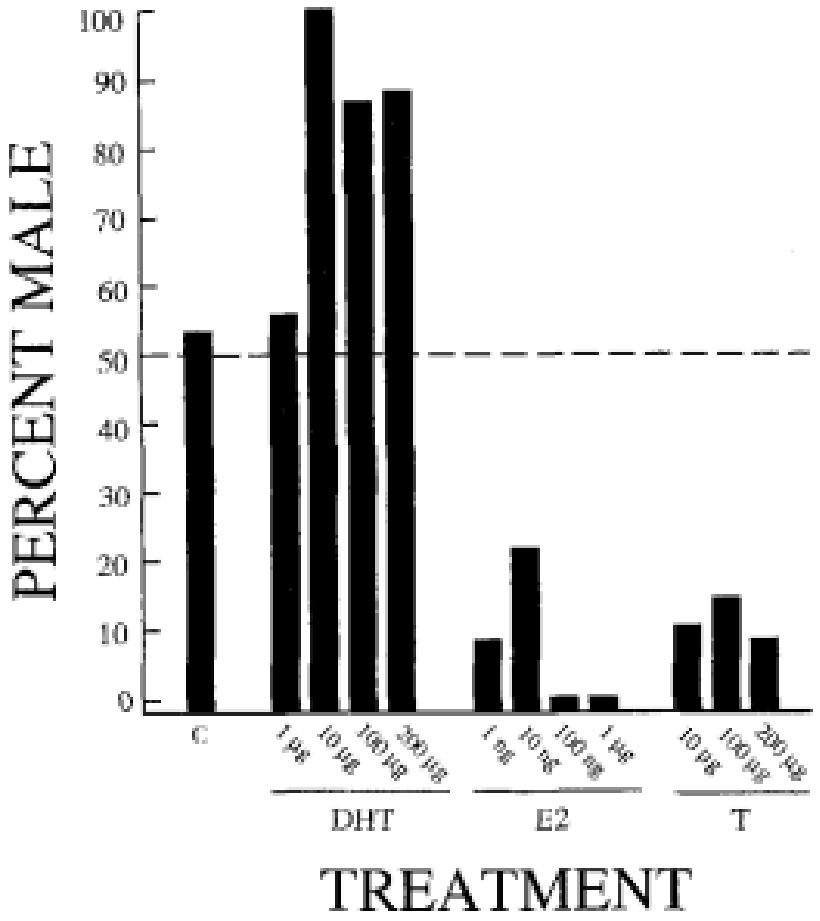
Female-producing temperature cue

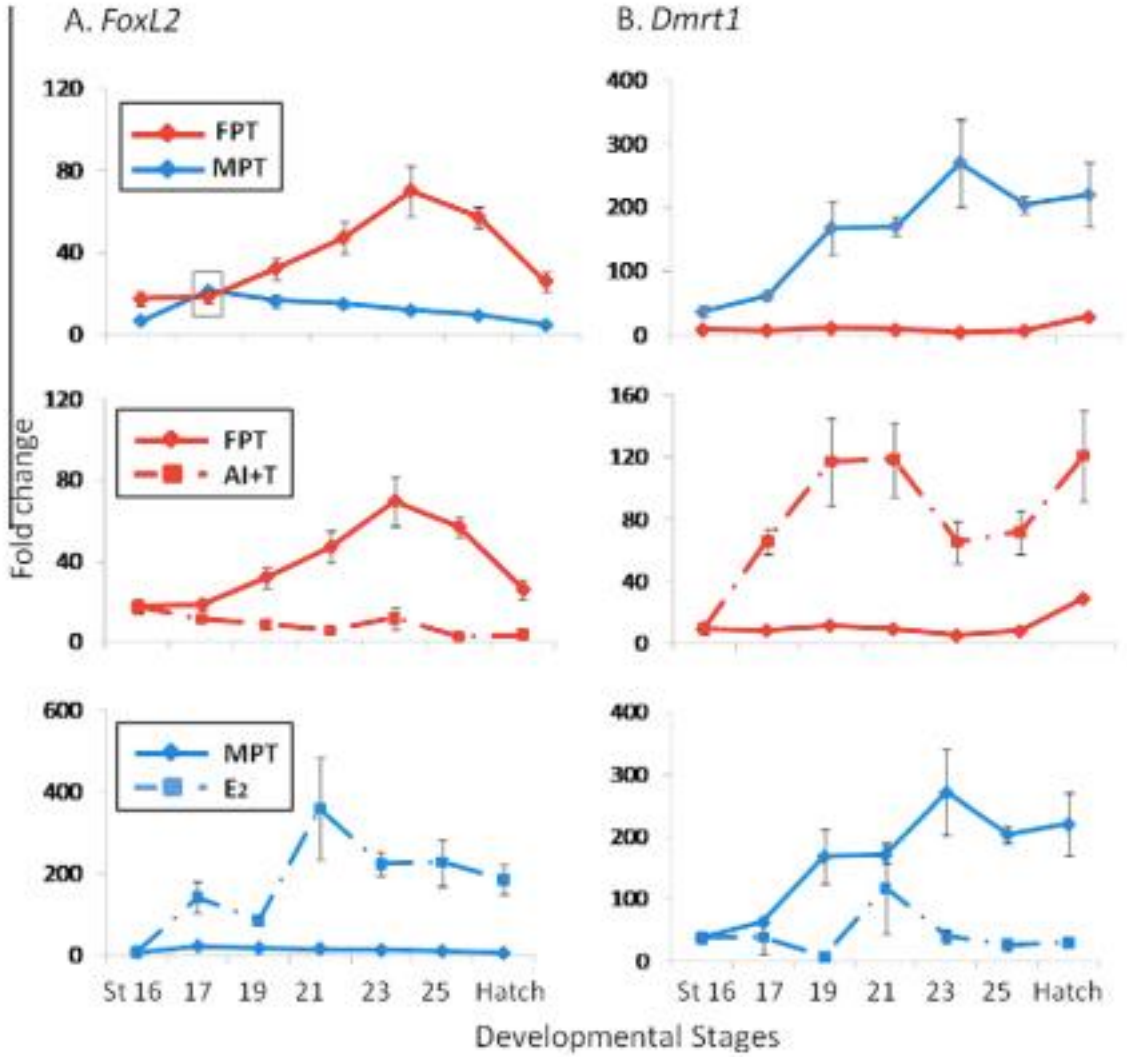


Ovarian development

♀

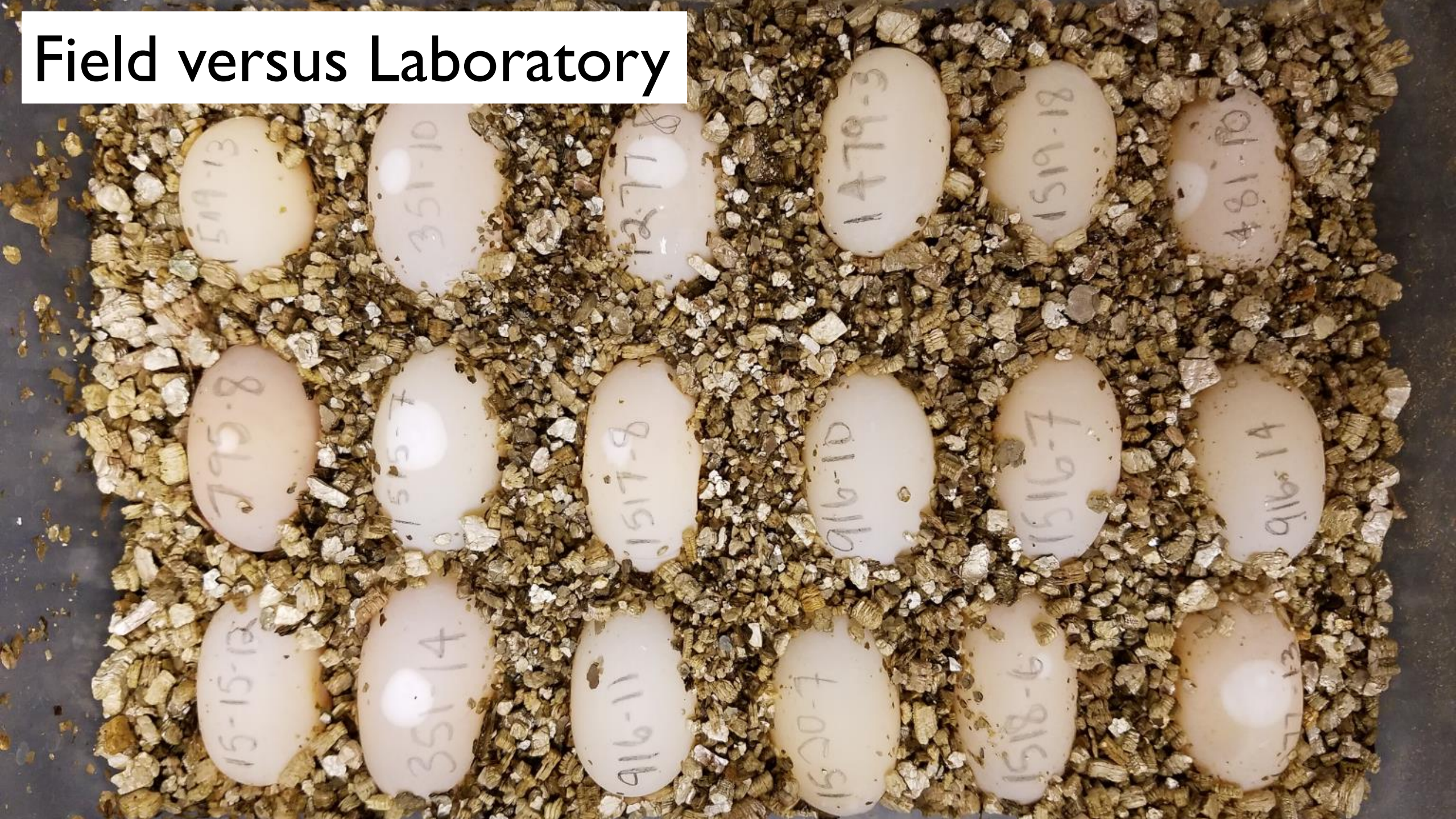
Steroid hormones





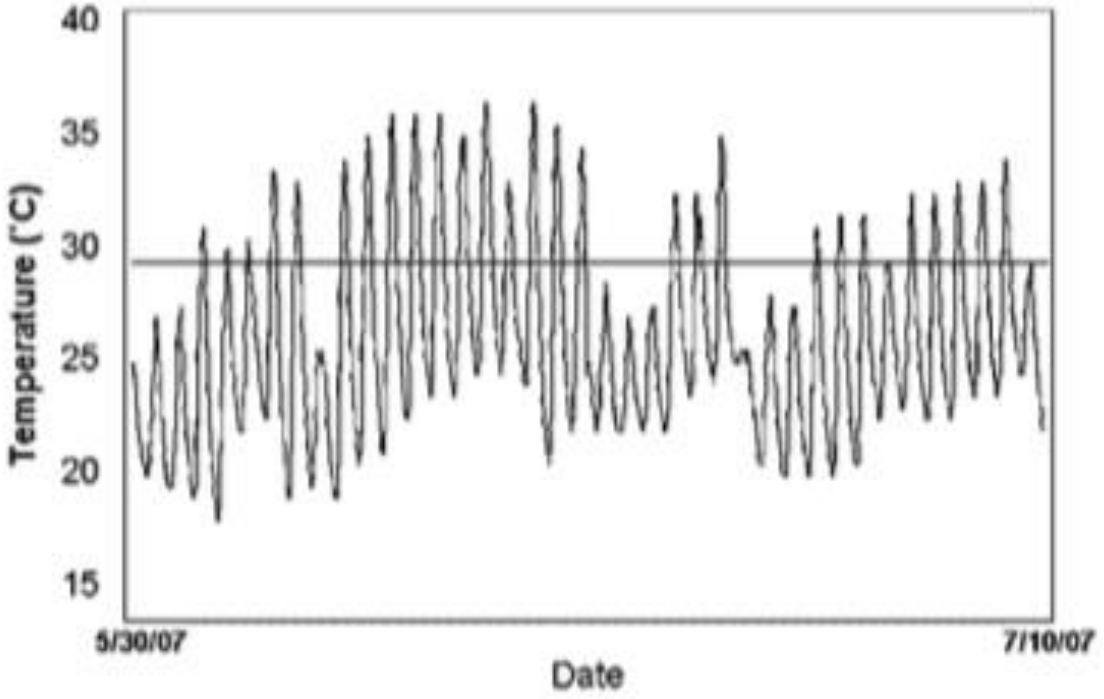
Crews et al. 1994; Matsumoto and Crews 2012

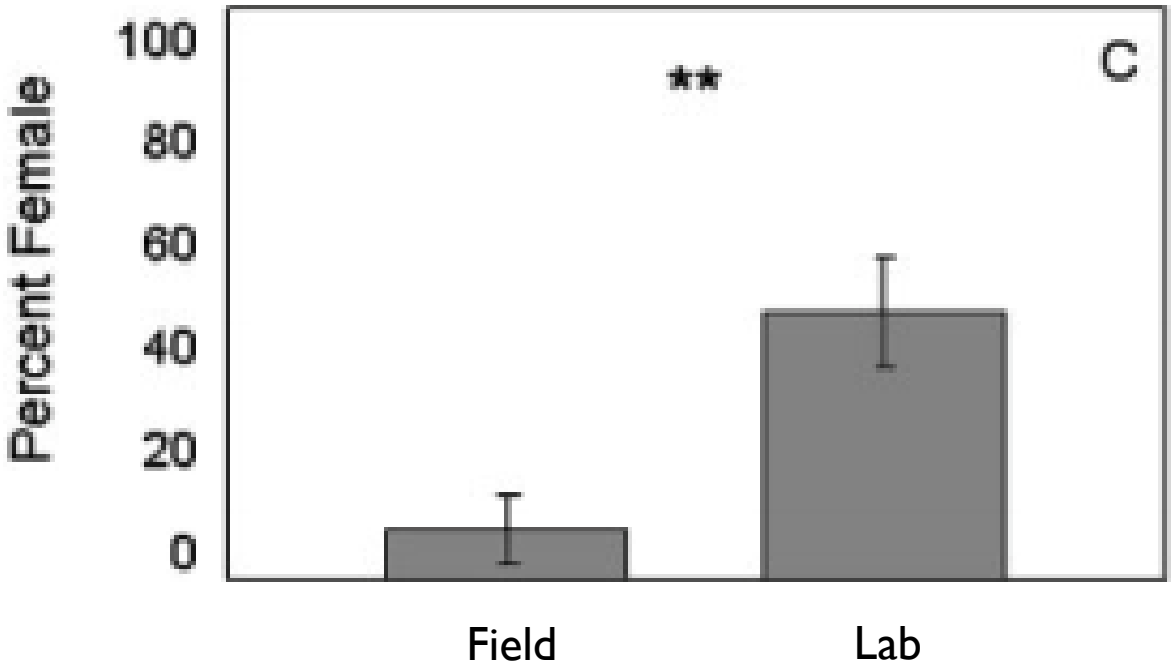
Field versus Laboratory



Constant versus Fluctuating

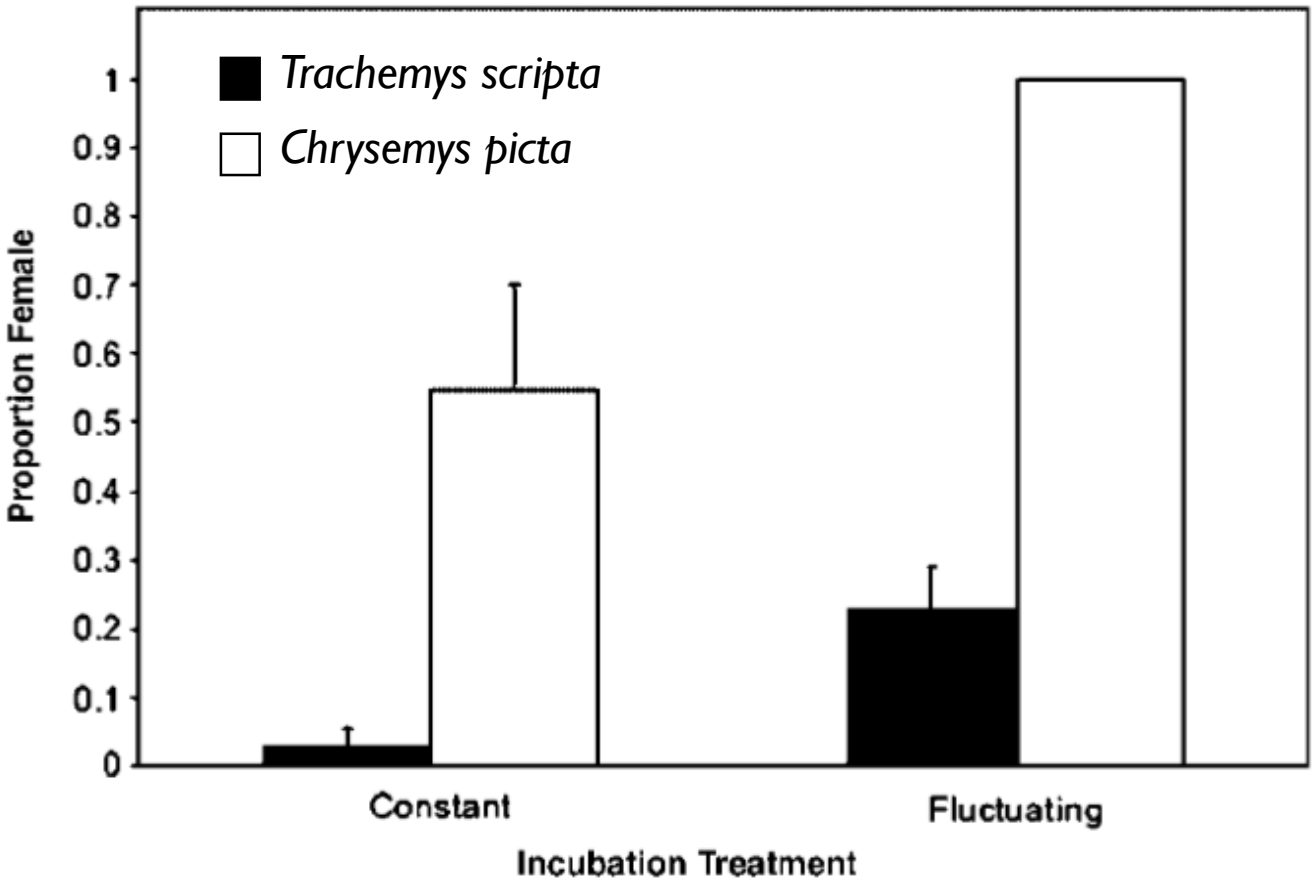
- Daily/seasonal fluctuations





Paitz et al. 2010

Average temperatures are poor predictors of sex ratios

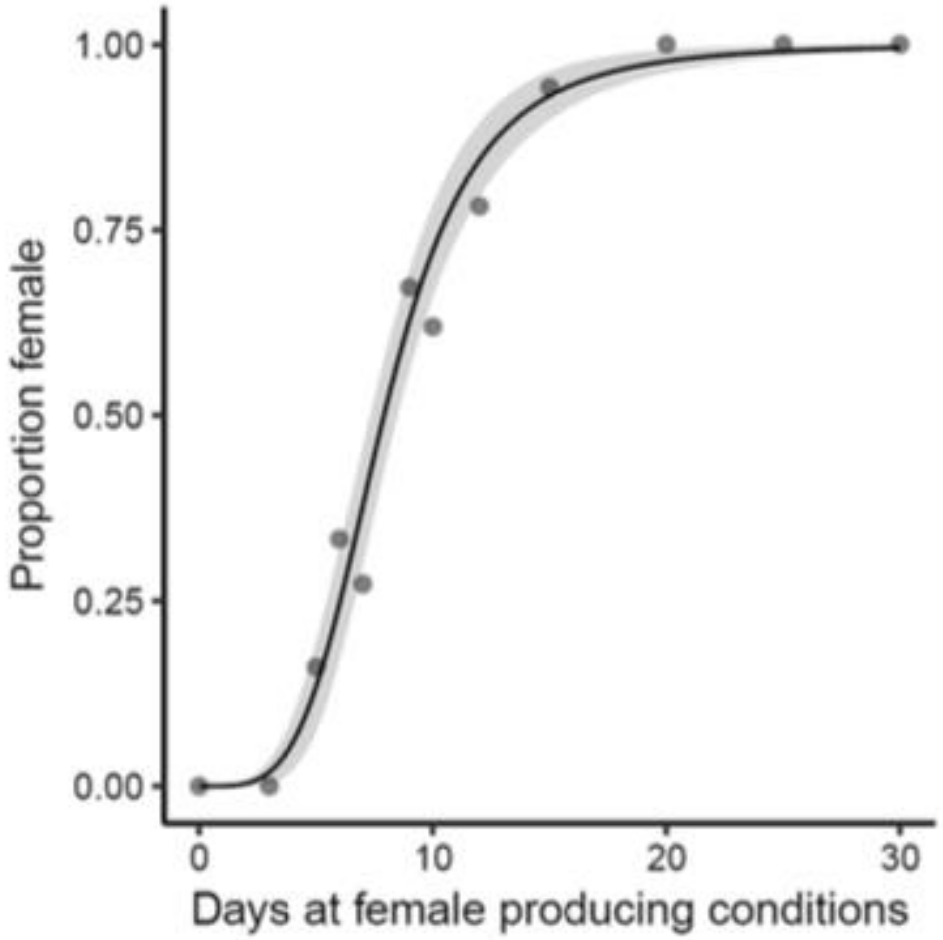


28.5 °C

28.5 ± 3 °C

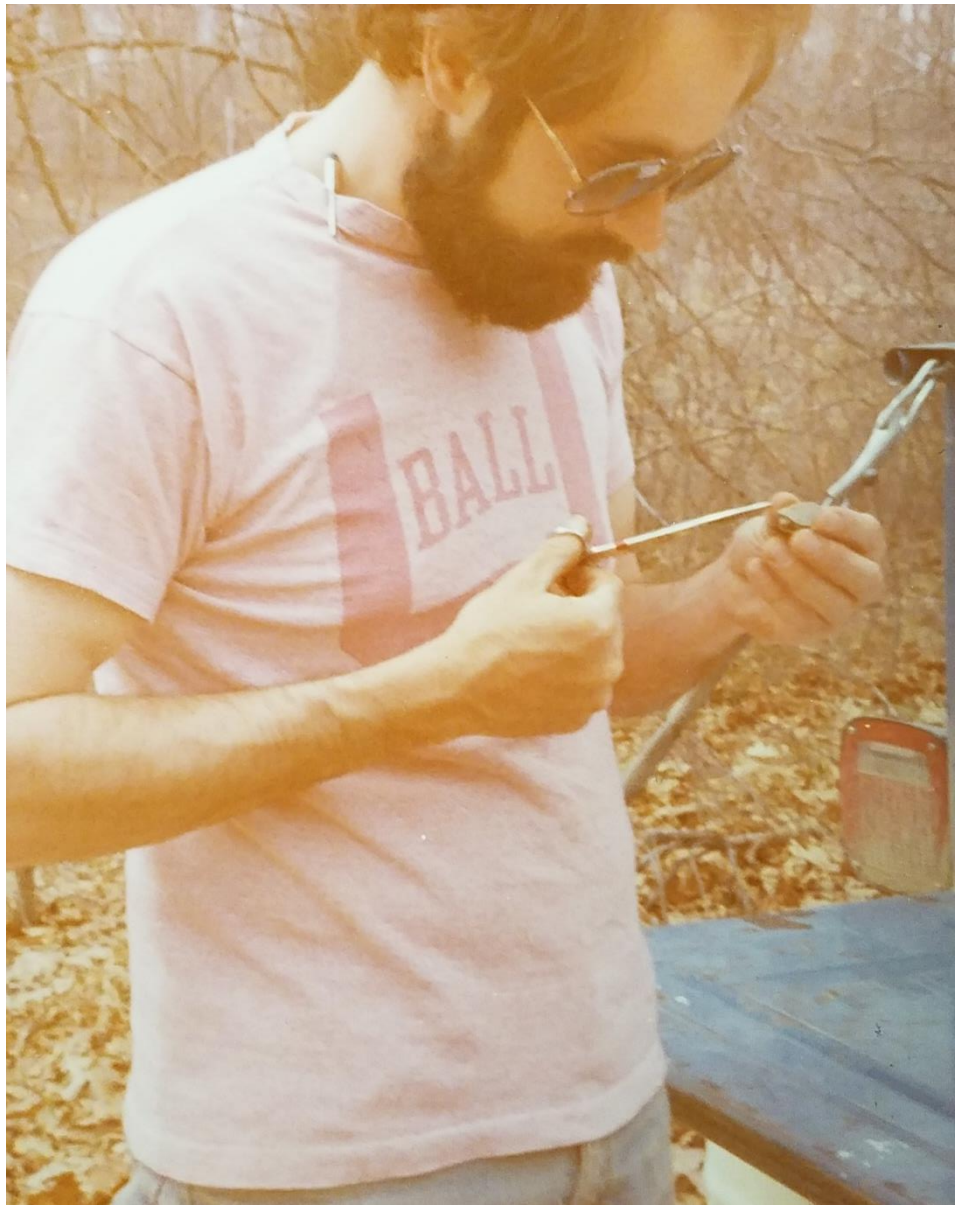
Les et al. 2007

Heat waves affect sex ratios



Carter et al. 2018

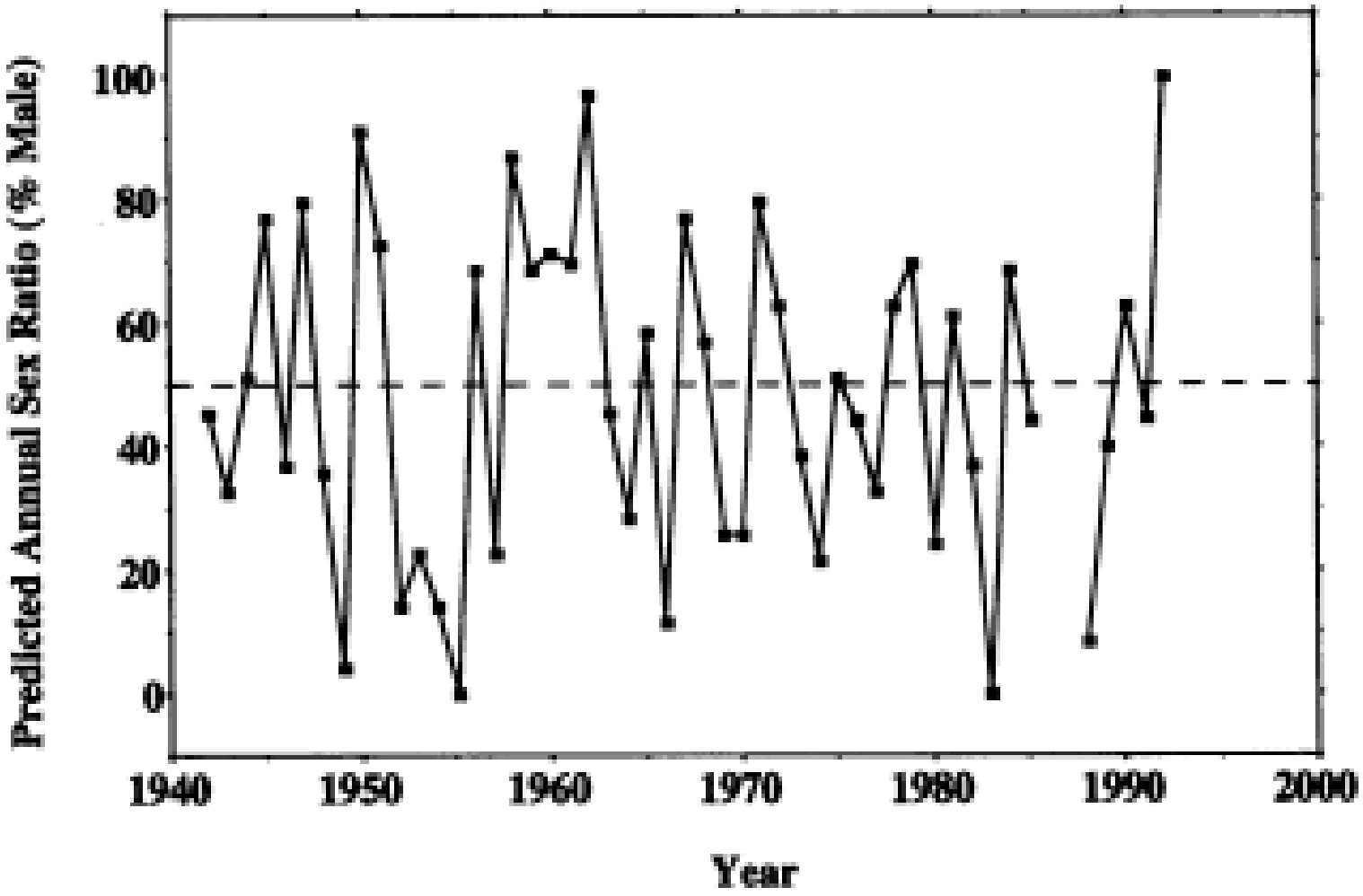
Current Understanding



1978

Natural Fluctuations

Chrysemys picta:



Janzen 1994

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

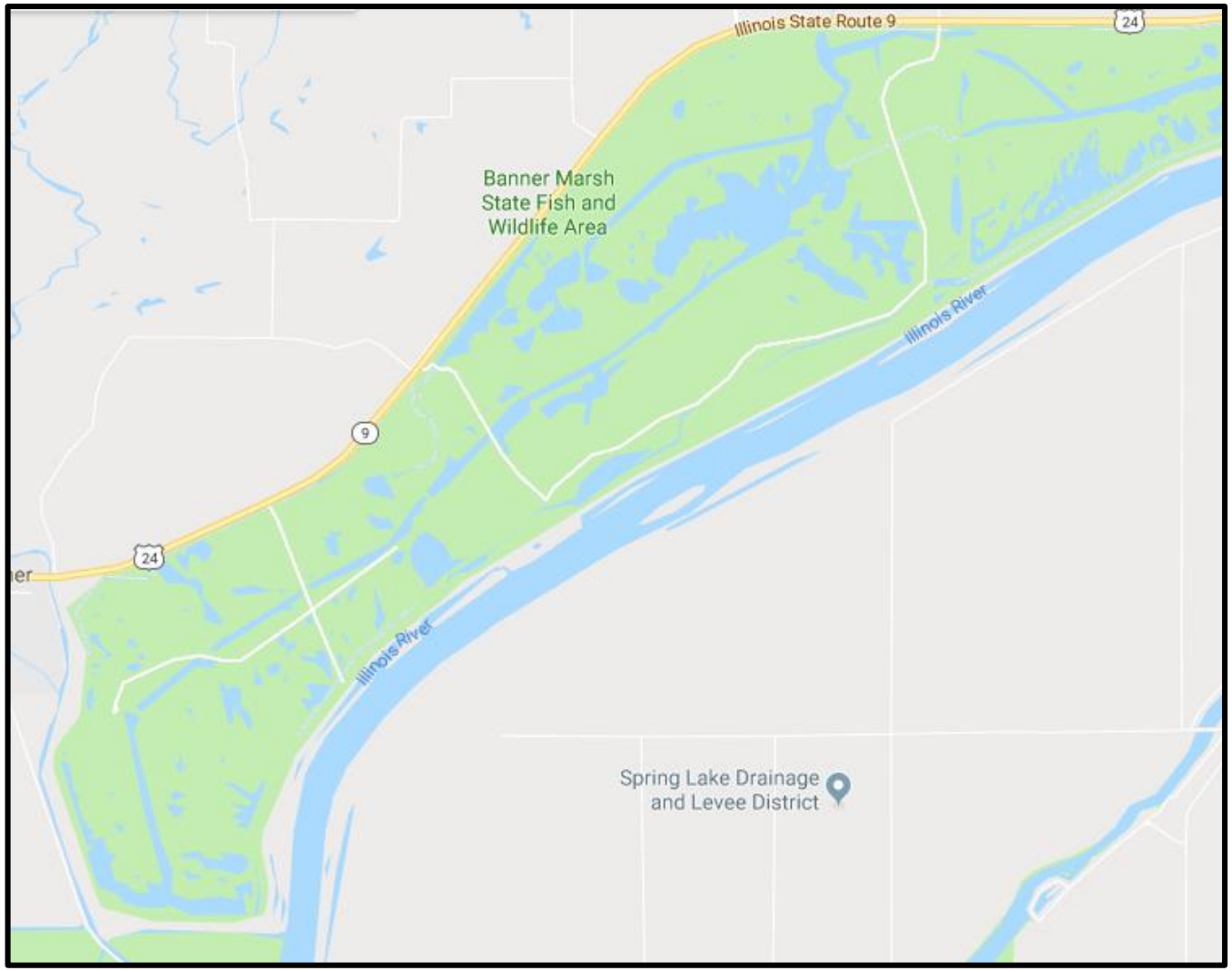
- Behavioral Plasticity
  - **Nesting phenology***
    - *Chrysemys picta* nesting 27 days earlier on average over 13 year period (*Schwanz and Janzen 2008*)
  - Spatial changes in nesting
    - Lacks plasticity (*Refsnider and Janzen 2012*; *Refsnider 2013*)
- Evolve a sensitivity to a different pivotal temperature
- Migrate to cooler climate to balance sex ratios
  - Depends on water body distributions

Overarching Hypothesis

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.*

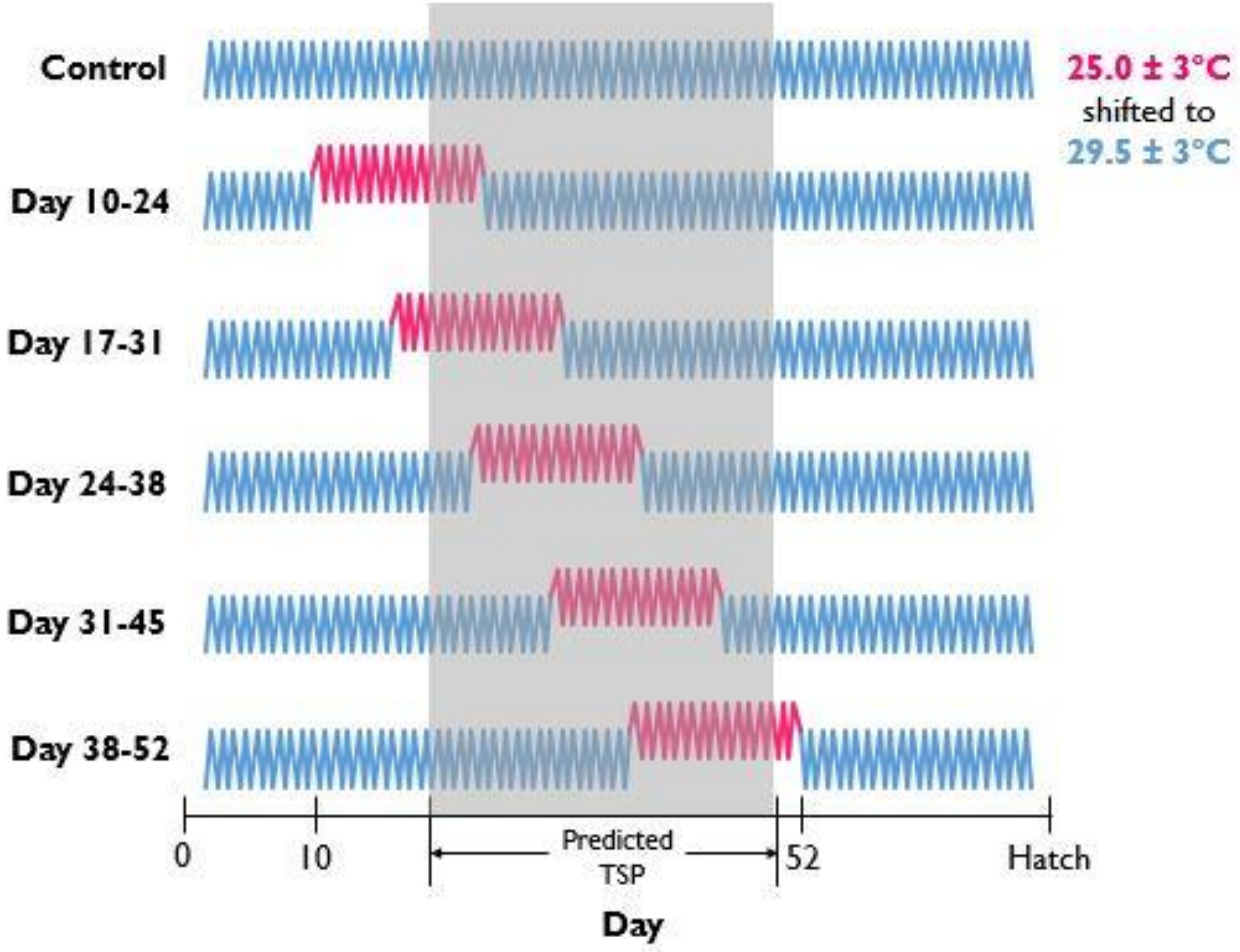
Prediction 2*: A heat wave applied during the TSP will trigger relatively higher levels of aromatase expression and relatively lower levels of Dmrt1 expression.*

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

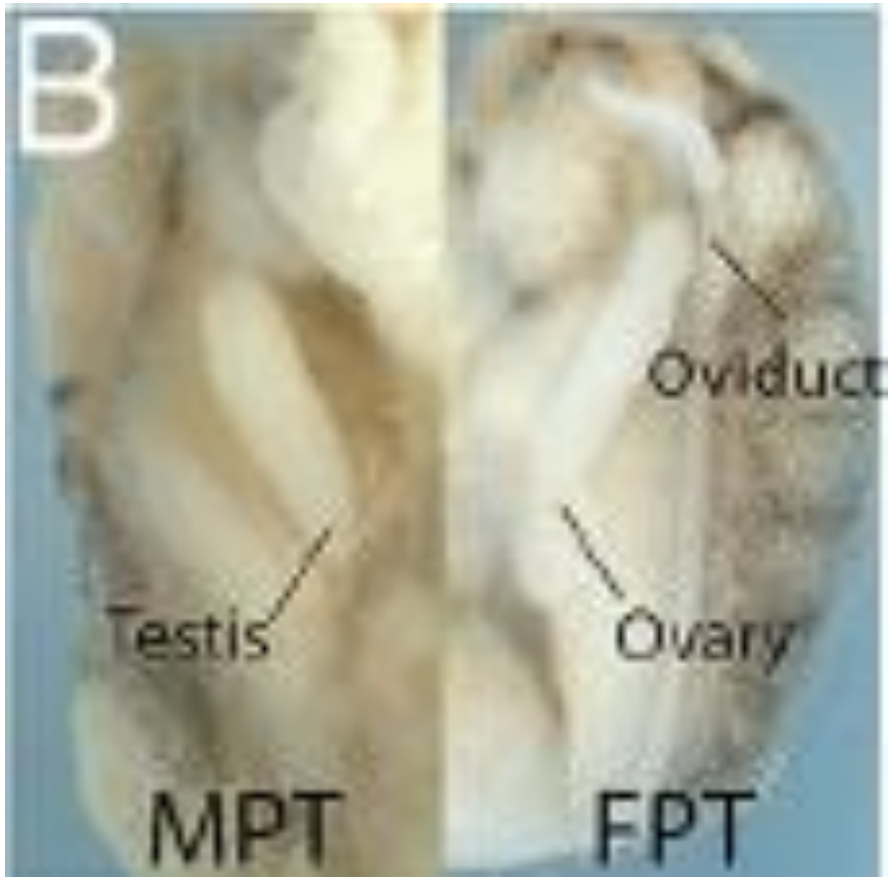
- Male producing condition:
  25 ± 3°C
  - "Baseline"
- Female producing condition:
  29.5 ± 3°C
  - "Heat wave"
- 15-day heat wave varied temporally
- Eggs: gravid female (oxytocin)/nest
- Group eggs to avoid clutch/box/incubator effects
- 20 eggs per treatment (sex ratio) 15 extra eggs in control and 24-38 (qPCR)



Illinois Climate Network 2015; Carter et al. 2017

Hypothesis 1- Methods (continued)

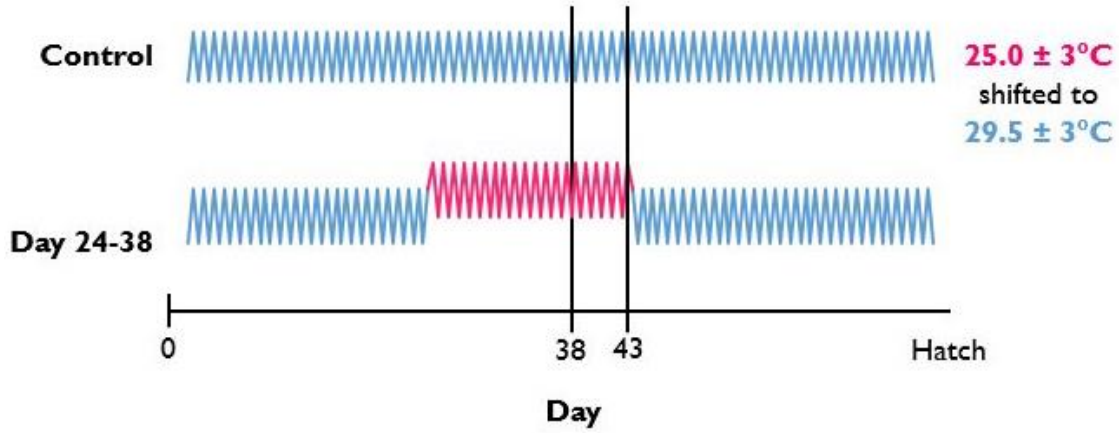
- Macroscopic gonad examination after euthasol injection



Matsumoto et al. 2012

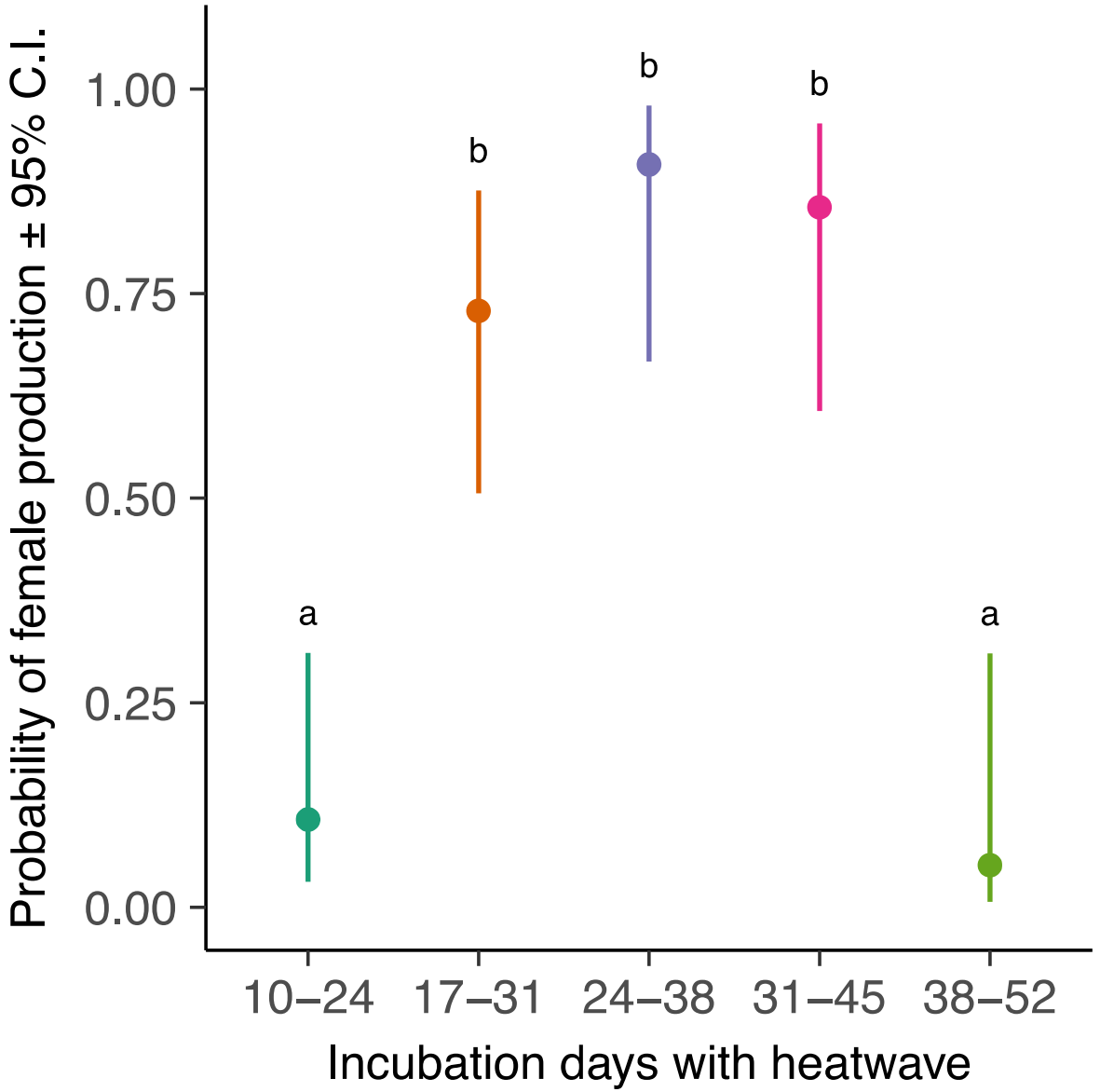
Hypothesis 1- qPCR

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



Primer Name	Sequence	Reference
Forward *Gapdh*	GGC TTT CCG TGT TCC AAC TC	Ge et al. 2017
Reverse *Gapdh*	GAC AAC CTG GTC CTC CGT GTA TC	Ge et al. 2017
Forward *Aromatase*	CGA CAT GGA CTT TGC ATC ACA	Ramsey et al. 2007
Reverse *Aromatase*	GAA CCA TCA TCT CCA ACA CAC ACT GGT TC	Ramsey et al. 2007
Forward *Dmrt1*	CAA CTA CTC CCA ATA CCA GAT GGC	Shoemaker et al. 2007
Reverse *Dmrt1*	GGC TTC GCA GGC TGT TTT TC	Shoemaker et al. 2007

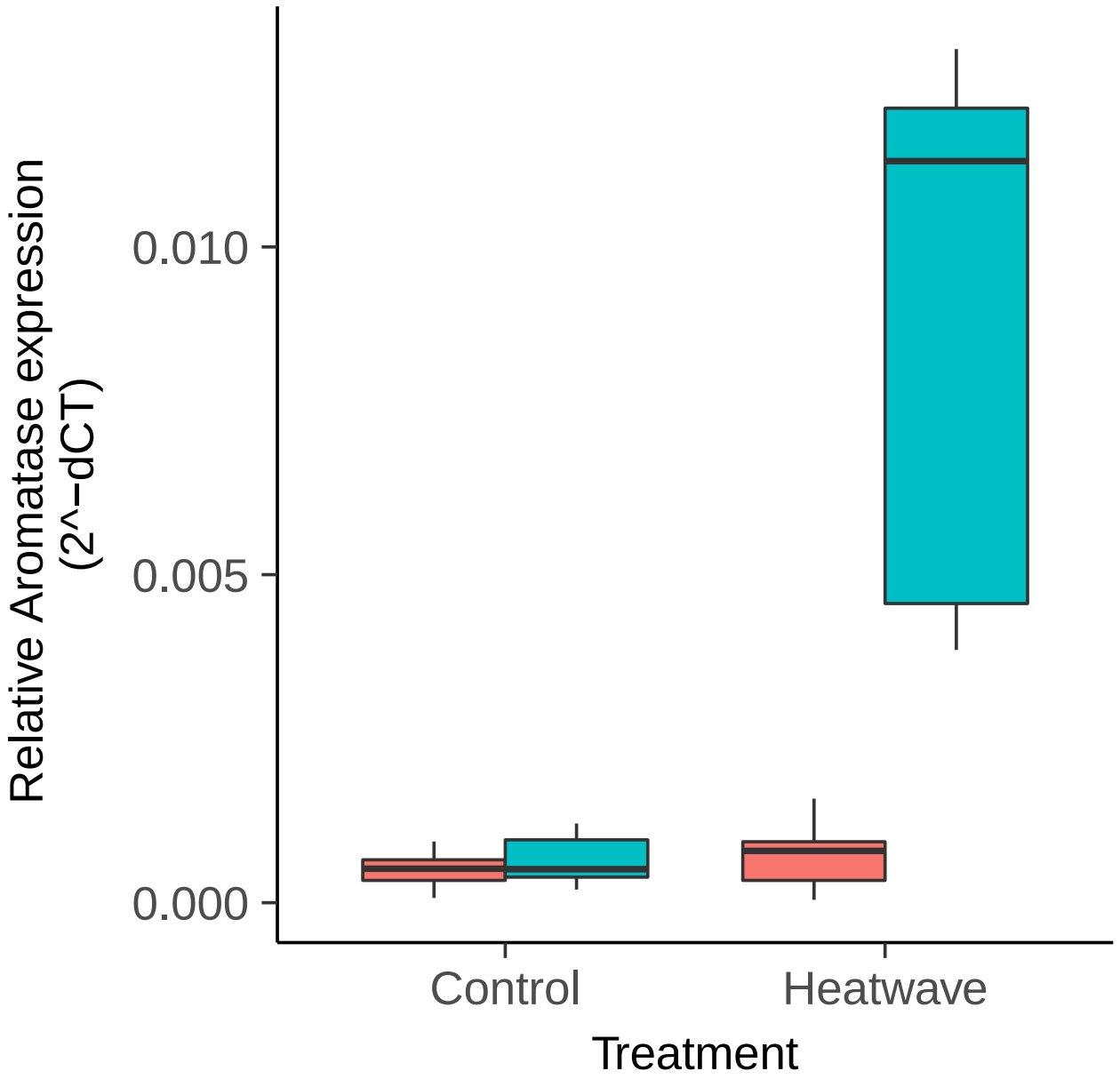
Sex ratio results:



Heatwave treatment timing: $\chi^2 = 29.324$, df = 4, $p < 0.001$

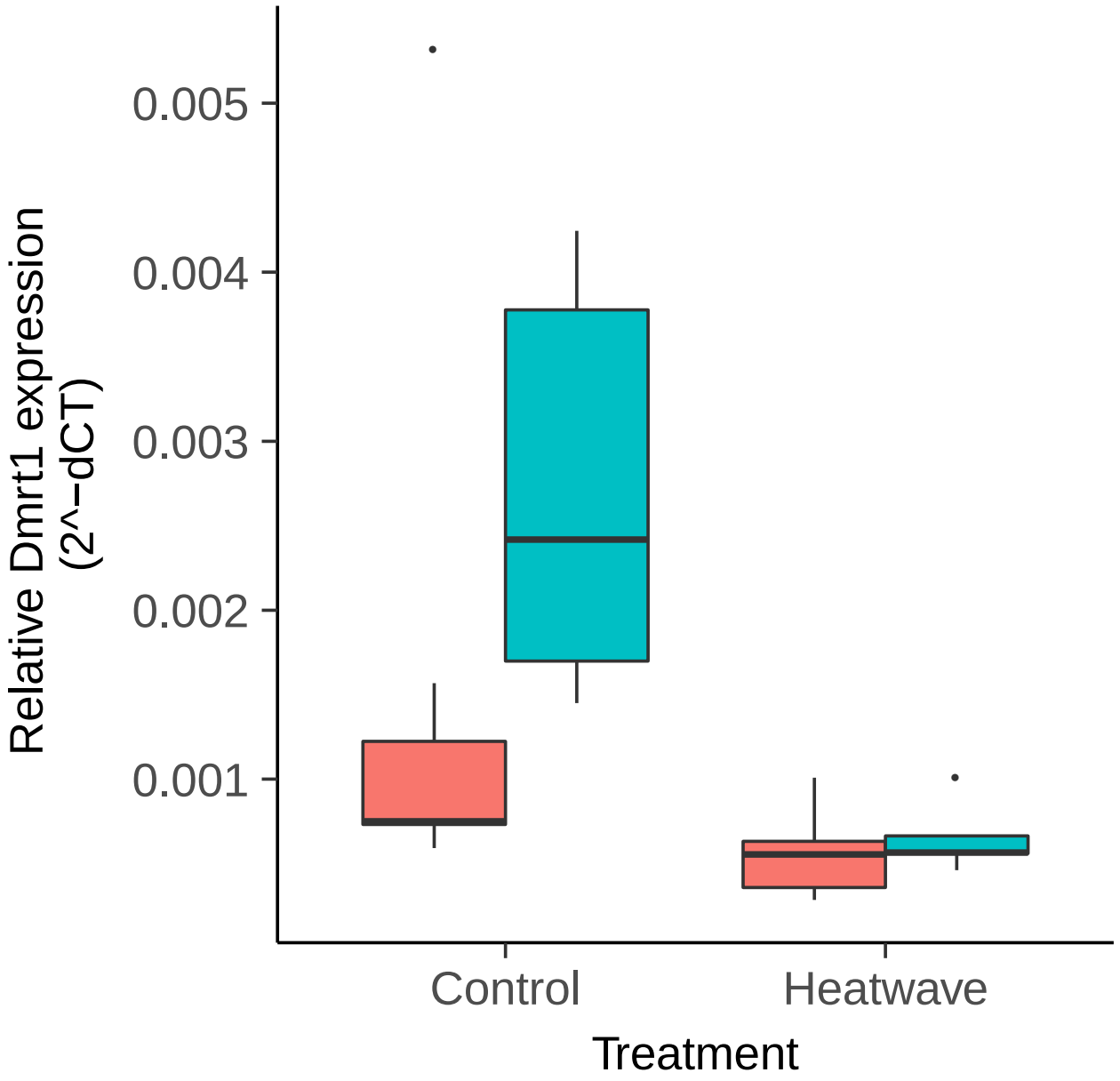
Middle third?

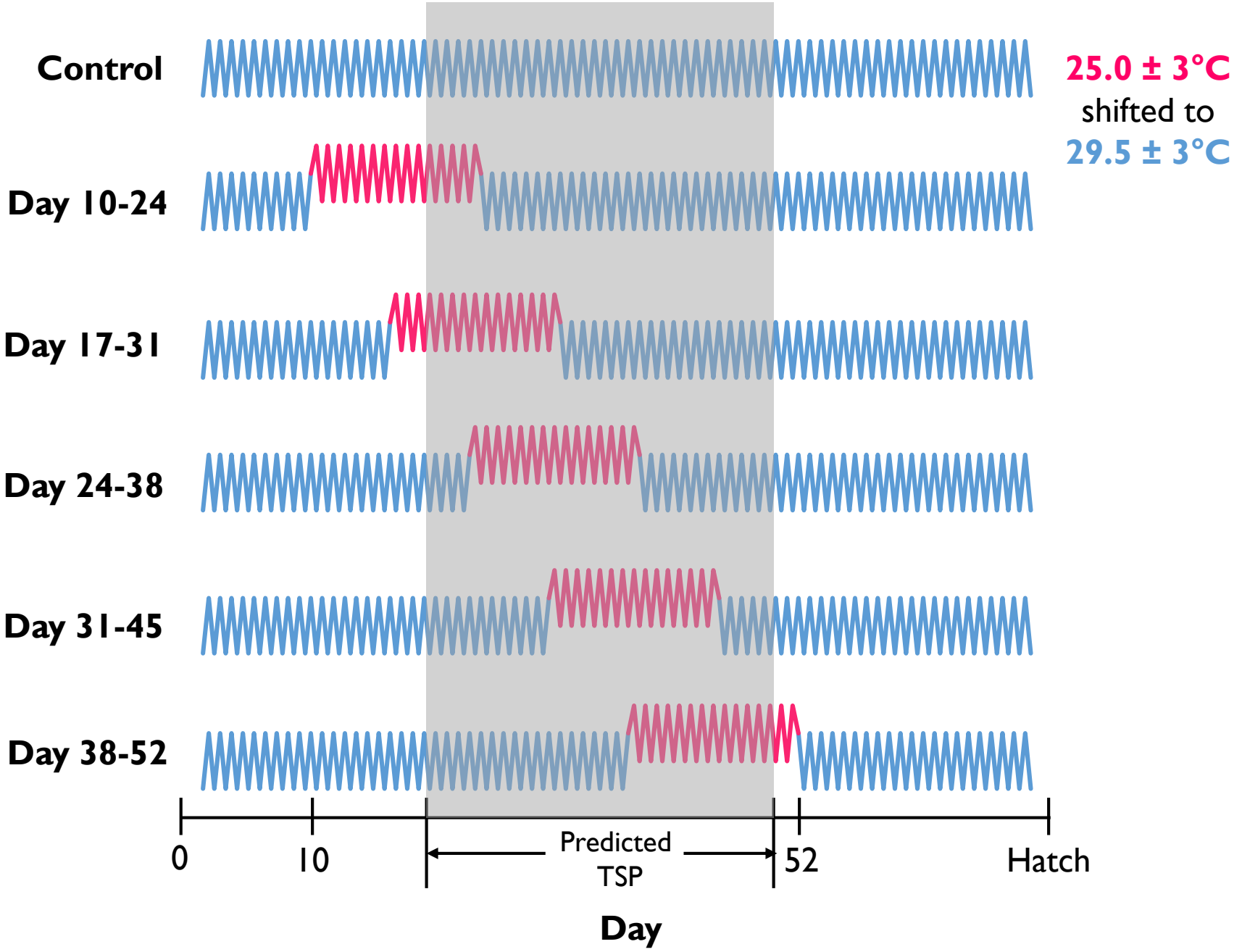
Treatment	Avg. Middle Third	% Female	Stat. Differences
Control	≈28-56	0%	
Heat wave 10-24	≈24-48	12%	a
Heat wave 17-31	≈24-48	72%	b
Heat wave 24-38	≈24-48	89%	b
Heat wave 31-45	≈25-50	84%	b
Heat wave 38-52	≈25-50	6%	a

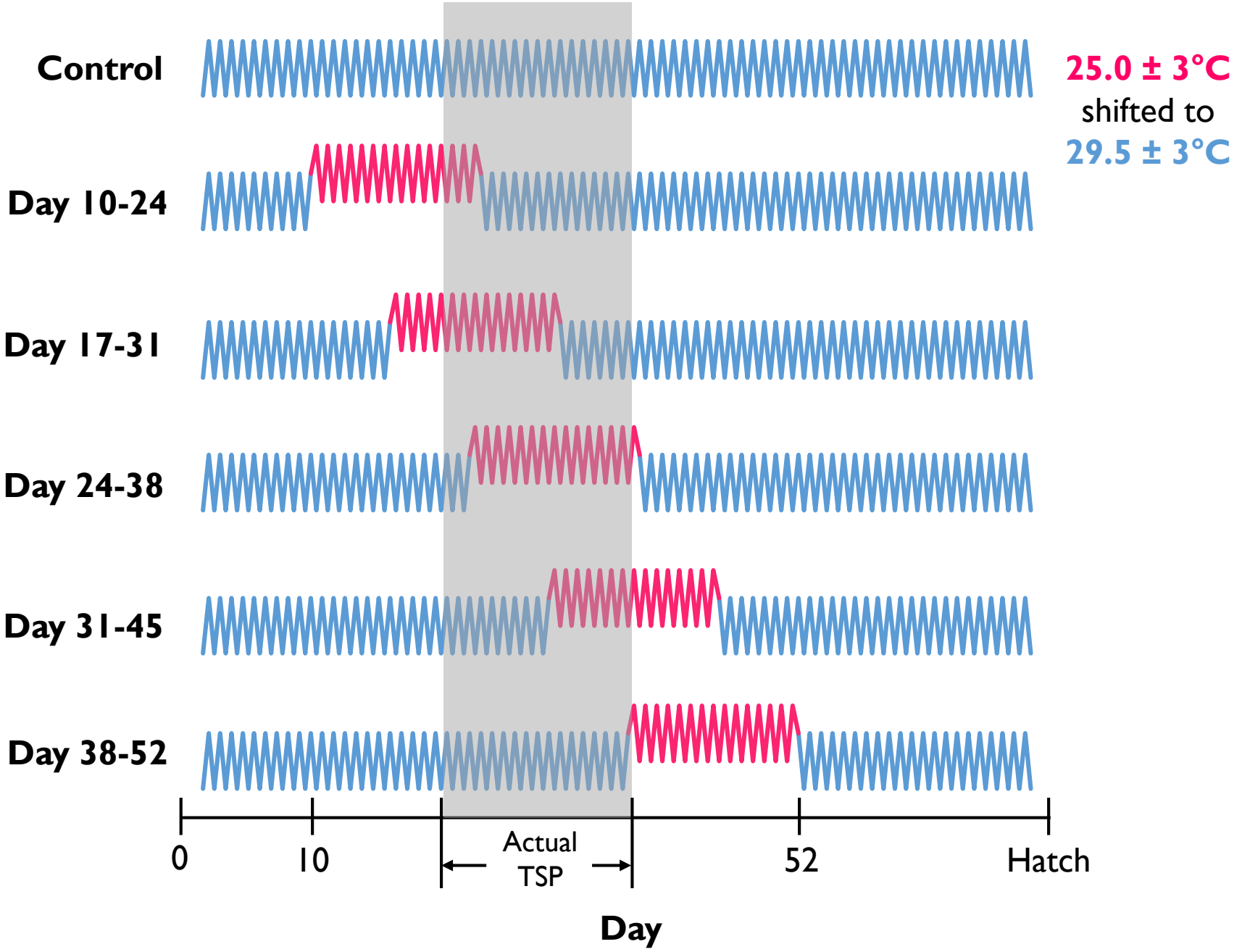


Aromatase	df	F value	p value
Treatment	1	11.32	p = 0.003 **
Day	1	15.16	p < 0.001 ***
Treatment*Day	1	11.37	p = 0.003 **

Dmrt1	df	F value	p value
Treatment	1	30.11	p < 0.001 ***
Day	1	15.42	p < 0.001 ***
Treatment*Day	1	6.53	p = .0193 *




Control
Day 10-24
Day 17-31
Day 24-38
Day 31-45
Day 38-52
25.0 ± 3°C
shifted to
29.5 ± 3°C
0
10
Predicted
TSP
52
Hatch
Day


Control
Day 10-24
Day 17-31
Day 24-38
Day 31-45
Day 38-52
25.0 ± 3°C
shifted to
29.5 ± 3°C
0
10
Actual
TSP
52
Hatch
Day

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

Treatment	Avg. Middle Third	% Female	Stat. Differences
Control	≈28-56	0%	
Heat wave 10-24	≈24-48	12%	a
Heat wave 17-31	≈24-48	72%	b
Heat wave 24-38	≈24-48	89%	b
Heat wave 31-45	≈25-50	84%	b
Heat wave 38-52	≈25-50	6%	a

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

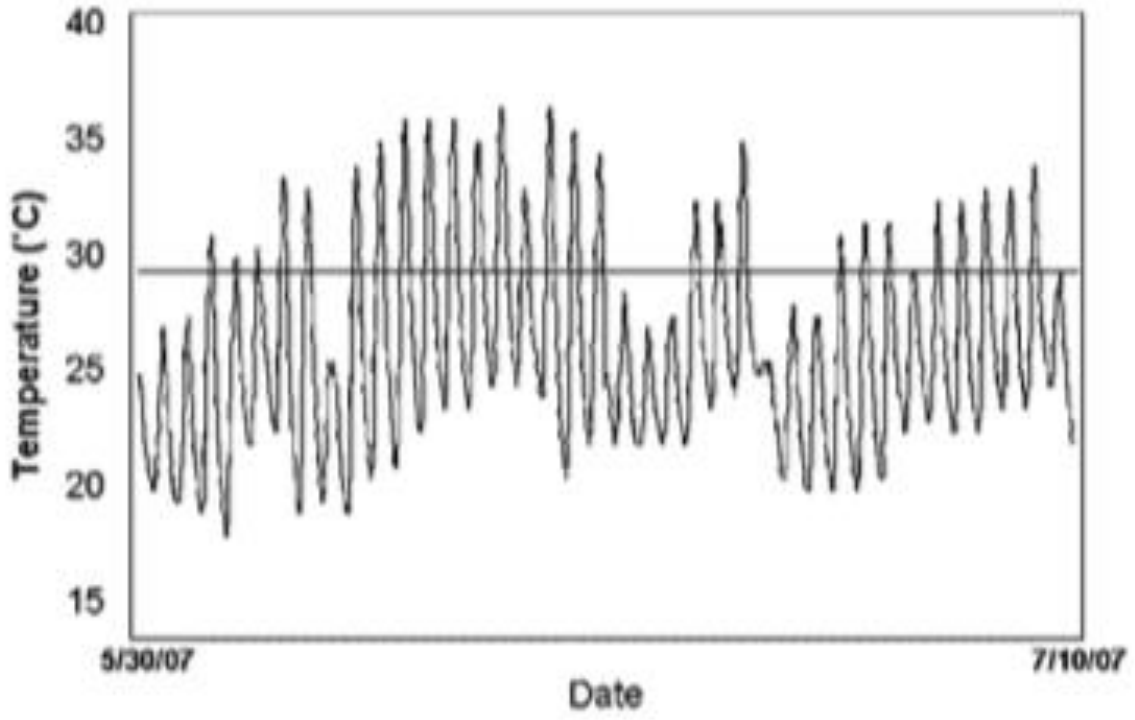
a. What sex ratios are being produced in nature?

Next Steps: Hypothesis 2

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

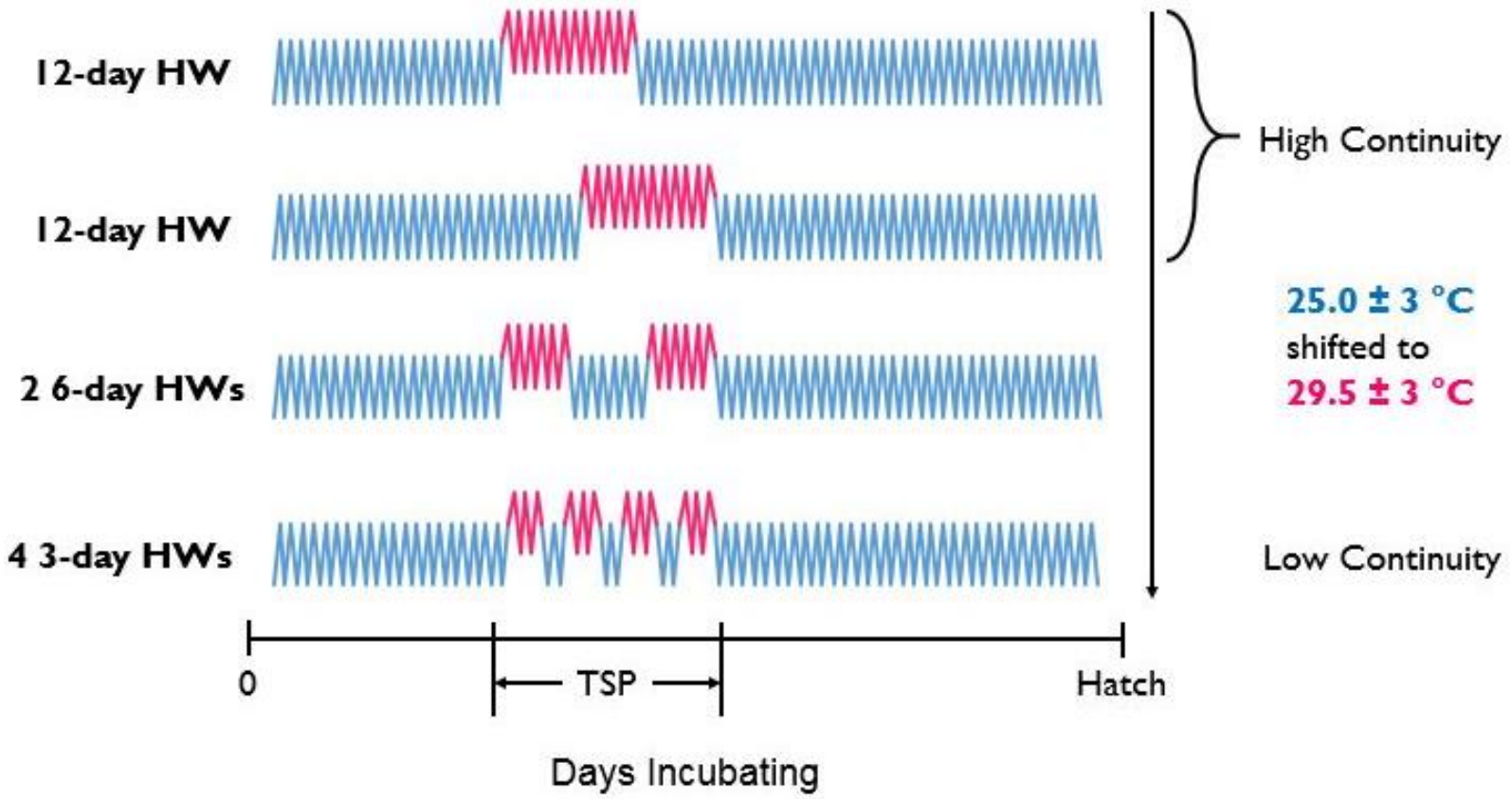
Prediction 1*: With hot days held constant, more continuous heat waves will produce more female-biased sex ratios.*

a. How does heat wave continuity affect sex determination?



Paitz et al. 2009

Hypothesis 2- Methods



Heat Wave Length (Days)	Proportion Females
8	24%
11	74%
14	94%
17	89%
20	90%
23	94%
26	90%
29	100%
32	100%
35	100%

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?

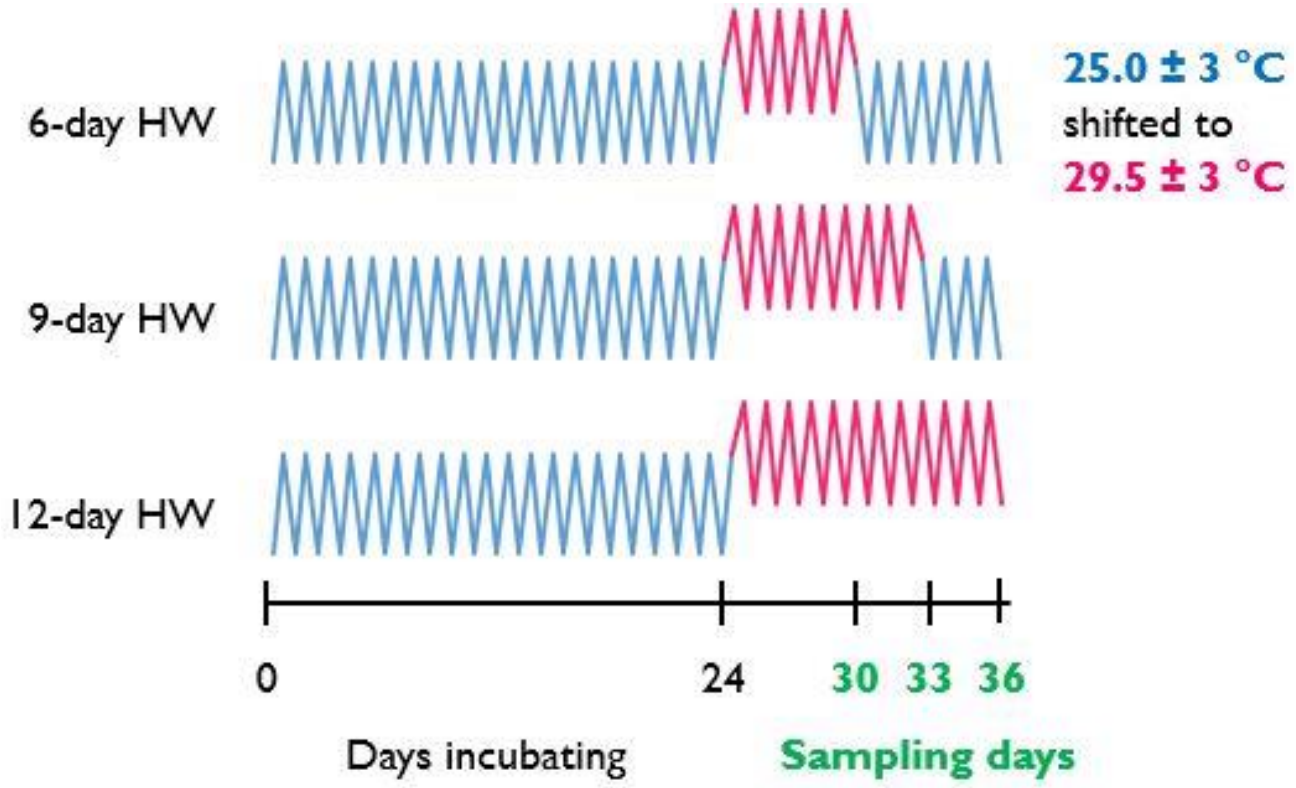
b. How does *Dmrt1* expression respond to a heat waves of varying lengths?

Heat Wave Length (Days)	Proportion Females
8	24%
11	74%
14	94%
17	89%
20	90%
23	94%
26	90%
29	100%
32	100%
35	100%

Carter et al. unpublished

Hypothesis 3- Methods

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




PINCH FREE ZONE
NO PINCHING

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Dr. Ben Sadd
Dr. Steven Juliano
Dr. Ryan Paitz

Community Outreach:

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Jennifer Trimble
Dr. Craig Gatto

Funding:

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