Joshua M. Hall[1] and Daniel A. Warner[1]

[1]Department of Biological Sciences, Auburn University, Auburn, AL

jmh0131@auburn.edu

Constantly fluctuating in an inconsistent way: comparing the effects of sinusoidal and naturally fluctuating incubation temperatures on embryo development

Abstract

Temperature is a commonly studied environmental factor influencing embryo development in oviparous ectotherms. Though most studies use constant temperature incubation conditions, researchers are aware of the effects of fluctuating temperatures on development. Daily-repeating sinusoidal fluctuations are now commonly used in studies of developmental plasticity; however, thermal fluctuations in natural nests are highly variable from day to day. Thus, using repeated, uniform fluctuations (e.g. sine waves) may still provide an incomplete picture of how embryos develop in the wild and generate inaccurate predictions of how species will respond to future thermal conditions (e.g. climate change). We used eggs from the brown anole lizard (*Anolis sagrei*) to test the effects of realistic nest temperature fluctuations vs constant temperatures and sinusoidal fluctuations in the lab. We used temperature data from nests to create 4 incubation treatments: a constant mean temperature, a daily-repeating sine fluctuation, a daily-repeating asymmetrical fluctuation (i.e. mean, hourly nest temperatures), and a treatment that allowed each day's thermal fluctuation to differ from all other days as in real nests. These 4 treatments were created for both early-season (March-April, relatively cool) and late-season (June-July, relatively warm) nest temperatures (2 by 4 factorial design; season x incubation treatment). We report results for developmental rates, physiology (VO_2 and heart rate), embryo survival, as well as morphology, performance, growth, and survival of hatchlings. By comparing the effects of several commonly used experimental thermal regimes with those of natural fluctuations, our study assesses the importance of using ecologically relevant incubation conditions when studying developmental plasticity in the laboratory.

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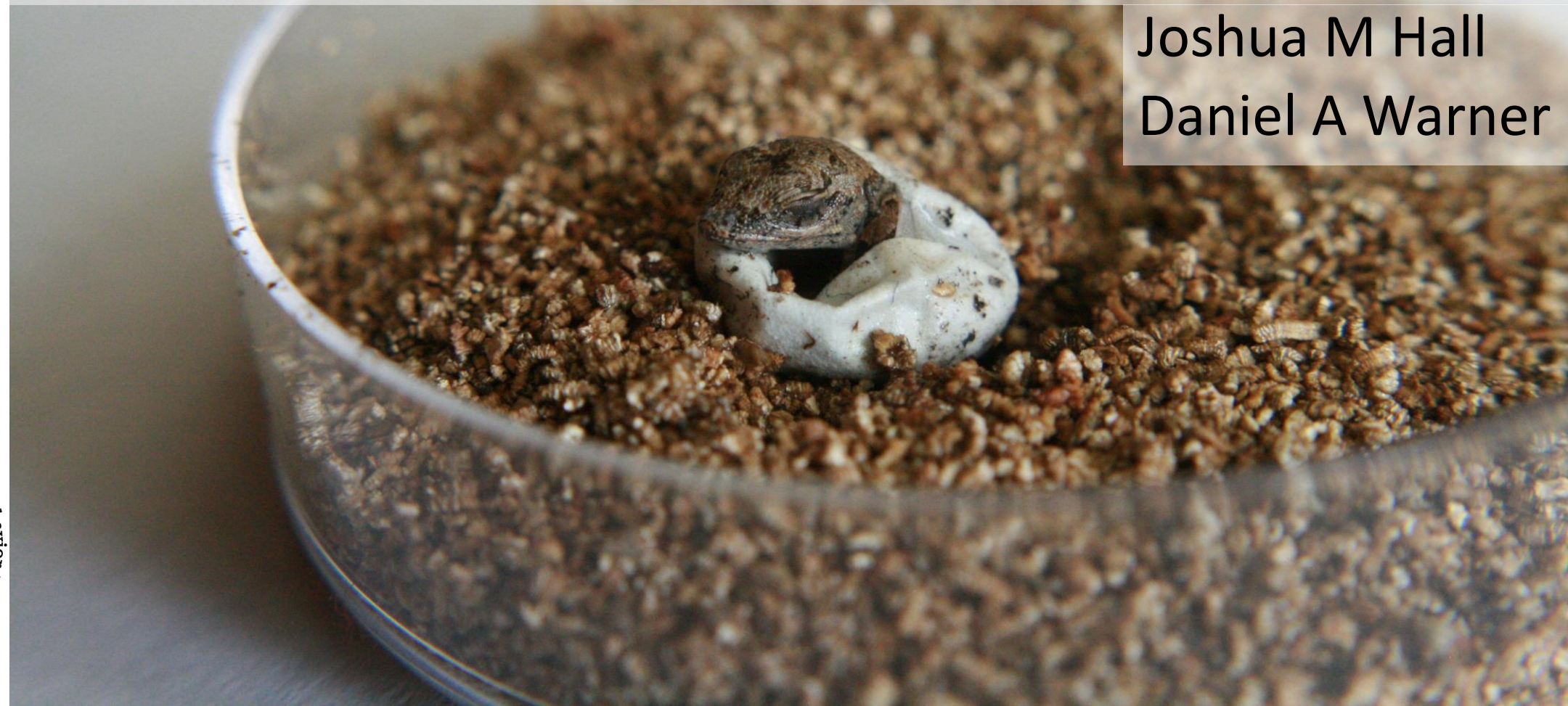
Constantly fluctuating in an inconsistent way: comparing the effects of sinusoidal and naturally fluctuating incubation temperatures on embryo development



Joshua M Hall
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WARNER LAB

Developmental plasticity

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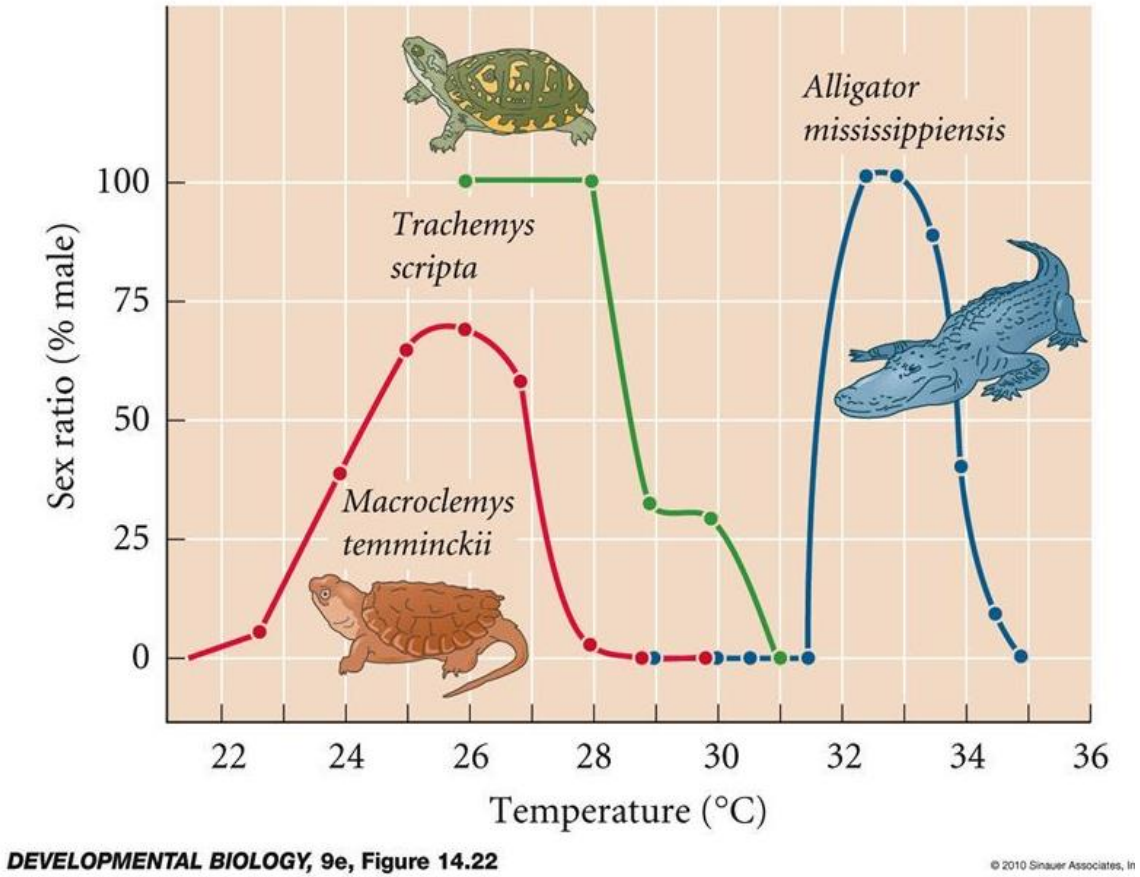
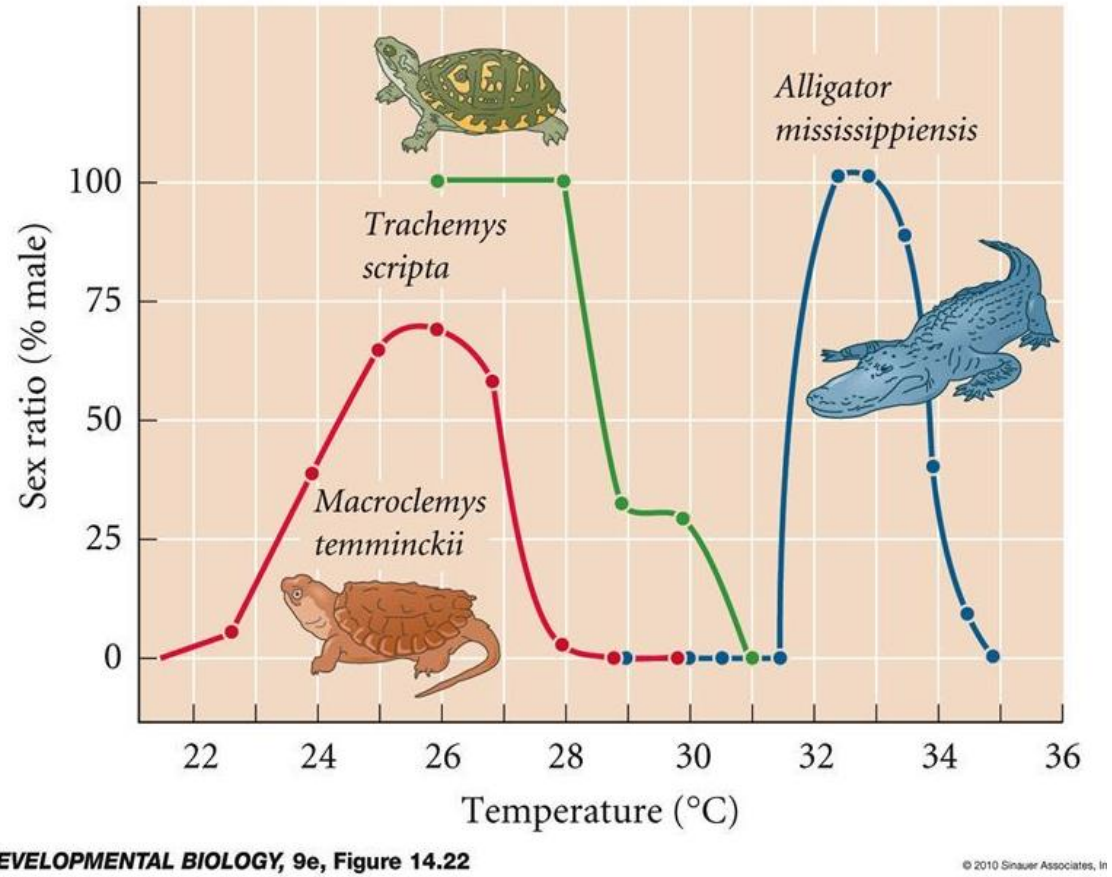


DEVELOPMENTAL BIOLOGY, 9e, Figure 14.22

Gilbert, 2018

Developmental plasticity

- Environment during embryo development can have lasting effects
- Lots of research on temperature and DP in reptiles



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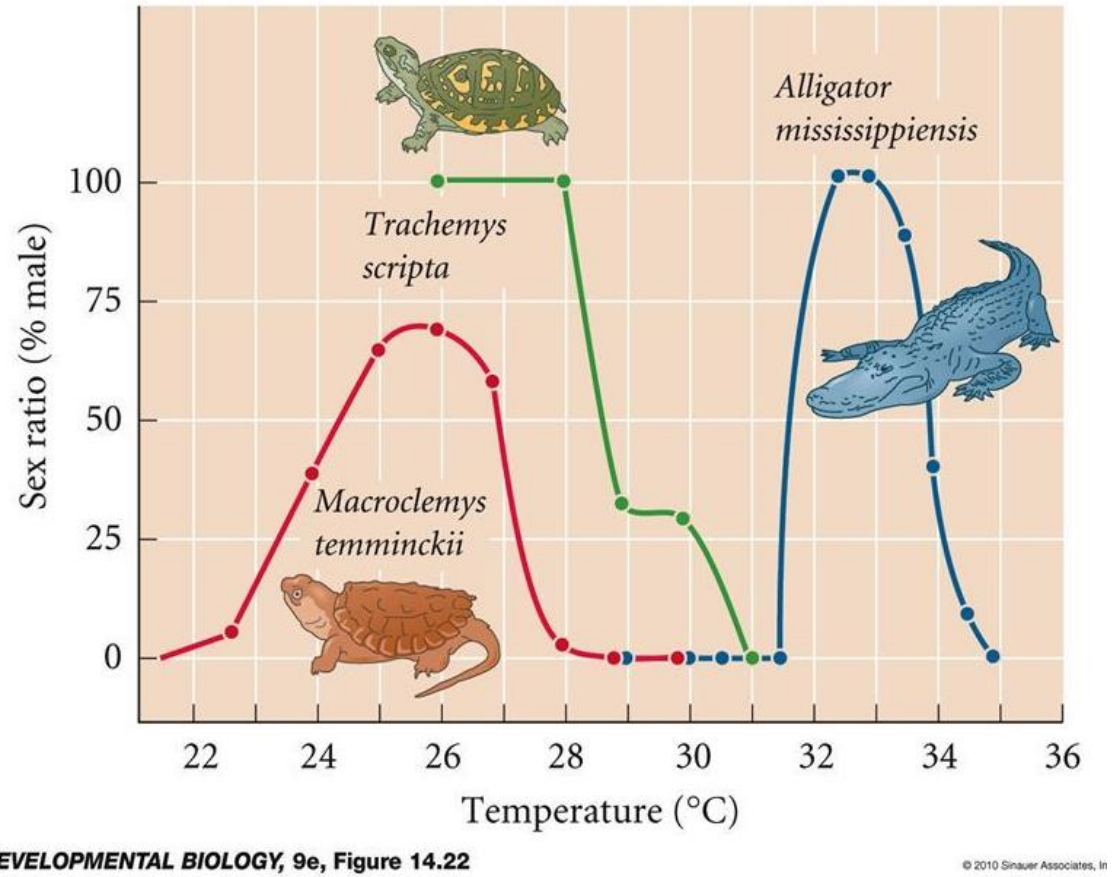

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While et al. 2018. J Exp Zool A

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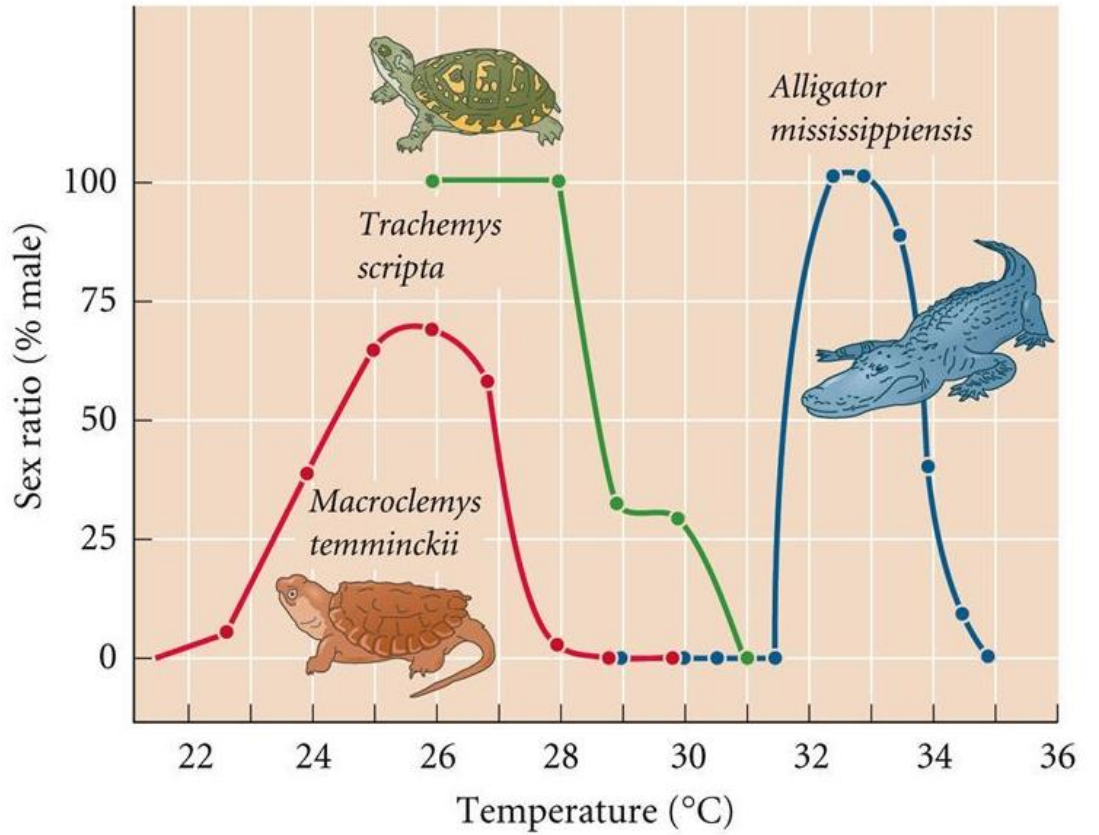

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Developmental plasticity

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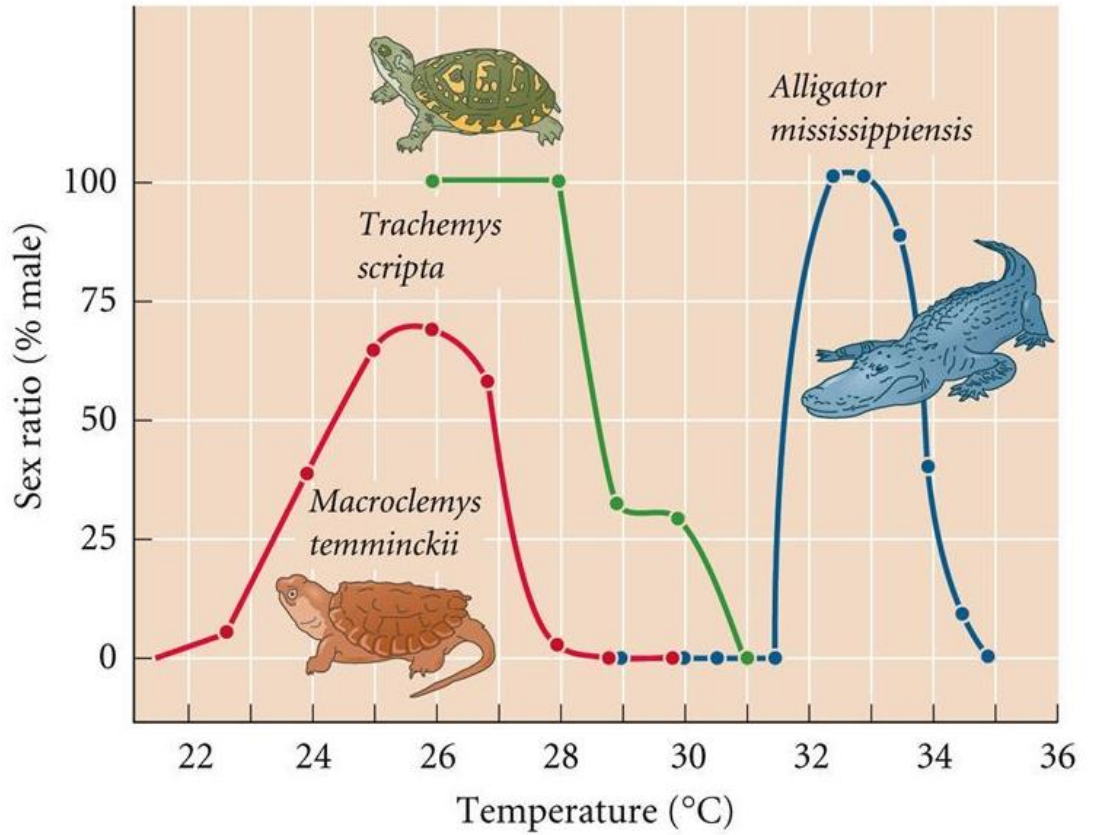
- Incubation regimes unrealistic



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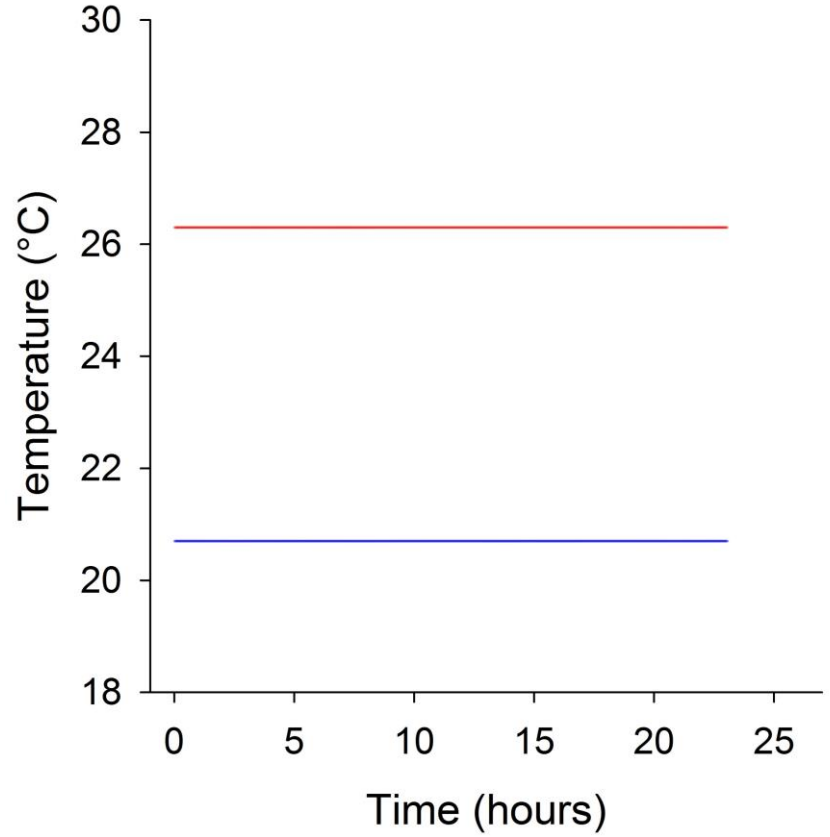
Unrealistic methods

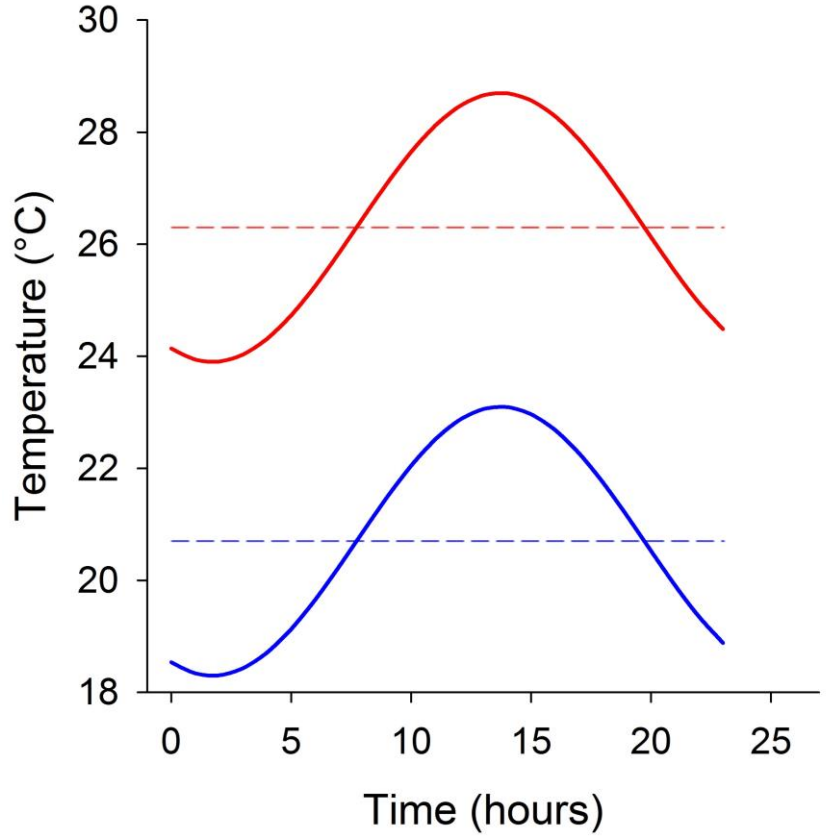
Unrealistic methods

- Constant temperature



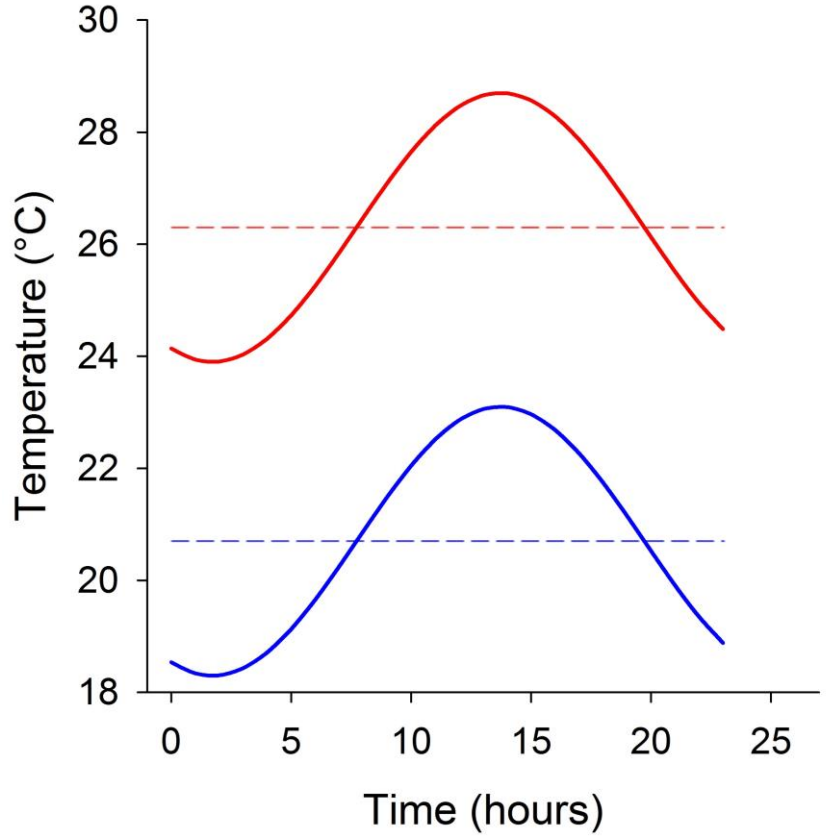
Unrealistic methods

- Constant temperature
- Repeated sinusoidal fluctuations



Unrealistic methods

- Constant temperature
- Repeated sinusoidal fluctuations
- Two problems



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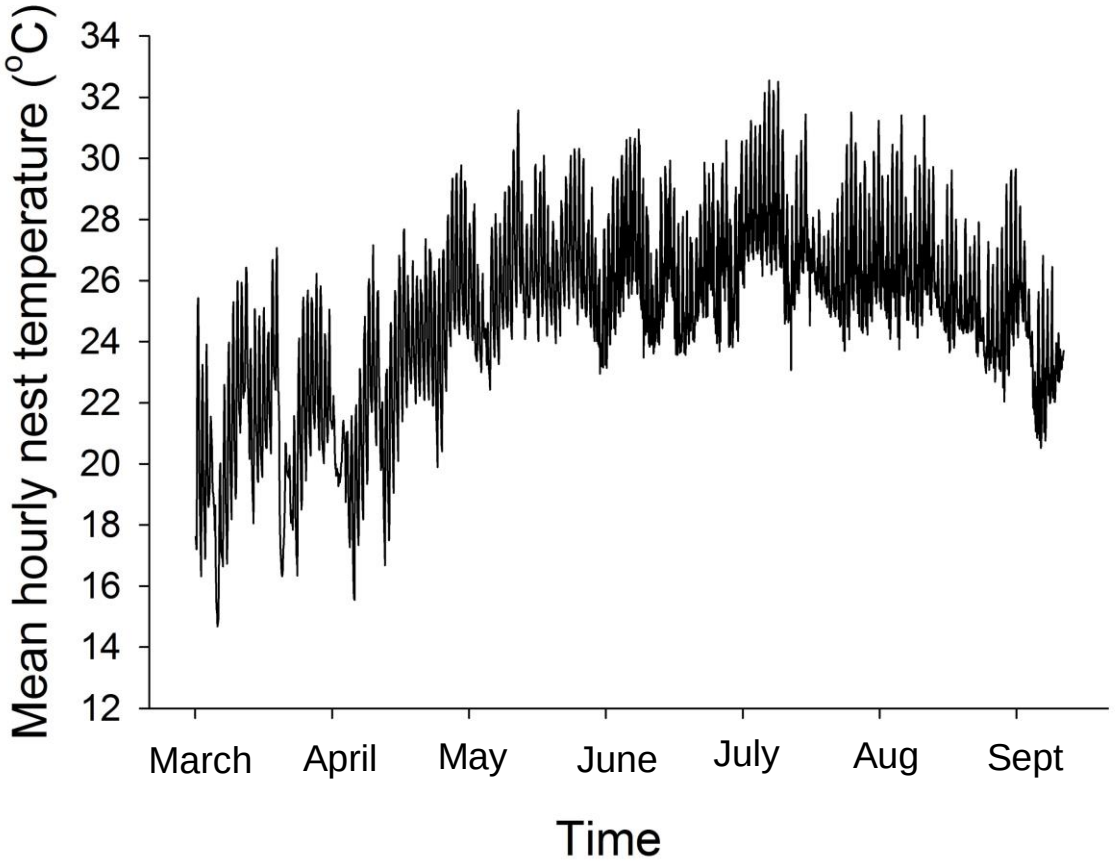

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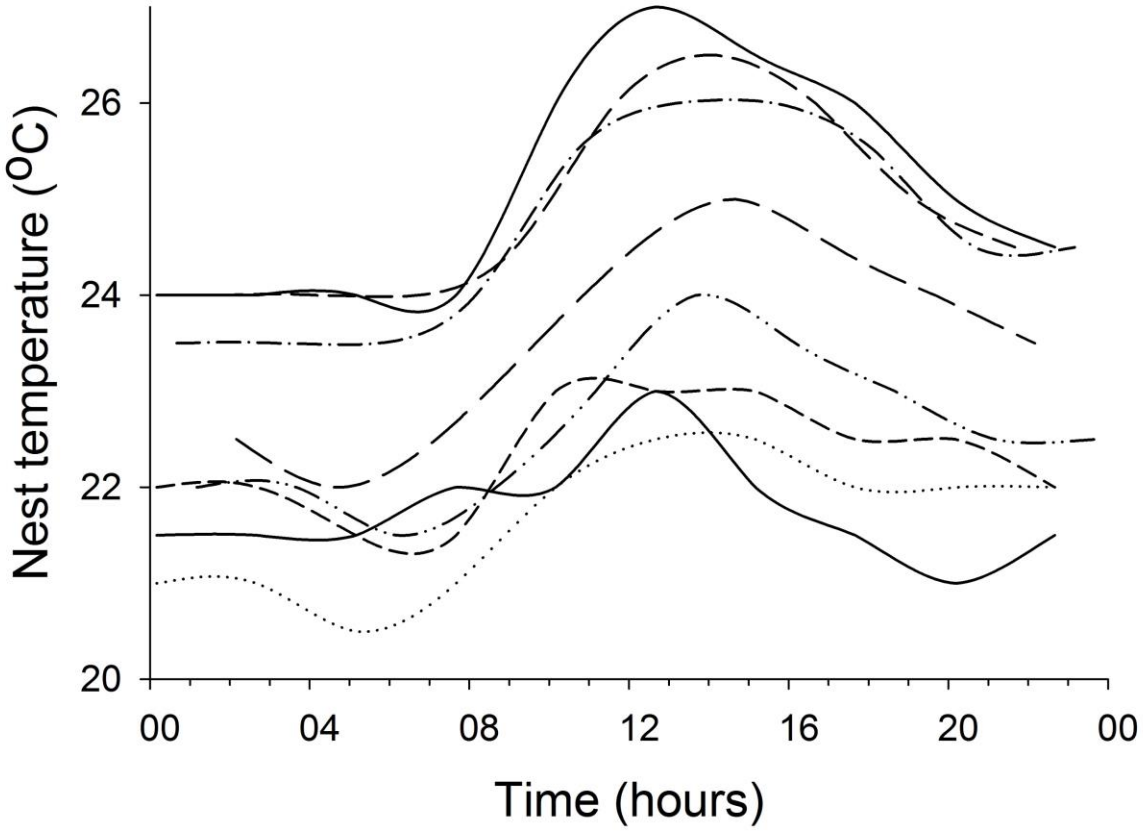

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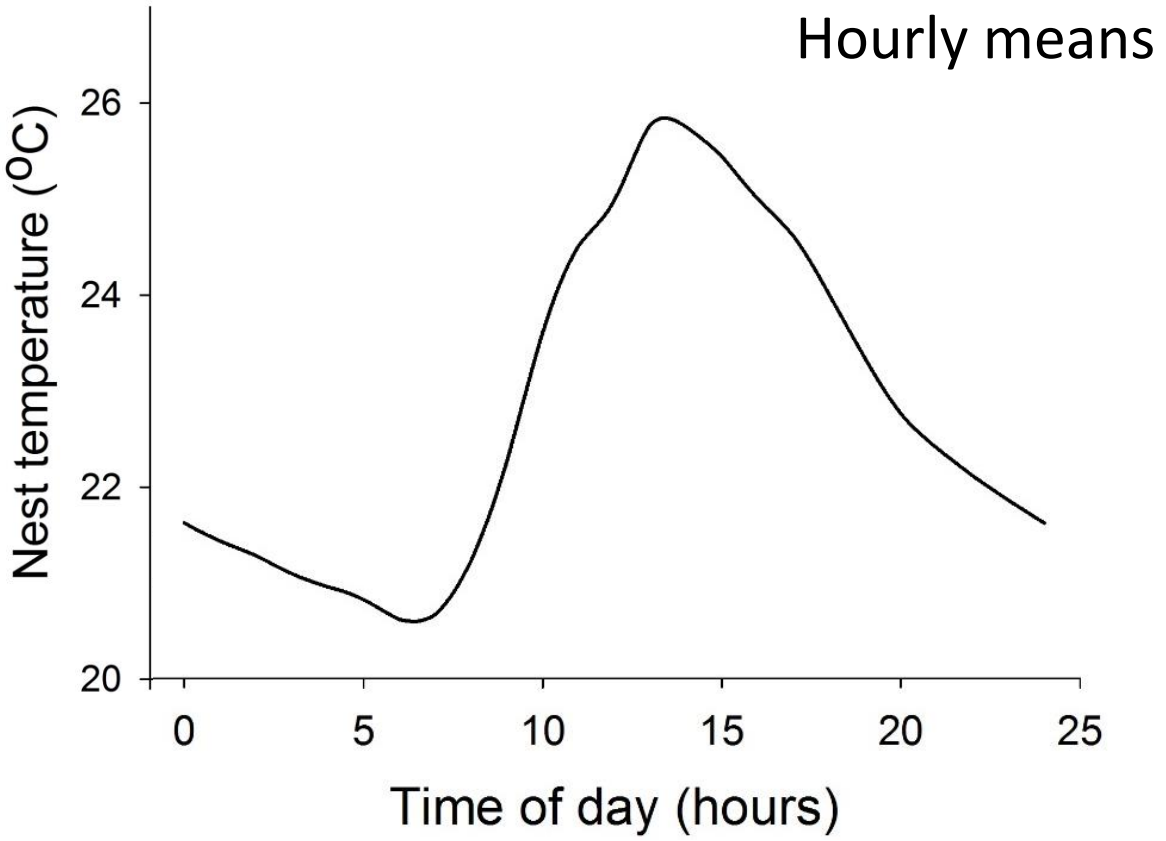


Hall & Warner. 2017. Biol J Linn Soc

Hulbert…Hall et al. 2017. J Exp Zool

Hall & Warner. 2018. J Exp Biol

Mitchell, Hall, Warner. 2018. Evol Ecol

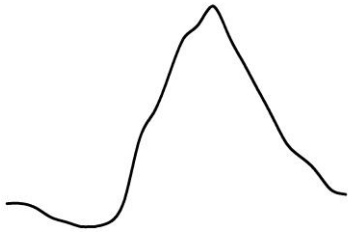
Hall et al. 2018. PBZ

Questions

Questions

- Sine waves vs hourly means?

VS



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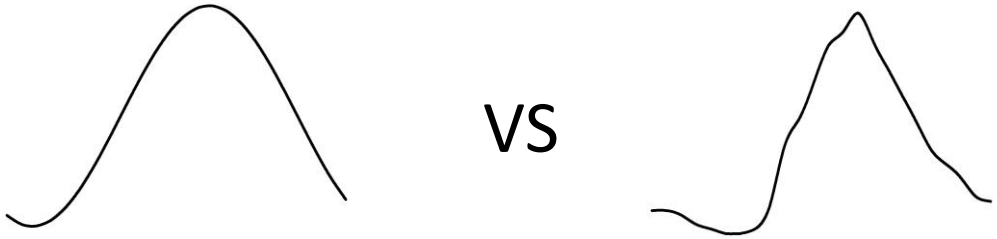

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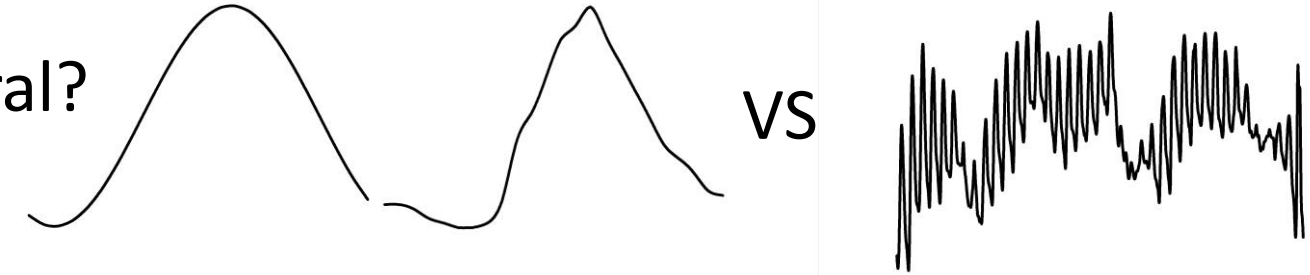

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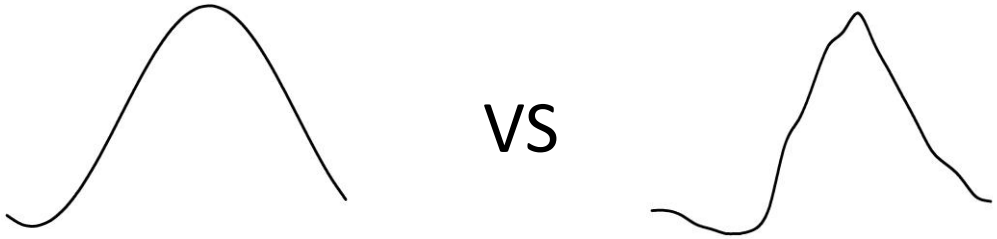

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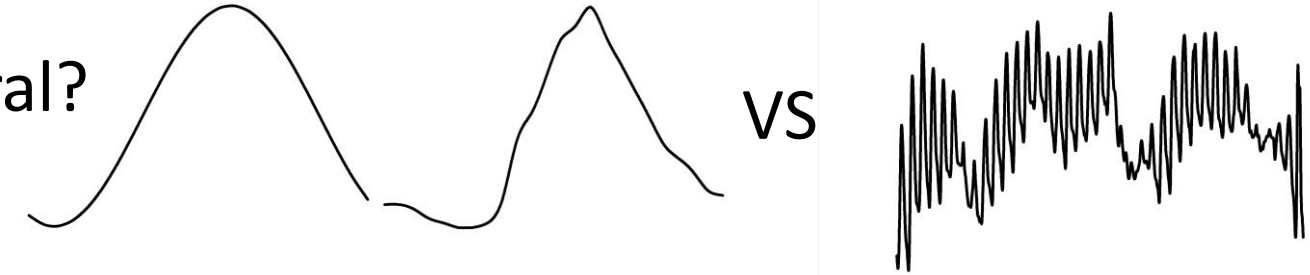

- Context-dependent effects?

Seasonal effects of incubation temperature

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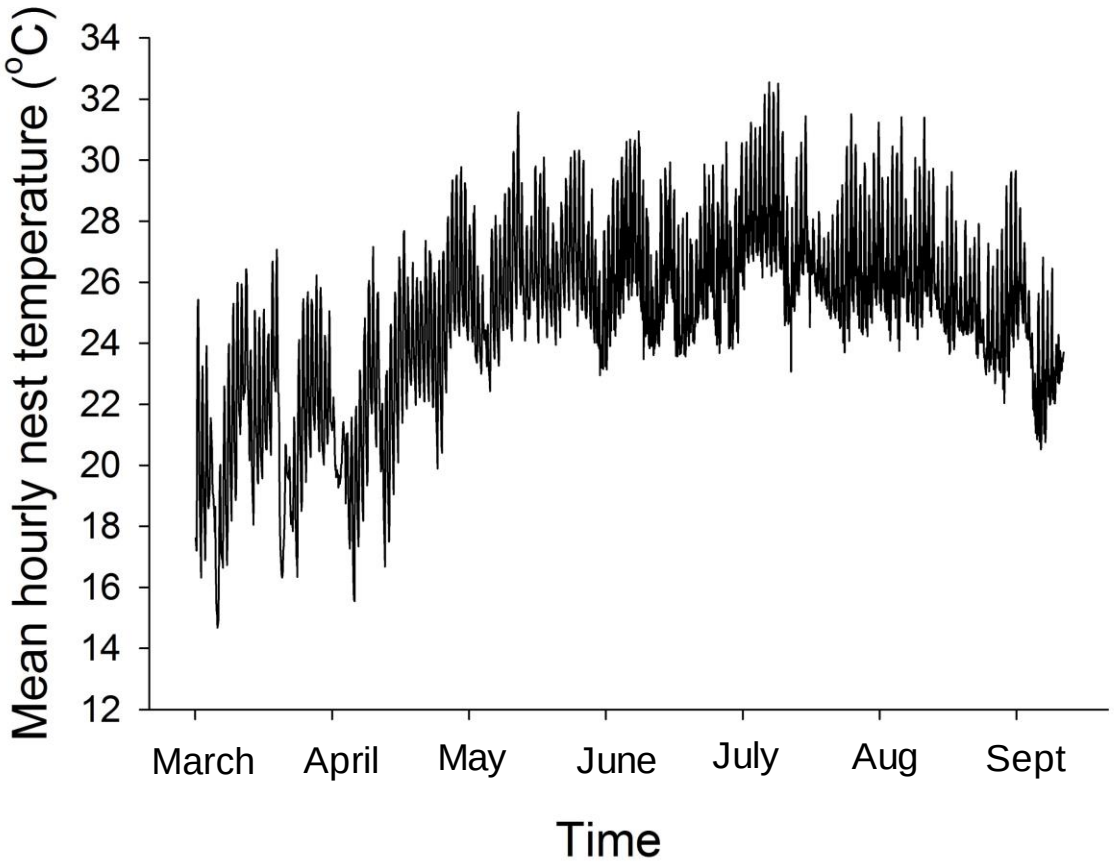
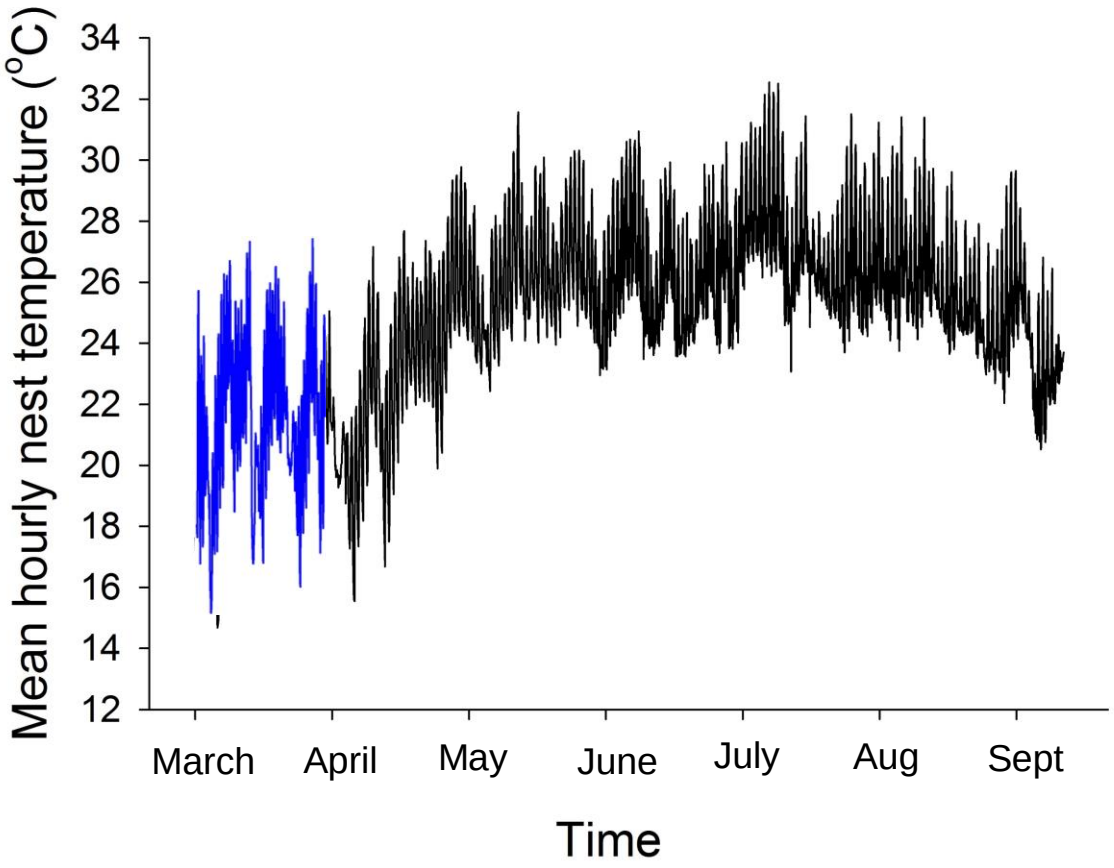


Pearson and Warner. 2018. *Proceedings R Soc B*

Seasonal effects of incubation temperature



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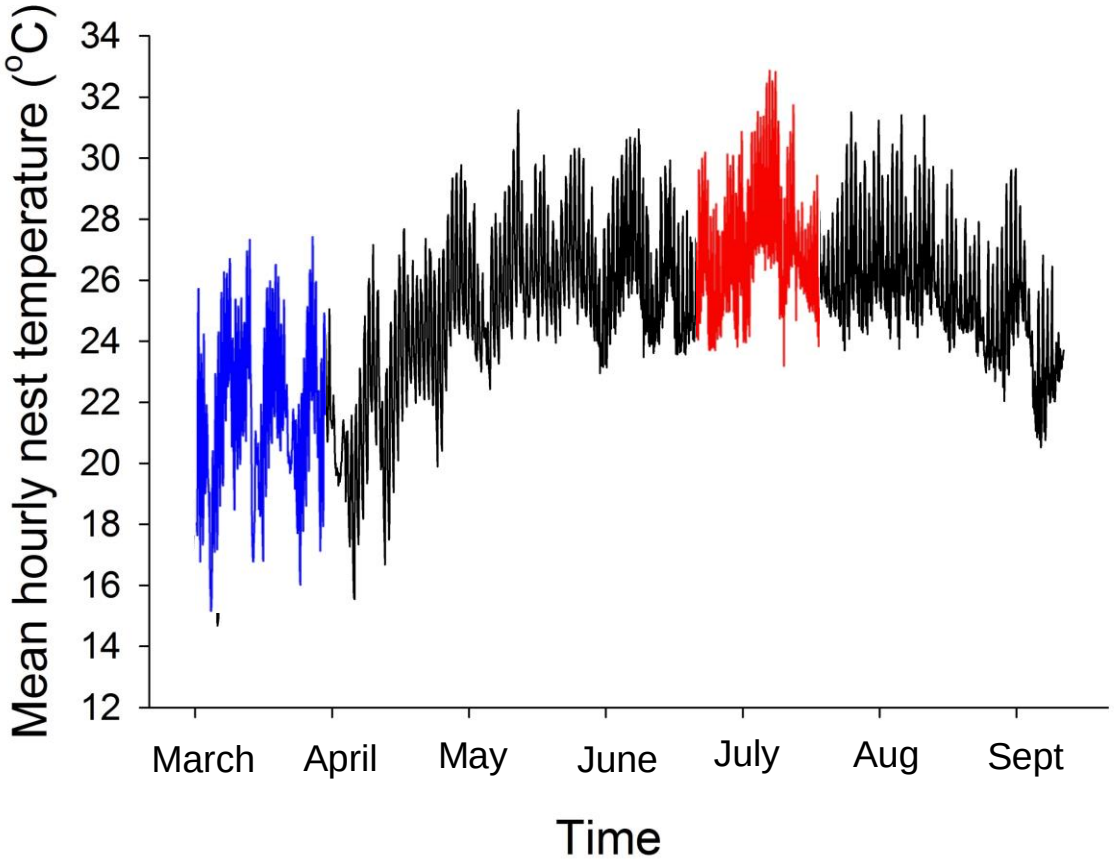

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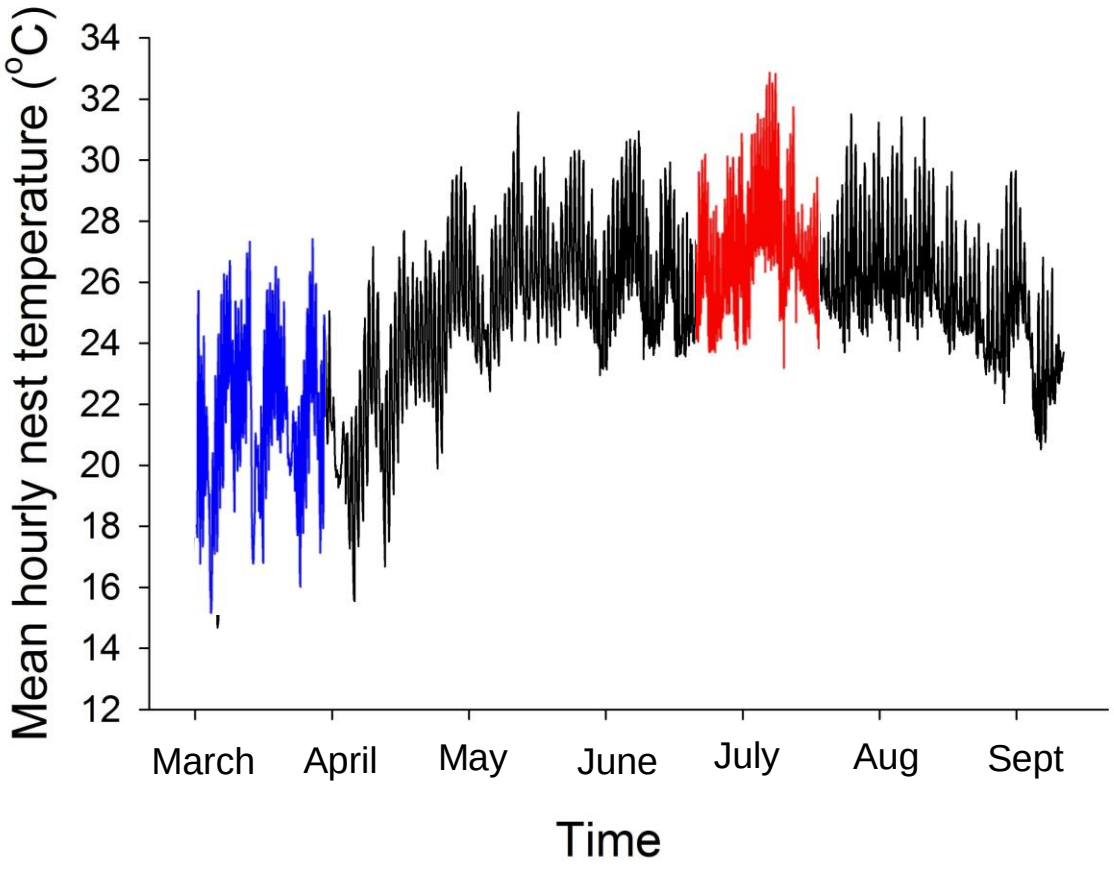
Methods



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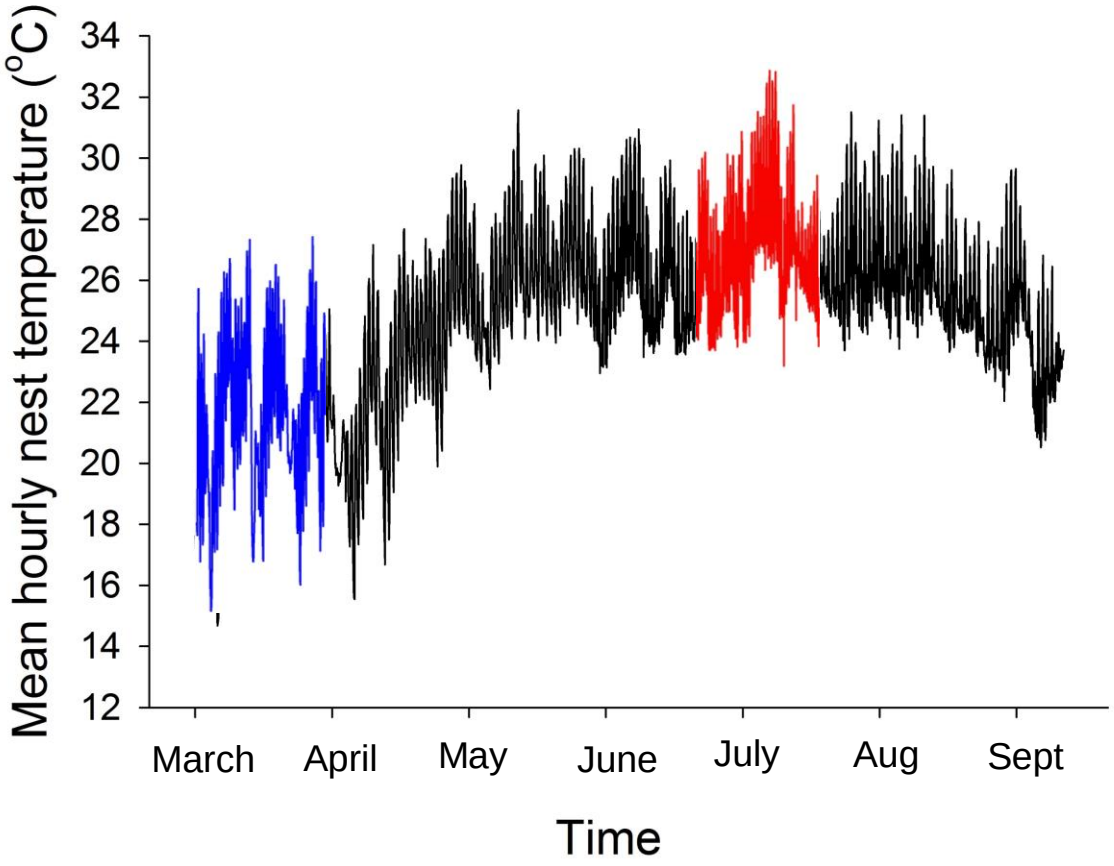
Mean hourly nest temperature (°C)
34
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March
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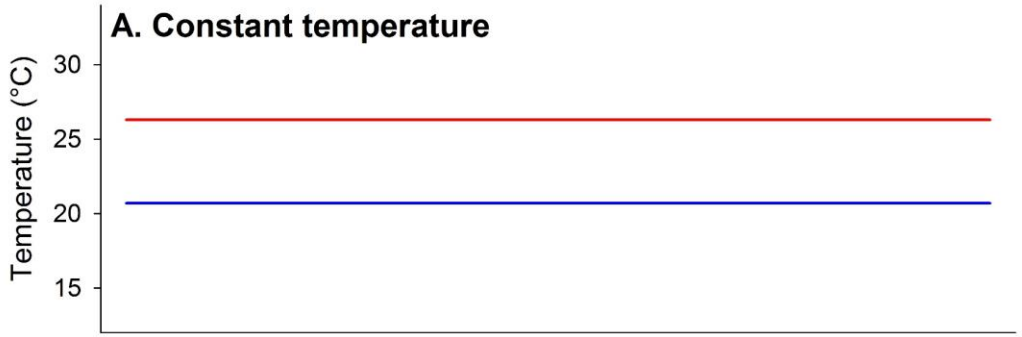
Time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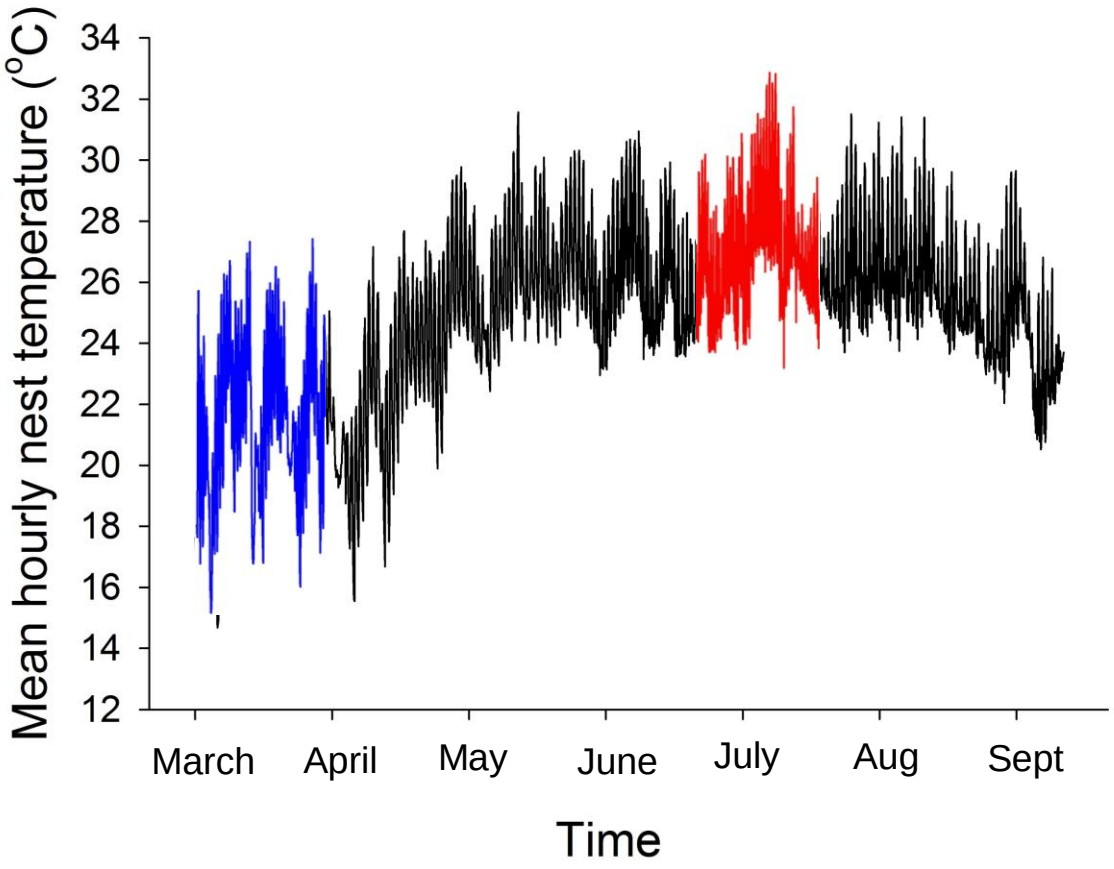
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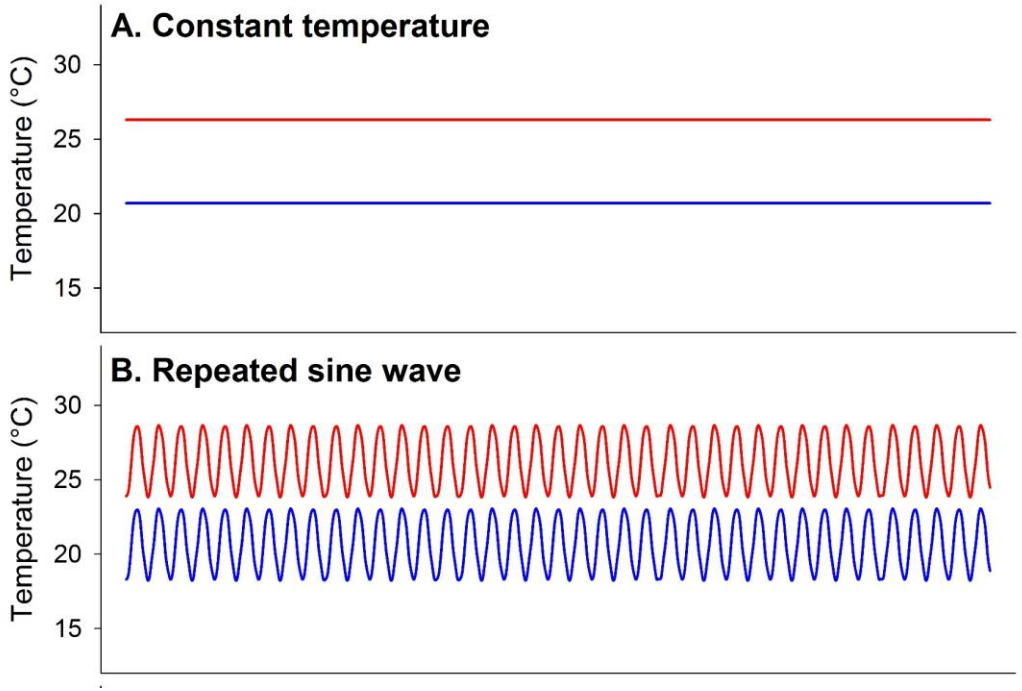
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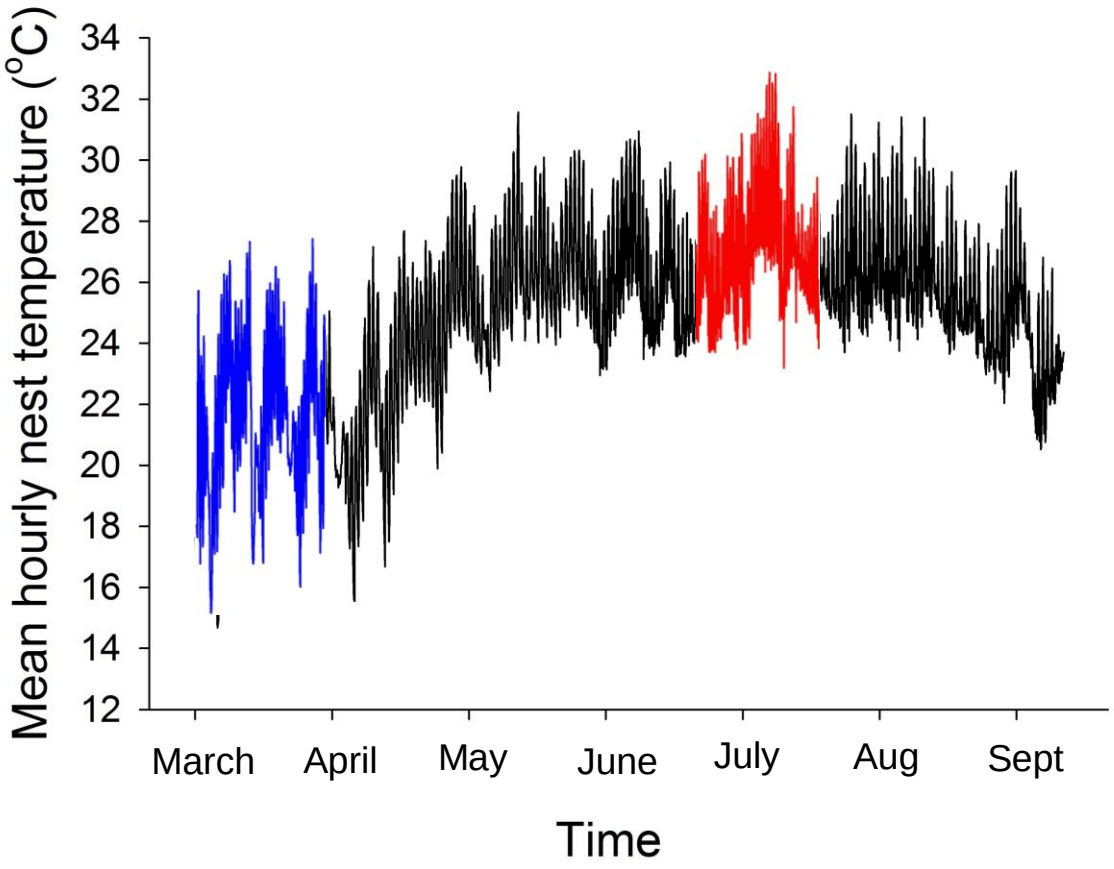
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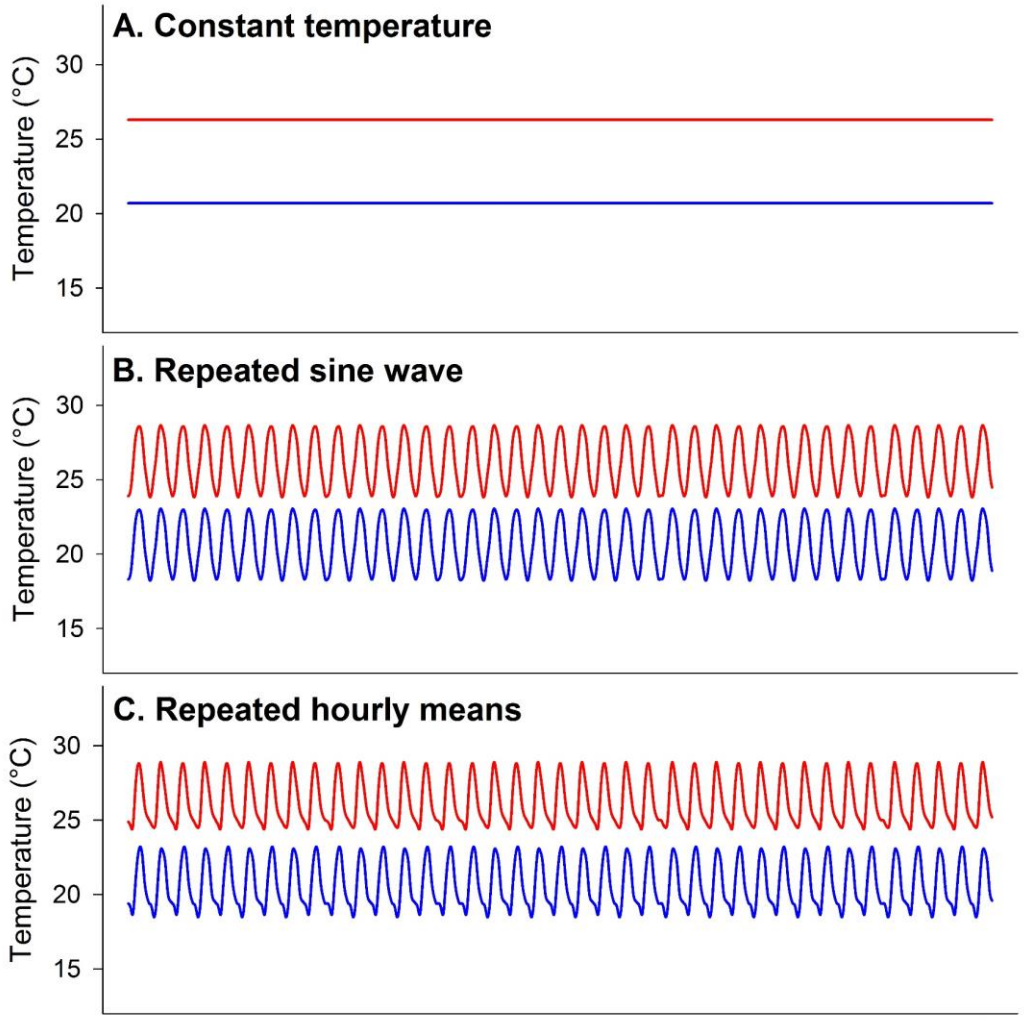
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C. Repeated hourly means
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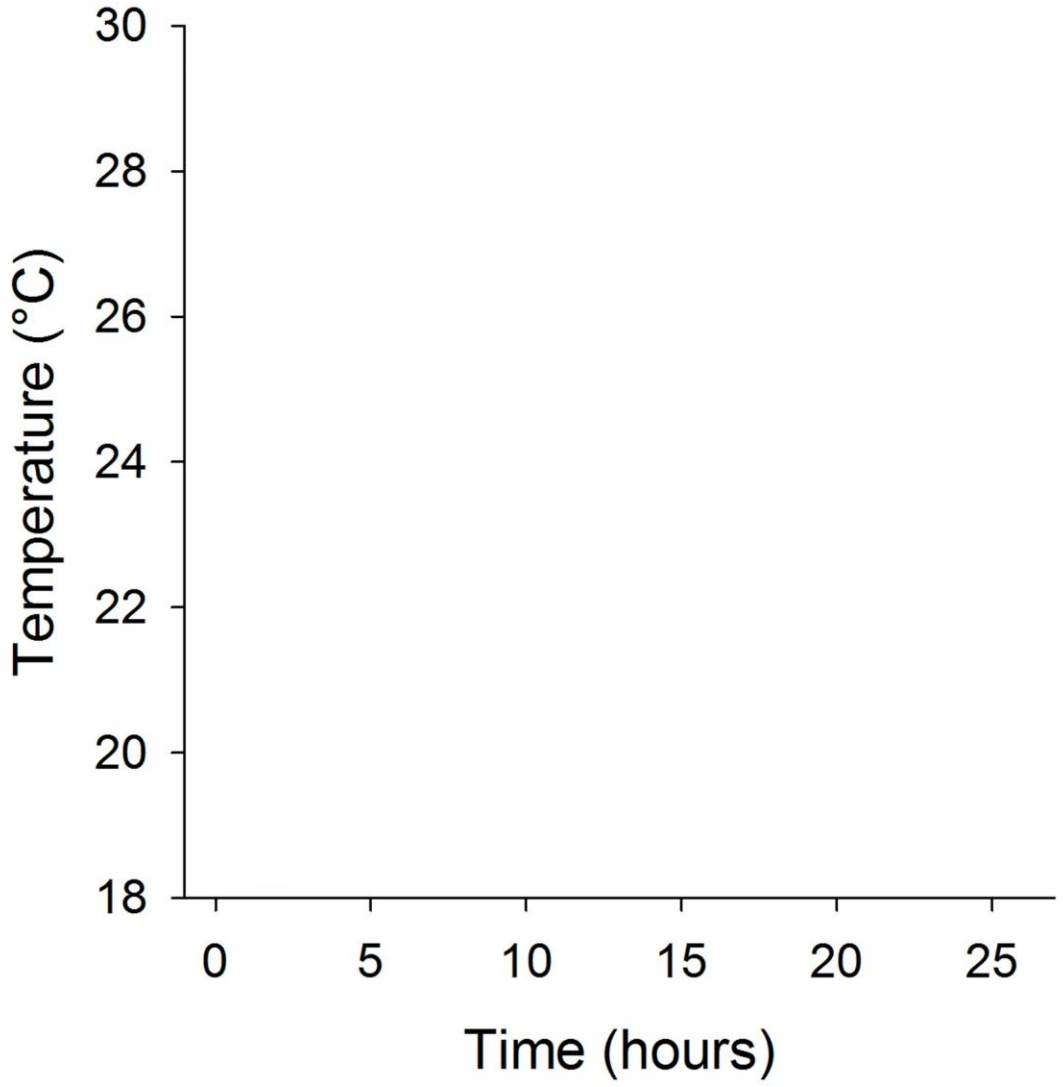
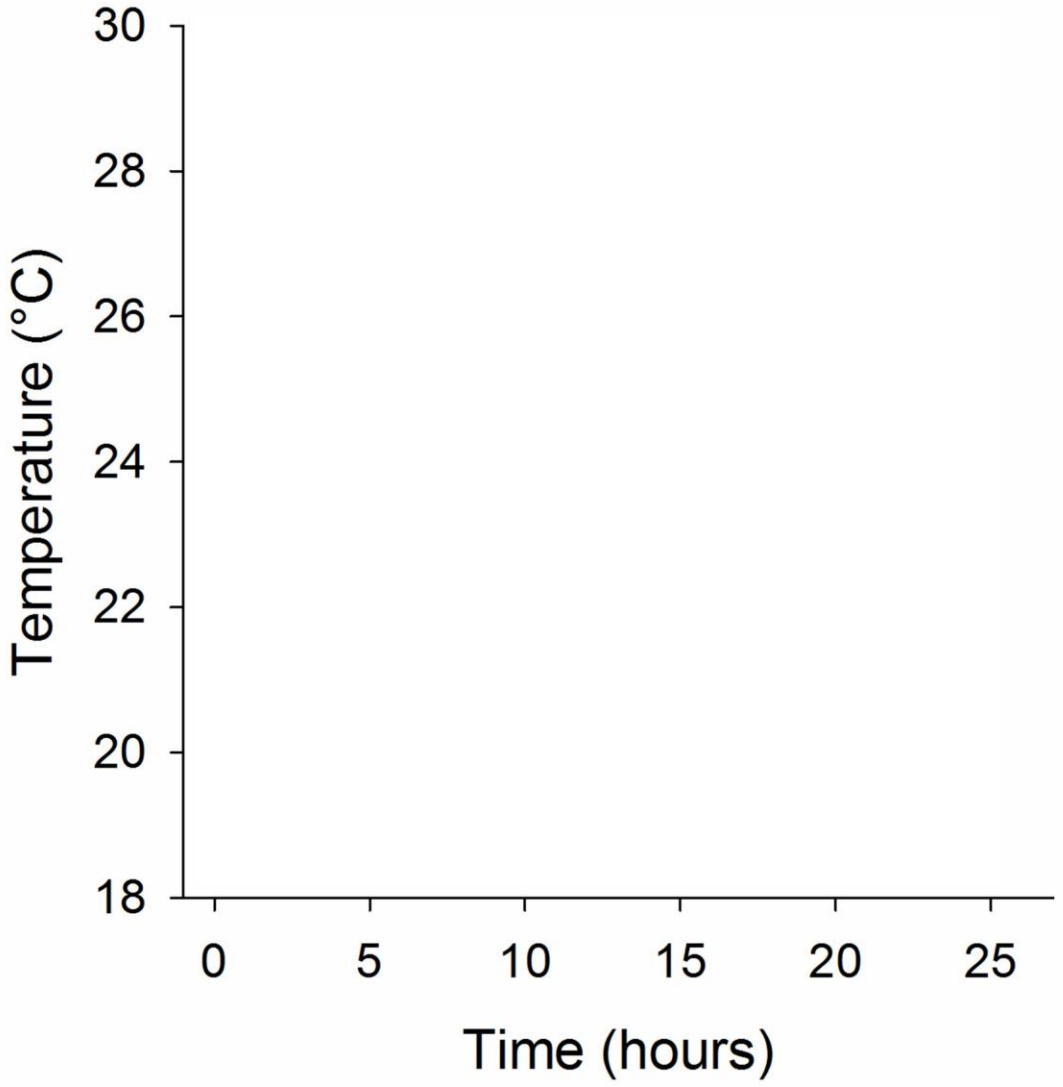
Sine Wave



Hourly Means



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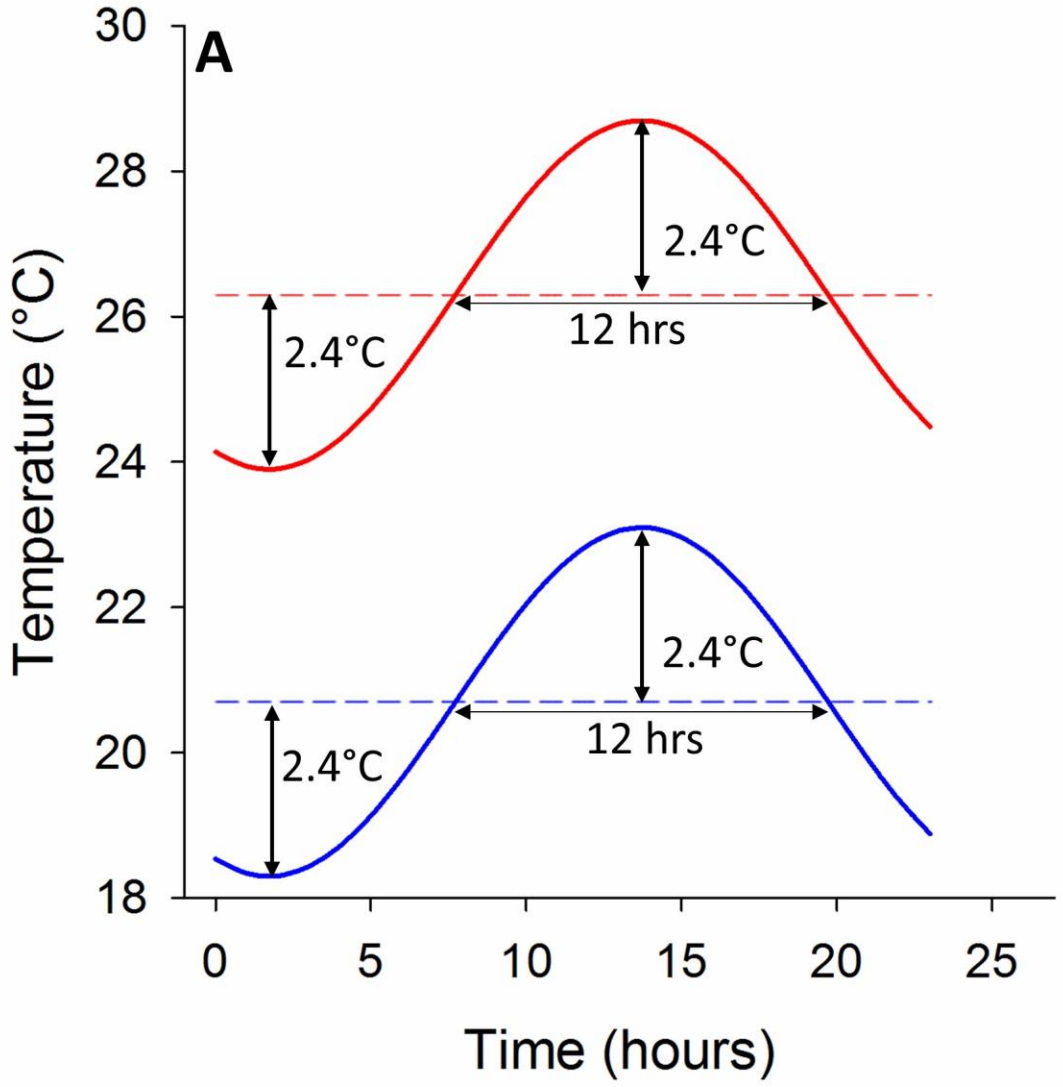

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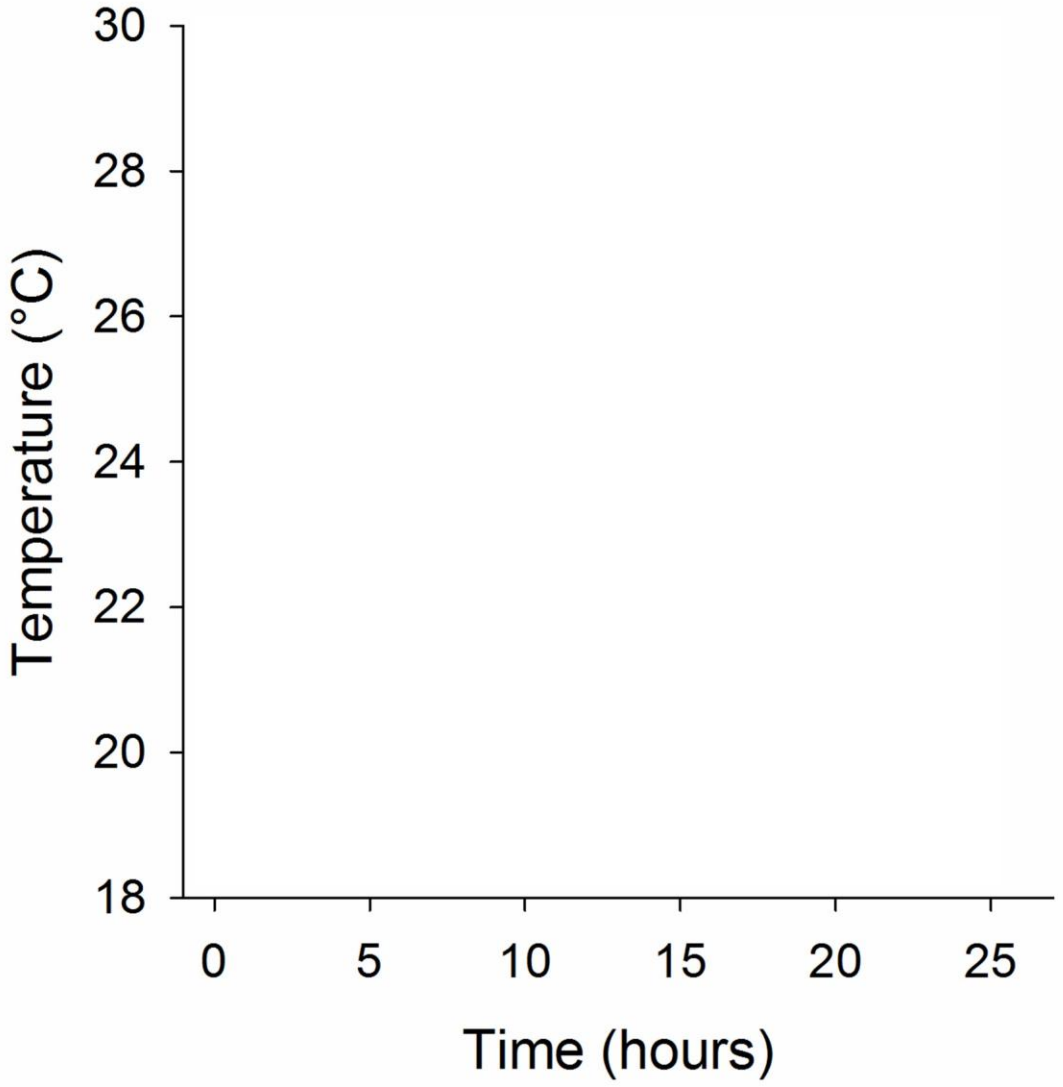

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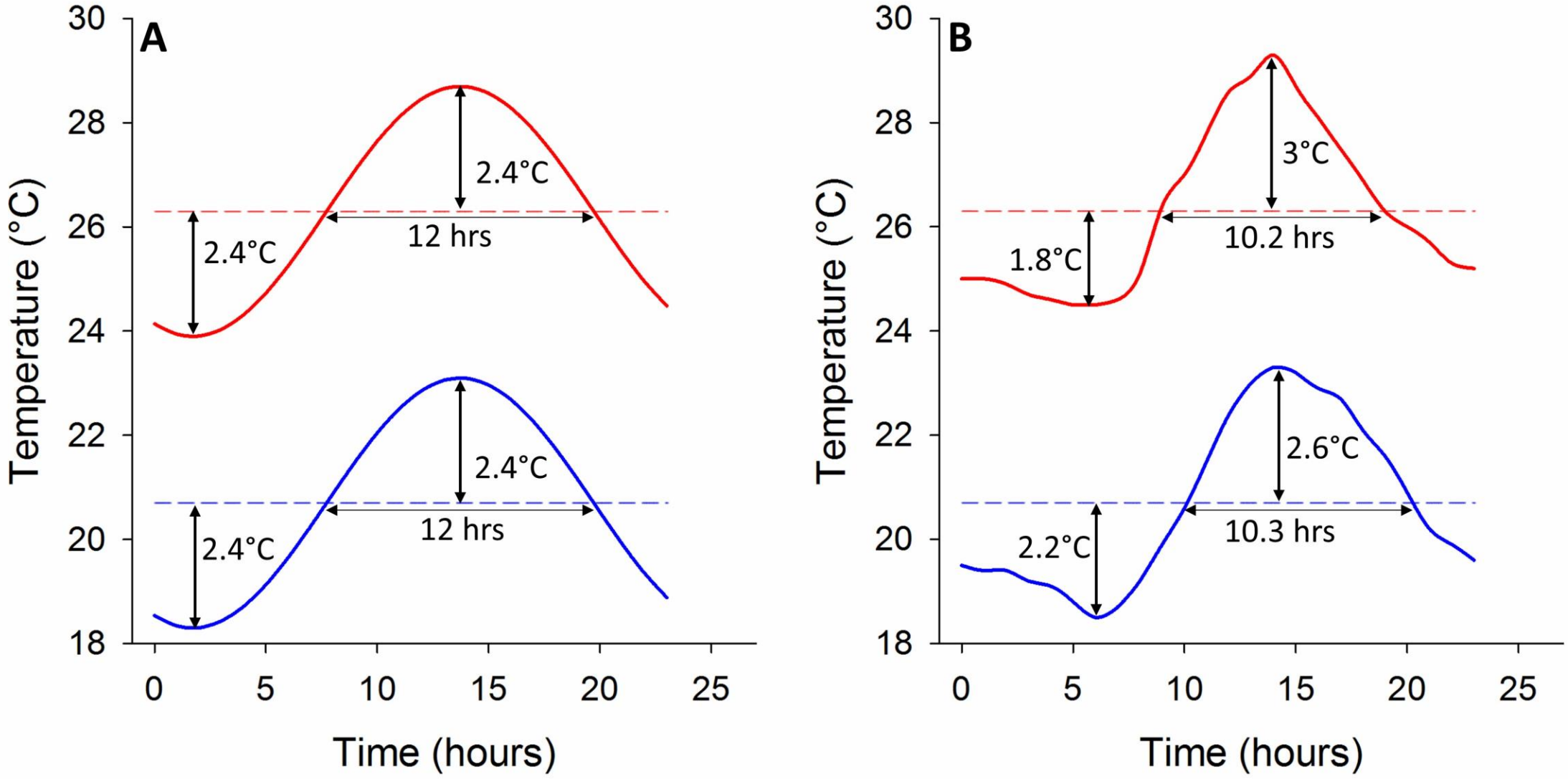

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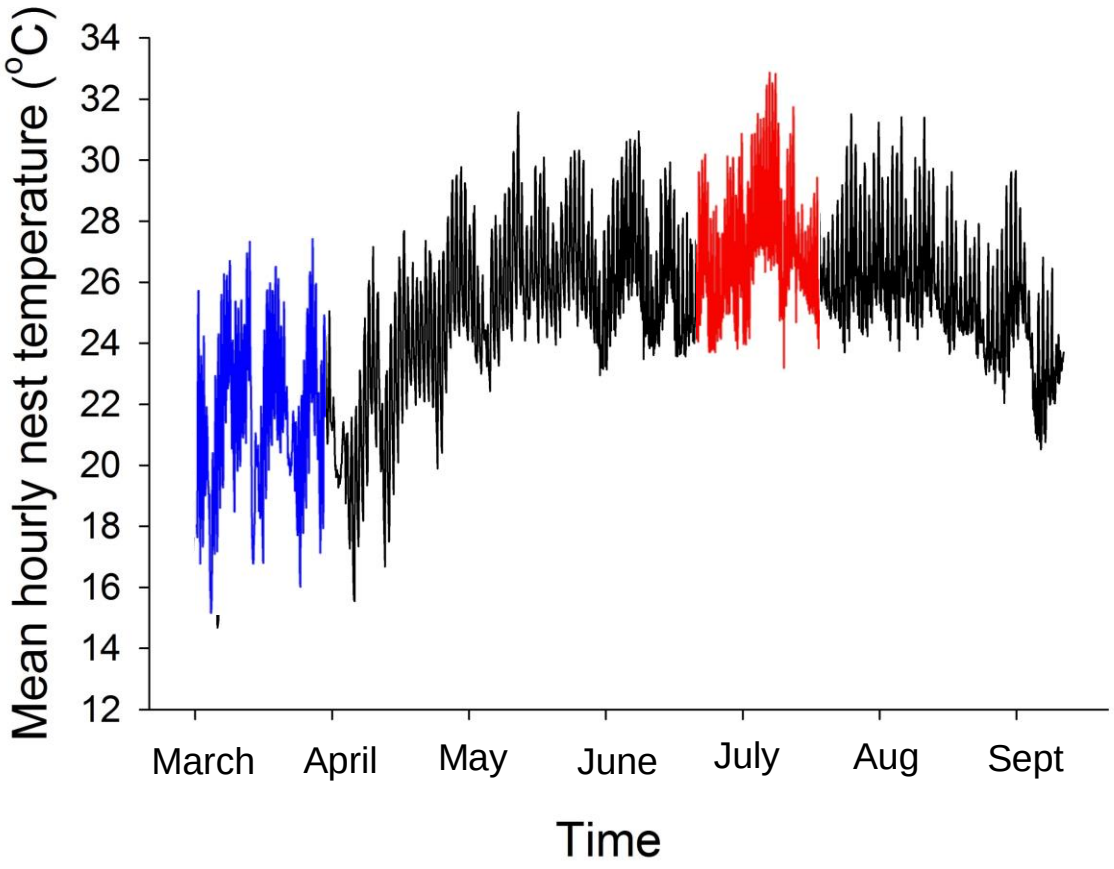
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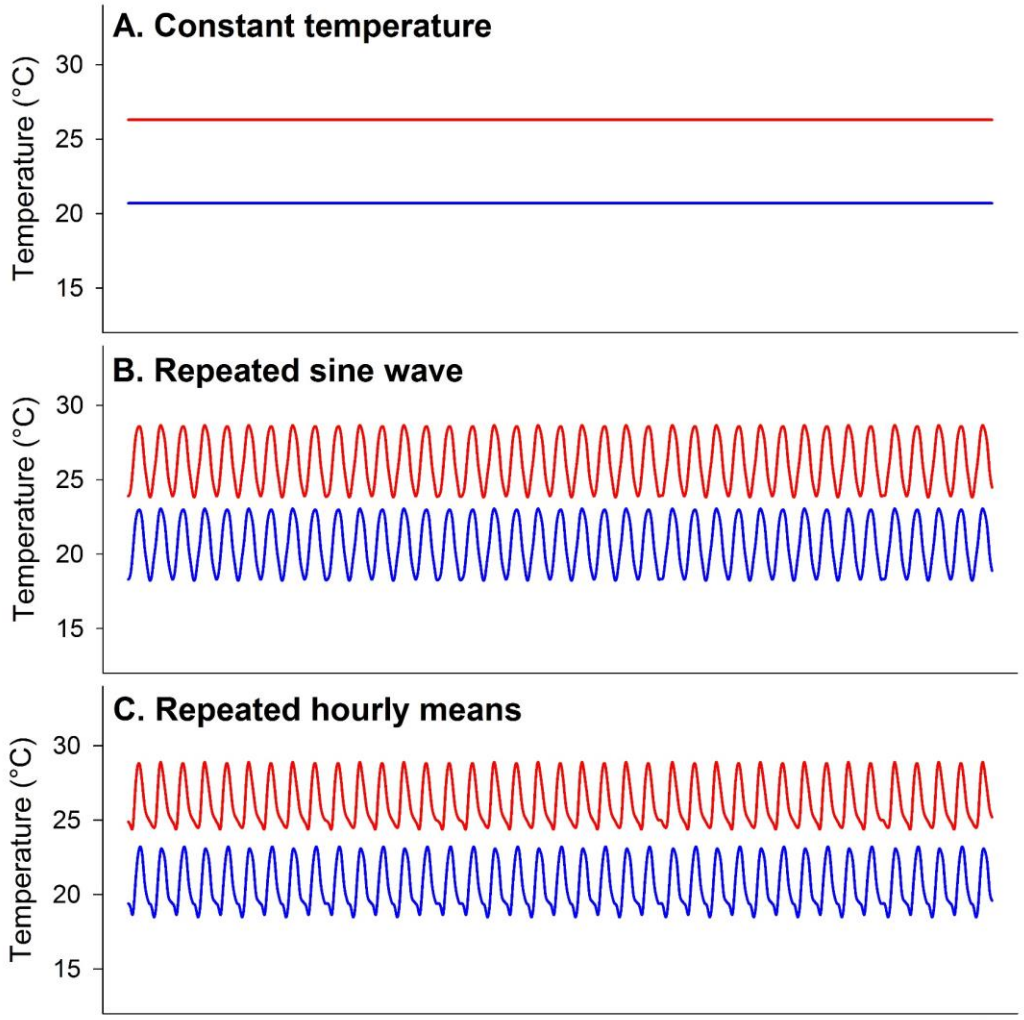
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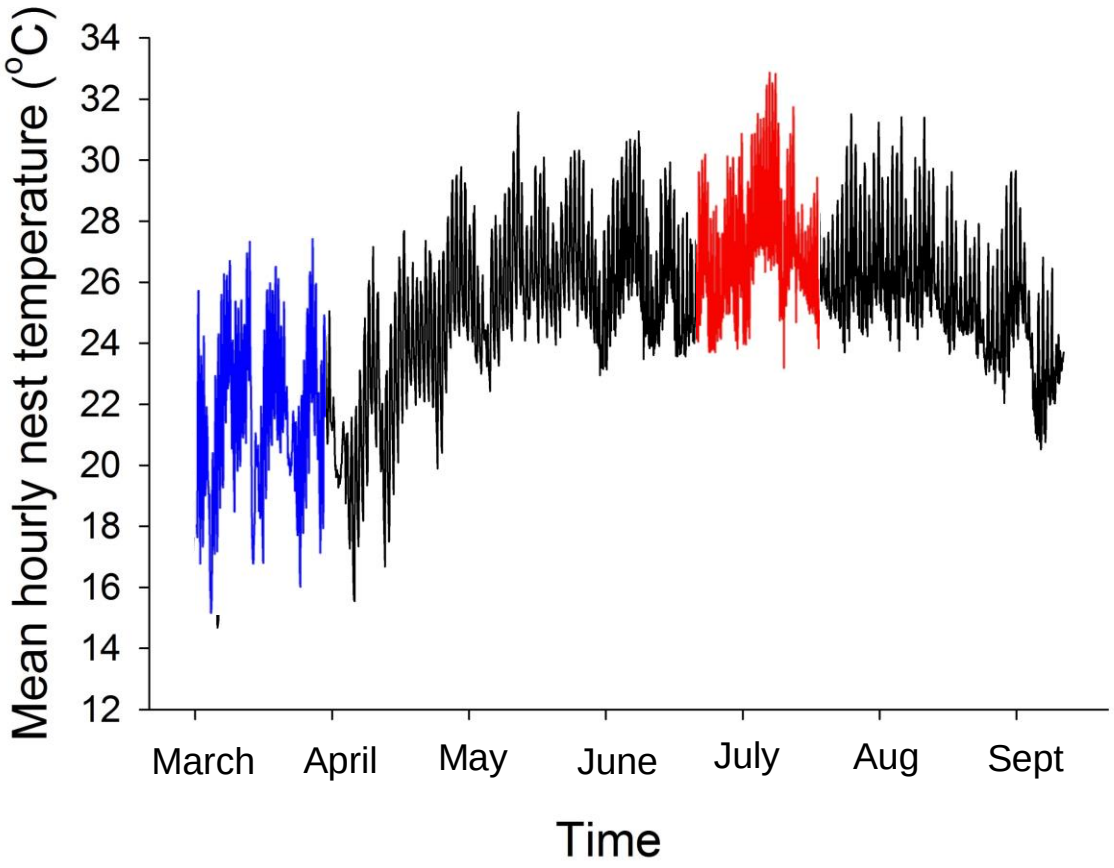
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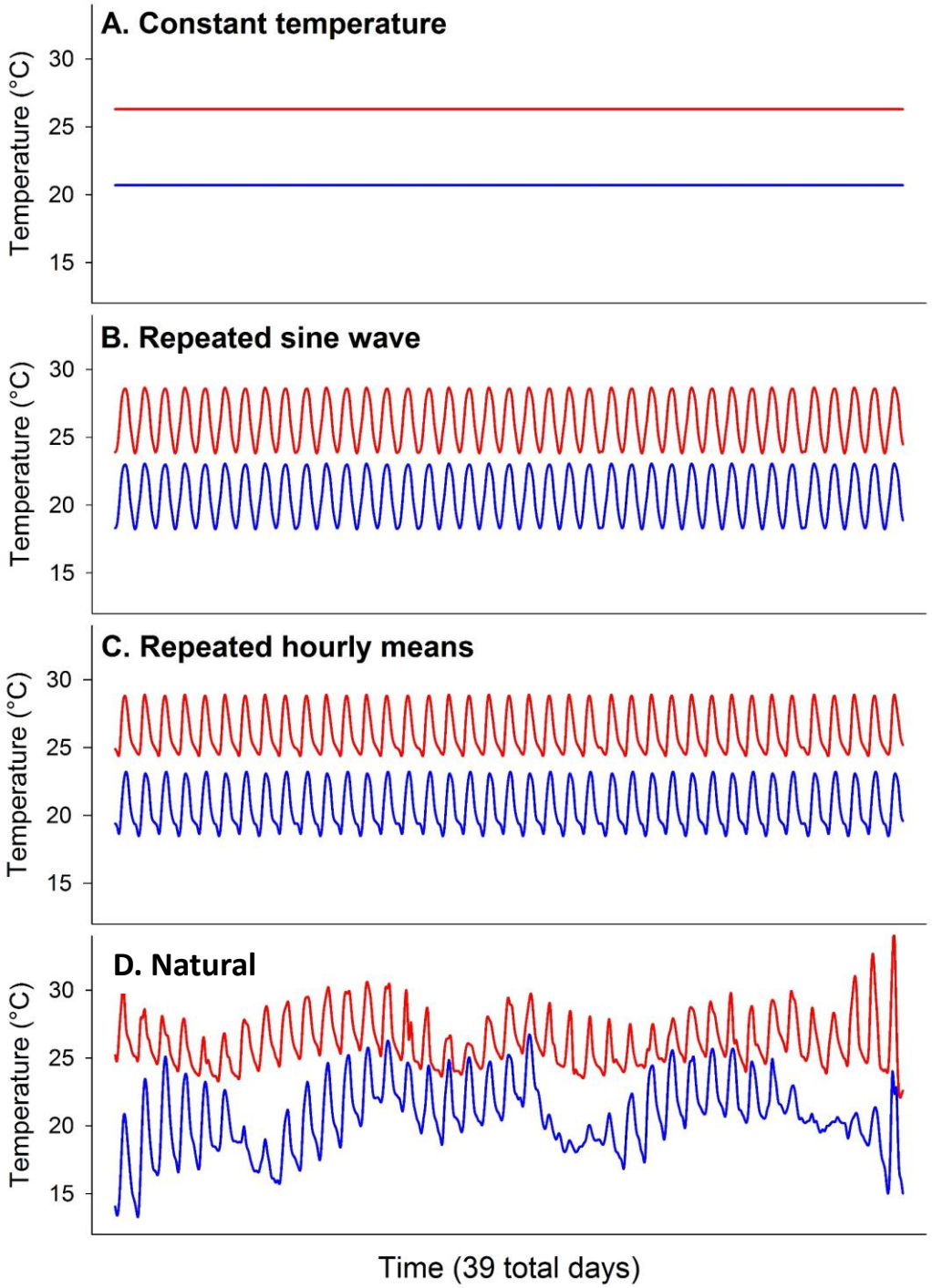
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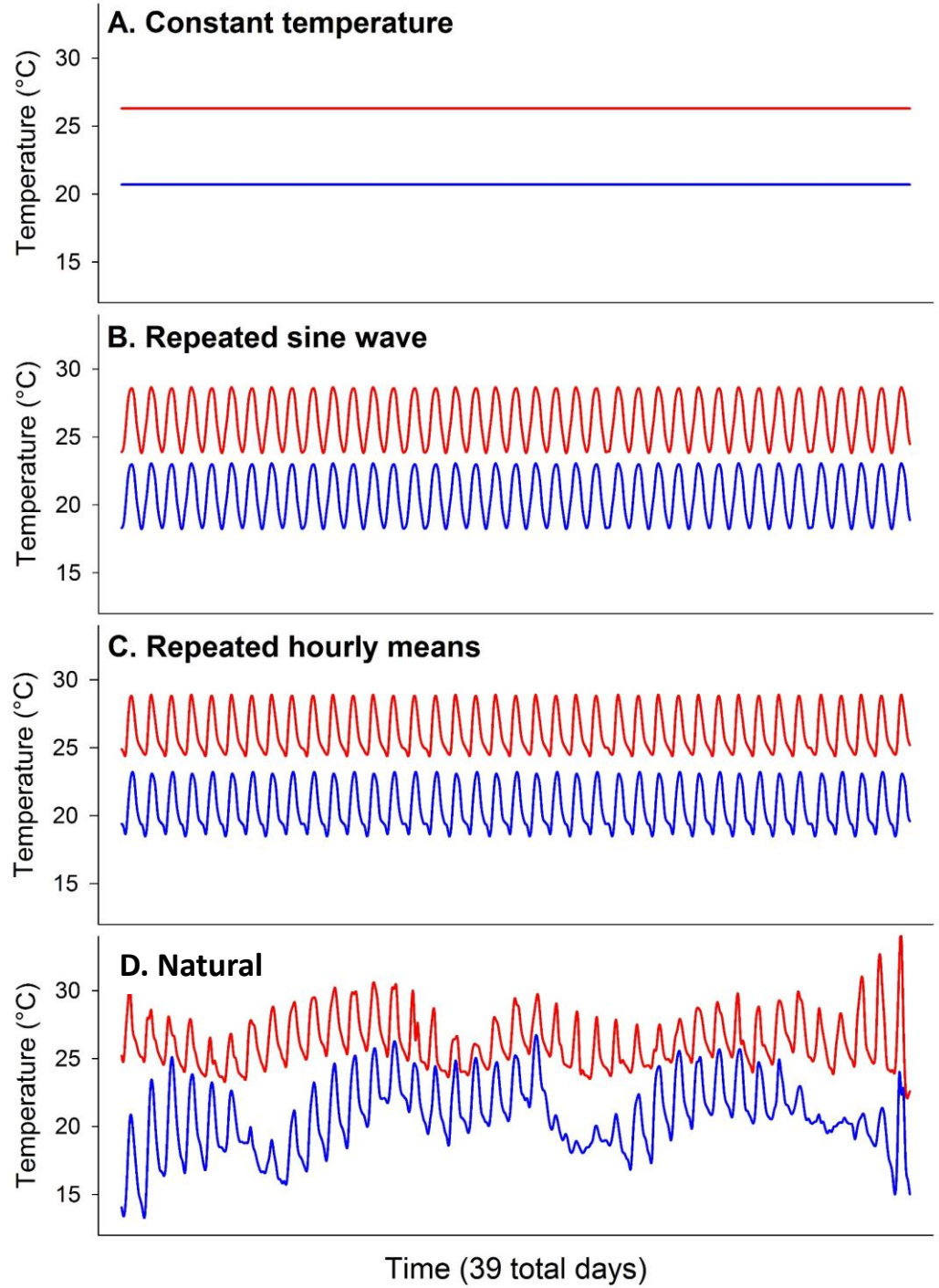
Time (39 total days)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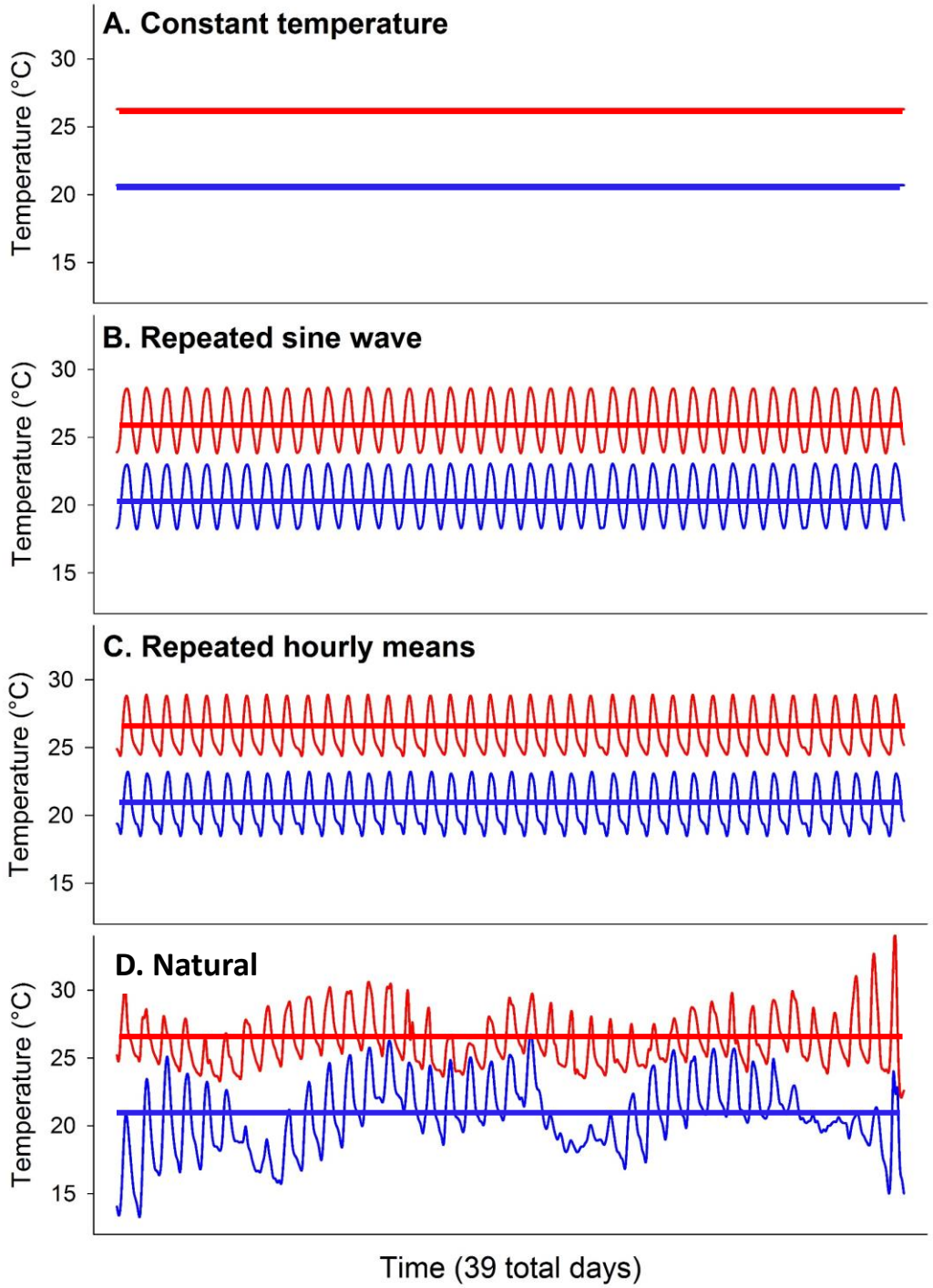
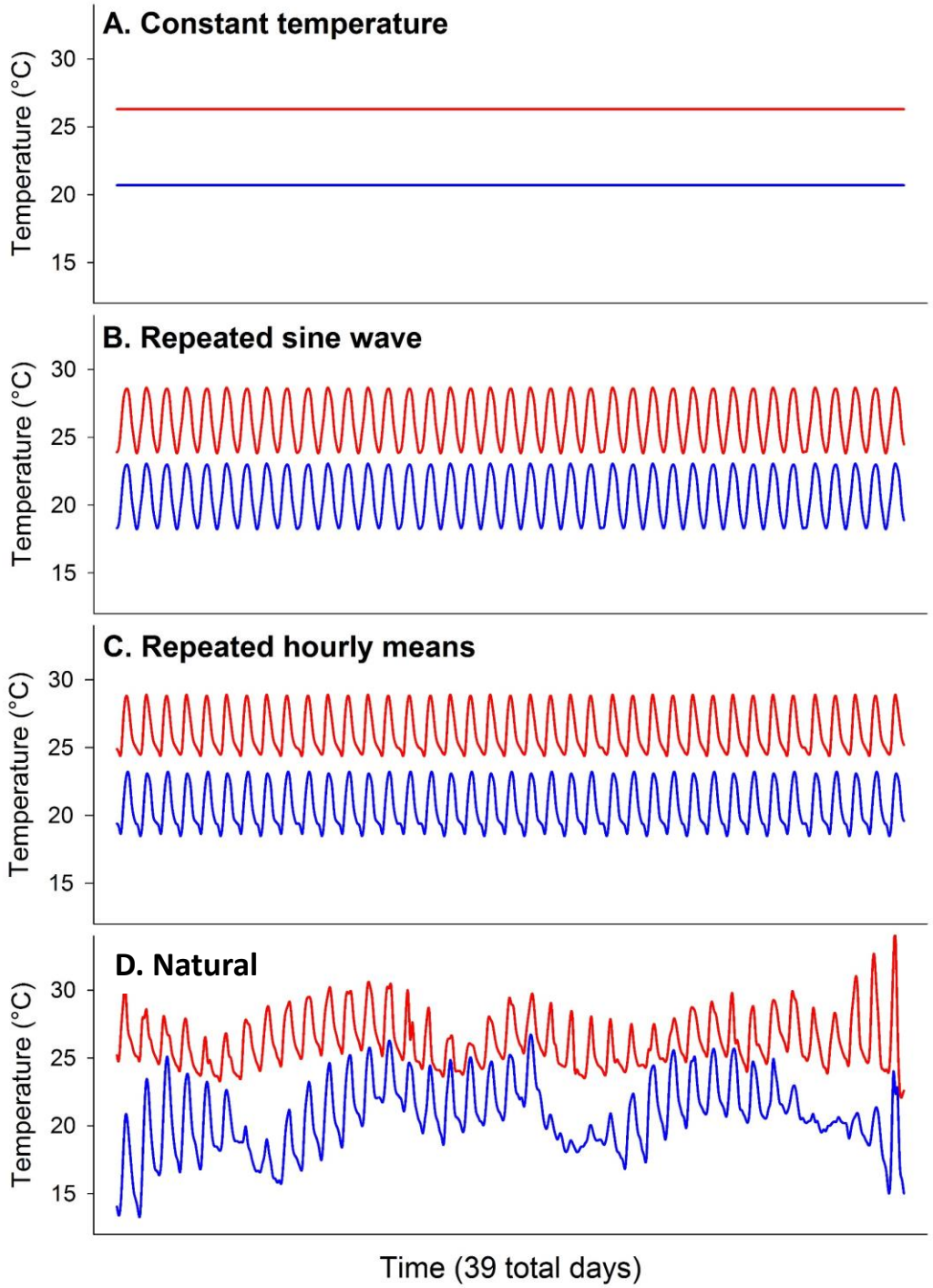
Mean temperatures are equal (within seasons)



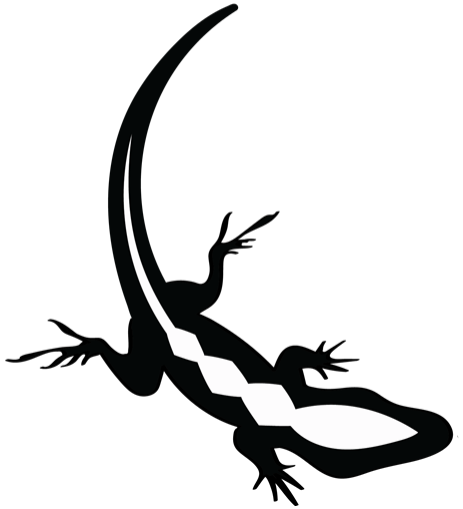
Mean temperatures are equal (within seasons)

Daily variation is same (4.8 °C)



Phenotypes

Embryos

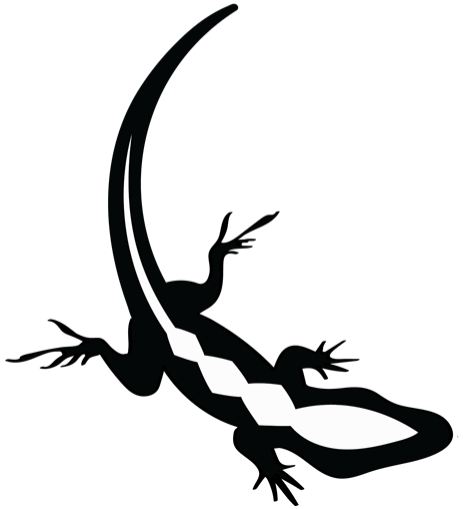


Hatchlings

Phenotypes

Embryos

- Survival
- Physiology
  - Developmental rate
  - Water uptake
  - Yolk conversion
  - Heart rate
  - Metabolism



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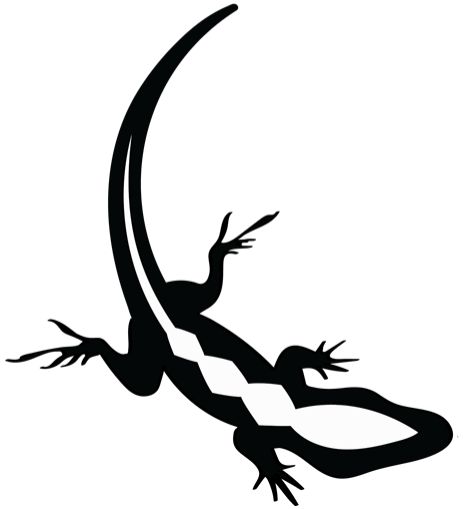


Hatchlings

- Survival (in the lab)
- Morphology
  - SVL and body mass
- Performance
  - Burst speed
  - Endurance
- Growth

Statistics

Statistics

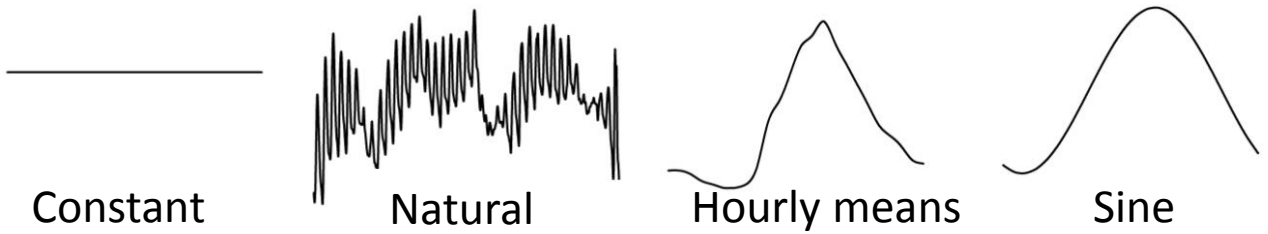
- Response Var ~

Statistics

- Response Var ~ Season (Early vs Late)

Statistics

- Response Var ~ Season + Incubation Treatment



Statistics

- Response Var ~ Season + Incubation Treatment + Interaction

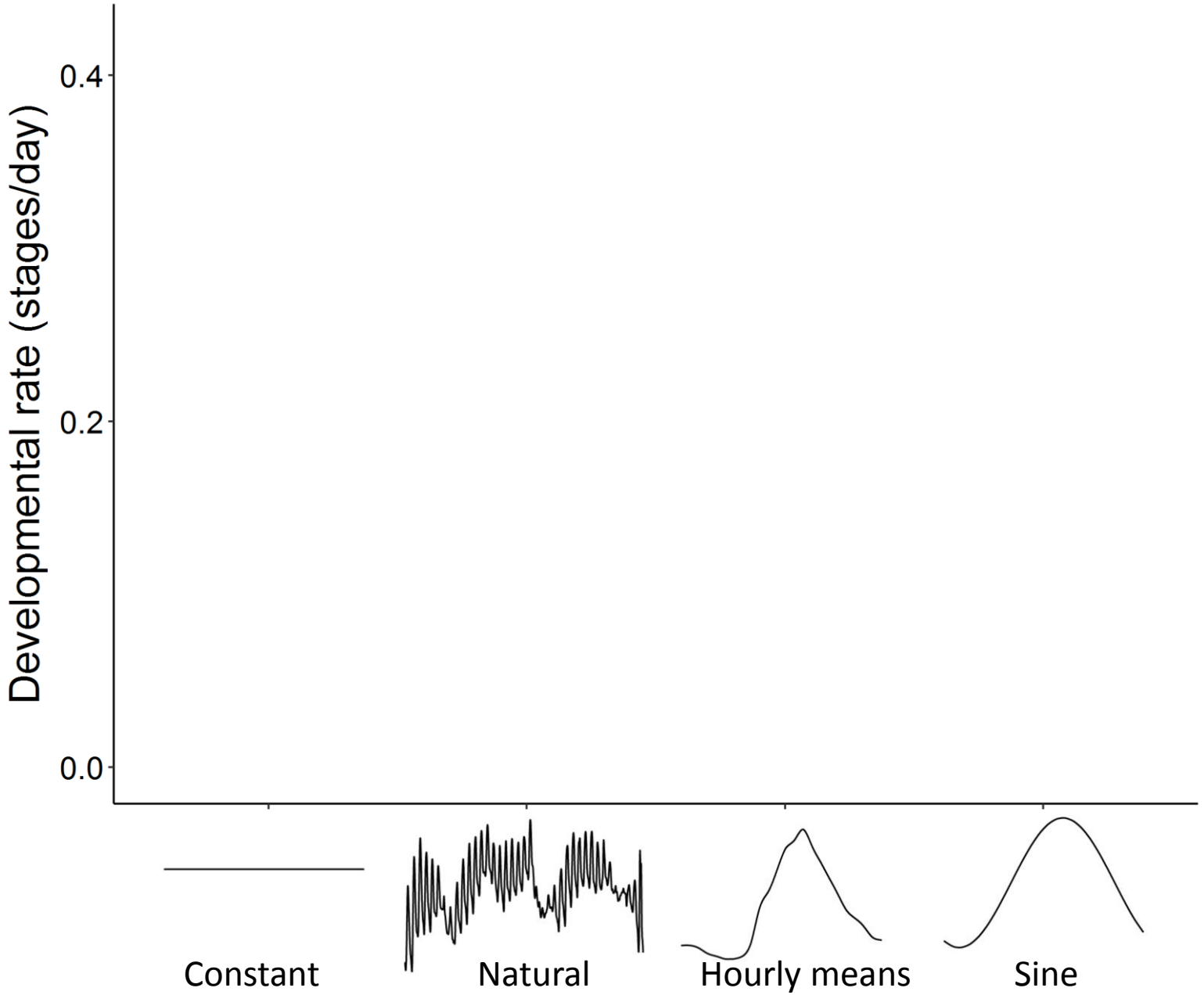
Statistics

- Response Var ~ Season + **Incubation Treatment** + **Interaction**

Effects on embryo development

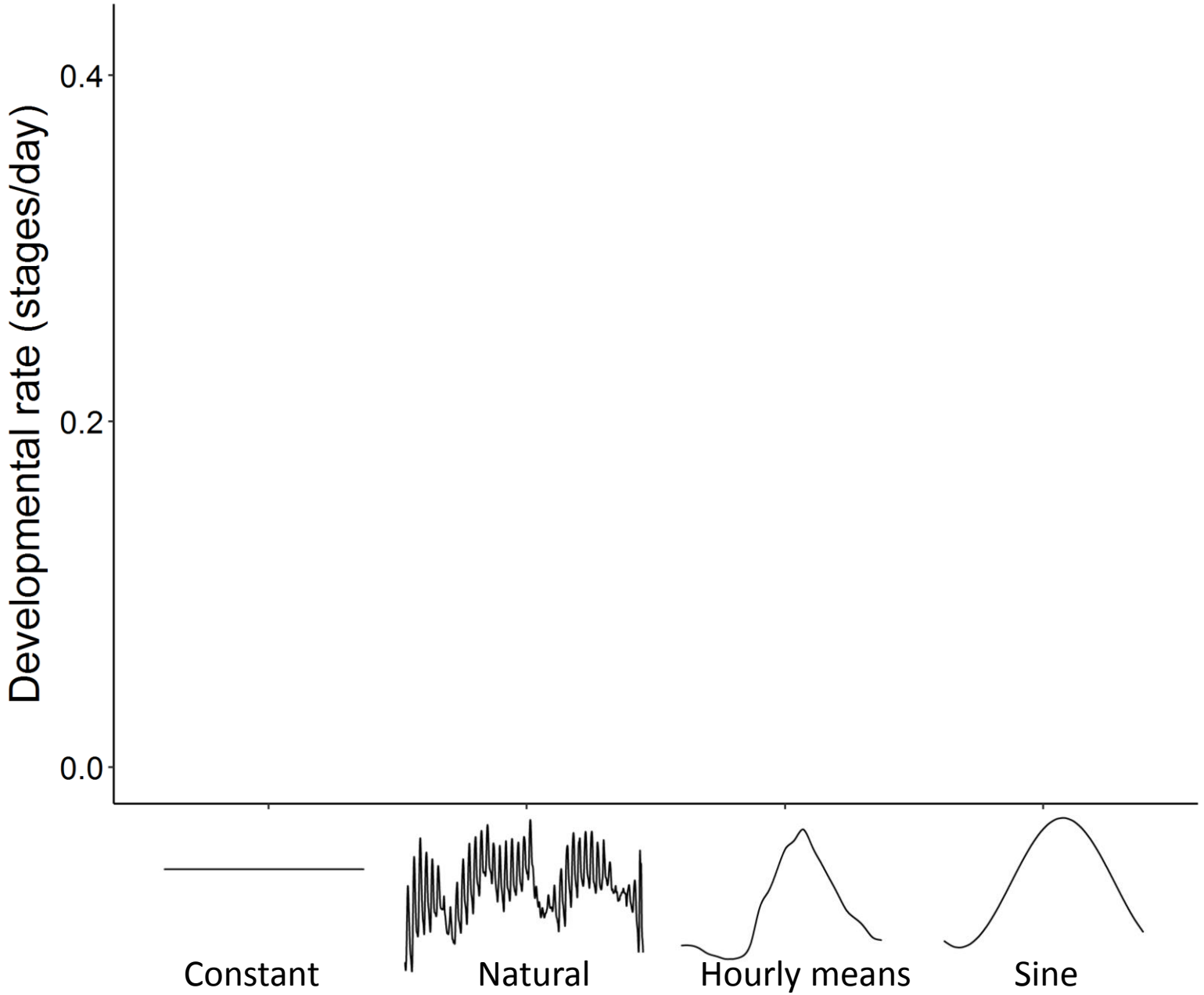
Developmental rate

	DF	F	p
Incubation Treatment			
Season X Treatment			



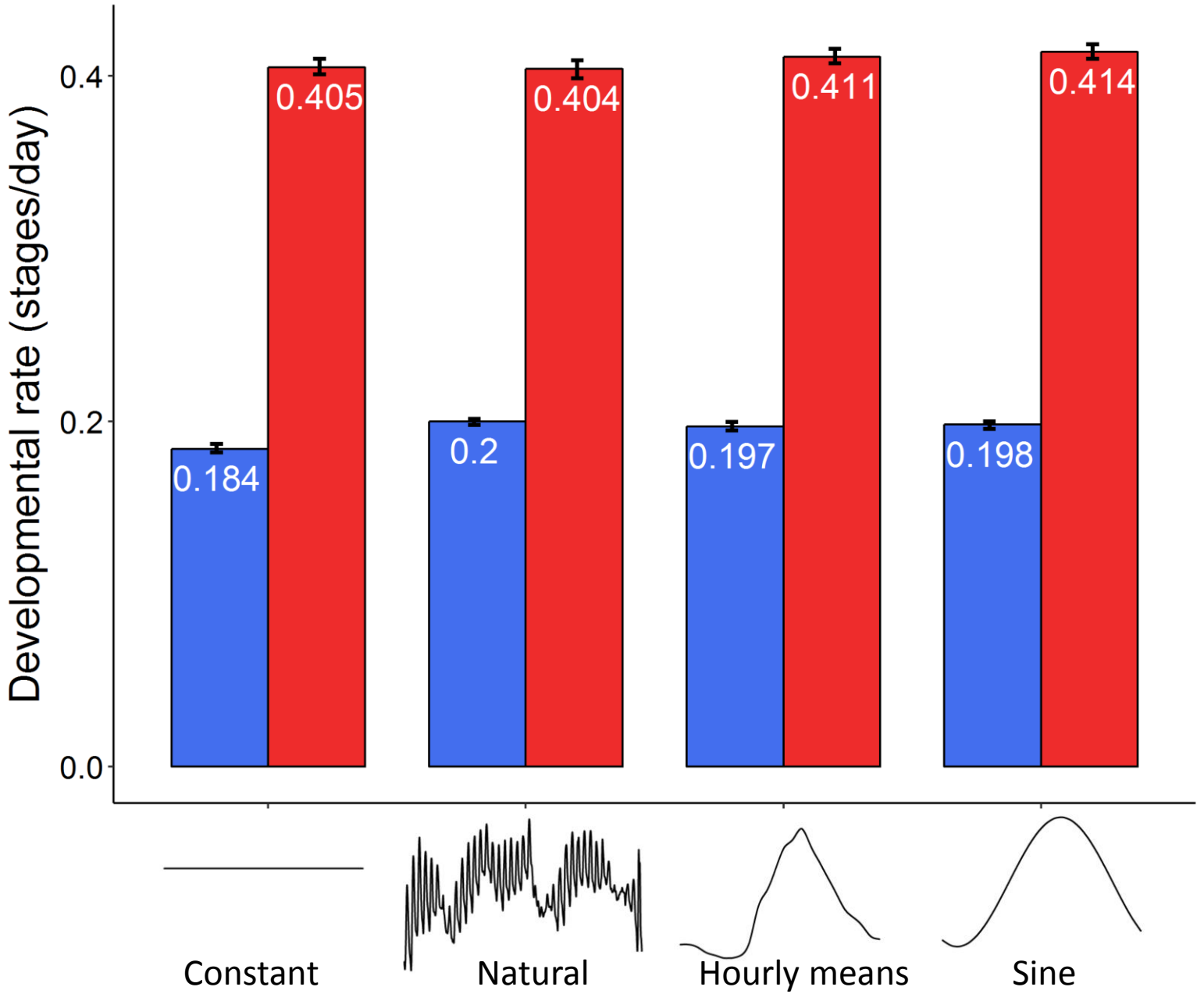
Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**



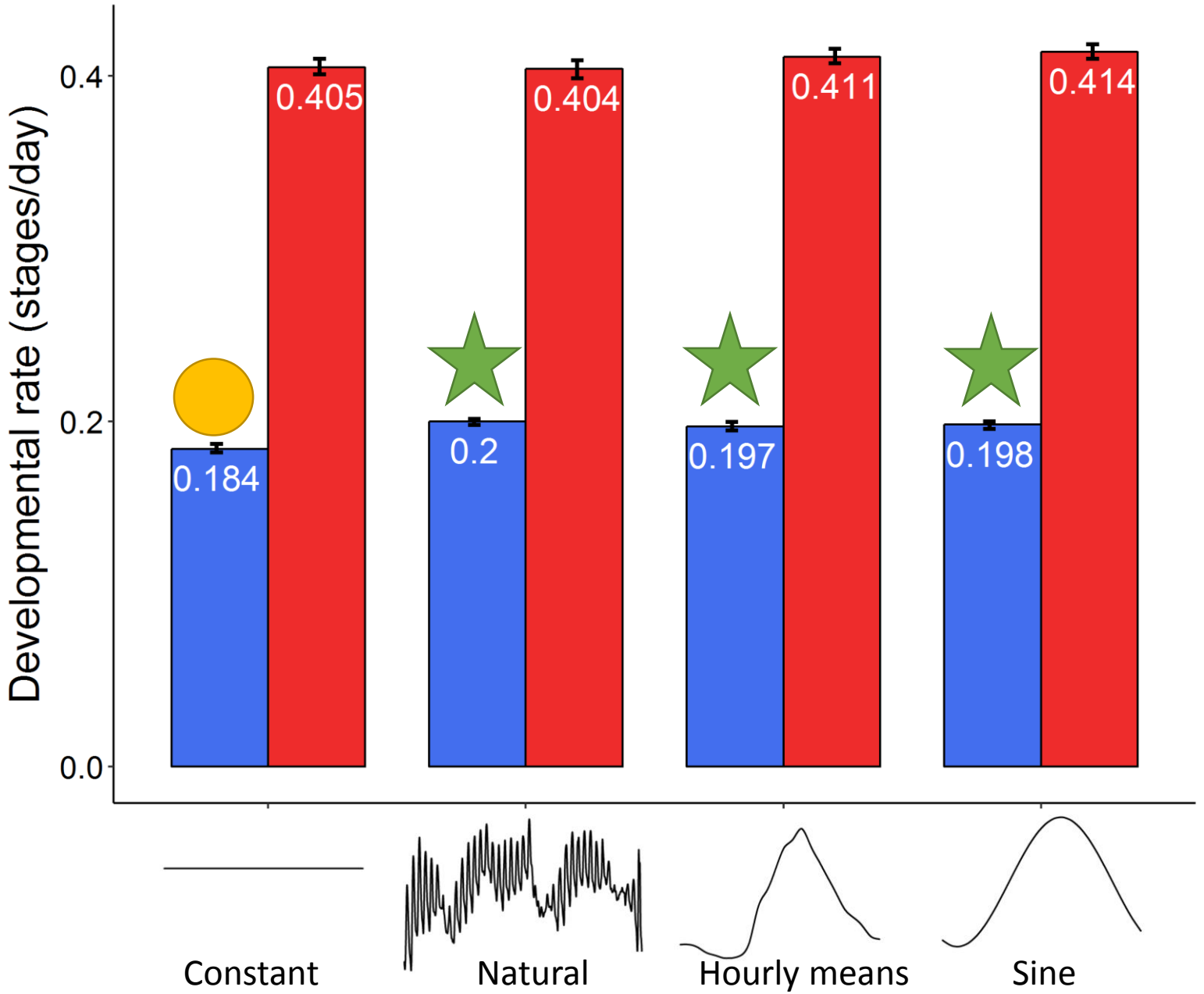
Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**



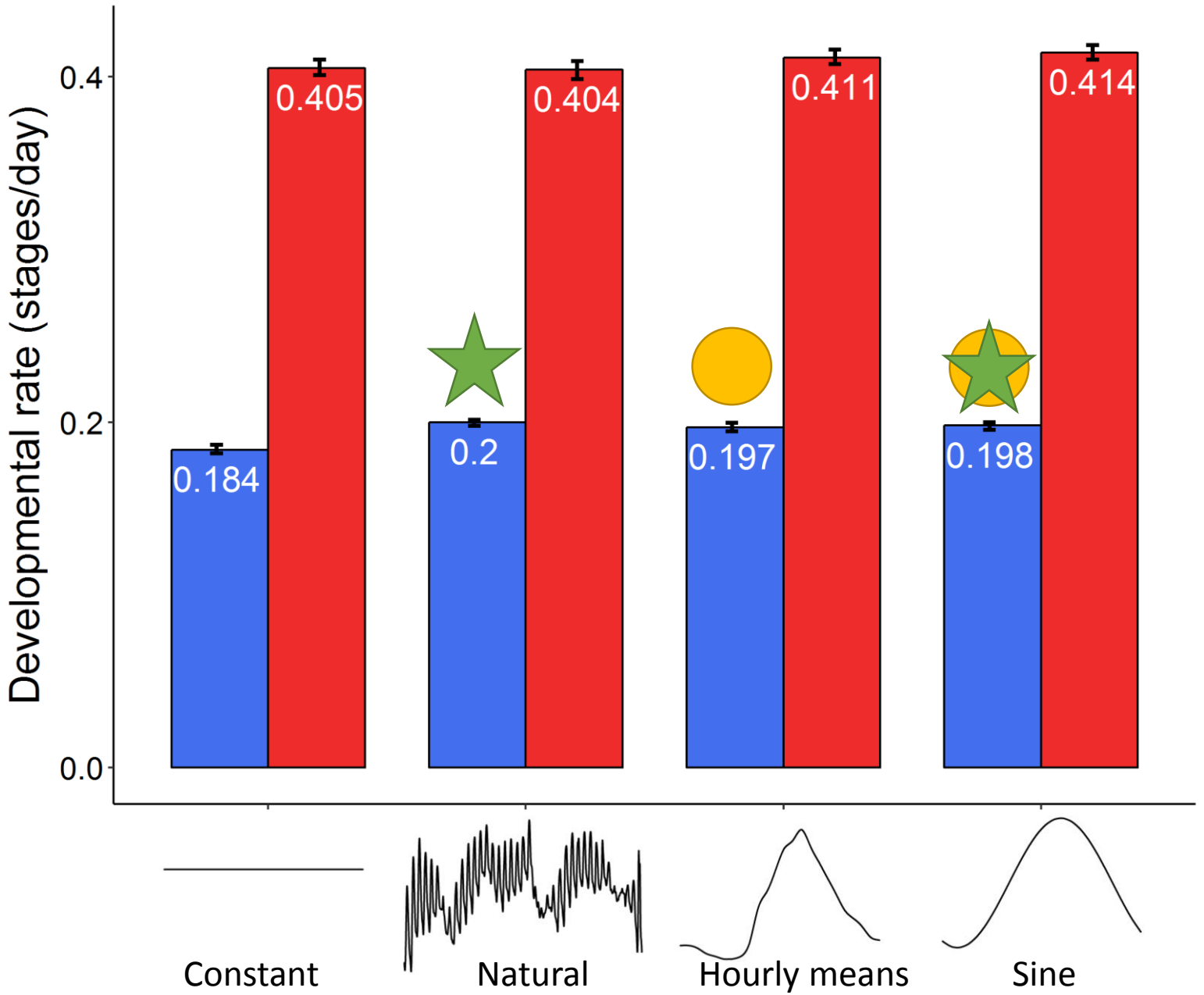
Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**



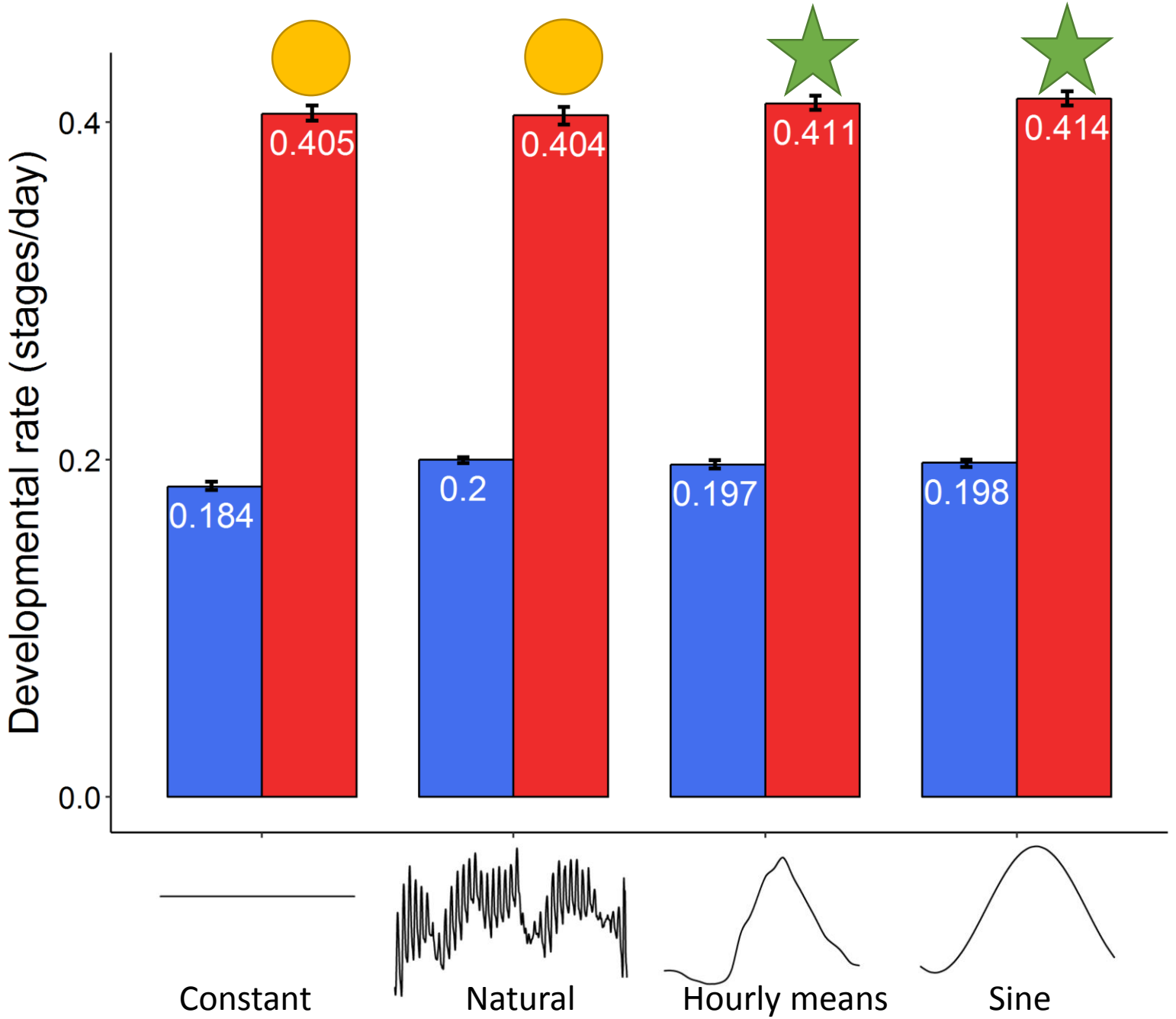
Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**



Developmental rate

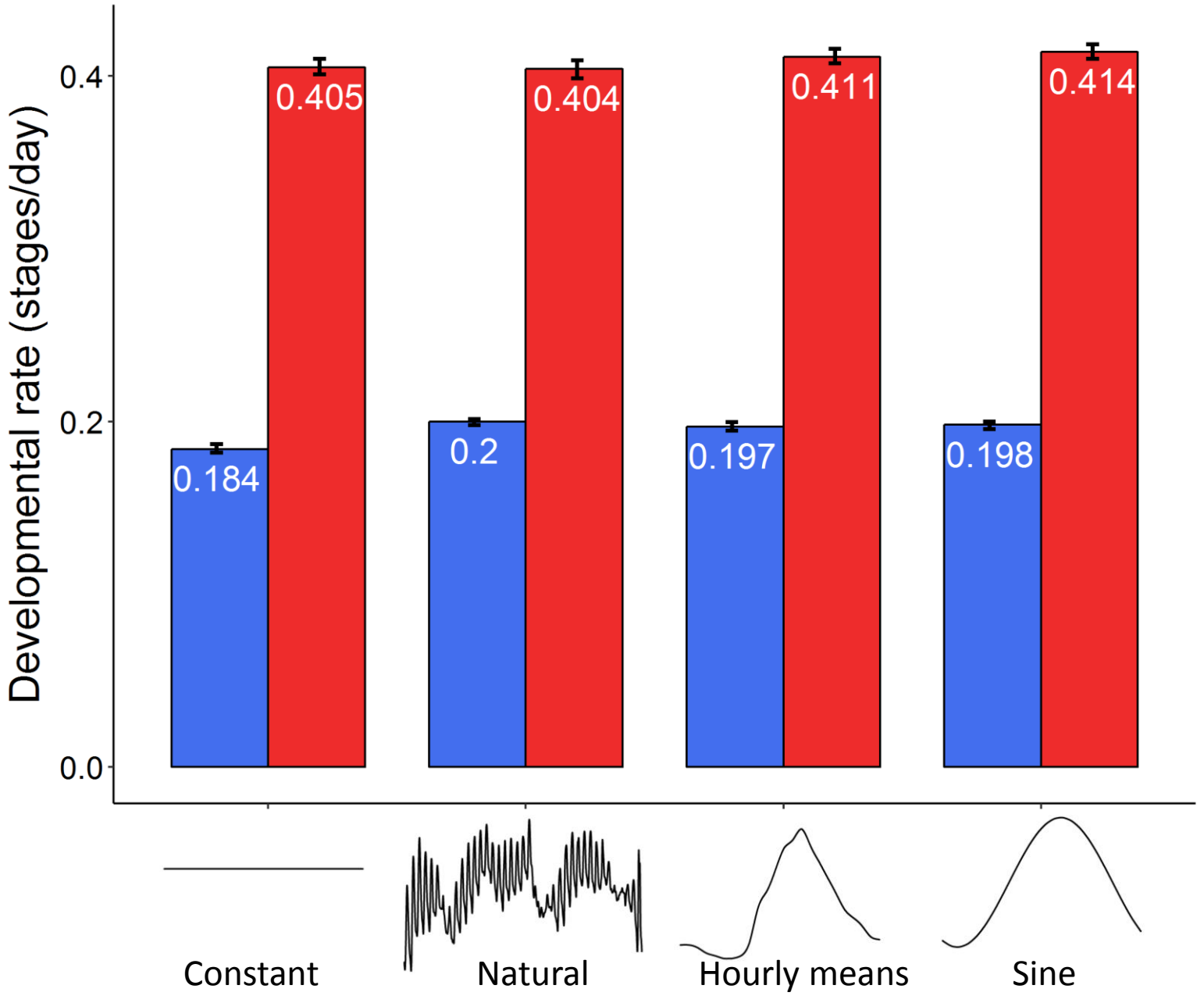
	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**



Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**

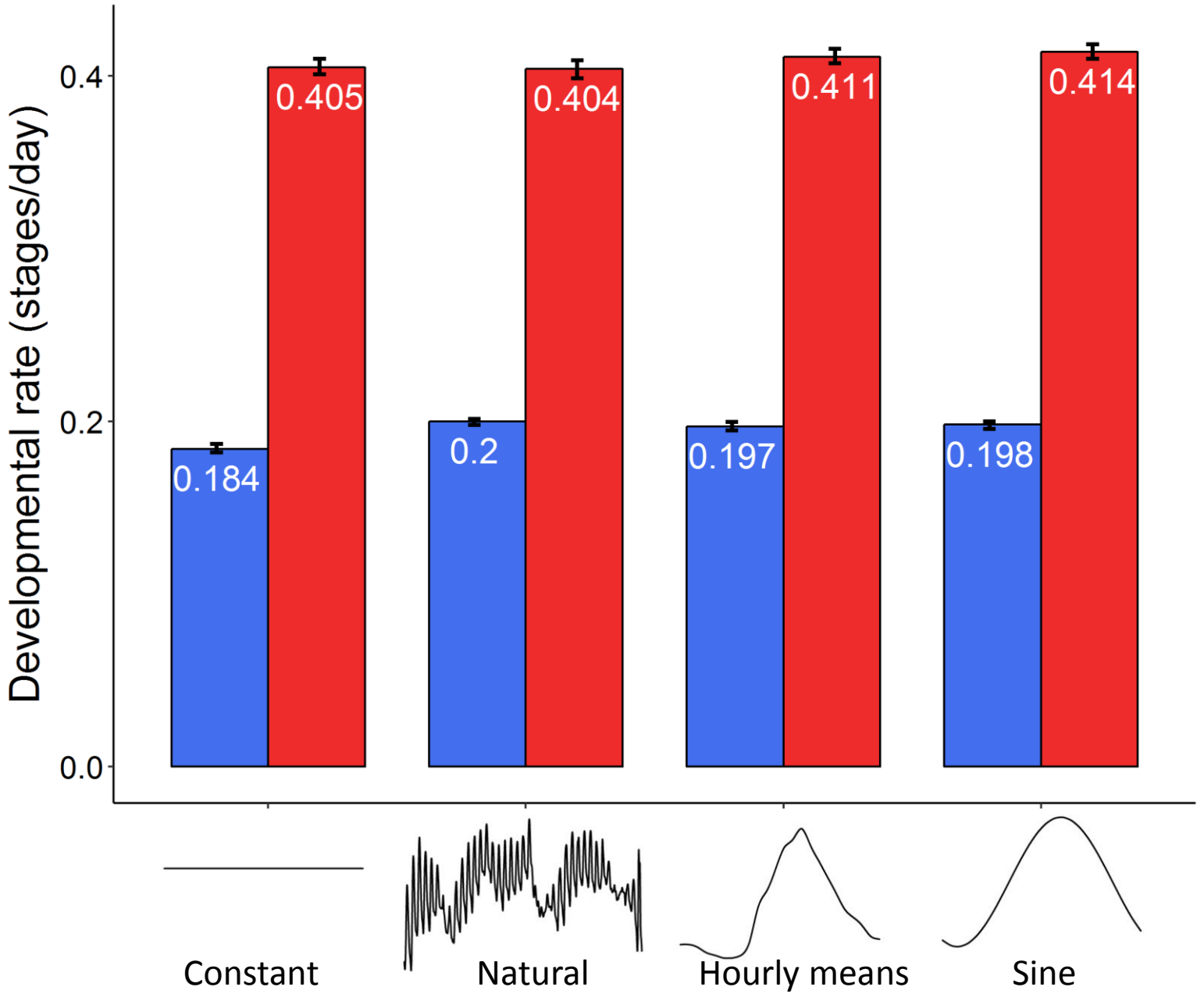
- Colder temperatures – natural treatment increases developmental rate



Developmental rate

	DF	F	p
Incubation Treatment	**3,293**	**47.73**	**<0.0001**
Season X Treatment	**3,293**	**21.93**	**<0.0001**

- Colder temperatures – natural treatment increases developmental rate
- Warmer temperatures – natural treatment slowed developmental rate



Embryo survival

	DF	χ^2	p
Incubation Treatment			
Season X Treatment			

Yolk conversion

	DF	F	p
Incubation Treatment			
Season X Treatment			

Water uptake

	DF	F	p
Incubation Treatment			
Season X Treatment			

Metabolic rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo heart rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo survival

	DF	χ^2	p
Incubation Treatment	3	6.11	0.11
Season X Treatment	3	2.74	0.43

Yolk conversion

	DF	F	p
Incubation Treatment			
Season X Treatment			

Water uptake

	DF	F	p
Incubation Treatment			
Season X Treatment			

Metabolic rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo heart rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo survival

	DF	χ^2	p
Incubation Treatment	3	6.11	0.11
Season X Treatment	3	2.74	0.43

Yolk conversion

	DF	F	p
Incubation Treatment			
Season X Treatment			

Water uptake

	DF	F	p
Incubation Treatment	3,88	1.64	0.18
Season X Treatment	3,88	1.31	0.28

Metabolic rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo heart rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo survival

	DF	χ^2	p
Incubation Treatment	3	6.11	0.11
Season X Treatment	3	2.74	0.43

Yolk conversion

	DF	F	p
Incubation Treatment			
Season X Treatment			

Water uptake

	DF	F	p
Incubation Treatment	3,88	1.64	0.18
Season X Treatment	3,88	1.31	0.28

Metabolic rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo heart rate

	DF	F	p
Incubation Treatment	3,70	0.90	0.45
Season X Treatment	3,70	0.34	0.80

Embryo survival

	DF	χ^2	p
Incubation Treatment	3	6.11	0.11
Season X Treatment	3	2.74	0.43

Yolk conversion

	DF	F	p
Incubation Treatment	3,69	1.07	0.37
Season X Treatment	3,69	0.20	0.90

Water uptake

	DF	F	p
Incubation Treatment	3,88	1.64	0.18
Season X Treatment	3,88	1.31	0.28

Metabolic rate

	DF	F	p
Incubation Treatment			
Season X Treatment			

Embryo heart rate

	DF	F	p
Incubation Treatment	3,70	0.90	0.45
Season X Treatment	3,70	0.34	0.80

Embryo survival

	DF	χ^2	p
Incubation Treatment	3	6.11	0.11
Season X Treatment	3	2.74	0.43

Yolk conversion

	DF	F	p
Incubation Treatment	3,69	1.07	0.37
Season X Treatment	3,69	0.20	0.90

Water uptake

	DF	F	p
Incubation Treatment	3,88	1.64	0.18
Season X Treatment	3,88	1.31	0.28

Metabolic rate

	DF	F	p
Incubation Treatment	1,87	0.48	0.70
Season X Treatment	1,87	0.58	0.63

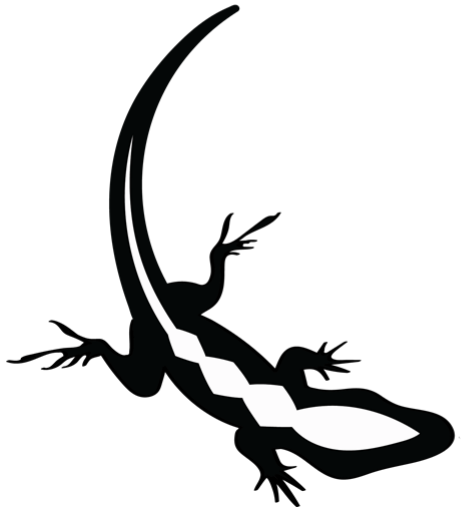
Embryo heart rate

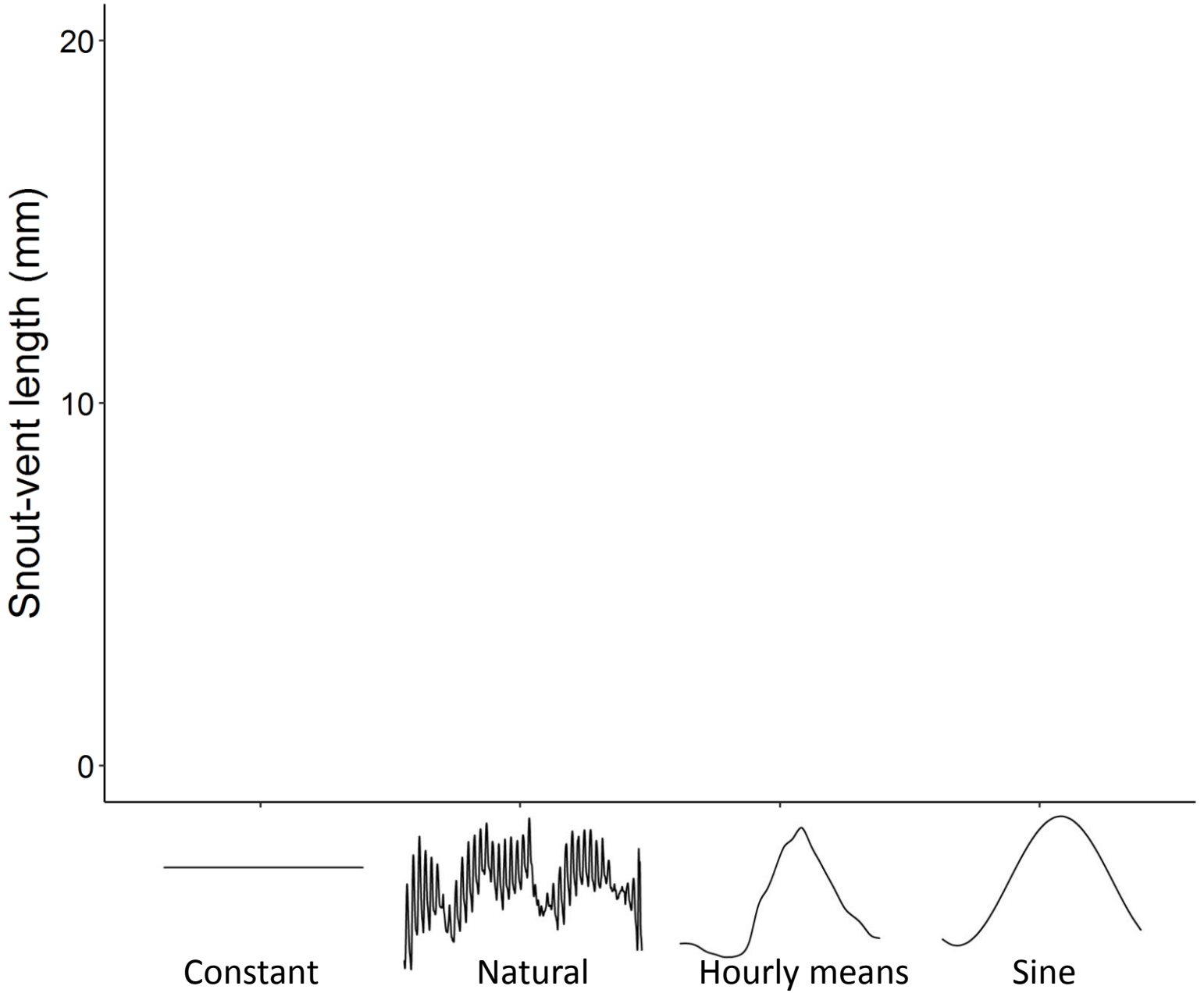
	DF	F	p
Incubation Treatment	3,70	0.90	0.45
Season X Treatment	3,70	0.34	0.80

Effects on hatchlings

Hatchling SVL

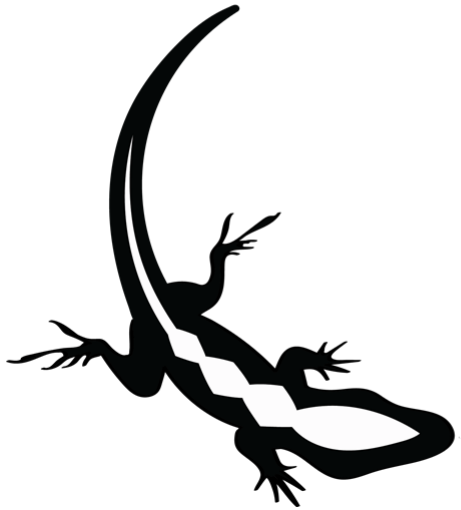
	DF	F	p
Incubation Treatment			
Season X Treatment			

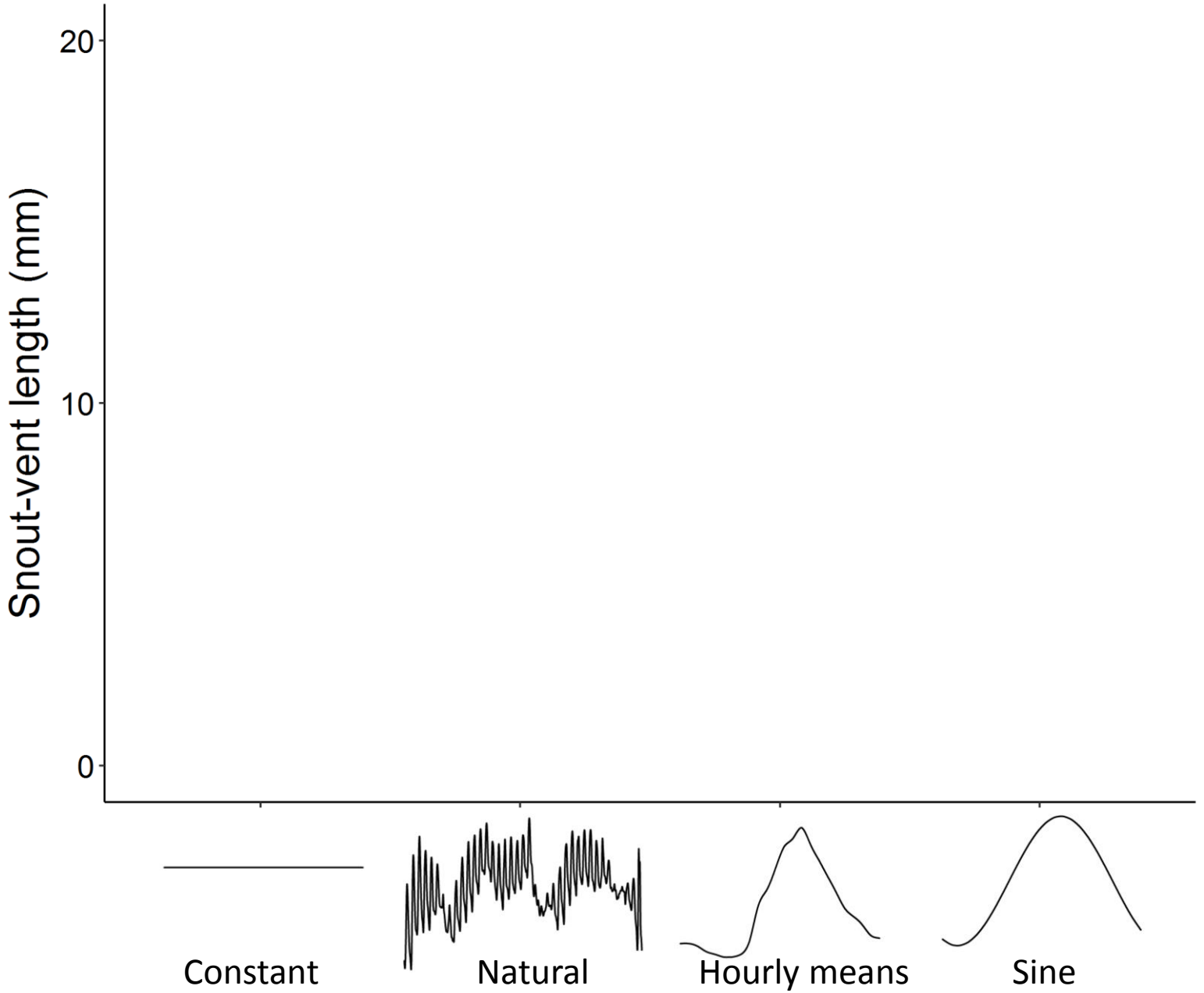




Hatchling SVL

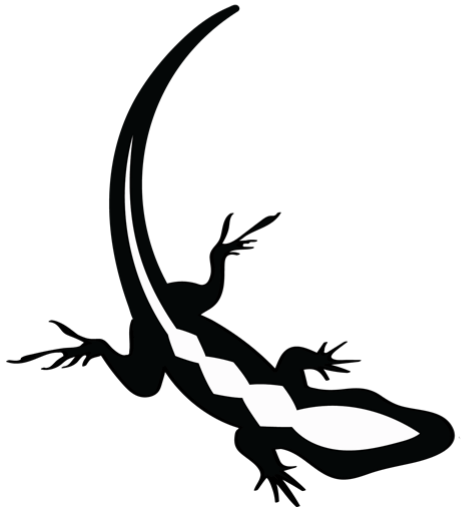
	DF	F	p
Incubation Treatment	**3,293**	**3.04**	**0.029**
Season X Treatment	3,293	1.2	0.31

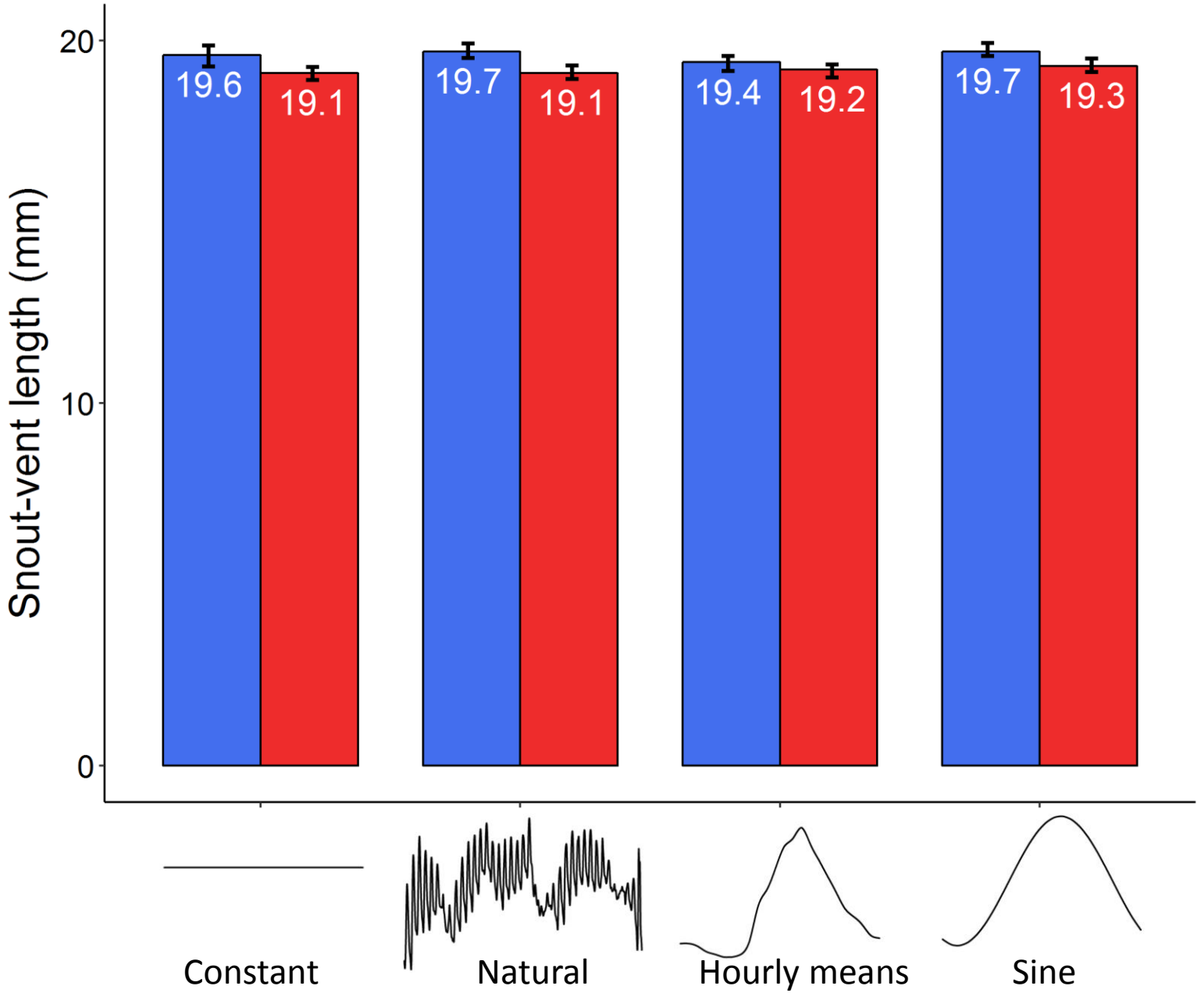




Hatchling SVL

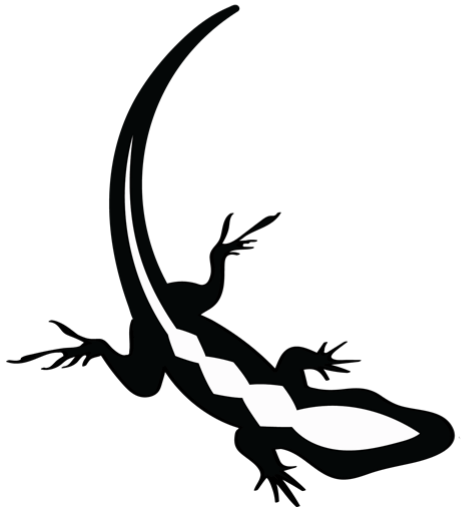
	DF	F	p
Incubation Treatment	**3,293**	**3.04**	**0.029**
Season X Treatment	3,293	1.2	0.31

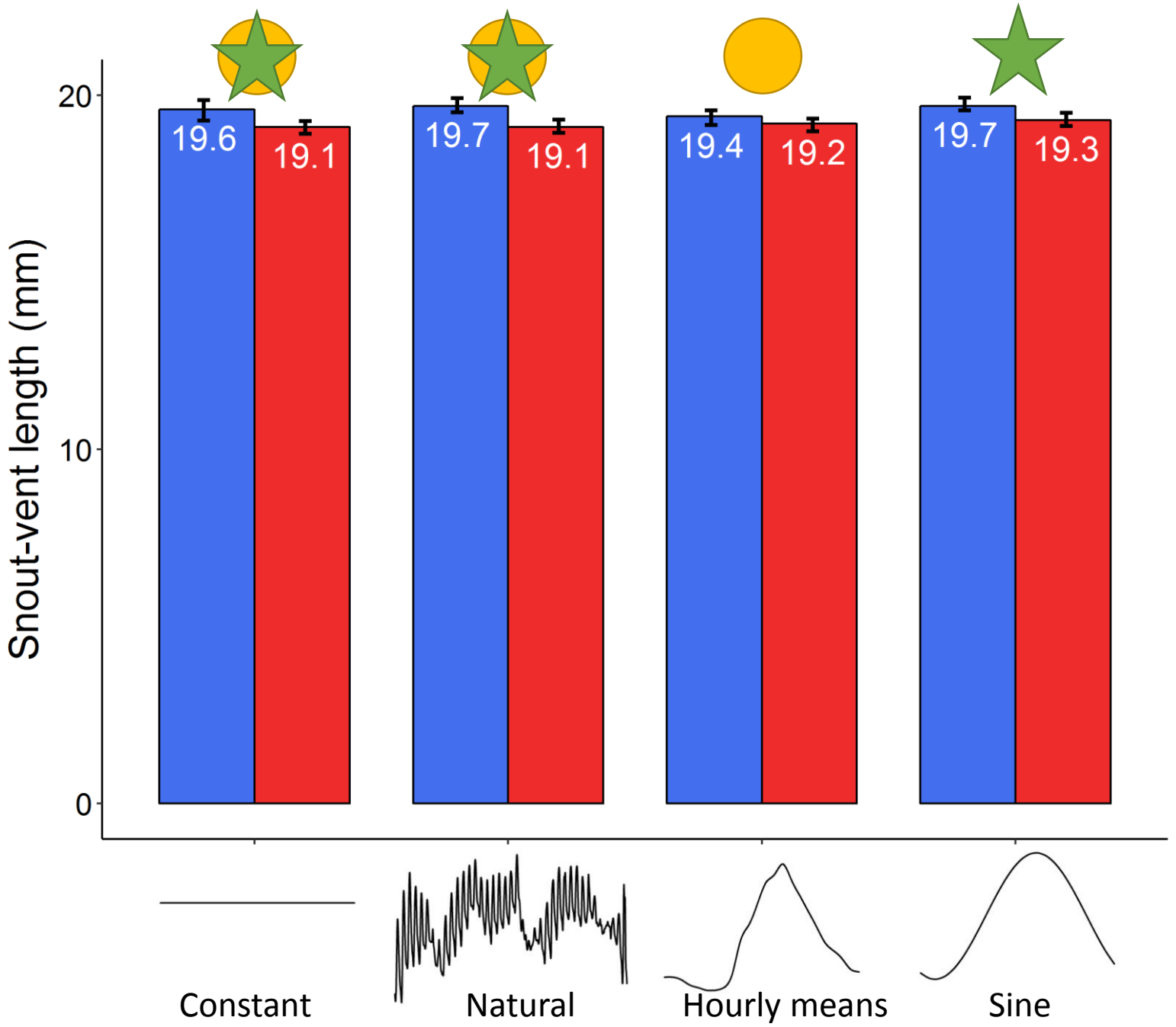




Hatchling SVL

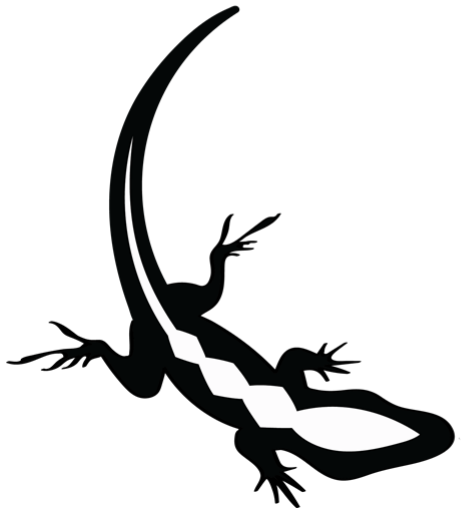
	DF	F	p
Incubation Treatment	**3,293**	**3.04**	**0.029**
Season X Treatment	3,293	1.2	0.31

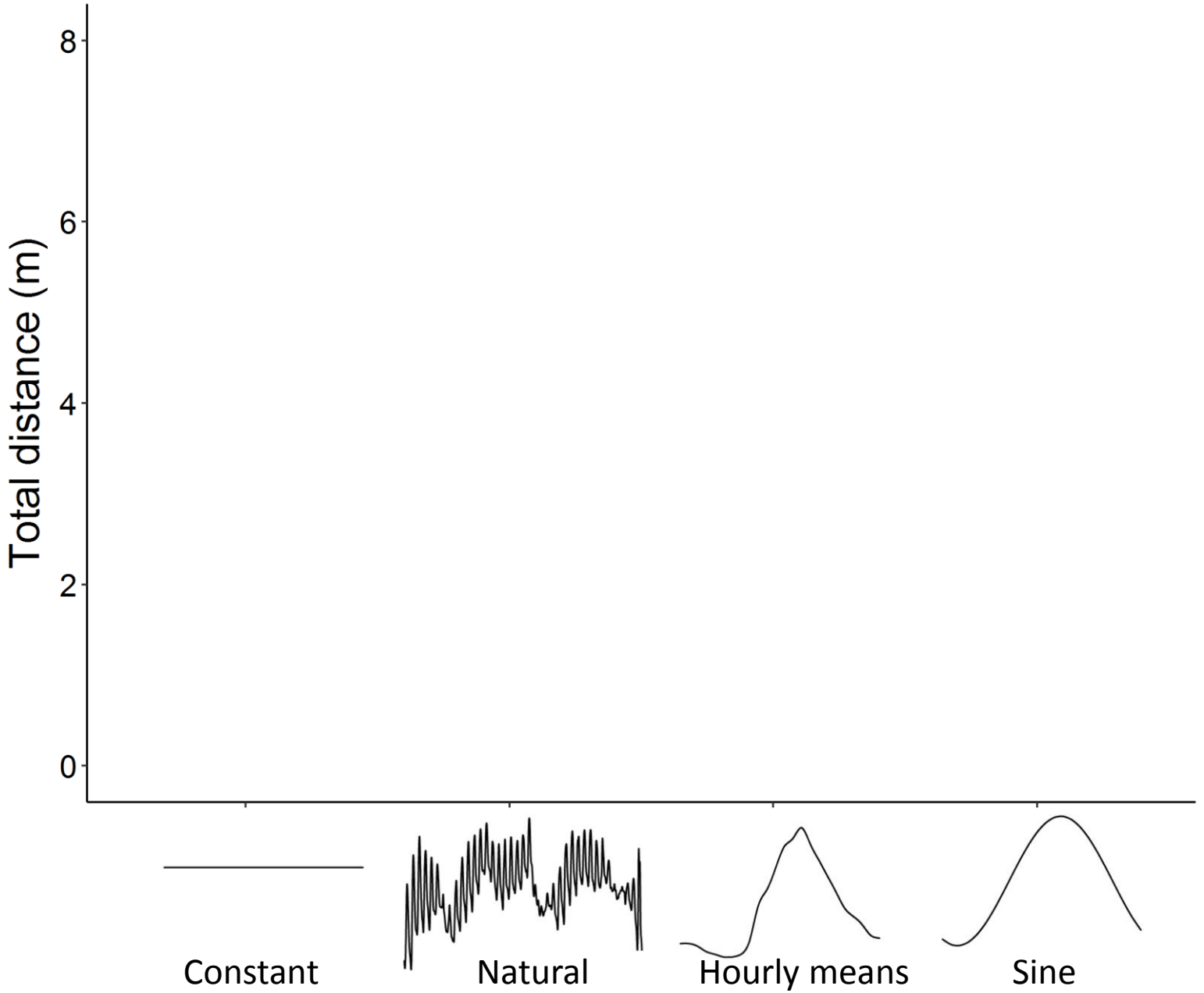




Endurance (distance)

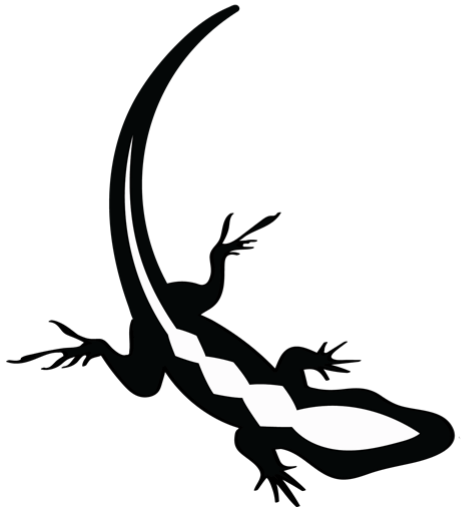
	DF	F	p
Incubation Treatment			
Season X Treatment			

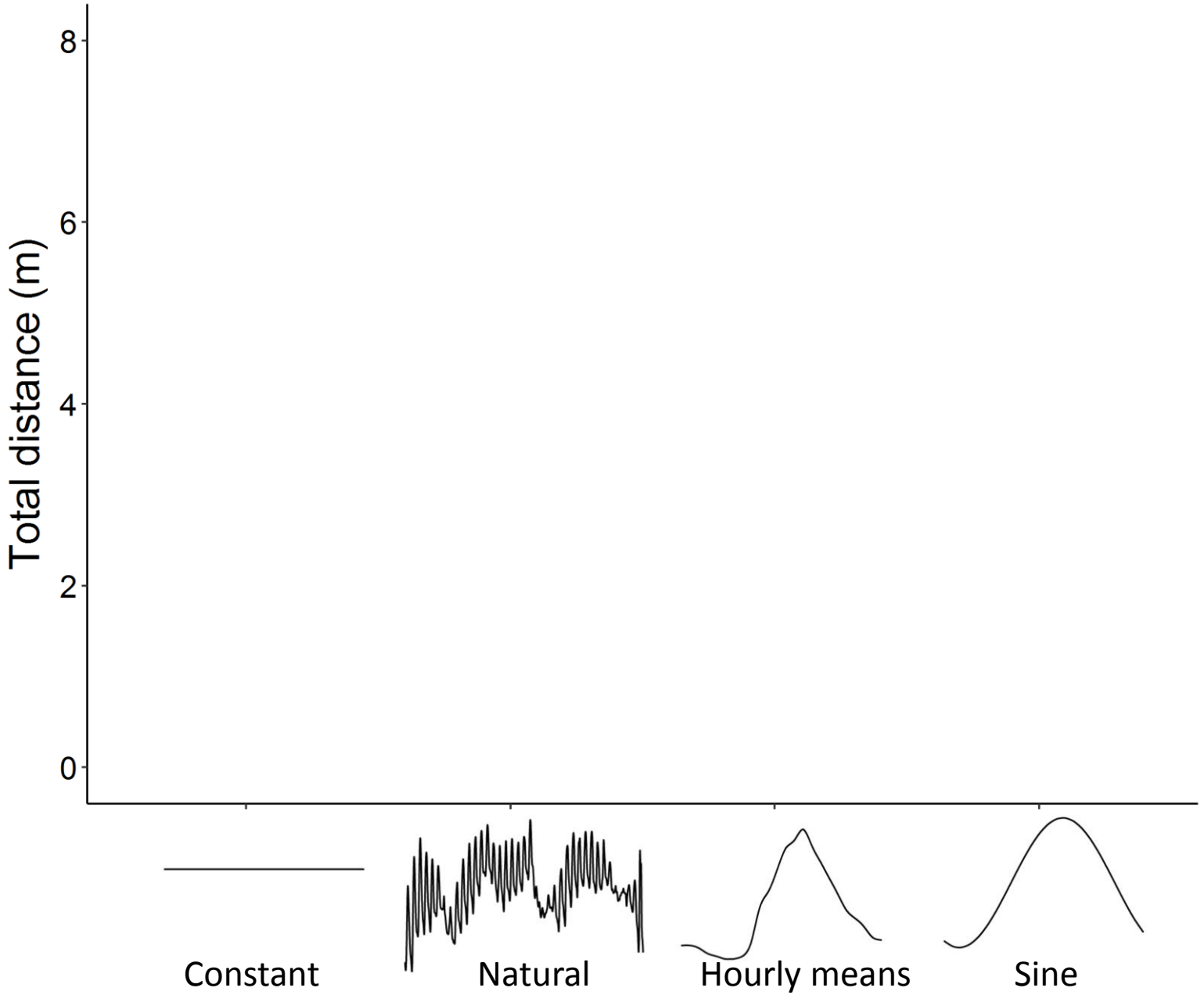




Endurance (distance)

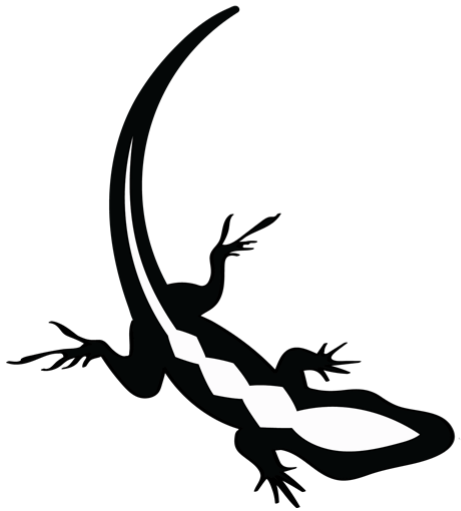
	DF	F	p
Incubation Treatment	**3,196**	**4.64**	**0.004**
Season X Treatment	**3,196**	**3.37**	**0.02**

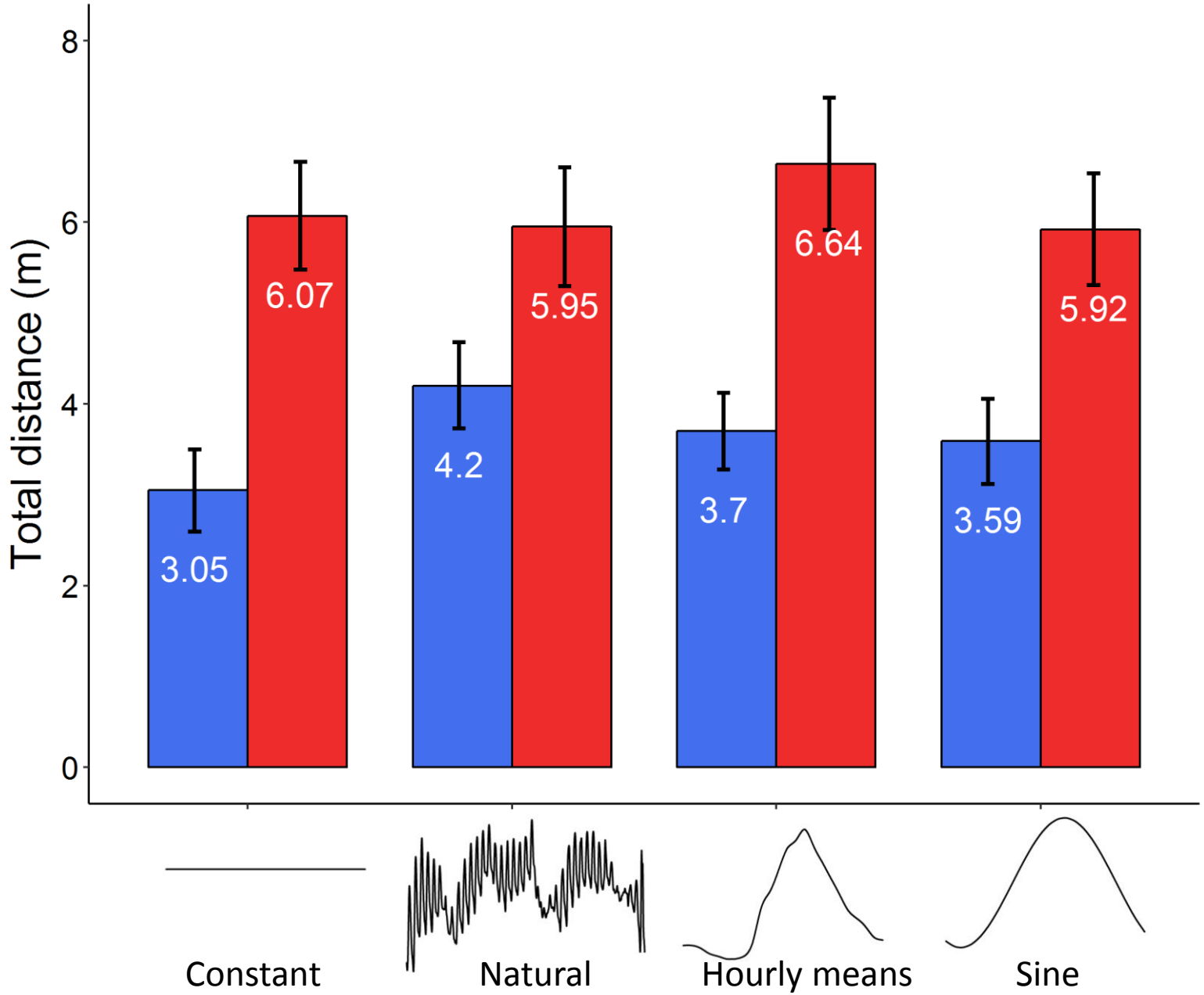




Endurance (distance)

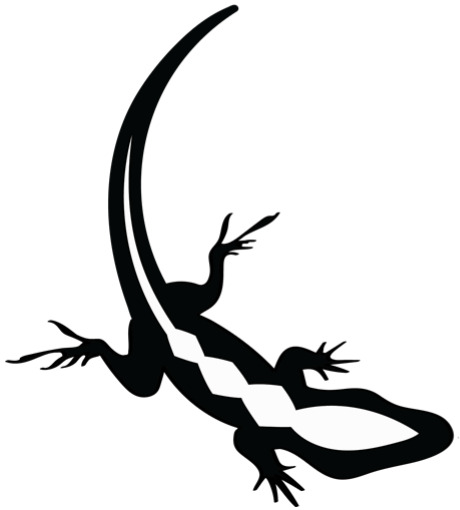
	DF	F	p
Incubation Treatment	**3,196**	**4.64**	**0.004**
Season X Treatment	**3,196**	**3.37**	**0.02**

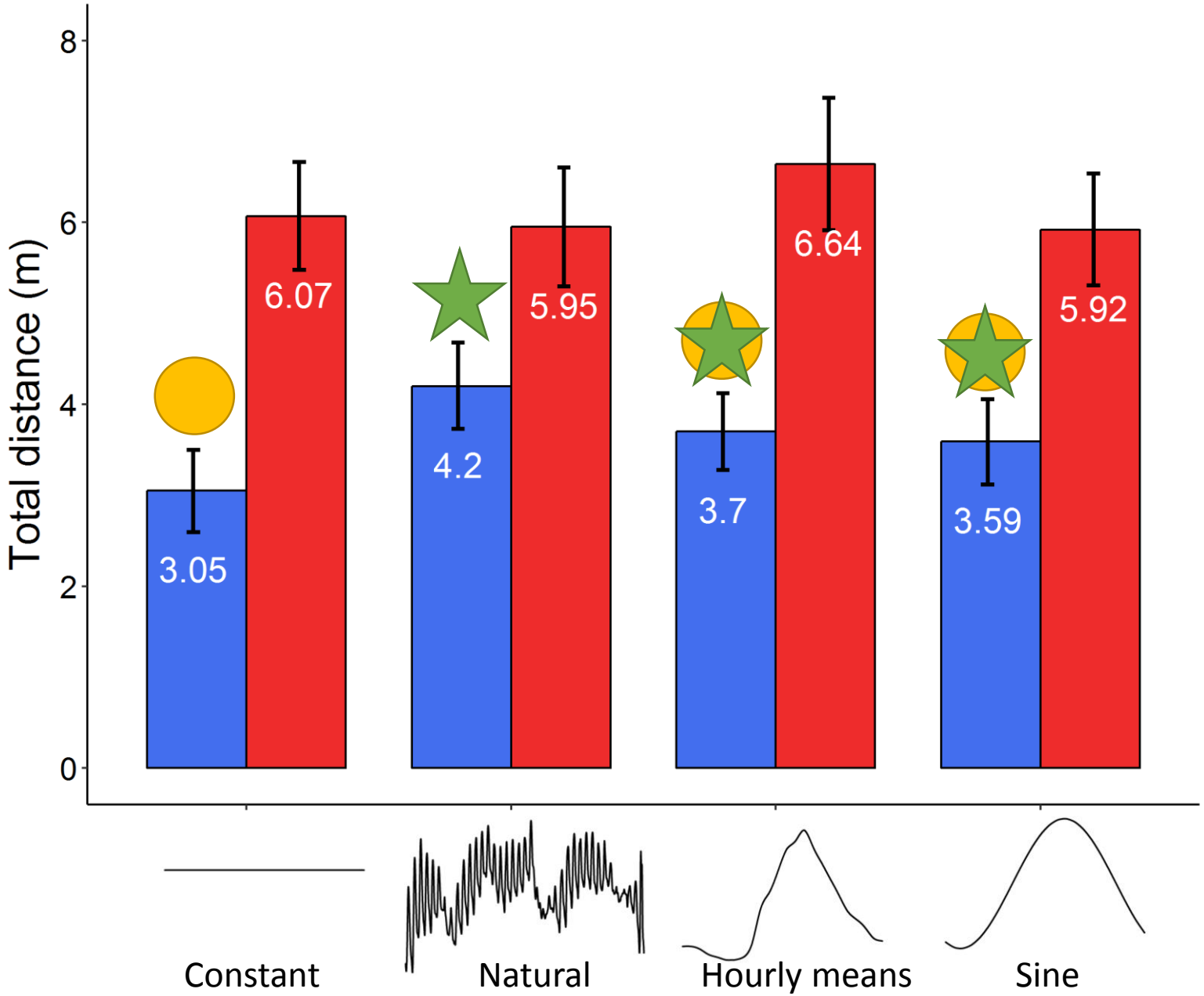




Endurance (distance)

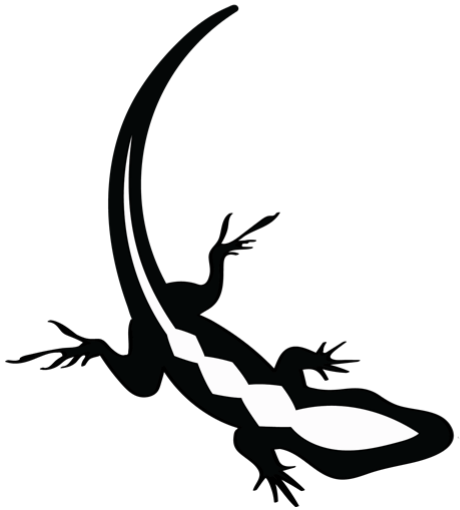
	DF	F	p
Incubation Treatment	**3,196**	**4.64**	**0.004**
Season X Treatment	**3,196**	**3.37**	**0.02**

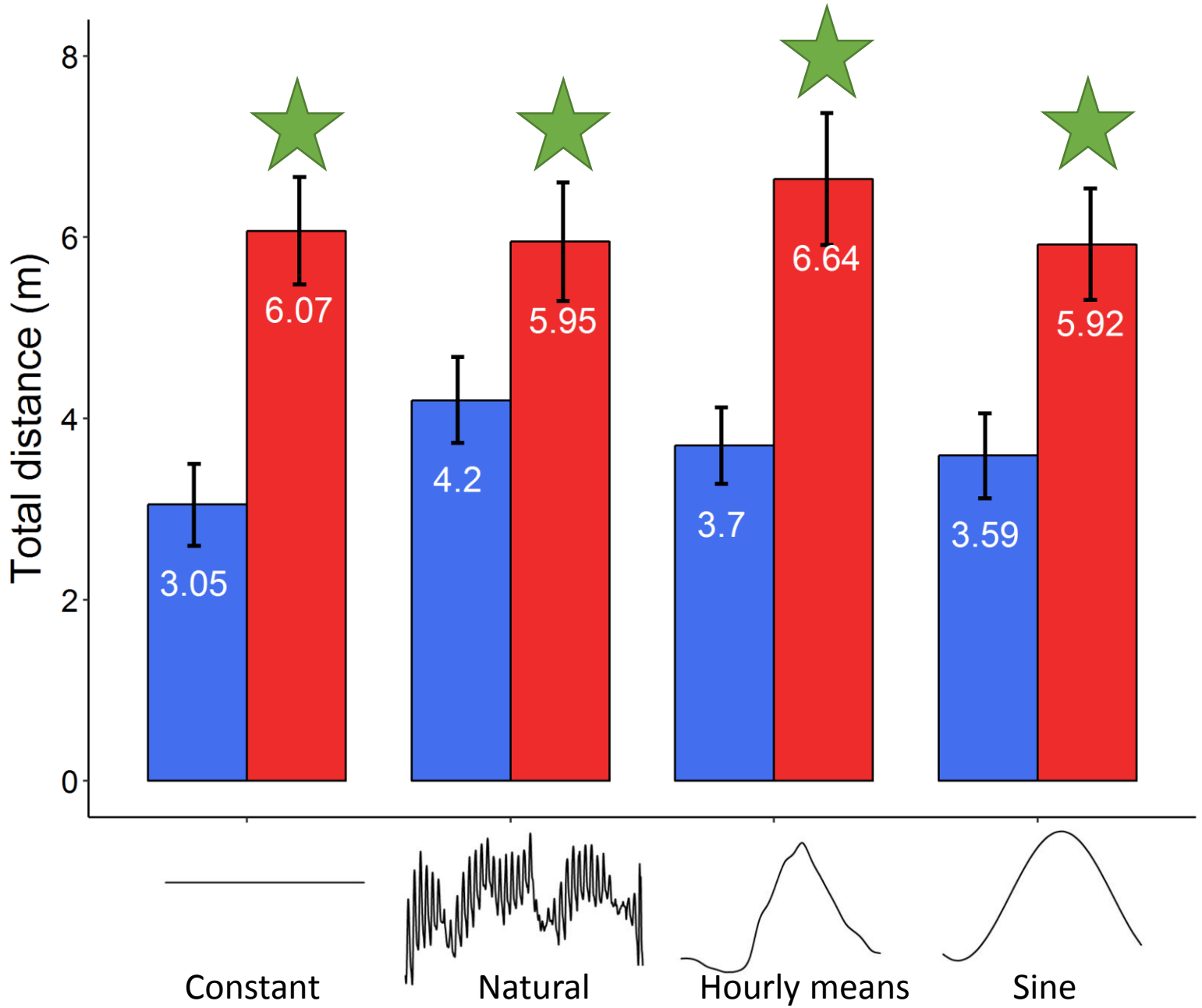




Endurance (distance)

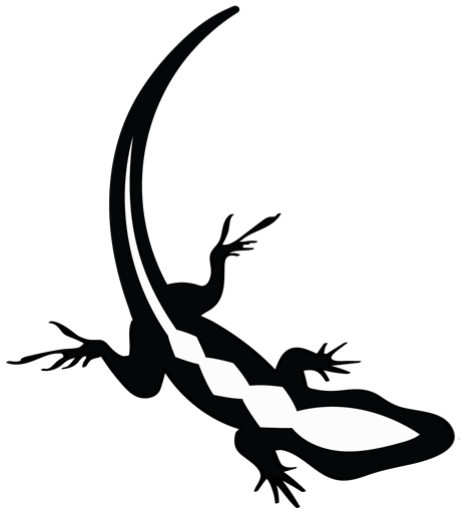
	DF	F	p
Incubation Treatment	**3,196**	**4.64**	**0.004**
Season X Treatment	**3,196**	**3.37**	**0.02**

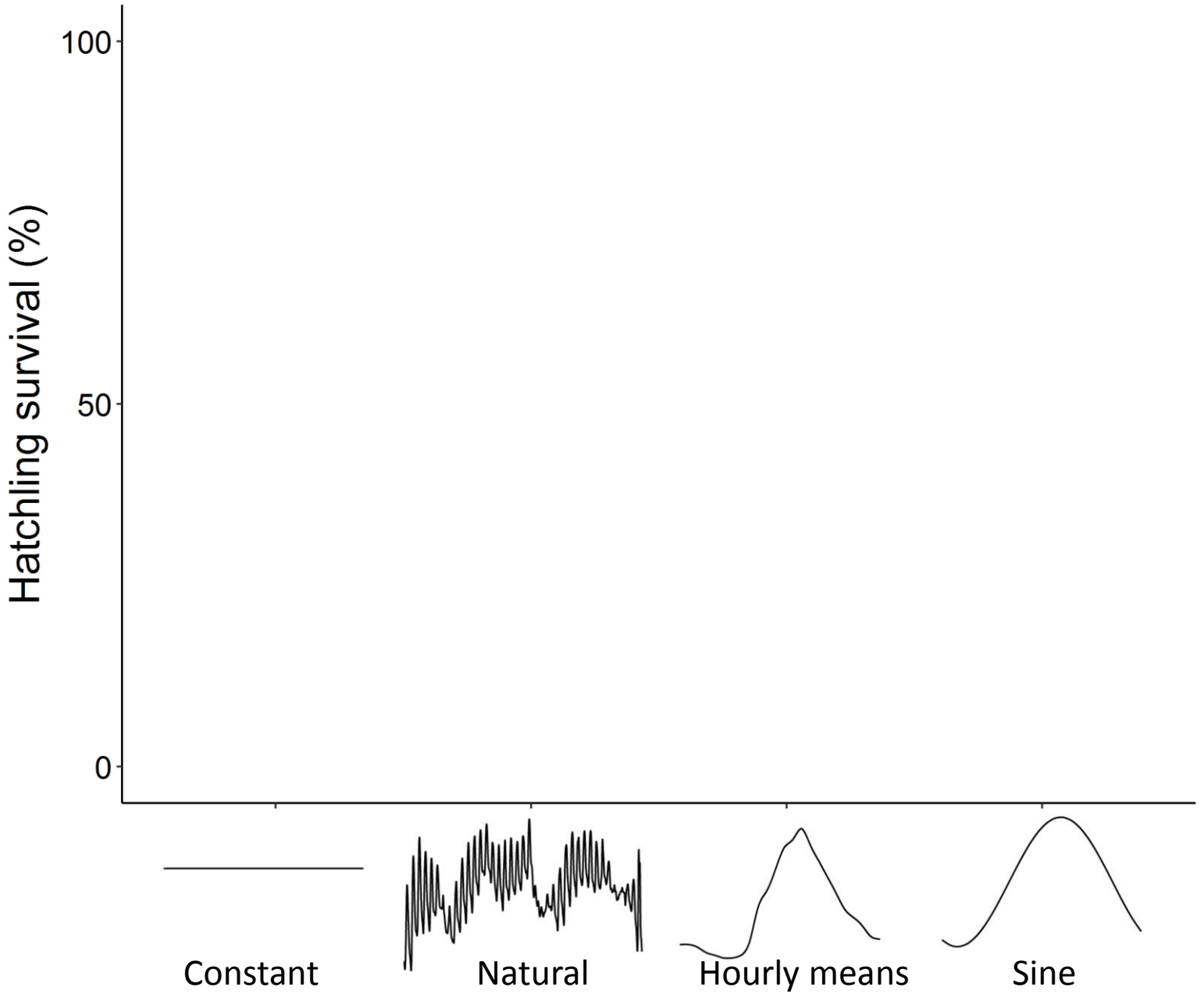




Hatchling survival

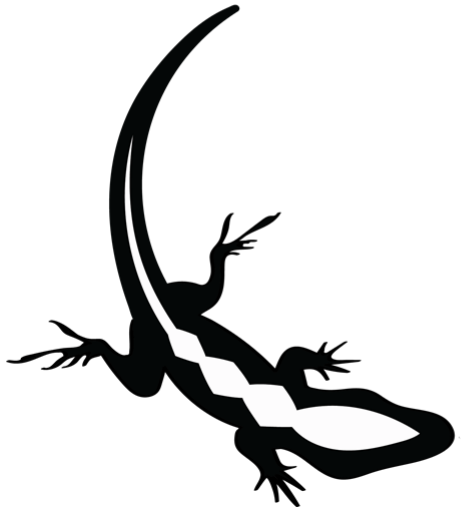
	DF	χ^2	p
Incubation Treatment			
Season X Treatment			

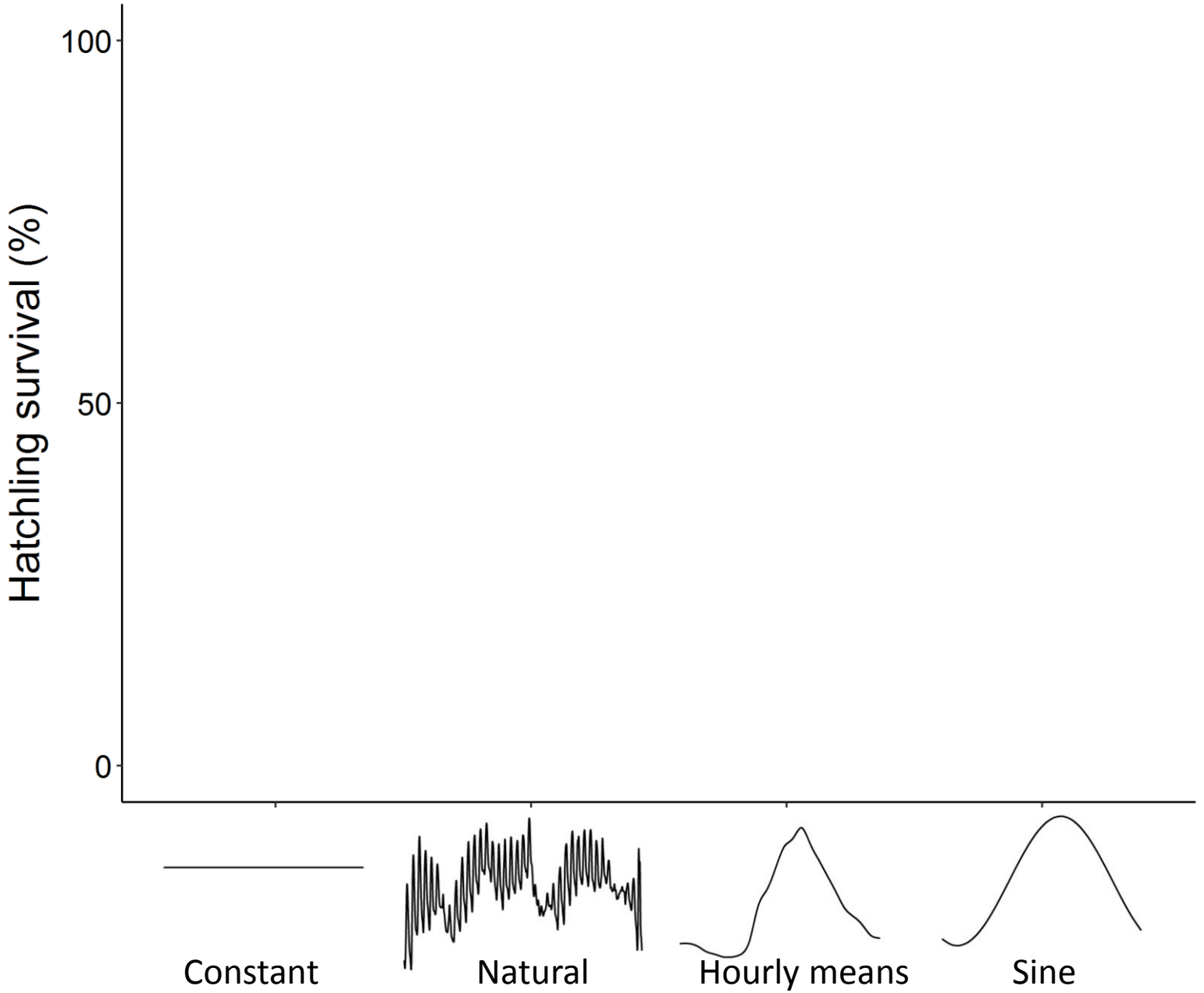




Hatchling survival

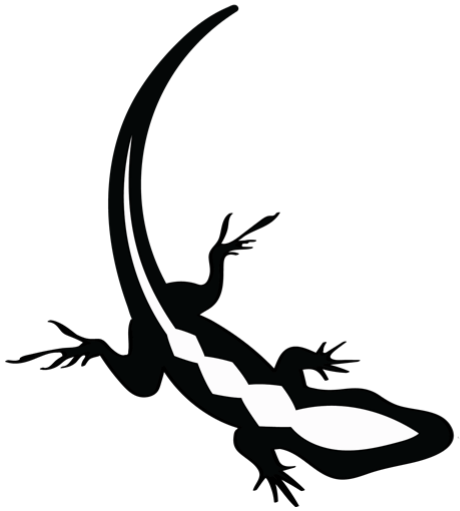
	DF	χ^2	p
Incubation Treatment	**3**	**14.62**	**0.002**
Season X Treatment	3	3.09	0.38

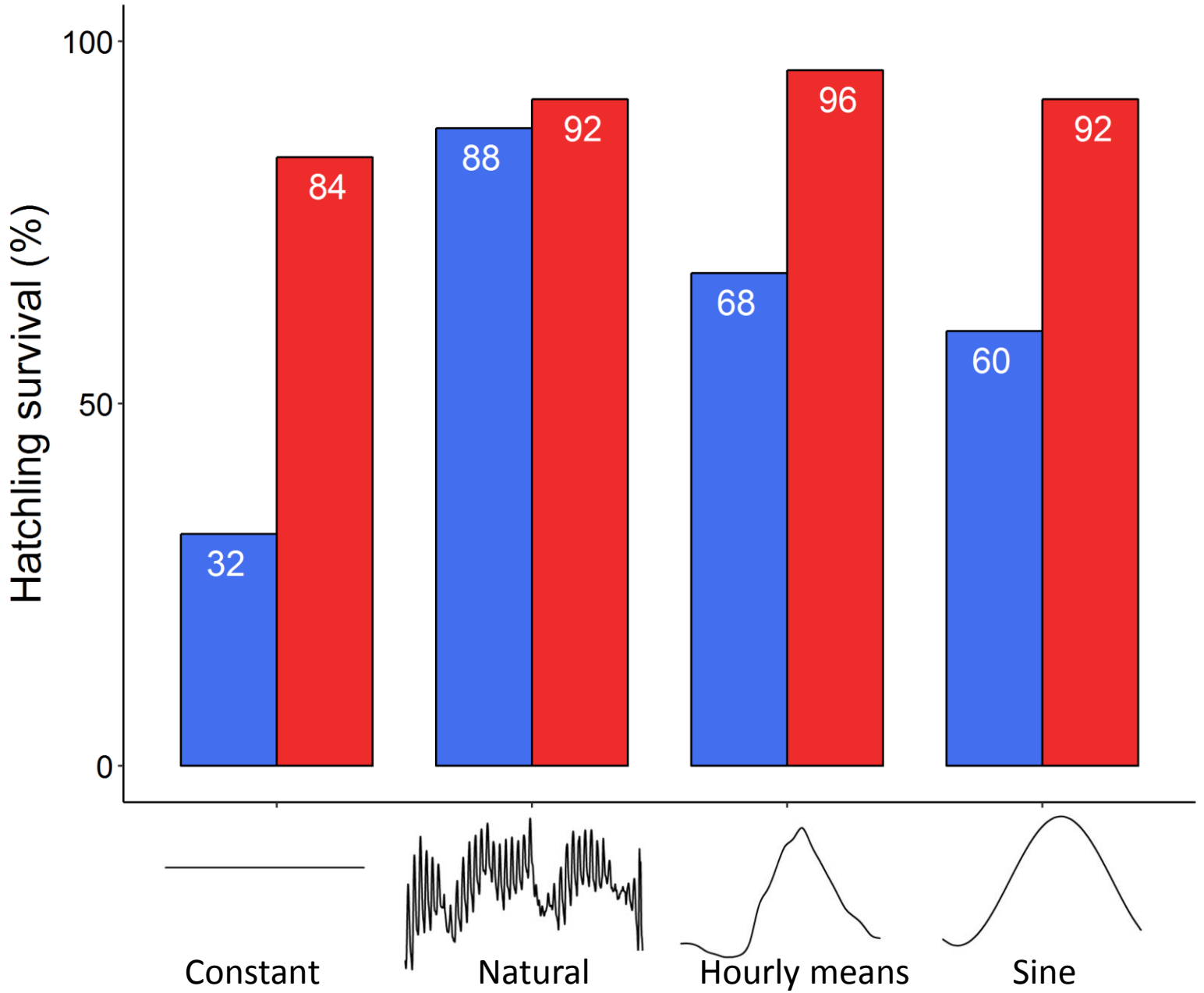




Hatchling survival

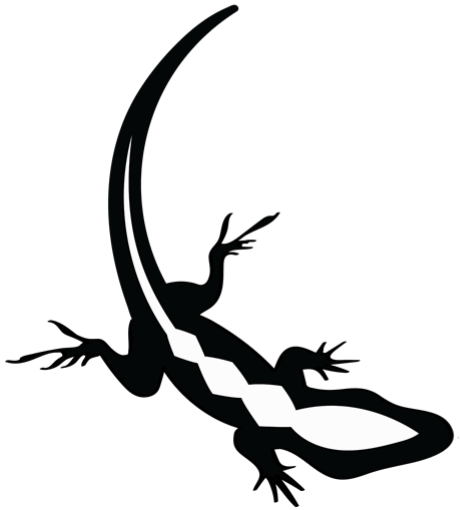
	DF	χ^2	p
Incubation Treatment	**3**	**14.62**	**0.002**
Season X Treatment	3	3.09	0.38

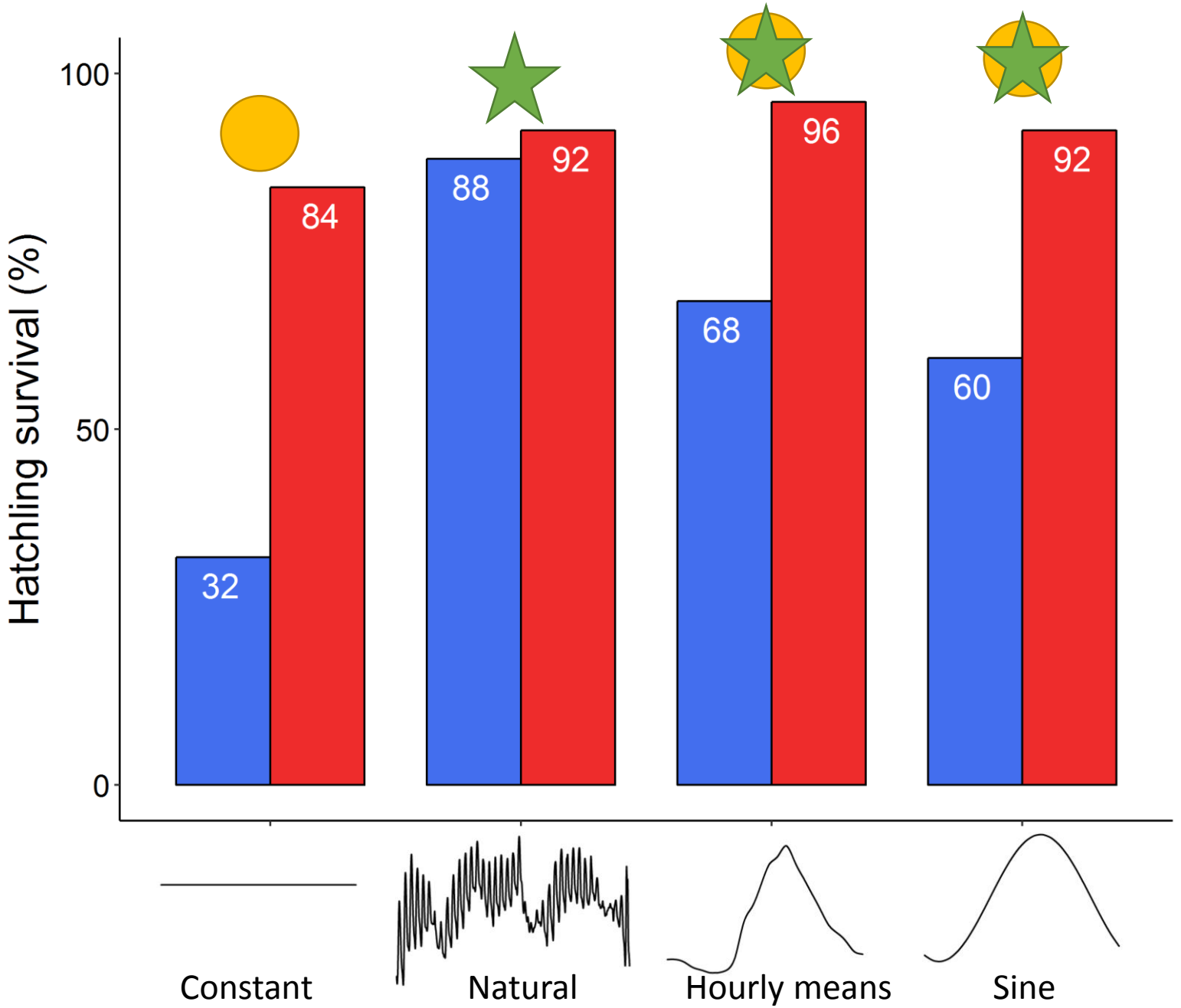




Hatchling survival

	DF	χ^2	p
Incubation Treatment	**3**	**14.62**	**0.002**
Season X Treatment	3	3.09	0.38

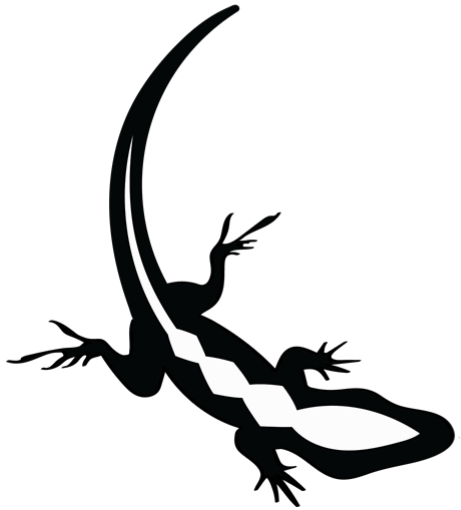


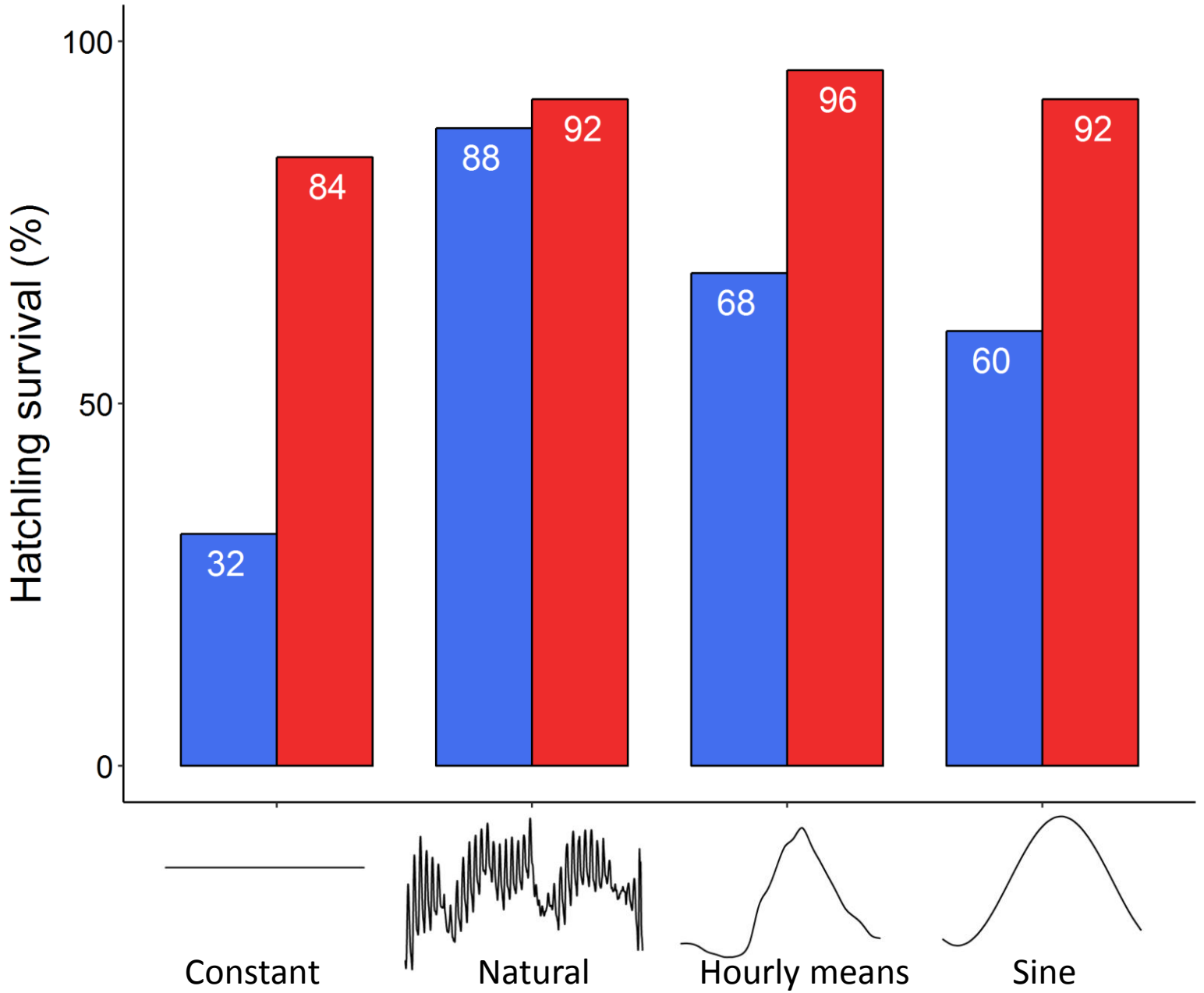


Hatchling survival

	DF	χ^2	p
Incubation Treatment	**3**	**14.62**	**0.002**
Season X Treatment	3	3.09	0.38

- Natural treatment improves hatchling survival at colder temperatures





Hatchling body mass

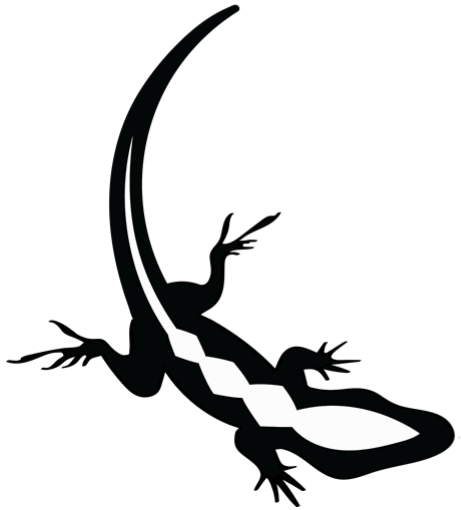
	DF	F	p
Incubation Treatment			
Season X Treatment			

Burst speed

	DF	F	p
Incubation Treatment			
Season X Treatment			

Hatchling growth

	DF	F	p
Incubation Treatment			
Season X Treatment			



Hatchling body mass

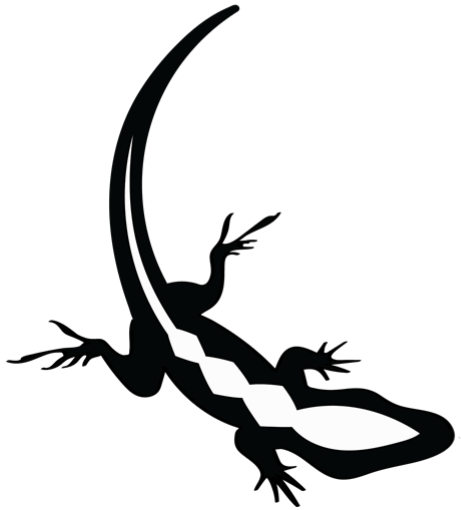
	DF	F	p
Incubation Treatment	3,293	0.96	0.41
Season X Treatment	3,293	0.89	0.45

Burst speed

	DF	F	p
Incubation Treatment			
Season X Treatment			

Hatchling growth

	DF	F	p
Incubation Treatment			
Season X Treatment			



Hatchling body mass

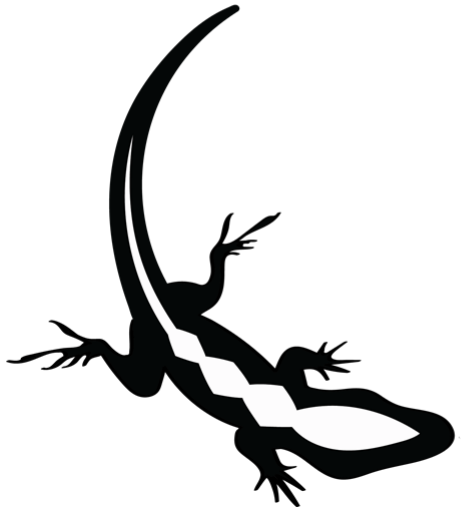
	DF	F	p
Incubation Treatment	3,293	0.96	0.41
Season X Treatment	3,293	0.89	0.45

Burst speed

	DF	F	p
Incubation Treatment	3,185	0.77	0.51
Season X Treatment	3,185	0.62	0.60

Hatchling growth

	DF	F	p
Incubation Treatment			
Season X Treatment			



Hatchling body mass

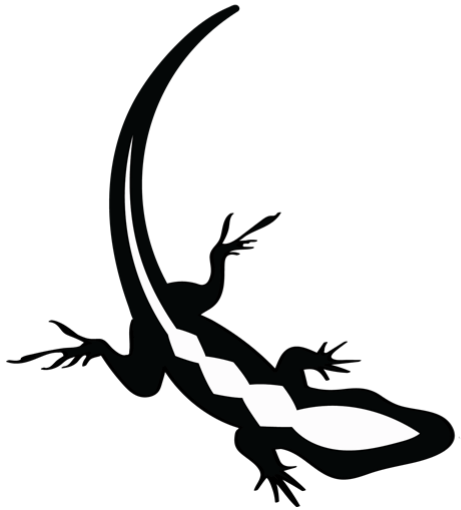
	DF	F	p
Incubation Treatment	3,293	0.96	0.41
Season X Treatment	3,293	0.89	0.45

Burst speed

	DF	F	p
Incubation Treatment	3,185	0.77	0.51
Season X Treatment	3,185	0.62	0.60

Hatchling growth

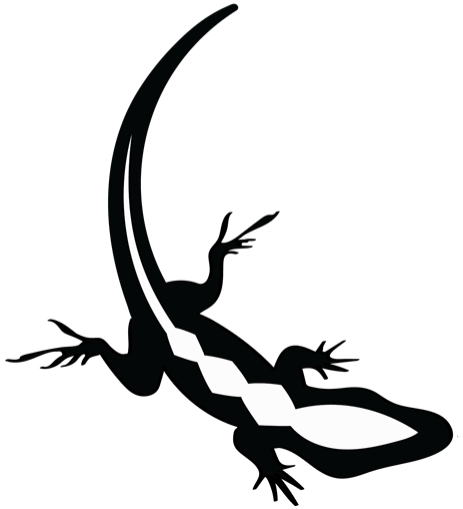
	DF	F	p
Incubation Treatment	3,62	0.30	0.83
Season X Treatment	3,62	0.12	0.95



Phenotypes

Embryos

- Survival
- Physiology
  - Developmental rate
  - Water uptake
  - Yolk conversion
  - Heart rate
  - Metabolism



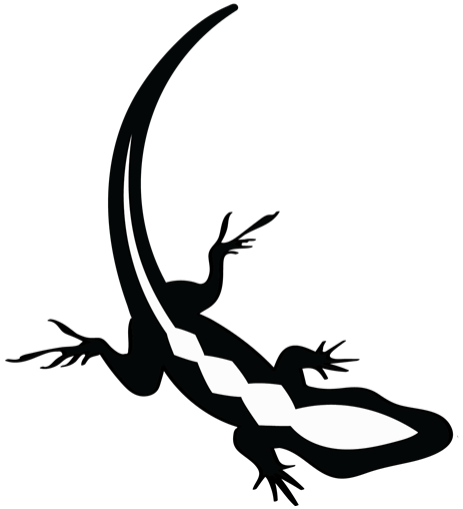
Hatchlings

- Survival (in the lab)
- Morphology
  - SVL and body mass
- Performance
  - Burst speed
  - Endurance
- Growth

Phenotypes

Embryos

- Survival
- **Physiology**
  - **Developmental rate**
  - Water uptake
  - Yolk conversion
  - Heart rate
  - Metabolism



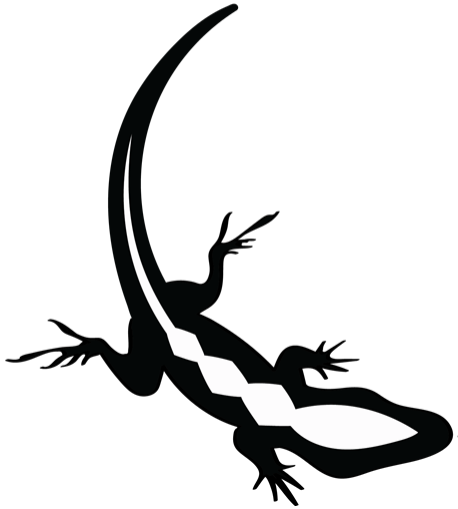
Hatchlings

- Survival (in the lab)
- Morphology
  - SVL and body mass
- Performance
  - Burst speed
  - Endurance
- Growth

Phenotypes

Embryos

- Survival
- **Physiology**
  - **Developmental rate**
  - Water uptake
  - Yolk conversion
  - Heart rate
  - Metabolism



Hatchlings

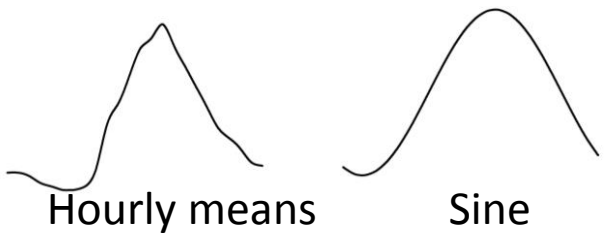
- **Survival (in the lab)**
- **Morphology**
  - **SVL** and body mass
- **Performance**
  - Burst speed
  - **Endurance**
- Growth

Conclusions

Suggestion

Conclusions

- Sine = Hourly means



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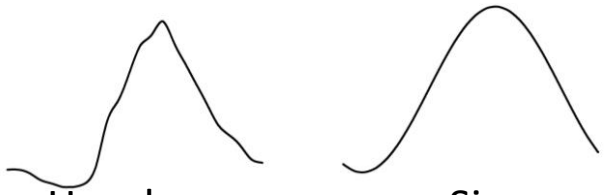

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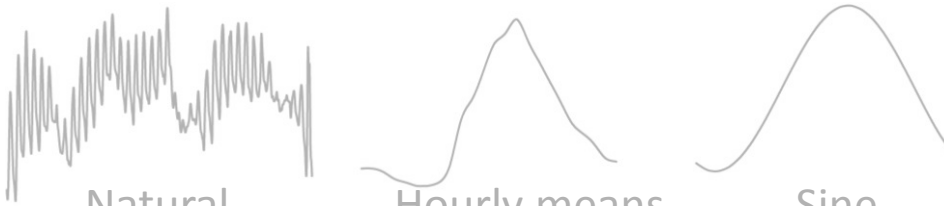

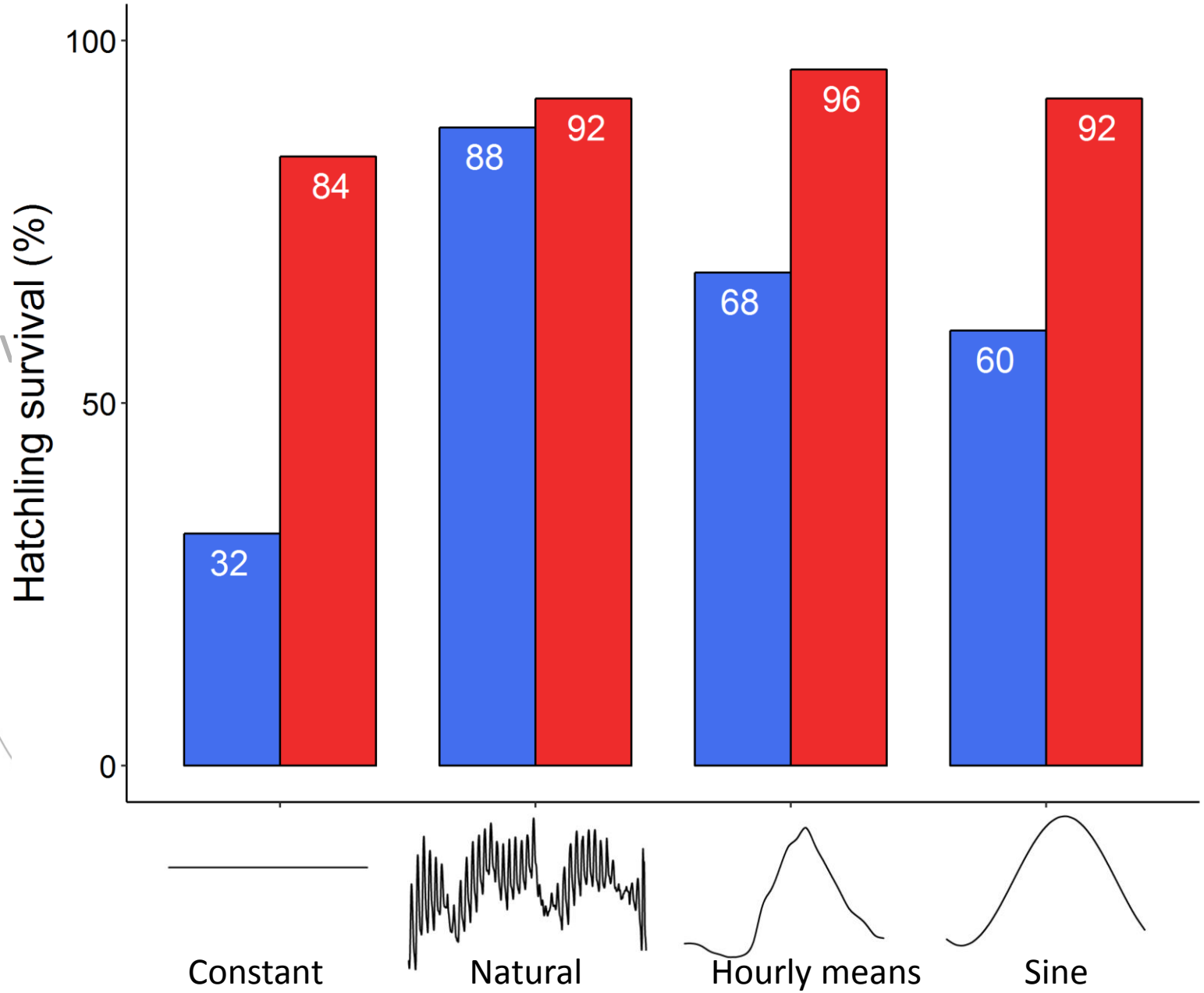

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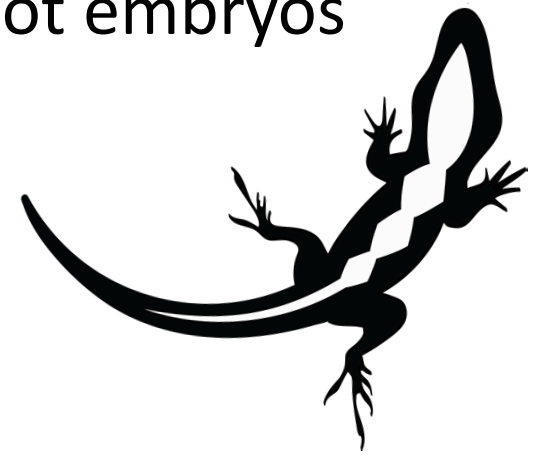

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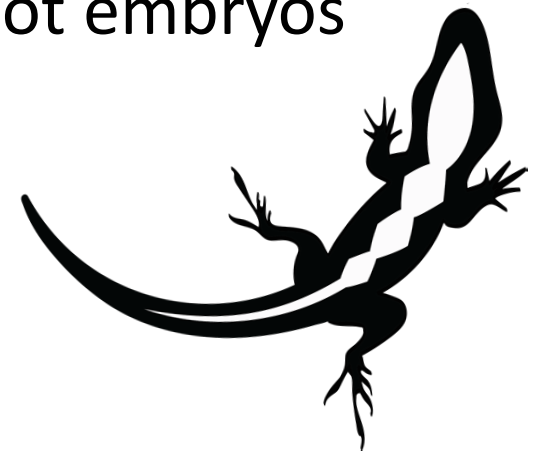

Suggestion

- Sine waves likely best, reproducible
- More context-dependent studies are required
- Researchers should replicate our methods in their own system
- Always measure hatchling performance, survival

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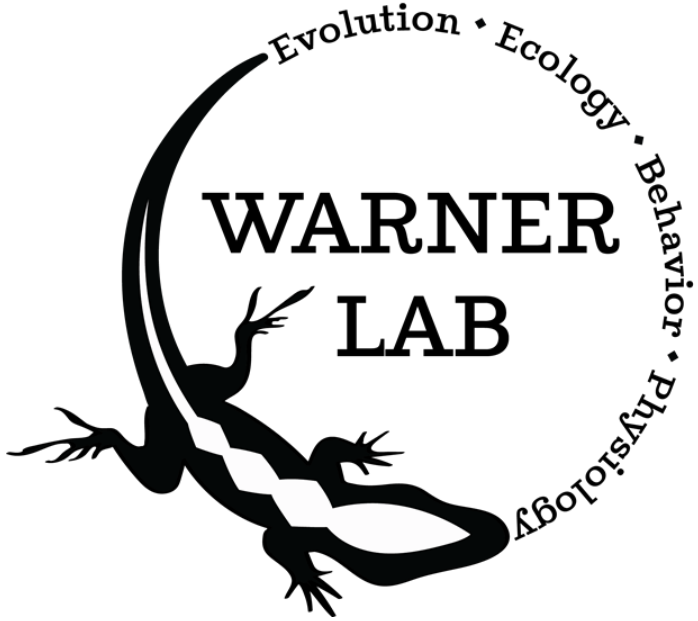

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