

# Thermo- vs. Photo-periodicity

D Buonaiuto, M Donahue, EM Wolkovich

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## 1. Introduction

- (a) Phenology is important
- (b) Experiments show phenology cued by temperature and photoperiod.
- (c) Interactions are important and need to be pursued.
- (d) Testing interactions is complex; we will consider photoperiod and forcing
- (e) Must be orthogonal

## 2. Experimental Designs for interactions

- (a) Each cue has possible 2 axes, intensity and period.
  - i. Photoperiod: usually concerned with period only
  - ii. Forcing, both period and intensity (temperature)
- (b) Three major experiment designs could be utilized.
  - i. Temp intensity and photo period
    - A. Pro: Simple, easy to make truly orthogonal
    - B. Con: Some literature says this is bad for temperature. (find it). As such is rarely done.
  - ii. Covary thermo- and photo-period (most common).
    - A. Pro: more accurate representation of forcing. Simulates nature so good for comparison to field.
    - B. Con: Non-orthogonal for GDH. Can determine between photo-period and thermo-period effects.
  - iii. Decouple thermo- and photo-period (never really done).
    - A. Pro: Orthogonal for GDH.
    - B. Con: New Non-orthogonal issue. Dawn T. (Find citations)
- (c) Design II will probably continue to be most popular. And we can and should use math to more accurately interpret our results.

## 3. Mathematical Predictions

- (a) Explain Data

- (b) Assumptions
    - i. Forcing is most important
    - ii. Others
  - (c) Show the math
  - (d) make predictions
4. Experimental Validation
- (a) Explain experiment two.
  - (b) Present comparison results

## Introduction

Phenology, the timing of life cycle transitions in organisms, is an important component of ecosystem structure and function (Chuine & Beaubien, 2001; Piao *et al.*, 2007; Cleland *et al.*, 2007). While phenology has been of interest to biologists for over a century, it has begun to receive increased attention in the recent decades as observed phenological shifts across a broad taxonomic spectrum have emerged as one of the most widely observed effects of anthropogenic climate change to date (Parmesan & Yohe, 2003; Root *et al.*, 2003; Menzel *et al.*, 2006; Wolkovich & Ettinger, 2014).

It has long been recognized that experimental manipulations in artificial environments are ideal for mechanistically characterizing phenological response to environmental fluctuations (Ettinger *et al.*; Primack *et al.*, 2015). This body of experimental work has demonstrated that temperature (winter chilling and spring forcing) and photoperiod are the primary cues of phenology for plants in the temperate/boreal zones (Rathcke & Lacey, 1985; Visser *et al.*, 2010; Forrest & Miller-Rushing, 2010).

Recent advances have demonstrated that plant phenological responses are nonlinear, due largely to interactions between cues (Flynn & Wolkovich, 2018; Laube *et al.*, 2014). This highlights the need for experiments to be designed to evaluate the strength of these interactions. To truly test interactions, treatments must be orthogonal: that is there must be more than one level of each treatment, and each level of each treatment must be crossed with every level of the other. This adds complexity to experimental setups, and experimental artifacts that interfere with interpretation of results are readily introduced (Wolkovich *et al.*, 2012). In this paper, we discuss a particular challenge that arises when investigating interaction between forcing temperature and photoperiod.

## Considering light and temperature in phenology experiments

For both light and temperature cues, there are two axes that can be manipulated in experiments. Then intensity of the cue (ie temperature of forcing or light intensity/wavelength) and the period (duration of temperature exposure or light periods). While light intensity has

been to shown to affect many aspects of organism’s biological activity including phenology (Brelsford & Robson, 2018; Cober *et al.*, 1996), the period of light exposure is generally considered to be the dominate light cue, and phenological research interests tends to focus on photoperiod rather than intensity (FindCitation). For temperature, though intensity is considered a major driver of phenology, the very common and successful model framework for quantifying temperature effects on phenology, the growing degree day or growing degree hour, integrates temperature with its period. Researchers must be thoughtful about how to incorporate these three main treatment axes(photo-period, theromo-period and thermo-intensity) in their experiments, and be wary of how these decisions will affect their experimental inference. There are three basic experimental designs (see figure 1 which are detailed in brief below:

### **Manipulation of Temperature intensity and photoperiod:**

The most simple design to test for a temperature x light interaction is to manipulate only one axis of each cue, for example, temperature intensity and light period. This would be implemented by applying high and low forcing treatments at constant temperatures, and crossing these with a long and short photo-period treatment at a constant light intensity (figure 1a).

The main advantage of this design is that is it simple to implement, maintains the orthogonality among the four treatment combinations (warm/short, warm/long, cool/short, cool/long) and allows for a very straight forward interpretation of the predictors. However, decades of work comparing the effect of constant vs. varying day night/night temperatures strongly suggest that constant temperatures are inadequate for capturing an accurate biological response (FindCitation), and may authors in fact recommend against this design. Instead, it has become virtually standard for researchers to include a diurnally varying thermo-period for forcing treatments. For example, rather than static high/low forcing treatments of 20°C and 16°C, researchers would use, a high forcing treatment of 24°/16°C and low 20°/12°C (day/night). (Ettinger *et al.*).

### **Coupled photo- and thermo-periodicity:**

Incorporating diurnal thermo-periodicity into experimental design require that researcher decide how to vary this periodicity relative to the experiments photo-period cycle. The vast majority of published experiments employ a design in which photo-and thermo-periodicity are synchronized as in (figure 1a) (Ettinger *et al.*). This design is perhaps the most intuitive, one, following the fact the photo- and thermo-period tend to co-vary in nature (FindCitation). This approximation of natural conditions may make this design the most useful for extrapolating inferences between experimental and observational studies. However, while it appears, to maintain orthogonality between treatments when considering temperature intensity alone, but, in this case, the forcing response is a product of both the intensity of the temperature cue and the duration of its exposure (growing degree hours). Thus, the coupling of photo- and thermo-period results in non-orthogonality between the four treatment combinations (see figure 2a). In this scenario, the effect of photo-period cannot be neatly distinguished from the effects of increased thermo-period under the longer day treatments,

making it impossible to accurately evaluate the relative importance of these cues, which is often a major goal of growth chamber experiments. This results in spurious interpretation of experimental result.

### **Decoupled photo- and thermo-periodicity:**

An alternative experimental design that incorporates thermo-periodicity into a temperature x photoperiod interaction experiment is decouples the experimental thermo- and photoperiods. This would be achieved by varying thermo-period on a constant schedule across all four treatment combinations (figure 1c). Though this experiment set up has not been widely, if ever, employed in growth chamber experiments (Ettinger *et al.*), this design restores full orthogonality to growing degree hour sums that make up the forcing treatment (figure 2a). But this obvious advantage for accurate statistical comparisons introduces a new biological artifact that may bias experimental results. *say more about this once we identify the sources about the importance of dawn temperature.* This possible biological interaction between also muddies statistical inferences when making cue comparisons.

As we have shown above, each design introduces its own biological or statistical artifacts that will bias the results of the experiments. We cannot conclusively solve the problems of true inference through modifying experimental designs, but rather, we must thoughtfully incorporate the implications of these design choices into our statistical metrics and interpretation of results. While we cannot conclusively suggest an optimal experimental design, we expect that because of its history and relationship to field conditions, a coupled photo-and thermo- period design will remain the most popular choice for phenological experiments in artificial environments. In the following section, we will use a public dataset to demonstrate how mathematical principles can be applied to tease apart the artifact introduced by this inherently imperfect experimental design and make more meaningful inferences about the cue interactions.

## **Math**

Our analysis is based on results from a large scale growth chamber experiment by Flynn & Wolkovich (2018), in which in addition to chilling and provenance, fully factorial light and temperature manipulations were applied to twig cuttings from 28 woody species. The authors determined a mean budburst sensitive to temperature of -9.5, and a mean sensitivity to photo-period of -4.5 with a weak, negative interaction between the two cues. We set out to calculate how much of the reported photo-period response could in actuality be driven by the latent differences in thermo-period between the long and short photo-period treatments.

1. State some assumptions. IE forcing is dominant cue
2. Do some math
3. Make a prediction.

# 1 Validation

1. How do we know if this is a good prediction? We are essentially predicting the expected difference between experimental design two and three.
2. We have 2 dataset. A subset of a Flynn & Wolkovich (2018) study were sampled at Harvard Forest, were forced at 18 day 12 night, with photoperiod treatments of 8 and 12 hours.
3. Another experiment by Buonaiuto and Wolkovich (unpublished data) also included these treatment levels, But, decoupled thermo-and photo-periodicity. What a happy accident.
4. We reanalyzed these datasets to compare the effects of coupling vs. uncoupling, see 2.

# 2 Wrap up

1. We can't say which design is absolutely best.
2. We expect design 2 will remain popular. This is okay because we have shown the design flaws are easiest to overcome mathematically.
3. We externally validated the math
4. Future studies should do this.

# References

- Brelsford, C.C. & Robson, T.M. (2018) Blue light advances bud burst in branches of three deciduous tree species under short-day conditions. *TREES-STRUCTURE AND FUNCTION* **32**, 1157–1164.
- Chuine, I. & Beaubien, E. (2001) Phenology is a major determinant of tree species range. *Ecology Letters* **4**, 500–510.
- Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. (2007) Shifting plant phenology in response to global change. *Trends in Ecology Evolution* **22**, 357 – 365.
- Cober, E., Tanner, J. & Voldeng, H. (1996) Soybean photoperiod-sensitivity loci respond differentially to light quality. *CROP SCIENCE* **36**, 606–610.
- Ettinger, A.K. *et al.* (????) Osprey .
- FindCitation (????) .
- Flynn, D.F.B. & Wolkovich, E.M. (2018) Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist* **219**, 1353–1362.

- Forrest, J. & Miller-Rushing, A.J. (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **365**, 3101–3112.
- Laube, J., Sparks, T.H., Estrella, N., Höfler, J., Ankerst, D.P. & Menzel, A. (2014) Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology* **20**, 170–182.
- Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kuebler, K., Bissolli, P., Braslavska, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, A., Defila, C., Donnelly, A., Filella, Y., Jatecza, K., Mage, F., Mestre, A., Nordli, O., Penuelas, J., Pirinen, P., Remisova, V., Scheifinger, H., Striz, M., Susnik, A., Van Vliet, A.J.H., Wielgolaski, F.E., Zach, S. & Züst, A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**, 1969–1976.
- Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37 EP –.
- Piao, S., Friedlingstein, P., Ciais, P., Viovy, N. & Demarty, J. (2007) Growing season extension and its impact on terrestrial carbon cycle in the northern hemisphere over the past 2 decades. *Global Biogeochemical Cycles* **21**.
- Primack, R.B., Laube, J., Gallinat, A.S. & Menzel, A. (2015) From observations to experiments in phenology research: investigating climate change impacts on trees and shrubs using dormant twigs. *ANNALS OF BOTANY* **116**, 889–897.
- Rathcke, B. & Lacey, E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* **16**, 179–214.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature* **421**, 57–60.
- Visser, M.E., Caro, S.P., van Oers, K., Schaper, S.V. & Helm, B. (2010) Phenology, seasonal timing and circannual rhythms: towards a unified framework. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**, 3113–3127.
- Wolkovich, E.M., Cook, B.I., Allen, J.M., Crimmins, T.M., Betancourt, J.L., Travers, S.E., Pau, S., Regetz, J., Davies, T.J., Kraft, N.J.B., Ault, T.R., Bolmgren, K., Mazer, S.J., McCabe, G.J., McGill, B.J., Parmesan, C., Salamin, N., Schwartz, M.D. & Cleland, E.E. (2012) Warming experiments underpredict plant phenological responses to climate change. *Nature* **485**, 494 EP –.
- Wolkovich, E.M. & Ettinger, A.K. (2014) Back to the future for plant phenology research. *New Phytologist* **203**, 1021–1024.

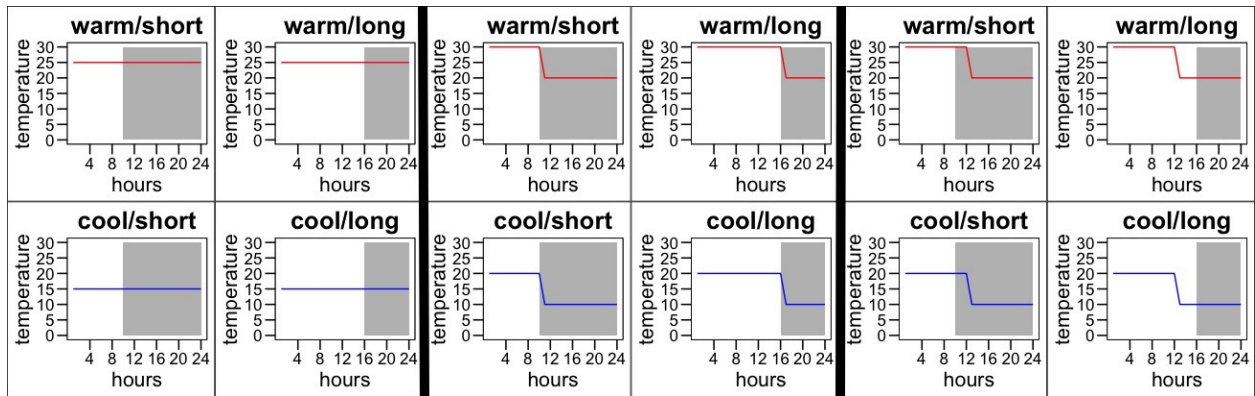


Figure 1: Experimental treatments

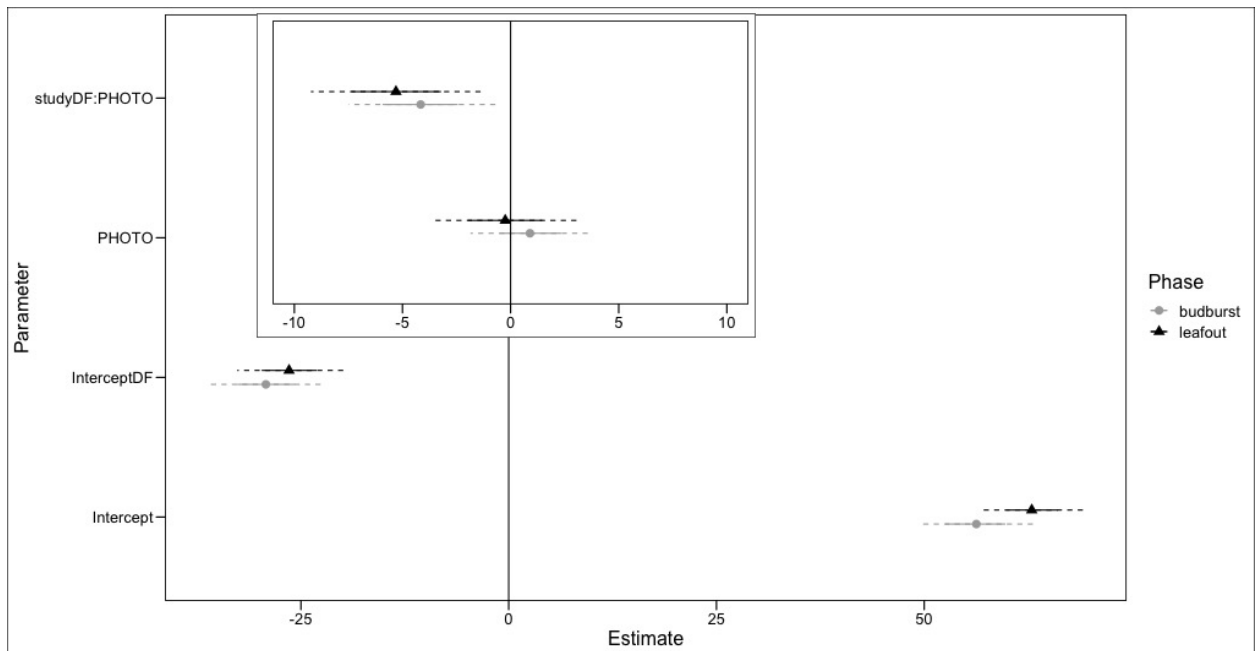


Figure 2: Experimental comparison all species

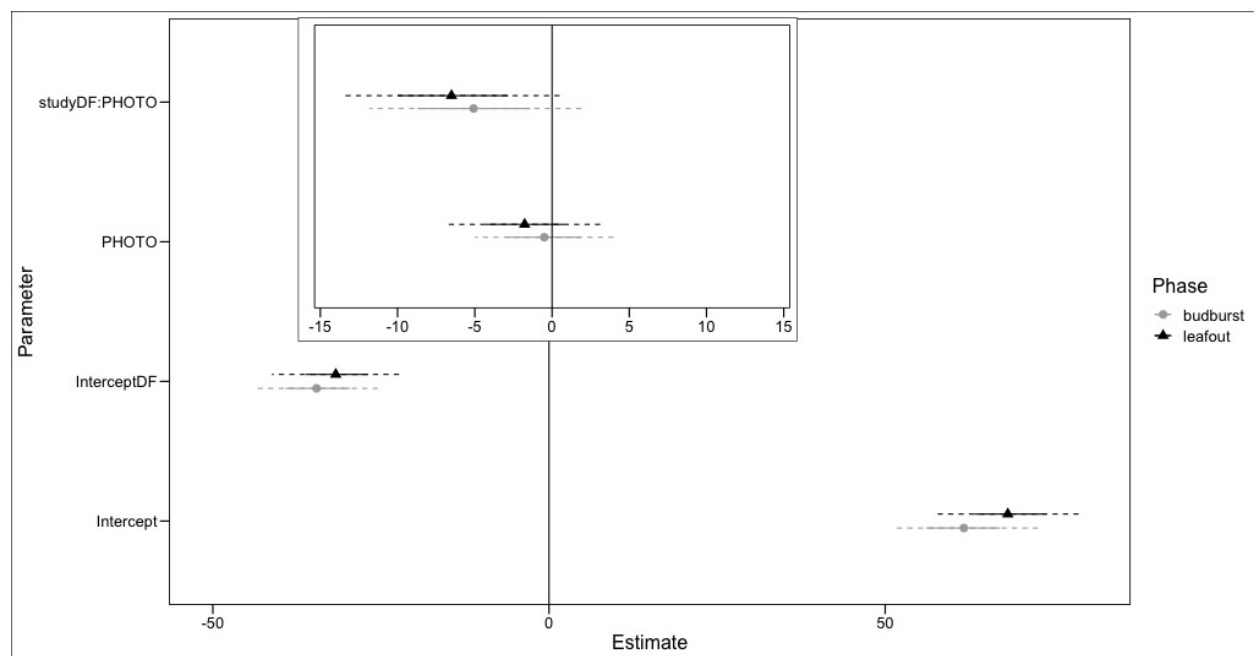


Figure 3: Matching species only