

Dear Dr. Ellison,

Please consider this manuscript, “Experimental designs for testing the interactive effects of tempera- ture and light in ecology: the problem of periodicity”, as a “ Perspective” article in *Methods in Ecology and Evolution*.

Experiments in growth chambers or other controlled environments are a powerful tool for quanti- fying the individual and interactive e ects of environmental cues on numerous biological processes. These studies have t remendously advanced our understanding of both fundamental eco-physiology and applied ecological forecasting (Osmond *et al.*, 2004). Yet, because experimentalists must balance ecological realism wit h statistical inference, experimental e ort wit h statistical power, and account for the e ects of unmanipulated or unmeasured variables (Scheiner & Gurevitch, 2001), seemingly small choices about experimental design can generate signi cant di erences in outcomes.

Using almost a century-wort h of experiments, and the phenology of woody plants as a case study (Wolkovich *et al.*, 2019), our submission highlights how a commonly used experimental design aimed to partition the e ects of temperature and photoperiod on spring phenology results in the incorrect est imation of cue e ects. This occurs in studies that couple the periodicity of light and temperature t reatments, which surreptit iously introduces experimental covariation. Notably, we examine the lit er- ature and nd that up to 40% of phenology studies have this issue, which may in part explain why the relative importance of photoperiod to spring phenology is currently a topic of signi cant controversy in the phenology lit erature (Koerner & Basler, 2010; Chuine *et al.*, 2010; Kourner & Basler, 2010; Zohner *et al.*, 2016; Way & Montgomery, 2015).

In this submission, we identify the extent of this problem by combining data simulations and an al- gebraic solut ion wit h a comparative analysis of published studies. Importantly, we provide guidance for alternative experimental designs that can overcome this stat ist ical issue. While we use spring phenology as a case study, we believe that our submission would be of broad interest to the readers of *Methods in Ecology and Evoltion*, as it is relevant to any branch of ecology or evolutionary biology where light or temperature controls a biological response (e.g., Franklin, 2009; Brown *et al.*, 2014; Casal & Quuest a, 2018).

The main text of this manuscript is 2,948 words in lengt h and it contains four gures. It is co-authored by M. Donohue and E.M. Wolkovich, and is not under consideration elsewhere. We hope that you will nd it suitable for publicat ion in *Methods in Ecology and Evolution*, and look forward to hearing from you.

Sincerely,

Daniel Buonaiuto