The ARNOLD ARBORETUM of HARVARD UNIVERSITY

Dear Dr. Ferry,

Please consider this manuscript, "Experimental designs for testing the interactive effects of temperature and light in ecology: the problem of periodicity", as a "Commentary" article in *Functional Ecology*.

Experiments in growth chambers or other controlled environments are a powerful tool for quantifying the individual and interactive effects of environmental cues on numerous biological processes. These studies have tremendously advanced our understanding of both fundamental eco-physiology and applied ecological forecasting (Osmond *et al.*, 2004). Yet, because experimentalists must balance ecological realism with robust inference, experimental effort with statistical power, and account for the effects of unmanipulated or unmeasured variables (Scheiner & Gurevitch, 2001), seemingly small choices about experimental design can generate significant differences in outcomes.

Using almost a century-worth 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 effects of temperature and photoperiod on spring phenology results in the incorrect estimation of cue effects. This occurs in studies that couple the periodicity of light and temperature treatments, which surreptitiously introduces experimental covariation. Notably, we examine the literature and find 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 significant controversy in the phenology literature (Koerner & Basler, 2010; Chuine *et al.*, 2010; Körner & 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 algebraic solution with a comparative analysis of published studies. Importantly, we provide guidance for alternative experimental designs that can overcome this statistical issue. While we use spring phenology as a case study, these experimental design issues arise for studies in both aquatic and terrestrial systems and across a broad range of plant and animal taxa (Stewart *et al.*, 2013). We believe that our submission would be of broad interest to the readers of *Functional Ecology*, as it is relevant to any branch of ecology where light or temperature controls a biological response (e.g., Franklin, 2009; Brown *et al.*, 2014; Casal & Qüesta, 2018).

The main text of this manuscript is 3,023 words in length and it contains four figures. It is co-authored by M. Donahue and E.M. Wolkovich, and is not under consideration elsewhere. We hope that you will find it suitable for publication in *Functional Ecology*, and look forward to hearing from you.

Sincerely,

Daniel Buonaiuto

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