The ARNOLD ARBORETUM of HARVARD UNIVERSITY

Dear Dr. Ellison,

Please consider this manuscript "Experimental designs for testing the interactive effects of temperature and light in ecology and 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 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 statistical 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 experiments with 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 the 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 issues. 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 & Qüesta, 2018).

The main text of this manuscript is 2,942 words in length and it contains 4 figures. It is co-authored by M. Donohue and E.M. Wolkovich, and is not under consideration elsewhere. We hope that you will find it suitable for publication in *Methods in Ecology and Evolution*, and look forward to hearing from you.

Sincerely,

Daniel Buonaiuto

References

Brown, E.E., Baumann, H. & Conover, D.O. (2014) Temperature and photoperiod effects on sex determination in a fish. *Journal of Experimental Marine Biology and Ecology* **461**, 39–43.

Casal, J.J. & Qüesta, J.I. (2018) Light and temperature cues. *The New Phytologist* **217**, 1029–1034.

Chuine, I., Morin, X., Bugmann, H., Koerner, C. & Basler, D. (2010) Warming, photoperiods, and tree phenology. *Science* **329**, 277–278.

Franklin, K.A. (2009) Light and temperature signal crosstalk in plant development. *Current Opinion in Plant Biology* **12**, 63–68.

Koerner, C. & Basler, D. (2010) Phenology under global warming. *Science* **327**, 1461–1462.

Körner, C. & Basler, D. (2010) Response—warming, photoperiods, and tree phenology. *Science* **329**, 278–278.

Osmond, B., Ananyev, G., Berry, J., Langdon, C., Kolber, Z., Lin, G., Monson, R., Nichol, C., Rascher, U., Schurr, U., Smith, S. & Yakir, D. (2004) Changing the way we think about global change research: scaling up in experimental ecosystem science. *Global Change Biology* **10**, 393–407.

Scheiner, S.M. & Gurevitch, J. (2001) *Design and analysis of ecological experiments*. Oxford University Press.

Way, D.A. & Montgomery, R.A. (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment* **38**, 1725–1736.

Wolkovich, E.M., Ettinger, A.K., Flynn, D., Savas, T., Chamberlain, C., Buonaiuto, D. & Samaha, J. (2019) Observed spring phenology responses in experimental environments (OSPREE). doi:10.5063/F1CZ35KB.

Zohner, C.M., Benito, B.M., Svenning, J.C. & Renner, S.S. (2016) Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nature Climate Change* **6**, 1120–1123.