

The eco-evolutionary significance of
turion reactivation asynchrony in
greater duckweed, *Spirodela polyrhiza*

By

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Abstract

Environmental change is expected to accelerate, and responses to environmental variation are becoming more relevant. The study of adaptation to variable environments has been central in the study of Biology at least since Darwin's time, but most recent studies in eco-evolutionary dynamics do not focus on traits that are thought to evolve as a response to environmental variation. Specifically, studies of evo-to-eco pathways centred on traits that may be adaptive through the expression of variation are lacking. An appropriate system for such a study is the greater duckweed, *Spirodela polyrhiza*, which is a clonal floating plant that produces overwintering structures called turions. Despite their clonality, turions reactivate at wildly different times, ranging from the early onset of Spring to the end of Fall, and some do not reactivate for a few years. By spreading the risk of early/late freeze-overs across newly formed fronds, *S. polyrhiza* ensures the survival of their offspring in the event of an unpredictable, rare, and catastrophic event. As such, turion reactivation asynchrony is hypothesized to be a bet-hedging strategy. However, it also provides *S. polyrhiza* additional benefits that may be under selection. Here, I test the null hypothesis that the inherent advantages of turion reactivation asynchrony are sufficient to explain its evolution in north temperate ecosystems without recourse to bet-hedging strategies. To test this, I conducted a common garden experiment using *S. polyrhiza* and two other duckweed species under varying levels of reactivation asynchrony, measuring final population sizes and overall growth rates in the context of naturally variable conditions. Additional analyses were run to further examine the inherent costs and advantages of asynchrony. There was evidence of an additional cost

of asynchrony in which fronds simply grow more slowly than expected. In the short term, asynchronous reactivation appears to be a maladaptive trait. This provides indirect evidence suggesting that turion reactivation asynchrony is under stronger selection when environmental conditions are poor. These results are therefore consistent with diversification bet-hedging strategies.

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Introduction

Studies in evolutionary biology focus on how heritable traits in populations change across generations, whereas ecological studies focus on relationships among organisms and their environments at various scales, from population dynamics to energy flux at the biosphere level, and typically assume that traits remain constant over time. Traditionally, it was believed that evolution operated too slowly to affect ecological processes over short periods of time; however, recent work has shown that evolution occurs more rapidly than previously thought (Hendry & Kinnison, 1999; Reznick & Ghalambor, 2001; Kinnison & Hairston, 2007; Hendry, Farrugia & Kinnison, 2008; Reznick, Losos & Travis, 2019). The study of eco-evolutionary dynamics is a relatively new field in biology and is based on the idea that evolutionary changes in natural populations affect ecological processes and that populations adapt to new ecological conditions, all on contemporary, or ecological time scales (Kinnison & Hairston, 2007; Pelletier, Garant & Hendry, 2009; De Meester et al., 2019). As the name implies, studies on eco-evolutionary dynamics combine concepts of both fields to understand the bi-directional relationship of ecology and evolution, or how ecological and evolutionary processes influence one another. The “eco-to-evo” direction in this relationship, where ecological conditions affect organismal adaptation, has been a central theme in evolutionary biology at least since Darwin’s time. Less recognized, however, is the pathway in which the evolution of traits in a given species results in changes to ecosystem function; henceforth “evo-to-eco” (Becks et al., 2010; Therry et al., 2019; Sentis et al., 2020; Leduc et al., 2021). Furthermore, trait evolution may affect ecology in such a way that it causes further evolutionary change, resulting in a feedback loop (Becks et al., 2012; Turcotte, Reznick & Hare, 2013; Croll, Egas, &

Smallegange, 2019). Eco-evolutionary dynamics is increasingly recognized as an important perspective for its contributions to understanding applied problems in fields like medicine (Stearns, 2018; Baptiste et al., 2021; Gogarten, 2022) and conservation biology (Kinnison & Hairston, 2007; Correa et al., 2015; Kreitzman et al., 2018; Zhao et al., 2022).

Many studies of the evo-to-eco pathway have focused on adaptation in a particular trait or in response to a specific environmental alteration; however, environments are expected to be more variable with increasing effects of climate change (Bousset & Chèvre, 2013; Bargués-Ribera & Gokhale, 2020). Adaptive responses to variability, rather than to particular environments, have long been recognized as central to species persistence (e.g., Lewontin & Cohen, 1969; Gillespie, 1977; Seger & Brockmann, 1987; Schreiber, 2015), and take on new relevance in the context of climate change, given that many species would respond to these changes in variable and unpredictable ways (Davis, Shaw & Etterson, 2005). Despite recent progress in our understanding of rapid evolution and its effects on ecology (Ezard, Côté, & Pelletier, 2009; Palkovacs et al., 2009; Hendry, 2013), which will be imperative in predicting shifts in ecosystem function, significant knowledge gaps in the study of evo-to-eco pathways remain.

Hendry (2019) specifies deficiencies in the study of evo-to-eco pathways, including areas that have yet to be addressed. The first is that most studies in eco-evolutionary dynamics are highly controlled, precluding a real understanding of natural environments, which are much more variable. Second, current studies tend to analyze per-capita effects of individuals or groups with a constant population density, rather than including density-

dependence in their design. It is likely that key species in given communities may be affected by evolutionary pathways directly, which in turn affects the rest of the community. While most studies focus on a single species to effectively isolate evo-to-eco effects, this underestimates community-wide effects on evolution if not paired with additional species interactions. Finally, most studies observe the effects of evolutionary change on ecological change, but it is also possible to see evolutionary change cause ecological stability instead. In addition to those listed by Hendry (2019), one knowledge gap that is largely unrecognized is that, even for controlled mesocosm studies of evolutionary effects in simple communities, the focus has been on the evolution of a particular trait, or evolution under a prescribed environmental change, and not on traits that respond to fluctuating environmental conditions.

Adaptive evolutionary responses to environmental variation differ from “simple” traits in that they may include the expression of variable phenotypes within genotypes, or the evolution of “safe” traits that reduce variance in fitness. Such responses include adaptive phenotypic plasticity, diversification bet hedging and conservative bet hedging. Phenotypic plasticity occurs when an organism responds appropriately to the state of its environment, and its evolution requires reliable cues associating environment with optimal traits (Nussey, Wilson, & Bromer, 2007; Reed, Schindler, & Waples, 2011). When the evolution of plasticity is constrained, or an environment fluctuates such that there are no reliable indicators of phenotype-fitness associations for future conditions, organisms are expected to evolve biological bet hedging strategies instead (Simons, 2011). Bet hedging occurs when organisms suffer decreased fitness in their typical conditions while

increasing relative fitness under rare but stressful conditions (Cohen, 1966; Simons, 2011). Conservative bet-hedging evolves when selection favours a trait that remains “safe” as opposed to one that maximizes potential fitness, and this can be compared to an insurance policy (Simons, 2011). This is a case where trait variance is minimized, however, in response to unpredictable environmental conditions. On the other hand, diversification bet-hedging spreads risk among an array of phenotypes, ensuring non-zero survival overall (Simons, 2011). While they appear to be maladaptive when observed for short periods of time, biological bet hedging strategies maximize long-term fitness by providing a significant advantage during rare unfavourable circumstances, maximizing geometric-mean fitness (Simons, 2002).

Bet hedging is relatively well understood theoretically and, although difficult to study (Simons, 2011), has been observed in a variety of organisms. For instance, strong evidence of dormancy being a mechanism to reduce the risk of reproductive failure in highly variable environments has been shown in the seeds of annual plant species (Tielbörger, Petruu & Lampei, 2012) and even the hatching of African killifish eggs (Pinceel et al., 2015). In the commonly studied bacterial system, *Escherichia coli*, exposure to both oxygen and trimethylamine oxide (TMAO) results in the bacteria increasing the expression of TMAO respiration as a strategy to spread the risk of sudden oxygen loss in the environment (Carey & Goulian, 2019). Bet-hedging is also shown to characterize the reproductive stages of the monocarpic perennial, *Lobelia inflata*, where the timing of bolting mismatches expected norms of reaction that would otherwise maximize reproduction (Simons & Johnston, 2003). In each case, bet hedging is shown as an optimal

strategy when conditions are unsuitable. That being said, studies that focus on their effects on ecology, i.e., evo-to-eco pathways, are lacking, and the importance of adaptations to environmental variance caused by climate change is increasingly being recognized (Davis, Shaw & Etterson, 2005). My thesis work will focus on the ecological effects of a variance trait that is believed to have evolved as a diversification bet hedging trait (Morris et al., 2020) and is known to evolve over contemporary time scales (Hitsman & Simons, 2020).

An appropriate system for the study of the evo-to-eco effects requires one or multiple variable traits that have the potential to influence ecology, and that may evolve rapidly. The aquatic plant, Greater duckweed, *Spirodela polyrhiza* (L.), fills these requirements. *S. polyrhiza* is among the smallest flowering plants known and can be found almost everywhere in the world except Arctic, Antarctic, as well as very humid and very dry climatic regions (Jacobs, 1947; Hillman, 1961; Laird & Barks, 2018; Bog, Appenroth & Sree, 2020). Duckweed has been used extensively across a variety of fields, such as in community ecology (Armitage & Jones, 2019), biofuel development, phytoremediation and ecotoxicological testing (Rahman et al., 2007; Appenroth et al., 2010; Ng & Chan, 2018; Singh, Pandey & Suthar, 2019). Duckweed species have been the subject of evolutionary biology studies because they are primarily clonal plants and are short lived (Laird & Barks, 2018). As *S. polyrhiza* reproduces on the pond surface, daughter fronds remain linked to the mother frond as colonies and are able to exchange photosynthates (Berrat-Segretain, 1996; Jin et al., 2021). With sufficient turbulence from external sources, such as wind, strong currents, or animal (including human) contact, colonies may

separate. This ‘fragmentation’ has been observed to increase the overall growth rate of *S. polyrhiza* populations, probably because separated fronds are able to use more of their resources for reproduction (Zhang et al, 2020).

Presumably as an adaptation to unpredictable environmental variation, greater duckweed produces turions, which are seedlike, quiescent overwintering bodies. These structures allow greater duckweed to survive in a dormant state during the colder months of the year (Jacobs, 1947), and reactivate throughout the following Spring. Turion production and reactivation occur over a wide time frame, i.e., asynchronously (Hitsman & Simons, 2020; Morris, Compton & Simons, 2020). Much of this variation occurs under constant environmental conditions within clones and is known to be generated by daughter frond birth-order (Mejbel & Simons, 2018). Smaller and, presumably, more unpredictable pond environments can also affect mean day to turion formation and reactivation (Compton, 2013), notably through plasticity to temperature, and through birth order effects (Morris, Compton & Simons, 2020). Furthermore, this pattern of variation differs across populations (Hitsman & Simons, 2020), where a gradient in the length of growing seasons across latitudes corresponds with the evolution of reaction norms characterized by earlier turion production at higher latitudes (Hitsman & Simons, 2020). This trait is therefore thought to evolve as a response to environmental variation and is known to be evolutionarily labile.

In addition to ensuring survival through unpredictable conditions in the year of production, asynchronous turion reactivation in *S. polyrhiza* is also thought to allow survival through rare freeze-over events at the pond surface during the first few weeks of

Spring in north temperate regions (Morris et al. 2020). Reactivating early when there is no freeze-over event grants the first few turions the “head-start” advantage of growing for longer periods of time with fewer competitors; however, although fronds produced later are not exposed to lethal freeze events, they have less time in the season to grow and reproduce, representing an inherent cost of asynchrony. If there were an optimal time for all turions to reactivate simultaneously without risk of freezing, this would undoubtedly provide *S. polyrhiza* an even greater advantage in maximizing any given genotype’s fitness. Nevertheless, the environmental cues are not reliable enough to have turions reactivate without the risk of a freeze-over. Based on the geometric-mean principle, “sacrificing” fitness under favourable circumstances should therefore be selected for and bet-hedging is expected to evolve (Simons, 2011).

Similar to effects of fragmentation, newly reactivated turions behave much like single fronds and exhibit high growth rates. Furthermore, each new turion enters the habitat without having been affected by direct inter- or intra-competition. “Fresh” new individuals are therefore expected to have higher potential fitness. With these growth advantages, an already established population is expected to increase more rapidly than a population that lacks turions, all else being equal, which may result in increased realized growth rates for *S. polyrhiza* in multi-species communities. The evolution of turion reactivation asynchrony in *S. polyrhiza* has the potential to affect coexisting species and is therefore an appropriate candidate trait for the study of evo-eco dynamics.

In this thesis I exploit this duckweed system to ask whether asynchronous turion reactivation in *S. polyrhiza* affects community dynamics. Given the potential interactions

with other species, I hypothesize that inherent advantages to asynchronous turion reactivation in the short-term are sufficient to explain the evolution of asynchrony, without recourse to explanations based on maximizing geometric mean fitness. These advantages may include increased overall apparent reproductive rates, increased individual growth due to starting as colonies of one frond similarly to fragmentation, or previously unobserved effects allowing *S. polyrhiza* to outcompete coexisting species.

An array of outdoor aquatic mesocosms was used to perform a common garden experiment in which the level of *S. polyrhiza* turion reactivation asynchrony was manipulated, and fitness (growth rates and final frond counts) were compared in treatments alongside combinations of two other duckweed species, *Lemna minor* and *Landoltia punctata*, in a factorial design. The experiment took place during the summer of 2022, when no freeze-over events were expected, thereby controlling for the benefits of bet-hedging. *S. polyrhiza* fronds were introduced into multispecies communities to simulate turion reactivation, while controlling for the simple exponential growth advantage/disadvantage of early/late turion reactivation (see supplementary material S1). Frond counts were used to compare overall fitness among reactivation treatments across communities. The null expectation is that the level of reactivation asynchrony has no effect on multispecies communities, where such possible effects include an increase or decrease in performance for either *S. polyrhiza* or any competing species. Alongside the main experiment, *S. polyrhiza* populations were grown with each extreme reactivation setting to measure the inherent cost of asynchrony in this system. An additional analysis was conducted in which growth rates from **synchronous** populations

of *S. polyrhiza* were used to simulate population growth given the realized introduction schedule of the **asynchronous** populations of *S. polyrhiza*. Overall predicted population counts differing from those observed would suggest an additional cost or benefit to asynchronous reactivation. If, contrary to our hypothesis, results show that there is no inherent advantage of asynchronous turion reactivation in the short-term, further study of this variance trait based on the geometric-mean fitness principle is warranted.

Materials and Methods

The focal duckweed species in this study is greater duckweed, *Spirodela polyrhiza*, and the competing species are the common duckweed, *Lemna minor*, and the dotted duckweed, *Landoltia punctata*. *L. minor* is more spherical than the larger *S. polyrhiza*. It is found in cooler, mostly oceanic regions of North America, Europe, Africa, and western Asia (Bog, Appenroth & Sree, 2020). *S. polyrhiza* and *L. minor* were collected in the Ottawa/Gatineau region, where they are commonly found (*S. polyrhiza*: Brewer Park, Ottawa (45° 23' 8.1" N, 75° 41' 12" W); *L. minor*: Nepean, Ottawa (45° 20' 31" N, 75° 51' 18" W)). *L. punctata*, previously known as *Spirodela punctata* (Landolt, 1986; Les & Crawford, 1999), is another member of the *Lemnoideae* subfamily (Bog, Appenroth & Sree, 2020). It is generally large relative to other duckweed species, roughly the same size as *S. polyrhiza*, and is named 'punctata' after the series of spots along its surface. It is found in north temperate regions similar to the Ottawa/Gatineau region and coexists with *L. minor* and *S. polyrhiza*. *L. punctata* was collected from the greenhouse stock population at Carleton University (45° 23' 2"N, 75° 41' 36" W).

The primary experiment was conducted from June 2 to June 15 in an array of fifteen 100 US gal. cattle tank mesocosms in the experimental gardens at Carleton University (45° 23' 01" N, 75° 41' 33" W) following the protocols from previous work on the same mesocosms system (Morris, Compton & Simons, 2018), notably rearing, mesocosm setup and nutrients. Stock populations of *S. polyrhiza* were reared in the lab in Appenroth's solution (Appenroth, Teller & Horin, 1996) with a 14/10 photoperiod. Each tank contained 24 floating rafts made from short segments of 6" diameter tubing fitted with buoyant insulation foam (Appendix 1). A layer of mesh was added to the bottom of

the rafts to keep fronds within the raft during periods of heavy winds or rain while also keeping reactivating turions, if any, from entering these populations from the bottom of the tank. Surface water temperature in each tank was monitored using temperature probes (model 107C) reporting to a CR6 datalogger (both from Campbell Scientific). Equal amounts of 0.6 – 0.4 – 0.4 fertilizer (sheep manure) were added to each mesocosm. Water levels were monitored and topped up as needed. Metal mesh frames and 30% shading cloth covered each mesocosm to prevent wildlife from getting in (e.g., birds, squirrels) and to avoid bleaching from overexposure to sunlight.

There were four unique combinations of species present among duckweed communities in this experiment: the first is *S. polyrhiza* without additional competitors; these are the reference populations used to define the appropriate times to introduce new fronds into each community based on their reactivation treatment, discussed below. The remaining three combinations were *S. polyrhiza* with *L. minor* (Spol + Lmin), *S. polyrhiza* with *L. punctata* (Spol + Lpun) and a final level with all three species present (Spol + Lmin + Lpun). 10 fronds of either *L. minor* or *L. punctata*, or both, were added to their respective multi-species community at the same time as *S. polyrhiza* in any given asynchrony setting. Starting frond counts of *S. polyrhiza* in any level of species interaction depends on the reactivation treatment for that community. The reference populations are referred to as 'Spol' since only *S. polyrhiza* is present.

The reactivation treatment consists of three settings: synchrony (**sync**), asynchrony (**async**), and an intermediate between the two extremes (**mid-async**). The starting populations in the **sync**, **mid-async**, and **async** treatments were 17, 11, and 4

fronds respectively (**Figure 1**). Recall that these are the starting counts such that each population is expected to reach an equal final population size across levels of turion reactivation, all else being equal (**Figure 2**). Each level of reactivation consists of its own “benchmark” frond counts, each of which represents a time at which reactivation occurring would result in the expected final frond count. While the design was to end with identical frond counts, the reference populations can be used to analyze differences between levels of reactivation should final frond counts differ once all reactivations have taken place.

Asynchrony was simulated by adding 3 fronds whenever the respective reference ‘*Spol*’ populations reached a benchmark frond count (**Table 1**). New fronds in the **mid-async** populations were introduced in a similar manner, where 4 fronds were added at each of their own benchmarks. No further introductions took place for the ‘synchrony’ populations. Tanks were evenly and randomly selected to begin the experimental phase either one day earlier or later to avoid potential bias of having every population start during optimal or unfavourable natural temperature conditions (see **supplementary S2**). Reference population size measurements took place every two to three days, and once the final introductions were made for the *asynchrony* treatments, the experimental phase was complete. While the aim was to end with the same final population sizes, the reference populations also allow calculation of expected frond counts based on their realized growth patterns under semi-natural conditions. At the end of the experiment, 24 replicates reached the point of final asynchronous reactivation.

Despite efforts to minimize differences in final frond counts across manipulated reference synchrony levels (*i.e.*, with the addition of reactivating fronds), differences are likely. The inclusion of reference treatments in each tank allows correction for the direct effects of synchrony on final frond counts in analyses of the interaction effects of synchrony and competition. However, this does not address the question of whether synchrony directly affects growth rates in *S. polyrhiza*. Growth across synchrony levels may be compared by using the “budding growth rates”; *i.e.*, reproduction of *S. polyrhiza* without the contribution of reactivation. Reference ‘sync’ populations, which grew exclusively by budding, can serve as a null expectation for budding growth rates in other populations in their respective mesocosms. Given known reactivation patterns in the ‘async’ populations, we can use budding growth rates from reference ‘sync’ populations in each mesocosm to calculate the final expected frond count, and therefore, the expected overall growth rates of any population. In this analysis, the ‘expected’ growth rates represent how *S. polyrhiza* should grow if budding growth rates (from reference ‘sync’ populations) are constant under ‘async’ reactivation settings. ‘Observed’ realized growth rates are simply the measured overall growth rates in ‘async’ populations during the experiment. In short, this is an indirect comparison of budding growth in asynchronous vs. synchronous reactivation settings. Since the focus here is on differences in growth between synchronous and asynchronous reactivation, only the ‘async’ and reference ‘sync’ populations were used. For completeness, competition is included as an effect in this analysis, and includes each species combination, including the reference

‘Spol’ (alone) populations. Differences in overall growth rate would indicate that the budding growth of fronds is unequal among levels of reactivation.

The extent of manipulated asynchrony under field conditions was dictated by realized growth rates. Based on these realized growth rates from the first trial, a second trial was conducted from July 23 to August 15 to enrich the study. The goal of this study was to observe *S. polyrhiza* reactivation under semi-natural conditions for a longer period of time and test for differences in population trends from the first trial. There was no mid-asynchrony treatment in this trial, and only twelve tanks were reused. ‘Async’ populations started with 4 fronds and had 2, 4, 8, 12, 12, 8 and 4 fronds added sequentially once their reference populations hit 5, 10, 20, 39, 80, 138 and 217 fronds respectively, ending at 217 fronds. Complete synchrony populations began with 20 fronds and were allowed to grow without intervention. Asynchronous reference populations were counted every two to three days, and fronds were added to the respective competing populations at the appropriate times, similarly to the first trial. Final frond counts for every population were measured when the respective reference populations reached 217 fronds.

The two main trials correct for the inherent numerical advantage of synchrony. An additional study was thus conducted to measure the inherent cost of asynchronous reactivation without controlling for timing itself, under the same experimental conditions alongside the second trial. ‘Sync’ and ‘async’ reactivation schedules were designed to have 20 turions introduced either all at once at the beginning of this side experiment (sync) or normally distributed over 20 days (async) with a slight right-skew to approximate reactivation patterns observed in the field (Compton, 2013). This normal distribution

refers to the quantity of fronds introduced being 1, 3, 4, 3, 2, 2, and then 1 per day for the next 5 days. Fitness was measured as final frond counts, and the main treatment is reactivation asynchrony. A “tank” variable was also included to account for among-tank variation due to temperature and nutrient differences.

Multi-way analyses of variance (ANOVAs) and t-tests were performed in R (ver. 4.2.2) to test for the effects of asynchrony, competition (categorical variables) and their interaction on population growth and predicted final frond counts (continuous variables). While surface area per frond was not measured in this study, this is another way that future work may estimate growth in *S. polyrhiza*. R code for each analysis is provided in appendices 2 through 6. Reactivation asynchrony, as the focal trait, is treated as a fixed effect. While an argument could be made for species present being a random effect, there are so few that coexist with *S. polyrhiza* in this region that the level of species present can be treated as a fixed effect. In these analyses, tanks are considered a random (blocking) effect because environmental variation is expected at this scale due to possible differences in nutrients and temperature across tanks. R code for these analyses is provided in appendices 2-6. Trial is included as a factor when all data is used in a single analysis. Post-hoc pairwise comparison tests were run in the main analyses to test for within-group differences following Bonferroni corrections. While performing Tukey tests was also an option, Bonferroni corrections allow for more conservative estimates. Similar analyses were performed with the data from the secondary study to measure the inherent cost of asynchronous reactivation in this system.

Results

Trial 1

In the first trial, 25 of 30 replicates successfully reached the final reactivation treatment and were thus included in analyses (**supplementary S3**). Loss of the five replicates (*i.e.*, failure to reach full experimental reactivation) was caused by either tearing of the mesh/buoyant foam of some rafts, or abnormally low growth, likely a result of obstruction by algae, in the reference populations.

The first step was to analyze the effect of reactivation synchrony treatment and overall competition on final frond counts and growth rates in the form of two-way ANOVAs (**appendix 1**). Overall competition has two levels: no competition (reference populations), and competition (species combinations lumped into a single competition level). The first analysis, in which final frond count is the response variable, finds significant effects of both reactivation treatment ($F(2, 277) = 73.69, p < 0.0001$) and overall competition ($F(1, 277) = 13.06, p < 0.001$), with evidence of an interaction effect ($F(2, 277) = 3.58, p < 0.05$). Frond counts for 'async', 'mid-async', and 'sync' reference populations were (mean $\pm$ SE) 54.92 ± 8.71 , 78.00 ± 22.77 and 91.72 ± 28.83 fronds per raft, respectively, at the termination of the trial. Similarly, the competing populations, across synchrony treatments in the same order, had (mean $\pm$ SE) 54.55 ± 12.69 , 70.31 ± 21.21 and 77.70 ± 22.95 fronds. A post-hoc test (with Bonferroni corrections) for differences among levels shows that all reference populations differ significantly (adjusted $p < 0.05$), and the competing 'async' population is significantly different from both 'mid-async' and 'sync' competing populations ($p < 0.0001$), while the latter two do not differ significantly ($p = 0.078$).

The same two-way ANOVA model, to test for effects of reactivation of *S. polyrhiza* and overall competition, was performed with growth rate as the response variable. Significant effects of reactivation ($F(2, 277) = 688.23, p < 0.0001$) and competition treatments were identified ($F(1, 277) = 11.63, p < 0.001$). No interaction effect was found ($F(2, 227) = 2.21, p = 0.111$). Final mean (+SE) growth rates for 'async', 'mid-async', and 'sync' reference populations were 0.257 ± 0.029 , 0.198 ± 0.034 , and 0.161 ± 0.037 fronds per day, respectively. Similarly, competing populations of each reactivation treatment, in the same order, had growth rates (+SE) 0.256 ± 0.033 , 0.188 ± 0.039 and 0.146 ± 0.037 fronds per day. A post-hoc test shows that mean growth rates differ among levels of synchrony for both reference and competing populations (adjusted $p < 0.001$ for each comparison).

To follow up on the previous analyses using the simple presence/absence of competitors, the two-way ANOVAs were refined to include each of the 4 unique species combinations as levels of competition (categorical). The ANOVA for effects of reactivation treatment and competition on final frond counts shows a significant effect of reactivation ($F(2, 271) = 76.01, p < 0.0001$) and a significant effect of competition ($F(3, 284) = 5.14, p < 0.01$). In addition, a significant interaction effect was identified ($F(6, 271) = 3.363, p < 0.01$). A post-hoc test was then run to analyze where differences occurred. A closer look at the within-group differences shows that the 'Spol' (reference) population is significantly different from treatments 'Spol + Lmin + Lpun' ($p < 0.01$) and 'Spol + Lpun' ($p < 0.05$). These differences are highlighted in **figure 3A**, and the final frond counts for *S. polyrhiza* in this analysis are summarized in **table 1**.

The two-way ANOVA that tests for the effects of reactivation treatment and competition (with all 4 unique levels) on growth rates shows a statistically significant effect of reactivation ($F(2, 271) = 719.16, p < 0.0001$), competition ($F(3, 271) = 5.05, p < 0.01$) and their interaction ($F(6, 271) = 3.34, p < 0.01$). A similar pattern of growth rates across levels of asynchrony was found here as in the first growth rate analysis, where 'async' $r >$ 'mid-async' $r >$ 'sync' r (**figure 3B**). The mean growth rates (+SE) are summarized in **table 2**.

Budding growth rate analyses

A two-way ANOVA was run to test for growth differences attributable to synchrony and competition treatments using budding growth rates (see Methods for explanation of budding growth rates and **appendix 3**). The calculations for expected overall growth rates of asynchronous populations are based on budding growth rates observed in synchronous reference populations and are summarized in **supplementary S4**. While no signal was found from competition ($F(3, 167) = 0.439, p = 0.726$), an effect of synchrony treatment was evident ($F(1, 167) = 71.77, p < 0.0001$). No interaction effect was present ($F(3, 167) = 0.737, p = 0.531$). Mean observed overall growth rates (0.248 ± 0.031) were roughly 8.2% lower than the expected values (0.270 ± 0.027 ; **figure 4**).

Both trials

For the second trial, only 6 complete replicates of 24 reached the final round of reactivation and could be included in analyses (**supplementary S5**). During this period of the growing season thick layers of algae grew near the water surface. Despite attempts at algae removal, most duckweed communities grew poorly. The replicates that achieved

final reactivation grew for 17 – 23 days. Although the two trials had slightly different design, for completeness they may be combined into a single analysis with the addition of the categorical effect, “trial.” Because trial 2 includes two synchrony levels, the “mid-async” from trial 1 was omitted from this analysis. In these analyses, ‘tank’ is nested within ‘trial’.

To analyze the effects of reactivation and competition on final frond counts across trials, a three-way ANOVA was conducted (**appendix 4**). All three main effects were significant (Reactivation: $F(1, 227) = 79.4, p < 0.0001$; Competition: $F(3, 227) = 4.4, p < 0.01$; Trial: $F(1, 227) = 1287.1, p < 0.0001$). The only significant interaction effect was that between trial and reactivation treatment ($F(1, 227) = 12.9, p < 0.001$). The same analysis as that for frond count was run for growth rates, and included the effects of reactivation treatment, competition, and trial. Reactivation and trial have significant effects on growth rates in this system (Reactivation: $F(1, 227) = 506.7, p < 0.0001$; Trial: $F(1, 227) = 4.77, p < 0.05$), whereas competition showed no significant effect on growth rates ($F(3, 227) = 1.89, p = 0.132$). An interaction between trial and reactivation treatment was also found ($F(3, 227) = 4.94, p = 0.027$).

Inherent cost of asynchrony

The above studies attempt to test for inherent costs of asynchrony by controlling for the more obvious fitness effects of reactivating early or late. A final two-way ANOVA was run to analyze among-tank variation and asynchrony effects on fitness in a side experiment (**supplementary S6**) that did not account for direct effects of early/late timing (**appendix 5**). ‘Tank’ in this case is considered a fixed effect. This analysis shows a

significant effect of synchrony treatment ($F(1, 24) = 180.65, p < 0.001$), with means ($\pm$ SE) 49.13 ± 41.00 and 143.96 ± 94.12 fronds for asynchrony and synchrony treatments respectively. Significant among-tank variation ($F(11, 24) = 27.348, p < 0.001$), and a significant interaction between synchrony treatment and tank ($F(11, 24) = 7.359, p < 0.001$) were also found.

Discussion

The objective of this study was to assess whether the inherent advantages of asynchrony in *S. polyrhiza* turion reactivation are sufficient to explain its evolution without recourse to bet hedging. Through the lens of an evo-to-eco pathway, effects of competition with other duckweed species were also tested. Final frond counts and growth rates were measured to test for effects of reactivation asynchrony as well as competition. Additional analyses regarding the budding growth rates and inherent costs of asynchrony were also run.

Budding growth rate analysis

Since the observed reference sync populations reproduce exclusively by budding, they should therefore be representative of budding rates within mesocosms. Thus, expected overall growth rates were calculated in an analysis that tests budding growth rates in asynchronous settings. Recall that 'observed' in this analysis refers to the overall growth rate measured in the async populations, while 'expected' refers to overall growth rates calculated based on the budding rates retrieved from the reference sync populations. Given that reactivation patterns identical to those in the observed asynchronous populations were used to derive the expected values, any discrepancies could only come from differences in budding growth rates in asynchronous populations. While async budding rates were not directly measured in the experiment, the fact that asynchronous *S. polyrhiza* populations overall grew slower than expected by 8.22% allows us to infer that budding growth rates in asynchronous populations were significantly lower than in synchronous populations. This would translate to an overall twofold numbers difference in a period of one month, and this would only increase over longer periods of

time. Overall growth rate in the asynchronous populations of this study is therefore likely to have decreased relative to synchronous populations as a result of this previously unobserved cost of asynchronous reactivation in budding growth.

Final frond count analyses

The reference populations allowed for the correction of any discrepancies between reactivation trials. An effect of reactivation was evident in the main study where final frond counts were higher for synchronous populations in competing and reference settings. The differences among reference populations can additionally be seen as the null expectation for each level of competition. Having the same effect of reactivation across levels of competition shows that species present do not have a strong effect on *S. polyrhiza* populations in this study. Evidence was found suggesting that competition could impact final frond counts, and the post-hoc analysis points to a difference between reference populations and the three-species competition level in synchronous populations. This difference was not found in mid-async and async treatments.. Competition among duckweed is commonly observed (Barrat-Segretain, 1996; Armitage & Jones, 2019), and this work indicates that the evolution of asynchrony is costly and more so in the context of competition. While it is also possible that sync populations simply began with too many fronds to reach equal final frond counts as async or mid-async populations, a cost in terms of budding growth rate is likely to have impacted these values. Future work may narrow in on budding growth with more measurements of *S. polyrhiza* for a higher resolution analysis of population trends.

Overall growth rate

The analysis centered on overall growth rates shows that growth is highest for async populations and decreases as reactivation becomes more synchronous. This pattern was expected since synchronous populations are the only ones to not receive additional fronds by reactivation while mid-async populations experienced an intermediary level of reactivation. Having observed a significant effect of competition in growth rate analyses, we may expect to observe stronger competition among fronds in natural pond systems. Higher levels of density are known to affect duckweed due to limiting resources such as nutrients and light when fronds overlap one another, impacting overall growth (Armitage & Jones 2019; Zhang et al., 2020). The overall increase in growth rate as well as a decrease in budding growth rate suggests that there may be a notable exchange between reproduction strategies in asynchronous populations.

Both trials; effects on frond count and overall growth rate

After the main experiment was complete, it was recognized that a higher level of asynchrony could be achieved to enrich the study. A second trial was then run, but due to abnormally high algae growth, not enough replication within tanks was achieved to run the budding growth rate analysis with this new dataset. The pH was likely too high for duckweed but just right for algae to outcompete duckweed in these populations (Szabó et al., 2005), and for this reason, only the analyses of overall growth and final frond counts could be conducted to test if the effects on these response variables would differ among trials. What was found, however, was that frond counts and growth rates behaved much the same way in Trial 2 as they did in Trial 1. Reactivation seems to benefit synchronous

populations over asynchronous ones while there are no strong effects of competition. The only difference was that the advantage of synchronous populations in terms of frond counts was higher in trial 2 than in trial 1. The significant interaction effect in this analysis shows that the advantage of synchronicity for final frond counts seems to increase over longer periods of time. Since it was still 'sync' populations that had the apparent advantage, the results from each trial in terms of frond counts are qualitatively identical. For growth rates, the effects of competition were statistically non-significant when both trials were lumped together in the analysis. This may be due to competing species having the same starting population sizes across trials while *S. polyrhiza* had more than double the frond counts to begin and end with in this portion of the experiment. Relatively, this would be a case where competition was artificially decreased in Trial 2, and having adjusted the competing populations similarly may have resulted in similar analyses to Trial 1. Future work would benefit from also considering variation at the level of competing species.

Side experiments; advantages and costs of asynchrony

The above studies attempt to test for inherent costs to asynchrony while controlling for fronds reactivating later than others. An additional and simple experiment was run to measure the fitness advantage of synchronous reactivation as opposed to asynchrony without controlling for these timing disadvantages. In this analysis, the fitness of any given frond decreases the later it reactivates, while reactivating synchronously as early as possible provides a higher advantage in absence of unfavourable environmental conditions. This demonstrates an inherent cost of reactivating asynchronously: losing

time in which to reproduce. Accounting for this cost was the primary reason to control for reactivation timing and initial frond counts. Though the quantities were arbitrary, ensuring that the same number of fronds was introduced to each reactivation treatment allows for this example of an inherent cost of asynchronous reactivation to take place.

Alternative explanation

In terms of the original hypothesis, the inherent advantages of asynchrony, notably the increased overall growth rate, come with an additional cost of decreased budding growth rates and a disadvantage in frond counts relative to a synchronous reactivation pattern. This reduces the maximum fitness potential of *S. polyrhiza* in the short term where synchronous populations can achieve higher frond counts earlier in the season, which is consistent with diversification bet-hedging strategies (A. Simons, 2011; S. Schreiber, 2015). The prevalence of asynchrony despite the apparent costs indicates that this study provides indirect evidence that asynchrony evolved as a bet-hedging strategy.

Fortuitous test of the advantage of asynchrony in rare unfavourable conditions

Given that this study only considered favourable environmental conditions, the evolution of synchronicity would be expected to occur. However, rare freeze-over events can occur early in the Spring in north temperate regions, affecting duckweed species (Morris et al, 2020). The benefits of asynchrony during rare unfavourable events were not originally part of the experimental design, but fortuitous circumstances arose in which this could be tested. Specifically, a period of abnormally low growth across all populations

took place and populations declined rapidly. This prompted a reset of the experiment, but some of these populations were kept to test and measure the advantage of asynchronous reactivation (**appendix 6**). Synchronous reference populations that experienced unfavourable conditions were compared to asynchronous populations from the same mesocosms after resetting the water and nutrients for each mesocosm, but only asynchronous populations continued to experience reactivation. While this was not a freeze-over event, the question is centered on how asynchrony is beneficial during periods of unfavourable conditions overall. Under these conditions, asynchrony is shown to have a much higher fitness over synchronicity by avoiding the negative impacts on its fitness (see **supplementary S7**). This result supports the notion that asynchrony evolved as a diversification bet-hedging strategy because the advantage of asynchrony only outweighs its costs when there are strongly unfavourable conditions. As such, we may expect asynchronous turion reactivation to be favoured in the long-term by maximizing geometric mean fitness.

Known sources of variation

Given that this study focuses on a trait evolved as a response to environmental variation, it's important to recognize some of the potential sources of additional variation that do not originate from reactivation asynchrony itself. For instance, it is known that higher densities promote intraspecific competition effects (Zhang et al., 2020). This goes beyond interspecific competition in the sense that the presence of additional layers of *S. polyrhiza* may hinder its own growth in natural populations. To reiterate, designing to end the experiment before a point where density effects may cause negative density effects

allows for more accurate inferences on the effects of reactivation itself. In addition to negative effects at higher densities, there is also the possibility of allee effects being present, i.e., positive reinforcement of growth at higher population densities. While this study was not designed to tease apart these effects, future work would benefit from including density as a factor in the experimental design to test for positive and negative effects of higher population densities.

It is also known that birth order and turion phenology evolve across populations (Hitsman & Simons, 2020). Since the reactivation treatment was based on introducing randomly selected fronds from a stock population in similar conditions, it is unlikely that birth order had an impact on the study. Meanwhile, using a single clone for the stock population also removes possible genetic components. Similarly, every frond that was introduced into the mesocosms was separated from its mother/sibling fronds to control for the potential effects of fragmentation. *S. polyrhiza* fronds have been observed to grow faster when separated from its colonies due to the release from having to spend nutrients and resources to maintain their connective ramets (Zhang et al., 2020), and this effect has also been observed in other stoloniferous plants (Wang et al., 2014). Overall, the controlling for these potential sources of variation ensures that the main effects originate from reactivation or competition.

Conclusion

This study has shown that asynchrony has two particular costs: one is the inherent cost of time in which to reproduce, and the second is a previously unobserved cost in budding growth rates. The benefits of synchronicity shown here highlight that an even

stronger benefit to asynchrony is more likely to be present given that it is the naturally observed phenotype in *S. polyrhiza* turion reactivation. In terms of the original hypothesis, these additional costs suggest that diversification bet-hedging is a more suitable explanation for the evolution of asynchrony. By chance, I was able to test this during a period of abnormally low growth in the mesocosms where asynchrony resulted in much higher fitness, providing a unique and important piece of evidence for the evolution of asynchrony as a bet-hedging strategy in *S. polyrhiza*. Future work on this system may center on effects of density, budding growth rates over time, and fitness benefits in a longer time frame. While difficult to perform experiments over longer time frames, theoretical frameworks may also be of use with known parameters under specific conditions. In terms of environmental unpredictability, this study takes the understanding of variation traits one step forward.

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Tables

Reactivation	Competition	Final frond count
<u>async</u>	<i>Spol + Lmin</i>	57.38 ± 15.38
	<i>Spol + Lpun</i>	55.13 ± 13.75
	<i>Spol + Lmin + Lpun</i>	51.28 ± 7.48
<u>mid-async</u>	<i>Spol + Lmin</i>	64.84 ± 21.42
	<i>Spol + Lpun</i>	75.60 ± 20.55
	<i>Spol + Lmin + Lpun</i>	70.48 ± 21.10
<u>sync</u>	<i>Spol + Lmin</i>	85.12 ± 23.13
	<i>Spol + Lpun</i>	74.29 ± 20.09
	<i>Spol + Lmin + Lpun</i>	73.38 ± 24.41

Table 1. Summary of *S. polyrhiza* final frond counts across reactivation and non-reference competition treatments in Trial 1.

Reactivation	Competition	Growth rate (day ⁻¹)
<u>async</u>	<i>Spol + Lmin</i>	0.235 ± 0.051
	<i>Spol + Lpun</i>	0.228 ± 0.045
	<i>Spol + Lmin + Lpun</i>	0.227 ± 0.048
<u>sync</u>	<i>Spol + Lmin</i>	0.148 ± 0.032
	<i>Spol + Lpun</i>	0.149 ± 0.029
	<i>Spol + Lmin + Lpun</i>	0.148 ± 0.031

Table 2. *S. polyrhiza* overall growth rates across reactivation and non-reference competition treatments in Trial 1.

Figures

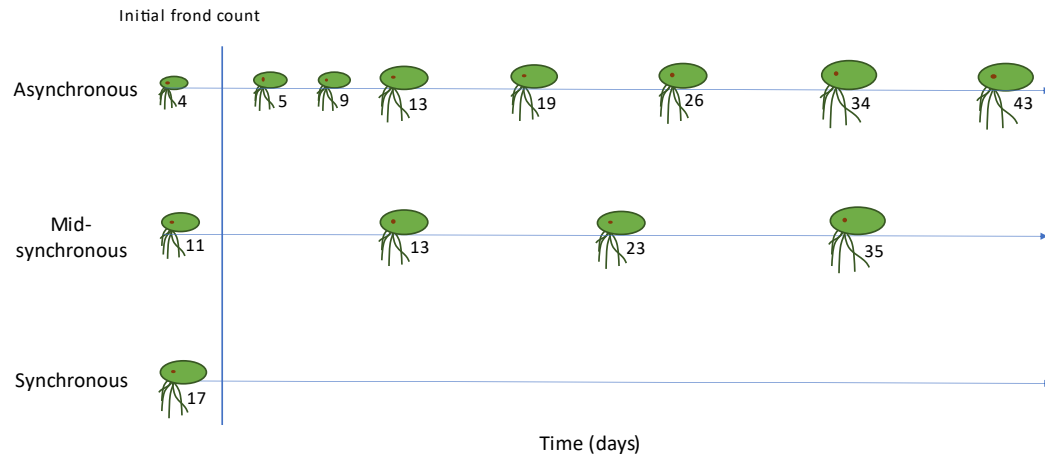


Figure 1. Frond count benchmarks for scheduling of reactivation for the three synchrony treatments. Shown are the frond counts for the *S. polyrhiza* reference populations that indicate the appropriate times at which the competing communities will have new *S. polyrhiza* fronds introduced such that final population sizes are expected to be equal. ‘**Async**’ populations will have 3 fronds added when each benchmark is reached. Similarly, ‘**mid-async**’ populations will receive 4 fronds at each benchmark. Final counts are expected to be 46 across reactivation treatments using this schedule, calculated using growth rates observed for *S. polyrhiza* in Appenroth’s nutrient medium (Appenroth, Keller & Horin, 1996) under laboratory conditions of 18°C and 14/10 photoperiod (Morris & Simons, 2018).

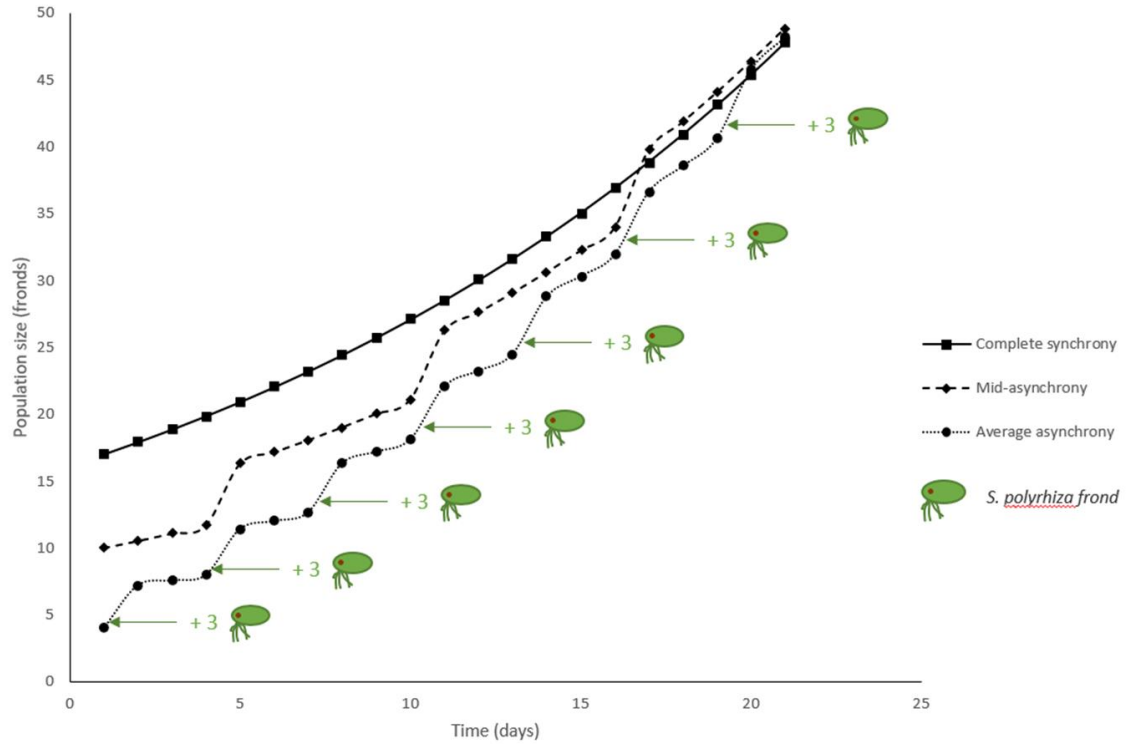


Figure 2. Expected *S. polyrhiza* population trends calculated using growth rate $r = 0.053$ (measured in the lab at 18°C ; see **supplementary S1**) with reactivation schedules for each reactivation treatment (async, mid-async, sync). Shown are the additions of *S. polyrhiza* at each benchmark frond count for 'async' populations. 'Mid-async' populations will experience fewer reactivations with more fronds. Final population sizes are expected to be 46 at day 21 under these conditions.

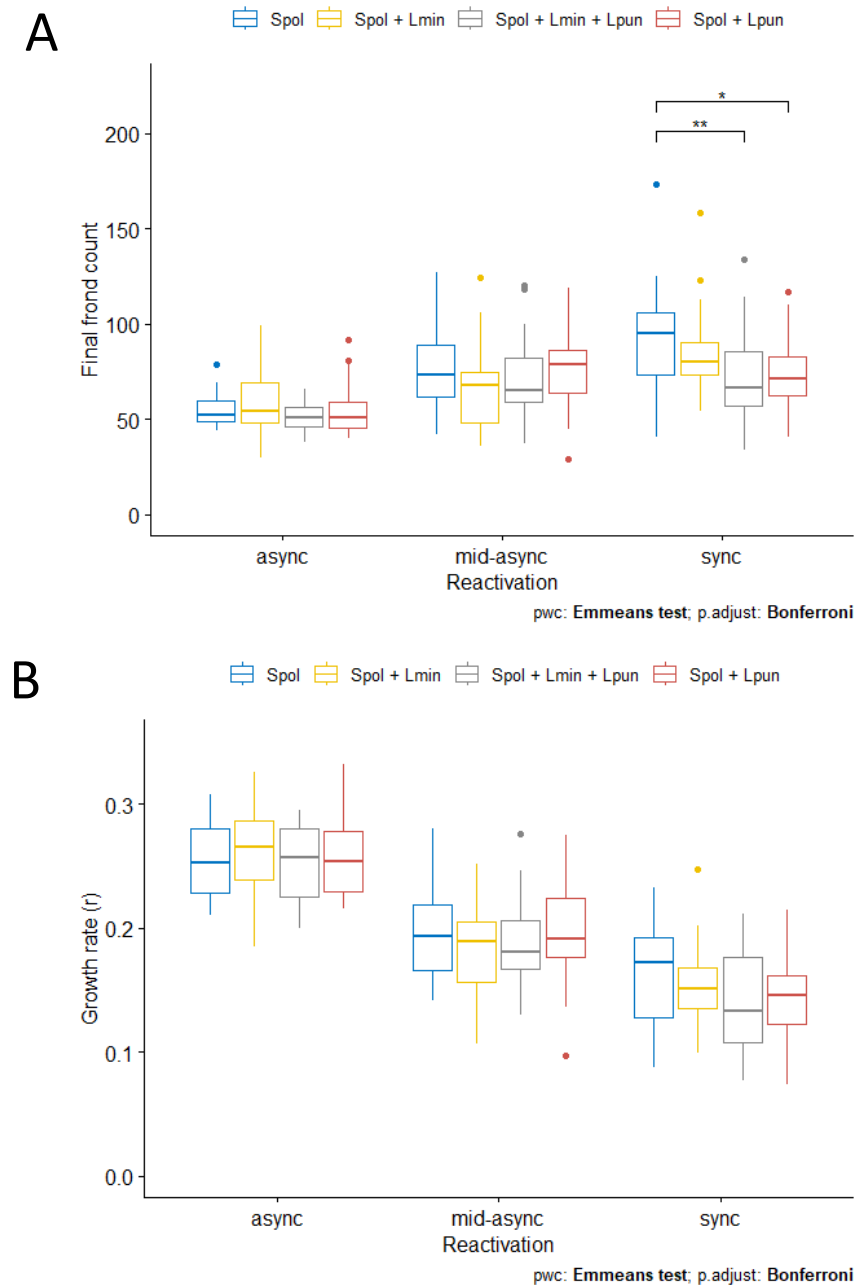


Figure 3. *S. polyrhiza* final frond counts (A) and growth rates (B) in response to competition and reactivation treatments in Trial 1. Shown are the effects of reactivation and competition on frond count (left) and on growth rates (right). Significance values for pairwise comparisons plotted: **** <0.0001 , *** <0.001 , ** <0.01 , * <0.05 , and non-significant within-group differences ($p > 0.05$) are not plotted.

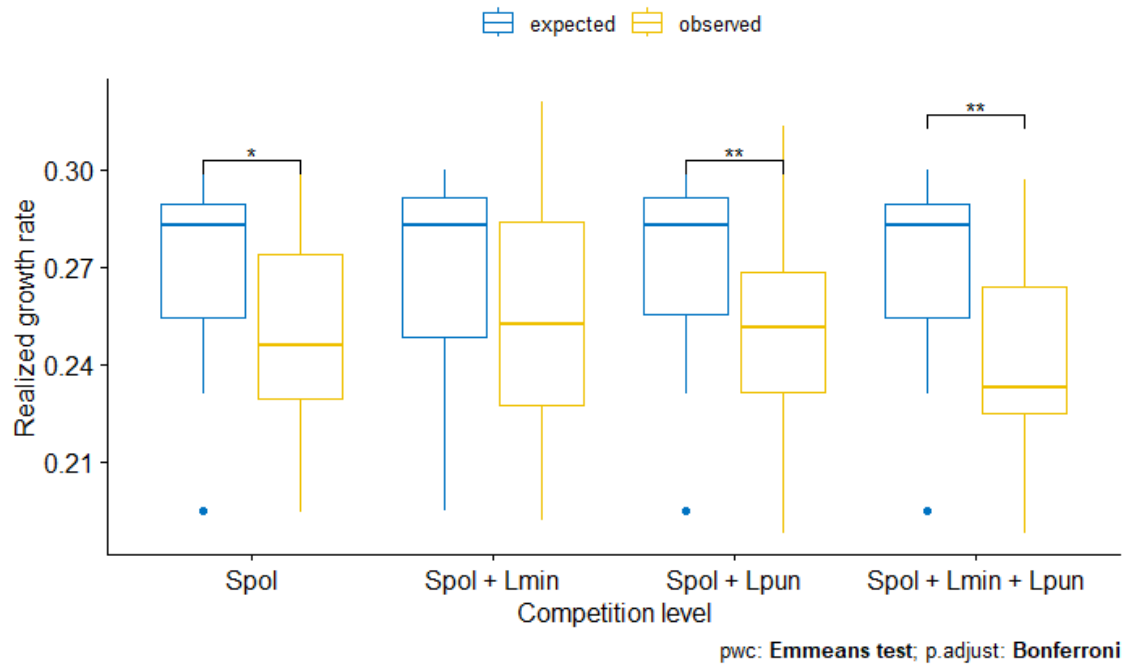


Figure 4. Expected (red) and observed (blue) overall growth rates of asynchronous *S. polyrhiza* populations in trial 1. Expected growth rates for every population that included an asynchronous treatment for *S. polyrhiza* were calculated based on budding growth rates (**sync** reference populations) and the reactivation patterns from the respective 'async' populations in each tank. Recall that reference 'sync' populations grew exclusively by budding. All else being equal, observed growth rates should be identical to expected values. Significance values for pairwise comparisons plotted: **** <0.0001 , *** <0.001 , ** <0.01 , * <0.05 , and non-significant within-group differences ($p > 0.05$) are not plotted.

Appendices



Appendix 1. Photo of one of the many floating rafts used in the main and side experiments. 24 were used in each mesocosm to house the duckweed communities throughout the main experiment.

Appendix 2: Trial 1 analyses

Dataset required: 'S3. Trial 1 data.csv'

2023-05-05

```
library(jtools)
library(lmerTest)
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##      lmer
## The following object is masked from 'package:stats':
##
##      step
library(tidyr)
##
## Attaching package: 'tidyr'
## The following objects are masked from 'package:Matrix':
##
##      expand, pack, unpack
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##      filter, lag
## The following objects are masked from 'package:base':
##
##      intersect, setdiff, setequal, union
library(readr)
library(ggplot2)
library(ggpubr)
## Registered S3 methods overwritten by 'broom':
##      method                from
```

```

## tidy.glht jtools
## tidy.summary.glht jtools
library(lme4)
library(emmeans)
library(rstatix)

##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
## filter
library(nlme)
##
## Attaching package: 'nlme'
## The following object is masked from 'package:dplyr':
##
## collapse
## The following object is masked from 'package:lme4':
##
## lmList
library(readODS)
## Warning: package 'readODS' was built under R version 4.2.3
d1 <- read.csv(choose.files()) #choose 'S3. Trial 1 analyses data.csv'. May need to save .ods file as .csv.
d1$rf <- as.numeric(d1$rf)
d1$tank <- as.character(d1$tank)
d1$d$c.rf <- as.numeric(d1$d$c.rf)
d1 <- d1[!is.na(d1$rf),]
summary(d1)

##      tank      comp      guide      species
## Length:591 Length:591 Length:591 Length:591
## Class :character Class :character Class :character Class :character
## Mode :character Mode :character Mode :character Mode :character
##
##
##
##
## asynchrony reactivation raft timing

```

```

## Length:591      Length:591      Min.   : 1.0      Length:591
## Class :character Class :character 1st Qu.: 79.5      Class :character
## Mode  :character Mode  :character Median :201.0      Mode  :character
##                                     Mean   :183.8
##                                     3rd Qu.:276.5
##                                     Max.   :360.0
##
## starting.pop      day.0           day.1           day.2
## Min.   : 4.00      Min.   : 4.00      Min.   : 4.00      Min.   : 4.00
## 1st Qu.:10.00      1st Qu.:10.00      1st Qu.:10.00      1st Qu.:10.00
## Median :10.00      Median :10.00      Median :10.00      Median :10.00
## Mean   :10.17      Mean   :10.13      Mean   :10.17      Mean   :11.96
## 3rd Qu.:10.00      3rd Qu.:10.00      3rd Qu.:10.00      3rd Qu.:15.00
## Max.   :17.00      Max.   :17.00      Max.   :17.00      Max.   :27.00
##                                     NA's   :401      NA's   :352      NA's   :239
## day.4            day.6           day.11          day.13
## Min.   : 0.00      Min.   : 6.00      Min.   : 0.00      Min.   : 0.00
## 1st Qu.: 9.50      1st Qu.:16.00      1st Qu.: 0.00      1st Qu.: 0.00
## Median :13.00      Median :22.00      Median : 44.00      Median : 0.00
## Mean   :13.17      Mean   :23.74      Mean   : 41.39      Mean   : 15.74
## 3rd Qu.:19.00      3rd Qu.:30.00      3rd Qu.: 64.00      3rd Qu.: 23.00
## Max.   :46.00      Max.   :56.00      Max.   :158.00      Max.   :173.00
##
## rf              finalpop
## Min.   :0.02186      Min.   : 13.00
## 1st Qu.:0.11440      1st Qu.: 34.00
## Median :0.16292      Median : 54.00
## Mean   :0.16535      Mean   : 56.44
## 3rd Qu.:0.21231      3rd Qu.: 73.00
## Max.   :0.33146      Max.   :173.00
##
dsp1 <- select(filter(dl, species == "sp"), !starts_with("day"))
head(dsp1)
## tank      comp      guide species asynchrony reactivation raft timing
## 1      1      Spol reference      sp      A1      async      1      start

```

```

## 2      1      Spol + Lmin competing      sp      A1      async      7      st
art
## 3      1      Spol + Lpun competing      sp      A1      async     13      st
art
## 4      1 Spol + Lmin + Lpun competing      sp      A1      async     19      st
art
## 5      1      Spol reference      sp      A2      async      2      st
art
## 6      1      Spol + Lmin competing      sp      A2      async      8      st
art

##      starting.pop      rf finalpop
## 1      4 0.2505526      49
## 2      4 0.2525729      50
## 3      4 0.2583998      53
## 4      4 0.2564949      52
## 5      4 0.2740840      62
## 6      4 0.2691243      59

dspcomp1 <- select(filter(dsp1, guide == "competing"), !starts_with("day"))
head(dspcomp1)

##      tank      comp      guide species asynchrony reactivation raft tim
ing
## 1      1      Spol + Lmin competing      sp      A1      async      7      st
art
## 2      1      Spol + Lpun competing      sp      A1      async     13      st
art
## 3      1 Spol + Lmin + Lpun competing      sp      A1      async     19      st
art
## 4      1      Spol + Lmin competing      sp      A2      async      8      st
art
## 5      1      Spol + Lpun competing      sp      A2      async     14      st
art
## 6      1 Spol + Lmin + Lpun competing      sp      A2      async     20      st
art

##      starting.pop      rf finalpop
## 1      4 0.2525729      50
## 2      4 0.2583998      53
## 3      4 0.2564949      52
## 4      4 0.2691243      59
## 5      4 0.2564949      52
## 6      4 0.2583998      53

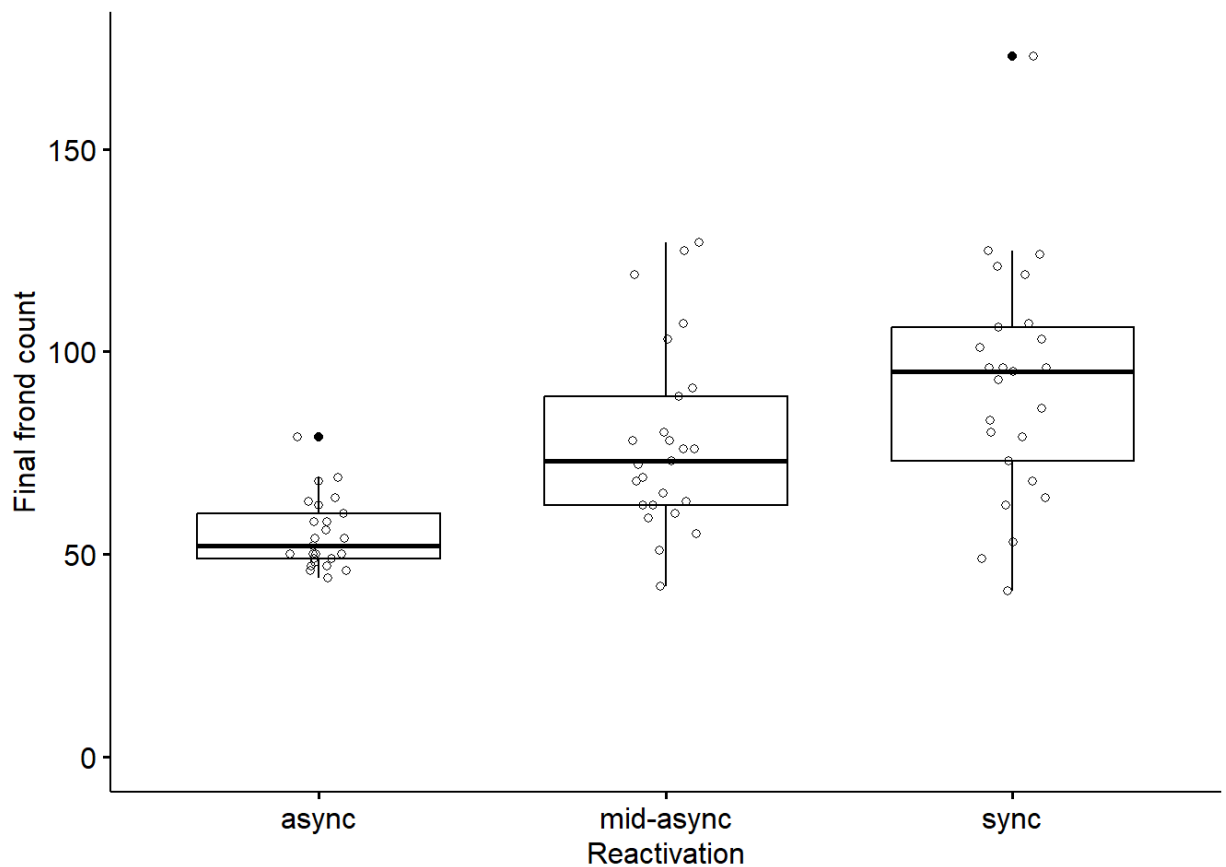
dspref1 <- select(filter(d1, guide == "reference"), !starts_with("day"))
ggboxplot(dspref1, x = "reactivation", y = "finalpop",

```

```

    order = c("async", "mid-async", "sync")) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  ylim(0,175) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Final frond count") +
  theme(legend.position="none")

```



```

group_by(dsprefl, reactivation) %>%
  summarise(
    count = n(),
    mean = mean(finalpop, na.rm = TRUE),
    sd = sd(finalpop, na.rm = TRUE)
  )
## # A tibble: 3 × 4
##   reactivation count mean    sd

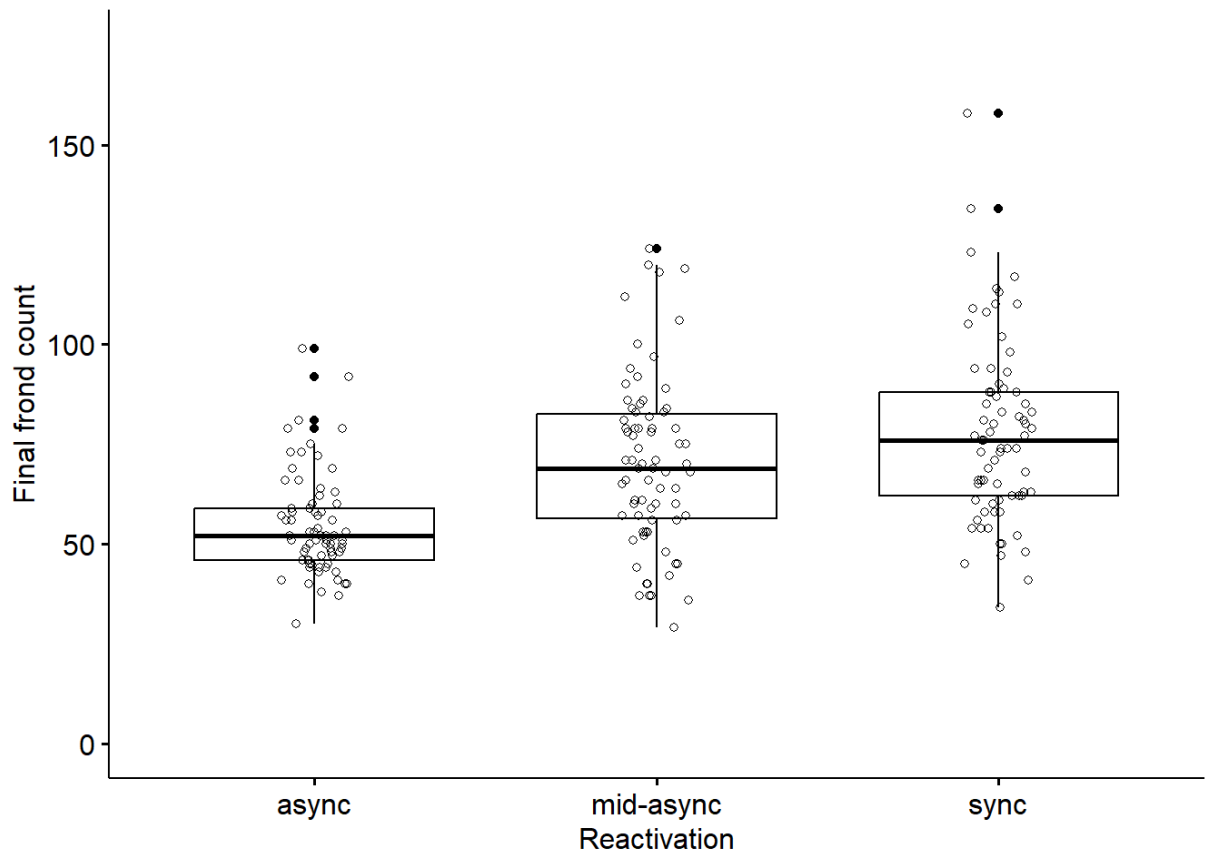
```

```
##      <chr>          <int> <dbl> <dbl>
## 1 async           25  54.9  8.71
## 2 mid-async       25   78   22.8
## 3 sync            25  91.7 28.8

anoval <- aov(finalpop ~ reactivation + Error(tank), data = dspref1)
summary(anoval)

##
## Error: tank
##           Df Sum Sq Mean Sq F value Pr(>F)
## Residuals 13  15391    1184
##
## Error: Within
##           Df Sum Sq Mean Sq F value    Pr(>F)
## reactivation  2  17293    8647    27.1 4.47e-09 ***
## Residuals    59  18822     319
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ggboxplot(dspcompl, x = "reactivation", y = "finalpop",
          order = c("async", "mid-async", "sync")) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  ylim(0,175) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Final frond count") +
  theme(legend.position="none")
```



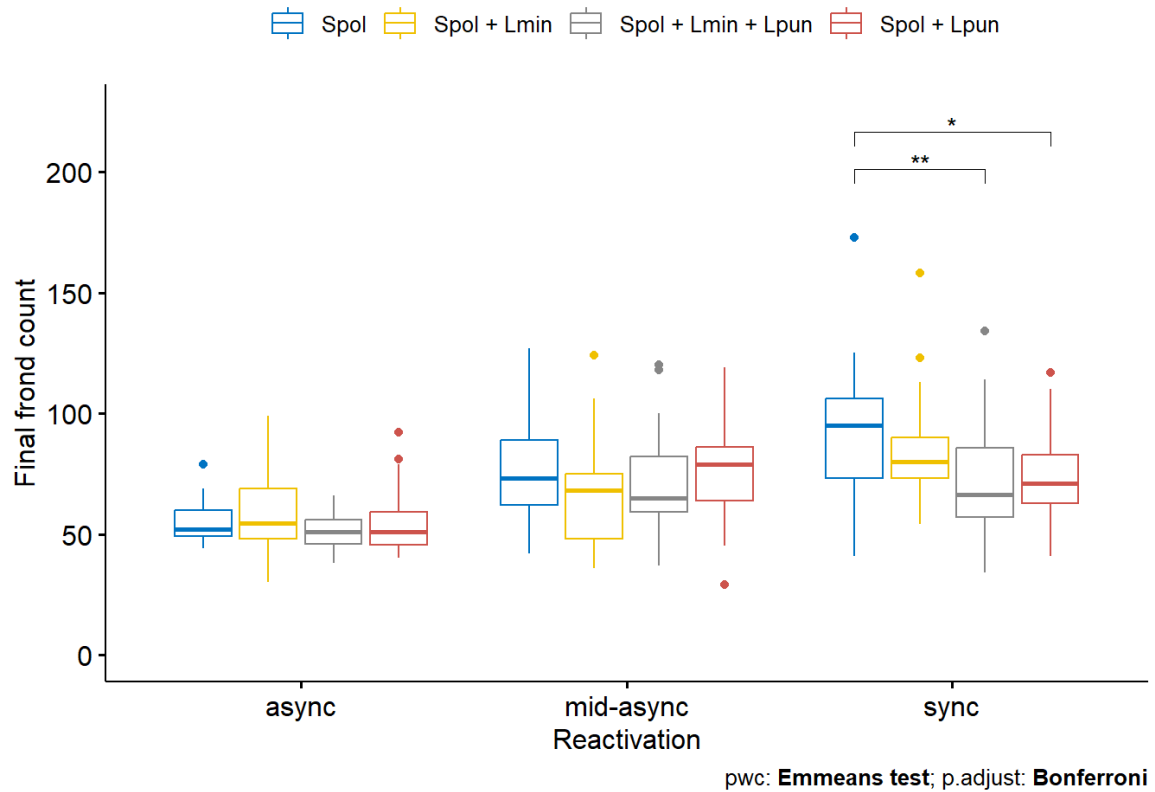
```
group_by(dspcompl, reactivation) %>%
  summarise(
    count = n(),
    mean = mean(finalpop, na.rm = TRUE),
    sd = sd(finalpop, na.rm = TRUE)
  )
## # A tibble: 3 × 4
##   reactivation count  mean    sd
##   <chr>         <int> <dbl> <dbl>
## 1 async           73  54.5  12.7
## 2 mid-async       75  70.3  21.2
## 3 sync           73  77.7  23.0
anova1 <- aov(finalpop ~ reactivation + Error(tank), data = dspcompl)
summary(anova1)
##
## Error: tank
##
##           Df Sum Sq Mean Sq F value Pr(>F)
```

```
## reactivation 2 8152 4076 1.584 0.249
## Residuals 11 28313 2574
##
## Error: Within
##
## Df Sum Sq Mean Sq F value Pr(>F)
## reactivation 2 20709 10355 46.08 <2e-16 ***
## Residuals 205 46062 225
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
res.aov <- dsp1 %>% anova_test(finalpop ~ reactivation * comp)
res.aov
## ANOVA Table (type II tests)
##
## Effect DFn DFd F p p<.05 ges
## 1 reactivation 2 284 45.077 9.96e-18 * 0.241
## 2 comp 3 284 3.105 2.70e-02 * 0.032
## 3 reactivation:comp 6 284 2.131 5.00e-02 * 0.043
pwc <- dsp1 %>%
  group_by(reactivation) %>%
  emmeans_test(finalpop ~ comp, p.adjust.method = "bonferroni") %>%
  add_xy_position(x = "reactivation")
pwc
## # A tibble: 18 × 15
## reactivation term .y. group1 group2 df stati...1 p p.adj p.a
dj...2
## <chr> <chr> <chr> <chr> <chr> <dbl> <dbl> <dbl> <dbl> <chr>
## 1 async comp final... Spol Spol ... 284 -0.431 0.667 1 ns
## 2 async comp final... Spol Spol ... 284 0.645 0.519 1 ns
## 3 async comp final... Spol Spol ... 284 -0.0360 0.971 1 ns
## 4 async comp final... Spol ... Spol ... 284 1.07 0.286 1 ns
## 5 async comp final... Spol ... Spol ... 284 0.391 0.696 1 ns
## 6 async comp final... Spol ... Spol ... 284 -0.675 0.501 1 ns
## 7 mid-async comp final... Spol Spol ... 284 2.33 0.0204 0.122 ns
## 8 mid-async comp final... Spol Spol ... 284 1.33 0.184 1 ns
## 9 mid-async comp final... Spol Spol ... 284 0.425 0.671 1 ns
## 10 mid-async comp final... Spol ... Spol ... 284 -1.00 0.318 1 ns
```

```

## 11 mid-async      comp  final... Spol ... Spol ... 284 -1.91 0.0575 0.345 ns
## 12 mid-async      comp  final... Spol ... Spol ... 284 -0.907 0.365 1 ns
## 13 sync           comp  final... Spol   Spol ... 284 1.17 0.243 1 ns
## 14 sync           comp  final... Spol   Spol ... 284 3.22 0.00144 0.00864 **
## 15 sync           comp  final... Spol   Spol ... 284 3.06 0.00245 0.0147 *
## 16 sync           comp  final... Spol ... Spol ... 284 2.06 0.0403 0.242 ns
## 17 sync           comp  final... Spol ... Spol ... 284 1.90 0.0585 0.351 ns
## 18 sync           comp  final... Spol ... Spol ... 284 -0.159 0.874 1 ns
## # ... with 5 more variables: y.position <dbl>, groups <named list>, x <dbl>,
## #   xmin <dbl>, xmax <dbl>, and abbreviated variable names 'statistic,
## #   ^p.adj.signif
ggboxplot(
  dspl, x = "reactivation", y = "finalpop",
  color = "comp", palette = "jco" ) +
  stat_pvalue_manual(pwc, hide.ns = TRUE) +
  ylim(0,225) +
  theme(legend.title=element_blank()) +
  labs(x = "Reactivation",
       y = "Final frond count",
  #   subtitle = get_test_label(res.aov, detailed = TRUE),
  #   caption = get_pwc_label(pwc)
  )

```



```
group_by(dspl, reactivation, comp) %>%
  summarise(
    count = n(),
    mean = mean(finalpop, na.rm = TRUE),
    sd = sd(finalpop, na.rm = TRUE)
  )
## `summarise()` has grouped output by 'reactivation'. You can override using the
## `.groups` argument.
## # A tibble: 12 × 5
## # Groups:   reactivation [3]
##   reactivation comp          count mean    sd
##   <chr>         <chr>         <int> <dbl> <dbl>
## 1 async        Spol             25  54.9  8.71
## 2 async        Spol + Lmin       24  57.4 15.4
## 3 async        Spol + Lmin + Lpun 25  51.3  7.48
## 4 async        Spol + Lpun       24  55.1 13.8
## 5 mid-async    Spol             25   78   22.8
```

```

## 6 mid-async      Spol + Lmin          25  64.8 21.4
## 7 mid-async      Spol + Lmin + Lpun    25  70.5 21.1
## 8 mid-async      Spol + Lpun           25  75.6 20.5
## 9 sync           Spol                  25  91.7 28.8
## 10 sync          Spol + Lmin           25  85.1 23.1
## 11 sync          Spol + Lmin + Lpun    24  73.4 24.4
## 12 sync          Spol + Lpun           24  74.3 20.1

finalpop.async.anova1 <- aov(finalpop ~ reactivation * guide + Error(tank), data = dsp1)

summary(finalpop.async.anova1, digits = 5)

##
## Error: tank
##
##           Df Sum Sq Mean Sq F value Pr(>F)
## reactivation  2   9329    4665   1.284  0.315
## Residuals    11  39953    3632
##
## Error: Within
##
##           Df Sum Sq Mean Sq F value    Pr(>F)
## reactivation    2  35958   17979  73.687 < 2e-16 ***
## guide           1   3187    3187  13.062 0.000358 ***
## reactivation:guide  2   1745     872   3.576 0.029299 *
## Residuals      277  67585     244
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

finalpop.async.anova1 <- aov(finalpop ~ reactivation * comp + Error(tank), data = dsp1)

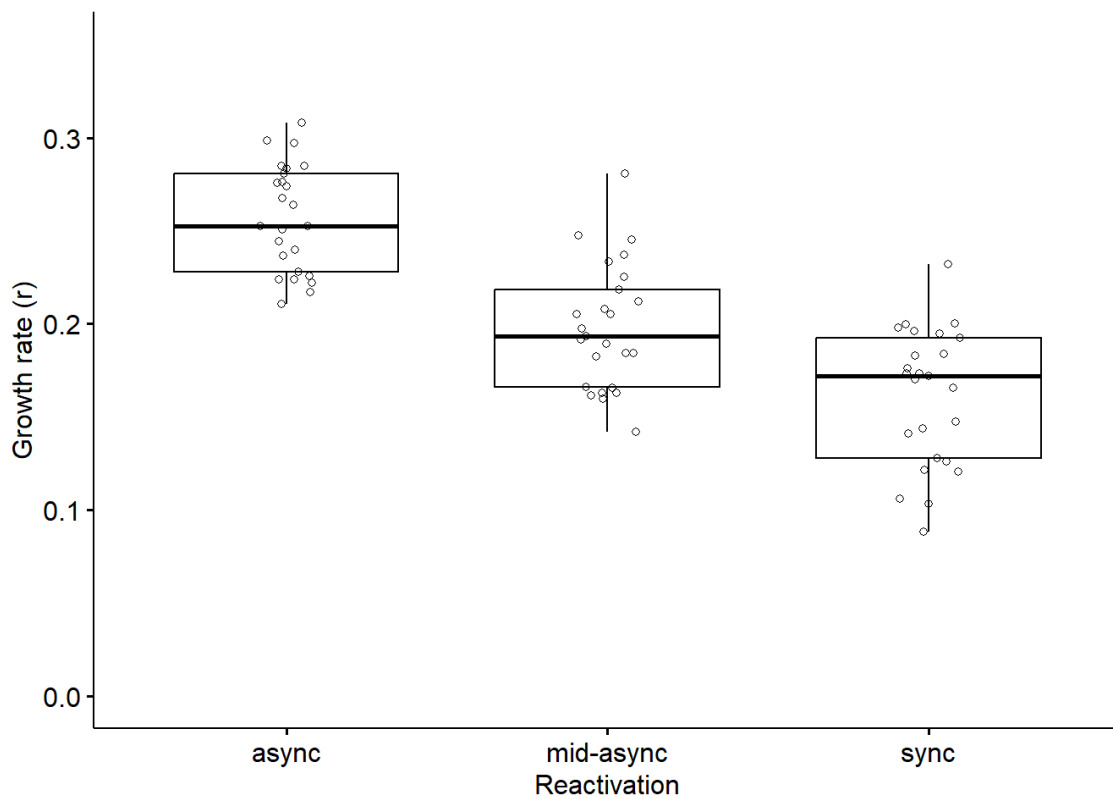
summary(finalpop.async.anova1, digits = 5)

##
## Error: tank
##
##           Df Sum Sq Mean Sq F value Pr(>F)
## reactivation  2   9329    4665   1.207  0.343
## comp         2   5159    2580   0.667  0.537
## Residuals    9  34794    3866
##
## Error: Within
##
##           Df Sum Sq Mean Sq F value    Pr(>F)
## reactivation    2  35958   17979  76.011 < 2e-16 ***

```

```
## comp          3    3645    1215    5.137 0.00180 **
## reactivation:comp  6    4773     795    3.363 0.00326 **
## Residuals      271   64099     237
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ggboxplot(dsprefl, x = "reactivation", y = "rf",
          order = c("async", "mid-async", "sync")) +
  ylim(0,0.35) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Growth rate (r)" +
    theme(legend.position="none")
```

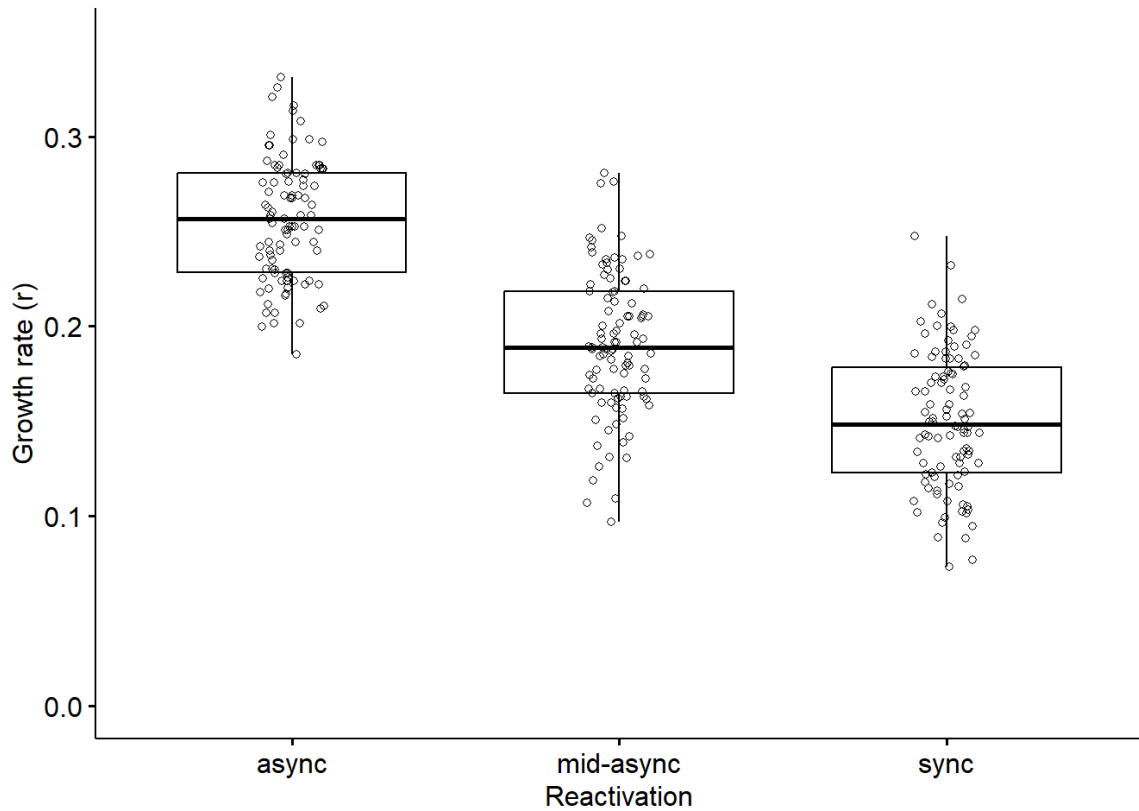


```
group_by(dsprefl, reactivation) %>%
  summarise(
    count = n(),
```

```

    mean = mean(rf, na.rm = TRUE),
    sd = sd(rf, na.rm = TRUE)
  )
## # A tibble: 3 × 4
##   reactivation count mean    sd
##   <chr>          <int> <dbl> <dbl>
## 1 async          25 0.257 0.0288
## 2 mid-async      25 0.198 0.0336
## 3 sync           25 0.161 0.0369
anova1 <- aov(rf ~ reactivation+ Error(tank), data = dspref1)
summary(anova1)
##
## Error: tank
##           Df Sum Sq Mean Sq F value Pr(>F)
## Residuals 13 0.05652 0.004348
##
## Error: Within
##           Df Sum Sq Mean Sq F value Pr(>F)
## reactivation  2 0.1158 0.05791   147.9 <2e-16 ***
## Residuals    59 0.0231 0.00039
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
ggboxplot(dsp1, x = "reactivation", y = "rf",
           order = c("async", "mid-async", "sync")) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  ylim(0,0.35) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Growth rate (r)" ) +
  theme(legend.position="none")

```



```
group_by(dsp1, reactivation) %>%
  summarise(
    count = n(),
    mean = mean(rf, na.rm = TRUE),
    sd = sd(rf, na.rm = TRUE)
  )
## # A tibble: 3 × 4
##   reactivation count  mean    sd
##   <chr>         <int> <dbl> <dbl>
## 1 async           98 0.256 0.0320
## 2 mid-async      100 0.191 0.0375
## 3 sync           98 0.150 0.0360
anova1 <- aov(rf ~ reactivation+ Error(tank), data = dsp1)
summary(anova1)
##
## Error: tank
##           Df  Sum Sq Mean Sq F value Pr(>F)
## reactivation  2 0.06674  0.03337   1.992  0.183
```

```
## Residuals      11 0.18425 0.01675
##
## Error: Within
##              Df Sum Sq Mean Sq F value Pr(>F)
## reactivation   2 0.5560 0.27800   657.6 <2e-16 ***
## Residuals     280 0.1184 0.00042
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

res.aov <- dsp1 %>% anova_test(rf ~ reactivation * comp)
res.aov
## ANOVA Table (type II tests)
##
##              Effect DFn DFd      F      p p<.05    ges
## 1      reactivation   2 284 228.082 8.44e-60      * 0.616
## 2              comp   3 284   1.638 1.81e-01      0.017
## 3 reactivation:comp   6 284   1.121 3.50e-01      0.023

pwc <- dsp1 %>%
  group_by(reactivation) %>%
  emmeans_test(rf ~ comp, p.adjust.method = "bonferroni") %>%
  add_xy_position(x = "reactivation")

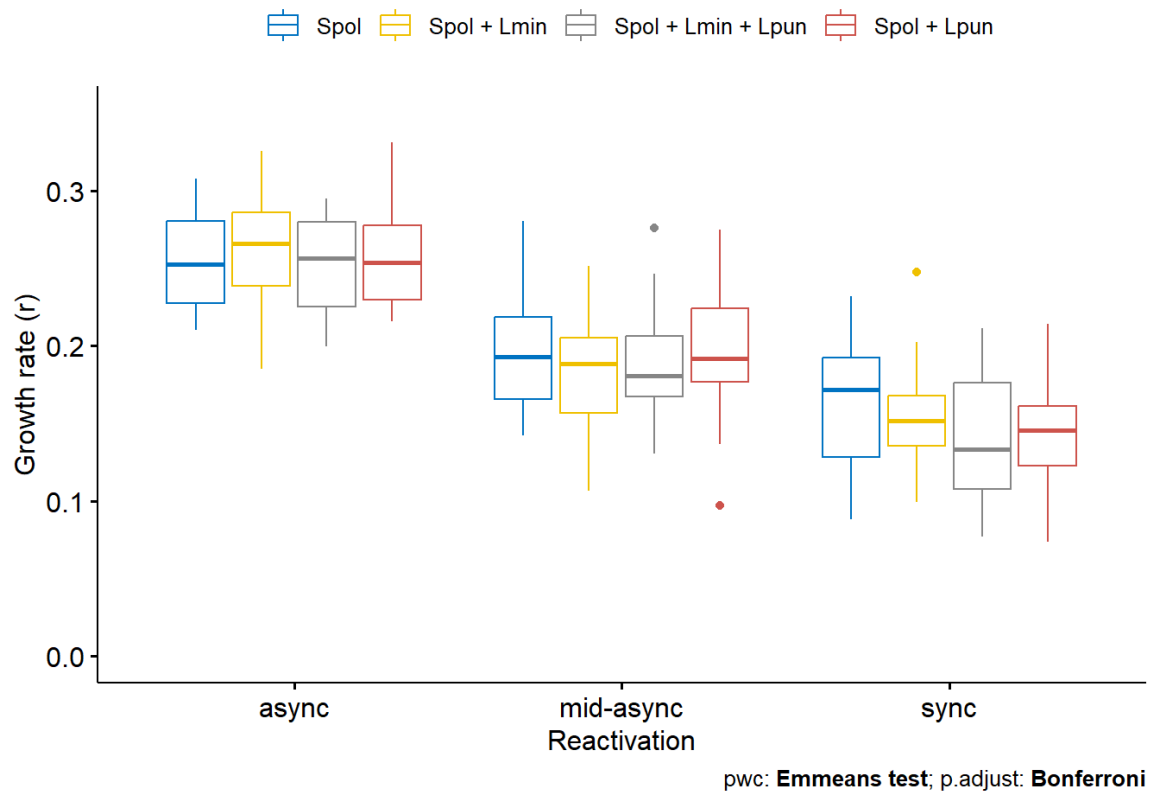
pwc
## # A tibble: 18 × 15
##   reacti...1 term .y. group1 group2 df stati...2 p p.adj p.adj...3 y.p
##   os...4
##   <chr>   <chr> <chr> <chr> <chr> <dbl> <dbl> <dbl> <dbl> <chr> <
##   dbl>
## 1 async  comp  rf    Spol  Spol ... 284 -0.411 0.681 1 ns 0
##   .345
## 2 async  comp  rf    Spol  Spol ... 284 0.647 0.518 1 ns 0
##   .363
## 3 async  comp  rf    Spol  Spol ... 284 -0.0382 0.970 1 ns 0
##   .380
## 4 async  comp  rf    Spol ... Spol ... 284 1.05 0.294 1 ns 0
##   .397
## 5 async  comp  rf    Spol ... Spol ... 284 0.369 0.712 1 ns 0
##   .415
## 6 async  comp  rf    Spol ... Spol ... 284 -0.679 0.498 1 ns 0
##   .432
## 7 mid-asy... comp  rf    Spol  Spol ... 284 1.84 0.0664 0.398 ns 0
##   .295
```

```

## 8 mid-asy... comp rf      Spol  Spol ... 284 0.999 0.318 1      ns      0
.313
## 9 mid-asy... comp rf      Spol  Spol ... 284 0.278 0.781 1      ns      0
.330
## 10 mid-asy... comp rf      Spol ... Spol ... 284 -0.843 0.400 1      ns      0
.347
## 11 mid-asy... comp rf      Spol ... Spol ... 284 -1.56 0.119 0.713 ns      0
.365
## 12 mid-asy... comp rf      Spol ... Spol ... 284 -0.721 0.471 1      ns      0
.382
## 13 sync      comp rf      Spol  Spol ... 284 0.525 0.600 1      ns      0
.262
## 14 sync      comp rf      Spol  Spol ... 284 2.20 0.0286 0.172 ns      0
.280
## 15 sync      comp rf      Spol  Spol ... 284 1.77 0.0773 0.464 ns      0
.297
## 16 sync      comp rf      Spol ... Spol ... 284 1.68 0.0940 0.564 ns      0
.314
## 17 sync      comp rf      Spol ... Spol ... 284 1.25 0.211 1      ns      0
.332
## 18 sync      comp rf      Spol ... Spol ... 284 -0.423 0.673 1      ns      0
.349

## # ... with 4 more variables: groups <named list>, x <dbl>, xmin <dbl>,
## #   xmax <dbl>, and abbreviated variable names 1reactivation, 2statistic,
## #   3p.adj.signif, 4y.position
ggboxplot(
  dspl, x = "reactivation", y = "rf",
  color = "comp", palette = "jco") +
  stat_pvalue_manual(pwc, hide.ns = TRUE) +
  ylim(0,0.35) +
  theme(legend.title=element_blank()) +
  labs(x = "Reactivation",
       y = "Growth rate (r)",
  #   subtitle = get_test_label(res.aov, detailed = TRUE),
  #   caption = get_pwc_label(pwc)
  )

```



```
group_by(dspl, reactivation, comp) %>%
  summarise(
    count = n(),
    mean = mean(rf, na.rm = TRUE),
    sd = sd(rf, na.rm = TRUE)
  )
```

`summarise()` has grouped output by 'reactivation'. You can override using the
`.groups` argument.

A tibble: 12 × 5

Groups: reactivation [3]

	reactivation	comp	count	mean	sd
	<chr>	<chr>	<int>	<dbl>	<dbl>
## 1	async	Spol	25	0.257	0.0288
## 2	async	Spol + Lmin	24	0.261	0.0381
## 3	async	Spol + Lmin + Lpun	25	0.250	0.0298
## 4	async	Spol + Lpun	24	0.257	0.0317
## 5	mid-async	Spol	25	0.198	0.0336

```

## 6 mid-async      Spol + Lmin      25 0.180 0.0409
## 7 mid-async      Spol + Lmin + Lpun 25 0.189 0.0365
## 8 mid-async      Spol + Lpun      25 0.196 0.0381
## 9 sync           Spol             25 0.161 0.0369
## 10 sync          Spol + Lmin      25 0.156 0.0327
## 11 sync          Spol + Lmin + Lpun 24 0.139 0.0376
## 12 sync          Spol + Lpun      24 0.144 0.0342
rf.async.anova1 <- aov(rf ~ reactivation * guide + Error(tank), data = dsp1)
summary(rf.async.anova1)

##
## Error: tank
##
##           Df  Sum Sq Mean Sq F value Pr(>F)
## reactivation  2 0.06674 0.03337   1.992  0.183
## Residuals    11 0.18425 0.01675
##
## Error: Within
##
##           Df Sum Sq Mean Sq F value    Pr(>F)
## reactivation    2 0.5560 0.27800 688.279 < 2e-16 ***
## guide           1 0.0047 0.00470  11.630 0.000745 ***
## reactivation:guide  2 0.0018 0.00089   2.214 0.111187
## Residuals      277 0.1119 0.00040
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
rf.async.anova1 <- aov(rf ~ reactivation * comp + Error(tank), data = dsp1)
summary(rf.async.anova1)

##
## Error: tank
##
##           Df  Sum Sq Mean Sq F value Pr(>F)
## reactivation  2 0.06674 0.03337   1.801  0.220
## comp         2 0.01752 0.00876   0.473  0.638
## Residuals    9 0.16673 0.01853
##
## Error: Within
##
##           Df Sum Sq Mean Sq F value    Pr(>F)
## reactivation    2 0.5560 0.27800 719.156 < 2e-16 ***
## comp           3 0.0059 0.00195   5.054 0.00201 **
## reactivation:comp  6 0.0077 0.00129   3.340 0.00343 **

```

```
## Residuals      271 0.1048 0.00039
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix 3: Budding growth rate analyses

Dataset required: 'S4. Budding growth rate analyses.csv'

11/28/2022

```
library(jtools)
library(lmerTest)
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##      lmer
## The following object is masked from 'package:stats':
##
##      step
#library(rethinking)
library(tidyr)
##
## Attaching package: 'tidyr'
## The following objects are masked from 'package:Matrix':
##
##      expand, pack, unpack
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##      filter, lag
## The following objects are masked from 'package:base':
##
##      intersect, setdiff, setequal, union
library(readr)
library(ggplot2)
library(ggpubr)
## Registered S3 methods overwritten by 'broom':
```

```

##      method          from
##      tidy.glht        jtools
##      tidy.summary.glht jtools
library(lme4)
library(report)
library(emmeans)
library(rstatix)

##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
##      filter
library(nlme)
##
## Attaching package: 'nlme'
## The following object is masked from 'package:dplyr':
##
##      collapse
## The following object is masked from 'package:lme4':
##
##      lmList
library(lme4)
library(readODS)

## Warning: package 'readODS' was built under R version 4.2.3

d <- read.csv(choose.files()) #choose 'S4. Budding growth rate analyses data.csv'. May need to save .ods file as .csv.

d$tank <- as.factor(d$tank)
d$community <- as.factor(d$community)

dcomp <- select(filter(d, species.interaction != "Spol"), !starts_with("day"))
res.aov <- d %>% anova_test(r ~ asynchrony * species.interaction)
res.aov

## ANOVA Table (type II tests)
##
##              Effect DFn DFd      F      p p<.05    ges
## 1              asynchrony    1 180 27.036 5.39e-07    * 0.131
## 2      species.interaction    3 180  0.287 8.35e-01      0.005
## 3 asynchrony:species.interaction    3 180  0.278 8.41e-01      0.005

```

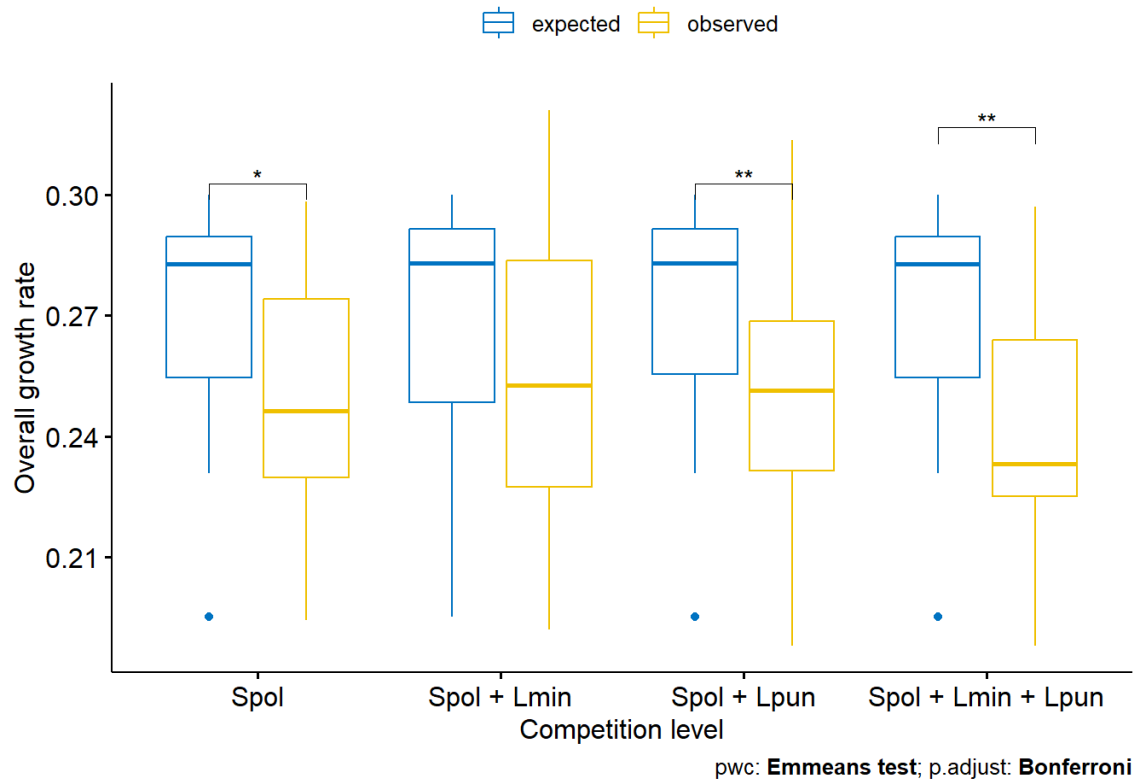
```
pwc <- d %>%
```

```
group_by(species.interaction) %>%
```

```
emmeans_test(r ~ asynchrony, p.adjust.method = "bonferroni") %>%
add_xy_position(x = "species.interaction")

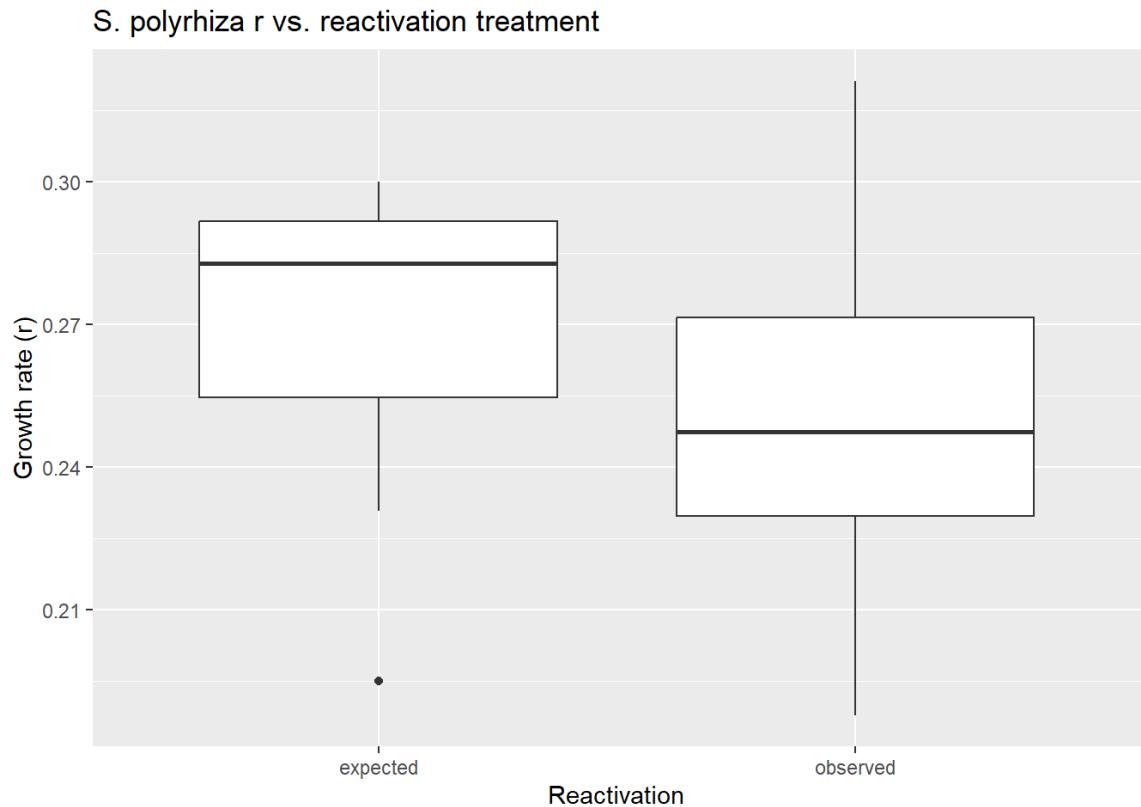
pwc
## # A tibble: 4 × 15
##   species.inte...1 term   .y.   group1 group2    df stati...2      p   p.adj p.a
dj...3
##   <chr>          <chr> <chr> <chr>  <chr>  <dbl>  <dbl>  <dbl>  <dbl> <ch
r>
## 1 Spol          asyn... r     expec... obser...  180    2.52 0.0125  0.0125  *
## 2 Spol + Lmin   asyn... r     expec... obser...  180    1.89 0.0597  0.0597  ns
## 3 Spol + Lmin +... asyn... r     expec... obser...  180    3.32 0.00108 0.00108 **
## 4 Spol + Lpun   asyn... r     expec... obser...  180    2.62 0.00952 0.00952 **
## # ... with 5 more variables: y.position <dbl>, groups <named list>, x <dbl>,
## #   xmin <dbl>, xmax <dbl>, and abbreviated variable names
## #   1species.interaction, 2statistic, 3p.adj.signif

ggboxplot(
  d, x = "species.interaction", y = "r",
  color = "asynchrony", palette = "jco" ) +
  stat_pvalue_manual(pwc, hide.ns = TRUE) +
#   ylim(0.18,0.35) +
  theme(legend.title=element_blank()) +
  labs(x = "Competition level",
       y = "Overall growth rate",
#       subtitle = get_test_label(res.aov, detailed = TRUE),
       caption = get_pwc_label(pwc)
  )
```



```
summary(aov(data=d, r ~ asynchrony * species.interaction + Error(tank)))
##
## Error: tank
##               Df  Sum Sq  Mean Sq F value Pr(>F)
## species.interaction  2 0.00548 0.002741   0.317  0.735
## Residuals           11 0.09518 0.008653
##
## Error: Within
##               Df  Sum Sq  Mean Sq F value  Pr(>F)
## asynchrony      1 0.02317 0.023171  71.769 1.2e-14 ***
## species.interaction      3 0.00042 0.000142   0.439   0.726
## asynchrony:species.interaction  3 0.00071 0.000238   0.737   0.531
## Residuals           167 0.05392 0.000323
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
ggplot(d, aes(x=asynchrony, y=r, )) +
#   scale_x_discrete(limits = c("one","two","three")) +
```

```
geom_boxplot() +
  labs(title = "S. polyrhiza r vs. reactivation treatment",
        x = "Reactivation",
        y = "Growth rate (r)")
```



```
summary(aov(data=d, r ~ asynchrony + Error(tank)))
##
## Error: tank
##           Df Sum Sq Mean Sq F value Pr(>F)
## Residuals 13  0.1007  0.007743
##
## Error: Within
##           Df Sum Sq Mean Sq F value Pr(>F)
## asynchrony  1 0.02317 0.023171   72.81 7e-15 ***
## Residuals 173 0.05506 0.000318
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
mean.finalpop.interaction <- d %>%
```

```

group_by(asynchrony) %>%
  summarise(
    mean.rf = mean(r),
    sd = sd(r, na.rm = TRUE)
  )
mean.finalpop.interaction
## # A tibble: 2 × 3
##   asynchrony mean.rf    sd
##   <chr>      <dbl> <dbl>
## 1 expected    0.270 0.0270
## 2 observed    0.248 0.0307

```

Appendix 4: Analyses with both trials

Dataset required: 'S5. Data for both trials.csv'

2023-05-05

```
library(jtools)
library(lmerTest)
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##      lmer
## The following object is masked from 'package:stats':
##
##      step
library(tidyr)
##
## Attaching package: 'tidyr'
## The following objects are masked from 'package:Matrix':
##
##      expand, pack, unpack
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##      filter, lag
## The following objects are masked from 'package:base':
##
##      intersect, setdiff, setequal, union
library(readr)
library(ggplot2)
library(ggpubr)
## Registered S3 methods overwritten by 'broom':
##      method          from
```

```

## tidy.glht jtools
## tidy.summary.glht jtools
library(lme4)
library(readODS)

## Warning: package 'readODS' was built under R version 4.2.3

d <- read.csv(choose.files()) #choose 'S5. Data for both trials.csv'. May need
to save .ods file as .csv.

d$rfr <- as.numeric(d$rfr)
d$tank <- as.character(d$tank)
d$trial <- as.factor(d$trial)
#$d$c.rf <- as.numeric(d$c.rf)
d <- d[!is.na(d$rfr),]
summary(d)

##      tank      comp      guide      species
## Length:486    Length:486    Length:486    Length:486
## Class :character Class :character Class :character Class :character
## Mode  :character Mode  :character Mode  :character Mode  :character
##
##
##
## reactivation      timing      days      starting.pop
## Length:486        Length:486    Min.   : 9.00    Min.   : 4.00
## Class :character  Class :character 1st Qu.:10.00   1st Qu.: 4.00
## Mode  :character  Mode  :character  Median :11.00   Median :10.00
##                                     Mean   :11.94   Mean   :10.58
##                                     3rd Qu.:13.00   3rd Qu.:17.00
##                                     Max.    :22.00   Max.    :20.00
##      finalpop      rf      finalcomp      trial
## Min.   : 13.00    Min.   :0.02186    Min.   :0.09868    1:391
## 1st Qu.: 42.00    1st Qu.:0.11642    1st Qu.:0.32144    2: 95
## Median : 58.00    Median :0.16302    Median :0.45247
## Mean   : 83.79    Mean   :0.16989    Mean   :0.50206
## 3rd Qu.: 96.00    3rd Qu.:0.22390    3rd Qu.:0.63515
## Max.   :411.00    Max.   :0.33146    Max.   :1.00000

dsp <- select(filter(d, species == "sp"), !starts_with("day"))
head(dsp)

```

```

##      tank      comp      guide species reactivation timing starting.pop finalp
op
## 1      1      Spol reference      sp      async start      4
49
## 2      1      Spol reference      sp      async start      4
62
## 3      1      Spol reference      sp      sync start      17      1
19
## 4      1      Spol reference      sp      sync start      17
95
## 5      1 Spol + Lmin competing      sp      async start      4
50
## 6      1 Spol + Lmin competing      sp      async start      4
59

##          rf finalcomp trial
## 1 0.2505526 1.0000000      1
## 2 0.2740840 1.0000000      1
## 3 0.1945910 1.0000000      1
## 4 0.1720664 1.0000000      1
## 5 0.2525729 0.4201681      1
## 6 0.2691243 0.4573643      1

dspcomp <- select(filter(d, guide == "competing"), !starts_with("day"))

dspref <- select(filter(d, guide == "reference"), !starts_with("day"))
#head(dspref)

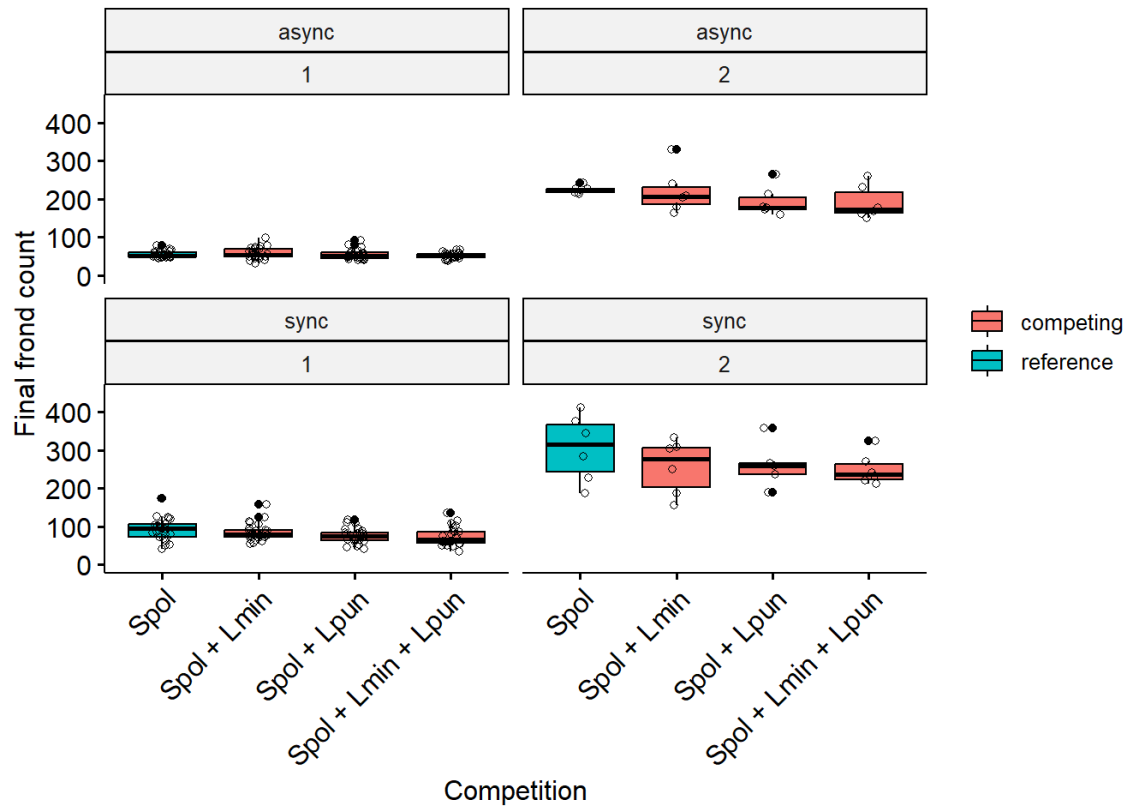
```

#three-way interaction with TRIAL ####finalpop ~ reactivation * comp * trial

```

ggboxplot(dsp, x = "comp", y = "finalpop", fill = "guide",
           order = c("Spol", "Spol + Lmin", "Spol + Lpun", "Spol + Lmin + Lpun")
) +
  facet_wrap(~reactivation + trial) +
  ylim(0,450) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  labs(x = "Competition",
       y = "Final frond count") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  theme(legend.position="right") +
  theme(legend.title=element_blank())

```



```
group_by(dsp, reactivation, comp, trial) %>%
  summarise(
    count = n(),
    mean = mean(finalpop, na.rm = TRUE),
    sd = sd(finalpop, na.rm = TRUE)
  )

## `summarise()` has grouped output by 'reactivation', 'comp'. You can override
## using the `.groups` argument.
## # A tibble: 16 × 6
## # Groups:   reactivation, comp [8]
##   reactivation comp          trial count mean    sd
##   <chr>         <chr>         <fct> <int> <dbl> <dbl>
## 1 1 async      Spol             1      25  54.9  8.71
## 2 2 async      Spol             2       6 224   10.9
## 3 3 async      Spol + Lmin      1      24  57.4 15.4
## 4 4 async      Spol + Lmin      2       6 221.  59.6
## 5 5 async      Spol + Lmin + Lpun 1      25  51.3  7.48
## 6 6 async      Spol + Lmin + Lpun 2       6 191.  44.4
```

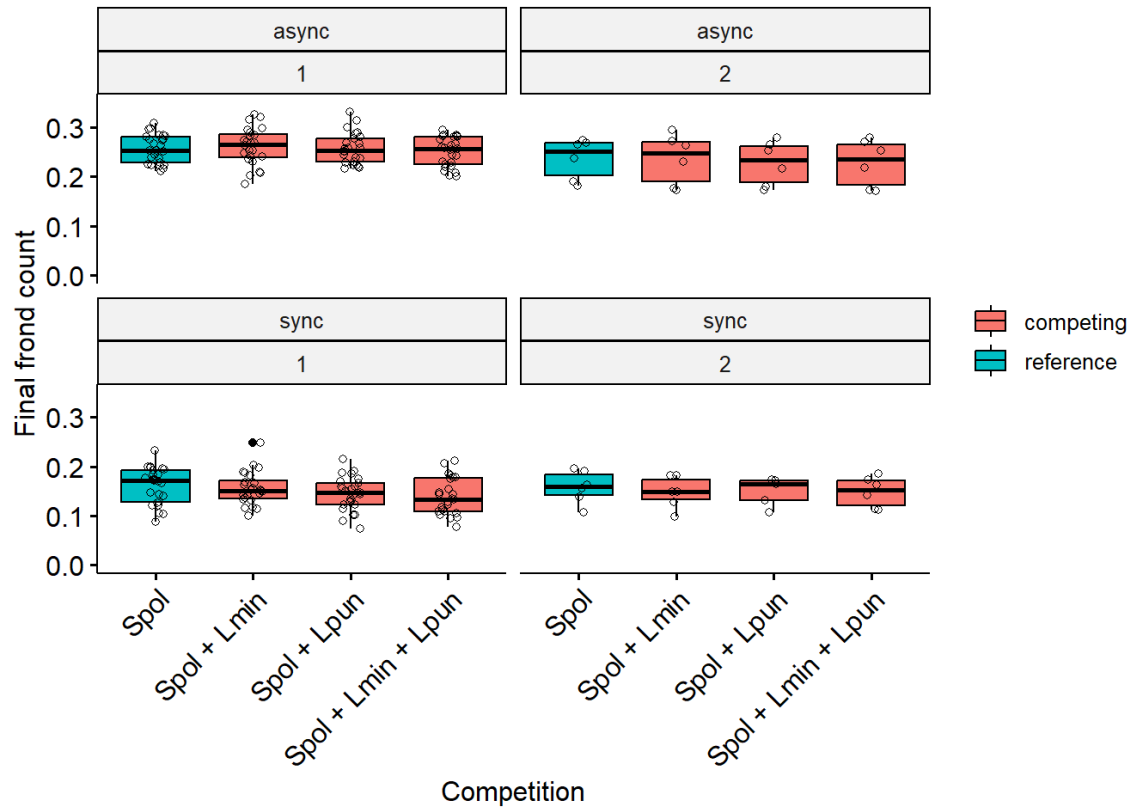
```
## 7 async      Spol + Lpun      1      24  55.1 13.8
## 8 async      Spol + Lpun      2       6 194.  39.0
## 9 sync       Spol            1      25  91.7 28.8
## 10 sync      Spol            2       6 305   87.2
## 11 sync      Spol + Lmin      1      24  85.0 23.6
## 12 sync      Spol + Lmin      2       6 256.  71.4
## 13 sync      Spol + Lmin + Lpun 1      24  73.4 24.4
## 14 sync      Spol + Lmin + Lpun 2       6 249.  41.2
## 15 sync      Spol + Lpun      1      25  74.9 19.9
## 16 sync      Spol + Lpun      2       5 262.  60.9

anova <- aov(finalpop ~ trial * reactivation * comp, data = dsp)
summary(anova)

##              Df Sum Sq Mean Sq F value    Pr(>F)
## trial              1 1086257 1086257 1287.045 < 2e-16 ***
## reactivation        1   67015   67015   79.402 < 2e-16 ***
## comp                3   11058    3686    4.367 0.005172 **
## trial:reactivation   1   10879   10879   12.889 0.000405 ***
## trial:comp           3    6300    2100    2.488 0.061243 .
## reactivation:comp    3    3118    1039    1.231 0.299073
## trial:reactivation:comp 3    2385     795    0.942 0.421158
## Residuals          227  191586     844
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
###rf ~ reactivation * comp * trial
```

```
ggboxplot(dsp, x = "comp", y = "rf", fill = "guide",
           order = c("Spol", "Spol + Lmin", "Spol + Lpun", "Spol + Lmin + Lpun")
) +
  facet_wrap(~reactivation + trial) +
  ylim(0,0.35) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  labs(x = "Competition",
       y = "Final frond count") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  theme(legend.position="right") +
  theme(legend.title=element_blank())
```



```
group_by(dsp, reactivation, comp, trial) %>%
  summarise(
    count = n(),
    mean = mean(rf, na.rm = TRUE),
    sd = sd(rf, na.rm = TRUE)
  )

## `summarise()` has grouped output by 'reactivation', 'comp'. You can override
## using the `.groups` argument.
## # A tibble: 16 × 6
## # Groups:   reactivation, comp [8]
##   reactivation comp          trial count  mean    sd
##   <chr>         <chr>         <fct> <int> <dbl> <dbl>
## 1 async       Spol             1      25 0.257 0.0288
## 2 async       Spol             2       6 0.236 0.0409
## 3 async     Spol + Lmin      1      24 0.261 0.0381
## 4 async     Spol + Lmin      2       6 0.235 0.0510
## 5 async     Spol + Lmin + Lpun 1      25 0.250 0.0298
## 6 async     Spol + Lmin + Lpun 2       6 0.227 0.0480
```

```

## 7 async      Spol + Lpun      1      24 0.257 0.0317
## 8 async      Spol + Lpun      2       6 0.228 0.0451
## 9 sync       Spol            1      25 0.161 0.0369
## 10 sync      Spol            2       6 0.158 0.0332
## 11 sync      Spol + Lmin      1      24 0.156 0.0334
## 12 sync      Spol + Lmin      2       6 0.148 0.0322
## 13 sync      Spol + Lmin + Lpun 1      24 0.139 0.0376
## 14 sync      Spol + Lmin + Lpun 2       6 0.148 0.0310
## 15 sync      Spol + Lpun      1      25 0.144 0.0338
## 16 sync      Spol + Lpun      2       5 0.149 0.0289

anova <- aov(rf ~ reactivation * trial * comp, data = dsp)
summary(anova)

##              Df Sum Sq Mean Sq F value Pr(>F)
## reactivation      1 0.6201  0.6201 506.743 <2e-16 ***
## trial              1 0.0058  0.0058   4.766 0.0300 *
## comp              3 0.0069  0.0023   1.890 0.1321
## reactivation:trial  1 0.0060  0.0060   4.942 0.0272 *
## reactivation:comp   3 0.0018  0.0006   0.504 0.6802
## trial:comp          3 0.0004  0.0001   0.120 0.9482
## reactivation:trial:comp 3 0.0006  0.0002   0.164 0.9207
## Residuals         227 0.2778  0.0012
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Appendix 5: Inherent Disadvantage of Asynchrony

Dataset required: 'S6. Inherent disadvantage data.csv'

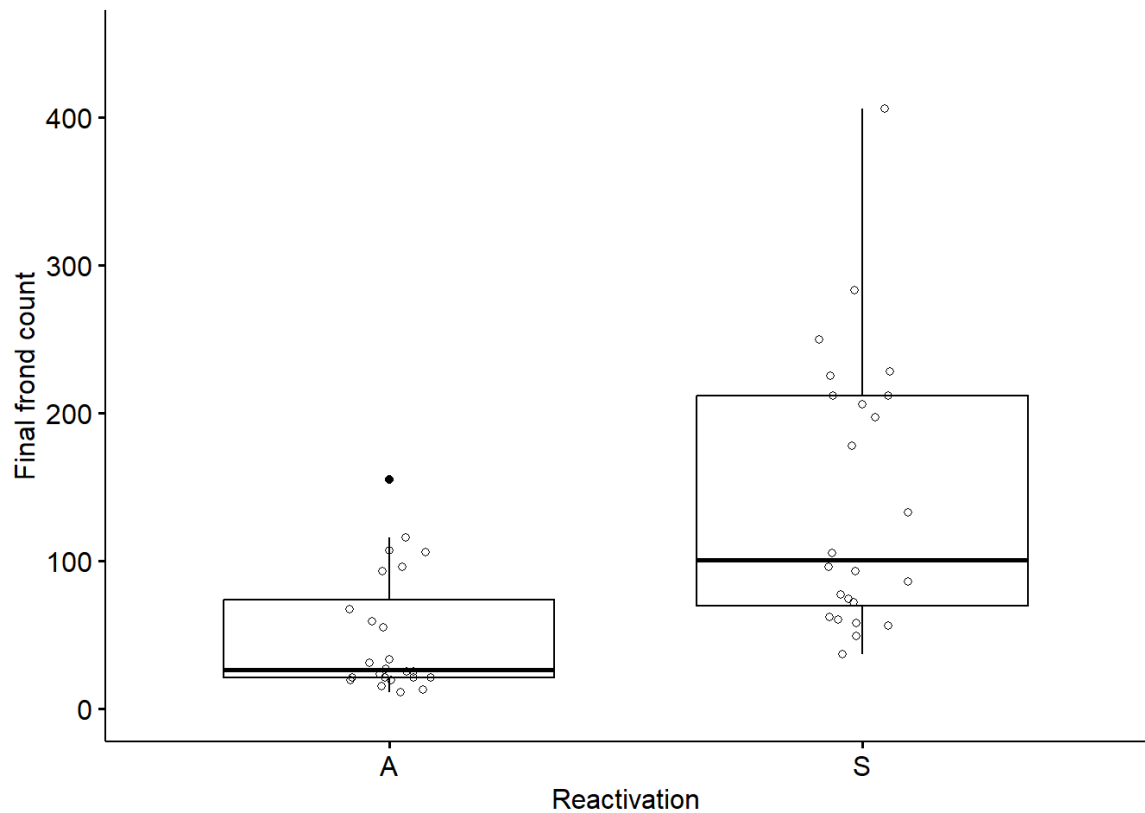
2023-05-05

```
library(jtools)
library(lmerTest)
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##      lmer
## The following object is masked from 'package:stats':
##
##      step
library(tidyr)
##
## Attaching package: 'tidyr'
## The following objects are masked from 'package:Matrix':
##
##      expand, pack, unpack
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##      filter, lag
## The following objects are masked from 'package:base':
##
##      intersect, setdiff, setequal, union
library(readr)
library(ggplot2)
library(ggpubr)
## Registered S3 methods overwritten by 'broom':
##      method          from
##      tidy.glht       jtools
```

```
## tidy.summary.glht jtools
library(lme4)
library(emmeans)
library(rstatix)

##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
## filter

d <- read.csv(choose.files()) #Choose 'S6. Inherent disadvantage data.csv'. May
need to save .ods file as .csv.
ggboxplot(d, x = "timing", y = "pop",
           order = c("A", "S")) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  ylim(0,450) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Final frond count") +
  theme(legend.position="none")
```



```
group_by(d, timing) %>%
  summarise(
    count = n(),
    mean = mean(pop, na.rm = TRUE),
    sd = sd(pop, na.rm = TRUE)
  )
## # A tibble: 2 × 4
##   timing count mean    sd
##   <chr>  <int> <dbl> <dbl>
## 1 A         24  49.1  41.0
## 2 S         24 144.   94.1
group_by(d, tank, timing) %>%
  summarise(
    count = n(),
    mean = mean(pop, na.rm = TRUE),
    sd = sd(pop, na.rm = TRUE)
  )
## `summarise()` has grouped output by 'tank'. You can override using the
```

```
## `.groups` argument.
## # A tibble: 24 × 5
## # Groups:   tank [12]
##   tank      timing count  mean    sd
##   <chr>    <chr>  <int> <dbl> <dbl>
## 1 ELEVEN    A         2   20    1.41
## 2 ELEVEN    S         2  49.5 17.7
## 3 FIFTEEN   A         2   25     0
## 4 FIFTEEN   S         2  86.5 13.4
## 5 FIVE       A         2   12    1.41
## 6 FIVE       S         2  96.5 51.6
## 7 FOURTEEN  A         2   27    5.66
## 8 FOURTEEN  S         2   65   12.7
## 9 NINE       A         2   18    4.24
## 10 NINE      S         2  53.5  6.36
## # ... with 14 more rows
t.test(pop ~ timing, data = d, var.equal = FALSE)
##
## Welch Two Sample t-test
##
## data:  pop by timing
## t = -4.5253, df = 31.426, p-value = 8.12e-05
## alternative hypothesis: true difference in means between group A and group S
## is not equal to 0
## 95 percent confidence interval:
##  -137.55049  -52.11618
## sample estimates:
## mean in group A mean in group S
##           49.1250           143.9583
```

Appendix 6: Advantage in stressed conditions

Dataset required: 'S7. Advantage in stressed conditions data.csv'

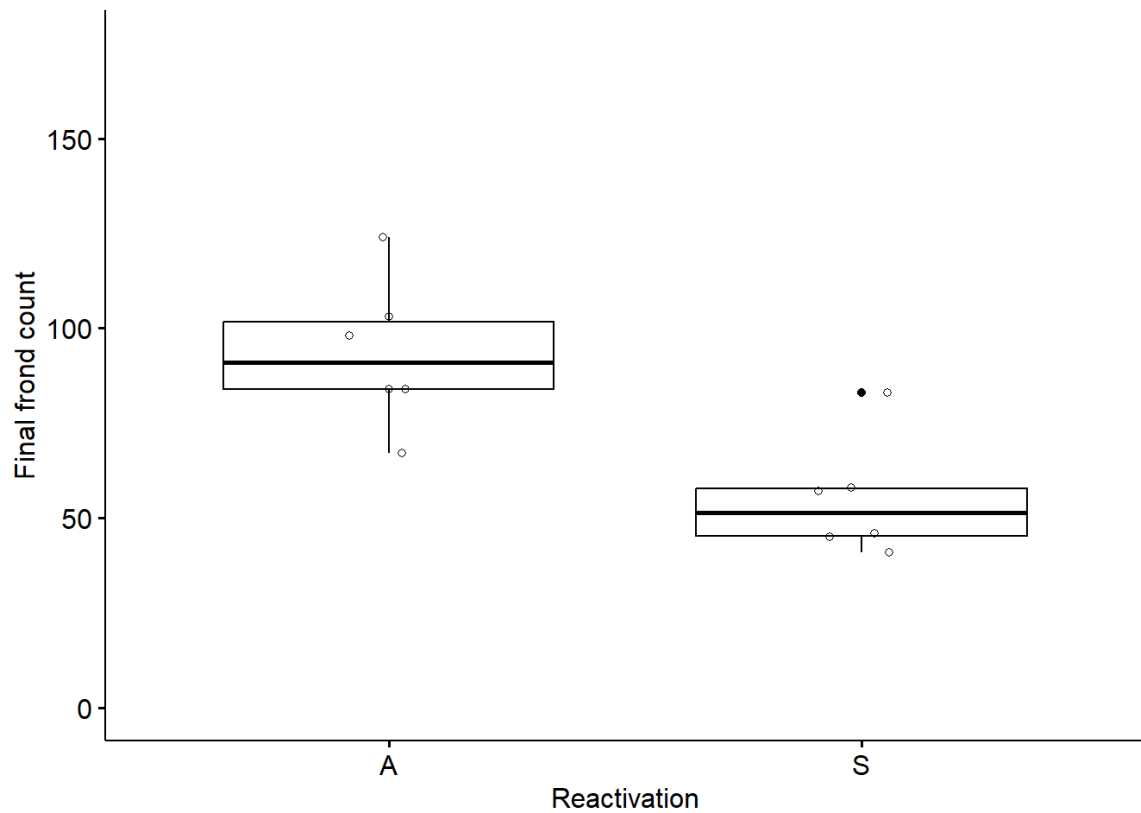
2023-05-05

```
library(jtools)
library(lmerTest)
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##      lmer
## The following object is masked from 'package:stats':
##
##      step
#library(rethinking)
library(tidyr)
##
## Attaching package: 'tidyr'
## The following objects are masked from 'package:Matrix':
##
##      expand, pack, unpack
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##      filter, lag
## The following objects are masked from 'package:base':
##
##      intersect, setdiff, setequal, union
library(readr)
library(ggplot2)
library(ggpubr)
## Registered S3 methods overwritten by 'broom':
##      method          from
```

```
## tidy.glht jtools
## tidy.summary.glht jtools
library(lme4)
library(emmeans)
library(rstatix)

##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
## filter

d <- read.csv(choose.files()) #Choose 'S7. Advantage in stressed conditions dat
a.csv'. May need to save .ods file as .csv.
ggboxplot(d, x = "timing", y = "pop",
           order = c("A", "S")) +
  geom_point(cex = 1.5, pch = 1.0, position = position_jitter(w=0.1,h=0)) +
  ylim(0,175) +
  labs(
    #title = "S. polyrhiza guide and competing populations",
    x = "Reactivation",
    y = "Final frond count") +
  theme(legend.position="none")
```



```
group_by(d, timing) %>%
  summarise(
    count = n(),
    mean = mean(pop, na.rm = TRUE),
    sd = sd(pop, na.rm = TRUE)
  )
## # A tibble: 2 × 4
##   timing count mean    sd
##   <chr>  <int> <dbl> <dbl>
## 1 A           6  93.3  19.6
## 2 S           6   55   15.3
group_by(d, tank, timing) %>%
  summarise(
    count = n(),
    mean = mean(pop, na.rm = TRUE),
    sd = sd(pop, na.rm = TRUE)
  )
## `summarise()` has grouped output by 'tank'. You can override using the
```

```
## `.groups` argument.
## # A tibble: 6 × 5
## # Groups:   tank [3]
##   tank   timing count  mean    sd
##   <chr>  <chr>  <int> <dbl> <dbl>
## 1 ELEVEN A         2  82.5 21.9
## 2 ELEVEN S         2   70  18.4
## 3 SEVEN  A         2   84    0
## 4 SEVEN  S         2   52  8.49
## 5 THREE  A         2 114. 14.8
## 6 THREE  S         2   43  2.83
t.test(pop ~ timing, data = d, var.equal = FALSE)
##
## Welch Two Sample t-test
##
## data:  pop by timing
## t = 3.7726, df = 9.4471, p-value = 0.004033
## alternative hypothesis: true difference in means between group A and group S
## is not equal to 0
## 95 percent confidence interval:
##  15.51247 61.15420
## sample estimates:
## mean in group A mean in group S
##           93.33333           55.00000
```