esa

ECOSPHERE

Duration and timing interactions of early-life stress and the potential for recovery

JACK SEIFARTH [1,3] HIDETOSHI INAMINE [1] ANGUS BUCKLING [2] AND KATRIONA SHEA [1,†]

[1]*Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16801 USA*
[2]*Department of Biosciences, University of Exeter, Penryn Campus, Penryn TR10 9FE UK*

Citation: Seifarth, J., H. Inamine, A. Buckling, and K. Shea. 2021. Duration and timing interactions of early-life stress and the potential for recovery. Ecosphere 12(7):e03620. 10.1002/ecs2.3620

Abstract. Stress comprises three sequential stages: the event (stressor), which leads to an initial effect on a focal organism (stress), followed by the organism's ultimate response (recovery or not). Using this framework, we test how the duration and timing of a stressor individually and interactively affect the initial and asymptotic organism outcomes. We applied either long or short water restrictions either early or later in the life of *Lepidium sativum*. While several of these water restriction treatments caused a noticeable initial effect, all were able to recover fully, except for those exposed to a long early water restriction. While long early stressors led to decreased height, a growth model revealed that both long and short early stressors changed the rate of growth and prolonged the inflection point, findings that would have been missed had effect and response not been analyzed separately. Both the stressor and the magnitude of the initial effect in part explained the change in the growth model parameter estimates. Overall, timing, duration, and, more importantly, the interaction of these two temporal aspects of stress significantly affects an organism's initial response to a stressor and its ability to ultimately recover from stress. Our findings highlight the importance of explicitly studying the interactions of stressor aspects and of examining the immediate stress effects, as well as long-term responses.

Key words: disturbance; duration; early-life stress; garden cress; *Lepidium sativum*; stress; stress interaction; timing.

Received 19 November 2020; revised 9 February 2021; accepted 17 February 2021; final version received 14 April 2021. Corresponding Editor: Debra P. C. Peters.

Copyright: © 2021 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

[3]Present address: Department of Epidemiology, Columbia University Mailman School of Public Health, New York, New York 10032 USA.

† **E-mail:** k-shea@psu.edu

INTRODUCTION

Stress is ubiquitous in nature. Stressors affect organisms across the tree of life including animals, plants, and bacteria. As a result, there is an immense body of research variously addressing the causes and consequences of insults to organisms (Selye 1956, Mustroph et al. 2010, O'Mahony et al. 2014, Tungjitwitayakul et al. 2015, Doffo et al. 2017). We here argue that this can most usefully be separated into three stages: the cause (stressor), which generates an immediate effect (stress), followed by an ultimate response (recovery, or not) in the focal organism (Rykiel 1985, Shea et al. 2004, Borics et al. 2013).

The time when a stress response is examined changes the conclusions drawn if the initial effect differs from the ultimate response. A stressor can cause a direct effect on the long-term response and/or an immediate stress effect. This short-term effect can then itself mediate the ultimate response. In this manner, an organism's final growth is partly explained by the stressor, and partly by the recovery immediately following the

stressor. Without a cause-effect-response framework, this process cannot be parsed out from a single, ultimate response.

In general, the ultimate response to stress is a decrease in fitness, such as a decrease in growth, fecundity, or survival of the individual or its offspring. A key body of work focuses on whether the duration of the stressor affects these responses. Chronic stressors are generally considered as long in duration, in contrast with acute stressors (Barker 1997, Romero 2004, Engle et al. 2008, Miller et al. 2012). In many cases, the longer a stressor lasts, the more damaging it is (Szira et al. 2008, Martin 2009), but there is the potential for acclimation to chronic stress (Romero 2004). Furthermore, short-term stress responses (e.g., in response to a natural enemy) may in fact enhance fitness (Monaghan and Haussmann 2015).

There is also a growing body of literature on the role of the timing of a stressor in the life cycle of an organism. An organism responds to stressors throughout its life cycle, but the immediate effects and ultimate responses may differ depending on the timing of the stressor (Robertson et al. 2001, Szira et al. 2008, Lavery et al. 2009, La Pierre et al. 2011, Akiyama and Ågren 2012, Dietrich and Smith 2016, Nakahama et al. 2016, Li and Pennings 2017, McMunn 2017, Holan et al. 2018). There is a growing recognition that very early life history traits and events may play a major role in determining species' ecological dynamics and function (Larson et al. 2020). Specifically, stressors at early stages of an organism's life can generate larger effects than the same stressor later in life (Glynn et al. 2001, Simčič et al. 2015, Tungjitwitayakul et al. 2015).

Thus, the same stressor can generate significantly different responses depending on its timing or duration (McCormick et al. 2015). Furthermore, a recent meta-analysis (Eyck et al. 2019) examining the effects of early-life stress across metazoans suggests that the individual responses to stressors were in part explained by the interaction of the timing and the duration of the stressor (Eyck et al. 2019). Despite this insight, there is a dearth of empirical studies on stressor interactions (but see, e.g., McCormick et al. 2015).

We present here a study specifically designed to address the potential for interactions between the timing and duration of a stressor. We experimentally manipulate timing and duration of water restriction independently and together to understand both the immediate effect on the individual organism and the subsequent recovery response of the organism. We argue that such studies can be conducted with any type of organism and would be equivalently relevant to animals, bacteria, plants, etc. Here, we focus on a deliberately simplified laboratory plant system to examine the interactions of timing and duration using the stressor, stress, initial response, ultimate response framework, providing a prototype for studies in more complex, natural systems.

We test water restriction as a *cause* of stress in *Lepidium sativum* L., a forb commonly referred to as garden cress. Our reference state is provided by control plants that are watered daily. Treatment plants are denied water either early or late, and the duration of restriction is either long or short, allowing us to attribute differences in growth to timing and duration of the stressor. We vary these aspects of stress while holding the frequency, daily intensity, and spatial extent of the water restriction constant. We hypothesize that (1) early stressors have a larger impact on plant growth (as plants have less mass and are still actively growing); (2) long stressors are more harmful than short stressors; and (3) the interaction between early and long stressors is more harmful than the additive effects of each individual stressor. Data on plant height immediately after the stressor provide information on the initial stress *effects*. Plant growth data are then analyzed using growth models that illuminate the different *responses* of the individuals. We further hypothesize that the ultimate response will be determined by both the stressor itself and the initial effect of that stressor.

Methods

Growth experiment

Garden cress *L. sativum* L. (Brassicaceae) was grown from seeds (Johnny's Selected Seeds) under 18–6 h light-dark cycle at 25°C and 75% humidity. Ten seeds were evenly sown on 4 mm thick paper towels in two rows in an 8 × 14 cm transparent container with ventilation. No other growth medium was provided. All experiments

were conducted in a growth chamber (Percival Scientific, Perry, Iowa, USA), and the location of each container within the growth chamber was randomized with respect to treatment. Cress grows to 2–3 cm over ten days in this laboratory setting under well-watered conditions and remains at this height for at least five more days.

Ten containers were each assigned to one of the following five water restriction treatments: control (no restriction), long early, long late, short early, or short late water restriction. Ten plants were grown in each container. Except for the control, each treatment had water withheld for one or two days (short and long treatments, respectively) at day 1 or 7 of the experiments (early and late treatments, respectively). Each container received 10 mL of deionized water on watering days, starting at day 0. Any excess water from the previous day was drained from each container before adding the daily watering treatment.

Each seedling's shoot length (defined here as the length of the stem from the radicle to the leaves) was measured daily for 10 d. On day 10, we also recorded root length (defined here as the straightened length of the longest individual root) and branching growth pattern of the root (binary branching index recorded as branched or not branched) via destructive sampling, to understand changes in root morphology associated with different root foraging behavior for water among treatments. Shoot length, root length, and branching index were averaged across all plants in a container.

We recorded the initial biomass of all 10 seeds before sowing in each container (no significant difference in initial biomass was found across containers). The total dry root biomass and dry shoot biomass for each container were measured at the end of the experiment, after desiccating the samples in an incubator at 50°C for two days and removing the seed coats.

Statistical analyses

To assess the initial *effect* of the treatments, we used *t*-tests to compare the shoot heights a day after water was withheld to those in the controls on the same day. To assess the ultimate responses to each treatment, we performed ANOVA ($n = 10$ containers for each treatment) on final shoot height (measured on day 10), root length, dry shoot biomass, and dry root biomass using the R package matrixStats (Bengtsson 2020). When treatments differed significantly at $P = 0.05$, we used Tukey's HSD to compare means.

To further determine which temporal aspect of the stressor was the source of differences among treatments, we fit linear models for shoot height, root length, and biomass against stressor duration, timing, and their interaction. We similarly fit linear models on key resource allocation metrics (Ostonen et al. 2007): R:S (defined as root biomass: shoot biomass) and specific shoot length and specific root length (both defined as length per mass). The ratios show if stressed individuals change strategies to allocate additional biomass to different physiological systems. The branching index was assessed using a general logistic mixed effects model with treatment as a fixed effect and container a random effect using the R package lme4 (Bates et al. 2015). The branching index provides a more detailed view of how water restrictions may affect the root system structure. To assess if the magnitude of the initial effect of the stressor in any way determined the ultimate height, shoot height immediately following the water restriction was included as a covariate along with treatment in a linear model. Since the water restriction ended on different days, each treatment was compared only to the control for this analysis. All statistical analyses were done in R (R Development Core Team 2020).

Growth model parameter estimation

Growth models were fit to the individually tracked shoot length data to assess the daily growth of the plants in response to the different stressors. We considered five widely used growth models (Linear, Loglinear, Logistic, Gompertz, and Von Bertalanffy; Paine et al. 2012) and used Akaike information criterion (AIC) scores to compare them (Akaike 1974). The Gompertz (Gompertz 1825; Eq. 1) and von Bertalanffy models fit our growth data far better than the other models (Appendix S1: Table S1). Because the parameters in the Gompertz model are easier to interpret biologically in our experimental context, we present analyses based on this model. Analyses performed based on the von Bertalanffy model yielded similar results (Appendix S1:

Fig. S1). We compared parameter estimates for the Gompertz model across treatments to parse the underlying growth processes,

$$\text{Height}(t) = A\exp(-\exp(-k(t-T_i))), \quad (1)$$

where A is the asymptotic height, k is the growth rate, and T_i is the inflection point. The asymptotic height represents the shoot height where growth ceased (the ultimate response of the plant). The growth rate represents how quickly the plants approached their asymptotic height. The inflection point represents the time point when the plants had the maximum growth rate.

We fit the growth model to data from each container, which provided ten sets of parameter estimates for each treatment. We compared parameter estimates across treatment by performing ANOVA ($n = 10$ for each container) on each of the three parameters. When treatments were significantly different at $P = 0.05$, we performed Tukey's HSD. The model was separately fit to each individual plant and averaged to obtain a container estimate, which qualitatively yielded a similar result (Appendix S1: Fig. S2). The impacts of timing, duration, and the interaction of timing and duration on these parameters were assessed using a linear model.

Results

Both early water restriction treatments generated immediate effects on plant height: Plants were 45% shorter after a short early stressor ($P = 2.96 \times 10^{-9}$, df = 10.99, $t = -15.98$) and 78% shorter after a long early stressor ($P = 9.87 \times 10^{-13}$, df = 13.19, $t = -24.69$) compared to the control (Fig. 1). The later water restrictions had no significant immediate effects on shoot height, with short late restrictions ($P = 9.96 \times 10^{-1}$, df = 17.61, $t = 3.99$) and long late restrictions ($P = 1.07 \times 10^{-1}$, df = 17.40, $t = -1.29$) not differing significantly from the control (Fig. 1).

However, there was only one water restriction treatment that impacted the ultimate growth response: long early (Fig. 2). Individuals grown

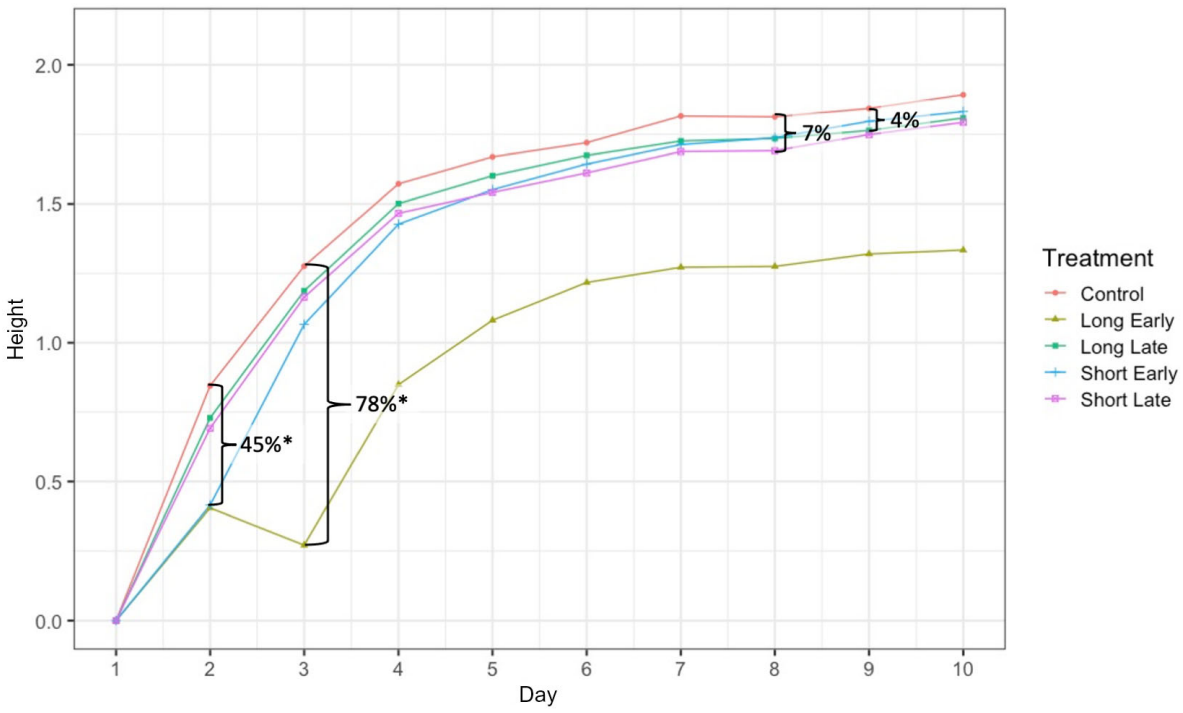


Fig. 1. The average height for each treatment over time. Brackets show the percent decrease in each treatment at the end of its water restriction compared to the control, with asterisks indicating significance at $P = 0.05$.

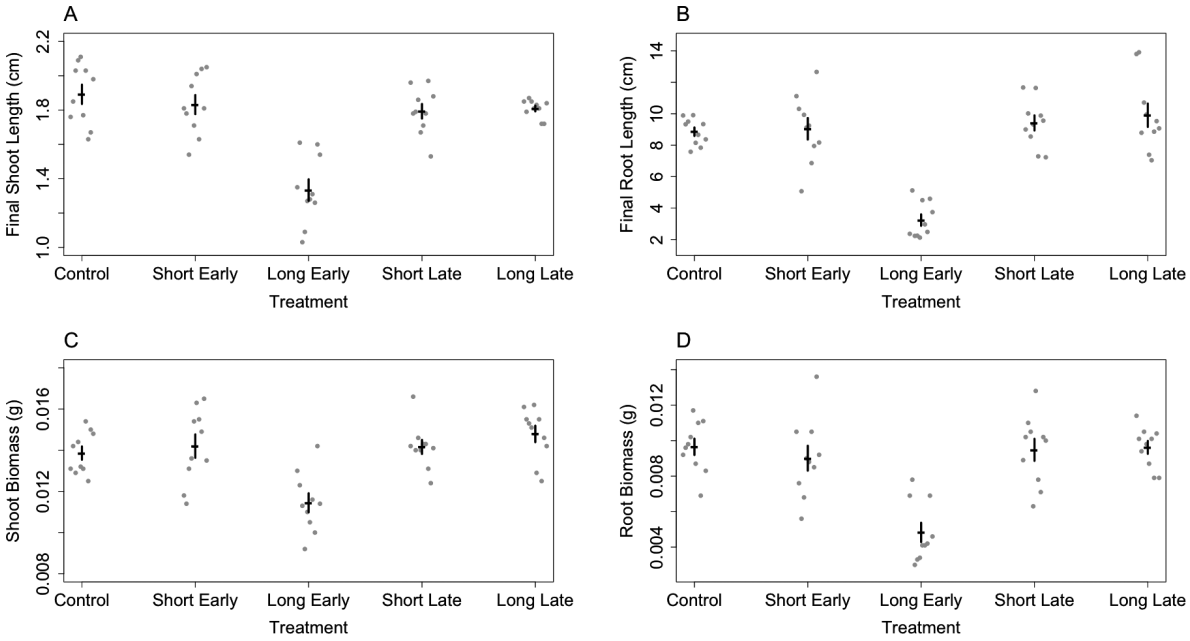


Fig. 2. (A) Final shoot length, (B) root length, (C) shoot biomass, and (D) root biomass plotted by treatment ($n = 10$). The value for each container is shown by jittered points, the average within the treatment as a horizontal bar, and the standard error as the vertical bar.

under a long early stressor had decreased final shoot height (ANOVA $P = 9.67 \times 10^{-10}$, df = 45, $F = 20.73$; TukeyHSD $P < 1.0 \times 10^{-15}$), final root length (ANOVA $P = 3.66 \times 10^{-11}$, df = 45, $F = 25.84$; TukeyHSD $P < 1.0 \times 10^{-15}$), dry shoot biomass (ANOVA $P = 1.55 \times 10^{-5}$, df = 45, $F = 9.24$; TukeyHSD $P = 0.002$), and dry root biomass (ANOVA $P = 1.38 \times 10^{-7}$, df = 45, $F = 14.24$; TukeyHSD $P = P = 1.60 \times 10^{-6}$). Other treatments did not significantly differ from the control. However, treatment alone cannot account for the final height. The height immediately following the water restriction, as well as the treatment, both predicted the ultimate height for long early plants (immediate height: $P < 1.0 \times 10^{-15}$, treatment: $P = 4.96 \times 10^{-5}$, df = 197, $F = 258.6$) and short early plants (immediate height: $P < 1.0 \times 10^{-15}$, treatment: $P = 8.28 \times 10^{-7}$, df = 197, $F = 77.04$). For short late and long late, the immediate heights significantly predicted ultimate height, but treatment did not.

The differences between treatments could be attributed to the timing of the stressor, the duration of the stressor, and, crucially, the interaction between the two. For final shoot height, root length, and dry root biomass, the differences between treatments are a result of the timing,

Table 1. The coefficients of the linear model for timing, duration, and their interaction among the different treatments for observed and modeled variables.

	Treatments		
Measurements	Timing	Duration	Interaction
Shoot height	0.154***	−0.170***	0.257***
Root length	2.490***	−1.900***	3.149***
Shoot biomass	0.00117***	−0.00075*	0.00169***
Root biomass	0.00186***	−0.00143**	0.00216***
Parameters (Gompertz)			
A, asymptotic height	0.119**	−0.131**	0.216***
k, growth rate	0.100*	−0.0575	0.105•
T_i, time of fastest growth	−0.500***	0.240***	−0.366***

Note: A positive value indicates an increase between the two treatments (i.e., early to late and short to long).

*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.
• $P < 0.1$.

duration, and interaction of the timing and duration of the stressor (Table 1). For dry shoot biomass, timing and the interaction between timing and duration are significant, but duration itself is not significant at $P = 0.05$. For all significant coefficients, the response to later timing and the interaction is positive, while the response to longer duration is negative; moving from an early to late water restriction increases growth, whereas moving from a short to long restriction decreases growth.

The R:S biomass ratio was significantly smaller for plants that experienced a long early restriction (ANOVA $P = 6.42 \times 10^{-6}$, df = 45, $F = 10.1$; TukeyHSD $P = 1.28 \times 10^{-5}$; Fig. 3A). The specific shoot length differed significantly between treatments (ANOVA $P = 0.0318$, df = 45, $F = 2.91$; Fig. 3B), with long early plants having a shorter specific shoot length than control plants (TukeyHSD $P = 0.021$). The specific root length was significantly shorter for long early plants compared to plants in the other treatments (ANOVA $P = 2.19 \times 10^{-3}$, df = 45, $F = 4.94$; TukeyHSD $P = 0.0907$; Fig. 3C). Long early plants were significantly more likely to have branching roots than plants in all other treatments as determined by the branching index general mixed effects model ($P = 6.97 \times 10^{-7}$, df = 493, $z = 4.96$; Fig. 4).

In order to better capture the differential growth responses to the treatment, we fit the Gompertz growth model to our shoot height data and compared the parameter estimates across treatments. Asymptotic height (A), inflection point (T_i), and growth rate (k) all differed among the treatments (Fig. 5). Individuals that experienced the long early stressor had a decreased asymptotic height (A), when compared to the other treatments including the control (ANOVA $P = 1.52 \times 10^{-06}$, df = 45, $F = 11.57$; TukeyHSD $P = 3.10 \times 10^{-06}$; Fig. 5A). Long early stressors decreased the growth rate (k), when compared to the control (ANOVA $P = 0.011$, df = 45, $F = 3.68$; TukeyHSD $P = 0.0094$; Fig. 5B). The inflection point (T_i), was increased more in both long early and short early treatments than in the control and late treatments (ANOVA $P = 4.59 \times 10^{-15}$, df = 45, $F = 44.37$; Fig. 5C) with plants that experienced long early water restriction being delayed by almost a day more than those in the short early treatment (TukeyHSD $P = 1.00 \times 10^{-06}$).

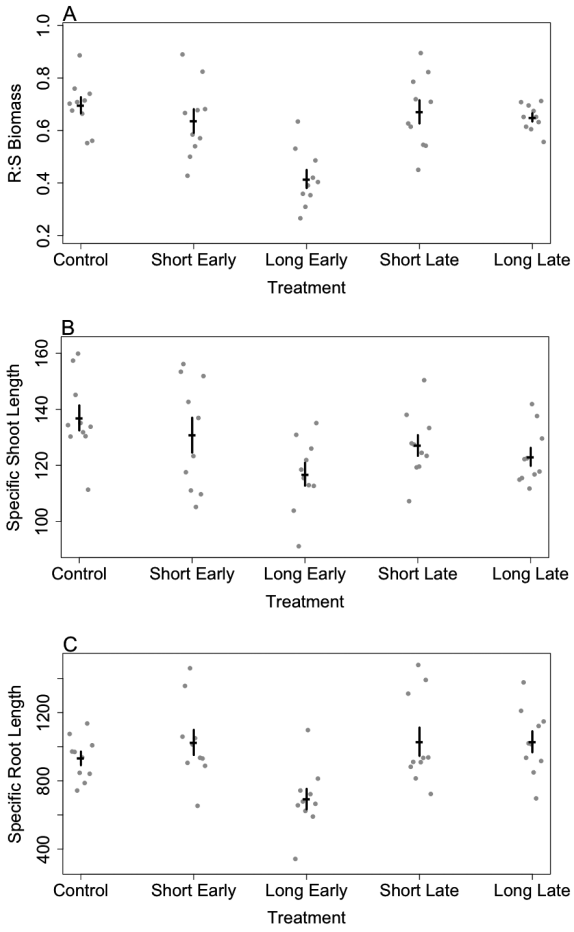


Fig. 3. (A) Root: shoot biomass, (B) specific shoot length, and (C) specific root length ratios ($n = 10$ containers). The value for each container is shown by jittered points, the average within the treatment as a horizontal bar, and the error as the vertical bar.

To understand which temporal aspect was responsible for response differences, the interaction effects were calculated for the model parameter estimates. The differences between treatments for the asymptotic height were significant for timing, duration, and their interaction (Table 1). For the inflection point, timing, duration, and the interaction were also all significant contributors to the differences observed between treatments. For the growth rate, k, timing alone explains the differences, as duration and the interaction are not significant at $P = 0.05$.

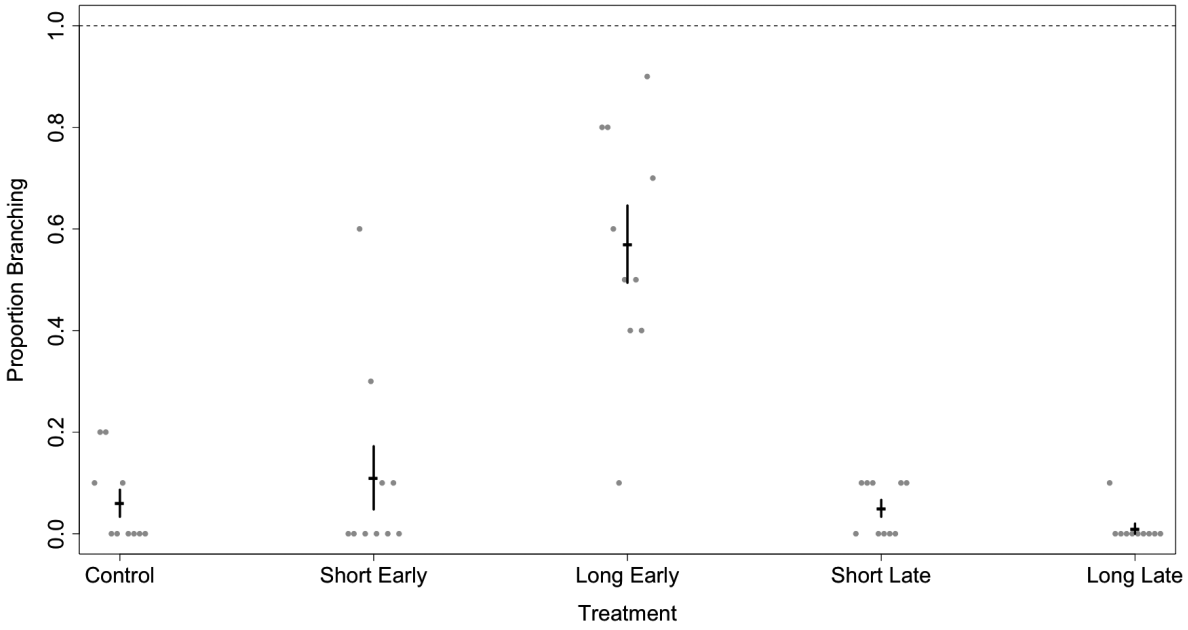


Fig. 4. The average proportion of individuals that possess branching roots per container ($n = 10$) ± standard error of the mean with jittered points for each container. The value for each container is shown by jittered points, the average within the treatment as a horizontal bar, and the error as the vertical bar.

Discussion

Stress occurring early in life generated significant decreases in plant height immediately following the release of the water restriction. However, plants under a short early water restriction responded by recovering to heights reached by control plants, while those that experienced restriction for an extended duration (long early) reached lower asymptotic heights. Plant heights immediately following the stressor (the effect) were significantly shorter for short late but not for long late as compared to the control, but even the significant effect was very small. In both cases, the responses (plants heights, etc., at the end of the experiment) for late stressors did not differ significantly from the control. The ultimate response of individuals was in part determined by the initial effect of the stressor. Even though the immediate effects of the stressor differ between treatments, the long early stressor was the only treatment to exhibit an altered long-term response (shorter shoot height, shorter root length, less shoot biomass, and less root biomass).

The timing of this stressor mattered; the same stress occurring earlier in the life of an individual decreased growth and/or ultimate height more than the same stress occurring later in life. Duration of the stressor also mattered, as a longer water restriction was more harmful than a more acute one. Importantly, the interaction between these two temporal variables was also significant. We recommend that multiple possible aspects of a stressor, and their interactions, be simultaneously examined to avoid errors in inference (Zhang and Shea 2012, McCormick et al. 2015). Similarly, if multiple stressors are present in a system, their interactions must also be considered (Davis et al. 1999).

The long early restriction changed the growth pattern and stress response of cress. This was determined using two methods: dynamical growth modeling as assessed by the Gompertz growth model and resource allocation as assessed by the growth partitioning ratios. We interpreted the parameters of the Gompertz growth model to understand how certain stages of the recovery differed among treatments. The Gompertz growth model tracked daily growth

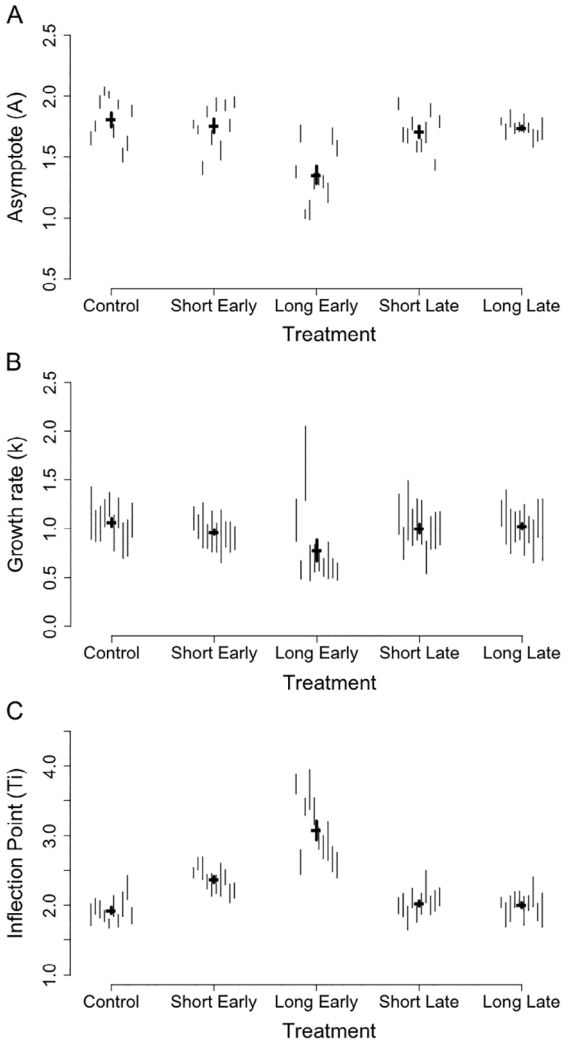


Fig. 5. Gompertz parameter estimates. The jittered, light gray vertical lines are the Gompertz model parameter estimates for each container along with its standard error. Vertical black lines represent the average for a treatment ± standard error among $n = 10$ containers, and the horizontal line represents the average across containers within a treatment.

well, as confirmed by the close agreement between the asymptotic height parameter and the empirically measured final shoot height. The inflection point (T_i, the time when a seedling is growing the fastest) occurred 2–3 days into growth for most treatments. However, the early stressors delayed inflection points compared to the other treatments, with plants grown under long early water restriction delaying a full day and those experiencing short early restrictions delaying less than a day (Fig. 5). Furthermore, the growth rate (k) was decreased by the long early water restriction compared to the other treatments. An early restriction initially stunts growth (by 45% in short early and by 78% in long early); however, it does not necessarily decrease growth in the long term when compared to the baseline control. Plants experiencing a short early stressor recovered to the same asymptotic heights (A) as all other treatments, while long early individuals grew to a shorter final height. Their response to the stressor was slower and of a lesser magnitude than those exposed to a short early stressor. Taken together, the initial effects and parameter values reveal when a stressor is detrimental and for how long its effects linger.

Resource allocation patterns attributed to stress were elucidated through the root:shoot (R:S) ratio, specific root length, specific shoot length, and branching index. In addition to delayed growth response and decrease in overall height, individuals grown under long early treatments had a decreased R:S biomass ratio. The specific shoot length was decreased for long early plants compared to control plants. Plants in the long early treatment therefore allocate more biomass into shoots than roots but are less able to convert this biomass into new shoot growth. Furthermore, within the root system, the growth strategy was altered. First, the specific root length for individuals in the long early treatment decreased compared to the plants in the other treatments, consistent with other studies (de Vries et al. 2016). Second, in the long early water restriction treatment, the roots exhibited a branching pattern with many lateral roots that were shorter in length as compared to those of plants in the other treatments, where individuals had one long root with few branches. Taken together, it seems that plants under stress in their early-life stages invest more resources into new, fine lateral roots rather than lengthening their longest root.

Our results join a growing body of work that suggests paying greater attention to interactions of stressor aspects (such as timing and duration) will be fruitful (Miller et al. 2012, Zhang and Shea 2012, McCormick et al. 2015, Eyck et al. 2019). We suggest that joint consideration of the timing and duration of stressors helps to

determine which stressors require mitigation and which stressors individuals may be able to overcome without intervention. Organisms may be able to withstand long-duration stressors, so long as they do not coincide with early life history events. Thus, initial differences in the effects of a stressor may or may not translate into longer-term differences in responses (Metcalfe and Monaghan 2001). Short early stressors appeared to temporarily harm the plants as seen by the delayed inflection point and stunted growth, yet they were able to recover to the same asymptotic height as the other treatments. Other systems also show that delays in growth or initial stress effects early in life do not always result in decreases in growth later in life (Grindstaff and Merrill 2017, Michel et al. 2018). Since plants experiencing short early stressors can recover, this raises the possibility that those experiencing long early water restrictions could also recover if proper interventions are applied. Our system would be ideal to test potential suitable mitigation strategies due to its high tractability and demonstration that some individuals that display initial effects will, in fact, recover on their own. By measuring individuals daily, we demonstrate that the ultimate response is affected by both the stressor and its immediate effect.

Stress affects organisms of all types (Del Giudice et al. 2018). We demonstrate that analyzing stress as a sequential process, by parsing stress into immediate effects and ultimate responses, will allow us to identify stressors that are most likely to impact organisms in the short and long term. This will also make it easier to investigate the effects of other interacting factors, such as interactions of water stress with nutrients and other resources (Gessler et al. 2017). Similar approaches that analyze perturbations using a cause, effect, and response framework to assess the influence of multiple disturbance aspects are also being found useful at the community and ecosystem levels (Shea et al. 2004, Mitchell et al. 2016), suggesting that this conceptual framework can be used to understand general responses to perturbations across different biological levels of organization.

In order to fully understand a stress response, studies must also examine multiple stressor aspects (including timing, duration, intensity, frequency, and extent; Miller et al. 2012, McCormick et al. 2015) as interactions between these aspects can significantly affect outcomes and interpretation (Zhang and Shea 2012, McCormick et al. 2015, Eyck et al. 2019). An improved understanding of such interactions and their time-dependence will deepen our mechanistic understanding of stress-related processes in the plethora of organisms affected by stress.

Acknowledgments

We gratefully acknowledge funding from the joint US National Science Foundation and the UK National Environment Research Council grant 155644 to KS and AB and support from an NSF REU supplement. We thank Joe Keller, Ciara Hovis, Britta Teller, Emily Howerton, Ishan Phadke, Shouli Li, Elyse Johnson, Trine Ready, Kimberlyn Nelson, and other Shea laboratory members for valuable discussions. We declare no conflicts of interest. Author contributions: KS and AB conceived the ideas; JS, KS, and HI designed the methodology; JS collected the data; JS and HI analyzed the data; JS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Literature Cited

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Akiyama, R., and J. Ågren. 2012. Magnitude and timing of leaf damage affect seed production in a natural population of *Arabidopsis thaliana* (Brassicaceae). PLOS ONE 7:e30015. https://doi.org/10.1371/journal.pone.0030015

Barker, D. J. P. 1997. Fetal nutrition and cardiovascular disease in later Life. British Medical Bulletin 53:96–108.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walke. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bengtsson, H. 2020. matrixStats: Functions that apply to rows and columns of matrices (and to vectors). R package version. https://CRAN.R-project.org/package=matrixStats

Borics, G., G. Várbíró, and J. Padisák. 2013. Disturbance and stress: different meanings in ecological dynamics? Hydrobiologia 711:1–7.

Davis, M. A., K. J. Wrage, P. B. Reich, M. G. Tjoelker, T. Schaeffer, and C. Muermann. 1999. Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. Plant Ecology 145:341–350.

de Vries, F. T., C. Brown, and C. J. Stevens. 2016. Grassland species root response to drought: consequences for soil carbon and nitrogen availability. Plant and Soil 409:297–312.

Del Giudice, M., C. L. Buck, L. E. Chaby, B. M. Gormally, C. C. Taff, C. J. Thawley, M. N. Vitousek, and H. Wada. 2018. What is stress? A systems perspective. Integrative and Comparative Biology 58:1019–1032.

Dietrich, J. D., and M. D. Smith. 2016. The effect of timing of growing season drought on flowering of a dominant C4 grass. Oecologia 181:391–399.

Doffo, G. N., S. E. Monteoliva, M. E. Rodríguez, and V. M. C. Luquez. 2017. Physiological responses to alternative flooding and drought stress episodes in two willow (*Salix* spp.) clones. Canadian Journal of Forest Research 47:174–182.

Engle, K. M., T.-S. Mei, M. Wasa, and J.-Q. Yu. 2008. Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. Journal of Neuropsychiatry and Clinical Neurosciences 45:788–802.

Eyck, H. J. F., K. L. Buchanan, O. L. Crino, and T. S. Jessop. 2019. Effects of developmental stress on animal phenotype and performance: a quantitative review. Biological Reviews 94:1143–1160.

Gessler, A., M. Schaub, and N. G. McDowell. 2017. The role of nutrients in drought-induced tree mortality and recovery. New Phytologist 214:513–520.

Glynn, L. M., P. D. Wadhwa, C. Dunkel-Schetter, A. Chicz-DeMet, and C. A. Sandman. 2001. When stress happens matters: effects of earthquake timing on stress responsivity in pregnancy. American Journal of Obstetrics and Gynecology 184:637–642.

Gompertz, B. 1825. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philosophical Transactions of the Royal Society of London B: Biological Sciences 182:513–585.

Grindstaff, J. L., and L. Merrill. 2017. Developmental corticosterone treatment does not program immune responses in zebra finches (*Taeniopygia guttata*). Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 327:262–272.

Holan, J. R., C. K. King, and A. R. Davis. 2018. Comparative copper sensitivity between life stages of common subantarctic marine invertebrates. Environmental Toxicology and Chemistry 37:807–815.

La Pierre, K. J., S. Yuan, C. C. Chang, M. L. Avolio, L. M. Hallett, T. Schreck, and M. D. Smith. 2011. Explaining temporal variation in above-ground productivity in a mesic grassland: the role of climate and flowering. Journal of Ecology 99:1250–1262.

Larson, J. E., B. L. Anacker, S. Wanous, and J. L. Funk. 2020. Ecological strategies begin at germination: traits, plasticity and survival in the first 4 days of plant life. Functional Ecology 34:968–979.

Lavery, P. S., K. McMahon, M. Mulligan, and A. Tennyson. 2009. Interactive effects of timing, intensity and duration of experimental shading on *Amphibolis griffithii*. Marine Ecology Progress Series 394:21–33.

Li, S., and S. C. Pennings. 2017. Timing of disturbance affects biomass and flowering of a saltmarsh plant and attack by stem-boring herbivores. Ecosphere 8: e01675.

Martin, L. B. 2009. Stress and immunity in wild vertebrates: timing is everything. General and Comparative Endocrinology 163:70–76.

McCormick, G. L., K. Shea, and T. Langkilde. 2015. How do duration, frequency, and intensity of exogenous CORT elevation affect immune outcomes of stress? General and Comparative Endocrinology 222:81–87.

McMunn, M. S. 2017. The timing of leaf damage affects future herbivory in mountain sagebrush (*Artemisia tridentata*). Ecology 98:1996–2002.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254–260.

Michel, E. S., S. Demarais, B. K. Strickland, and G. Wang. 2018. Birth date promotes a tortoise or hare tactic for body mass development of a long-lived male ungulate. Oecologia 186:117–128.

Miller, A. D., S. H. Roxburgh, and K. Shea. 2012. Timing of disturbance alters competitive outcomes and mechanisms of coexistence in an annual plant model. Theoretical Ecology 5:419–432.

Mitchell, P. J., et al. 2016. An ecoclimatic framework for evaluating the resilience of vegetation to water deficit. Global Change Biology 22:1677–1689.

Monaghan, P., and M. F. Haussmann. 2015. The positive and negative consequences of stressors during early life. Early Human Development 91:643–647.

Mustroph, A., S. C. Lee, T. Oosumi, M. E. Zanetti, H. Yang, K. Ma, A. Yaghoubi-Masihi, T. Fukao, and J. Bailey-Serres. 2010. Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. Plant Physiology 152:1484–1500.

Nakahama, N., K. Uchida, A. Ushimaru, and Y. Isagi. 2016. Timing of mowing influences genetic diversity and reproductive success in endangered semi-natural grassland plants. Agriculture, Ecosystems and Environment 221:20–27.

O'Mahony, S. M., et al. 2014. Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or

anxiety-related behaviors in male rats. Neuroscience 277:885–901.

Ostonen, I., et al. 2007. Specific root length as an indicator of environmental change. Plant Biosystems 141:426–442.

Paine, C. E. T., T. R. Marthews, D. R. Vogt, D. Purves, M. Rees, A. Hector, and L. A. Turnbull. 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods in Ecology and Evolution 3:245–256.

R Development Core Team 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robertson, A., P. Bacon, and G. Heagny. 2001. The responses of floodplain primary production to flood frequency and timing. Journal of Applied Ecology 38:126–136.

Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19:249–255.

Rykiel, E. J. 1985. Towards a definition of disturbance. Australian Journal of Ecology 10:361–365.

Selye, H. 1956. The stress of life. Revised edition. McGraw-Hill, New York, New York, USA.

Shea, K., S. H. Roxburgh, and E. S. J. Rauschert. 2004. Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecology Letters 7:491–508.

Simčič, T., D. Jesenšek, and A. Brancelj. 2015. Effects of increased temperature on metabolic activity and oxidative stress in the first life stages of marble trout (*Salmo marmoratus*). Fish Physiology and Biochemistry 41:1005–1014.

Szira, F., A. F. Bálint, A. Börner, and G. Galiba. 2008. Evaluation of drought-related traits and screening methods at different developmental stages in spring barley. Journal of Agronomy and Crop Science 194:334–342.

Tungjitwitayakul, J., N. Tatun, B. Vajarasathira, and S. Sakura. 2015. Expression of heat shock protein genes in different developmental stages and after temperature stress in the maize weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 108:1313–1323.

Zhang, R., and K. Shea. 2012. Integrating multiple disturbance aspects: management of an invasive thistle, *Carduus nutans*. Annals of Botany 110:1395–1401.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3620/full