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Preliminary analysis reveals sediment burial decreases mass loss and increases survival of the aquatic invasive plant *Hydrilla verticillata* following desiccation over short time scales

Bridgett N. Pickman* and Matthew A. Barnes

Texas Tech University, Department of Natural Resources Management, Lubbock, TX 79409, USA

Author e-mails: bridgett.pickman@ttu.edu (BNP), matthew.a.barnes@ttu.edu (MAB)

*Corresponding author

Abstract

Aquatic plants represent a key target in the management of biological invasions due to their ability to outcompete native species, interfere with human water use, and modify nutrient cycles. Studying desiccation tolerance of aquatic plants can improve understanding of their survival in habitats with fluctuating water levels, as well as response of invasive aquatic plants to management practices such as drawdowns. In a preliminary study, using *Hydrilla verticillata* as a case study, we desiccated plant fragments for 3 hours on top of sediment, buried beneath sediment, and in the absence of sediment in laboratory mesocosms to quantify the effects of sediment burial on survival and growth upon reintroduction to a common aquatic environment. In the absence of sediments, fragments experienced the greatest mass loss during desiccation and highest mortality rates upon reintroduction to the aquatic environment. Burial decreased plant fragment mass loss during desiccation, and upon return to a common aquatic environment, buried fragments demonstrated rates of new growth indistinguishable from undried control fragments. Overall, our results suggest that the presence of sediments can play an important role in the ability of aquatic plants to withstand desiccation stress at short time scales. Future studies should consider longer time scales and conditions most representative of field settings, as an understanding of sediments can inform management practices aimed at the control and eradication of hydrilla and other invaders.

Key words: aquatic weed, drawdown, drying, macrophyte, regeneration

Introduction

Aquatic plants represent a key target in the management of biological invasions. Aquatic plants provide plankton and fish with shelter and protection. Their role as producers in the food web can contribute to profound and cascading community impacts when invading aquatic plants displace their native counterparts (Capers et al. 2007). Aquatic invasive plants have also demonstrated the ability to affect the abiotic as well as the biotic environment. For example, invasive species are known to modify nutrient cycles and productivity (Kelly and Hawes 2005). Aquatic invasive plants influence human interaction with aquatic environments, especially by negatively impacting the ecosystem goods and services provided by those environments such as nutrient cycling and erosion control (Gordon 1998). Other impacts of ecosystem services include impeding access for human recreational activities, as well as adverse effects on irrigation practices and potable water production.

Anthropogenic dispersal of seeds, vegetative fragments, and other plant components as hitchhikers on trailered boats and other recreational equipment represents a primary dispersal pathway for a variety of species (Johnson et al. 2001; Rothlisberger et al. 2010). Therefore, several recent studies have examined whether species' response to and resistance of desiccation stress experienced during overland dispersal can provide insight into invasion potential (Jerde et al. 2012). Among recent insights, Barnes et al. (2013) demonstrated that desiccation responses are species specific, McAlarnen et al. (2012) demonstrated that different parts of the same plant (e.g. vegetative

fragments from apical or bottom portions of the plant) respond differently to desiccation stress, and additional work has demonstrated that variation in environmental conditions mediate plant response to desiccation stress (e.g. Bickel 2015).

Understanding desiccation resistance and response does not only benefit knowledge of aquatic invasive plant overland dispersal. Desiccation resistance and tolerance to fluctuating water levels represent critical species traits that determine plant communities in ephemeral streams, wetlands, tidally influenced waters, and other aquatic habitats where disturbance or regular water fluctuations dominate (Van Geest et al. 2005). Accordingly, desiccation resistance or tolerance may contribute to the ability of an aquatic plant species to invade such systems. Furthermore, one tool in the management of aquatic invasive plants is drawdown, a practice in which the water level is lowered to expose plant fragments to air, resulting in plant death (e.g. Poovey and Kay 1998). Unlike in the case of hitchhiking overland dispersal, in the cases of drawdown and fluctuating water levels, unique factors of the physical environment such as the presence of insulating sediments may influence the desiccation process.

There is yet much to be discovered of hydrilla's response to desiccation. Our study parallels the objectives of several others on the topic of hydrilla desiccation response, particularly due to the common usage of drawdowns for management purposes (Madsen 1997). Doyle and Smart (2001) observed hydrilla tubers under drawdown conditions to gauge tuber desiccation response. While this study was informative, it primarily focused on tubers and neglected hydrilla's ability to reproduce via stem fragmentation. Our present research effort aims to accumulate additional information on vegetative fragments that will inform effective drawdown practice and understanding of aquatic plant response to desiccation.

As a precursor to a large-scale analysis of desiccation during a drawdown event, we report here on a laboratory experiment at an abbreviated time scale and simplified environmental conditions to explore whether the presence or absence of sediment may affect desiccation rate and ultimate survival of *Hydrilla verticillata* (hydrilla). In particular, we inquired whether the presence of sediment slowed hydrilla desiccation rate, and whether this information could be used to predict the amount of biomass lost during desiccation, and ultimately survival. This evaluation can help inform management practices aimed at the control and eradication of hydrilla and other invasive species.

Methods

To investigate the effect of sediment presence on fragment desiccation, we conducted an experiment to measure the response of dioecious hydrilla fragments upon reintroduction to an aquatic environment following desiccation under different sediment conditions. Of Indo-Asian origin, hydrilla represents a common invasive aquatic plant that now occurs on every continent except Antarctica and has become especially problematic in Europe and North America. Where it has been introduced, hydrilla can grow dense monocultures that displace native vegetation, reduce water flow, and interfere with human water use, leading some to refer to the plant as "the perfect aquatic weed" (Langeland 1996). Recent species distribution models suggest that hydrilla has the potential to further extend its invaded range across North America (Barnes et al. 2014), so recent research has addressed the influence of desiccation on dispersal success, and here we conduct preliminary analysis on the influence of desiccation *in situ*.

We collected whole hydrilla plants for this study from four sample locations along the shore of Lake Pflugerville, Texas, USA (30.4392ºN; 97.5721ºW) by wading and collecting specimens directly from the littoral zone as well as via rake toss. The samples were transported in coolers and transferred to an indoor holding tank where they remained for 36 hours before beginning the study. We conducted desiccation experiments in an aluminum-framed, glass greenhouse at Texas Tech University (33.5843ºN; 101.8783ºW). Rooms within the greenhouse are individually climate-controlled, and the conditions during our experiment were 23.5 ± 2.6 °C and 64.2 ± 4.4% relative humidity. Desiccation treatments occurred in late afternoon, when plants received full, diffuse (not-direct) sunlight.

We began by cutting the apical ends of 80 *Hydrilla verticillata* plants into 20 cm fragments for use in three desiccation treatments plus an undried control group (N = 20 samples per treatment). At the beginning of the experiment, samples were blot-dried with a towel, and we recorded the mass of each before they were placed into their respective treatment containers (50 cm × 34 cm × 14 cm, approx. 24 L). Dry sand represented the sediment in our experiment, as it did in a similar study by Doyle and Smart (2001). We placed 20 samples on top of 2 cm of sand, buried 20 beneath 7 cm of sand, and placed 20 on the bottom of their mesocosm without sand present. A final 20 samples served as an undried control group.

Fragments air-dried for 3 hours. To ensure each fragment received identical desiccation conditions (besides the imposed sediment treatment), we rotated the locations of the containers within the greenhouse

on an hourly basis. We measured mass again follo-wing desiccation to calculate change in biomass due to the imposed treatments. All samples (i.e. undried control and desiccation treatment samples) were then placed into individual 16-oz (approx. 473 mL) jars with 2-mm^2 nylon mesh lids. We submerged jars in a common aquatic environment (300 gal [approx. 1136 L] structural foam stock tank filled with reverse osmosis water, pH = 7.6) with a pump to aerate the water and ensure circulation among individual jars. A pilot experiment using red dye released at one end of a tank confirmed that this setup allows water to move throughout the tank and into all jars (Jerde et al. 2012, M.A. Barnes personal observation). Following the design of Jerde et al. (2012), individual jars with mesh lids allowed us to track individual fragment performance within a common environment while simultaneously allowing for sufficient water exchange and circulation between and within jars. For the follo-wing 3 weeks, survival and the presence or absence of new vegetative or root growth was noted. The jars were moved haphazardly around the common environ-ment at each weekly inspection. To provide one measurement of fragment growth during the experiment, after the four weeks in the common environment, we again blot-dried fragments with a towel and recorded fragment mass.

During the initial plant desiccations and associated analysis, we noticed that a small amount of sand stuck to the buried fragments and fragments desiccated on top of sand. To correct for any potential influence of residual sand on post-desiccation treatment mass, we cut a separate group of 20 samples into 20 cm frag-ments, blot dried, and weighed them. We then placed 10 samples on top of 2 cm sand and buried 10 beneath 7 cm of sand and weighed the fragments again to determine the immediate difference in mass resulting from fragment contact with sand. The mean value (mean ± standard deviation; top fragments = 0.07 ± .10 g; buried fragments = 0.32 ± .31 g) was subtracted from final mass measurements to account for minor sand effects. All statistical methods and results reported herein refer to data corrected in this manner.

We applied logistic regression to test whether pro-portion mass lost influenced survival. Next, we calcu-lated area under the receiver operating characteristic curve (AUC) to assess model performance, where AUC = 0.5 indicates the model predicts outcomes no better than random, and AUC ≥ 0.7 indicates strong predictive power (Hosmer and Lemeshow 2000). Proportion data were arcsine square root transformed to meet the assumptions of ANOVA. Given that mass lost proved to be a significant predictor of fragment survival (see Results), we applied ANOVA followed by a Tukey Test to identify desiccation treatment effect on proportion of mass lost (a poten-
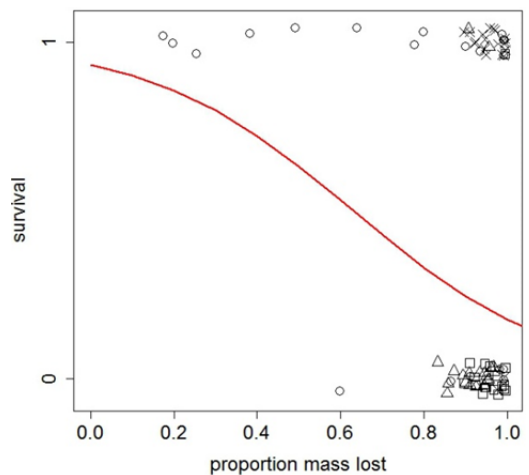
Figure 1. Across all treatments, proportion mass lost proved to be a reliable predictor of survival (logistic regression p = 0.0007; AUC = 0.8026). In our experiment, plants could only survive (i.e. y-axis value of 1) or die (i.e. 0), but data are "jittered" on y-axis to improve visibility. Surviving fragments included 2 of 20 no-sediment treatment fragments (denoted by triangles), 1 of 20 fragments on top of sediment (denoted by squares), 14 of 20 buried fragments (denoted by circles), and 18 of 20 undried control fragments (denoted by x).

tially more informative- continuous rather than binary-response). We also applied ANOVA and Tukey Tests to assess the relationship between desiccation treatment and subsequent growth within the common aquatic environment.

Results

We designed an experiment to compare the effects of desiccation in the presence and absence of sediment. To ensure that differences between plant performance were the result of our imposed desiccation treatments, we first had to confirm that all fragments began the experiment with statistically indistinguishable initial masses. The initial masses of treatment and control fragments did not significantly differ from one another (ANOVA, $F_{3,76}$ = 2.18, p = 0.0973). Following desiccation and regardless of desiccation conditions (i.e. sediment treatment), percent mass lost proved to be a reliable predictor of survival (logistic regression p = 0.0007; AUC = 0.8026; Figure 1); fragments that lost a greater proportion of their initial mass were less likely to survive the study. Logistic regression predicted that over 61% mass loss was required before fragments became more likely to die than to survive. However, some variation occurred across the range of mass losses seen in this experiment, and we observed death of 2 control fragments and survival of multiple fragments despite > 90% mass loss.

After determining that mass loss predicted fragment survival, we asked whether our imposed sediment treatments influenced the rate of mass loss. ANOVA confirmed that mass loss did differ depending on sediment treatments in our study ($F_{2,57} = 8.137$, $p < 0.001$; Figure 2). The proportion of mass lost was greatest in fragments without sediment and on top of sediment, although a post-hoc Tukey Test indicated that there was not a difference between these two treatments ($p = 0.3246$). Buried fragments lost significantly less mass than both those without sediment (Tukey Test $p = 0.0410$) and those on top of sediment (Tukey Test $p = 0.0006$). Notably, although the mean and median mass loss observed was lower in buried fragments compared to fragments on top of sediment or in the absence of sediment, some buried fragments still lost mass at similar rates to other treatments, and the difference between treatments was driven by the tail or skewed portion of buried fragments that lost less mass than in other treatments (Figure 2).

To determine how fragments performed upon reintroduction to an aquatic environment, we measured new growth after 4 weeks in the common environment. Treatment did significantly influence growth (ANOVA, $F_{3,76} = 7.383$, $p = 0.0002$). Upon reintroduction, fragments without and on top of sediment demonstrated virtually no growth, while the buried fragments had new growth comparable to the undried control after 4 weeks (Tukey Test $p = 0.9999$; Figure 3).

Discussion

Desiccation of aquatic plants can play a critical role in their invasion biology, as the ability to withstand or tolerate desiccation stress influences overland dispersal, establishment potential in habitats with fluctuating water levels, and survival in the face of management efforts such as drawdown. We used laboratory experiments to demonstrate that the presence of sediment impacts the desiccation response of the invasive aquatic plant hydrilla. In our experiment, burial beneath sediment slowed desiccation rate, which we speculate was due to an insulating effect the sediment provides, hindering evaporation and the loss of mass during the drying period. We observed a threshold mass loss of 61% to influence survival; beyond this mass loss threshold, plant fragments were more likely to die than to survive upon reintroduction to an aquatic environment. Sediments can influence desiccation response in hydrilla and possibly other aquatic invasive plants, which re-emphasizes the importance of considering local environmental conditions to maximize research and management practices.
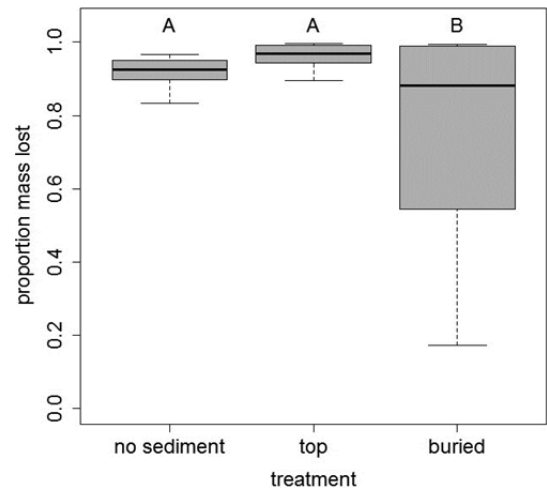
Figure 2. Treatment and control fragments had similar initial masses (ANOVA $p = 0.0973$), but mass change during desiccation differed between treatments ($p = 0.0008$). Buried fragments differed from fragments without sediment (Tukey Test $p = 0.0410$) and fragments on top of sediment ($p = 0.0006$), and no difference occurred between fragments without and on top of sediment ($p = 0.3246$). Letters above boxes denote statistical relationships.
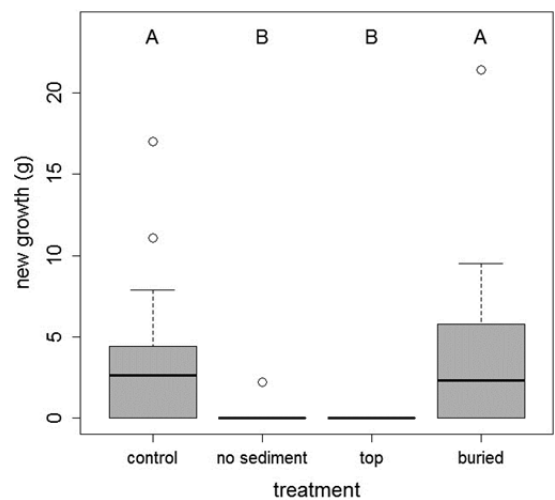
Figure 3. Treatment also influenced growth in the 4 weeks following reintroduction to an aquatic environment (ANOVA $p = 0.0002$). Buried fragments grew similarly to undried controls (Tukey Test $p = 0.9999$) and significantly more than top ($p = 0.0054$) and no-sediment ($p = 0.0085$) fragments. Letters above boxes denote statistical relationships.

Ours is not the first study to examine the desiccation of aquatic plant fragments and draw connections between this phenomenon and invasion potential. Both Evans et al. (2011) and Jerde et al. (2012) previously quantified desiccation rates in Eurasian water milfoil *Myriophyllum spicatum*, and Brucker-hoff et al. (2015) examined desiccation in both

Eurasian water milfoil and curly-leaf pond weed *Potamogeton crispus*. Barnes et al. (2013) surveyed a more extensive list of aquatic plants. All of these previous studies emphasized the role that desiccation plays in overland dispersal of aquatic plants. Our study distinguishes itself from these previous efforts by considering the influence of sediment burial on mass loss and survival *within* simulated aquatic or semi-aquatic environments as well as how unique factors of the physical environment such as the presence of insulating sediments may influence the desiccation process.

Our study does compare more directly with several others, particularly due to the considerable invasive history of our focal organism hydrilla (Langeland 1996) and the historic use of drawdown to manage aquatic invasive plants (Madsen 1997). Doyle and Smart (2001) observed the desiccation of hydrilla tubers in drawdown conditions and found that for every 1% decline in moisture, there was approximately a 2% decline in tuber viability. Despite a year-long drawdown, they concluded that no significant desiccation stress to the tubers buried beneath sediment occurred. Although our study occurred at a much shorter timescale, the conclusion of Doyle and Smart (2001) is consistent with our finding that plants buried beneath sediment experienced slower desiccation rates than those sitting on the sediment surface. The generalizability of this pattern is strengthened by the fact that we observed vegetative plant fragments while Doyle and Smart (2001) used tubers, and McAlarnen et al. (2012) demonstrated that different plant parts may display different desiccation responses. The recent surge in interest in studying desiccation of aquatic plant fragments to understand aquatic plant invasion (e.g. Evans et al. 2011; Jerde et al. 2012; Barnes et al. 2014; Bickel 2015; Bruckerhoff et al. 2015), should not undermine the importance of other structures, such as subterranean turions or tubers, for conveying desiccation resistance and invasion potential to aquatic plants.

In another study similar to our own, Poovey and Kay (1998) observed hydrilla under drawdown conditions in the presence of different hydrosoils and found that hydrilla biomass, number of tubers, tuber weights, ratio of tuber numbers to unit biomass, and ratio of tuber weight per unit biomass were each significantly affected by both length of drawdown as well as hydrosoil type. Poovey and Kay (1998) also observed an interaction between drawdown and hydrosoil type, leading them to conclude that characteristics of the hydrosoil present in the drawdown zone may influence management success. We only examined one soil type (i.e. sand) in our experiment, but future examinations of different soil types and combinations of soils could further benefit management efforts and research aimed at understanding desiccation of invasive and other aquatic plants in habitats where drawdown or other temporary desiccation events may occur. We observed the desiccation response of hydrilla in a climate-controlled greenhouse, but future studies could expand on these findings by replicating the experiment under field conditions. Manipulation of depth of sediment may also play a role in desiccation response, as we only observed a single fixed depth. Examination of these factors may better inform drawdown practices to account for desiccation rates of plant fragments buried and rooted at different depths beneath the sediment surface.

Dry sand represented the sediment in our experiment, and we found it well-suited as such due to its unforgiving nature in comparison with soil. Fragments buried in soil would most likely lose moisture at a slower rate than those buried in sand due to a higher sediment moisture content. Future studies that consider different sediment types or sediments with varying moisture contents would further add to our understanding of plant desiccation stress. Our experiment lasted 3 hours, whereas drawdowns for the management of invasive aquatic species usually last for months or longer. Even with the harsh sediment and relatively brief drying time we saw significant evidence that the presence of sediment influences mass loss and ultimate survival. Thus, if this experiment were carried out in the real world we could anticipate that increased soil moisture would provide even more protection against desiccation, although the extended drawdown period could very well counteract the insulative impact of the soil. Further investigation would be required to verify if our results were a direct result of the low moisture content of the substrate, yet we have our doubts that the rate at which fragments of each treatment lose mass would fluctuate substantially even in moist sediment. Future studies should expand on our experimental design by performing drawdowns *in situ* to further explore aquatic plant fragment desiccation response. These studies could elaborate on our findings by extending the duration of the drawdown to a month or longer, as well as substituting more complex substrates in lieu of sand.

Overall, we believe our study contributes to the growing narrative that desiccation response represents an important element in our understanding of aquatic plant invasions, and we have re-emphasized the fact that overland dispersal is not the only occurrence in which this knowledge can contribute to the management of biological invasions. Future drawdown

practices could be increased in effectiveness by understanding the influence of local conditions and species-specific desiccation responses. For example, our observations suggest that tilling or other efforts to expose buried vegetative fragments may benefit overall impacts of drawdown on invasive plant communities. Furthermore, this knowledge may guide future management practices of native aquatic species in littoral zones with naturally fluctuating water levels. Finally, although our research contributes to a growing literature on the influence of desiccation on aquatic plant fragments, our study design can also contribute to understanding of desiccation, fluctuating water levels, and management practices such as drawdown on other invasive aquatic species.

Acknowledgements

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