Note

Viability of Aquatic Plant Fragments following Desiccation

Matthew A. Barnes, Christopher L. Jerde, Doug Keller, W. Lindsay Chadderton, Jennifer G. Howeth, and David M. Lodge*

Desiccation following prolonged air exposure challenges survival of aquatic plants during droughts, water drawdowns, and overland dispersal. To improve predictions of plant response to air exposure, we observed the viability of vegetative fragments of 10 aquatic plant species (*Cabomba caroliniana, Ceratophyllum demersum, Elodea canadensis, Egeria densa, Myriophyllum aquaticum, Myriophyllum heterophyllum, Myriophyllum spicatum, Potamogeton crispus, Potamogeton richardsonii,* and *Hydrilla verticillata*) following desiccation. We recorded mass loss, desiccation rate, and plant fragment survival across a range of air exposures. Mass loss accurately predicted viability of aquatic plant fragments upon reintroduction to water. However, similar periods of air exposure differentially affected viability between species. Understanding viability following desiccation can contribute to predicting dispersal, improving eradication protocols, and disposing of aquatic plants following removal from invaded lakes or contaminated equipment.

Nomenclature: Brazilian egeria, *Egeria densa* Planch.; common elodea, *Elodea canadensis* Michx.; coontail, *Ceratophyllum demersum* L.; curlyleaf pondweed, *Potamogeton crispus* L.; Eurasian watermilfoil, *Myriophyllum spicatum* L.; fanwort, *Cabomba caroliniana* Gray; hydrilla, *Hydrilla verticillata* (L. f.) Royle; parrotfeather, *Myriophyllum aquaticum* (Vell.) Verdc.; Richardson's pondweed, *Potamogeton richardsonii* (A. Bennett) Rydb.; variable-leaf watermilfoil, *Myriophyllum heterophyllum* Michx.

Key words: Dispersal, invasion, macrophyte, management, prediction.

Desiccation represents a critical challenge to the survival of plants (reviewed in Alpert 2000; Bewley 1979; Hsiao 1973). Among plants, those species that make their homes in the aquatic environment may be particularly threatened during periods of water loss. Desiccation of aquatic macrophytes can occur routinely for species that occur in intertidal regions (Chu et al. 2012) or areas that experience seasonal drought (Westwood et al. 2006) or other water drawdowns (Barrat-Segretain and Cellot 2007). Aquatic plants also experience desiccation during dispersal across terrestrial expanses as they travel as hitchhikers on terrestrial or semiaquatic organisms (Figuerola and Green 2002; Vaschoenwinkel et al. 2008) or recreational boats (Johnson et al. 2001). In fact, despite the potential for desiccation stress, dispersal of vegetative aquatic plant fragments as hitchhikers on trailered boats has been implicated as a major invasion pathway for aquatic plants (Rothlisberger et al. 2010).

Broadly, two plant physiological characteristics influence the success of hitchhiking vegetative plant fragments: the ability of plants to survive fragmentation (Kimbel 1982) and the subsequent ability of fragments to resist or tolerate the desiccation and other stressors of the pathway (Evans et al. 2011; Jerde et al. 2012; Johnstone et al. 1985; McAlarnen et al. 2013). We compared desiccation rates and postdesiccation survival of vegetative fragments across 10 aquatic plant species, emphasizing differences between plant physical forms and fragment lengths as well as

variable desiccation time periods. Quantifying aquatic plant species viability following desiccation can contribute to predicting dispersal, improving invasive species eradication protocols, and disposing of weeds following removal from invaded lakes or contaminated equipment.

Materials and Methods

Study Organisms. We studied the desiccation rates and viability following air exposure of vegetative fragments of 10 aquatic plant species (Table 1). All species live fully submersed lifestyles, though the emergent apical ends of *Myriophyllum aquaticum* (Vell.) Verdc. (parrotfeather) were also included in this study. Selected plant species represented a variety of different physical forms including plants with divided ($n = 5$) vs. entire ($n = 5$) leaf margins. Most were field-collected, but three species were purchased from online retailers due to limited local distribution (Table 1). We stored plants in aerated aquaria filled with well water for no more than 48 h before conducting experiments. Only apical ends of vegetative fragments were used in experiments, and all fragments were inspected to assure minimal physiological stress (i.e., obvious tissue damage or decomposition) existed prior to use.

Desiccation Rate. We hypothesized the rate at which plant fragments lose water during air exposure would be a strong predictor of survival following reintroduction to an aquatic environment. To determine desiccation rate of each plant species, we followed the methods of Jerde et al. (2012).

Briefly, we monitored mass loss of individual fragments of each species when positioned on a rigid mesh platform in front of a box fan. An "active" desiccation method differs from air-drying methods employed in previous studies of aquatic plant desiccation (e.g., Johnstone et al. 1985), and we believe our approach provides a more realistic simulation of the desiccation stress experienced by plant fragments during situations such as hitchhiking overland dispersal. Indeed, in a pilot experiment on 20-cm (7.9-in) lengths of *Cabomba caroliniana* Gray (fanwort), fragments exposed to active (i.e., fan) drying desiccated at a significantly faster rate than passively dried fragments (Mann-Whitney U test, P = 0.0102).

The experiments occurred in a climate-controlled solarium with temperatures approximately 25 ± 1 C standard deviation (SD) (77 ± 1.8 F), 40 ± 8% SD relative humidity, and a dew point of approximately 11 C. For each species, we began experiments by blotting dry (to standardize initial fragment conditions) 15 replicate fragments of both 10 and 20 cm in length. We recorded the mass of individual fragments at the beginning of each trial and every 15 min for 2 h or until fragments reached a constant mass. Following the approach of Jerde et al. (2012), we fit an exponential decay function to the repeated measurements for each individual fragment to estimate desiccation rate. State law prohibited us from transporting *Hydrilla verticillata* (L. f.) Royle (hydrilla), so desiccation rate data for this species were collected near the collection site in the Ohio River, where local conditions were approximately 21 C and 41% relative humidity.

We performed an ANOVA to test for differences in desiccation rate between species. In addition to testing for differences between plant species, we hypothesized that plants with divided leaf margins would demonstrate higher desiccation rates than plants with entire leaf margins due to an increased surface area : volume ratio promoting transpiration and water loss. Treating leaf type as a fixed effect with species as a random factor allowed for testing the significance in desiccation rate between leaf types. All statistical analyses were performed in R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria).

Viability following Desiccation. Using a different set of desiccated fragments, we tracked the viability of fragments for each species across variable desiccation times. We applied 0-h (i.e., no-desiccation control), 1-h, or 3-h desiccation treatments as previously described, placed fragments into individually labeled 0.9-L (1-qt) canning jars with 2-mm^2 (0.003-in^2) nylon mesh lids, and placed jars in a 900-L plastic trough of circulating well water. The trough was housed in the same solarium where desiccation occurred, so recovering fragments received approximately 14 h of direct natural sunlight daily. Jars allowed us to monitor fate of individual fragments in a common

Table 1. List of plant species used in this study.[a]

Species	Leaf margin	Native range	Invasive range	Collection site
Cabomba caroliniana Gray	Divided	North America, South America	Australia, Asia, Canada	Purchased
Ceratophyllum demersum L.	Divided	North America, possibly United Kingdom	Uncertain in temperate regions around the globe	St. Joseph River, South Bend, IN
Egeria densa Planch.	Entire	South America	Australia, Asia, Europe, New Zealand, North America, southern Africa	Purchased
Elodea canadensis Michx.	Entire	North America	Africa, Australia, Europe, Central America, Russia, South America,	Stone Lake, LaPorte, IN
Hydrilla verticillata (L.f.) Royle	Entire	Australia, Asia, possibly Europe	Africa, North America, South America, possibly reintroduced in Australia, Asia, and Europe	Ohio River, OH
Myriophyllum aquaticum (Vell.) Verdc.	Divided	South America	Africa, Australia, Europe, North America	Purchased
Myriophyllum heterophyllum Michx.	Divided	North America	Europe	Pinhook Lake, South Bend, IN
Myriophyllum spicatum L.	Divided	Eurasia, northern Africa	North America, southern Africa	St. Joseph River, South Bend, IN
Potamogeton crispus L.	Entire	Africa, Australia, Eurasia	North America, New Zealand	Pinhook Lake, South Bend, IN
Potamogeton richardsonii (A. Benn.) Rydb.	Entire	North America	Unknown	North Chain Lake, South Bend, IN

[a] Native and invasive ranges summarized from Invasive Species Specialist Group (2012).

environment. We monitored fragments weekly until recovery or death. For *C. caroliniana*, *Egeria densa* Planch. (Brazilian egeria), *Elodea canadensis* Michx. (common elodea), *M. aquaticum*, *Myriophyllum heterophyllum* Michx. (variable-leaf watermilfoil,), *Myriophyllum spicatum* L. (Eurasian watermilfoil), and *Potamogeton richardsonii* (A. Bennett) Rydb. (Richardson's pondweed), we followed the convention of Jerde et al. (2012) of using presence of roots as an indicator of viability. We are confident in this metric, because of 783 fragments of all species that formed roots in this experiment, only one single *Elodea canadensis* fragment died within a 6-wk observation period after forming roots. The plant *Ceratophyllum demersum* L. (coontail) does not form roots, so viability was determined based on the survival of plant fragments for 10 wk. Survival was assessed based on the maintenance of rigid structure and green pigmentation (dead fragments became brown detritus on the bottom of jars). Unlike the other plants in our study, *Potamogeton crispus* L. (curlyleaf pondweed) senesces in early summer, coinciding with the approximate timing of our collection, so none of our observed fragments formed roots. We therefore measured *P. crispus* viability by monitoring for formation of turions. Finally, because state law prohibited us from transporting *H. verticillata*, we could not collect viability data to relate to previously collected desiccation rate data. For each other species, we calculated the percentage of fragments that remained viable following each desiccation time period, and differences were identified by nonoverlapping 95% confidence intervals.

We hypothesized that mass loss due to desiccation would predict viability. To test this prediction, we produced a separate logistic regression for each plant species to model fragment fate (a binomial response: recovery or death) as a function of percentage of mass loss. We assessed the goodness of fit of each logistic regression model by calculating the area under the receiver operating characteristic curve (AUC), where AUC = 0.5 indicates that the model predicts outcomes no better than random, and AUC ≥ 0.7 indicates acceptable levels of predictive power (Hosmer and Lemeshow 2000). AUC was calculated using the pROC package in R (Robin et al. 2011).

Finally, we extracted from each logistic regression model a threshold percentage of mass loss (i.e., the inflection point of the logistic model) that signified that a plant fragment was more likely to die than recover upon reintroduction to an aquatic environment. We also calculated the mass loss percentage in each model corresponding to 10% viability (i.e., 90% probability of death).

Results and Discussion

Across all plant species, the relationship between desiccation time and plant fragment mass followed an exponential decay, beginning with rapid mass loss and
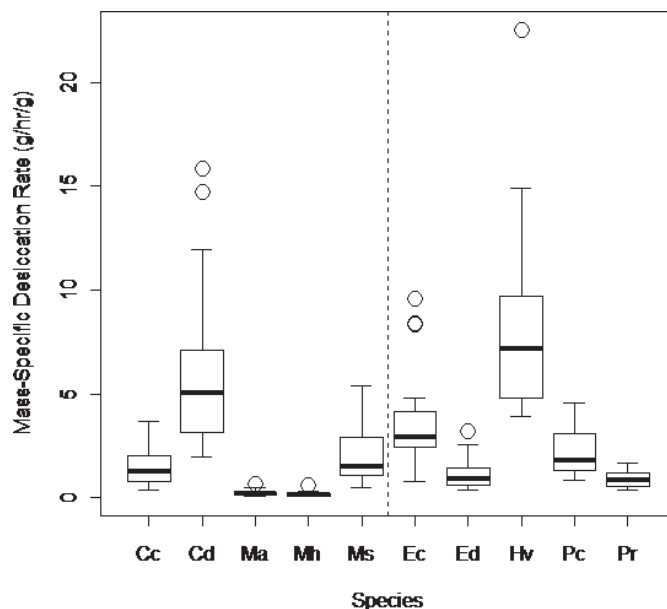

Figure 1. Comparison of desiccation rate between species. Box and whisker plots depict the distribution of data around the median desiccation rate for each species. Open circles represent statistical outliers for each species. Species abbreviations: *Cc* = *Cabomba caroliniana*, *Cd* = *Ceratophyllum dermersum*, *Ma* = *Myriophyllum aquaticum*, *Mh* = *M. heterophyllum*, *Ms* = *M. spicatum*, *Ed* = *Egeria densa*, *Ec* = *Elodea canadensis*, *Hv* = *Hydrilla verticillata*, *Pc* = *Potamogeton crispus*, and *Pr* = *P. richardsonii*. Dashed vertical line separates plants with divided leaves (left) from plants with entire leaves (right). *N* = 15 fragments for each species.

followed by decelerating mass loss as plant fragments approached their final dry mass. Desiccation rates varied markedly between species (Figure 1). *Myriophyllum heterophyllum* exhibited the slowest average desiccation rate at 0.16 g h^{-1} (0.006 oz h^{-1}) when normalized for original fragment mass, and *Hydrilla verticillata* was the fastest drying plant, losing an average of 9.96 g h^{-1} when normalized for original fragment mass. ANOVA verified that desiccation rates differed significantly between plant species ($P = 0.01$; Figure 1).

Contrary to prediction, no difference in desiccation rates occurred between species with entire leaf margins vs. divided leaf margins (ANOVA, $P = 0.61$). Across all plant species, viability decreased as a function of desiccation time as expected. However, the nature of this relationship differed between species, as evidenced by comparison of 95% confidence intervals of the mean viability (Figure 2). Several species, including *Cabomba caroliniana, Ceratophyllum demersum, Egeria densa, M. aquaticum,* and *M. heterophyllum* experienced 100% viability among control (i.e., nondesiccated) fragments. *Cabomba caroliniana, Ceratophyllum demersum, M. spicatum,* and *P. crispus* all experienced 100% mortality following 3 h of desiccation (Figure 2).
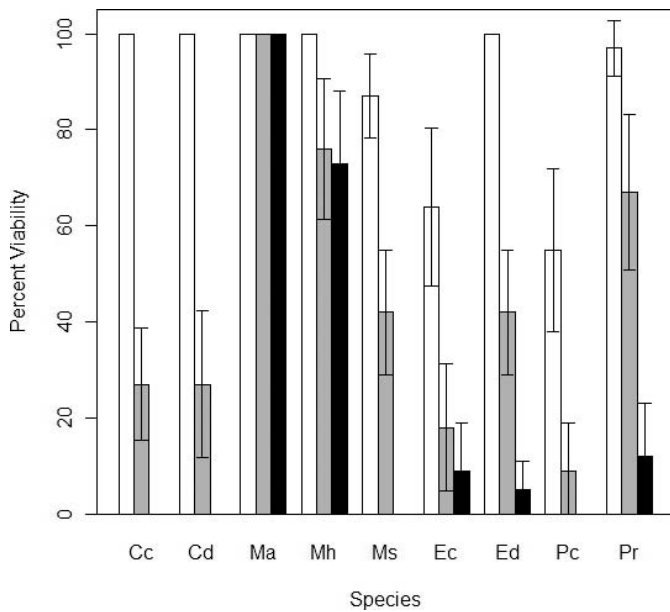
Figure 2. Percent variability of plant fragments of each species following 0 (white bars), 1 (gray bars), and 3 h (black bars) of desiccation. Error bars represent 95% confidence intervals. Species abbreviations: *Cc = Cabomba caroliniana, Cd = Ceratophyllum demersum, Ma = Myriophyllum aquaticum, Mh = M. hetero-phyllum, Ms = M. spicatum, Ed = Egeria densa, Ec = Elodea canadensis, Pc = Potamogeton crispus,* and *Pr = P. richardsonii.*

Mass loss was a significant (all logistic regression models P < 0.01; Table 2) and accurate (all AUC > 0.8; Table 2) predictor of viability following desiccation for each species. The threshold where plant fragments are equally likely to die as they are to remain viable varied between species, ranging from ≤ 15% mass loss in *Elodea canadensis* and *P. crispus* to approximately 80% for *M. aquaticum* and *M. heterophyllum.*

On average, logistic models predicted that approximately 90% mass loss corresponded to a 90% probability of death following desiccation, although the models predicted that several species required total mass loss to achieve a 90% probability of death, reflecting the potential for survival of aquatic plant fragments that can occur even at extreme levels of desiccation. Species desiccation rate provided a strong predictor of viability following 1 h of desiccation (P = 0.02, $R^2 = 0.58$, Figure 3), supporting the hypothesis that plant species that desiccate faster have lower probability of survival following air exposure.

In this experiment, we focused on desiccation of vegetative fragments of aquatic plants, but other plant structures such as the thick tubers produced by *H. verticillata* (Langeland 1996) may be even more tolerant of desiccation. Furthermore, we examined desiccation under one set of controlled environmental conditions, but changes in temperature, humidity, or wind speeds will alter outcomes through varied desiccation rate. Our experimental setup (i.e., individual vegetative fragments arranged for drying on a mesh screen) represented relatively harsh conditions compared to the conditions under which some plant fragments may experience air exposure, such as dispersing in bunches on recreational equipment (Johnson et al. 2001) or among sediments during a water drawdown (Barrat-Segretain and Cellot 2007). Although the specific desiccation rates and resulting viabilities observed in this study have limited applicability to many real-world situations, a clear lesson has emerged from our data: aquatic plant desiccation should be considered on a species-specific basis.

A framework for assessing the vulnerability of ecosystems to invasion by aquatic weeds must consider dispersal modes (i.e., how propagules move between sites; Wilson et al. 2009) and pathways (i.e., between which sites propagules

Table 2. Description of the logistic regression models developed for each aquatic plant species.[a]

Species	N	Model slope	Model intercept	Model P-value	AUC[b]	Mass loss resulting in 50% viability	Mass loss resulting in 10% viability
						——————%——————	
Cabomba caroliniana	164	−6.81	3.96	< 0.001	0.95	58	90
Ceratophyllum demersum	121	−8.52	5.74	0.009	0.95	67	93
Egeria densa	165	−3.65	1.89	< 0.001	0.87	52	100
Elodea canadensis	110	−3.43	0.50	< 0.001	0.84	15	79
Myriophyllum aquaticum	96	−3,418.00	2,719.00	< 0.001	1.00	80	80
Myriophyllum heterophyllum	121	−5.70	4.49	< 0.001	0.82	79	100
Myriophyllum spicatum	143	−5.31	2.40	< 0.001	0.86	45	87
Potamogeton crispus	121	−3.88	0.27	< 0.001	0.86	7	64
Potamogeton richardsonii	121	−5.55	3.27	< 0.001	0.88	59	99

[a] Model slope and model intercept describe characteristics of each model. *N* indicates number of plant fragments observed to develop the model for each species. Mass loss indicating 50% death corresponds to the inflection point of each logistic regression model.

[b] Abbreviation: AUC, area under the receiver operating characteristic curve; this provides a measure of goodness of model fit.
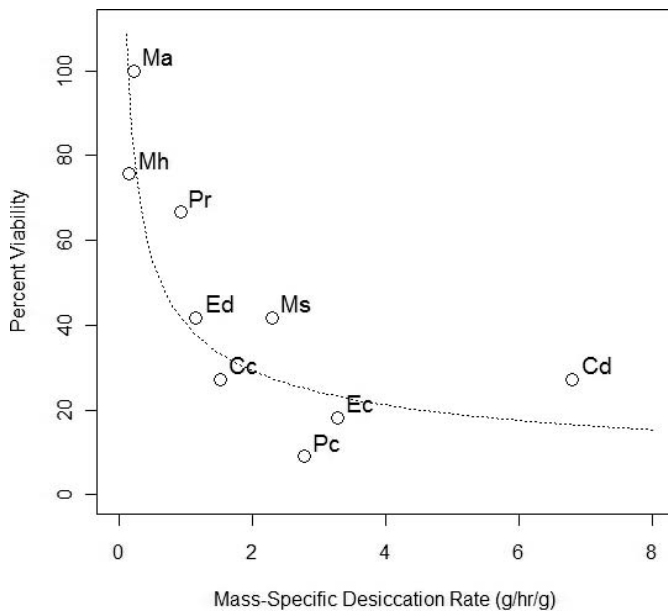
Figure 3. Mass-specific species desiccation rate provided a strong predictor of survival following 1 h of desiccation. The model (dotted line) is described by the following equation: % Viability = $40.478(\text{Desiccation Rate})^{-0.466}$. Data points are labeled with the following species abbreviations: *Cc* = *Cabomba caroliniana*, *Cd* = *Ceratophyllum demersum*, *Ma* = *Myriophyllum aquaticum*, *Mh* = *M. heterophyllum*, *Ms* = *M. spicatum*, *Ed* = *Egeria densa*, *Ec* = *Elodea canadensis*, *Pc* = *Potamogeton crispus*, and *Pr* = *P. richardsonii*.

are most likely to move; Vander Zanden and Olden 2008). Furthermore, determining which ecosystems will support a population upon introduction and predicting what negative impacts an introduced species will have on its environment also comprise fundamental aspects of a framework for managing biological invasions (Vander Zanden and Olden 2008). Continued study of life history and physiology of aquatic plants like our own examination of viability following desiccation will greatly improve our ability to place invaders and potential invaders in an appropriate management framework.

Acknowledgments

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