NOTES

Simulated overland transport of Eurasian watermilfoil: survival of desiccated plant fragments

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INTRODUCTION

Biological invasions occur when species are introduced beyond their historical range, establish self-sustaining populations, and spread, sometimes with severe ecological and economic consequences (Sakai et al. 2001). Human movement provides an opportunity for aquatic plants and animals to travel rapidly over land between otherwise isolated water bodies (Johnson et al. 2001, Drury and Rothlisberger 2008). The biology of some aquatic invasive plants makes them particularly likely to disperse via anthropogenic pathways, as many species undergo fragmentation, an asexual process by which part of the plant becomes detached from its roots (Madsen et al. 1988). Fragments tend to accumulate in shallow waters and along the shoreline and near boat launches, which leads to their entanglement in boat trailers and motors (Johnson et al. 2001, Rothlisberger and Lodge 2011), and can then lead to transport to new water bodies as "hitchhikers" (Johnstone et al. 1985).

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an aquatic invasive plant that spreads via fragmentation and has a history of negative environmental and economic impact (Smith and Barko 1990). To reduce the probability of egress of plant fragments on boats and trailers, it is important to know whether all fragments pose an equal risk or whether some fragments are too small or from a suboptimal part of the plant to survive drying and transport. Here, we simulated the drying effects of overland transport of Eurasian watermilfoil and examined the effects of desiccation on the survival of fragments of different lengths and types (apical shoots or basal stems). This research benefits management efforts by characterizing the different risk of invasion posed by Eurasian watermilfoil fragments with diverse characteristics.

MATERIALS AND METHODS

Eurasian watermilfoil was collected in August of 2009 from the St. Joseph River in South Bend, Indiana (42° N, 86° W).

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To characterize the desiccation rate of Eurasian watermilfoil, 15 replicate plant fragments of either 5 cm or 10 cm, and either apical shoots or basal stems (defined as >30 cm below the apical end) were placed on a rigid mesh platform in front of a fan at to simulate overland transport. Jerde et al. (2012) found that anemometer readings taken at multiple locations where macrophytes foul on the back of boat trailers indicated that a vehicle traveling at 89 km/h (55 miles/h) could expose fragments to average wind speeds of less than 16 km/h. The fan used in this experiment generated airspeeds of approximately 12.1 km/h (SD 2.4), falling within the range observed by Jerde et al. (2012). The platform was rotated 180 degrees every 30 min. We recorded fragment mass every 15 minutes and dried the plant segments for 2 hours or until a constant mass was obtained. Regulated environmental conditions in the solarium used during the experimental period were similar to Jerde et al. (2012), with the average temperature of 25°C and relative humidity of 40%.

We estimated desiccation rate of each plant fragment following the methods of Jerde et al. (2012) in which the data are modeled by an exponential decay function, $y(t) = ae^{b*t} + c$, where $y(t)$ is the measured mass at time t, a is the initial mass of the milfoil fragment, and c is the final fragment mass after oven-drying at 50°C for a minimum of 1 week. We calculated and interpreted b as the estimated desiccation rate. The mean desiccation rate was compared between length and type treatments with a two-way analysis of variance (ANOVA).

We conducted a second drying experiment to characterize Eurasian watermilfoil recovery following partial desiccation. In a fully-crossed experiment examining the effect of length (5 cm or 10 cm) and type (apical shoots or basal stems), 20 replicates of each treatment were dried for 60 minutes. As a control, we also included 20 fragments from each treatment without desiccation. We recorded the initial wet biomass and put each plant fragment into an individual jar with a mesh lid and placed jars in a 900 L trough of circulating well water, which allowed us to track individual fragments in a common environment. Fragments grew in full sunlight in a climate-controlled greenhouse, with air temperatures maintained between 20-25° C. Day lengths ranged from 13 hours of sunlight to 10.5 hours of sunlight by the conclusion. We monitored replicates weekly for 8 weeks, noting death or root formation.

Dead fragments (i.e. loss of structure and green pigment) were removed from the experiment.

At the end of the 8 week experiment, for those fragments that grew roots, we counted the number of emerging root segments, measured total root length, recorded final wet biomass, and calculated percent mass change of each fragment during the experiment. A two-way ANOVA was used to test for treatment effects on percent biomass change, number of emerging roots, and total root length. For all statistical analyses, the data were transformed if necessary to satisfy the assumptions of ANOVA.

To determine whether the results of the previous experiment were due to real differences between fragment type and lengths, and not simply an artifact of differences in the average number of nodes between fragments of different treatment groups, we repeated the previously described experiments holding the number of nodes, rather than fragment length, constant. Through visual inspection, we determined an average 10 cm apical shoot fragment had 7 nodes. We then measured, dried, and placed 7-node fragments from the apical end or bottom in jars with mesh lids, and submerged them in a common environment as previously described. This variation of the experiment did not change the experimental outcome, so we present only the results of the first two experiments in the following section.

RESULTS AND DISCUSSION

The type and length of the fragment both significantly affected the desiccation rate (type $p < 0.001$; length $p < 0.001$; Mean Regression $R^2 = 0.79$). Specifically, the 5 cm fragments dried out more quickly (mean $\pm$ SE $= 4.06 \pm 0.31$ g/hr/initial g biomass) than the 10 cm fragments (mean $\pm$ SE $= 1.97 \pm 0.12$ g/hr/initial g biomass), and the fragments from the bottom (mean $\pm$ SE $= 3.71 \pm 0.35$ g/hr/initial g biomass) dried out more quickly than fragments from the apical end of the plant (mean $\pm$ SE $= 2.32 \pm 0.18$ g/hr/initial g biomass). Overall, the greatest biomass loss occurred in the beginning of the drying period and the rate of biomass loss declined over time. These results occurred in a climate-controlled greenhouse. Climatic factors, particularly humidity, would likely affect desiccation rate, although we anticipate both type and length contribute greatly to the mean desiccation rate.

Our experiments on fragment survival following desiccation indicated that the difference in desiccation rates translated to differences in survival. Desiccation ($p < 0.001$), fragment length ($p = 0.028$) and type of fragment ($p < 0.001$) all significantly affected the percent biomass change of fragments after the 8 week treatment time (Figure 1A). All basal stems except for control fragments died (Figure 1B-C). Most fragments lost biomass over the course of the experiment (Figure 1A). One notable exception to this trend was 5 cm apical shoot control treatment fragments, which exhibited positive biomass change (12%). The mass loss of fragments can be attributed to the fact that for most fragments the original biomass of the fragment died over the 8 week experiment, and the final biomass we measured was the product of new growth. Fragments that did not experience desiccation accumulated more biomass and had longer roots than those produced by dried fragments ($p < 0.001$); apical shoot ends had more emerging roots ($p < 0.001$, Figure 1B) and greater total
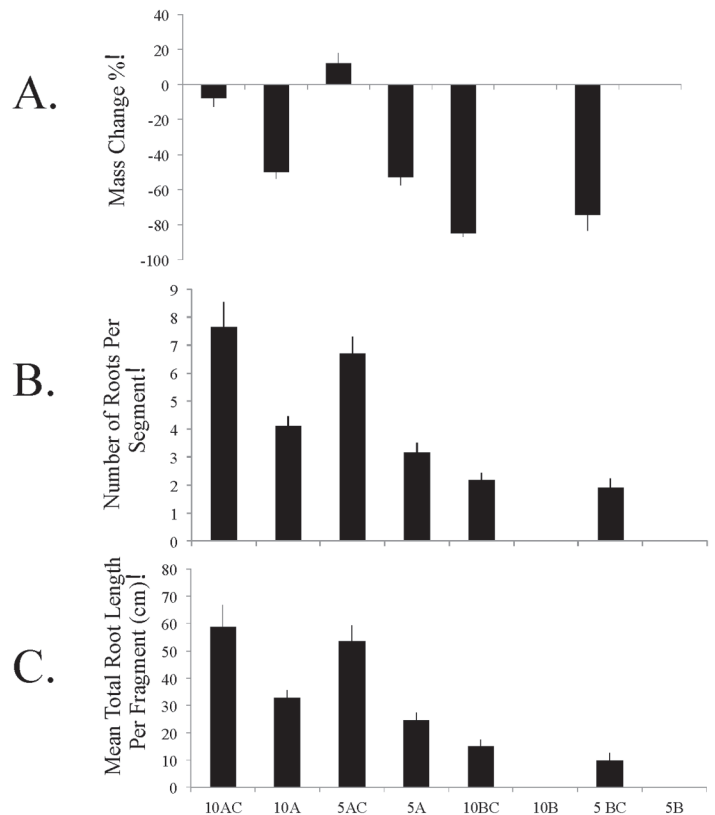

Figure 1. Responses 8 weeks after return to water by dried fragments of different lengths (5 or 10 cm) and types (A= apical shoots; B = basal stems C = control fragments). Error bars indicate ± 1 Standard Error. Dried basal stem fragments of 5 and 10 cm lengths died. **(A)** Percent biomass change; **(B)** Average number of emerging roots per fragment; **(C)** Mean total root length per fragment.

root length ($p < 0.001$, Figure 1C) than basal stems. Thus, even though plant fragments grew over the course of the experiment, they demonstrated net biomass loss. This finding may indicate the importance of dispersal early in the growing season to maximize opportunity for growth and probability of establishment in the receiving environment.

Fragment length and type both influenced fragment survival (Figure 2). In our experiment, we considered the formation of roots to be synonymous with survival, as we never had any plants die after forming roots. More apical shoots formed roots than basal fragments (Figure 2). A greater percentage of longer fragments also grew roots than shorter fragments, and control fragments again fared better than dried fragments. While differences exist, dried apical shoot fragments of both lengths survived, which suggests apical milfoil fragments may have the potential to survive overland transport and reestablish in new bodies of water. From these results we conclude that fragment type is the most important factor in predicting survival after desiccation.

When we repeated the experiment holding the number of nodes constant (7 nodes), rather than varying length, we observed similar results (data not shown) suggesting that the observed difference between the apical shoots and basal stems was not due to the presence of more nodes at the top of the plant due to condensed spacing, but rather to physiological differences between the apical and bottom ends.
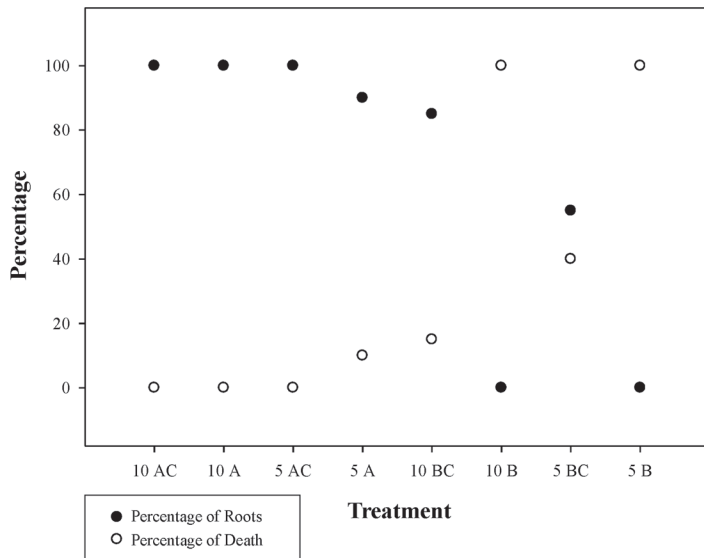
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Figure 2. Percent fragment survival over the course of the experiment by dried fragments of different lengths (5 or 10 cm) and types (A= apical shoots; B = basal stems C = control fragments).

Regeneration from a bud (apical end) after fragmentation is common for both free-floating and submerged plants (Sculthorpe 1967). Additionally, the apical end of the plant has relatively short internode spaces with greener leaves whereas the bottom of the plant has larger internode distances and often more decomposed leaves. The dense packing of leaves from the apical end of Eurasian watermilfoil and the possible aggregation of many fragments may act as insulation against drying, holding in water and thus aiding survival. Indeed, Jerde et al. (2012) found that clumped watermilfoil, mimicking >25cm fragments wrapped around a boat propeller, desiccated more slowly than single strand fragments, resulting in increased survival. Desiccation decreased the likelihood of fragment survival, but longer fragments and apical shoots fared better than shorter, bottom fragments. This hypothesis is consistent with the findings of Evans et al. (2011) and Jerde et al. (2012) who found that increasing desiccation exposure decreased survival of Eurasian watermilfoil fragments. Our results are also consistent with previous work (not including desiccation) in which apical fragments and larger fragments experienced higher relative growth rates and higher regeneration and colonization abilities when compared with fragments without an apical tip (Riis et al. 2009).

Current estimates suggest that two-thirds of boaters do not always take the necessary steps to rid their boats of possible exotic organisms (Rothlisberger et al. 2010), so the continued spread of invasive plants and other species is probable. As the data presented here suggest, small fragments, particularly with apical buds, can survive the desiccation experienced during overland transport and establish in new bodies of water.

ACKNOWLEDGMENTS

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