Fragment viability and rootlet formation in Eurasian watermilfoil after desiccation

Celia Ann Evans[1], K. M. Forrest[2], D. L. Kelting[3], L. E. Steblen[2]

Comment [AAA1]: You did not measure viability, only regrowth.

ABSTRACT

Eurasian watermilfoil often invades aquatic ecosystems in North America via fragment transport from infested lakes to uninfested water bodies by watercraft and boat trailers. While fragments transported on watercraft and trailers are likely introduced to new water bodies in various stages of desiccation, surprisingly little is known about the desiccation tolerance and subsequent viability of Eurasian watermilfoil. We conducted *in-situ* and laboratory experiments, during the growing season in 2010 to examine 1) the rate at which Eurasian watermilfoil desiccates, 2) the likelihood of new growth and rootlet formation in control fragments and fragments that had been desiccated for 3, 6, 18, 24 and 48 hours, and; ~~,~~ 3) time until new growth and rootlet formation in the different treatment groups. We found that desiccation over time fit a Michaelis-Menten type function on which 87% and 96% desiccation occurred after just 3 and 6 hours respectively and 100% desiccation of milfoil ~~strands~~ fragments occurred at approximately 13 hours under laboratory conditions. Based on a logistic regression model, desiccation significantly reduced the likelihood of fragment viability from 98% in control fragments to 2% in fragments that were completely (100%) desiccated in the laboratory experiment. Desiccation also increased the time until new growth and rootlet formation. In control treatments, 20% of Eurasian watermilfoil nodes produced new growth (via lateral bud growth) after 5 weeks and 90% of those produced rootlets. ~~We learned that while~~Although desiccation significantly reduced Eurasian watermilfoil fragment viability, a small proportion of fragments that were 100% dried were still viable and able to form rootlets.

Key Words*: desiccation tolerance, logistic regression, drying, watermilfoil physiology*

INTRODUCTION

Eurasian watermilfoil (*Myrophyllum spicatum* L.) is a submersed rooted, aquatic perennial that continues to invade and negatively influence recreational activity and alter the structure of littoral zone ecosystems across a wide geographic distribution outside of its native range. In the Adirondack Park of Northern New York State, the Adirondack Park Invasive Plant Program (APIPP) reported 79 lakes infested with aquatic invasive plants in 2010, 55 of which were reported to contain Eurasian watermilfoil, making it the most common aquatic invasive plant in the Adirondack region (T. Smith, personal communication, November 22, 2010). Working in collaboration with APIPP, the Adirondack Watershed Institute of Paul Smith's College manages a spread prevention initiative called the Watershed Stewardship Program, wherein Stewards work at boat launches in the Adirondack Park to inspect watercraft, collect data on boater demographics, and educate boaters about the ways in which they can reduce the likelihood of transporting invasive species from lake to lake. While doing this work, Stewards regularly pull fragments of aquatic plants off of boats and trailers. Stewards stationed at 7 boat launches in 2008 and 8 boat launches in 2009 (for varying numbers of days per week) identified and removed 21 and 12 Eurasian watermilfoil fragments (in those years, respectively) from boats and trailers preparing to launch into lakes without Eurasian watermilfoil populations (Watershed Stewardship Program 2008 and 2009). These fragments were in various stages of desiccation.

Dispersal of Eurasian watermilfoil within lakes occurs primarily by stolon growth and secondarily by fragmentation; seeds are thought to be a relatively unimportant means of dispersal (Madsen and Smith 1997). Autofragmentation occurs in mid-late summer when biomass is greatest in the top 20 cm of growth. Some nodes develop rootlets and begin to abscise from the plant below and can be carried by currents to surrounding areas to settle and establish.

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Allofragmentation occurs from disturbance such as boat motors, paddles, wind etc. that breaks fragments free from rooted stems and similarly allows establishment of new colonies. In a Texas study, fragmentation was responsible for 26% of the spread of Eurasian watermilfoil within the study ponds, while in Lake George, NY, 46% of fragments that settled in the sediment established as new plants (Madsen and Smith 1997).

Long distance dispersal of Eurasian watermilfoil from one water body to another appears to be caused mainly by the transfer of fragments on water craft and water craft trailers. In a New Zealand study nearly 20% of the aquatic wetland flora were introduced species, and the inter-lake movement of boats was almost exclusively the cause of the transfer of aquatic weeds. Johnstone et al. (1985) reported that none of the 5 invasive species they were studying were found in lakes with no boating or fishing activity.

Once Eurasian watermilfoil has established in a lake it is rarely possible to eradicate it through management efforts. Among other methods, benthic matting (Mayer 1978) and hand pulling operations (Kelting and Laxson 2010) have been shown to be effective at significantly reducing milfoil density. In Upper Saranac Lake, NY in the Adirondack Park, after 2 years of intensive hand harvesting, Eurasian watermilfoil was reduced to <5% cover for over 90% of the littoral zone. The cost of a program like this is astronomical, however, and not economically feasible in most cases. In the context of the above information, efforts to prevent the initial invasion are likely the best option for uninfected lakes.

A study conducted in the Great Lakes Region showed that while high pressure boat washing and visual inspection reduced the amount of macrophytes introduced to water bodies by boats by 88%, only about 1/3 of registered boaters always take these precautions (Rothlisberger et al. 2010)~~, suggesting that there is much work still to be done to educate the boating public~~

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Comment [AAA6]: I think these two paragraphs can be condensed together. I would not spend much time introducing Autofragmentation etc. Spend more time on the long distance (i.e. the ability) dispersal because that is the point of the paper.

Comment [AAA7]: Remove, this does not fit with the scope of the paper.

Comment [AAA8]: This should be included with long distance dispersal above as a means of dispersal and introduction.

~~with the hope of changing behaviors.~~ Surprisingly little published information exists about how drying or desiccation influences the viability of aquatic invasive plant fragments. Approximately <0.1% of angiosperms have been shown to be desiccation tolerant (Alpert 2000). A New Zealand study showed that survivorship of fragments decreased greatly with ~~%~~ percent water loss and that there were differences in desiccation tolerance among the aquatic macrophyte species in that study (Johnstone et al. 1985). ~~In the only information we could find on the effects of desiccation in Eurasian watermilfoil,~~ Barnes et al. (2009) reported that desiccation of Eurasian watermilfoil after 1 hour and 3 hours was 70% and 90% respectively. ~~and that fragments that were coiled as they dried were substantially less dry after the same time period.~~ In most plants, particularly the higher plants (i.e. angiosperms) sufficient drying results in death. ~~-~~The term desiccation tolerance is used to describe the condition in which the adults of the species (not just the inactive stages such as seeds or spores) can tolerate drying. ~~Approximately <0.1% of angiosperms have been shown to be desiccation tolerant (Alpert 2000), though it is more common in bryophytes (Proctor 2000).~~

The level of desiccation tolerance in Eurasian watermilfoil has ~~not been yet~~yet to be established. ~~but t~~The rapid spread of this common invasive plant in North America via boats and boat trailers suggests that tolerance of plant tissue or dormant lateral buds to desiccation is a likely characteristic of at least some proportion of individuals in the species. Understanding the levels of tolerance of aquatic plants to desiccation is critical in being able eventually model the probability of new invasions. Indeed, a better understanding of how drying affects growth and development of Eurasian watermilfoil will provide valuable information for managers and educators as well.

In order to understand the viability of Eurasian watermilfoil, after different degrees of drying, we set out to determine, 1) the rate at which desiccation occurs in Eurasian watermilfoil, 2) the proportion of fragments or nodes likely to form rootlets in undesiccated (control) fragments and in fragments that had been desiccated for 3, 6, 18, 24 and 48 hours, and, 3) the length of time it takes for rootlets to form in the different treatment groups.

MATERIALS AND METHODS

During the summer of 2010, we conducted two *in- situ* experiments and one in lab experiment to determine the viability of Eurasian watermilfoil after different drying times resulting in varying levels of desiccation. The two in-situ experiments were conducted in Eurasian watermilfoil infested lakes: Second Pond (44.282755, -74.184237) and Little Lake Colby (44.329988, -74.151621), both located in the Saranac River watershed in the northern Adirondack Park of New York State.

Field Experiments. Fragments of Eurasian watermilfoil were harvested from ~~infected~~ infested lakes in the northern Adirondack Park in the vicinity of Paul Smiths, NY where beds were easily accessible by canoe, or where hand harvesting operations were being conducted. Sixty ~~strands~~ fragments of Eurasian watermilfoil, each 10 nodes long were selected for each experimental trial. Individual strands were measured, patted dry and weighed. The samples were then laid out to air dry in a low humidity, room temperature laboratory for 3, 6, 18, 24, or 48 hours, with ten replicate strands in each treatment. After the sample groups had dried, and after they were re-weighed, each individual replicate was marked by loosely tying short lengths of embroidery thread between the second and third node on each end, so we could track the progress of individual strands. The control treatments consisted of 10 fragments that were patted dry, weighed, measured and put immediately back into lake water.

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Comment [AAA9]: How long did they dry?

Six, 50cm X 40cm X 40cm cages were constructed out of 1 cm hardware cloth zip ties. These cages were placed at Second Pond (6/24/10 to 7/15/10) and in Little Lake Colby (7/23/10 to 8/27/10) in a sandy area of the littoral zone for 4 and 5 weeks respectively. The cages were submerged to just below the surface in about 75 cm of water in the littoral zone attached to narrow wooden stakes with zip ties.

The cages were checked and data collected at weeks 2 through 4 at Second Pond, and weeks 1 through 5 weeks at Little Lake Colby to determine viability of strands using proportion of new growth and rootlet formation as indices. It should be noted that due to desiccation damage to plant tissue, and various amounts of wave action, fragments from desiccation treatments were lost over time from cages in the field experiments and so qualitative data are presented here, rather than statistical analyses (see *Observation of plant tissue integrity and growth* section).

Laboratory experiment. We conducted a laboratory experiment using the same drying treatments and methods. After weighing, measuring and drying we placed 10 Eurasian watermilfoil fragments (each with 10 nodes) from each treatment into clear plastic basins containing water from Lower St. Regis Lake in a temperature controlled laboratory (around 21ºC) under grow lights set at a height of approximately 1.3m above the basins. The lights were set to a cycle of 16 hours on and 8 hours off. Water levels in the basins were marked, and at least 1/5 of the water was changed every 3-5 days with freshly collected lake water to increase aeration and provide new nutrients. Starting at day 7, strands were examined and data collected on new growth and rootlet development for 5 weeks.

Desiccation controls. In 2009 (a preliminary study) and 2010, ten (each) additional 10-node fragments were used as a desiccation control, to determine the total % water by weight in milfoil strands so we could determine the % of total desiccation for each strand in each of the

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drying treatments. In each year these fragments were weighed and placed in an oven set at 45°C for 48 hours or until no further mass loss. Percent water weight was determined as: ((fresh mass – oven dry mass)/fresh mass) *100. There was no significant difference between % water weight of Eurasian watermilfoil strands between years so 2009 and 2010 data were combined to determine the mean.

Data Analysis. Drying of fragments in 2009 and in 2010 showed that Eurasian watermilfoil is 88.9 (± 0.35 SD) and 88.2 (± 1.4 SD) ~~and percent~~percent water by weight respectively (not significantly different across years). These data were used to determine the percent of total desiccation for each fragment that occurred as a function of the drying time. Because we subtracted the percent mass loss of each fragment from the average percent water weight of the strands in the desiccation control trials, percent total desiccation is presented with 95% confidence intervals that occasionally exceed 100%. We decided that this would be the ~~most honest and~~ appropriate way to display the data on percent desiccation even though plants could not, in reality, be >100% desiccated.

Percent desiccation due to drying time was not different in either of the two field experiments or the laboratory experiment, therefore desiccation data from the three trials were pooled to analyze fragment drying rates. We used logistic regression on laboratory data to determine the probabilities of fragments producing new growth and fragments producing rootlets in the different drying treatment. We developed full and reduced logistic regression models to look at the effect of drying treatment, experimental time and the interaction between drying and time on production of new growth (indicating viability). All statistics were done using Mini-tab (version 15). We present qualitative data for evidence of rootlet production in laboratory and field experiments because sample size was too small for logistic regression and also for growth

Comment [AAA10]: Was the laboratory study conducted in 2009 as well? If not you can not include those data with data collected in 2010 and compare to your treatments.

Comment [AAA11]: How did other studies present % desiccation data? It would make sense to use their approach and cite their paper.

Comment [AAA12]: You cannot pool data collected from field studies with data collected in a controlled laboratory setting.

Comment [AAA13]: You said in the previous sentence that you pooled the lab data with field data, and now are analyzing only lab data?

Comment [AAA14]: More description is needed regarding the use of the logistic model approach, especially with the use of a full and reduced model. This approach will not be intuitive to most readers.

in *in-situ* pond studies since logistic regression was not valid due to loss over time of fragments from cages in the lakes.

RESULTS AND DISCUSSION

Desiccation of Eurasian watermilfoil *due to drying.* We fit a Michaelis-Menten type function to the relationship between percent total desiccation and drying time (Figure 1). The strands were 87% desiccated after just 3 hours of drying under our laboratory conditions of room temperature and low humidity. After 6 hours of drying, the percent desiccation of ~~strands~~ fragments ranged between 93 and 99 percent. After 18 hours of drying and beyond, all measurable water was lost from the ~~strands~~fragments. Using the equation for the fitted function we estimate that 100% desiccation occurs at approximately 13 hours.

Eurasian watermilfoil dried quickly under the conditions in this study. Rapid drying has been associated with desiccation tolerance, especially with bryophytes, but also in vascular plants (Gaff 1997). It has been proposed that rapid loss of water reduces damage that can occur during rehydration and that the greatest amount of damage to plant tissues may be sustained at intermediate dryness levels (Alpert 2000). Our data for desiccation rates are very close to those reported for Eurasian watermilfoil by Barnes et al. (2009) in which 3 hours of drying resulted in 90% desiccation.

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Fragment viability - effect of drying treatment and time on new growth in the laboratory. Full and reduced logistic regression models showed that drying treatment alone significantly reduced the likelihood of new growth on desiccated milfoil fragments ($z = -7.24$, p ~~=~~ < 0.0001?, reduced model, Table 1). Using the reduced model in Table one, we predicted the probability of new growth. Control fragments had a probability of 0.98 of producing new growth while fragments dried for only 3 hours resulting in 87% desiccation had significantly reduced

probability of ~~viability~~ new growth of 0.06 and completely desiccated plants had a probability of viability of 0.02 (Table 2). The confidence intervals for desiccation percentages greater than 0 are large, so a larger sample size is needed to narrow the confidence limits around the probability for dry fragments. Regardless, there is a probability greater than zero of highly desiccated fragments producing growth.

No loss of tissue could occur over time in the laboratory experiment, which provided insight to the loss of fragments over time in the field experiments. Control treatment fragments were buoyant for the entire period of the experiment while fragments from the 3 hour drying treatment were initially buoyant and then began to sink in week 3. All other treatments were not buoyant after drying as noted above. The only disturbance in laboratory basins was the changing of water every 3 to 5 days. Even this minimal disturbance began to break apart the most desiccated strands within 2-3 weeks. After 4 weeks of no evidence of new growth in the 18 through 48 hour drying treatments (100% desiccated) we were about to end the experiment, when we observed new growth and rootlet development in both the 18 and 48 hour drying treatments.

Observation of plant tissue integrity and growth after drying in pond experiments. The same disintegration of plant tissue we observed in the laboratory experiment lead to the loss of fragments or partial fragments from cages in the field over time (aided in Second Pond by heavy wave action).

After 2 weeks in Second Pond the cages were checked and partial fragments remained in the control, 3, 6 and 18 hour drying treatments. There was no new growth in any of the treatments, and fragments were longest and most abundant in the control treatment, shorter and fewer in the 3 hour drying treatment, and even smaller and fewer fragments after 6 and 18 hours

Comment [AAA16]: Make into two sentences.

Comment [AAA17]: Reword... Maybe...A larger sample size would have likely reduced the variability in our study, thereby increasing the accuracy of our model. None the less, there is a probability of highly desiccated Eurasian watermilfoil to grow new lateral buds and rootlets .

of drying. There were no fragments remaining in the 24 hour and 48 hour drying treatments. After three weeks, the control treatment contained fewer and shorter fragments; however a proportion of remaining fragments showed new growth, some with rootlets. The three hour treatment also showed new growth, however in a reduced proportion of remaining fragments and with no rootlet production. Only a few partial fragments remained in the 6 hour drying treatment with none showing new growth. After 4 weeks, only several short fragments remained in the control cage.

After just one week in the Little Lake Colby experiment the control treatment showed new growth. The other treatments had no new growth and appeared to be in the process of disintegrating. In the second week the Little Lake Colby control group had new growth on all 10 fragments, some with rootlet growth. The 3 hour drying treatment had one fragment with new growth after two weeks, and all the rest of the samples showed no new development. By the third week rootlets appeared on 8 of 10 control fragments and new growth was found on every fragment. Also a second strand in the 3 hour drying treatment had new growth. By the fourth week the 6, 18, 24, and 48 hour drying treatments no longer contained fragments due to disintegration of tissue and loss from cages.

Effect of desiccation on node viability in the laboratory. Table 3 presents the data for proportion of 100 nodes (10 fragments of 10 nodes each) in each drying treatment in the laboratory in which we observed new growth and the proportion of those which formed rootlets. Because all fragments remained in the basins for the entire experimental time, unlike the *in-situ* experiments, we could calculate the proportion of viability on a per node basis which allows us to think about viability in plant fragments of different lengths (# of nodes). New growth began in the first week in the control and 3 hour (87% desiccation) drying treatments. The proportion

Comment [AAA18]: This section and subsequent data tables need to be removed. Qualitative data does not add to the paper. You could revise and shorten this section and report some major observations from the field to lend support for your lab trial (as the lab trial is the strength of this paper).

of nodes with new growth increased through time in the control treatment. In the 3 hour drying treatment the new growth observed after the first week was not observed again until week 5 (day 33) with a proportion of viable nodes of only 0.01. No growth was observed in the 6 hour (96%) and 18, 24, and 48 hour (100% desiccation) drying treatments until week 5 when the proportion of viable nodes in the 6, 18 and 48 hour drying treatments were each 0.01.

Rootlet production began in the control treatment in the 3rd week of the experiment where the proportion of nodes with new growth forming rootlets was 0.3. By the end of the experiment the proportion of nodes forming new growth in the control was 0.2 and the proportion of those nodes that formed rootlets was 0.9. These data suggest that at least 20% of Eurasian watermilfoil nodes contain viable dormant lateral buds. All fragments we used had the apical tip and between 5 – 10 cm of the upper stem removed because it was difficult to count the crowded nodes of the tips. The removal of the apical tips likely released some dormant lateral buds. However the process of desiccation *per se* may initiate physiological changes leading to growth in some aquatic plant buds (Malek 1981). To be able to move forward and predict the likelihood that desiccated fragments will be viable when introduced to new lakes we need to learn more about the ratio of lateral buds/node (Johnstone et al. 1985) and mechanisms of lateral bud growth initiation. If release from apical dominance is partially or mostly responsible for initiation of bud growth, then the likelihood of viability will be different for fragments that include the terminal growth and those that have had that terminal growth removed.

Of the other 4 treatments that produced one instance of new growth, two (3 and 18 hour drying treatments) did not produce a rootlet during the experiment but the other two (6 and 48 hour drying treatments) each showed rootlet growth. It should be noted that new growth and rootlet development were delayed until after week 4 in these treatments. If we present these data

in desiccation categories, as we did for the regression analysis, the 96% desiccation (6 hour drying) had a 0.01 probability of a node producing new growth and the 100% desiccation (18, 24, and 48 hour) showed new growth and rootlet development, a proportion of 0.007 (2 out of 300 nodes were viable). These data represent a valuable first estimate and could be useful in initial predictions of time until invasion of new lakes where boater traffic and incidents of fragment transport and number of nodes of Eurasian watermilfoil are available.

Both the quantitative and qualitative analyses show a significant reduction in viability as a function of desiccation. However, even after long desiccation time and 100% loss of measurable water some small fraction of fragments (nodes within fragments) were able to produce new growth. ~~Our data suggest that once new growth is initiated, rootlets usually follow.~~ The initiation of new growth in Eurasian watermilfoil does not appear to be the rehydration of leaf tissue, but rather the rehydration of dormant lateral buds which produce new stems and rootlets. Eurasian watermilfoil does not appear to have a high tolerance to desiccation; however, some lateral buds can withstand full drying and eventually produce new growth. This is similar to what Johnstone et al. (1985) found for several aquatic invasive species they studied in New Zealand, where ~~T~~they reported that after 50% mass loss of fragments~~,~~ all leaves on the fragment died, but fragments still were able to grow from lateral buds.

Mechanisms of tolerance to desiccation probably include both cellular and sub-cellular level responses to oxidative damage and possibly mechanisms that reduce physical damage to cell membranes when desiccated cells begin to lose turgor (Alpert 2000). If there is variability within Eurasian watermilfoil populations for tolerance to desiccation, then fragments that ultimately are successful in colonizing new lakes that are transported via watercraft will be those that can withstand desiccation. It seems that long distance transport of milfoil ~~strands~~ fragments

Comment [AAA19]: Again, the level of qualitative data needs to be reduced. You can report the major observations in the text to support your logistic model, but 3 paragraphs and a table is too much to devote to these data with no statistical analyses.

Comment [AAA20]: Qualitative analyses do not show significant reductions.

Comment [AAA21]: Citation?

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could create a strong selection pressure against fragments of plants that are not tolerant to desiccation, potentially resulting in substantially increased desiccation tolerance in subsequently colonized lakes.

In conclusion, Eurasian watermilfoil fragments dry out quickly, at least under the conditions in our study (room temperature and relatively low humidity). After 3 hours, fragments contained only an average of 13% of the original moisture. Loss of tissue integrity and a significant reduction in viability are associated with desiccation. Decreased viability was a function of percent desiccation and was statistically significant and also shown qualitatively by both the reduction in production of new growth and rootlets and also by the longer time it took for the development of new growth and rootlets in dryer fragments. Though, ~~While this is good news, and the likelihood of growth and root production of a desiccated or partially desiccated fragment is much reduced, it is not, however, eliminated. Our~~ our data suggest that for fragments that are 100% dry there is still a probability of 0.02 ~~probability~~ of new growth ~~and that the new growth will likely form rootlets~~. Moreover, one of the incidences of rootlet formation in the 100% desiccation group was in the 48 hour drying treatment. Based on our desiccation rate curve full desiccation occurred in 13 hours, so this tissue was viable after having been fully desiccated for 35 hours. This suggests that at least a small amount of Eurasian watermilfoil dormant lateral buds are highly desiccation tolerant.

Our data can not estimate the likelihood of establishment of fragments once introduced to a new lake, since environmental variables such as lake sediment texture, nutrient composition, and light environment (Grace and Wetzel 1978) will also play a role in the establishment of any viable Eurasian watermilfoil fragments. ~~Nor can our data be used to determine a minimum drying time to reduce viability to zero: 1) because our treatments did not result in zero viability,~~

Comment [AAA24]: You do not know if "tolerance" is a genetic trait here. It could be due to fragment size, carbohydrate storage, age of the plant from which the fragment came, life stage, environment, locality, etc. there are a number of plausible explanations rather than genetic. Carbohydrate content maybe the most plausible as starch and TNC concentrations will be greater in different sections of the plant over the season as it pertains to the life history characteristics of the plant. I think this needs further discussion rather than suggesting "tolerance" is strictly genetic.

Comment [AAA25]: regrowth

Comment [AAA26]: Confusing

Comment [AAA27]: This would have tested for viability. If you would have taken your fragments after drying and data collection, potted them, and placed them in mesocosms under the same lab conditions you could have developed a viability curve.

Comment [AAA28]: Life history and carbohydrate allocation patterns will also play a role in fragment regrowth.

~~and 2) because drying conditions in the environment where fragments are clinging to watercraft may be very different than in this study~~However, our data represent a "worst case" scenario for Eurasian watermilfoil fragments, in that, we completely dried the fragments. Under field condions, ~~F~~fragments transported along with watercraft in wells, on bunks of trailers and other locations ~~may be~~are likely kept more moist during transport. ~~to a greater or lesser degree~~. Furthermore, Barnes et al. (2009) found that coiled Eurasian watermilfoil fragments desiccated much more slowly than uncoiled fragments suggesting that the position of fragments on trailers and equipment may be more important than length of time out of water. Additional research is needed to ~~More work is needed to~~ better understand the relationships between desiccation and fragment growth~~viability~~ under different environmental conditions with respect to life history characteristics ~~and in different populations of Eurasian watermilfoil~~. ~~It will be valuable to consider how this information can be applied in modeling the likelihood of new Eurasian watermilfoil colony establishment from lake to lake.~~

In the meantime, the results of this study can be used to emphasize the need for continued vigilance on the part of educators and boaters, as fragments that look, feel and are dry may indeed still be viable. In order to reduce invasion of Eurasian watermilfoil into new lakes, the inspection and removal of all plant material (regardless of the observed apparent condition) and careful boat washing are critical practices.

Comment [AAA29]: This can be reworded and added into the discussion somewhere.

ACKNOWLEDGEMENTS

We would like to acknowledge Adirondack Watershed Institute, Watershed Stewardship Program Director Eric Holmlund, Ph.D. for his comments on the manuscript and for the initial desire to begin to answer the questions addressed herein. Corey Laxson provided valuable input to the manuscript development. Jeanne Ashworth also helped with the field and laboratory work. This project was partially supported by a grant from the U.S. Department of the Interior, Fish and Wildlife Service.

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Comment [AAA30]: Not cited in the text.

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Comment [AAA31]: Not cited in text.

Comment [AAA32]: Not cited in text.

FOOTNOTES

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379 **TABLES**

380 Table 1. Results of logistic regression analysis on the probability of fragments producing new growth as a function of percent fragment
381 desiccation and incubation days for a laboratory incubation study.

382

Predictor	Coefficient	S.E. Coefficient	Z Statistic	P value	Odds Ratio	Confidence Intervals for the Odds Ratio	
						Lower 95% CI	Upper 95% CI
			Model 1				
Constant	1.696	1.818	0.93	0.351			
Desiccation	-0.085	0.024	-3.50	0.000	0.92	0.88	0.96
Days	0.139	0.125	1.11	0.268	1.15	0.90	1.47
Desiccation x Days	-0.0004	0.0014	-0.27	0.790	1.00	1.00	1.00
			Model 2				
Constant	4.031	0.970	4.15	0.000			
Desiccation	-0.079	0.011	-7.24	0.000	0.92	0.91	0.94

Table 2. Probability and 95% confidence intervals for probability of fragments producing new growth as a function of percent fragment desiccation for a laboratory study predicted using the logistic regression model 2 in Table 1.

Desiccation %	Probability	95% Confidence	
		Lower	Upper
0	0.98	0.89	1.00
87	0.06	0.00	0.72
96	0.03	0.00	0.61
100	0.02	0.00	0.55

Table 3. Data from a laboratory experiment examining the new growth and rootlet development over time after exposure to drying which lead to different degrees of desiccation in European Water Milfoil (*Myriophyllum spicatum*) Each drying treatment contained 10 replicate strands, each 10 nodes long, resulting in 100 nodes per drying treatment. Qualitative data are presented as proportion of nodes rather than fragments that grew and rooted. NA = not applicable because in those treatments/time there is no new growth that could form rootlets.

Experimental day	Drying Treatment (hours)	% Desiccation	Proportion of nodes with new growth	Proportion of new growth with rootlets
7	0	0	0.09	0.00
	3	87	0.01	0.00
	6	96	0.00	NA
	18	100	0.00	NA
	24	100	0.00	NA
	48	100	0.00	NA
14	0	0	0.14	0.00
	3	87	0.00	NA
	6	96	0.00	NA
	18	100	0.00	NA
	24	100	0.00	NA
	48	100	0.00	NA
	0	0	0.17	0.30

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20	3	87	0.00	NA
	6	96	0.00	NA
	18	100	0.00	NA
	24	100	0.00	NA
	48	100	0.00	NA
26	0	0	0.17	0.71
	3	87	0.00	NA
	6	96	0.00	NA
	18	100	0.00	NA
	24	100	0.00	NA
	48	100	0.00	NA
33	0	0	0.20	0.9
	3	87	0.01	0.0
	6	96	0.01	1.0
	18	100	0.01	0.0
	24	100	0.0	NA
	48	100	0.01	1.0

FIGURE LEGENDS

Figure 1. The average percent fragment desiccation versus drying time with bars showint + one standard error of the mean, n=30. A Michaelis-Menten function was fit to the data and the equation shows that drying time explained 95% of the variation in desiccation%, with 100% desiccation occurring in about 13 hours drying time. Dashed lines are upper and lower 95% confidence intervals for the predicted desiccation %.

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405 FIGURES

406 Figure 1.