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How long do shoot fragments of hydrilla (*Hydrilla verticillata*) and Eurasian watermilfoil (*Myriophyllum spicatum*) remain buoyant?

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ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum* L.) and monoecious hydrilla [*Hydrilla verticillata* (L.f.) Royle] are aggressive submersed aquatic invasive plants that continue to spread into northern tier states. Although floating fragments have been identified as a key pathway for intra- and interlake dispersal of several submersed invasive species, the length of time these fragments remain buoyant has not been reported. Through a series of small-scale greenhouse trials, we evaluated the time required for monoecious and dioecious hydrilla, and Eurasian watermilfoil fragments to sink to the bottom of the container. We evaluated multiple-sized fragments (4 to 20 cm) and found slight differences in settling times for increasing fragment sizes across all three species. Fragments remained buoyant for monoecious hydrilla between 1 and 2 d, Eurasian watermilfoil between 2 and 4 d, and dioecious hydrilla between 2 and 6 d. Exposure of 12-cm monoecious hydrilla fragments to minimal aeration designed to break the water surface tension increased buoyancy (3 d) when compared to static conditions (2 d). Combining multiple intertwined fragments (12 cm) to create a small mat did not change settling times. Results suggest species differences in buoyancy/settling times, yet fragments of monoecious hydrilla and Eurasian watermilfoil did not remain buoyant beyond 3 d. Root formation was noted within 3 to 6 d of monoecious hydrilla settling to the bottom. Future trials to evaluate fragment success in establishing in different sediment and light conditions are recommended. The current results can be of use in dispersal models and provide additional information for risk assessments.

Key words: aquatic plant fragments, hydrochorous dispersal, invasive aquatic plants, monoecious hydrilla, root development.

INTRODUCTION

Hydrilla [*Hydrilla verticillata* (L.f.) Royle] is an aggressive submersed aquatic invasive species that has been described as the "perfect aquatic weed" (Langeland 1996). It is a rooted, submersed macrophyte that can rapidly expand and occupy large expanses of lakes and reservoirs. This ability for extensive growth can negatively impact recreation, flood control capacity, and aesthetics. Control programs can be costly and typically rely on the use of registered herbicides or stocking of triploid grass carp (Colle 2014, Netherland 2014). There are two biotypes of hydrilla in the United States with monoecious (M.) hydrilla spreading in the mid-Atlantic and northern tier states and dioecious (D.) hydrilla largely found in southern Gulf Coast states. M. hydrilla emerges from tubers each spring (Harlan et al. 1985, Sutton et al. 1992, Owens et al. 2012) and growth is characterized by rapid lateral expansion, followed by steady growth towards the water surface (Van 1989). The dioecious biotype tends to persist as a perennial and growth is characterized by rapid growth to the water surface. Eurasian watermilfoil (*Myriophyllum spicatum* L., hereafter called EWM) represents a northern invasive plant that also grows rapidly to the water surface, forming dense surface canopies, and produces significant fragments via both disturbance and autofragmentation (Madsen 2014). The rapid spread of hydrilla and EWM is often attributed to the ability to produce copious vegetative shoot fragments that aid in dispersal of the plant.

Given the recent range expansion of M. hydrilla into northern tier water bodies, there is increasing interest in factors that can promote the spread of this plant. Fragmentation and subsequent hydrochorous dispersal likely represent a key mechanism for spread within and between interconnected waters. Although hydrilla and several other invasive plants are reported to rapidly disperse via this mechanism, there is limited documentation to describe this process. Barrat-Segretain and Bornette (2000) have described hydrilla as having an "always ready" strategy, which means that fragments that are disturbed by mechanical removal, animal feeding, or human activities float to become established in a new area. Fragments as small as 1 or 2 nodes (a few cm) are capable of regrowth and establishment at new sites (Langeland and Sutton 1980, Madsen and Smith 1999).

Although there are numerous reports of hydrilla and EWM dispersing via floating fragments (Sculthrope 1967, Haller et al 1976, Langeland and Sutton 1980, Pieterse 1981, Spencer and Ksander 1991, Sutton 1996, Madsen and Smith 1999), there are no data regarding the length of time that these propagules can remain afloat. It has been hypothesized that fragments can stay afloat for several days or weeks and travel great distances (Barrat-Segretain 1996). The

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ability to stay afloat is likely related to aerenchyma tissue (air chambers) that mediates internal gas exchange of aquatic plants (Sculthorpe 1967, Jung et al. 2008).

Hydrilla and EWM are already present in many water bodies in the United States, yet the potential for additional spread remains significant. Simple determination of how long these plant fragments remain buoyant might provide insight regarding potential differences between species, as well as information that can be used in dispersal models and risk assessments that are being developed to predict potential spread of submersed invasive plants.

The objective of this study was to determine the duration that fragments of three invasive plants to change from positive to negative buoyancy under various scenarios. Hypotheses tested included: 1) buoyancy is different between species, 2) increasing the size of fragment will increase the time they remain buoyant, 3) disruption of the water's surface will decrease the time a fragment stays afloat, 4) intertwining multiple fragments together will increase the time fragments stay afloat, and 5) buoyancy will be different in the summer versus in the fall.

MATERIALS AND METHODS

All studies were conducted under greenhouse conditions at the University of Florida Center for Aquatic and Invasive Plants (UF CAIP), Gainesville, FL. Apical shoot fragments of M. hydrilla, D. hydrilla, and EWM were collected from stock culture tanks at the UF CAIP. Uniform-sized fragments were washed to remove epiphytic debris and allowed to acclimate for 1 h prior to placement in study containers. Initial evaluations compared buoyancy in 900- versus 90- versus 19-L containers and determined that tank size did not impact longevity of buoyancy for any of our test species (data not shown). Utilization of the 19-L containers allowed for a large number of treatments and replicates in a relatively small space. The 19-L containers were filled with well water.

Influence of fragment size on buoyancy

Five fragment lengths (4, 8, 12, 16, and 20 cm) for M. hydrilla, D. hydrilla, and EWM were collected on 2 June 2015. Ninety 19-L containers (five fragment lengths by three species by six replicates) were prepared, and a single fragment was placed in each container and then checked daily. The number of days required to change from positive to negative buoyancy was recorded. Negative buoyancy was determined when the fragment rested on the bottom of the container. The study was then repeated on 9 June 2015. Water temperatures were recorded every 6 h using an Onset® HOBO Water Temp Pro v2 data logger.[1] Fall trials were initiated 5 October and again on 17 October 2015.

Impact of aeration on hydrilla fragment buoyancy

On 29 June 2015, 12-cm fragments of M. hydrilla were collected and placed into 10 (19-L) containers that were kept under static conditions, and another 10 containers had an air stone, connected to a small pump positioned near the water's surface to disrupt the water's surface tension. Air flow was kept to a minimum because the objective was to simply break the surface tension. Containers were checked daily to monitor change from positive to negative buoyancy. This study was repeated on 14 July 2015.

Impact of multiple hydrilla fragments on buoyancy

Fragments (12 cm) of M. hydrilla were intertwined in bundles of 1, 2, 4, or 8 fragments and placed on the water surface of 19-L containers. The intertwined bundles simulate small floating mats of hydrilla. Each bundle was replicated five times and checked daily to determine the time to change from positive to negative buoyancy. This study was conducted on 29 June and repeated on 14 July 2015.

Root formation

Documenting the timing of root development on M. hydrilla fragments was also of interest. Therefore, experiments were initiated on 27 July 2015, 20 (19-L) containers had a single 12-cm fragment of M. hydrilla placed on the water's surface. Lids were placed on half of the containers to reduce light intensity to $< 20\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$. All containers were kept under static conditions. Fragments were checked daily to monitor root development over the course of 14 d and root lengths were determined at 7 and 14 d. This study was repeated on 10 August 2015.

Statistics

Data were subjected to ANOVA and means were separated using the least squared means method ($P = 0.05$). When no significant treatment by experiment interactions were observed for the repeated studies, the data were pooled for analysis. A difference in response between hydrilla biotypes in the fall trials was noted and data are presented separately.

RESULTS

All fragments removed from parent plants were initially buoyant. Floating fragments of both hydrilla biotypes and EWM started off in a horizontal position. A few hours prior to sinking, the hydrilla apical shoots usually became vertical while the excised end remained at the water's surface. It was observed that that factors such as small air bubbles associated with flowers or limited algae growth tended to increase buoyancy.

Influence of fragment size on buoyancy—summer

According to Rstudio software, the M. hydrilla and EWM fragments remained buoyant on average between 1.9 and 2.5 d (Figure 1A and 1C, respectively). In contrast, D. hydrilla remained buoyant for an average of 4.4 d (Figure 1B). Whereas increasing fragment size was associated with increased duration of buoyancy in the summer trials, the magnitude of these differences remained small. In general,

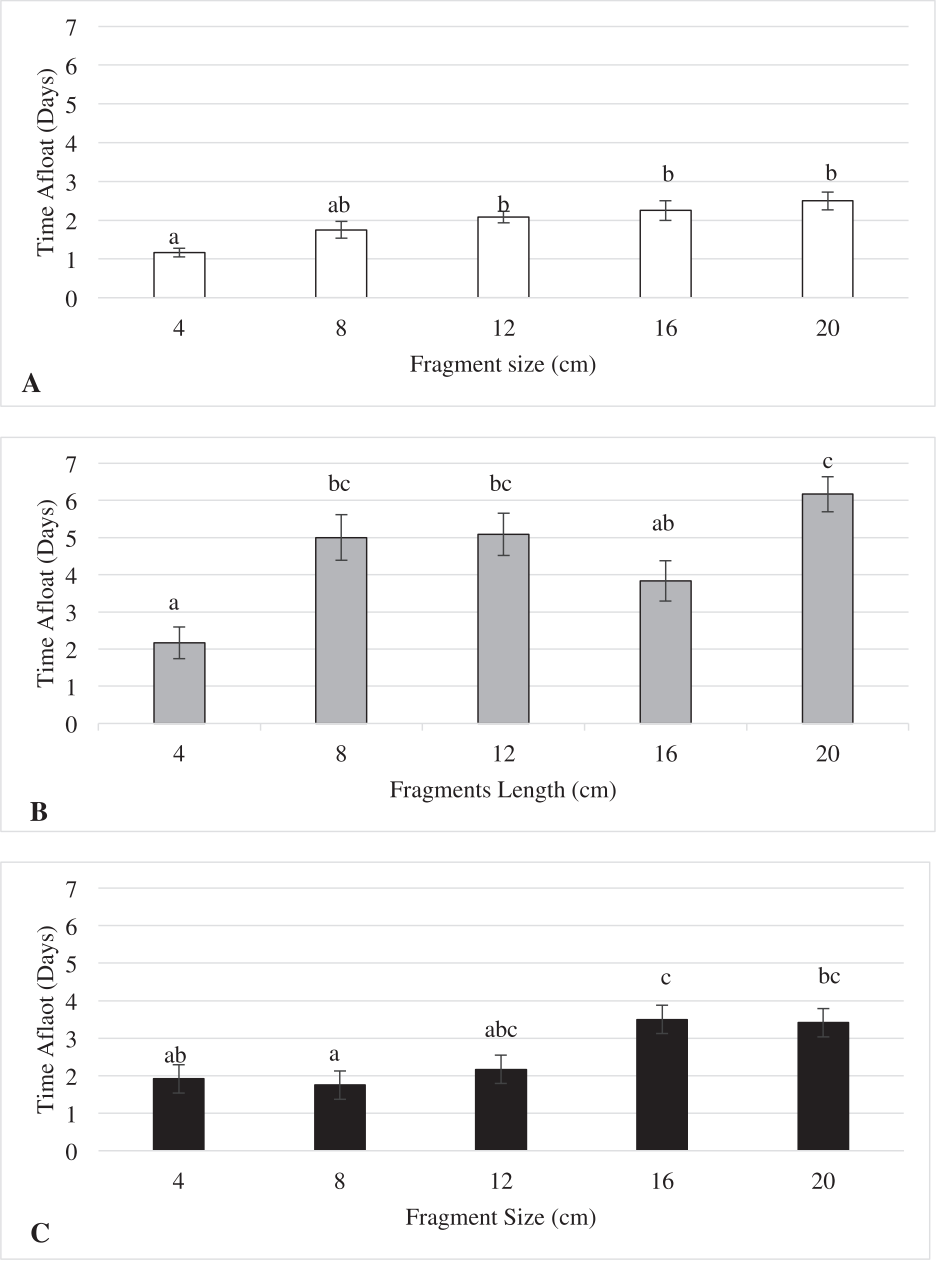

Figure 1. The time in days for fragments of different length (4, 8, 12, 16, or 20 cm) of (A) monoecious (M.) hydrilla, (B) dioecious (D.) hydrilla, and (C) Eurasian water milfoil (EWM) to change from a positive to negative buoyancy in June (summer). Letters indicate significant differences in sinking times within a plant species for each fragment length evaluated as derived from a lsmeans test. Bars represent the mean of six replicates ± SE.

buoyancy of M. hydrilla increased from 1 to 2 d, D. hydrilla increased from 2 to 6 d, and EWM increased from 1.5 to 3.5 d. The average water temperature was 25.3 C and 26.8 C for trials 1 and 2, respectively.

Influence of fragment size on buoyancy—fall

M. hydrilla remained buoyant an average of 5.6 and 3 d for trials 1 and 2 (Figure 2A), whereas D. hydrilla remained buoyant an average of 3.7 and 1.9 d for trials 1 and 2 (Figure 2B). Despite differences between trials in this study we did not observe differences in time of buoyancy for different fragment lengths of hydrilla. EWM remained buoyant the longest with an average of 5.4 d combined for trials 1 and 2 (Figure 2C). Average water temperatures were 24.7 C and 23.5 C for trials 1 and 2, respectively. Both M. and D. hydrilla in the second fall trial sank in half the time compared to plants in the first fall trial. There was a difference (P value < 0.001) between summer and fall trials, with M. hydrilla and EWM taking longer to sink in the fall, whereas D. hydrilla sank faster.

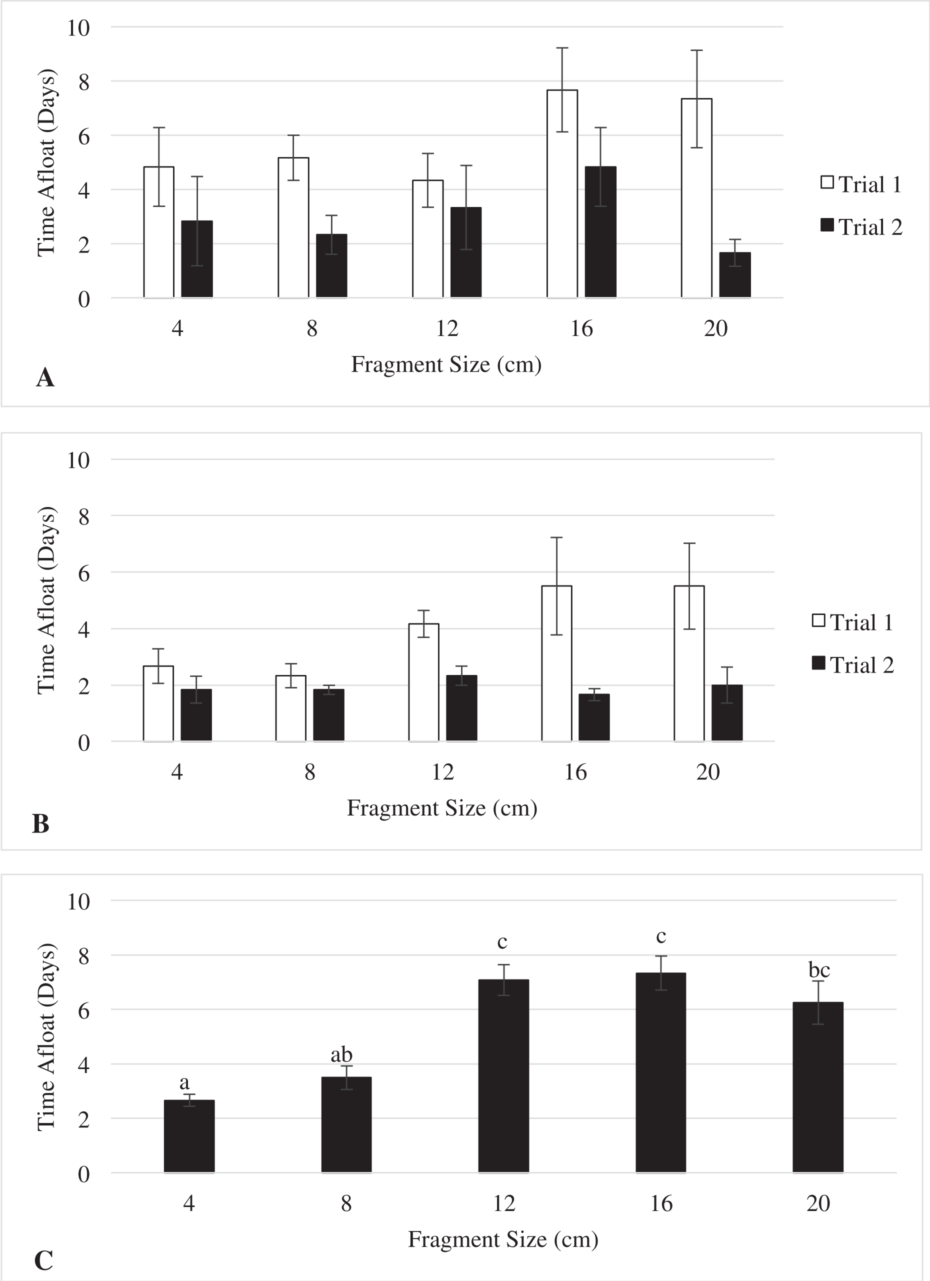

Figure 2. The time in days for fragments of different length (4, 8, 12, 16, 20 cm) of (A) monoecious (M.) hydrilla, (B) dioecious (D.) hydrilla, and (C) Eurasian water milfoil (EWM) to change from a positive to negative buoyancy in October (fall) . Letters indicate significant differences in sinking times between each length as derived from a lsmeans test. M. and D. hydrilla trials were significantly different and are shown separately. EWM trials were not significantly different, so data were combined. Bars represent the mean of six replicates ± SE.

Impact of aeration on fragment buoyancy

The M. hydrilla fragments in the aerated containers remained afloat longer (3 d) compared to the fragments in the static treatment (2 d) (Figure 3). We hypothesized that disrupting surface tension would enhance rates of sinking, yet these results suggest the opposite effect. The average water temperature over the duration of was 26 C and 27.8 C for trials 1 and 2, respectively. In these trials, we provided light aeration to insure breaking of surface water tension, and we did not evaluate a wide range of aeration rates. Under field conditions, fragments would be expected to encounter a wide range of turbulence, and this should be evaluated in future trials.

Influence of multiple fragments on buoyancy

The number of intertwined M. hydrilla fragments had no effect on the sinking time (Figure 4). We hypothesized that multiple fragments might increase buoyancy; however, these

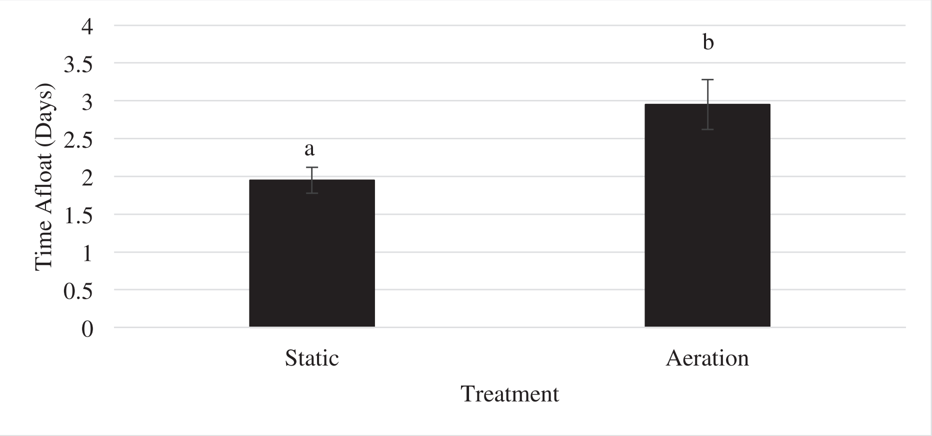

Figure 3. The time in days for 12-cm fragments of monoecious (M.) hydrilla to change from a positive to negative buoyancy while being exposed to either aerated or static conditions. Different letters indicate significant difference between treatments as derived from a lsmeans test. Bars represent the mean of 10 replicates ± SE.

results indicate that small clusters of plants sank at the same rate as individual fragments. The average water temperature over the studies was 26 C and 27.8 C for both trials, respectively.

Root formation

Root formation on M. hydrilla fragments was noticed approximately 4 to 6.6 d after placement in containers under high light conditions. Fragments exposed to reduced light conditions formed roots at 3 to 4 d after placement in the containers (Table 1). Root formation did not occur until the plant fragments had sunk to the bottom, and then they formed quickly. Total root length in trial 1 was 109 and 181 mm for full light and reduced light at 7 d, and 235 and 417 mm at 14 d, respectively. Root length in trial 2 was 157 and 359 mm for full and reduced light at 7 d, and 189 and 692 mm at 14 d, respectively. Fragments under reduced light formed roots significantly faster in both trials 1 ($P = 0.048$) and 2 ($P = < 0.001$).

DISCUSSION

Despite multiple papers citing fragments as a key means of dispersal, we are not aware of any authors who have quantitatively evaluated the time that plant fragments remain buoyant or the mechanisms that result in fragments becoming negatively buoyant. Barrat-Segretain (1996) speculated that fragments of submersed aquatic plants could stay afloat for days to weeks. Our results suggest that individual fragments or small clusters of fragments of EWM and M. hydrilla are likely to sink within 1 to 2 d during the summer, whereas D. hydrilla remained buoyant up to two to three times longer. The opposite was observed during the fall where M. hydrilla and EWM remained buoyant longer than D. hydrilla. Differences between species buoyancy and fragment size were relatively minor. Based on the hydrilla fragments turning to a vertical position before they sank (i.e., part of the fragment was still on the surface of the water) we speculated that surface tension was keeping it afloat; however, addition of aeration to remove surface tension had an opposite effect. In some cases, large rafts of

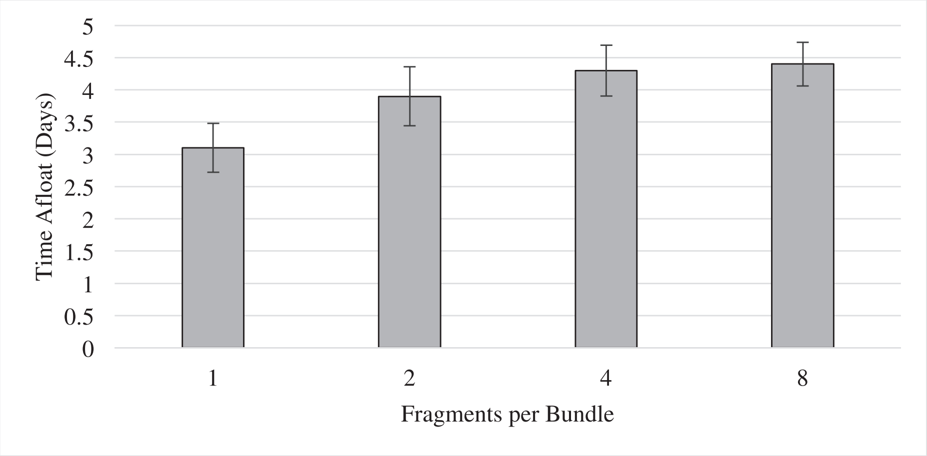

Figure 4. The time in days for different-sized bundles (1, 2, 4, or 8 fragments) of monoecious (M.) hydrilla to change from a positive to negative buoyancy. There were no significant differences between the different-sized bundles indicated by an ANOVA. Bars represent the mean of five replicates ± SE.

TABLE 1. TIME IN DAYS (D) FOR 12-CM FRAGMENTS OF MONOECIOUS HYDRILLA (M. HYDRILLA) TO PRODUCE ROOTS AFTER PLACEMENT IN 19-L TANKS UNDER FULL-LIGHT AND REDUCED-LIGHT (< 20 μMOL M^{-2} SEC^{-1}) CONDITIONS. LETTERS INDICATE SIGNIFICANT DIFFERENCE BASED ON LSMEANS MEANS SEPARATION TEST. UPPER AND LOWER CASE LETTERS CORRESPOND WITH SEPARATE TRIALS.

Treatment	Trial	Root Formation (d ± SE)
Full light	1	6.6 (0.9) A
Reduced light	1	4.4 (0.5) B
Full light	2	4.0 (0.0) a
Reduced light	2	3.2 (0.1) b

uprooted shoot material can be observed floating in water bodies following various disturbance events (especially late in the growing season). Our results would not likely be predictive of the behavior of these large mats, but they should provide reasonable estimates associated with the buoyancy of small fragments that are often produced in high numbers on a daily basis during the height of the growing season.

The difference in hydrilla buoyancy between the two studies in October was not expected. Growth rates are changing and carbohydrates are being mobilized as hydrilla shoots are being stimulated to produce turions and tubers under these shortened photoperiods (Meadows 2013). The faster sinking rates in the second fall study suggest a change in the condition of both biotypes of hydrilla. Differences in buoyancy between summer and fall trials were expected; however, an explanation for increased buoyancy times of M. hydrilla and EWM and decreased buoyancy times for D. hydrilla will require further study.

The rapid formation of roots in floating fragments increases the likelihood of establishment into new areas (Harlan et al.1985, Pennington and Sytsma 2016). The majority of fragments formed roots after they sank to the bottom of the container. In the Pennington and Sytsma (2016) study, it was noted that all but three stems of Brazilian egeria (*Egeria densa* Planch), 8 to 12 and 18 to 22 cm, formed roots while floating on the surface, although none of their fragments sank in the 11-wk trial. At this time we do not know why there was a difference between Brazillian egeria in the Pennington and Sytsma (2016) study and the two species (hydrilla and EWM) in the present study. Formation of roots while hovering just above the sediments can aid in establishment in shallow waters where roots can come into contact with the sediment surface even if the fragment is still floating (Pennington and Sytsma 2016). In this study, greatly reducing the amount of light available stimulated roots to grow twice as long as those produced in full light.

Future recommendations include: 1) developing protocols to determine factors that influence success of fragment establishment with an emphasis on sediment composition, nutrition, and light environment; 2) influence of time of year and temperature on buoyancy; and 3) evaluation of the dynamics associated with larger fragments and floating mats.

Although results from these studies are straightforward, they do provide quantitative data for improving dispersal models and risk assessments. In lieu of broad assumptions, these basic studies provide information for how these fragments can move within and between waterbodies. Based on these results, it is unlikely an individual fragment could result in truly long-distance in-water dispersal; however, the ability to remain afloat for 2 to 3 d could result in a new founder colony far from the existing bed.

SOURCE OF MATERIALS

[1] Onset® HOBO Water Temp Pro v2 data logger, Onset Computer Corporation. 470 MacArthur Blvd. Bourne, MA 02532.

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