Retention of viability by fragmented invasive Crassula helmsii, Elodea canadensis and Lagarosiphon major

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Abstract
Invasive aquatic macrophytes tend to reproduce and spread through vegetative means, often via fragmentary propagules. Dispersal among aquatic sites may occur overland via attachment to various vectors, or within river systems by directional water currents. However, for many species the relationship between fragment size and resumption of growth is unknown. Here, we assessed resumption of growth of apical and mid-stem fragments of invasive Crassula helmsii, Elodea canadensis and Lagarosiphon major. Proportionally, apical fragments tended to more readily resume growth than mid-stem sections, especially for E. canadensis and L. major (80–100%). However, viability did not scale linearly with increasing fragment size, which suggests that fragment size is not a singular determinant of propagule fitness. Nevertheless, longer fragments generally produced greater numbers of shoots and roots, but root production significantly differed among species and was determined through an interaction between plant section, species and fragment length. Overall, all species produced new shoots and roots from fragments as small as 10 mm. C. helmsii mid-stem fragments standardised by node counts did not display new growth (up to 10 nodes), while E. canadensis tended to show greater shoot and root production with increasing node counts. It is evident that a medium to high proportion of small fragmentary propagules of these invasive macrophytes can retain viability. These data have clear implications for understanding the dispersal of these invasive species and their management. Specifically, cutting and dredging may increase rather than decrease infestations, especially in downstream directions. Thus, in the absence of adequate fragment containment, current short-term control strategies may in fact be counterproductive.

Keywords
aquatic macrophyte, biosecurity, dispersal, fragment degradation, invasive alien species, minimal viable propagule
1  |  INTRODUCTION

Invasive freshwater aquatic macrophytes have been shown to adversely affect freshwater ecosystems and represent a considerable management burden (e.g., Cuthbert et al., 2021; Hussner et al., 2017). Successful dispersal and establishment of invasive macrophytes are largely dependent on fragmentary propagule survival and viability in relation to root and shoot production (Coughlan, Cuthbert, Kelly, & Jansen, 2018; Heidbüchel, Sachs, Hamzehian, & Hussner, 2020). Generally, the abundance and reproductive quality of propagules that are introduced increase the likelihood of viable propagules colonising suitable environments (see Stringham & Lockwood, 2021 for discussion). While zoohchorous and anthropogenic vectors can facilitate overland transport between habitats (Coughlan et al., 2018; Rothlisberger, Chadderton, McNulty, & Lodge, 2010), fragmentary propagules can also be spread through directional water currents within river systems, that is, hydrochory (Sameel, 2013). Vegetative propagules can remain buoyant for several months, and can be rapidly dispersed over considerable distances within streams and rivers (e.g., up to 5 km; Heidbüchel et al., 2020). For many species of macrophytes, fragmentation is frequently a result of mechanical disturbances including anthropogenic activities and grazing animals (e.g., Crane et al., 2021; Rothlisberger et al., 2010). In particular, fragmentation and dispersal can also be caused by poor management practices. For example, machinery used to control or remove invasive macrophytes may contribute to increased fragmentation (Hussner et al., 2017), and even overland dispersal if adequate biosecurity protocols are not employed (e.g., Coughlan, Cuthbert, & Dick, 2020; Mohit, Johnson, & Arnott, 2021).

Currently, the extent to which fragment size affects propagule survival and viability (i.e., resumption of growth) is poorly documented (Coughlan et al., 2018; Heidbüchel, Kuntz, & Hussner, 2016; Heidbüchel, Sachs, Stanik, & Hussner, 2019). Although a broadly linear relationship is often assumed between fragment size and viability, with larger fragments displaying greater capacity for resumption of growth (e.g., Heidbüchel et al., 2016, 2019), the fragmentation tolerances of many invasive macrophytes remain unknown. Nevertheless, evidence suggests that exceptionally small and even single node stem fragments of many invasive macrophytes can retain viability (e.g., Hussner, 2009; Heidbüchel et al., 2016 and citations therein). Here, we determined relationships between fragment size and resumption of growth for three invasive macrophytes: Crassula helmsii (Kirk) Cockayne; Elodea canadensis Michx.; Lagarosiphon major (Ridley) Moss. Apical and mid-stem sections were assessed for viability. Fragment size was separately assessed considering length and the number of nodes. We hypothesised that species-specific thresholds exist, whereby fragment size influences propagule fitness. In particular, we anticipated that a minimum fragment size threshold is required for the resumption of growth, below which fragments do not retain viability. Further, we expected apical sections to show greater resumption of growth than mid-stem sections, given the presence of apical buds (meristematic tissue).

2  |  METHODS

Details of specimen collection and cultivation are provided in Appendix S1.

2.1  |  Fragmentation protocol

Fragmentary propagules were cut from mature, unbranched stems of C. helmsii, E. canadensis and L. major. Both apical and mid-stem sections were separately assessed for viability at lengths of 10, 20, 30, 50 and 100 mm (n = 5 per treatment). This sample size was balanced across the entire range of fully factorial experimental groups, and allowed for sufficient statistical power to find significant effects. While apical sections included the apical bud, mid-stem sections were harvested from at least 60 mm below the apical bud. Separately, for C. helmsii and E. canadensis mid-stems, fragmentary propagules were also harvested in relation to node counts of 1, 2, 3, 5 and 10 (n = 5 per treatment). Viability of L. major as a function of node-length was not assessed as this species lacks distinct nodes. Fragments were cut immediately above the first node at the upper end and immediately below that of the lower.

Fragments were harvested as required and briefly maintained in lake water-filled aquaria (≤30 min). Following this, each plant fragment was randomly selected from the aquaria and excess media gently removed by manually spinning individual fragments, 10 times in both directions, within a handheld centrifugal spinner. Wet-biomass was then recorded for each fragment; length was also recorded for fragments selected by node count (Tables S1 and S2 in Appendix S1). Following this, each fragment was kept in an individual glass beaker containing 500 ml lake water for 42 days; resumption of growth generally occurs within <28 days (e.g., Kuntz, Heidbüchel, & Hussner, 2014). Water lost through evaporation was replenished with lake water weekly. Specimens were maintained constantly at 16 ± 1°C. Cool, white light was supplied by fluorescent strips at 190 μmol m⁻² s⁻¹ (Skye SKP 215; Llandrindod Wells, Powys, UK) on a 12:12 hr light-to-dark regime.

On completion of the 6-week experimental period, survival and viability of each fragment were assessed. First, a biodegradation scale (0–10; Crane et al., 2019) was used to assess tissue degradation of the fragmentary propagules, whereby those that displayed new shoot and/or root growth were considered viable. Finally, a count of new shoots and roots was recorded.

2.2  |  Statistical analysis

Separate analyses for fragment length and node count assessments were performed, with viability considered in relation to degradation score, shoot number or root number. Degradation scores were analysed using proportional odds logistic regression (Venables & Ripley, 2002) considering species, starting length (or node count) and plant section (for length analysis), as well as their interactions,
with fragment biomass included as a covariate. The proportional odds assumption was tested by comparing AIC between ordinal and multinomial models. Shoot and root numbers were analysed using Poisson generalised linear models according to the same variables. Residual over/under-dispersion was tested statistically (Kleiber & Zeileis, 2008). A backward stepwise deletion procedure was followed such that final models included only significant terms (Crawley, 2007), with analysis of deviance used to compute coefficients. Tukey-adjusted estimated marginal means were used post hoc (Lenth, 2020). All analyses were computed in R v.4.0.2 (R Core Team, 2020).

3 | RESULTS

3.1 | Minimum viable length

Plant species displayed significantly different degradation scores according to section, resulting in a significant two-way interaction ($\chi^2 = 10.99$, df = 2, $p = .004$). L. major always displayed the least degradation (all $p < .01$), whereas only apical sections of C. helmsii were significantly more degraded than E. canadensis ($p < .05$; Table S1, Appendix S1). There was a significant effect of fragment length on degradation score according to species ($\chi^2 = 15.58$, df = 8, $p = .04$). L. major was significantly less degraded than C. helmsii and E. canadensis at all lengths (all $p < .05$) except for 10 mm E. canadensis fragments ($p > .05$). In turn, C. helmsii was only significantly more degraded than E. canadensis at 10 mm ($p < .01$). Degradation significantly decreased with increasing fragment biomass of the fragment ($\chi^2 = 8.15$, df = 1, $p = .004$). The plant section and fragment length interaction was not significant ($\chi^2 = 2.26$, df = 4, $p = .69$).

Resumption of growth was observed for all species, fragment lengths and plant sections with the exception of mid-stem sections of C. helmsii and E. canadensis for lengths of 10 and 30 mm, respectively (Figure 1). There was no significant effect of fragment length on shoot generation among tested species ($\chi^2 = 3.59$, df = 4, $p = .47$), however, the longest fragment length (100 mm) tended to produce the most shoots. Nevertheless, all species produced shoots from fragments as small as 10 mm (Table S1, Appendix S1). Shoot production differed, however, among species ($\chi^2 = 30.53$, df = 2, $p < .001$); C. helmsii produced significantly fewer shoots than L. major or E. canadensis (both $p < .001$), with a similar shoot production for L. major and E. canadensis ($p > .05$). Plant section also significantly affected shoot production ($\chi^2 = 38.76$, df = 1, $p < .001$), with apical sections tending to produce over twice as many shoots as mid-stems. Shoot production was significantly positively related to biomass of the fragment ($\chi^2 = 23.55$, df = 1, $p < .001$). There were no significant interaction effects (all $p > .05$).

Similarly, root production was evidenced by L. major and E. canadensis at sizes $\geq 10$ mm, while C. helmsii only produced roots at $\geq 20$ mm (Table S1, Appendix S1). All two-way interaction terms were significant (species $\times$ section: $\chi^2 = 110.10$, df = 2, $p < .001$; species $\times$ length: $\chi^2 = 66.18$, df = 8, $p < .001$; section $\times$ length: $\chi^2 = 12.97$, df = 4, $p = .01$), indicating emergent effects among taxa, lengths and stem sections. While L. major and E. canadensis exhibited significantly greater apical root production than mid-stem sections (both $p < .001$), the inverse was true for C. helmsii ($p < .001$). Root production by L. major substantially increased with increasing fragment length.

**Figure 1** Resumption of growth for fragmentary propagules of Crassula helmsii, Elodea canadensis and Lagarosiphon major [Color figure can be viewed at wileyonlinelibrary.com]
compared with both other species, which remained low and relatively similar for C. helmsii and E. canadensis (Table S1, Appendix S1). Biomass of the fragment significantly affected root production ($\chi^2 = 9.05$, df = 1, $p = .003$).

3.2 Minimum viable node count

Degradation scores differed between the species among node counts ($\chi^2 = 11.13$, df = 4, $p = .03$), whereby degradation of C. helmsii was significantly higher at a node count of 10 ($p < .001$; Table S2, Appendix S1). Biomass of the fragment did not significantly affect degradation between these species ($\chi^2 = 1.43$, df = 1, $p = .23$).

Resumption of growth was only observed for E. canadensis (Figure 1), as C. helmsii produced neither new shoots nor roots at any of the assessed node numbers and was thus removed from the analysis (Table S2, Appendix S1). For E. canadensis, shoot production increased significantly with node numbers ($\chi^2 = 12.20$, df = 4, $p = .02$), with shoot production highest at node counts of 10 (Table S2, Appendix S1). Root production in E. canadensis was also significantly affected by node numbers ($\chi^2 = 23.27$, df = 4, $p < .001$), again being substantially highest at node counts of 10 (Table S2, Appendix S1). Biomass of the fragment did not significantly influence shoot ($\chi^2 = 0.31$, df = 1, $p = .58$) or root ($\chi^2 = 0.09$, df = 1, $p = .77$) production.

4 DISCUSSION

Invasive C. helmsii, E. canadensis and L. major produced new shoots and roots from fragments ≥10 mm, except for root production by C. helmsii, which only occurred in fragments ≥20 mm. The proportion of fragments that retained viability (i.e., show resumption of growth) was generally high, especially for apical fragments of E. canadensis and L. major (80–100%). However, viability retention did not scale linearly with increasing fragment length, suggesting that fragment length alone does not govern propagule fitness for the assessed size range. Nonetheless, biomass was a significant predictor of viability.

While previous studies have indicated that larger fragments show greater retention of viability (e.g., Heidbüchel et al., 2016, 2019), their assessments are generally based on fragments ≥50 mm (but see Coughlan et al., 2018; Hussner, 2009). In the present study, apical fragments produced more shoots than mid-stem sections in all three species tested. Further, longer fragments tended to produce a greater number of shoots. Although apical sections tended to produce more roots than mid-stem fragments, this was not observed for C. helmsii. Longer fragments also generally produced more roots, excepting apical sections of C. helmsii and mid-stem fragments for E. canadensis, with the length to root production relationship being inconsistent. Surprisingly, mid-stem C. helmsii fragments standardised by node counts did not display any new growth, despite counts of 5 and 10 nodes resulting in fragment lengths ranging from 24 to 55 mm. However, E. canadensis showed greater shoot and root production with increasing node counts.

Although the present study did not determine the minimum fragment size needed to act as a viable propagule, our results suggest that apical fragments of all three species may retain viability at sub-centimetre fragment lengths. While new growth may emerge from sub-centimetre mid-stem fragments of E. canadensis and L. major, we demonstrated fragmentary propagules of C. helmsii measuring <20 mm appear incapable of resumption of root growth (but see Hussner, 2009). Nonetheless, the resumption of shoot growth evidenced by 10 mm C. helmsii could indicate that root growth resumption is equally possible with a longer timescale or greater sample size than employed here (6 weeks and $n = 5$). The enhanced capacity of apical sections to produce new growth may be explained by the presence of meristematic tissue within apical buds, promoting the development of new tissue (Barrat-Segretain, Bornette, & Hering-Vilas-Bôas, 1998). However, apical sections of C. helmsii may have produced less new growth than E. canadensis and L. major as these potentially emergent sections of C. helmsii may be optimised for out-of-water growth. Overall, as a decrease in fragment size can be associated with a reduced likelihood of propagule viability, we anticipate that sub-centimetre fragmentary propagules will display a further reduction in their capacity for resumption of growth than the fragment size ranges assessed by the present study. While physical disturbances can increase fragmentation rates, E. canadensis appears to have a greater potential for downstream dispersal in stream and river systems given its ability to more readily fragment under relatively modest water flow conditions compared to L. major (see Redekop, Hofstra, & Hussner, 2016), despite generally greater levels of growth resumption being detected for fragmentary propagules of L. major. Currently, velocity induced fragmentation data for C. helmsii do not appear to be readily available in the literature. However, it should be noted that C. helmsii may also spread via seed production (e.g., D’hondt et al., 2016). The number of fragments generated by different management activities also needs to be determined, as well as the effectiveness of methods employed to contain fragments (e.g., mesh nets and containment booms).

The present study cannot predict the likelihood of in situ establishment or the growth of fragmentary propagules into adult plants. Further, possible low nutrient concentrations, a lack of water movement and a sub-optimal temperature regime in the experimental setup may have expedited degradation. In particular, while all three of the assessed species will tolerate waters as cool as 10°C, optimal temperatures for growth can be as high as 20–25°C (CABI, 2021). As a result, the present study may underestimate retention of viability. Accordingly, future research should assess long-term growth patterns of these invasive macrophytes, as well as the effects of a range of biotic and abiotic context-dependencies, such as seasonal change, water flow rates, water quality and temperature parameters (e.g., Heidbüchel et al., 2020; Hoffmann, Raeder, & Melzer, 2014). Although a variety of similar studies have employed relatively low replication of experimental treatments due to non-independence of data (e.g., Hoffmann et al., 2014; Kuntz et al., 2014), the increased
replication by the independent and fully factorial design of the current study gave both unequivocal significant and non-significant results, supporting the elucidation of the true biological patterns from the present study. In addition, the retention of viability by fragmentary propagules following both overland or hydrochorous dispersal should be considered (Coughlan et al., 2018). However, it can be speculated that the smaller the fragment, the higher the likelihood of overland dispersal due to ease of adherence to passive vectors such as boating equipment as well as reduced visual detection by human observers (Rothlisberger et al., 2010). Therefore, the conclusion that smaller fragments are nearly as viable as larger ones is of some concern, even when grown at less than optimal conditions. Further to this issue, future studies will need to determine the desiccation tolerance of these small fragments (e.g., Coughlan et al., 2018). The present study thus has major implications for management practices concerning C. helmsi, E. canadensis and L. major. Specifically, control practices reliant on cutting and mechanical removal of aquatic macrophytes will need to avoid excessive fragmentation of macrophytes and remove all stem sections >5 mm long. However, complete removal of fragments sized <5 mm may be exceedingly difficult, therefore, new integrated practices of mechanical, chemical and biological control should be developed. Thus, many current short-term management strategies, such as cutting, may in fact be counterproductive. Finally, rigorous biosecurity protocols should be enforced to ensure equipment is thoroughly decontaminated to limit secondary spread (Coughlan et al., 2020; Crane et al., 2019; Mohit et al., 2021). Biosecurity assessments should seek to determine the minimum viable size for propagules of invasive species, with differences in viability evidenced here among species and plant sections.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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