Aquatic Botany 162 (2020) 103204

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Dead and gone: Steam exposure kills layered clumps of invasive curly waterweed *Lagarosiphon major*

Neil E. Coughlan[a,b,*], Fearghail Armstrong[a,b], Ross N. Cuthbert[a,b], Lawrence E. Eagling[a,b], Louise Kregting[b,c], Jaimie T.A. Dick[a,b], Hugh J. MacIsaac[d], Kate Crane[a,b]

[a] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland, UK
[b] Queen's Marine Laboratory, Queen's University Belfast, 12-13 The Strand, Portaferry, BT22 1PF, Northern Ireland, UK
[c] School of Natural and Built Environment, Queen's University Belfast, Belfast, BT9 5BN, UK
[d] Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada

ARTICLE INFO

Keywords:
Biosecurity
Decontamination
Fragment degradation
Invasive species management
Secondary dispersal
Spread-prevention

ABSTRACT

Population suppression and eradication of invasive, alien macrophytes can be complex, costly and labour intensive, therefore prevention of further spread is an essential aspect of management. However, following the physical removal of entangled clumps of plant material adhering to anthropogenic vectors including outboard engines, guidelines for appropriate disposal are often unclear, inadequate, or non-existent. Here, we explore use of direct steam exposure to cause complete degradation of layered clumps of invasive curly waterweed *Lagarosiphon major* (Ridley) Moss. Clumps were arranged as three, stacked 15 × 15 cm layers, with 40 ± 0.1 g of entangled stems per layer, to which steam was directly applied downwards onto the top layer. The top surface area was divided into nine subsections to ensure an even application of steam per 5 × 5 cm for durations of 5, 10, 30, 60, or 120-sec, equivalent to 0.75, 1.5, 4.5, 9, or 18-min steam applications. Ten seconds of exposure caused total degradation of top and middle layers, while up to 30-sec was required for the bottom layer. For shorter exposures, new growth - if it occurred - was evidenced by a single new shoot of < 5 mm in length following 28-days of recovery. Conversely, control specimens displayed excellent survival and production of new growth. We suggest that the simple, yet highly efficacious technique of steam exposure can be used to improve prevent spread of invasive macrophytes.

1. Introduction

Invasive alien species (IAS) are a serious threat to native biodiversity, ecological functioning, and the economic and recreational value of freshwater ecosystems (Ricciardi and MacIsaac, 2011; Booy et al., 2017; Piria et al., 2017). Through the formation of expansive underwater stands of vegetation, submerged invasive macrophytes can have a detrimental effect on the physical, chemical and biological processes of freshwater ecosystems (Schultz and Dibble, 2012; Hussner, 2014). Although fragmentary propagules of invasive macrophytes can be dispersed overland between isolated waterways (Rothlisberger et al., 2010; Coughlan et al., 2017), overland transportation of clumped material by anthropogenic vectors, such as angling equipment, nets and recreational boats, is responsible for many invasion events (Johnstone et al. 1985). Once established, management options for population suppression are often complex, expensive, resource-intensive, damaging to non-target species, and are frequently unsuccessful (Hussner et al., 2017; Beric and MacIsaac, 2015). Therefore, preventing invader spread is considered the most cost-effective and productive strategy for mitigating negative impacts (Booy et al., 2017; Coughlan et al., 2019).

Although a variety of biosecurity protocols designed to suppress invasive macrophyte populations (Beric and MacIsaac, 2015) and curtail further spread (Crane et al., 2019; Cuthbert et al., 2019) are available, their efficacy has been called into question (Coughlan et al., 2019; Cuthbert et al., 2019). For example, the 'Check, Clean, Dry' procedure is widely advertised to prevent invader spread. The effectiveness of this protocol is dependent on complete hand-removal of organisms found adhering to equipment, particularly in relation to clumps of plants. However, once removed, guidelines for effective disposal of plant material are often unclear or non-existent. Typically, plant material is either returned to the water, casually left to

* Corresponding author at: Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland, UK.
E-mail address: neil.coughlan.zoology@gmail.com (N.E. Coughlan).

https://doi.org/10.1016/j.aquabot.2020.103204
Received 4 October 2019; Received in revised form 8 January 2020; Accepted 11 January 2020
Available online 18 January 2020

accumulate on the ground, discarded in trash receptacles, or moved to a designated area for biodegradation through a mixture of desiccation and natural decomposition. Reintroduction into water is clearly inappropriate, while haphazard accumulations on the ground could also allow for some secondary dispersal. Further, secure trash receptacle facilities, or appropriate waste management thereafter, may not be available. For example, it may not be permissible to dispose of large quantities of viable waste plant material in a general trash receptacle. In addition, transport and disposal of potentially viable invasive plant biomass may be prohibited by law except under strict licencing conditions (e.g. EU Regulation, 1143/, 2014). In these cases, most waterway users will be legally prevented from knowingly moving waste material away from its site of origin.

Incorrect or inadequate disposal of IAS can lead to further spread (Coughlan et al., 2019; Cuthbert et al., 2019). For example, clumps of invasive macropyhtes may become entangled on equipment (e.g. outboard motor or boat trailer) at an invaded site and subsequently be transported to non-invaded destinations, and this may serve to introduce the species to the uninvaded site. In some instances, plant material removed from equipment may not be returned to the waterway, but rather left to desiccate and decompose. Although desiccation can limit the survival of macrophyte fragmentary propagules, surviving individuals may resume growth even following lengthy exposure to adverse conditions (Jerde et al., 2012; Bruckerhoff et al., 2015; Coughlan et al., 2018). In particular, clumped layers of stems could be especially resistant to desiccation than single stems given their lower surface area to volume ratio (Bruckerhoff et al., 2015). Accordingly, there is an urgent need to establish effective, efficient, and environmentally-friendly protocols that facilitate thorough decontamination and optimal invader biomass disposal. Crane et al. (2019) determined that a ten second steam treatment caused complete degradation of apical fragmentary propagules for seven invasive macrophyte species, owing to thermal shock. Their study also highlighted the need to determine the minimal steam exposure time required to effect degradation of entangled and clumped plant material, as larger fragments are likely to have a greater capacity for growth resumption following steam exposure (Jiang et al., 2009). In particular, Crane et al. (2019) argued that large clumps of plant material composed of long stems coiled into several layers, would exhibit increased resistance to steam applications in a fashion similar to desiccation resistance.

Curly waterweed, *Lagarosiphon major*, (Ridley) Moss 1928, is an invasive canopy-forming submerged macrophyte that can establish vast monocultures that are difficult to control (Caffrey et al., 2010). Native to South Africa, in the Northern Hemisphere *L. major* displays overwinter growth and can achieve substantial biomass under conditions not possible for many native species (Martin and Coetzee, 2014). Like many other invasive macrophytes, *L. major* predominantly spreads *via* vegetative fragments, which have a high survival potential (Redekop et al., 2016; Coughlan et al., 2018). Notably, *L. major* is listed as a Species of Union Concern in the European Union, which requires Member States to prevent its spread, and control or eradicate existing populations (EU Regulation, 1143/, 2014). Here we seek to improve spread-prevention and disposal protocols by assessing the efficacy of steam treatments to cause complete degradation of layered clumps of *L. major*, as a model invasive macrophyte species.

2. Methods

2.1. Sample collection and cultivation

Lagarosiphon major was collected from Lough Corrib, Co. Galway, Ireland (53°26'36.9"N; 9°19'17.5"W) and transported in source water to Queen's University Marine Laboratory, Portaferry, Northern Ireland, UK. A cultivated stock of *L. major* was then maintained within a 2000 L aerated aquarium filled, and topped up, with locally sourced lake water (Lough Cowey: 54°24′41.79″N; 5°32′25.96″W), without inclusion of any substrate. The aquarium was stored outdoors and was subject to natural daylight and ambient temperatures. *L. major* was visually observed to display excellent survival and growth over a six month cultivation period prior to experimentation (NEC & KC *per. obs.*).

2.2. Steam exposure

Stems of *L. major*, including branched stems and apical tips, were harvested from the aquarium. Excess water was gently shaken from the plant material, until dripping ceased. A wet-weight of 40 ± 1 g was then established for coiled clumps of mixed stems with lengths between ~10–30 cm. Clumps were placed into flat, plastic mesh-bags (15 × 15 cm; mesh pores = 1.5 × 2 mm), so that the entire area of the mesh-bag contained plant material. Once filled, each mesh-bag had a layer thickness of 15–20 mm. Bagged *L. major* was then briefly maintained within dechlorinated tap-water prior to experimental use (< 30 min). Following this, while still dripping, damp mesh-bags were stacked to create a triple layer formation to simulate macrophyte accumulation around boat propellers or on boat trailers. Rather than applying steam with a supporting surface directly behind the stacked layers, mesh-bags were elevated by 10 cm using a rigid mesh sheet. This thus allowed steam to potentially pass completely through the stacked clumps, while preventing heat transfer to any underling surface such as stone, concrete or metal, which may aid or inhibit the efficacy of steam treatment.

To ensure that the entire upward-facing surface area of the mesh-bags received the same steam exposure time, stacked layers were overlaid with a metal quadrat consisting of nine grid-squares (15 × 15 cm; grid squares 5 × 5 cm). Each 5 × 5 cm grid-square was directly exposed to a continuous jet of steam (≥ 100 °C; 350 kPa; Karcher® SC3 Steam Cleaner) at a distance of 2–3 cm from the spout for: 5, 10, 30, 60, or 120-sec. When all nine-grid squares are considered as a whole, this equates to 0.75, 1.5, 4.5, 9, or 18-min steam application for the entire upward facing surface area of the stacked clumps. The steam jet was continuously moved over the area of a single grid-square for the exposure period. This process was repeated in a randomised fashion until all grid-squares had been treated by a single exposure time, e.g. all five seconds or all ten seconds, per stacked clump. Controls were allowed to air-dry for an 18-min period, and were otherwise handled like treated groups. All treatments were replicated three times (i.e. 3 sets of 3 stacked layers).

Immediately following steam exposure, *L. major* was removed from the mesh-bags and placed separately into clear plastic containers: 12 L × 8 W × 12 H cm; high-density polyethene. To prevent additional thermal shock, *L. major* was allowed to cool for a 15-min period. Then, one litre of aerated locally sourced lake water was added to each container (~ 12 °C). All containers were stored in the laboratory at 16 ± 1 °C, under a 12:12 light to dark regime of 190 μmol m^{-2} s^{-1} (Skye PAR Special: SKP 210/S 37156). Tissue degradation and the presence of new growth were assessed at 7-days following steam exposure using a modified version of the degradation scale proposed by Crane et al. (2019: see Table 1). Rather than assessing degradation of an individual fragmentary propagule, we applied it to the entire clump of plant material (i.e. contents of each layered bag). Given that certain steam-treated samples displayed new growth, all samples (including controls) were retained for 28-days to confirm survival and assess potential re-growth, under the laboratory condition outlined above. Kruskal-Wallis rank sum tests were used to assess effectiveness of steam exposure at inducing degradation for each individual layer, separately. Data analyses were performed using R v3.4.4 (R Core Development Team, 2018).

3. Results

Steam exposures lasting five seconds caused substantial but not complete degradation of the stacked clumps of macrophytes. However, the top layer showed complete and statistically significant degradation

Table 1
Degradation scale describing visual tissue biodegradation stages and/or resumption of growth for aquatic macrophyte clumps (see Crane et al., 2019 for fragmentary propagules).

Pathway choice	Description of clump	Score
No shoot and/or root growth present (A)		
	A1. Complete degradation	10
	A.2. More than or equal to 90 % clump degradation	9
	A.3. More than or equal to 50 % clump degradation	8
	A.4. All leaves exhibiting paling or browning	7
	A.5. Paling or browning affecting any leaves	6
	A.6. Degradation at fragmentation sites	5
Shoot and/or root growth present (B)		
	B.1. More than or equal to 90 % clump degradation	4
	B.2. More than or equal to 50 % clump degradation	3
	B.3. All leaves exhibiting paling or browning	2
	B.4. Paling or browning affecting any leaves	1
	B.5. Degradation at fragmentation sites	0

Table 2
Median degradation score describing visual biodegradation stages and/or resumption of growth for layered clumps of invasive *Lagarosiphon major* at 7-days post exposure to direct steam treatments (n = 3: see Table 1). Minimum and maximum range scores presented in parenthesis, when applicable. Shaded region delineates complete degradation. Controls were allowed to air-dry for a 15-min period.

Layer	Exposure Time (sec)					
Exposure time per 5 × 5 cm (s):	Control	5	10	30	60	120
Exposure time per 15 × 15 cm (m):		0.75	1.5	4.5	9	18
1st : Top	1	4 (4–10)	10	10	10	10
2nd : Middle	1	10 (4–10)	10	10	10	10
3rd : Bottom	1	2	4 (2–10)	10	10	10

following steam treatments of ten seconds or longer, per 5 × 5 cm (i.e. score = 10: K-W: χ^2 = 14.875, df = 5, P = 0.01: Table 2). Similarly, steam treatments of ten seconds or longer caused significant degradation of all plant material in the second layer (K-W: χ^2 = 14.500, df = 5, P = 0.01). In the third layer, significant degradation of all plant material occurred following exposure of 30 sec or longer, per 5×5 cm (K-W: χ^2 = 15.558, df = 5, P = 0.01). For steam treatments lasting five or ten seconds, some viable clumps produced only one single new shoot per steam treated clump, with no new roots being formed. In these cases, we observed new shoot growth of < 5 mm in length, per clump following the entire 28-day recovery period (i.e. score < 5; Crane et al., 2019). Contrastingly, all control groups displayed excellent survival, and viability following the entire 28-day recovery period, with little degradation observed. Therefore, all control sample scores = 1 (Table 2).

4. Discussion

Steam treatment can cause complete degradation of *L. major* clumps. Following ten seconds of steam exposure, total degradation of the top and middle layers was observed, while the bottom layer required up to thirty seconds. If new growth occurred, it did so by a single new shoot with minimal growth, and only at lower intensity applications of steam, i.e. five and ten seconds per 5 × 5 cm. Thus steam treatments seem to be an effective tool to improve biosecurity protocols for the disposal of entangled and layered clumps of invasive *L. major*. Further, direct steam exposure also caused complete degradation of fragmentary propagules for a variety of invasive macrophytes following ten seconds of exposure (Crane et al., 2019). Although Crane et al. (2019) examined relatively large apical fragmentary propagules for seven invasive macrophytes (35–185.6 mm; 0.11–0.86 g), this study highlights that large, layered clumps of coiled plants can also be effectively killed following exposure to steam. However, morphological and physiological differences of different macrophyte species may aid resistance to steam treatment, especially for layered plants. For example, species with more rigid stems or dense fronds may better insulate internal clump biomass than less rigid or dense structures, therefore requiring use of longer exposure times to achieve complete clump mortality. For instance, species such as *Ceratophyllum demersum* - which possess relatively rigid stem and frond structures - may require longer steam exposure than less robust species, such as *Egeria densa*. Further, emergent stems produced by some submerged macrophyte species, may also require longer steam exposure times to facilitate penetration of a potentially thicker outer cuticle for destruction of the meristematic tissue underneath. Overall, as untreated clumps returned to a waterbody can theoretically fragment into numerous viable propagules, steam exposure appears to be a robust method for causing mortality of macrophyte clumps. In addition, complete degradation could potentially be achieved with shorter exposure times at distances > 3 cm from the spout by employing industrial steam cleaners capable of producing higher temperatures and greater water pressure (e.g. ≥ 180 °C, 10–12 bar) than the domestic household steamer used in this study (Coughlan et al., 2019; Crane et al., 2019).

Although other biosecurity procedures, such as immersion in water of 45 °C for 15-min, can result in 100 % mortality for some IAS (Anderson et al., 2015; Coughlan et al., 2019), the practicality of upscaling some procedures for the disposal of large clumps of plant material is questionable. In addition, while chemical methods are somewhat effective at inducing invader mortality, the application of these methods for macrophyte disposal remain unclear and unreliable (Cuthbert et al., 2018, 2019). Equally, the environmental impact of large spills and improper chemical disposal requires consideration. Despite the demonstrated efficacy of direct steam exposure to cause mortality in invasive macrophytes, its possible effect on equipment also requires consideration since potential damage could deter anglers and boaters from utilising steam treatments as a biosecurity method. Accordingly, further assessment of non-target steam effects needs to be assessed. Having confirmed the efficacy of steam treatments to induce necrosis of *L. major*, species-specific susceptibilities to steam exposure requires examination.

Steam applications also have the potential to be used as part of population suppression and eradication strategies. For instance, in many waterways, invasive macrophytes are cut and removed from the system but their disposal is often a strictly controlled, labour-intensive and expensive process (Hussner et al., 2017). The integration of steam technology onto commercially-operating weed-cutting boats could facilitate improved control of invasive plants. In particular, mechanical harvesting and cutting of macrophytes can produce large amounts of fragmentary propagules (Hussner et al., 2017), which can fall from weed-cutting boats prior to the harvest being deposited on-shore. However, if harvested material could be steamed on-board vessels, any fragments that manage to slip back into the waterway will have been killed. In addition, steam treatments could improve on-land disposal of invasive plant material by preventing possible spread of viable fragments.

Overall, the results presented here further highlight the use of steam treatment as an effective tool for invader disposal and decontamination of equipment, with negligible risk to the environment and end-users once appropriate and risk-assessed application procedures are implemented. For example, industrial steaming devices could be installed at designated biosecurity stations, such as points of waterway entry and exit (e.g. angling locations and marinas: Coughlan et al., 2019; Crane et al., 2019). Steam decontamination facilities, operated by a trained attendant, could greatly reduce the transfer of IAS in a cost-effective, environmentally-friendly, yet highly successful way. Once physically removed from equipment and steamed, invasive plant material could disposed above the waterway flood line, and allowed to safely decompose. Accordingly, the adoption of steam decontamination and

invader disposal techniques should be incorporated into water-user Codes of Practice, and promoted by relevant biosecurity campaigns, stakeholder groups, and practitioners.

Author statement

NEC proposed the study; NEC and KC designed the experiments; NEC, KC, FA and LE conducted the experiments; NEC and RNC performed data analysis; all authors contributed to the interpretation of results and the writing of the manuscript, which was led by NEC.

Acknowledgments

NEC and JTAD acknowledge support from the Irish Environmental Protection Agency project "Prevention, control and eradication of invasive alien species" (2015-NC-MS-4). RNC acknowledges support from Department for the Economy, Northern Ireland. KC is supported through contributions from Queen's University Belfast, the University of Windsor and McGill University. JTAD acknowledges funding received from NERC, while HJM acknowledges NSERC Canada. Thanks also to James Dickey and Briar Dick for stimulating discussions. We thank two anonymous reviewers for helpful comments.

References

Anderson, L.G., Dunn, A.M., Rosewarne, P.J., Stebbing, P.D., 2015. Invaders in hot water: a simple decontamination method to prevent the accidental spread of aquatic invasive non-native species. Biol. Invasions 17, 2287–2297.

Beric, B., MacIsaac, H.J., 2015. Determinants of rapid response success for alien invasive species in aquatic ecosystems. Biol. Invasions 17, 3327–3335.

Booy, O., Mill, A.C., Roy, H.E., Hiley, A., et al., 2017. Risk management to prioritise the eradication of new and emerging invasive non-native species. Biol. Invasions 19, 2401–2417.

Bruckerhoff, L., Havel, J., Knight, S., 2015. Survival of invasive aquatic plants after air exposure and implications for dispersal by recreational boats. Hydrobiologia 746, 113–121.

Caffrey, J.M., Millane, M., Evers, S., Moran, H., Butler, M., 2010. A novel approach to aquatic weed control and habitat restoration using biodegradable jute matting. Aquat. Invasions 5, 123–129.

Coughlan, N.E., Kelly, T.C., Davenport, J., Jansen, M.A., 2017. Up, up and away: bird--mediated ectozoochorous dispersal between aquatic environments. Freshw. Biol. 62, 631–648.

Coughlan, N.E., Cuthbert, R.N., Kelly, T.C., Jansen, M.A., 2018. Parched plants: survival and viability of invasive aquatic macrophytes following exposure to various desiccation regimes. Aquat. Bot. 150, 9–15.

Coughlan, N.E., Cuthbert, R.N., Dickey, J.W.E., Crane, K., Caffrey, J.M., Lucy, F.E., Davis, E., Dick, J.T.A., 2019. Better biosecurity: spread-prevention of the invasive Asian clam, *Corbicula fluminea* (Müller, 1774). Manag. Biol. Invasions 10, 111–126.

Crane, K., Cuthbert, R.N., Dick, J.T., Kregting, L., MacIsaac, H.J., Coughlan, N.E., 2019. Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. Biol. Invasions 21, 1311–1321.

Cuthbert, R.N., Coughlan, N.E., Crane, K., Caffrey, J.M., MacIsaac, H.J., Dick, J.T., 2018. A dip or a dab: assessing the efficacy of Virasure® Aquatic disinfectant to reduce secondary spread of the invasive curly waterweed *Lagarosiphon major*. Manag. Biol. Invasions 9, 259–265.

Cuthbert, R.N., Crane, K., Dick, J.T., Caffrey, J.M., MacIsaac, H.J., Coughlan, N.E., 2019. Die hard: impact of aquatic disinfectants on the survival and viability of invasive *Elodea nuttallii*. Aquat. Bot. 154, 11–17.

EU Regulation 1143/2014, 2014. European Union Regulation No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the Prevention and Management of the Introduction and Spread of Invasive Alien Species.

Hussner, A., 2014. Long-term macrophyte mapping documents a continuously shift from native to non-native aquatic plant dominance in the thermally abnormal River Erft (North Rhine-Westphalia, Germany). Limnologica-Ecol. Manag. Inland Waters 48, 39–45.

Hussner, A., Stiers, I., Verhofstad, M.J.J.M., Bakker, E.S., et al., 2017. Management and control methods of invasive alien aquatic plants: a review. Aquat. Bot. 136, 112–137.

Jerde, C.L., Barnes, M.A., DeBuysser, E.K., Noveroske, A., Chadderton, W.L., Lodge, D.M., 2012. Eurasian watermilfoil fitness loss and invasion potential following desiccation during simulated overland transport. Aquat. Invasions 7, 135–142.

Jiang, J., An, S., Zhou, C., Guan, B., Sun, Z., Cai, Y., Liu, F., 2009. Fragment propagation and colonization ability enhanced and varied at node level after escaping from apical dominance in submerged macrophytes. J. Integr. Plant Biol. 51, 308–315.

Martin, G.D., Coetzee, J.A., 2014. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. Aquat. Bot. 114, 1–11.

Piria, M., Copp, G.H., Dick, J.T.A., Duplić, A., et al., 2017. Tackling invasive alien species in Europe II: threats and opportunities until 2020. Manag. Biol. Invasions 8, 273–286.

Redekop, P., Hofstra, D., Hussner, A., 2016. *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. Aquat. Bot. 130, 45–49.

Ricciardi, A., MacIsaac, H.J., 2011. Impacts of biological invasions on freshwater ecosystems. In: Richardson, D.M. (Ed.), Fifty Years of Invasion Ecology: the Legacy of Charles Elton. Blackwell Publishing Ltd, New York, USA, pp. 211–223.

Rothlisberger, J.D., Chadderton, W.L., McNulty, J., Lodge, D.M., 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries 35, 121–132.

Schultz, R., Dibble, E., 2012. Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. Hydrobiologia 684, 1–14.