

Research Article

Tomorrow Never Dies: biodegradation and subsequent viability of invasive macrophytes following exposure to aquatic disinfectants

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Abstract

Aquatic invasive alien species (IAS) negatively impact freshwater ecosystems worldwide. As suppression and eradication of established invader populations are often complex, costly and resource-intensive, the prevention of further invader spread is considered a key aspect of proactive management measures. Although broad-spectrum aquatic disinfectants have been suggested as a suitable decontamination mechanism to enhance invader spread-prevention strategies, inconsistencies concerning their effectiveness are reported within the literature. Here, we examine the use of two aquatic disinfectants, which were developed to kill damaging microbes, to induce substantial degradation of the apical fragmentary propagules of five invasive macrophytes: *Crassula helmsii* (Kirk) Cockayne; *Egeria densa* Planchon; *Elodea canadensis* Michx; *Hydrocotyle ranunculoides* Linnaeus; *Lagarosiphon major* (Ridley) Moss. Apical fragments were exposed to 0% (0 g L⁻¹), 2% (20 g L⁻¹) or 4% (40 g L⁻¹) solutions of Virkon® Aquatic and Virasure® Aquatic, for submergence treatments of five, fifteen, thirty or sixty minutes. After 28 days, degradation of treated fragments was significantly greater than that of control groups, particularly for 4% solutions and longer exposure times. Despite this, sustained viability in relation to shoot and/or root regrowth was exhibited by almost all plant species. However, new shoot growth rates were significantly reduced following exposure to all treatments. At matched concentrations, there was no significant difference between the two disinfectants. Overall, it appears that the examined aquatic disinfectants will not curtail the spread of these invasive macrophytes. Yet, longer submergence times, multiple applications and synergistic effects of different biosecurity treatments may enhance preventative measures against further spread and this requires investigation.

Key words: fragment degradation, invasive alien species, secondary dispersal, spread-prevention, Virasure® Aquatic; Virkon® Aquatic

Introduction

Globally, aquatic invasive alien species (IAS) have adversely altered the biodiversity, ecological functioning, and economic and social value of freshwater ecosystems (Ricciardi and MacIsaac 2010; Piria et al. 2017). Notably, invasive macrophytes can negatively impact the biotic and abiotic processes of freshwater systems, which frequently results in the detrimental modification of habitats, community dynamics and species assemblages (Schultz and Dibble 2012; Kuehne et al. 2016; Lu et al. 2018). Moreover, invasive macrophytes often represent a considerable management burden, as large stands can escalate flood frequencies, devalue adjacent properties, and inhibit recreational and commercial activities (Hussner et al. 2017). Although management options for effective suppression and eradication of established IAS populations are available, these are often complex, expensive and resource-intensive endeavours, which can be damaging to non-target species (Caffrey et al. 2010; Hussner et al. 2017; Coughlan et al. 2018b, 2019b).

Freshwater ecosystems are especially susceptible to the influx of damaging IAS due to the presence of numerous transport pathways and a plethora of associated vectors (Dudgeon et al. 2006; Ricciardi and MacIsaac 2010; Coughlan et al. 2017a). For example, anthropogenic activities such as angling and boating, and the ornamental plant and aquatic pet trades, have facilitated a substantial number of IAS introductions (Rothlisberger et al. 2010; Gallardo and Aldridge 2013; Anderson et al. 2014). Accordingly, as established invaders are notoriously difficult to control, the prevention of further IAS spread is now widely recognised as a vital means of reducing invader impacts (Coughlan et al. 2018a, 2019a; Crane et al. 2019; Cuthbert et al. 2018, 2019a; Shannon et al. 2018). Indeed, the concept of spread-prevention is integral to the Convention on Biological Diversity, and is now strongly emphasised in national, e.g. EC (Birds and Natural Habitats) Regulations SI 477/2011, and international policy and legislation, such as EU Regulation 1143/2014, New Zealand Biosecurity Strategy, Great Britain Non-Native Species Strategy (EC 2011; EU 2014; GBNNSS 2015; PGNZ 2016).

Biosecurity campaigns such as *Check, Clean, Dry* in New Zealand and Great Britain have attempted to prevent invader spread through increased public awareness and the provision of practical decontamination guidance (Anderson et al. 2015; Piria et al. 2017; Coughlan et al. 2018a, 2019a). In essence, the campaign promotes the use of systematic checks of potential vectors such as footwear, clothing, nets, watercraft, trailers and vehicles, which is then followed by the physical removal of adhering organisms through cleaning procedures. However, despite highlighting the need to thoroughly clean equipment, recommendations concerning appropriate methods of disinfection are deficient. Finally, following systematic decontamination, extended drying times are recommended as a best

practice protocol. Although simple and effective, extended drying times can be difficult to incorporate into daily working practices (Anderson et al. 2015; Sutcliffe et al. 2018). Additionally, the application of many proposed spread-prevention techniques are limited due to poor practicality, time restraints, lack of known efficacy, expense, and undesirable non-target effects (Barbour et al. 2013; Piria et al. 2017; Coughlan et al. 2018a, 2019a; Crane et al. 2019). Therefore, the further development of simple but expeditious spread-prevention protocols remain a management priority.

Although broad-spectrum aquatic disinfectants have been confirmed to kill damaging pathogenic microbes within laboratory tests and under various field conditions, variability of in-field conditions can substantially reduce the known efficacy of recommended treatments (Tidbury et al. 2018). Despite such concerns, aquatic disinfectants have been suggested as a suitable method for preventing the spread of IAS, as disinfection through submergence in known chemical solutions can induce substantial invader mortality (Barbour et al. 2013; Cuthbert et al. 2018, 2019b). In particular, the provision of in-field biosecurity stations may provide effective decontamination of water users' equipment (Coughlan et al. 2019a; Crane et al. 2019; Cuthbert et al. 2019a). For example, as a biosecurity procedure, small items of equipment such as nets, waders and paddles could be completely submerged in disinfection baths, while spray applications may be more suitable for larger items, e.g. boats and trailers. However, further experimentation is required to assess the efficacy of such an approach.

Broad-spectrum aquatic disinfectants, such as Virkon® Aquatic and Virasure® Aquatic, are generally used in aquaculture for the control of a wide range of bacteria, viruses and fungi (Stockton-Fiti and Moffitt 2017). Currently, however, these disinfectants are being increasingly used for decontamination of equipment by recreational water users and responsible authorities, such as government agencies. Accordingly, the legal issues concerning the use of broad-spectrum aquatic disinfectants as *in situ* biosecurity agents for non-microscopic and invasive organisms (e.g. herbicide or insecticide) will need to be addressed (Cuthbert et al. 2018, 2019a; Sebire et al. 2018). Yet the risk of toxicity to non-target aquatic organisms *via* residues and spills is considered to be low, with adherence to best-practice protocols (see Stockton-Fiti and Moffitt 2017). However, somewhat conflicting evidence is presented in the literature concerning the success of these oxidising agent-based disinfectants to inhibit the spread of invasive macrophytes at solution concentrations of 1% (10 g L⁻¹), 2% (20 g L⁻¹) or 4% (40 g L⁻¹) for exposure times of up to five minutes (see Cuthbert et al. 2018, 2019a). Accordingly, assessment of longer exposure times is required.

Numerous invasive aquatic macrophytes predominantly reproduce and spread through vegetative propagation, particularly *via* apical fragmentary propagules (Umetsu et al. 2012; Li et al. 2015; Redekop et al. 2016). Within aquatic environments, plant fragmentation frequently occurs through either

Table 1. Study species, source site locations and invaded range. See www.cabi.org/isc/ for further species specific information.

Species	Common name	Source site	Invasion Range
<i>Crassula helmsii</i> (Kirk) Cockayne	Australian swamp stoncrop/ New Zealand Pigmyweed	Lough Beg 54°47'28.6"N; 6° 28'27.1"W	Europe, North America, invasive in native range
<i>Egeria densa</i> (Planch.) Casp.	Leafy elodea	Artificial Pond Dominican College, Portstewart 55°10'54.1"N; 6°43'18.3"W	Europe, North America, Central America, Caribbean, Oceania
<i>Elodea canadensis</i> Michx.	Canadian waterweed	Mill Pond, Tully Mill 54°15'32.34"N; 7°42'50.88"W	South America, Europe, Africa, Asia, Oceania, invasive in native range
<i>Hydrocotyle ranunculoides</i> L.f.	Floating pennywort	Aire and Calder Navigation 53°45'12.6"N; 1°25'57.9"W	Europe; Australia, invasive in native range
<i>Lagarosiphon major</i> (Ridl.) Moss	African elodea/ African curly waterweed	Artificial Pond Portadown Golf Club 54°24'14.6"N; 6°24'51.3"W	Europe, Australia, New Zealand, potentially invasive in native range

self-induced autofragmentation or allofragmentation, whereby fragmentation is a result of disturbance, such as changes in water velocity, sediment mobility, animal or anthropogenic activity (Bakker et al. 2016). Although most fragments will likely be dispersed within hydrologically connected systems, overland dispersal is a frequent occurrence (Johnson et al. 2001; Rothlisberger et al. 2010; Coughlan et al. 2017b). In most cases, overland transport is facilitated through the adherence of fragmentary propagules to recreational equipment, boats, vehicles and trailers (Johnson et al. 2001; Rothlisberger et al. 2010). Currently, however, there is a lack of information concerning the efficacy of various biosecurity procedures that are thought to inhibit the spread of fragmentary propagules for a variety of invasive aquatic macrophytes (Coughlan et al. 2018a; Cuthbert et al. 2018, 2019a). Therefore, to better inform spread-prevention practices, it is necessary to quantify the subsequent viability (i.e. regeneration by production of new shoot or root growth) of invasive macrophyte fragmentary propagules following exposure to biosecurity treatments, including disinfection.

Here, we examine the efficacy of two aquatic disinfectants, Virkon® Aquatic and Virasure® Aquatic, to induce plant tissue biodegradation, reduce the number of new roots and shoots produced, and decrease new shoot growth rates of fragmentary propagules for five invasive macrophytes: *Crassula helmsii* (Kirk) Cockayne; *Egeria densa* Planchon; *Elodea canadensis* Michx; *Hydrocotyle ranunculoides* Linnaeus; and *Lagarosiphon major* (Ridley) Moss. Effects on apical fragmentary propagules of each species were examined with respect to disinfectant concentration and exposure time.

Materials and methods

Sample collection and cultivation.

All species were collected throughout Northern Ireland (NI) from a variety of sites, other than *H. ranunculoides* which was collected in Great Britain (Table 1). All species obtained in NI were collected as whole plants, excluding the buried roots and rhizomes, and transported in source water to Queen's

Table 2. Summation of species mean fragmentary propagule lengths and weights, and exposure times (minutes) to aquatic disinfectants, for 0% (0 g L⁻¹), 2% (20 g L⁻¹) and 4% (40 g L⁻¹) solutions of Virkon® Aquatic and Virasure® Aquatic. Degradation assessment points (i.e. recovery days post exposure) for each focal species are given. Control samples were not exposed to aquatic disinfectants.

Species	Mean (± SE) length mm	Mean (± SE) weight g	Exposure times	Assessment point
<i>Crassula helmsii</i>	100 ± 0	0.15 ± 0.01	5 m, 15 m, 30 m	7 d, 14 d, 21 d, 28 d
<i>Egeria densa</i>	100 ± 0	0.92 ± 0.04	5 m, 15 m, 30 m	7 d, 14 d, 21 d, 28 d
<i>Elodea canadensis</i>	100 ± 0	0.39 ± 0.01	5 m, 15 m, 30 m	7 d, 14 d, 21 d, 28 d
<i>Hydrocotyle ranunculoides</i>	160 ± 0	0.49 ± 0.01	5 m, 15 m, 30 m, 60 m	2 d, 7 d, 21 d
<i>Lagarosiphon major</i>	100 ± 0	1.62 ± 0.05	5 m, 15 m, 30 m	7 d, 14 d, 21 d, 28 d

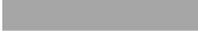
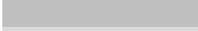
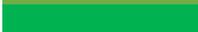
Marine Laboratory (QML), Portaferry, NI. Similarly, whole plants of *H. ranunculoides*, with their associated roots and rhizomes, were transported in source water to the University of Leeds, Great Britain. Each of the species collected in NI were separately maintained in the laboratory within aerated aquaria, filled with locally sourced pond water (Lough Cowey: 54°24'41.8"N; 5°32'256.0"W), under a 16 hr light and 8 hr darkness regime at *circa* 12 °C. Water was exchanged on a weekly basis. All species were visually observed to display excellent survival and sustained growth during a cultivation period of three months. Similarly, *H. ranunculoides* was kept in an aerated aquarium with source water, which was supplemented *ad hoc* with de-chlorinated tap-water, at 14 °C under a 12:12 hr light-dark regime. All waste invasive plant material was destroyed by autoclaving.

Efficacy of Virkon® Aquatic and Virasure® Aquatic solutions

The efficacy of Virkon® Aquatic (Antec Int. DuPont) and Virasure® Aquatic (Fish Vet Group) was examined using 2% (20 g L⁻¹), 4% (40 g L⁻¹) disinfectant solutions, and a 0% (0 g L⁻¹) control. In all cases, submergent apical fragments were harvested from mature plants, and cut from unbranched sections of stem. Based on available plant material, an arbitrary fragment length of 100 mm was chosen. However, in the case of *H. ranunculoides* emergent apical fragments, of length 160 mm, were used. All fragments were harvested as required and briefly maintained (< thirty minutes) in de-chlorinated tap-water (*circa* 6–8 °C) prior to experimental use. Plant fragments were randomly selected from these holding aquaria and excess liquid was gently removed by manually spinning individual fragments, ten times in both directions, using a handheld centrifugal spinner. Fragment weight was recorded (Table 2).

Fragmentary propagules of each species were then independently submerged in 2% and 4% solutions of Virkon® Aquatic or Virasure® Aquatic for a period of five, fifteen or thirty minutes, and in the case of *H. ranunculoides*, due to the use of potentially more robust emergent apical fragments, sixty minutes. All treatment combinations were replicated in triplicate, i.e. *n* = 3. All solutions were made using dechlorinated tap-water. Control groups were likewise submerged in dechlorinated tap water (i.e. a 0% solution) for the same exposure times. Post-exposure, all samples were

Table 3. Degradation scale describing visual tissue biodegradation stages and/or resumption of growth for aquatic macrophyte fragmentary propagules (see Crane et al. 2019). Colour codes relate to the graphical representation of results in Figures 1 and 2.

Score	Description	Colour code
10	Complete degradation.	
9	No new shoot and/or root growth present with more than or equal to 90% stem degradation.	
8	No new shoot and/or root growth present with more than or equal to 50% stem degradation.	
7	No new shoot and/or root growth present with all leaved exhibiting paling or browning.	
6	No new shoot and/or root growth present with paling or browning affecting any leaves.	
5	No new shoot and/or root growth present with degradation at fragmentation site.	
4	New shoot and/or root growth present with more than or equal to 90% stem degradation.	
3	New shoot and/or root growth present with more than or equal to 50% stem degradation.	
2	New shoot and/or root growth present with all leaves exhibiting paling or browning.	
1	New shoot and/or root growth present with paling or browning affecting any leaves.	
0	New shoot and/or root growth present with degradation at fragmentation site.	

submerged in dechlorinated water and gently washed clean for a two minute period; this was repeated twice (see Cuthbert et al. 2019a). All fragments were then immediately placed within individual plastic magenta vessels containing *circa* 300 ml of locally sourced pond water or dechlorinated water in the case of *H. ranunculoides*. Excepting *H. ranunculoides*, the fragmentary propagules were then housed under standard growth conditions of 18 °C, with 16:8 hr light-dark regime. *H. ranunculoides* was kept at 14 °C under a 12:12 hr light-dark regime. In all cases, water loss due to evaporation was replenished as required.

Fragmentary tissue degradation and retention of viability, as evidenced by the presence of new shoot or root growth, was assessed at 7, 14, 21 and 28 days following exposure to disinfectants, excepting *H. ranunculoides*, which was examined at 2, 7 and 21 days. To accomplish this, the novel degradation scale described by Crane et al. (2019; see Table 3) was used. The scale is comprised of eleven distinct categories (0–10, inclusive). The categories are designed to allow simple visual assessment of fragment viability and degradation (score 0–4), fragment survival, whereby no meaningful degradation or indication of viability has occurred (score 5), and various levels of degradation without evidence of viability shown (score 6–10). In addition, to assess comparable differences in fragment viability, a count of new shoots and roots, and a measurement of new shoot lengths were recorded for *C. helmsii*, *E. densa*, *E. canadensis* and *L. major* upon completion of the experiment.

Statistical methods

All statistical analyses were performed using R version 3.4.4 (R Core Team 2018). Using the degradation scale (Table 3), we examined the visually-scored survivability and viability of plants using a mixed ordinal regression model facilitated through the *ordinal* package in R (Christensen 2018). The proportional odds assumption was satisfied. Plant tissue degradation scores were modelled with respect to *treatment* (5 levels: control, two

aquatic disinfectants and two concentrations), *exposure time* (3 levels), *plant species* (4 levels) and *observation period* (4 levels). We integrated a random effects structure to account for repeated measures over the observation period ($n = 4$). *Hydrocotyle ranunculoides* degradation was analysed individually owing to its separate assessment and difference in predictor variable levelling, with respect to *treatment* (5 levels), *exposure time* (4 levels) and *observation period* (3 levels).

Counts of root and shoot growth were analysed separately using generalised linear models (GLMs) assuming a Poisson error distribution for all plants, excepting *H. ranunculoides*. Where residuals were found to be overdispersed relative to degrees of freedom, a quasi-Poisson family was employed. Root and shoot growth were modelled with respect to *treatment* (5 levels), *exposure time* (3 levels) and *plant species* (4 levels).

Viability of plants in relation to the length of new shoot growth, except *H. ranunculoides*, was analysed using beta regression with the *betareg* package in *R* (Cribari-Neto and Zeileis 2010), whereby relative growth rate (RGR; following Van Echelpoel 2016) was modelled with respect to *treatment* (5 levels), *exposure time* (3 levels), and *plant species* (4 levels). Data were transformed to adjust zero values in the dataset, where no resumption of growth occurred, in order to meet model assumptions:

$$y_t = (y(n - 1) + 0.5)/n \quad (1)$$

where y_t is the transformed output and n is the sample size. In all models, backward stepwise deletion was performed in order to satisfy the minimum adequate model, where non-significant terms and interactions were excluded *via* analysis of deviance (Crawley 2013). Tukey's comparisons were used for *post hoc* analyses using *lsmeans* (Lenth 2016).

Results

For *C. helmsii*, *E. densa*, *E. canadensis* and *L. major*, fragmentary propagules displayed viability post-treatment with both Virkon® Aquatic and Virasure® Aquatic (Figure 1). Complete degradation was only exhibited by *E. densa* fragmentary propagules, particularly where exposed to the highest concentration solutions for the maximum exposure duration (4% solutions for fifteen or thirty minutes). However, although sustained viability, as evidenced by shoot or root regrowth, was demonstrated by *C. helmsii*, *E. canadensis* and *L. major*, scaled degradation significantly increased with treatment ($\chi^2 = 163.53$, $df = 4$, $P < 0.001$). Moreover, treated plants exhibited significantly greater degradation than control groups (0% solutions), irrespective of disinfectant concentration (all $P < 0.001$). Overall, treatment at 4% concentrations of Virkon® Aquatic or Virasure® Aquatic induced significantly greater degradation than 2% concentrations (all $P < 0.01$), and there was no difference between the two products at matched

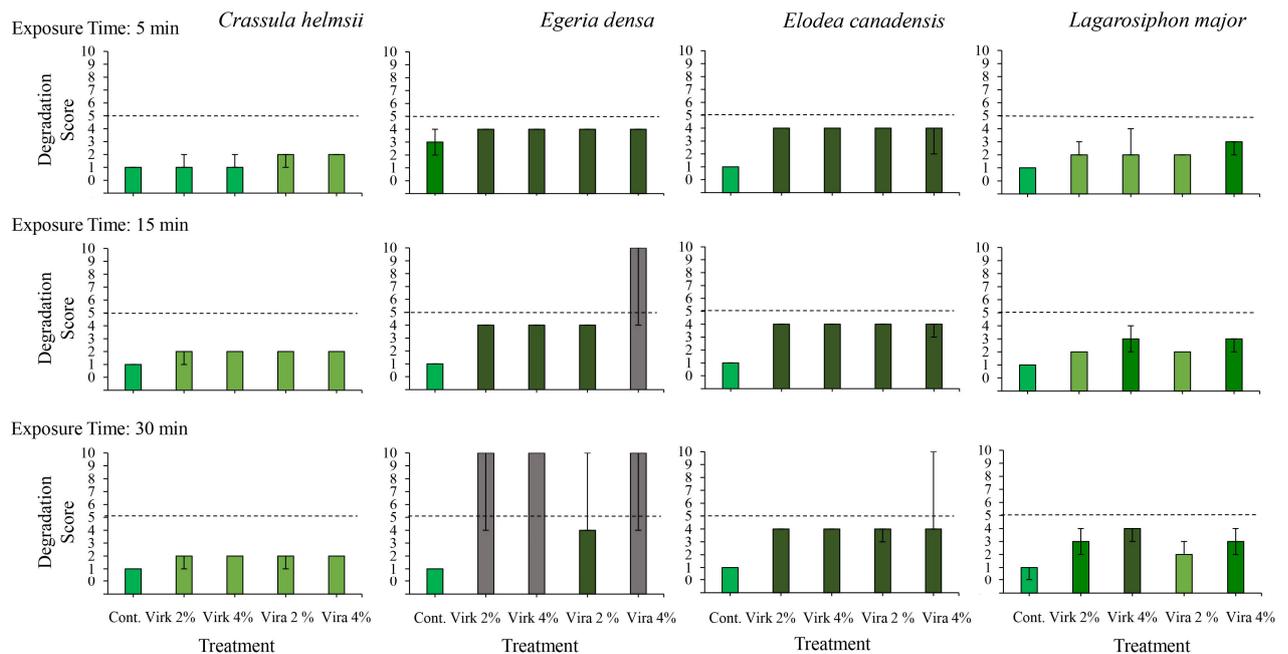


Figure 1. Median degradation score depicting visual biodegradation stages and/or resumption of growth for four different species of macrophyte fragmentary propagules at 28 days post exposure to aquatic disinfectants, for 0% (0 g L^{-1}), 2% (20 g L^{-1}) and 4% (40 g L^{-1}) solutions of selected aquatic disinfectants. Fragments were submerged for five, fifteen or thirty minutes ($n = 3$ per treatment). Bars signify minimum and maximum scores attained. The dashed line highlights a score of 5, which indicates no meaningful deterioration of the plant tissues or resumption of growth has occurred. Scores of 0–4 portray incremental levels of degradation, while noting the presence of sustained viability. Scores of 6–10 denote plant tissue degradation stages that lack viability in relation to the resumption of new growth. See Table 3 for description of the score categories. Cont. = Control; Virk = Virkon[®] Aquatic; Vira = Virasure[®] Aquatic.

concentrations (all $P > 0.05$). However, viability was not inhibited by disinfectant treatments in the majority of cases (see Table 3; Figure 1).

Scaled degradation following treatment was significantly different among *C. helmsii*, *E. densa*, *E. canadensis* and *L. major* ($\chi^2 = 188.99$, $df = 4$, $P < 0.001$). In particular, *E. densa* exhibited significantly greater degradation than any other plant species (all $P < 0.001$). In turn, *E. canadensis* was significantly more degraded than *L. major* ($P < 0.001$), whilst *C. helmsii* was significantly less degraded than either *E. canadensis* or *L. major* (both $P < 0.001$). Overall, greater exposure times increased degradation ($\chi^2 = 36.51$, $df = 2$, $P < 0.001$), with degradation between each examined incremental exposure time increasing significantly (all $P < 0.01$). Degradation also significantly increased over the duration of the monitoring period ($\chi^2 = 56.30$, $df = 3$, $P < 0.001$). Furthermore, a significant “treatment \times species \times time” interaction effect ($\chi^2 = 82.54$, $df = 24$, $P < 0.001$) was recorded. This reflected various interactive complexities, such as high occurrence of total fragmentary propagule degradation (score of 10) exhibited by *E. densa* at 4% disinfectant treatments with an exposure of fifteen minutes or longer. All other plant fragmentary propagules demonstrated consistent survival and viability through resumption of growth over the observation period.

For *H. ranunculoides*, although complete degradation was not achieved following any treatment, the use of aquatic disinfectant significantly increased plant degradation overall ($\chi^2 = 91.04$, $df = 4$, $P < 0.001$; Figure 2).

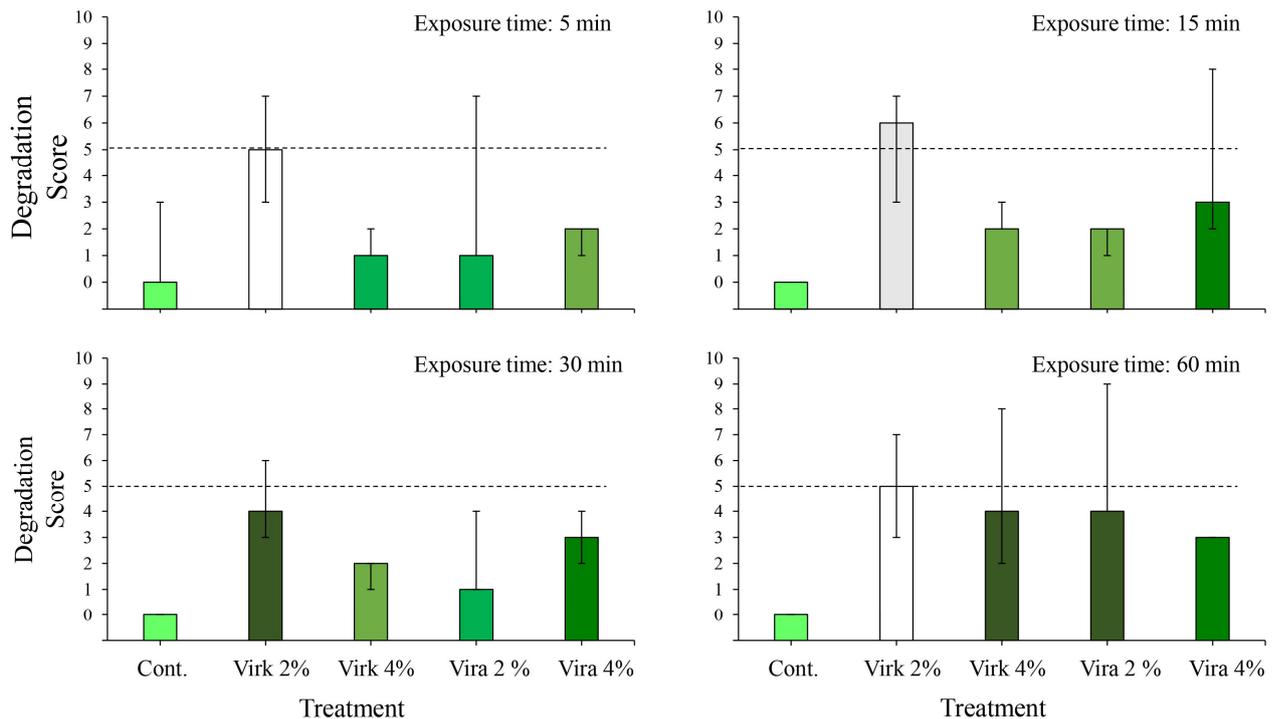
Hydrocotyle ranunculoides


Figure 2. Median degradation score depicting visual biodegradation stages and/or resumption of growth for fragmentary propagules of *Hydrocotyle ranunculoides* at 21 days post exposure to aquatic disinfectants, for 0% (0 g L⁻¹), 2% (20 g L⁻¹) and 4% (40 g L⁻¹) solutions of selected aquatic disinfectants. Fragments were submerged for five, fifteen, thirty or sixty minutes ($n = 3$ per treatment). Bars signify minimum and maximum scores attained. The dashed line highlights a score of 5, whereby no meaningful deterioration of the plant tissues or resumption of growth has occurred. Scores of 0–4 portray incremental levels of degradation, while noting the presence of sustained viability. Scores of 6–10 denote plant tissue degradation stages which lack of viability in relation to the resumption of new growth. See Table 3 for description of the score categories. Cont. = Control; Virk = Virkon[®] Aquatic; Vira = Virasure[®] Aquatic.

All disinfectant treatments significantly increased degradation in comparison to control groups (all $P < 0.001$). Although treatment with disinfectant frequently inhibited new growth of *H. ranunculoides*, survival was often evidenced through sustained stem vitality (Table 3). Degradation was significantly influenced by treatment exposure time ($\chi^2 = 16.55$, $df = 3$, $P < 0.001$); sixty minute exposures yielded significantly greater degradation than all other exposure times (all $P < 0.05$), whilst there were no significant differences between shorter exposure times (all $P > 0.05$). Degradation of *H. ranunculoides* also differed significantly over the course of the monitoring period ($\chi^2 = 128.82$, $df = 2$, $P < 0.001$), demonstrating recovery *via* reduced degradation scoring of treated plants over the monitoring period overall (all $P < 0.001$).

Viability in relation to shoot and root counts was also exhibited by all plant species irrespective of treatment and exposure time. Root counts were significantly affected by disinfectant treatments ($F_{4, 175} = 6.52$, $P < 0.001$), and differed significantly between plant species ($F_{3, 172} = 115.39$, $P < 0.001$; Figure 3). *Crassula helmsii* displayed the greatest root production between the four focal plants. Contrastingly, only one *E. densa* replicate exhibited root regrowth following treatment for fifteen minutes or longer. Root

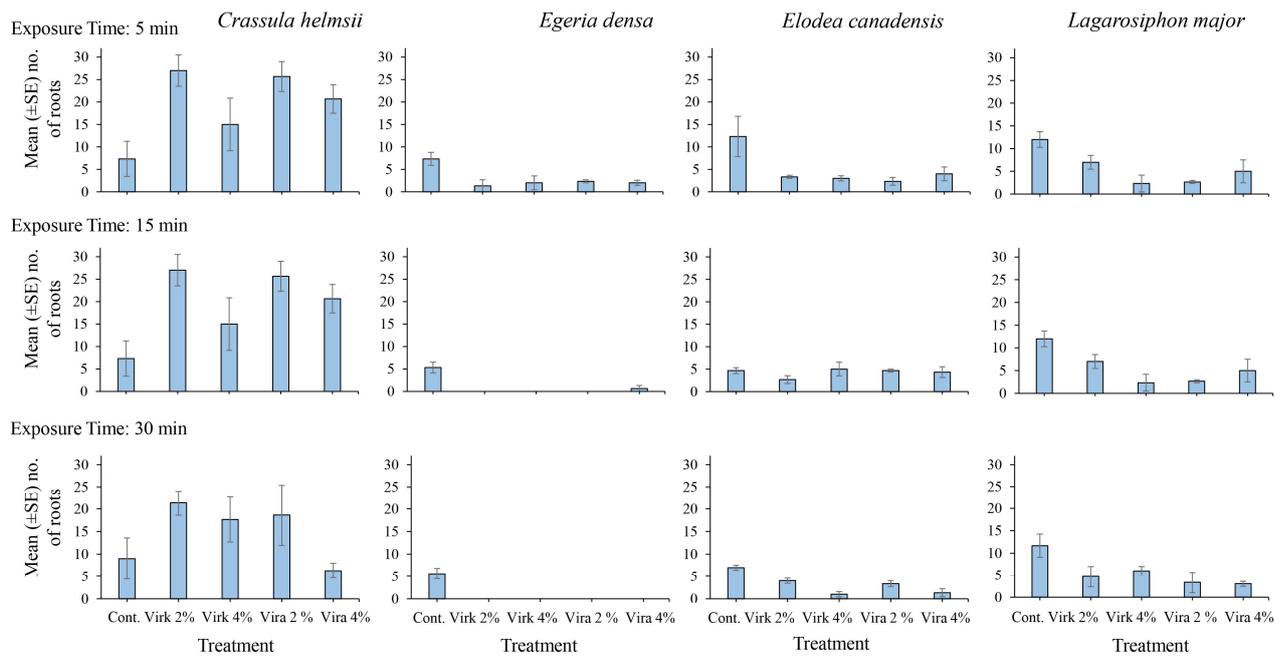


Figure 3. Mean (\pm SE) count of new roots for macrophyte fragmentary propagules at 28 days post exposure to aquatic disinfectants, for 0% (0 g L^{-1}), 2% (20 g L^{-1}) and 4% (40 g L^{-1}) solutions of selected aquatic disinfectants. Fragments were submerged for five, fifteen or thirty minutes ($n = 3$ per treatment). Cont. = Control; Virk = Virkon[®] Aquatic; Vira = Virasure[®] Aquatic.

generation was also significantly lower following longer exposures times ($F_{2, 170} = 7.96$, $P < 0.001$). In addition, there was a significant “treatment \times species \times time” interaction effect ($F_{24, 120} = 1.65$, $P < 0.05$). This reflected significant variation between plants amongst disinfectant treatment groups, whilst root counts across control plant species did not significantly differ following five and thirty minute exposures (all $P > 0.05$).

Overall, new shoot counts were not significantly affected by disinfectant treatment ($\chi^2 = 3.26$, $df = 4$, $P > 0.05$). However, new shoot counts differed significantly between plant species ($\chi^2 = 159.77$, $df = 3$, $P < 0.001$), with *C. helmsii* and *E. canadensis* producing the greatest number of new shoots of the four focal plants (all $P < 0.001$; Figure 4). Moreover, exposure time did not significantly influence shoot production ($\chi^2 = 4.14$, $df = 2$, $P > 0.05$). However, there was a significant “treatment \times species” interaction effect ($\chi^2 = 38.91$, $df = 12$, $P < 0.001$), wherein the higher number of new shoots produced by *C. helmsii* was conditional to the disinfectant treatment. Specifically, although *C. helmsii* produced significantly more new shoots than *L. major* or *E. densa* under all disinfectant treatments (all $P < 0.05$), there were no significant differences compared to *E. densa* or *L. major* in control groups (both $P > 0.05$). On the other hand, new shoot growth for *C. helmsii* and *E. canadensis* did not significantly differ under any disinfectant treatment (all $P > 0.05$), but *E. canadensis* growth was significantly greater than *C. helmsii* in controls ($P < 0.01$). Further, new shoot growth of *E. canadensis* was significantly greater than *L. major* in all treatments ($P < 0.05$), except the Virkon[®] Aquatic 4% treatment ($P > 0.05$).

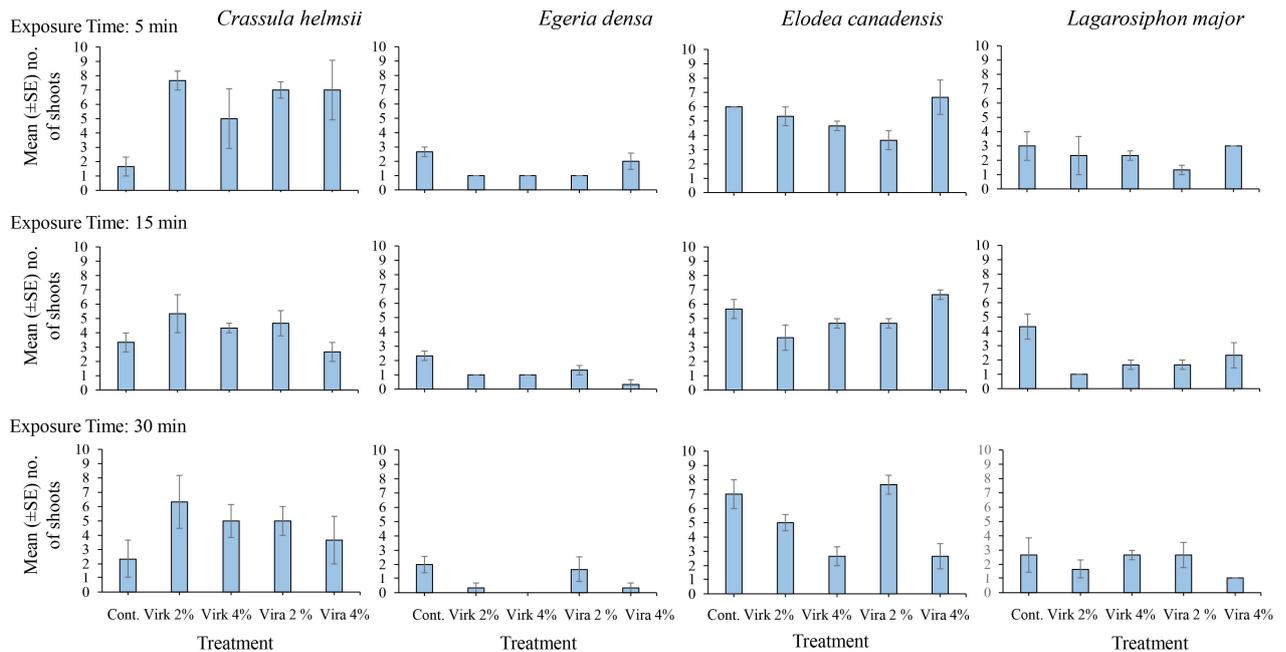


Figure 4. Mean (\pm SE) count of new shoots for macrophyte fragmentary propagules at 28 days post exposure to aquatic disinfectants, for 0% (0 g L^{-1}), 2% (20 g L^{-1}) and 4% (40 g L^{-1}) solutions of selected aquatic disinfectants. Fragments were submerged for five, fifteen or thirty minutes ($n = 3$ per treatment). Cont. = Control; Virk = Virkon[®] Aquatic; Vira = Virasure[®] Aquatic.

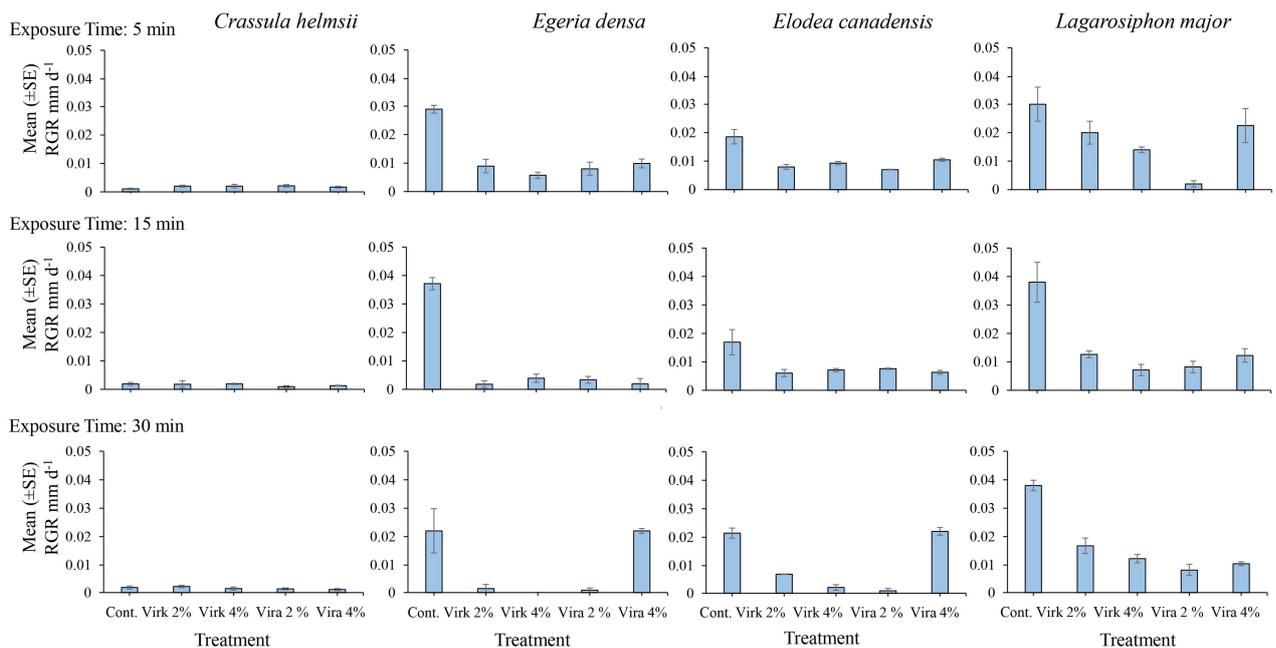


Figure 5. Mean (\pm SE) relative growth rate for new shoot growth produced by macrophyte fragmentary propagules at 28 days post exposure to aquatic disinfectants, for 0% (0 g L^{-1}), 2% (20 g L^{-1}) and 4% (40 g L^{-1}) solutions of selected aquatic disinfectants. Fragments were submerged for five, fifteen or thirty minutes ($n = 3$ per treatment). Cont. = Control; Virk = Virkon[®] Aquatic; Vira = Virasure[®] Aquatic.

New shoot RGR was significantly reduced by treatment ($F_{4, 119} = 9.74$, $P < 0.001$; Figure 5), reflecting lower regrowth across all disinfectant treated plant species relative to the controls (all $P < 0.001$). Yet, there were no significant overall differences in shoot RGR amongst disinfectant treatments, regardless of concentration (all $P > 0.05$). Relative growth rates also differed significantly between species overall ($F_{3, 119} = 22.58$, $P < 0.001$),

wherein *C. helmsii* displayed significantly lower RGR of new shoots than all other species (all $P < 0.001$), and *L. major* exhibited greater regrowth than *E. canadensis* or *E. densa* (both $P < 0.01$). However, the lesser regrowth of *C. helmsii* was only consistently significant compared to all other plants across control groups (all $P < 0.01$), and was more similar under disinfectant treatments, driving a significant “treatment \times species” interaction ($F_{12, 119} = 2.47$, $P < 0.01$). Relative growth rates of new shoots were not significantly affected by exposure time ($F_{3, 119} = 2.16$, $P > 0.05$).

Discussion

In the present study, the ability of two aquatic disinfectants, Virkon® Aquatic and Virasure® Aquatic, to induce plant tissue degradation, decrease the number of new roots and shoots produced, and reduce the RGR of new shoots, was examined for fragmentary propagules of selected prolific invasive macrophytes. Although 2% and 4% solutions of both aquatic disinfectants induced substantial degradation of the original fragmentary propagule, all species retained viability in relation to shoot and/or root regrowth, even following submergence in 4% solutions for exposure times of thirty minutes or longer. Although 1% solution of Virasure® Aquatic has previously been observed to induce complete degradation and prohibit viability of *L. major* fragmentary propagules following a two minute submergence (Cuthbert et al. 2018), the present study indicates that the examined broad-spectrum aquatic disinfectants will not be capable of curtailing its resumption of growth.

Similarly, Cuthbert et al. (2019a) observed that, whilst different propagule stages of *Elodea nuttallii* (Planchon) H. St. John, 1920 (i.e. apical tip and mid-stem) displayed substantial and sustained degradation following a five minute submergence in 1% and 4% solutions of both Virkon® Aquatic and Virasure® Aquatic, all *E. nuttallii* fragments subsequently demonstrated viability through resumption of shoot or root growth. Interestingly, complete necrosis of *L. major* was recorded by Cuthbert et al. (2018) for fragmentary propagules of a smaller size (50 mm) than those examined by the present study (100 mm). Although both studies examined fragment lengths of a size range considered capable of surviving overland transport (Barrat-Segretain et al. 1998; Coughlan et al. 2018a), larger fragments are known to display a greater capacity for retention of viability (Jiang et al. 2009; Hoffmann et al. 2015). Therefore, this may have enabled resumption of growth by the longer fragmentary propagules examined by this study. Although the examined oxidising agent-based disinfectants adversely impacted treated fragments, it appears that only outer cell integrity was negatively affected in most cases, with regrowth being produced from meristematic cells.

Morphological and physiological differences among the examined species may have influenced resumption of growth following exposure to aquatic disinfectant treatments. For example, *E. densa* was noticeably less

robust than the other examined species, which all displayed more rigid stem and leaf structures. These morphological differences could explain the lower levels of degradation shown by fragments of *C. helmsii*, *E. canadensis*, *H. ranunculoides* and *L. major*. Although only submerged fragments of *C. helmsii* were used in this study, this species is capable of producing emergent stems. These emergent shoots are more robust than the submerged leaves and have a thicker outer cuticle. As such, this ability may explain the greater numbers of new roots and shoots produced by *C. helmsii*. Conversely, this greater investment in the number of new growth structures produced coincided with a lower overall RGR for shoots. Although the number of new growth structures produced may increase the likelihood of establishing a new plant, beyond the indicators of viability documented in this study, a further assessment of subsequent colonisation abilities is still required. Interestingly, while apical fragments of *H. ranunculoides* were less structurally rigid than those of *C. helmsii*, *E. canadensis*, and *L. major*, *H. ranunculoides* generally displayed lower degradation scores than *E. canadensis*. As apical fragments of *H. ranunculoides* are emergent, while those produced by *E. canadensis* are submerged, the observed rates of degradation may reflect additional morphological (e.g. permeability of cuticle layer) or physiological-related differences between these species.

As discussed by Cuthbert et al. (2019a), abiotic conditions such as nutrient-rich pond water may promote sustained fragment viability in comparison to dechlorinated tap-water. This may, in part, explain why more substantial degradation of *L. major* was observed by Cuthbert et al. (2018), and demonstrates important context-dependencies which may influence the efficacy of biosecurity protocols. However, this phenomenon was not observed in the present study in relation to the placement of *H. ranunculoides* in dechlorinated tap-water following chemical exposure. In addition, a more favourable light intensity of 200–250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and warmer temperature conditions (18 °C) used by Cuthbert et al. (2019a), and for the present study, may have promoted greater resumption of growth by certain macrophytes. Accordingly, further examination of the effect of biotic and abiotic conditions on the survival and resumption of growth of different sized fragmentary propagules, following exposure to broad-spectrum aquatic disinfectants, is needed.

Although higher disinfectant concentrations and extended exposures times may result in a further reduction of fragment viability, an observed lack of disinfectant solubility beyond a 5% solution may inhibit achievement of these increased concentrations in the field (Cuthbert et al. 2019a). Moreover, such solutions may also potentially represent an environmental or user health concern, as the highest concentration of a 4% solutions of Virasure® Aquatic is only recommended for thermal fogging purposes by the manufacturer (Cuthbert et al. 2019a). Currently, the use of 1% solutions are recommend by both manufactures for surface disinfection

and submergence treatments, such as footbaths. However, solutions at double and quadruple this recommendation did not necessarily inhibit the production of new growth following maximum exposure of thirty or sixty minutes. In addition, as biosecurity applications should be non-time-consuming to encourage maximum participation (Sutcliffe et al. 2018), exposure times beyond thirty minutes may not be practical.

Nevertheless, broad-spectrum aquatic disinfectants, which are widely used within aquaculture, may still provide an effective umbrella decontamination treatment for multiple taxonomic groups, such as invertebrates, bacterial, fungal and viral pathogens (Barbour et al. 2013; Cuthbert et al. 2019b). However, the susceptibility of all IAS transportable life history stages to biosecurity treatments requires further examination. Yet, if a treatment can induce complete invader mortality at its most robust life stage, it will also likely do so at more vulnerable growth phases (Coughlan et al. 2019a). Despite the clear need for further assessment, the use of disinfectants as part of biosecurity practices, which incorporate the procedural steps of visual inspection and the physical removal of any adhering organisms, may synergistically provide improved decontamination of equipment (Cuthbert et al. 2019a; Joyce et al. 2019), particularly as plant material will often become noticeably entangled around equipment as a large clump (e.g. nets, outboard motors and boat anchors). In such scenarios, chemical applications alone will not be capable of causing mortality of adhering plants, which will need to be physically removed. However, as other damaging invaders can contaminate equipment and even plant material, e.g. invertebrates and pathogens, chemical decontamination procedures will be an advantageous edition to spread-prevention strategies. Moreover, as small fragmentary propagules or a single invertebrate or pathogen can be exceedingly difficult to visually detect, but sufficient to establish new invader populations (Bickel 2015; Coughlan et al. 2017c), further examination of the effectiveness of broad-spectrum aquatic disinfectants to achieve complete decontamination of equipment is required. In particular, the use of chemical disinfectants in combination with other approaches merits consideration. For example, the efficacy of disinfection treatments could possibly be improved by incorporating additional cleaning protocols, such as immersion in hot water (≥ 45 °C; Anderson et al. 2015), direct steam exposure (Coughlan et al. 2019a; Crane et al. 2019; Joyce et al. 2019) and extended drying times (Coughlan et al. 2018a; Shannon et al. 2018). In doing so, future evaluation of alternative treatments should provide for more effective and practical decontaminating procedures. Further, the synergistic effects of various applications could possibly provide for greater efficacy.

Despite a lack of common approaches to biosecurity, spread-prevention of invaders has become integral to both national and international IAS management strategies (Caffrey et al. 2014; Piria et al. 2017). Yet, although

national and international legislation concerning spread-prevention has been implemented (e.g. EU Regulation 1143/2014) invaders continuing to spread at an unprecedented rate, even between unconnected waterbodies (Caffrey et al. 2016; Seebens et al. 2017). Therefore, innovative biosecurity protocols that maximise prevention of further invader spread remain an urgent priority to protect biodiversity in aquatic systems (Crane et al. 2019; Cuthbert et al. 2019b; Joyce et al. 2019). Ideally, in order to engage maximum participation by all water users, biosecurity protocols should, *inter alia*, utilise materials that are readily available, be relatively easily applied with no specialist training required, be non-time-consuming, inexpensive, and environmentally friendly (Stebbing et al. 2011; Anderson et al. 2015; Coughlan et al. 2018a, 2019a; Crane et al. 2019; Sutcliffe et al. 2018). Although disinfectants, such as Virkon® Aquatic and Virasure® Aquatic, have been developed to induce mortality of bacterial, fungal and viral pathogens, confirmation of their ability to kill damaging parasites, such as the invasive salmon fluke *Gyrodactylus salaris*, under in-field conditions is required (Tidbury et al. 2018; Cuthbert et al. 2019a). Finally, whilst the findings presented here indicate that aquatic disinfectants can induce substantial degradation of fragmentary propagules, confirmation of the concentrations and exposure times needed to prevent resumption of fragment growth is still required.

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Author contributions

KC and NEC proposed the study; KC, NEC and RNC designed the experiment; KC, NEC, RNC, EMC and SJB conducted the experiment; LE, ERCS and CS provided substantial technical support; RNC performed data analysis; all authors contributed to the interpretation of results and the writing of the manuscript, which was led by NEC.

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