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Die Hard: impact of aquatic disinfectants on the survival and viability of invasive *Elodea nuttallii*

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ABSTRACT

Aquatic invasive species (AIS) continue to adversely influence freshwater ecosystems worldwide. Management protocols designed to prevent further invader spread are essential, as control and eradication of established AIS populations are often complex, costly, resource-intensive, and can be relatively ineffective. Therefore, in-field biosecurity techniques designed to deliver effective decontamination of water users' equipment, e.g. angler's nets, footwear, and kayaks, are needed. Disinfection through brief submergence in chemical solutions may be beneficial. Although broad-spectrum, aquatic disinfectants have been recommended as suitable biosecurity agents, the ability of these chemicals to inhibit invader spread remains poorly understood. Here, we examined the effectiveness of two aquatic disinfectants, Virasure® Aquatic and Virkon® Aquatic, to reduce growth rates, induce biodegradation, and decrease shoot and root production at the fragmentary propagule stage of the prolific invasive macrophyte, *Elodea nuttallii* (Planchon) H. St. John, 1920. We examined the efficacy of both chemicals at submergence times of one, two and five minutes, using 0% (0 g L^{-1}), 1% (10 g L^{-1}) and 4% (40 g L^{-1}) disinfectant solutions. Both apical and mid-stem fragmentary sections were examined separately. A biodegradation scale was applied to visually assess tissue degradation and/or resumption of growth. Although *E. nuttallii* displayed substantial and sustained degradation after all disinfection treatments, all fragments demonstrated viability through resumption of shoot or root growth over the observation period. Therefore, at the examined concentrations and exposure times, it appears that these broad-spectrum aquatic disinfectants are not capable of curtailing the spread of invasive *E. nuttallii*. However, longer submergence times, multiple applications and synergistic effects of different biosecurity treatments could potentially prevent further *E. nuttallii* spread and this requires investigation.

1. Introduction

Aquatic invasive species (AIS) represent a serious threat to the biodiversity, ecological functioning, economic and social value of freshwater ecosystems worldwide (Caffrey et al., 2014; Piria et al., 2017). In particular, invasive macrophytes are known to negatively affect the physical, chemical and biological processes of freshwater ecosystems (Hussner, 2014; Kuehne et al., 2016). Moreover, invasive macrophytes are often a substantial economic and management burden, as large mono- or polyspecific swards can escalate flood frequencies, precipitate water quality deterioration, reduce invertebrate and fish diversity, and inhibit recreational and commercial activities (Caffrey et al., 2011; Schultz and Dibble, 2012; Hussner et al., 2017).

In many instances, management options for effective suppression and eradication of established AIS populations are complex, costly, resource-intensive and damaging to non-target species (Caffrey et al., 2011; Piria et al., 2017; Coughlan et al., 2018c). Therefore, spread-prevention is considered key to mitigating further invader impacts (Booy et al., 2017; Coughlan et al., 2018b; Crane et al., 2018; Cuthbert et al., 2018). However, due to their exposure to a plethora of natural

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and anthropogenic vectors, freshwater systems remain highly susceptible to invader spread, colonisation, establishment and reinvasion (Rothlisberger et al., 2010; Banha et al., 2017; Coughlan et al., 2017a,c). Accordingly, there is an urgent need for innovative and simple prevention protocols that maximise inhibition of invader spread, but remain user and environmentally friendly (Sutcliffe et al., 2018; Coughlan et al., 2018c; Crane et al., 2018; Shannon et al., 2018). Moreover, spread-prevention tactics must balance efficacy with cost, legislative barriers and non-target effects (Cuthbert et al., 2018).

Currently, although control measures to suppress invader populations can be successful (Hussner et al., 2014; Beric and MacIsaac, 2015; Caffrey et al., 2018), there often exists only a limited understanding of the relative efficacies of recommended spread-prevention techniques (Barbour et al., 2013; Piria et al., 2017; Coughlan et al., 2018b; Crane et al., 2018). In particular, while broad-spectrum aquatic disinfectants (Barbour et al., 2013; Cuthbert et al., 2018), desiccation (Coughlan et al., 2017b, 2018b), hot water (Anderson et al., 2015; Shannon et al., 2018), and steam applications (Crane et al., 2018) have been suggested as suitable mechanisms to limit AIS spread, species-specific susceptibility requires confirmation. Further, although aquatic disinfectants have proven successful against a variety of invasive Mollusca and macrophyte species (e.g. Barbour et al., 2013; Stockton-Fiti and Moffitt, 2017; Cuthbert et al., 2018), their impact on different life history stages is not always clear (Coughlan et al., 2018a).

Elodea nuttallii (Planchon) H. St. John, 1920, is a submerged invasive macrophyte originating from North America (Cook and Urmi-König, 1985; Champion et al., 2010). Invasive in Europe, Asia, Australia and certain areas of North America, this rooted perennial aquatic macrophyte typically inhabits lakes, ponds and slow-moving rivers (Champion et al., 2010; Schwoerer and Morton, 2018). *Elodea nuttallii* can tolerate a broad range of environmental conditions, hybridise with native counterparts, and can rapidly dominate invaded ecosystems through formation of dense monospecific stands (Cook and Urmi-König, 1985; Champion et al., 2010; Zehnsdorf et al., 2015; Thouvenot and Thiébaut, 2018). Although research suggests that *E. nuttallii* may be less detrimental to European wetlands than previously thought (see Kelly et al., 2015), population suppression to minimize disruption of navigation, flood risk, water extraction and recreational activities, remains a substantial and costly management concern (Hussner, 2012; Hussner et al., 2017). For example, in the North American State of Alaska, *Elodea* infestations are thought to cause an economic loss of circa $100 million per year to recreational floatplane pilots and commercial freshwater fisheries (Schwoerer and Morton, 2018). Moreover, *E. nuttallii* is listed as a European Union Invasive Alien Species of Union Concern (EU Regulation 1143/2014), and appropriate management actions to inhibit its further spread are a legal requirement.

Like many invasive macrophytes, *E. nuttallii* predominantly spreads by vegetative propagation, particularly via vegetative fragments, which have a high survival potential (Hoffmann et al., 2015; Coughlan et al., 2018b). Due to disturbance events, e.g. changes in water velocity, grazing by herbivorous animals and anthropogenic activity, in situ fragmentation of macrophytes such as *E. nuttallii* is a frequent occurrence (Riis et al., 2009; Bakker et al., 2016). Although fragments generally remain within the aquatic medium, fragmentary propagules are capable of surviving overland dispersal between hydrologically unconnected sites (Barnes et al., 2013; Coughlan et al., 2018b). However, different plant sections can display a differential capacity for regeneration, colonisation and rate of growth, which can be linked to factors such as fragment size and apical dominance (Cline, 1991; Riis et al., 2009). As the application of broad-spectrum aquatic disinfectants has previously successfully induced degradation of macrophyte fragmentary propagules (Cuthbert et al., 2018), decontamination of water users' equipment through brief disinfectant soaking exposures may help reduce further invader spread.

Aquatic disinfectants such as Virasure® Aquatic and Virkon® Aquatic are being increasingly used by recreational water users and responsible authorities, e.g. government agencies, for decontamination of equipment. However, little data concerning the efficacy of these oxidising agents to inhibit the spread of macrophtye propagules currently exists (Cuthbert et al., 2018). Here, we examine the efficacy of Virasure® Aquatic and Virkon® Aquatic at concentrations of 1% (10 g L^{-1}) and 4% (40 g L^{-1}), for submergence treatments at one, two and five minutes, to reduce growth rates, induce plant tissue biodegradation, and limit new shoot and root growth of both apical and mid-stem sections of *E. nuttallii*. Use of 1% solutions are recommend by both manufactures for surface disinfection and submergence treatments, e.g. footbaths. However, we have also arbitrarily chosen to quadruple this recommendation to assess any differential effects of increased concentration. In addition, to encourage maximum participation, biosecurity applications should be non-time-consuming (Sutcliffe et al., 2018). Therefore, we aim to assess the efficacy of relatively brief exposure times.

2. Methods

2.1. Sample collection and cultivation

Elodea nuttallii was collected in Lough Erne, Northern Ireland (NI: 54° 17′ 07.89″ N; 7° 32′ 52.61″ W), and transported in source water to Queen's Marine Laboratory (QML), Portaferry, NI. The plant stock was maintained in the laboratory in aerated aquaria, filled with locally sourced pond water (Lough Cowey: 54° 24' 41.79" N; 5° 32' 25.96" W). The stock was cultured at circa 13 °C under a 16 h light and 8 h darkness regime, with a light intensity of 200 – 250 μmol·m^{-2}·s^{-1}, supplied by cool white fluorescent lamps. Water was exchanged on a weekly basis. Prior to experimentation, *E. nuttallii* was allowed to acclimate to laboratory conditions for seven days, and displayed a high level of survival and sustained growth during an overall cultivation period of two months. All waste invasive plant material was destroyed by autoclaving.

2.2. Efficacy of Virasure® Aquatic and Virkon® Aquatic solutions

The efficacy of Virasure® Aquatic (Fish Vet Group) and Virkon® Aquatic (DuPont) was examined using 0% (0 g L^{-1}), 1% (10 g L^{-1}) and 4% (40 g L^{-1}) solutions made with dechlorinated tap water (n = 3 replicates per experimental group). Two differential fragmentary sections of *E. nuttallii*, apical and mid-stem, were individually examined. In both cases, fragments were standardised by a node count of ten. Mid-stem fragments were cut immediately above and below the final nodes; apical fragments were cut below their final node only. In all cases, apical fragments were harvested from mature, unbranched sections of plants. Initial fragment length was recorded, with a mean (± SE) apical fragment and mid-stem length of 6.2 ± 0.1 mm and 7.65 ± 0.1 mm, respectively. Fragments were harvested as required and briefly maintained (< 30 min) in dechlorinated tap water prior to experimental use.

Groups of three fragments, of a single section type, were utilised as an experimental unit. These groups were submerged in treatment solutions of Virasure® Aquatic or Virkon® Aquatic for a period of one, two or five minutes. Control groups were likewise submerged in dechlorinated tap water for the same exposure times. Post exposure, all samples were submerged in dechlorinated tap water and gently rinsed clean for a two-minute period to ensure loss of chemical. This process was repeated with a second cleaning station for a further two minutes. All fragments were immediately placed within individual cylindrical glass vessels, 200 mm H × 80 mm W, containing 250 ml of pond water. Following treatment, the standard conditions for fragmentary growth were 18 °C, with a 16:8 h light–dark regime at a light intensity of 200 – 250 μmol·m^{-2}·s^{-1}. Water loss due to evaporation was replenished as required.

Based on the biodegradation scale proposed by Cuthbert et al. (2018), a novel biodegradation scale was developed to monitor tissue degradation, fragment survival and subsequent viability, i.e.

Table 1
Biodegradation scale describing visual tissue degradation stages and/or resumption of growth for aquatic macrophyte fragmentary propagules (see Crane et al., 2018; Cuthbert et al., 2018).

Score	Description
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.

regeneration by production of new shoot or root growth (Table 1; Crane et al., 2018). The scale comprised 11 distinct score categories (0–10, inclusive) that allow for visual estimation of survivability alone (a score of 5), whereby the fragments display no degradation or resumption of growth, and both the retention (score 0–4) and lack (score 6–10) of viability in relation to various stages of tissue degradation (see Crane et al., 2018). Upon cessation, after 28 days, fragments were scored using the biodegradation scale. New shoot lengths, and a count of new shoots and roots, were recorded.

2.3. Statistical analyses

Statistical analyses were performed using R v3.4.2 (R Core Development Team, 2017). Relative growth rates (RGR) were analysed for new shoot lengths using ANOVA, as residuals were found to be normally distributed (Shapiro-Wilk test, $P > 0.05$) and homoscedastic (Bartlett's test, $P > 0.05$). Here, we calculated RGR using the total length of all new shoot growth displayed by every fragment within each triplicate replicate. RGR was averaged per day across the 28-day recovery period. Scaled degradation (Table 1) of *E. nuttallii* at the final observation point was analysed using proportional odds logistic regression as the parallel regression assumption was satisfied. We used the lowest scoring fragment (i.e. least degraded) of the triplicate within each replicate to derive individual data points for analysis. Then, raw regrowth counts of shoots and roots at the end of the observation period were analysed separately using generalised linear models (GLMs) assuming Poisson distributions of error and log links. Here, likelihood ratio tests (LRTs) were used to report the significance of factors to the dependent variables. Treatment (5 levels), exposure time (3 levels) and plant part (2 levels) were incorporated as explanatory variables in all models. We implemented second-order Akaike Information Criterion (AICc) rankings to select models, which minimised information loss. Post hoc tests were performed using Tukey's contrasts for significant effects in each model.

3. Results

All control plants exhibited high levels of survival and viability, evidenced through examination of their RGR alongside levels of plant tissue biodegradation, and sustained shoot and root growth in all instances (Figs. 1 and 2). RGR of new shoots differed significantly depending on treatment ($F_{4, 83} = 7.83$, $P < 0.001$). Treatment with either Virasure® Aquatic or Virkon® Aquatic at 4% concentrations significantly reduced growth rates compared to control groups (both $P < 0.001$; Fig. 1). Treatment with Virkon® Aquatic at 1% concentration was significantly more effective at reducing shoot growth compared to controls ($P = 0.04$), but a 1% solution of Virasure® Aquatic did not reduce growth compared to the control groups ($P = 0.25$). Exposure time also had a significant effect on overall shoot RGR ($F_{2, 83} = 4.18$, $P = 0.02$), wherein five minute exposures were significantly more effective than one minute exposures in reducing RGR of *E. nuttallii* ($P = 0.01$). Overall, there were no significant differences between one and two minute exposures to treatments ($P = 0.49$), nor two and five minute exposures ($P = 0.20$: Fig. 1). There was also no significant difference between apical and mid-stem sections of *E. nuttallii* for new shoot RGR ($F_{1, 82} = 0.61$, $P = 0.44$).

Although treatment with Virasure® Aquatic and Virkon® Aquatic significantly increased degradation of *E. nuttallii* ($\chi^2 = 37.28$, $df = 4$, $P < 0.001$: Fig. 1), all treated fragments demonstrated viability through resumption of shoot or root growth over the observation period (Fig. 2). This occurred despite consistent degradation to the original treated fragment. With the exception of exposure to 1% Virasure®, all disinfectant treatments, particularly 4% concentrations, displayed significantly greater degradation than control *E. nuttallii* groups (all $P < 0.05$). Overall, longer treatment exposure times significantly increased degradation of fragmentary propagules ($\chi^2 = 8.48$, $df = 2$, $P = 0.01$: Fig. 1). Whilst there were no significant differences between one and two minute exposures ($P = 1.00$), five minute exposures were significantly more effective than lower exposure durations (both $P = 0.02$). Furthermore, degradation was consistent between apical and mid-stem sections of the plant, as there was no significant effect of plant part on scaled degradation ($\chi^2 = 0.68$, $df = 1$, $P = 0.41$).

Shoot and root production was evidenced in all disinfectant-treated *E. nuttallii* groups (Fig. 2). However, treatment with Virasure® Aquatic or Virkon® Aquatic had a significant effect on shoot counts ($\chi^2 = 12.88$, $df = 4$, $P = 0.01$), with the 4% concentrations significantly reducing new shoot numbers compared to control groups (both $P = 0.03$). However, whilst exposure time had no significant effect on shoot counts overall ($\chi^2 = 4.06$, $df = 2$, $P = 0.13$), apical sections exhibited significantly higher numbers of shoot counts than mid-stem sections ($\chi^2 = 6.71$, $df = 1$, $P = 0.01$: Fig. 2). This effect was sustained across all treatment groups, as there was no significant 'treatment × plant part' interaction ($\chi^2 = 4.61$, $df = 4$, $P = 0.33$). Similarly, root counts were also significantly affected by disinfectant treatment ($\chi^2 = 46.33$, $df = 4$, $P < 0.001$), wherein all disinfectant-treated groups exhibited significantly lower root counts post-treatment than controls (all $P < 0.001$). As with shoots, significantly more roots were produced by apical *E. nuttallii* fragments than mid-stem sections ($\chi^2 = 12.17$, $df = 1$, $P < 0.001$). This effect was again sustained across all treatment groups as there was no significant 'treatment × plant part' interaction ($\chi^2 = 3.29$, $df = 4$, $P = 0.51$). On the other hand, exposure time to treatment had no significant effect on the generation of roots at the end of the observation period ($\chi^2 = 3.10$, $df = 2$, $P = 0.21$).

4. Discussion

Anthropogenic activities such as angling, boating, and the aquatic pet and ornamental plant trades have likely facilitated a substantial portion of damaging AIS introductions (Johnson et al., 2001; Rothlisberger et al., 2010; Banha et al., 2017; Dickey et al., 2018).

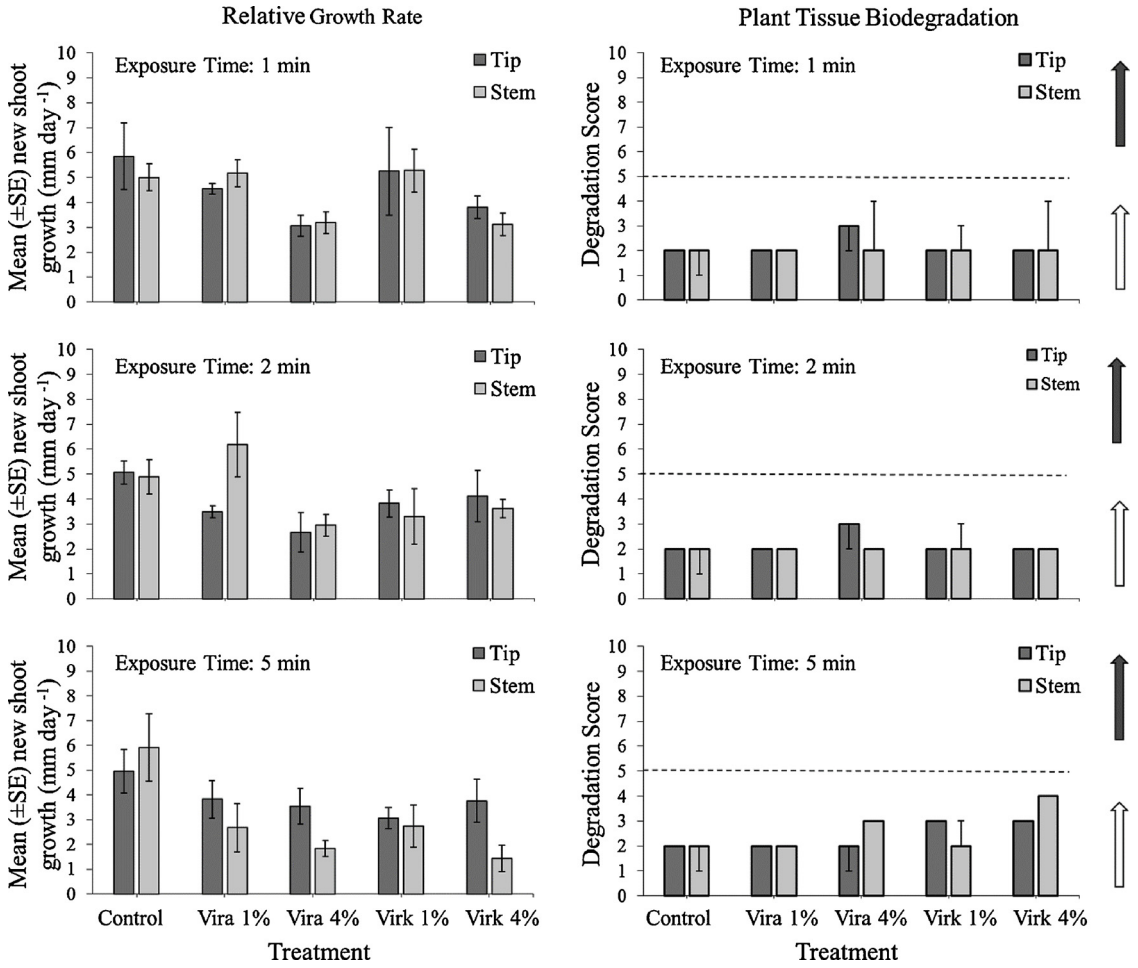

Fig. 1. Mean (± SE) relative growth rate and median Biodegradation Score (error bars signify minimum and maximum scores attained) for differential *Elodea nuttallii* fragmentary propagules, i.e. apical tip or mid-stem, following submersion in 0%, 1% or 4% solutions of selected aquatic disinfectants (n = 3). Fragments were submerged for one, two or five minutes. All plants were harvested after a recovery period of 28 days. Incremental degradation scores (see Table 1): 0–4 = increasing fragmentary degradation with viability displayed (white arrow); 5 = no degradation or viability displayed (dashed line); and 6–10 = increasing fragmentary degradation with no viability shown (grey arrow). Vira = Virasure® Aquatic; Virk = Virkon® Aquatic.

Accordingly, although the identity of vectors responsible for the dispersal of AIS are not always known (Caffrey et al., 2016; Coughlan et al., 2017c), spread-prevention through efficient, cost-effective and widely applicable biosecurity protocols has become integral to AIS management strategies (Anderson et al., 2014: Booy et al., 2017; Piria et al., 2017).

Previously, Cuthbert et al. (2018) observed that a two-minute submersion using 1% Virasure® Aquatic solution could achieve complete degradation of fragmentary propagules of *Lagarosiphon major* (Ridley) Moss, 1928, and argued that the chemical could effectively reduce the secondary spread of the species. As such, chemical treatment could therefore form an integral aspect of best-practice biosecurity protocols, through either soaking, mist spray or fogging of equipment, e.g. anglers' nets, kayaks, boats, water wells, vehicles and trailers. However, *L. major* may have been particularly susceptible to the acidity of the disinfectant as this species is more suited to and can even induce alkaline environments (Stiers et al., 2011). Although both aquatic disinfectants used in the present study induced substantial degradation of the original/parental *E. nuttallii* fragments, the examined concentrations and exposure times did not inhibit propagule viability, i.e. resumption of growth. Whilst higher treatment concentrations resulted in greater degradation of the fragmentary propagules, even the highest examined concentration of 4% failed to prevent resumption of shoot or root growth. Conversely, 4% concentrations of both disinfectants did reduce the number of new shoots, while all examined concentrations reduced new root production. Moreover, the growth rate of new shoots was restricted by 4% solutions of both disinfectants, especially at five minutes exposure. Ultimately, although the examined oxidising agent-based disinfectants adversely impacted treated fragments, it appears only outer cell integrity was negatively affected, with regrowth being produced from meristematic cells.

Higher concentrations and longer submergence times would likely result in substantial fragment degradation and inhibit subsequent viability. However, increased concentrations can be difficult to obtain, due to lack of chemical compound solubility beyond a 5% solution (RNC, KC and NEC per. obs.) and may represent an environmental or user health concern. In addition, longer submergence times may be impractical, especially in field scenarios. Currently, to prevent the spread of fish pathogens, the label information of both products endorse a 1% solution for footbaths and the surface disinfection of equipment. Additionally, 4% solutions of Virasure® Aquatic are recommended for thermal fogging purposes.

In the present study, we examined relatively large plant fragments as these are known to exhibit a greater capacity for regrowth (Jiang et al., 2009; Hoffmann et al., 2015; Coughlan et al., 2018b), but are within the threshold of propagules which can likely entangle with, and be transported overland by, anthropogenic vectors (Coughlan et al., 2018b). Moreover, the size range examined is thought to reduce inhibition of lateral growth driven through apical dominance (Cline, 1991). However, although the overall RGR of differential fragmentary

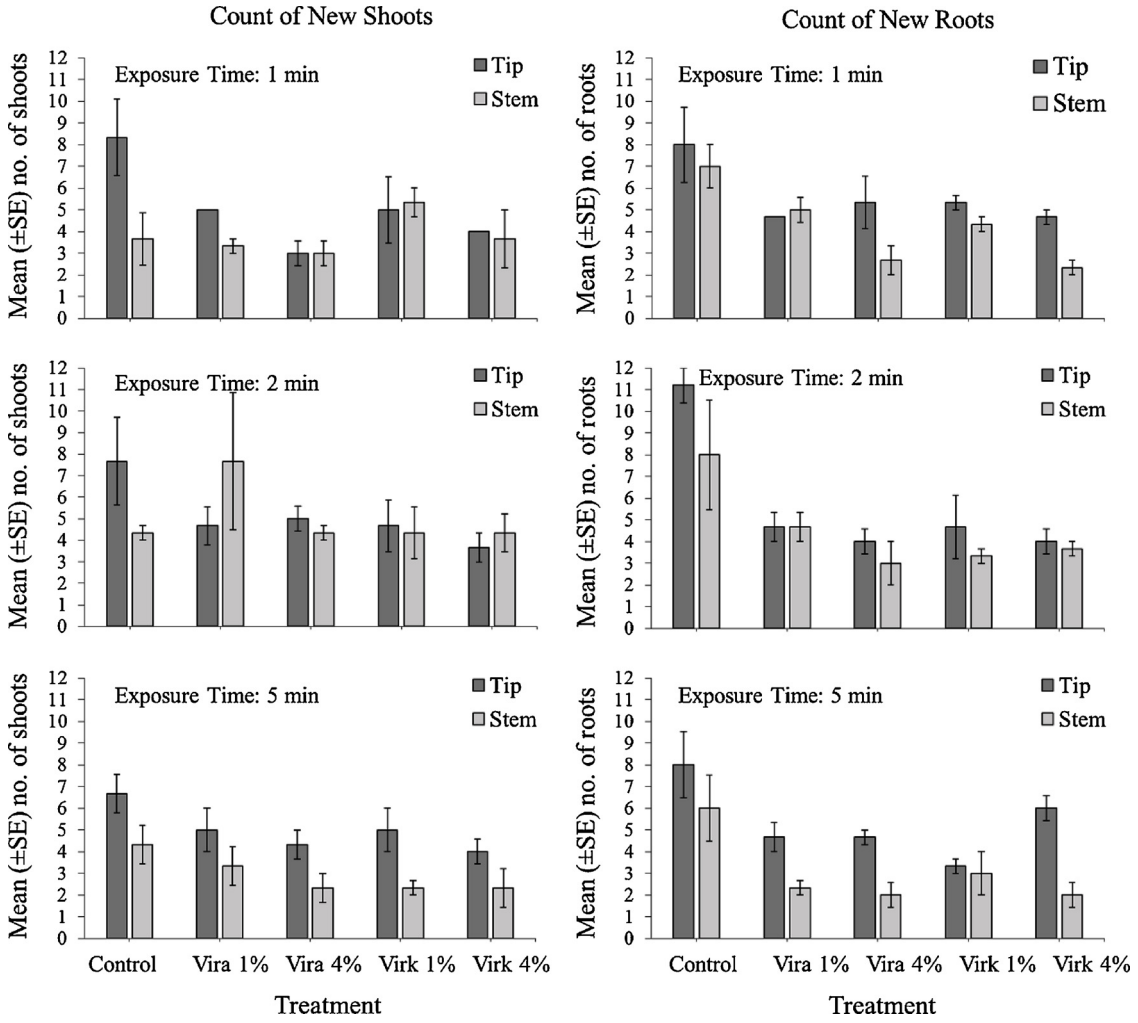

Fig. 2. Mean (± SE) count of new shoots and roots for differential *Elodea nuttallii* fragmentary propagules, i.e. apical tip or mid-stem, following submersion in 0%, 1% or 4% solutions of selected aquatic disinfectants (n = 3). Fragments were submerged for one, two or five minutes. All plants were harvested after a recovery period of 28 days. Vira = Virasure® Aquatic; Virk = Virkon® Aquatic.

sections did not differ, apical *E. nuttallii* fragments displayed greater numbers of both new shoots and roots, compared to mid-stem sections. This may indicate some retention of apical dominance. In contrast to the study performed by Cuthbert et al. (2018), *E. nuttallii* fragments were kept in pond water following exposure to the disinfectants rather than dechlorinated tap water. Although Cuthbert et al. (2018) did include substrate within their post treatment growth containers, the more nutrient-rich pond water may have benefited sustained viability in comparison to dechlorinated tap water. Moreover, the favourable light intensity of 200–250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and warmer temperature conditions (18 °C) used in the present study may have promoted resumption of growth by *E. nuttallii*. Species-specific characteristics and environmental variables such as nutrient availability and light intensity will influence macrophyte fragment survival and viability (Kuntz et al., 2014; Hoffmann et al., 2015). Therefore, deciphering the impacts of these additional environmental complexities on treatment viabilities is of particular importance given the range of environments in which AIS proliferate.

Spread-prevention campaigns, such as *Check, Clean, Dry* in New Zealand and Great Britain, aim to promote best-practice biosecurity protocols, designed to limit AIS spread, amongst water users (Anderson et al., 2014; Shannon et al., 2018). However, the overall efficacy of biosecurity treatments suggested within the wider literature can vary with inter- and intraspecific differences (Coughlan et al., 2018a; Crane et al., 2018). For example, while Virkon® Aquatic can induce circa 93% mortality of juvenile Asian clam, *Corbicula fluminea*, following a five-minute exposure, adults are largely resistant to aquatic disinfectants (Barbour et al., 2013; Coughlan et al., 2018a). Additional trials investigating the impacts of such disinfectant solutions on different AIS propagule stages, for existing and emerging invaders, should be considered (Cuthbert et al., 2018). However, if a treatment can induce complete invader mortality at its most robust life stage, it will also likely do so at more vulnerable life stages (Coughlan et al., 2018a). While risks of toxicity to other aquatic organisms via residues and spills is considered low with good practice (see Stockton-Fiti and Moffitt, 2017), additional assessments for potential non-target effects on native species, particularly macroinvertebrates, would be highly beneficial (Cuthbert et al., 2018).

Although physical removal of adhering organic material following visual inspection is undoubtedly beneficial, small organisms or propagules may not be observed, and therefore would require more thorough management protocols (Rothlisberger et al., 2010; Crane et al., 2018). Equally, while extended drying times can inhibit invader spread (Coughlan et al., 2018b), many water users rapidly and repeatedly move both short and long-distances between multiple freshwater sites (Anderson et al., 2014; De Ventura et al., 2016). Therefore, such protocols can be frequently difficult to incorporate into daily working practices (Sutcliffe et al., 2018; Shannon et al., 2018). Even though further examination of broad-spectrum disinfectants should continue, development of innovative but simple, user- and environmentally-

friendly protocols is urgently required (Crane et al., 2018; Shannon et al., 2018). In addition, the synergistic effects of multiple differential treatments, such as manual cleaning and disinfectant application combined with minimum drying times, should be explored further.

Biosecurity protocols will likely be improved with the use of broad-spectrum aquatic disinfectants. In particular, 1% Virkon S® kills damaging pathogens and parasites, such as the invasive salmon fluke, *Gyrodactylus salaris*, at a fifteen-minute exposure (Koski et al., 2016). Nevertheless, high concentrations of this chemical are not suitable for use near freshwaters (Sebire et al., 2018). However, although Virasure® Aquatic and Virkon® Aquatic are more suitable for aquatic environments as they contain inert ingredients, confirmation of their ability to induce *G. salaris* mortality is required. Overall, our results suggest that further examination of the efficacy of aquatic disinfectants to inhibit the spread of AIS is essential. Moreover, as the worldwide spread of AIS has rapidly escalated (Seebens et al., 2017), there is a pressing need to conduct these studies. Currently, clarification of legal issues concerning the licensed use of Virasure® Aquatic and Virkon® Aquatic as a biosecurity agent against invasive organisms, other than viruses, bacteria, fungi, and moulds, is urgently required. Equally, the incorporation of aquatic disinfectants within biosecurity management protocols requires immediate consideration by stakeholders, such as angling and sporting groups, policy makers and legislators.

Author contributions

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

Declaration of interest

The authors declare no conflicts of interest.

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