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ORIGINAL PAPER

Better off dead: assessment of aquatic disinfectants and thermal shock treatments to prevent the spread of invasive freshwater bivalves

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Abstract Biosecurity protocols designed to prevent further spread of invasive alien species have become a key component of invader management strategies. Yet, the species-specific efficacy of many biosecurity treatments are frequently unclear or unknown. Invasive quagga, *Dreissena bugensis*, and zebra mussels, *D. polymorpha*, are a serious threat to freshwater ecosystems worldwide. Here, we examine the effectiveness of immersion ($\leq$ 90 min) within 2% or 4% solutions for two commonly used disinfectants (Virasure® Aquatic and Virkon® Aquatic) to cause mortality of adult *Dreissena* bivalves. Further, we assessed the effectiveness of thermal treatments: steam spray ($\geq$ 100 °C; $\leq$ 120 s); hot air ($-$ 500 °C; $\leq$ 60 s); and dry ice exposure ($-$ 78 °C; $\leq$ 300 g; 15 min). Complete mortality of *D. polymorpha* was observed following exposure to both disinfectants for 90 min, at both concentrations. However, high but incomplete mortality (40–90%) was recorded for *D. bugensis* across disinfectant treatments. For both species, complete mortality was achieved following 30 s of steam. In addition, 10 s of hot air and 15 min

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exposure to 300 g of dry ice can both completely killed groups of *D. polymorpha*. Overall, although the disinfectants did not cause complete mortality, it appears that relatively brief exposure to thermal treatments could be used to curtail the further spread of *Dreissena* species.

Keywords Biosecurity · Decontaminate · Invasive alien species · *Dreissena bugensis* · *Dreissena polymorpha*

Introduction

Invasive alien species (IAS) can negatively impact freshwater ecosystems, as their presence frequently results in the detrimental alteration of biodiversity, ecological functioning, and the economic and social value of invaded waterways (Dudgeon et al. 2006; Miehls et al. 2009; Sousa et al. 2014). As management options for effective control and eradication of established invader populations are often complex, costly and resource-intensive, the prevention of further IAS spread is considered an essential component of an effective management strategy (Piria et al. 2017; Booy et al. 2017). Overland transport of aquatic IAS by anthrophonic vectors, including watercraft, boat-trailers, and angling equipment, remains an especially problematic mechanism of dispersal (De Venture et al. 2016). In particular, niche areas of equipment can be difficult to reliably decontaminate through manual cleaning alone, e.g., chain lockers and internal surfaces of pipework (Cahill et al. 2019). Accordingly, biosecurity measures designed to decontaminate vectors are needed (Caffrey et al. 2014). Although a variety of biosecurity protocols designed to prevent the introduction and secondary spread of IAS have been developed and tested, such as immersion in hot water (Anderson et al. 2015; Shannon et al. 2018), aquatic disinfectants (Cuthbert et al. 2018; 2019; Bradbeer et al. 2020), desiccation exposure (Anderson et al. 2015; Coughlan et al. 2018a), and hot water spray (Comeau et al. 2011), the species-specific and context-dependent relative efficacies of many spread-prevention practices are often unclear or unknown (Anderson et al. 2015; Coughlan et al. 2019a; Bradbeer et al. 2020). As a result, there remains an urgent need to confirm the effectiveness of these treatments for additional IAS, to inform application guidelines to better minimise the risk of further IAS spread (Shannon et al. 2018; Crane et al. 2019; 2020b; Coughlan et al. 2019a).

Although originally developed to kill damaging pathogenic microbes, broad-spectrum aquatic disinfectants such as Virasure® Aquatic and Virkon® Aquatic are frequently used to aid decontamination of equipment to prevent further IAS spread. Some aquatic disinfectants have been observed to both partially and completely kill IAS (e.g. Cuthbert et al. 2018; 2019; Sebire et al. 2018; Bradbeer et al. 2020), however, the species-specific susceptibility of invaders to disinfectant solutions still requires further confirmation, e.g. across exposure durations and solution concentrations (Crane et al. 2020b; Bradbeer et al. 2020; Coughlan et al. 2019a). In addition, thermal shock treatments (i.e. sudden exposure to extreme hot or cold temperatures) have also been proposed as a mechanism to enable improved decontamination of equipment for the reduction of IAS spread (e.g. Stebbing and Rimmer 2014; Shannon et al. 2018), as well as facilitating the on-going suppression of established populations (Coughlan et al. 2018b; 2019b). For example, applications of steam have been found to kill a number of invasive macrophyte (Crane et al. 2019) and invertebrate species (Bradbeer et al. 2020; Joyce et al. 2019; Cuthbert et al. 2020). However, further assessment of steam as a tool for IAS decontamination is still required, especially for the identification of optimal and species-specific treatments (Bradbeer et al. 2020; Crane et al. 2019). Moreover, as thermal shock treatments represent a promising research direction for the development of improved IAS control strategies (e.g. Coughlan et al. 2018b; 2019b), further examination of such treatments as tools for population suppression should be considered.

Invasive bivalve species, such as quagga mussel, *Dreissena bugensis* (Andrusov 1897), and zebra mussel, *D. polymorpha* (Pallas 1771), are considered a major threat to the function and biodiversity of freshwater ecosystems worldwide (Higgins and Vander Zanden 2010; Sousa et al. 2014; Karatayev et al. 2015). As dominant filter-feeders, invasive *Dreissena* species can alter ecosystem structure and function (Crane et al. 2020a), through increased water clarity and the physical modification of benthic habitats (Karatayev et al. 2015). Such changes can result in

zooplankton declines (Kissman et al. 2010), blooms of potentially toxic cyanobacteria (Knoll et al. 2008), and increased populations of both benthic invertebrates and submerged aquatic vegetation, resulting in benthic orientated food-web structures (Mayer et al. 2002; Zhu et al. 2006; Miehls et al. 2009). Further, invasive *Dreissena* species frequently display a high degree of physiological and ecological plasticity (Sousa et al. 2014), and have a remarkable capacity for anthropogenic (De Ventura et al. 2016) and even zoochorous dispersal (Coughlan et al. 2017). In addition, as biofouling organisms, *Dreissena* species can have a substantial negative economic impact by adhering to and damaging structures (Nakano and Strayer 2014). Accordingly, a mosaic of freshwater environments are susceptible to the introduction and establishment of these invasive bivalves, which can subsequently act as new source locations facilitating further invader spread (Sousa et al. 2014; Karatayev et al. 2015). Therefore, there is an urgent need to better prevent the initial transport and introduction of these damaging invaders.

In the present study, we examined the efficacy of two commonly used oxidising-agent based disinfectants, Virasure® Aquatic and Virkon® Aquatic, and various thermal treatments to cause mortality of *D. bugensis* and *D. polymorpha*. We assessed the effectiveness of immersion within disinfectant treatments at 2 and 4% concentration for both species at various exposure times. These concentrations were chosen, as unlike juvenile specimens (see Barbour et al. 2013), adult Asian clam, *Corbicula fluminea*, specimens have been observed to be largely resistant to 2 and 4% solutions of Virasure® Aquatic and Virkon® Aquatic (Coughlan et al. 2019a), while 2% solutions of Virkon® Aquatic have previously been used to kill adult specimens of *D. bugensis* (Stockton-Fiti and Moffitt 2017). Accordingly, given the inconsistencies reported between studies concerning the efficacy of these disinfectants to kill bivalves, we sought to provide a more in-depth assessment for the effectiveness of 2 and 4% solutions towards adult *Dreissena* species. Similarly, we assessed the effectiveness of relatively rapid applications of steam for both species. Finally, we determined the efficacy of both hot air exposure, i.e. 5–120 s, and commercially available dry ice pellets (i.e. solid CO_2 pellets at − 78 °C) to cause mortality of *D. polymorpha*. We hypothesised that greater disinfectant concentrations and longer exposure times will cause substantial, if not complete mortality of both *D. bugensis* and *D. polymorpha*. Equally, we predict that both steam and hot air induced thermal shock will cause mortality to the tested *Dreissena* species. Likewise, with the application of a large enough quantity, we expect dry ice to cause compete mortality of *D. polymorpha*.

Methods

Specimen collection and maintenance

For the assessment of disinfectant solutions and steam treatments, *D. bugensis* and *D. polymorpha* specimens were collected from Wraysbury River, Surrey, UK (51° 27′ 02.3″ N, 0° 31′ 18.4″ W) and Grafham Water, Cambridgeshire, UK (52° 17′ 31.2″ N, 0° 19′ 23.9″ W), respectively. Specimens were transported in source water to the University of Leeds, UK. Specimens were then housed in aerated aquaria filled with dechlorinated tap-water, at a constant temperature of 14 ± 1 °C under a 12:12 h light–dark regime. For examination of hot air and dry ice treatments, *D. polymorpha* specimens were collected from Lough Erne, Northern Ireland, UK (54° 17′ 07.89″ N; 7° 32′ 52.61″ W) and transported in source water to the Queen's Marine Laboratory, Northern Ireland, UK. These specimens were likewise maintained in aerated aquaria containing one-part source water and one-part dechlorinated tap-water, at a constant temperature of 13 ± 1 °C under a 12:12 h regime. In all cases, organisms were acclimated for one week prior to experimental use.

Immersion in aquatic disinfectant solutions

The efficacy of aquatic disinfectants Virasure® Aquatic (Fish Vet Group) and Virkon® Aquatic (Antec Int. DuPont) was examined using 2% (20 g L^{-1}), or 4% (40 g L^{-1}) disinfectant solutions, and a 0% (0 g L^{-1}) control. All solutions were made using dechlorinated tap water. Disinfectant solutions were assessed for four exposure times: 15, 30, 60, 90 min. Only actively-filtering individuals that responded to mechanical stimuli were selected for experimentation. Specimens collected from Surrey and Cambridgeshire were used in this experiment. See Table 1 for an overview of the experimental design.

Table 1 Mean (± SE) raw percentage mortality of *Dreissena bugensis* and *D. polymorpha* at 24 h following immersion in 2% (20 g L^{-1}) or 4% (40 g L^{-1}) disinfectant solutions, and a 0% (0 g L^{-1}) control, for various exposure times

Treatment	Concentration (%)	Exposure Time (min)			
		15	30	60	90
Immersion in disinfectant solutions D. bugensis					
Control	0	0	0	3.3 ± 3.3	0
Virasure® Aquatic	2	66.7 ± 8.8	56.7 ± 3.3	63.3 ± 6.7	70 ± 5.8
Virasure® Aquatic	4	46.7 ± 3.3	76.7 ± 3.3	73.3 ± 8.8	66.7 ± 6.7
Virkon® Aquatic	2	73.3 ± 3.3	73.3 ± 3.3	80 ± 5.8	73.3 ± 3.3
Virkon® Aquatic	4	46.7 ± 3.3	80 ± 10	46.7 ± 6.7	56.7 ± 8.8
D. polymorpha					
Control	0	0	0	0	0
Virasure® Aquatic	2	76.7 ± 3.3	96.7 ± 3.3	80 ± 5.8	*100*
Virasure® Aquatic	4	56.7 ± 14.5	86.7 ± 3.3	53.3 ± 6.7	*100*
Virkon® Aquatic	2	*100*	96.7 ± 3.3	90	*100*
Virkon® Aquatic	4	83.3 ± 6.7	70 ± 5.7	86.7 ± 6.7	*100*

All treatments were replicated three times. Numbers in italic font delineates complete mortality

In all cases, groups of ten bivalves were briefly maintained (< 30 min) in dechlorinated tap water prior to experimentation (mean ± SE, min.–max. specimen length for *D. bugensis* and *D. polymorpha*: 29.5 ± 0.1, 23.4–39.43 mm and 21.4 ± 0.1, 18.51–29.84 mm, respectively). Each species was examined separately. Treatment groups were submerged into disinfectant solutions for the allotted treatment period. Control groups were likewise immersed in dechlorinated tap water (i.e. 0% solution) for the same exposure times. Following experimental exposure, the groups were immediately extracted, re-submerged in dechlorinated water for a two-minute period to aid the removal of excess disinfectant; this was repeated twice (see Cuthbert et al. 2019). Specimen groups were then returned to 250 ml of dechlorinated bubbled water (14 °C; 12:12 h light–dark) for a 24 h recovery period, after which mortality was assessed. Specimens were considered dead if they were gaping, or if they offered no resistance to being teased apart with tweezers and did not reclose. All disinfectant treatments were replicated three times per concentration, species and exposure time (i.e. $n = 3$ groups of 10 animals per treatment).

Direct steam exposure

To examine the efficacy of steam treatments to cause mortality *D. bugensis* and *D. polymorpha* specimens, groups of ten specimens (30.3 ± 0.3, 25.45–37.14 mm and 21.3 ± 0.2, 17.48–26.95 mm, respectively) were 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 nozzle of the device for: 5, 10, 30, 60, or 120 s. Specimens collected from Surrey and Cambridgeshire were used in this experiment. See Table 2 for an overview of the experimental design. Each species was examined separately, and all treatments were replicated three times per species. All groups were briefly maintained (< 30 min) in dechlorinated tap water and extracted as needed. Groups were held within fine-meshed flat-bottomed sieves during steam exposure. After exposure, all specimens were cooled for a five-minute period to allow a gradual temperature reduction prior to being returned to water. Control specimens were held within fine-meshed flat-bottomed sieves and allowed to air-dry for a fifteen-minute period. Following this, specimen groups were returned to 250 ml of dechlorinated aerated water (14 °C; 12:12 h light–dark) for a recovery period of 24 h, after which mortality was assessed as above.

Exposure of Dreissena polymorpha to hot air

Dreissena polymorpha specimens were directly exposed to a continuous jet of hot air for: 5, 10, 30, or 60 s (− 500 °C; Bosch Heat Gun PHG 500–2). Specimens collected from Northern Ireland were used in this experiment. See Table 2 for an overview of the experimental design. Groups of ten *D. polymorpha* (20.45 ± 0.3, 16.6–25.83 mm) were briefly

Table 2 Mean (± SE) raw percentage mortality of *Dreissena bugensis* and/or *D. polymorpha* at 24 h following the application of thermal shock treatments, i.e. steam, hot air, or dry ice exposure

Treatment	Exposure Time (sec)					
	Control	5	10	30	60	120
Steam spray (≥ 100 °C)						
D. bugensis	3.3 ± 3.3	6.7 ± 6.7	73.3 ± 26.7	*100*	*100*	*100*
D. polymorpha	0	36.7 ± 3.3	96.7 ± 3.3	*100*	*100*	*100*
Hot air (– 500 °C)						
D. polymorpha	0	56.7 ± 8.8	*100*	*100*	*100*	–

	Treatment (g)			
	Control	100	200	300
Dry ice (− 78 °C) for 15 min				
D. polymorpha	0	71.1 ± 4.8	96.7 ± 1.9	*100*

All treatments were replicated three times. Numbers in italic font delineates complete mortality

maintained (< 30 min) in dechlorinated tap water and extracted as needed. Groups were placed as a loose clump on a flat plastic board, and exposed to hot air at a distance of 2–3 cm from the nozzle of the device. All treatments were replicated three times. Control specimens placed on a plastic board and were allowed to air-dry for a fifteen-minute period. After exposure, all specimens were cooled for a five-minute period, to allow gradual cooling prior to being returned to water. Following this, specimen groups were returned to 250 ml of dechlorinated aerated water (13 °C; 12:12 h light–dark) for a recovery period of 24 h, after which mortality was assessed as above.

Exposure of Dreissena polymorpha to dry ice

To assess the efficacy of dry ice to cause mortality of *D. polymorpha*, groups of thirty specimens (23.9 ± 0.2, 18.1–29.9 mm) were exposed to 100, 200 or 300 g of commercially available 9 mm dry ice pellets for fifteen minutes. Specimens collected from Northern Ireland were used in this experiment. See Table 2 for an overview of the experimental design. Controls groups were allowed to air-dry for a fifteen-minute period and dry ice was not added. All treatments were replicated three times. Groups of mussels were placed within cylindrical plastic containers (height, 234 mm; diameter, 180 mm; mussel density = 1179 ind. m^{-2}). The desired mass of the dry ice pellets was weighed and immediately added to the appropriate container. Dry ice pellets were distributed as evenly as possible over the entire base area of the container. Following exposure, specimens were immediately removed from the experimental container. Any specimens embedded within the dry ice were carefully removed by hand, using a small metal ice-pick and cool tap water (− 6 °C). Specimen groups were then returned to 600 ml of dechlorinated aerated water (13 °C; 12:12 h light–dark) for a 24 h recovery period, after which mortality was assessed.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test for differences in intraspecific bivalve shell lengths. Size differences were separately assessed for each experiment across all treatment groups in relation to disinfectant product used (i.e. Virasure® Aquatic, Virkon® Aquatic or control), exposure times for steam and hot air, and amounts of dry ice used. Means were pooled using all individuals within each replicate, for each respective treatment. Where residuals did not meet normality (Shapiro–Wilk test, $P < 0.05$) or homoscedasticity assumptions (Levene's test, $P < 0.05$), a log10 transformation was applied to normalise residuals and homogenise variance.

Binomial generalised linear models (GLMs) with logit links were used to examine bivalve mortality rates separately in each experiment. A fitting function was used within GLMs to account for instances of complete separation via the bias-reducing adjusted scores approach (Firth 1993; Kosmidis and Firth 2009; Kosmidis 2014). For each of the four experiments, models were structured initially as follows: (1) disinfectant, *mortality* − *treatment* (5 levels: control, 2% Virkon® Aquatic, 4% Virkon® Aquatic, 2%

Virasure® Aquatic, 4% Virasure® Aquatic) * *exposure* (4 levels: 15, 30, 60, 90 min) * *species* (2 levels: *D. bugensis*, *D. polymorpha*); (2) steam, *mortality* − *exposure* (6 levels: control, 5, 10, 30, 60, 120 s) * *species* (2 levels: *D. bugensis*, *D. polymorpha*); (3) hot air, *mortality* − *exposure* (five levels: control, 5, 10, 30, 60 s); and (4), dry ice, *mortality* − *treatment* (4 levels: control, 100, 200, 300 g).

An information theoretic approach via model averaging was used to identify predictors of substantial importance in determining mortality rates of bivalves within each experiment. All possible models were identified and ranked based on a second-order derivation of Akaike's information criterion (AICc) for small sample sizes (Burnham and Anderson 2002; Barton 2018). For all candidate models, ΔAICc was discerned as the difference in AICc between the best model and model *i*. Models with ΔAICc ≤ 2 were considered interchangeable (Burnham and Anderson 2002). The AICc model weight was discerned based on the weight of evidence that a given model was the best among all those considered. The relative variable importance (RVI) for each predictor was then calculated by the sum of weights (w_i) of models which contained the focal variable. Predictors with RVI near 1 are considered to have high importance (Burnham and Anderson 2002). Analysis of deviance was used to infer statistical significance of predictors in the top model. Where a significant interaction was found, Type III sums of squares were employed, whilst Type II sums of squares were implemented in the lack of a significant interaction (Langsrud 2003; Fox and Weisberg 2011). Estimated marginal means were used *post-hoc* for pairwise Tukey comparisons of significant predictors (Lenth 2018). All statistical analyses were performed in R v3.5.1 (R Core Development Team 2018).

Results

Disinfectant immersion

Total mortality was consistently observed in *D. polymorpha* following all 90-min disinfectant exposures, and following 15-min exposures to 2% Virkon® Aquatic. Otherwise, mortality in *D. polymorpha* varied between 42–100% for all other disinfectant exposures. Conversely, a maximum average of 80% mortality was observed in *D. bugensis* following disinfectant treatments. Controls for both species exhibited high survival (97–100%; Table 1). *Treatment*, *exposure* and *species* were of high importance in the top model (all RVI = 1; Table 3). Furthermore, the '*treatment* × *species*' and '*exposure* × *species*' interactions were of considerable importance (both RVI ≥ 0.99). A significant '*treatment* × *species*' term (GLM, $\chi^2 = 20.36$, df = 4, $P < 0.001$) reflected significantly greater mortality of *D. polymorpha* compared to *D. bugensis* following all disinfectant treatments (all $P < 0.05$), whilst interspecific mortality rates of control specimens were more similar ($P = 0.14$). The '*exposure* × *species*' interaction was also significant (GLM, $\chi^2 = 31.90$, df = 3, $P < 0.001$), with mortality rates of *D. polymorpha* significantly higher than *D. bugensis* following 90 min of exposure ($P < 0.001$), yet differences were less statistically clear under shorter disinfectant

Table 3 Model averaging results of binomial generalised linear models (GLMs) considering bivalve mortality rates in: (I) disinfectant immersion as a function of *treatment* (t: 5 levels), *exposure* (e: 4 levels) and *species* (s: 2 levels); (II) steam exposure as a function of *exposure* (e: 6 levels) and *species* (s: 2 levels); (III) hot air exposure as a function of *exposure* (e: 5 levels); and (IV) dry ice exposure as a function of *treatment* (t: 4 levels)

Experiment GLMs	Model	df	logLik	AICc	ΔAICc	w_i	Cum. w_i
Disinfectant immersion	t + e + s + t:s + e:s	16	− 134.33	305.94	0.00	0.99	0.99
Steam exposure	e + s	7	− 29.33	86.27	0.00	0.99	0.99
Hot air exposure	e	5	− 7.00	30.66	0.00	1.00	1.00
Dry ice exposure	t	4	− 11.22	36.15	0.00	1.00	1.00

ΔAICc is the difference between the focal model and the model with the lowest AICc, weight $w_i$is the probability that the focal model is the top model, while Cum. $w_i$denotes cumulative model weights. Models with high importance (ΔAICc ≤ 2) are shown here

exposures (all $P > 0.05$). For both *Dreissena* species, intraspecific shell lengths did not differ between disinfectant products (*D. bugensis*: ANOVA, $F_{2,\ 6} = 3.60$, $P = 0.09$; *D. polymorpha*: ANOVA, $F_{2,\ 6} = 4.17$, $P = 0.07$).

Steam exposure

For both species, total mortality was observed following steam exposures of $\geq$ 30 s (Table 3). Both the *exposure* and *species* terms were of high importance (RVI $\geq$ 0.99), whilst their interaction was relatively unimportant (RVI < 0.01; Table 3); mortality was higher following longer steam exposures and greater for *D. polymorpha* than *D. bugensis* (Table 2). Steam treatment caused significant mortality in bivalves (GLM, $\chi^2 = 334.11$, df = 5, $P < 0.001$), with exposures for $\geq$ 10 s causing significantly greater mortality than control or 5 s groups (all $P < 0.001$). Differences between 5 s exposures and control groups were not statistically clear ($P = 0.05$). Mortality rates of *D. polymorpha* were significantly higher than *D. bugensis* overall (GLM, $\chi^2 = 9.56$, df = 1, $P = 0.002$). For both *Dreissena* species, intraspecific shell lengths did not differ (*D. bugensis*: ANOVA, $F_{5,\ 12} = 0.47$, $P = 0.79$; *D. polymorpha*: ANOVA, $F_{5,\ 12} = 2.51$, $P = 0.09$).

Hot air exposure

Total mortality of *D. polymorpha* was found following hot air exposures for $\geq$ 10 s (Table 2). *Exposure* held high importance as a predictor variable (RVI = 1.00; Table 3). Accordingly, hot air applications caused significant mortality in *D. polymorpha* overall (GLM, $\chi^2 = 134.78$, df = 4, $P < 0.001$), with all exposures driving significant mortality compared to controls (all $P < 0.05$). Differences among hot air treatments were not statistically apparent (all $P > 0.05$). Specimen size did not differ among treatment groups (ANOVA, $F_{4,\ 10} = 2.20$, $P = 0.14$).

Dry ice exposure

Total mortality of *D. polymorpha* was exhibited following 300 g treatments (Table 2). Dry ice application was a highly important predictor (RVI = 1.00; Table 3), with *treatment* significantly influencing bivalve mortality rates (GLM, $\chi^2 = 320.32$, df = 3, $P < 0.001$). Mortality following dry ice exposure was always significantly higher than controls (all $P < 0.001$). In turn, 200 g and 300 g exposures caused significantly greater mortality than 100 g exposures (both $P < 0.05$); differences between 200 and 300 g applications were not statistically clear ($P = 0.57$). *Dreissena polymorpha* specimens selected for experimentation did not differ in size (ANOVA, $F_{3,\ 8} = 0.83$, $P = 0.51$).

Discussion

Aquatic disinfectants were partially successful for causing mortality in *D. polymorpha*, however mortality was only consistently achieved at the maximum exposure of 90 min. In contrast, thermal shock treatments were highly successful, resulting in complete mortality of the examined *Dreissena* species. Specifically, complete mortality was reliably achieved for both *Dreissena* species following 30 s of steam. Similarly, *D. polymorpha* displayed 100% mortality after exposure to hot air for 10 s and after a 15-min exposure to 300 g of dry ice. Accordingly, it appears that relatively brief exposure to steam, hot air and dry ice treatments could be used to curtail the further spread of the *Dreissena* species, rather than broad-spectrum aquatic disinfectants.

Immersion within solutions of Virasure® Aquatic or Virkon® Aquatic did not reliably cause mortality in adult *Dreissena*, other than for *D. polymorpha* specimens at the maximum 90-min period. However, in-field soaking durations of 90 min will be impractical for many water users, and does not represent an efficient means of decontamination. Furthermore, for both species, although high if not complete mortality was observed for almost all treatments, these data lack a clear consistency, and therefore, these treatments should be considered ineffective. This may, in part, be due to organism reaction to disinfectant exposure; at a higher concentration mussels may shut their shells quicker, or maintain their shells closed throughout the treatment, and are therefore exposed to less disinfectant. Similarly, inconsistent results have also been documented for other invasive bivalve species exposed to 2% and 4% solutions of Virasure® Aquatic or Virkon® Aquatic. Coughlan et al. (2019a) reported low mortality of *C. fluminea* (< 55%) following disinfectant exposures for up to 80 min (specimen

shell heights: 15–36 mm). However, Barbour et al. (2013) observed − 93% mortality of juvenile *C. fluminea* specimens (shell heights: 5.1–10 mm) following five-minute exposures to 2% solution of Virkon® Aquatic. Interestingly, inter- and intraspecific differences concerning disinfectant treatment efficacies, as further highlighted by the present study, could be reflective of species size class differences (Coughlan et al. (2019a). Therefore, the disparity between our results for specimens of shell length 29.5 ± 0.1 mm (23.4–39.43 mm) and the complete mortality of adult *D. bugensis* (5–20 mm) recorded by Stockton-Fiti and Moffitt (2017), following ≥ 10 min exposure to 2% Virkon® Aquatic, may be attributed to differences in size class of specimens used. Accordingly, further examination of size class related effects will need to be considered when determining the effectiveness of aquatic disinfectants to cause mortality of bivalve species. Equally, differences in duration of post treatment recovery periods may influence the proportion of dead bivalves recorded, with longer recovery periods facilitating improved determination (e.g. 72 h; Stockton-Fiti and Moffitt 2017). Overall, it appears that aquatic disinfectants will not effectively kill all adult *Dreissena* specimens within a relatively rapid 24 h period following treatment, which may allow for recovery and further dispersal. Interestingly, although the incidental observation of a release of a white cloudy film from shells during disinfectant exposure was also observed in the present study, rapid mortality was not observed. Likewise, following the recovery period, some shells of both *Dreissena* species displayed a bleached or translucent appearance, as described by Stockton-Fiti and Moffitt (2017), which is likely an artefact of low pH levels (− 2.5 pH) produced by the examined disinfectants.

For both *Dreissena* species, complete mortality was reliably achieved following steam exposure for ≥ 30 s. This result is consistent with the high levels of efficacy reported for steam spray treatments by a number of other studies, concerning bivalve (Coughlan et al. 2019a; Joyce et al. 2019), crustacean (Bradbeer et al. 2020), dipteran (Cuthbert et al. 2020) and macrophyte species (Crane et al. 2019; Coughlan et al. 2020). Similarly, exposure to a hot air jet for ≥ 10 s consistently caused complete mortality of *D. polymorpha* specimens, demonstrating the potential application of this novel treatment for improved biosecurity practices. Further, cold thermal shock caused by the application of 300 g of dry ice (− 78 °C) resulted in complete mortality of 30 *D. polymorpha* specimens (1179 ind. m^{-2}), following a 15-min exposure period. Although hot air and dry ice applications were not examined for *D. bugensis*, given the intensity of these thermal shock treatments, and that *D. bugensis* generally display weaker shells than *D. polymorpha* at locations where both species co-exist (Casper and Johnson 2010), we suspect that similar thermal exposure times could also be used to reliably achieve mortality of *D. bugensis*. For spread-prevention purposes, dry ice applications could be used to kill *Dreissena* species within niche areas that are difficult to manually clean, such as chain lockers and internal surfaces of pipework. Further, given that *Dreissena* species reside upon, rather than within substrates, thermal shock treatments of steam, hot air and dry ice applications could also be potentially used for population suppression in areas were mussels become exposed to air during instances of low water levels, such as water draw-down events levels. However, whilst promising, the efficacy of steam and hot air treatments to prevent further invader spread requires confirmation under field-conditions.

Overall, although exposure to broad-spectrum disinfectants did not reliably cause complete mortality, it appears that relatively brief exposure to steam, hot air and dry ice treatments could be used as part of effective and efficient biosecurity protocols to prevent further spread of the *Dreissena* species. Further, as treatment times are considered a barrier to good biosecurity practice (Sutcliffe et al. 2018), rapidly applied thermal treatments may prove to be highly beneficial, especially when combined synergistically with other cleaning methods, such as hand removal, brushing or scraping (Crane et al. 2019; Bradbeer et al. 2020). In principal, thermal treatments likely represent a particularly environmentally-friendly mechanism for IAS spread-prevention, as steam, hot air and dry ice will rapidly dissipate thermal energy into the surrounding air (Coughlan et al. 2018b; Joyce et al. 2019). Given that *Dreissena* species frequently exclude native species from invaded habitats (Sousa et al. 2014; Karatayev et al. 2015), targeted thermal shock treatments to suppress populations could also be preferable to mechanical and chemical population control methods, which can result in detrimental habitat alteration (e.g. dredging methods), wider

waterway impacts and have lingering effects (Sousa et al. 2014; Coughlan et al. 2018b; Crane et al. 2019).

Although confirmation of effectiveness under in-field conditions is still required (Tidbury et al. 2018), aquatic disinfectants can cause mortality of bacterial, fungal and viral pathogens (e.g. Jussila et al. 2014). Accordingly, biosecurity protocols will likely be improved with the use of broad-spectrum aquatic disinfectants (Cuthbert et al. 2019). Nevertheless, the efficacy of thermal treatments to inactivate bacterial, fungal and viral pathogens merits investigation. Further, the use of thermal treatments also negates the issues surrounding the use of chemical disinfectants, in relation to waste disinfectant disposal, run-off catchment, and legal uncertainties (Sebire et al. 2018; Bradbeer et al. 2020). Thermal treatments may also aid decontamination of equipment items that are problematic to otherwise manually clean such as niche areas or large complex structures, e.g. intake grates, chains, pipework, trailers and vehicles (Crane et al. 2019; Joyce et al. 2019). However, development of operational thermal treatments will require an assessment of risk in relation to potential damaging of equipment, such as vessel components, waterproof clothing, and existing anti-foul coatings (Joyce et al. 2019). Furthermore, health and safety requirements for users will also need to be considered. To achieve participation in decontamination by water users, the installation of cleaning facilities in the form of biosecurity stations at points of waterway exit and entry, e.g. angling stations and boat ramps, would be beneficial (Shannon et al. 2018; Crane et al. 2019). These stations could take the form of self-service, automated or trained operator-attended decontamination facilities could greatly reduce the transfer of IAS in a simple, cost-effective, environmentally-friendly, yet highly successful way (Coughlan et al. 2019a; Crane et al. 2019). At these stations, to prevent re-entry of IAS into waterways, runoff water and invader biomass would need to be contained; this can be achieved by the installation of an enclosed cleaning area with an interceptor. Further, promotion and adoption of these techniques by biosecurity campaigns, stakeholder groups, and practitioners should be encouraged (Davis et al. 2018; Sutcliffe et al. 2018). Furthermore, the requirement to perform and adhere to biosecurity protocols should be incorporated into relevant Codes of Practice (Coughlan et al. 2019a), with subsequent enforcement in relation to all water users.

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Author contributions NEC proposed the study; NEC, SJB, EMC and KC designed the experiments; NEC, SJB and EMC 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.

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