Research Article

The effectiveness of hot water pressurized spray in field conditions to slow the spread of invasive alien species

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Abstract

An array of vectors have been identified that pose a risk of spreading invasive alien species (IAS), from personal protective equipment to large equipment such as vehicles and boats. Biosecurity practices that remove and/or kill IAS reduce the risk of accidental spread. The effectiveness of biosecurity protocols suitable for large equipment is little tested and requires development. One widely-used biosecurity method for large equipment is high-pressure hot water spray machines. This study tests the effectiveness of high-pressure hot water spray to induce mortality in two invasive aquatic plants: floating pennywort (*Hydrocotyle ranunculoides*) and Australian swamp-stonecrop (*Crassula helmsii*); and two invasive invertebrates: killer shrimp (*Dikerogammarus villosus*) and zebra mussel (*Dreissena polymorpha*) in field conditions. IAS were exposed to hot water spray for a range of durations (5–15 seconds) and from a range of distances (10–30 cm). Further treatments of up to 90 seconds were applied to *C. helmsii*. Complete survival of *D. polymorpha, D. villosus* and *C. helmsii* was seen in all control treatments following exposure to cold water spray. Hot water spray caused complete mortality of *D. polymorpha* and *D. villosus* at 10 cm for 15 seconds, demonstrating the effectiveness of the hot water treatment in inducing mortality. However, treatments were less effective when applied at longer distances and shorter durations. In contrast, hot water spray was ineffective in causing mortality in *C. helmsii*, even at 90 seconds of exposure. Fragmentation and complete mortality was seen in *H. ranunculoides* following exposure to hot and cold water spray, therefore the pressure of the spray was associated with *H. ranunculoides* mortality. The use of hot water spray is effective against the aquatic invasive animals tested here, however to ensure complete mortality, the importance of both duration and distance of hot water spray application is highlighted. Hot water spray did cause complete mortality in *H. ranunculoides* but not in *C. helmsii*, therefore the need for treatment water containment and safe disposal is paramount to prevent spread of potentially viable propagules.

Key words: biosecurity, invasive non-native species, prevention, *Dikerogammarus villosus, Dreissena polymorpha, Hydrocotyle ranunculoides, Crassula helmsii*

Introduction

The impacts resulting from the introduction and spread of invasive alien species (IAS) are of growing concern for a range of stakeholders. The annual cost of IAS is estimated to be €20 billion in Europe (Kettunen et al. 2009) and £120 billion in the US (Pimentel et al. 2005), however these estimates are likely now conservative. Biodiversity impacts associated with invasions in the freshwater environment include disease introduction, ecosystem function alteration and predation of, and competition with native species (Pimentel et al. 2005; Hejda et al. 2009; Hulme 2014). Social and economic impacts such as increased flood risk and direct human health concerns have also been documented, presenting a considerable financial and environmental burden (Pimentel et al. 2005; Hulme 2014). Whilst controlling established populations is essential, the feasibility of complete eradication, especially in the freshwater environment is often limited (Vander Zanden et al. 2010). Preventing the spread of IAS is considered the most cost-effective method for management (Leung et al. 2002; Booy et al. 2017; Léger et al. 2017; Millett and Snyder-Beattie 2017). Preventative methods reduce the risk of both introductions of novel IAS, as well as limiting the further dispersion of already-established IAS into other areas. In line with the Aichi Biodiversity Targets (Target 9), the spread of freshwater IAS and associated dispersal corridors throughout Europe has been well documented over recent decades (Bij de Vaate et al. 2002; García-Berthou et al. 2005; Leuven et al. 2009; Gherardi 2010; Convention of Biodiversity 2020). Informed by this and with the aim of preventing further spread and introductions of IAS, regulation and framework have been introduced both at the international and national levels (e.g. EU Regulation, GB Strategy; UK Parliament Act 1981; European Union 2014; Department for Environment, Food and Rural Affairs 2015). Prevention of introduction and secondary spread is key to this regulation.

To introduce and implement effective prevention methods, the pathways and vectors of IAS need to be targeted. In the freshwater environment, vectors include fieldwork equipment, recreational equipment (e.g. angling gear and canoes), and boats (Johnson et al. 2001; Davidson et al. 2010; Anderson et al. 2014; Smith et al. 2020). Prevention methods aim to decontaminate such vectors by removing and/or killing attached IAS before transit and therefore reduce the risk of IAS spread; this is collectively termed "biosecurity". National campaigns have been introduced to inform stakeholders of effective biosecurity methods. These include: "Check Clean Dry" in the UK and New Zealand (Great Britain Non-Native Species Secretariat 2020; Biosecurity New Zealand 2020), "Clean Drain Dry" in the USA (Stop Aquatic Hitch Hikers 2020) and "Play Clean Go" in British Columbia, Canada (Invasive Species Council of British Columbia 2020). These campaigns centre around three stages to apply biosecurity to equipment: 1) visual inspection 2) cleaning and 3) drying. The second stage

of cleaning equipment is informed by studies testing a range of treatments including; freezing temperatures (McMahon et al. 1993; Coughlan et al. 2018, 2020b), saline treatments (Hofius et al. 2015), disinfectants (Watters et al. 2013; Moffitt et al. 2015; Cuthbert et al. 2018, 2019; Sebire et al. 2018; Bradbeer et al. 2020; Coughlan et al. 2020b; Crane et al. 2020), steam (Crane et al. 2018; Bradbeer et al. 2020; Coughlan et al. 2020a; b), and hot water (Morse 2009; Beyer et al. 2010; Comeau et al. 2011; Anderson et al. 2015; Shannon et al. 2018). The use of hot water relies on sudden thermal shock to induce IAS mortality. Studies testing hot water immersion have found high effectiveness against freshwater IAS when exposed to 45 °C water for 15 minutes (Anderson et al. 2015; Shannon et al. 2018). Whilst very useful for small equipment, large equipment may have limited capacity to be immersed, both because of the mechanics of moving such equipment as well as the risk that specific components may be damaged if immersed. High pressure hot water spray machines are widely used to clean large equipment, both from a maintenance and biosecurity perspective (Morse 2009; Comeau et al. 2011; Stebbing and Rimmer 2013). However, their effectiveness has been little tested. Two factors are combined to remove and/or kill IAS on pieces of equipment: water temperature and water pressure. The pressure of the spray may dislodge organisms and/or cause physical damage or induce mortality, and the thermal shock may induce mortality. Compared to other biosecurity methods, this method is relatively environmentally-friendly as it does not involve chemicals and the water run-off dissipates heat relatively quickly.

A limited number of studies have sought to assess the effectiveness of hot water spray machines to induce mortality in freshwater IAS (Morse 2009; Comeau 2011; Stebbing and Rimmer 2013; Watters 2014). No studies have assessed the effectiveness of hot water spray to induce mortality in any invasive plant species. The lack of studies, in particular in field conditions, in addition to absence of information on their effectiveness for plant IAS presents a clear knowledge gap that needs to be addressed to inform biosecurity guidance for fieldworkers.

This study focuses on four high impact IAS, two animals and two plants species (Figure 1). The spread of zebra mussel, *Dreissena polymorpha* (Pallas, 1771) and killer shrimp, *Dikerogammarus villosus* (Sowinsky, 1894) has sparked considerable concern in recent decades (Bij de Vaate et al. 2002; Rewicz et al. 2014; Gallardo and Aldridge 2015). *Dikerogammarus villosus* is invasive in many countries, detected in Italy in 2006 (Casellato et al. 2006), France in 2007 (Grabowski et al. 2007) and in the UK in 2010, where it remains an alert species (MacNeil et al. 2010; Great Britain Non-Native Species Secretariat 2020). Impacts of *D. villosus* include ecosystem function alteration and predation of native invertebrates and vertebrates (Dick et al. 2002; Krisp and Maier 2005; Jourdan et al. 2016; Taylor and Dunn 2017). There is a high risk of spread associated with *D. villosus* as
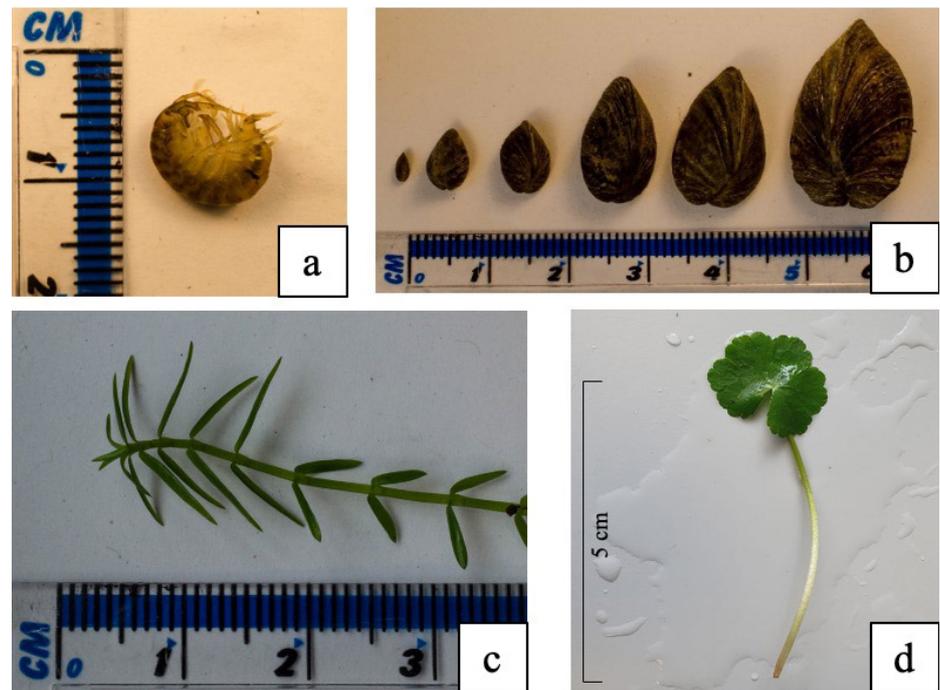
Figure 1. Photographs of four invasive alien species utilised in hot water spray effectiveness experiments; a. *Dikerogammarus villosus*, b. *Dreissena polymorpha*, c. *Crassula helmsii*, d. *Hydrocotyle ranunculoides*. Photographs by Chris Pollock (a, b, c) and Stephanie Bradbeer (d).

individuals are known to survive in damp conditions for up to 16 days, and can therefore be accidentally spread on large equipment such as boats (Pöckl 2009; Anderson et al. 2015). Impacts of established *D. polymorpha* include adhering to and damaging infrastructure, ecosystem function alterations and facilitating the spread of other IAS (MacNeil et al. 2008). Of both environmental and economic concern, adherence to surfaces can cause substantial costs in removal or replacement as well as causing habitat alterations (Karatayev et al. 2002; Connelly et al. 2007). This invasive mussel can similarly be spread by contaminated large equipment and is one of the main targets of watercraft check stations (e.g. Don't Move a Mussel in the USA and Canada, www.dontmoveamussel.ca/mussels/).

In addition to invasive invertebrates, there are an array of invasive plants that can monopolise an environment, compete with and exclude native plants and create flood risk and navigation issues (European Union 2014; Department for Environment, Food and Rural Affairs 2015). Native to the Americas, *Hydrocotyle ranunculoides* is listed as an EU species of Union concern and documented to be invasive in 9 member states (European Commission 2017). This rapidly growing plant generates large vegetative mats which prevent light penetration, resulting in anoxic conditions and the exclusion of native plants (European and Mediterranean Plant Protection Organization 2006). These mats also reduce water flow, which presents a considerable flood risk and water navigation issues (European and Mediterranean Plant Protection Organization 2006). *Crassula helmsii*, native to Australasia, was first introduced to the UK over a century ago and mainland Europe in the 1980s. It is categorised as still spreading (European

and Mediterranean Plant Protection Organization 2007). *Crassula helmsii* can prevent light penetration and negatively impact biodiversity and habitat structure (European and Mediterranean Plant Protection Organization 2007). These plants can regenerate from small fragments and therefore pose a considerable risk for accidental spread via vectors such as vehicles and boats. With the severe impacts associated with the presence of these freshwater invasive animal and plants, the need to identify methods that will prevent their spread is imperative. Despite being a widely used biosecurity method, evidence regarding the effectiveness of hot water spray machines to induce IAS mortality is limited. Such knowledge is needed to inform biosecurity guidelines, thus reducing the risk of IAS spread. It has been highlighted that whilst some recommendations exist for water temperatures that will kill certain IAS, the duration of exposure also needs to be specified (Morse 2009). Moreover, specifications such as distance the spray is applied from, also needs to be defined in recommendations as thermal dissipation of the water spray increases with a greater distance.

This study assesses the effectiveness of hot water spray to induce mortality in four IAS, including two previously untested plant species, *C. helmsii* and *H. ranunculoides*. The experimental design was to simulate the typical usage of hot water spray machines by stakeholders for cleaning equipment in the field. Given the temperature data from the preliminary study and the limited evidence for mortality of IAS due to hot water spray exposure, this study sought to use the highest machine-programmed temperature achievable, 90 °C. Here we consider two variables: the duration of exposure and the distance of spray. These variables reflect user behaviours that may be achievable to alter, thus definable in biosecurity guidance. It was predicted that closer distances and longer durations of hot water spray would result in high if not complete mortality of the IAS tested. This is likely to be due to both a higher maximum temperature and overall thermal exposure. Complete IAS survival of control cold water spray treatment demonstrates that whilst at closer distances, the pressure of the spray will be higher, in these treatments it does not induce mortality. Furthermore, it was predicted the susceptibility of the different IAS to mortality due to thermal spray exposure would differ.

Materials and methods

This study tested the effectiveness of high pressure hot water spray to induce mortality in four IAS, under field conditions. Treatments consisted of varying the duration of spray exposure and the distance the spray is applied from. Ambient temperature and the temperature of the water on-contact was recorded. To assess effectiveness of treatments, IAS mortality was measured at 1, 8, 24 and 72 hours after exposure. Invasive plant species were visually inspected 7 and 14 days post-exposure to assess degradation and root/shoot regeneration (see Crane et al. 2018; Cuthbert et al. 2019).

Experimental Organisms

All organisms were collected between September and November 2018. *Dikerogammarus villosus* and *D. polymorpha* were collected from Grafham Water, Cambridgeshire (Latitude 52.291993; Longitude −0.32329470), *C. helmsii* from Letchmire Pastures, Yorkshire (Latitude 53.741094; Longitude −1.3565728), and *H. ranunculoides* from Waterhaigh Woodland Park, Yorkshire (Latitude 53.752838; Longitude −1.4318973). Surface water temperature was measured at each collection site (12.0 °C, Grafham Water, 11.6 °C, Letchmaire Pastures, and 10.8 °C, Waterhaigh). Organisms were transported to the University of Leeds and stored in aerated tanks in a control temperature room (14 °C) with 12 hour light:dark cycle (07:00:19:00). To minimise the potential effect of stress due to collection and transport, all organisms were housed for at least 72 hours prior to experimentation and only visually healthy organisms were selected for experimentation (active filter-feeding *D. polymorpha* individuals, actively swimming *D. villosus* individuals, positive phototropism in *C. helmsii* and *H. ranunculoides* individuals).

Experimental Set Up

The experimental set up was used to simulate a typical usage of hot water spray machines by stakeholders for cleaning equipment in the field. Experiments were conducted in October and November 2018 in field conditions at an Environment Agency depot, Yorkshire, UK (Latitude 53.827866; Longitude −1.076799), using a Karcher HDS 7/10-4A hot water pressurised spray machine (herein, hot water spray machine). A 10 cm^2 bag (1 mm mesh) was attached to a metal backboard and the lance of the hot water spray machine held, according to the treatment, at a set distance between the end of the nozzle and the contact point of the mesh bag. The nozzle type was the standard model provided with the hot water spray machine and was set to the narrowest aperture. Enclosed within the mesh bag was one organism (IAS) and a fast-reacting temperature probe (Lascar EasyLog Thermistor Probe EL-USB-TP-LCD, accuracy ± 0.6 °C). The metal backboard was used to represent a large piece of equipment such as a boat or vehicle. Equipment made of metal will conduct thermal energy away from a contact point at a greater rate than other materials, therefore protocols found to be effective whilst using metal are likely also be effective on other materials such as foam, plastic or fibreglass. The mesh bag allowed for the containment of the invasive organism, avoided pooling of water and provided minimal shelter, as used in previous studies (for example Morse 2009; Comeau 2011; Stebbing and Rimmer 2013). Furthermore, the size of the mesh bag ensured equal application of the spray to the bag and its contents as well as being typical of equipment which may be cleaned using a hot water spray machine (e.g. large fishing nets).

Table 1. Average maximum on-contact water temperature (°C) for 15 seconds duration of hot water spray applied from 7 distances: 100, 75, 50, 40, 30, 20 and 10 cm and with 4 machine-programmed temperatures: 90, 80, 75, 60 °C (n = 2).

		Hot water spray machine temperature			
		60 °C	75 °C	80 °C	90 °C
	10 cm	54.4	60.6	63.4	67.4
	20 cm	48.3	51.4	58.5	59.0
	30 cm	41.8	51.5	50.2	55.9
Distance	40 cm	39.5	48.9	42.9	52.0
	50 cm	37.5	45.1	40.2	47.9
	75 cm	31.2	37.5	39.6	40.9
	100 cm	31.5	33.2	32.0	37.3

Preliminary study

A preliminary study was conducted to assess the on-contact temperature when hot water spray was applied from 7 different distances (10, 20, 30, 40, 50, 75 and 100 cm) and with 4 different machine-programmed temperatures (60, 75, 80 and 90 °C) in field conditions. All spray exposures were for a set duration of 15 seconds and at a constant pressure, 1600 PSI. An additional infrared temperature device (Fluke 566) was used to confirm temperature measurements of the fast-reacting probe (Lascar EasyLog Thermistor Probe EL-USB-TP-LCD). The preliminary study was conducted to inform on the variables (i.e. exposure distance and duration) that would be tested for IAS mortality assessments. Spray applied at closer distances resulted in higher temperatures; the highest machine temperature (90 °C) at the closest distance (10 cm) resulting in a maximum on-contact temperature of 67.4 °C (Table 1). The maximum temperature when spray was applied from a distance of ≥ 40 cm was ≤ 52.0 °C, decreasing to ≤ 37.6 °C at a distance of 100 cm (Table 1). These relatively low temperatures when the spray was applied from ≥ 40 cm, suggested that these treatments would be unlikely to result in high IAS mortality because previous studies have identified that immersion in > 45 °C water is required to induce IAS mortality (Anderson et al. 2015; Shannon et al. 2018). Therefore, the shorter distances of 10, 20 and 30 cm were considered for further experimentation as these resulted in higher maximum temperatures (Table 1). As there was a substantial difference between machine-program and temperature, the highest achievable machine-temperature (90 °C) was utilised for further experimentation.

Treatments

Experimental treatments for IAS exposure consisted of a constant machine-programmed temperature (90 °C) and pressure (1600 PSI) with the hot water spray applied from three distances: 10, 20 and 30 cm; and for three durations: 5, 10 and 15 seconds. Shorter exposure durations of spray than that tested in the preliminary study were included as it was considered that 15 seconds / 10 cm^2 was a substantial amount of time for practical guidance when scaling up to the area of a large piece of equipment.

These relatively short durations were informed by in field usage of hot water spray machines by stakeholders as well as research that has identified that the time required for biosecurity is an important factor for biosecurity uptake by stakeholders (Sutcliffe et al. 2018), therefore identifying treatments that require minimal time is desirable. Moreover, previous hot water spray studies have tested durations of 5 and 10 seconds, therefore allowing for ease of comparisons (Morse 2009; Comeau et al. 2011; Stebbing and Rimmer 2013). Control treatment consisted of cold water (15 °C) spray applied from the closest distance (10 cm) for the longest duration of exposure (15 seconds). Complete survival of control treatment individuals would demonstrate that pressure of the spray may dislodge IAS but not induce mortality. Additional exposure durations of 30, 60 and 90 seconds from a distance of 10 cm and 30 cm were conducted for *C. helmsii*, as no mortality was recorded in any of the ≤ 15 seconds treatments. Similarly, to test whether pressure from this longer duration induces mortality in *C. helmsii*, a further control was conducted; cold water (15 °C) spray from the closest distance (10 cm) for the longest duration of exposure (90 seconds). Twelve individual organisms were used per treatment (nine thermal treatments + control), totalling 120 individuals used of each species; 204 for *C. helmsii* including the additional treatments.

All exposure treatments took place in field conditions in October and November 2018. One day prior to exposure, organisms were measured, and one organism randomly placed in individual plastic containers (surface area 548 mm^2, vol 1917 mm^3) with 200 ml of aerated water and a mesh lid (surface area 528 mm^2, vol 1848 mm^3) and housed in a control temperature room (14 °C). Specimens of *D. villosus* were also given a glass bead to provide a refuge and prevent exhaustion from excess swimming. Measurements were taken of the wet weight of *D. villosus* (mean ± SE 0.133 ± 0.015 g; range 0.104–0.170 g) and *D. polymorpha* (1.291 ± 0.029 g; range 0.773–2.231 g) and the length and width of *D. polymorpha* (20.966 ± 0.157 mm; range 17.48–25.02 mm, 11.084 ± 0.114 mm; range 8.48–14.39 mm, respectively). All measurements of the organisms were not statistically different between treatments (ANOVA, $p = > 0.05$ for all). Fragments of *C. helmsii* and *H. ranunculoides* were made by cutting 50 mm of the stem and chlorophyll recorded using a chlorophyll meter as a proxy for plant health (Konica Minolta SPAD 502-Plus). The chlorophyll meter reader determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions, with a higher value indicating a healthier plant. Only mature plants with > 8.8 chlorophyll value and adult invertebrates were assessed as the adult stages have been shown to be less vulnerable than juveniles to environmental change/stressors (Dan et al. 2000; Coughlan et al. 2018; Sebire et al. 2018).

On the day of exposure, containers holding an individual IAS in 200 ml aerated water were transported to the Environment Agency depot and stored

Table 2. Ambient temperature (°C; mean, St. Dev., range) during experimental exposure of invasive alien species on each day.

Day	Species tested	Temperature (°C)		
		Mean	St Dev	Range
1	*Dikerogammarus villosus*	15.98	1.98	12.7–22.0
2	*Hydrocotyle ranunculoides*	16.57	3.03	11.5–23.7
3	*Dreissena polymorpha*	16.87	1.92	13.9–22.1
4	*Crassula helmsii*	15.36	2.22	9.9–20.2
5	*Crassula helmsii*	14.73	3.30	8.2 –22.0
6	*Crassula helmsii*	13.05	3.20	8.2–22.9
7	*Crassula helmsii*	8.40	3.26	4.6–16.8

on-site outdoors in a sheltered shaded location for the duration of the day (approx. 7 hours). The hot water spray machine was programmed (90 °C) and operated continuously for > 5 minutes prior to experimental use to allow all components to reach the desired working temperature. At the time of exposure, one individual organism was placed in the mesh bag attached to the metal backboard, also containing the temperature probe, and sprayed from a set distance for a set duration of time. After exposure, organisms remained within the mesh bag for 30 seconds and were then removed and emersed in a mesh-bottomed container for two minutes to allow for gradual cooling and to avoid a second thermal shock if immediately returned to water. After these two minutes, the individual was returned to the original container with 200 ml aerated water. Remaining in the mesh bag after exposure allowed for the assessment of temperature reduction following exposure, in addition to temperature increase during treatment exposure. To control for slight change in ambient temperature throughout the day, the first replicate of each of the 9 treatments were completed before continuing to the subsequent replicates and repeated until all 12 replicates of each treatment were completed. Experiments were conducted in a sheltered location and postponed if there was excessive wind or rain. Ambient temperature on each day during the time of experimental exposure was measured (Table 2) All treatments for one species were conducted within one day, other than *C. helmsii* which was tested over 2 days; additional experiments of *C. helmsii* (30, 60, 90 seconds exposure from 10 cm and 30 cm) were also tested over 2 days. Post-exposure, containers holding individual IAS were returned to the control temperature room (14 °C).

Mortality testing

To assess the effectiveness of hot water spray treatments, mortality was measured at 1, 8, 24 and 72 hours post-exposure, using methods described in previous studies (Anderson et al. 2015; Shannon et al. 2018). *Dreissena polymorpha* were identified as dead if the shells were gaping and did not close in response to physical stimulus (shell valves gently prodded with blunt-ended forceps; Morse 2009; Comeau et al. 2011). *Dreissena polymorpha* individuals with closed shells were tested for mortality by exerting slight

pressure from fingertips to open the shell (without damaging the organism) and individuals that did not immediately shut their shell after pressure was removed were identified as dead (Comeau et al. 2011). *Dikerogammarus villosus* were identified as dead if they did not move in response to stimulus (gently prodded with blunt-ended forceps) and were discoloured with pereopods not held underneath the body. Chlorophyll measurements of *C. helmsii* and *H. ranunculoides* were recorded using a chlorophyll meter (Konica Minolta SPAD 502-Plus) to assess plant health and mortality. Adjacent pairs of *C. helmsii* leaves were removed to take a chlorophyll measurement due to the mechanics of the reader. To record the chlorophyll readings at each of the 5 time-points (pre-exposure and 1, 8, 24 and 72 hours post-exposure), a pair of leaves were removed and chlorophyll measured. A preliminary experiment testing *C. helmsii* showed no significant difference in chlorophyll meter readings within a plant between leaf-pairings (ANOVA, p = 0.95, n = 35). Therefore, measurements taken from different locations on the plant at each time point would not show difference in readings due to those locations. Chlorophyll content was then used as a proxy for plant health, with measurements of < 0.8 being assumed dead (Dan et al. 2000). Additionally, plants were kept for 2 weeks post-exposure to observe any root and/or shoot regrowth following treatments (see Crane et al. 2018; Cuthbert et al. 2019).

Statistical Analysis

All statistical analyses were conducted in R (Version 3.6.3). Analysis of variance (ANOVA) was used to compare chlorophyll values of each leaf-pairing within an individual *C. helmsii* plant to validate sampling leaf-pairing at each mortality time point (n = 35). Separate ANOVAs were also conducted to compare the weight of *D. villosus* and *D. polymorpha* and the length and width of *D. polymorpha* between treatments (n = 12 per treatment, 10 treatments). Residuals were checked for normality and where non-normal, data was log transformed and the ANOVA re-run.

To assess IAS mortality across time between the hot water spray treatments and control cold water spray treatment, a Cox Proportional Hazards Regression model was run for each species. Due to the model being unable to compare a control with 0% mortality at all time-points, one replicate of the control treatments was redefined as "dead" at the last time point (72 hours post-exposure). This analysis was therefore more conservative and allowed for comparisons of the cold water spray control to the hot water spray treatments. The effect of hot water spray treatments on IAS mortality were then analysed without the control treatment data to allow for further analyses into the components of the treatments: "distance" and "duration" of spray application. For each species, a Cox Proportional Hazards Regression model was run to assess the effect of distance and duration on IAS mortality across time. Initially an interaction term

(distance × duration) was included and removed if found to be statistically non-significant. Models with/out an interaction term were compared using Akaike information criterion (AIC) tests. Significant distance and duration factors were subsequently analysed using log-link pairwise comparisons.

Following the assessment of treatment on IAS mortality, the on-contact water temperatures during treatments were explored. The temperature probe recorded the on-contact temperature every second, and values were extracted for during the exposure (5, 10 or 15 seconds), 5 seconds pre-exposure and 30 seconds post-exposure. Commencement of treatments were identified by a 1.0 °C/second increase. The temperature data for the three IAS exposure treatments (5, 10 and 15 seconds from 10, 20 and 30 cm, n = 36) were analysed. Additionally, analysis was also conducted for the temperature data from the further treatments of *C. helmsii* (30, 60 and 90 seconds from 10 and 30 cm, n = 12). The maximum water temperature was compared between treatments using an ANOVA. Models were checked for variance and normality of residuals. Where residuals were non-normal, data was log transformed and the ANOVA re-run and residual normality checked. Tukey HSD *posthoc* tests were performed to compare hot water spray treatments to the control cold water spray treatment. Following this, the control data was removed to allow for the components of the treatments, distance and duration, to be analysed in relation to the maximum temperature. Models were checked for normality of residuals and variance. Where non-normal, data was log transformed and the ANOVA re-run and checked for normality of residuals. Initially an interaction term was included (distance × duration) and removed if found to be statistically non-significant. Models with/out an interaction term were compared using AIC tests. Tukey HSD *posthoc* tests were run to identify significance between levels of factors (distance and duration).

To assess the total thermal exposure an area under the curve (AUC) analyses were run. Total thermal exposure was calculated using a baseline of 25 °C; temperature values greater than the baseline for the treatment exposure and 30 seconds post-exposure were summed to give a total thermal exposure. The control was not analysed as all thermal exposure values were 0 as temperature values were < 25 °C in all cases. Initially the effect of treatment on total thermal exposure was analysed using ANOVA with Tukey HSD *posthoc* tests. Models were checked for normality of residuals and variance. Where non-normal, data was log transformed and the ANOVA re-run and checked for normality of residuals and variance. Following this, the effect of the components of the treatments (distance and duration) on thermal exposure were analysed. Initially an interaction term (distance × duration) was included and removed if found to be statistically non-significant. Models with/out an interaction term were compared using an AIC tests. Tukey HSD *posthoc* tests were run to identify significance between levels of factors (distance and duration).

Table 3. Percentage mortality of (a) *Dikerogammarus villosus*, (b) *Dreissena polymorpha*, (c) *Crassula helmsii* and (d) *Hydrocotyle ranunculoides*, 24 hours after exposure to high-pressurised hot water spray (n = 12). Treatments consisted of hot-water spray (machine temperature, 90 °C) exposure for durations of 5, 10 and 15 seconds from a distance of 10, 20 and 30 cm (a, b, c), 30, 60 and 90 seconds from 10 cm and 30 cm (c) and hot water (machine temperature, 90 °C) and cold water (18 °C) spray for 5 seconds from a distance of 30 cm (d). Green shading indicates complete mortality.

a

Dikerogammarus villosus		Distance		
		10 cm	20 cm	30 cm
Duration	5 secs	100%	100%	75%
	10 secs	100%	100%	83%
	15 secs	100%	100%	83%

b

Dreissena polymorpha		Distance		
		10 cm	20 cm	30 cm
Duration	5 secs	58%	25%	17%
	10 secs	92%	50%	25%
	15 secs	100%	83%	33%

c

Crassula helmsii		Distance		
		10 cm	20 cm	30 cm
Duration	5 secs	0%	0%	0%
	10 secs	0%	0%	0%
	15 secs	0%	0%	0%
	30 secs	0%		0%
	60 secs	0%		0%
	90 secs	0%		0%

d

Hydrocotyle ranunculoides		Water Temperature	Distance, 30 cm
Duration	5 secs	Hot Water 90 °C	100%
	5 secs	Cold Water 18 °C	100%

Results

Hot water spray caused mortality in both invasive animal species tested, with higher mortality at longer durations and shorter distances. Complete mortality was achieved with hot water spray treatment applied for 15 seconds from 10 cm, for both *D. villosus* and *D. polymorpha*, 24 hours after exposure (Table 3a, b). All durations of spray applied from 10 cm and 20 cm caused 100% mortality in *D. villosus*, whilst spray applied from 30 cm resulted in high mortality (75–83%; Table 3a). *Dreissena polymorpha* was more resistant to hot water spray treatments. Complete mortality was only achieved at the longest duration (15 seconds) and the shortest distance (10 cm). High mortality of *D. polymorpha* (> 80%) was seen following hot water spray treatments of 10 seconds from 10 cm and 15 seconds from 20 cm (Table 3b). For both invasive animal species further mortality was observed 72 hours post exposure, however complete mortality was not achieved in any treatments that had incomplete mortality at 24 hours (see Table S1 for 72 hour mortality). Due to this, and for comparison to previous biosecurity studies (Comeau et al. 2011; Anderson et al. 2015; Shannon et al. 2018), here we report 24 hour mortality (Table 3). No mortality of *D. polymorpha* and *D. villosus* was seen in the cold water control spray treatment.

Table 4. Cox proportional hazards regression analysis for mortality of *Dikerogammarus villosus* and *Dreissena polymorpha* exposed to hot water spray treatments compared to the cold water spray (control) treatment. Grey shading indicates significance. The hazard ratio (exp(coef)) and range (lower.95 and upper.95) shows at a given point in time the risk of death compared to that of the control.

Distance (cm)	Duration (seconds)	*Dikerogammarus villosus*				*Dreissena polymorpha*			
		exp(coef)	Lower .95	Upper .95	p	exp(coef)	Lower .95	Upper .95	p
10	5	51.80	6.575	408.0	< 0.001	12.32	1.538	98.73	0.018
10	10	51.80	6.575	408.0	< 0.001	39.87	5.093	312.18	< 0.001
10	15	51.80	6.575	408.0	< 0.001	46.75	5.961	366.56	< 0.001
20	5	51.80	6.575	408.0	< 0.001	6.18	0.722	52.91	0.096
20	10	51.80	6.575	408.0	< 0.001	12.37	1.545	99.05	0.018
20	15	51.80	6.575	408.0	< 0.001	25.00	3.181	196.48	0.002
30	5	21.20	2.676	168.0	0.003	3.39	0.353	32.63	0.290
30	10	24.20	3.081	190.1	0.002	6.26	0.731	53.58	0.094
30	15	30.79	3.945	240.3	0.001	6.85	0.800	58.61	0.079

In contrast, complete survival was observed in *C. helmsii* for all treatments at all time-points (1, 8, 24, 72 hour) after exposure, including the additional treatments of 30, 60 and 90 seconds from 10 cm and 30 cm (Table 3c). There was also complete *C. helmsii* survival in cold water control treatments. Fragmentation of *H. ranunculoides* occurred when exposed to spray treatments, therefore treatments of 5 seconds exposure from 30 cm for hot water spray and cold water spray was tested. Both cold and hot water spray treatments resulted in 100% mortality of *H. ranunculoides* at all time-points after exposure (Table 3d). No recovery of *H. ranunculoides* was seen 14 days after exposure.

Mortality was significantly higher for all hot water spray treatments in comparisons with the control cold water spray treatment for *D. villosus* (Cox regression, all p = < 0.05; Table 4). Mortality was significantly higher for all hot water spray treatments in comparisons with the control cold water spray treatment for *D. polymorpha*, except for 4 treatments (Cox regression, Table 4). The hot water spray treatments that were not significantly different to the control cold water for *D. polymorpha* mortality were treatments from longer distances; 30 cm for 5 seconds, 10 seconds and 15 seconds and 20 cm for 5 seconds (p = 0.290, p = 0.094, p = 0.079, p = 0.096, respectively; Table 4). However, these hot water spray treatments had a hazard ratio > 1.0, therefore at a given time point had a greater risk of death compared to the control cold water spray treatment (Table 4). *Crassula helmsii* mortality was not analysed as there was 100% survival in all treatments, and therefore no variability in the data.

Further analysis was conducted into the effect of the components of the hot water spray treatments (distance and duration) on IAS mortality. For *D. villosus*, there was a significant effect of distance ($\chi^2 = 9.83$, p = 0.007) but not duration of spray ($\chi^2 = 0.19$, p = 0.909) on mortality. There was no significant interaction between distance and duration ($\chi^2 = 0.47$, p = 0.977) which was removed, thus improving the model (model 1 v model 2, AIC 794.21, 786.68). Pairwise comparisons found there was significantly lower *D. villosus* mortality in 30 cm treatments compared to 10 cm and 20 cm

Table 5. Pairwise comparison of cox proportional hazards regression model assessing the mortality of *Dreissena polymorpha* exposed to treatments of three durations of thermal spray applied from three distances. Grey shading indicates significance.

		10 cm			20 cm			30 cm		
		5 sec	10 sec	15 sec	5 sec	10 sec	15 sec	5 sec	10 sec	15 sec
10 cm	5 sec	–	–	–	–	–	–	–	–	–
	10 sec	0.042	–	–	–	–	–	–	–	–
	15 sec	0.011	0.982	–	–	–	–	–	–	–
20 cm	5 sec	0.307	0.009	0.002	–	–	–	–	–	–
	10 sec	0.982	0.055	0.013	0.307	–	–	–	–	–
	15 sec	0.256	0.404	0.307	0.042	0.305	–	–	–	–
30 cm	5 sec	0.091	0.003	0.001	0.492	0.091	0.011	–	–	–
	10 sec	0.307	0.007	0.002	0.982	0.307	0.040	0.503	–	–
	15 sec	0.423	0.018	0.008	0.982	0.404	0.085	0.476	0.982	–

treatments (p = 0.007, exp(coef) = 1.998, lower/upper .95 = 1.20–3.32, for both). For *D. polymorpha*, there was a significant effect of both distance (χ^2 = 22.63, p < 0.001), and duration of hot water spray (χ^2 = 16.25, p < 0.001) on mortality. There was no significant interaction between distance and duration (χ^2 = 1.470, p = 0.832) which was removed, thus improving the model (model 1 v model 2; AIC 549.43, 542.90). Pairwise comparisons identified significance between treatments (Table 5). Treatment applied from 10 cm for 15 seconds (closest distance and longest duration) showed significantly higher mortality compared to all treatments, except for treatments applied from 20 cm for 15 seconds (p = 0.31) and 10 cm for 10 seconds (p = 0.98). Where treatments were applied from 30 cm, the effect on *D. polymorpha* mortality was not significantly dependent on duration of treatment (p = > 0.05 for all).

Following the identification of the effect of hot water treatment on IAS mortality, we explored the maximum temperature and total thermal exposure during each treatment to confirm the thermal differences between treatments. The water temperature during exposure, 30 seconds post-exposure and 5 second pre-exposure was extracted (Figure 2).
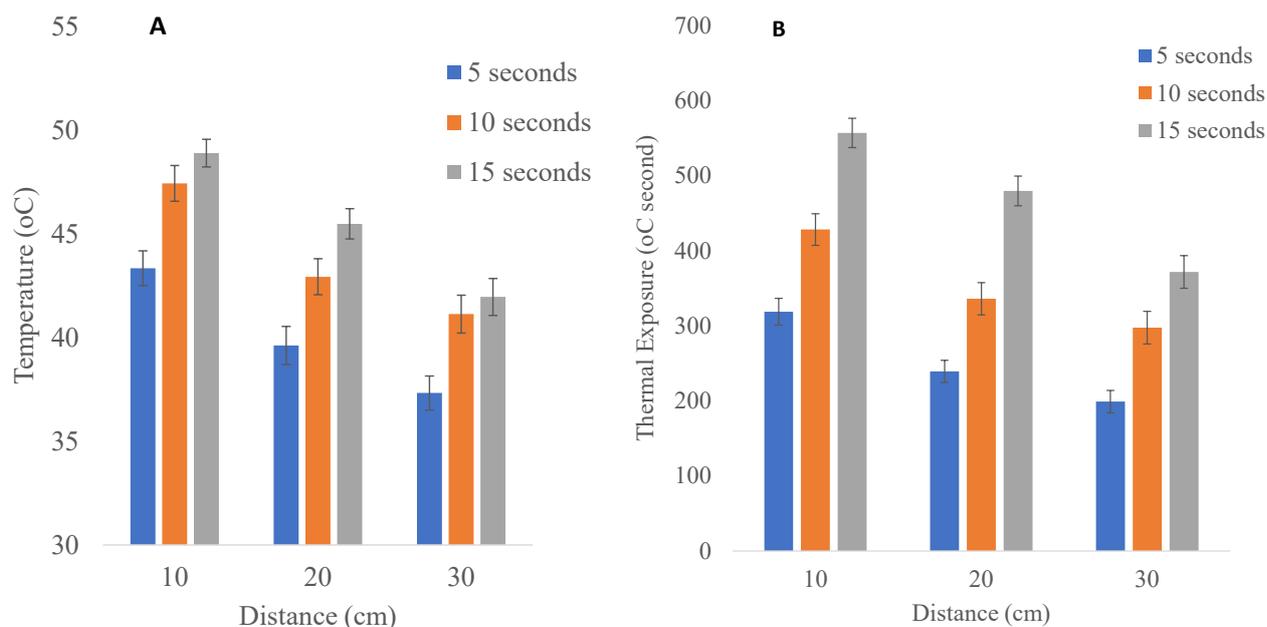
The average maximum temperatures of hot water treatments applied ranged from 37.3 °C ± 0.82 (SE) to 48.9 °C ± 0.67 (Figure 3a). There was a significant effect of treatment on the maximum water temperature (data log transformed, ANOVA, $F_{9, 350}$ = 202.8, p < 0.001). Tukey HSD *posthoc* test identified the water temperature of the control cold water treatment was significantly different to all hot water treatments (p = < 0.001 for all). Thus, the control data were removed from the data set to allow for the assessment of the effect of hot water treatments and their components, distance and duration on the water temperature. The maximum temperature was compared between hot water spray treatments using an ANOVA (data log transformed). There was a significant effect of treatment (ANOVA, $F_{8,315}$ = 19.95, p < 0.001) and *post-hoc* Tukey HSD analysis identified significant differences between individual treatments (Table S2). Treatment applied from 10 cm for 15 seconds (shortest distance and longest duration) had a significantly higher maximum temperature (48.9 °C ± 0.67) to all
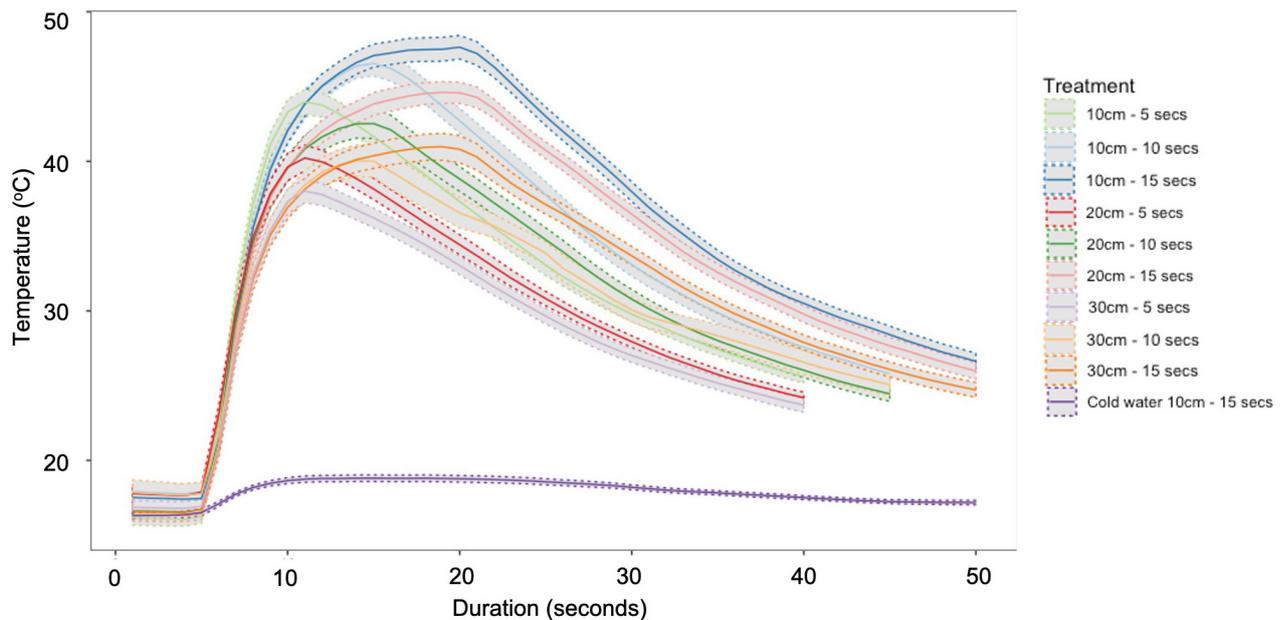
Figure 2. Average temperature of hot water spray treatment applied to *Dreissena polymorpha, Dikerogammarus villosus* and *Crassula helmsii* from three distances (10, 20, 30 cm) and for three durations (5, 10, 15 seconds) and control cold water treatment (from 10 cm for 15 seconds). Grey shading shows standard error of average (n = 36).

treatments (p < 0.001), except for treatment applied from 10 cm for 10 seconds (p = 0.959; 47.4 °C ± 0.87) and from 20 cm for 15 seconds (p = 0.868; 45.5 °C ± 0.73). Treatment applied from 30 cm for 5 seconds (longest distance for the shortest duration) had the lowest average maximum temperature (37.3 °C ± 0.82) and was significantly different to all treatments except for the 5 second treatment from 20 cm (39.62 °C ± 0.91; p = 0.461). We then explored the effect on maximum temperature of the components of treatment; distance and duration (data log-transformed). An interaction term of distance and duration was non-significant (ANOVA, $F_{4,315} = 0.285$, p = 0.089) which was removed, thus improving the model (model 1 v model 2, AIC = −998.45 v AIC = −1005.27). Both distance and duration of spray had a significant effect on the maximum temperature (ANOVA, distance: $F_{2,319} = 45.76$, p < 0.001, duration: $F_{2,319} = 34.20$, p < 0.001). Tukey HSD *posthoc* analysis found all distances were significant from one another (p < 0.001 for all). The shortest duration treatment, 5 second were significantly different from 10 second and 15 second treatments (Tukey HSD *posthoc,* p < 0.001, for both), whilst 10 second and 15 second treatments were only marginally significantly different (p = 0.047).

The total thermal exposure was calculated by summing the temperature (°C) during treatment and 30 seconds post-exposure, above a baseline of 25 °C, herein termed "thermal exposure". The control cold water spray treatments were not analysed with the hot water spray treatments as the water temperature during control exposure did not exceed 25 °C. As predicted, thermal exposure increased as spray application duration increased and/or distance decreased (Figure 3b). There was a significant effect of treatment on thermal exposure (ANOVA, $F_{8,314} = 34.47$, p < 0.001)

Figure 3. (A) Average maximum temperature, (B) Average thermal exposure (area under the curve), of hot water spray applied to *Dreissena polymorpha, Dikerogammarus villosus* and *Crassula helmsii* from three distances (10, 20, 30 cm) and for three durations (5, 10, 15 seconds). Error bars show standard error (n = 36).

and Tukey HSD *posthoc* analyses identified statistically significant differences between treatments (Table S3). Treatment applied from 10 cm for 15 seconds (closest distance and longest duration) had the highest thermal exposure (557.17 °C seconds ± 19.55), which was significantly different to all other treatments (p < 0.001 for all) except for treatment from 20 cm for 15 seconds (479.93 °C seconds ± 19.88, p = 0.120). Treatment applied from 10 cm for 5 seconds (318.92 °C seconds ± 17.97; closest distance and shortest duration) was not significantly different to the treatment applied from 30 cm for 10 seconds (297.72 °C seconds ± 21.71; p = 0.997) or 15 seconds (371.98 °C seconds ± 21.80; p = 0.589; furthest distance and longest duration). Analysis of the components of the treatment that found duration and distance of spray had a significant effect on thermal exposure (ANOVA, $F_{2,314} = 41.52$, p < 0.001; $F_{2,314} = 93.51$, p < 0.001, respectively). There was no significant interaction between duration and distance (ANOVA, $F_{4,314} = 1.268$, p = 0.282 which was removed, thus improving the model (model 1 v model 2, AIC = 3999.82 v 3997.00). Tukey HSD *posthoc* analyses identified significant differences in thermal exposure between each of the distances and each of the durations (p = < 0.001, for all).

Despite the lack of mortality of *C. helmsii* in the longer duration treatments, the maximum temperature and thermal exposure from hot water spray treatments applied from 10 cm and 30 cm for 30 seconds, 60 seconds and 90 seconds was explored. This highlighted that spray applied for > 30 seconds showed little difference in maximum temperature achieved. However, the distance which the hot water spray is applied from had a significant impact on maximum temperature achieved on contact (Figure 4a).
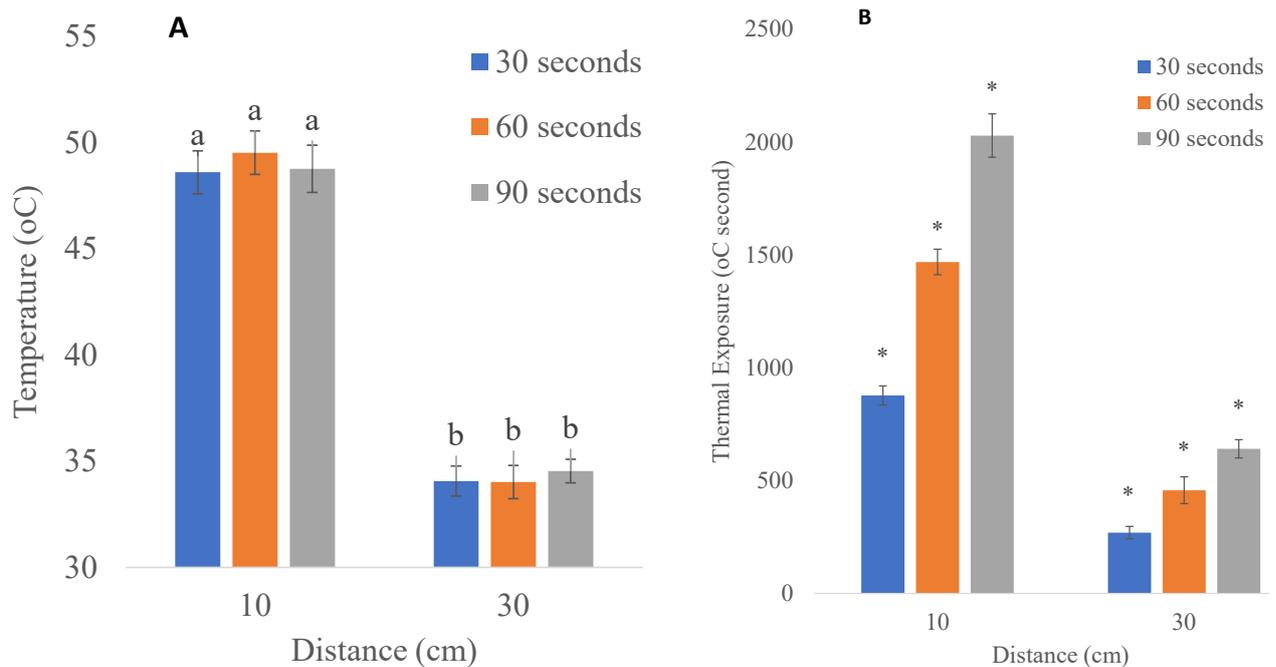
Figure 4. (A) Average maximum temperature, (B) Average thermal exposure (area under the curve), of hot water spray applied to *Crassula helmsii* from two distances (10 and 30 cm) for three durations (30, 60, 90 seconds). Error bars show standard error (n = 12), * show statistical significance and letters show treatments statistically the same to another.

The average maximum temperatures ranged from 34.0 °C ± 0.78 (30 cm for 60 seconds) to 49.5 °C ± 1.02 (10 cm for 60 seconds; Figure 4a). An outlier was identified and removed from the dataset (control treatment). There was a statistically significant effect of treatment on the maximum temperature (data log transformed, ANOVA, $F_{6,88}$ = 300.8, p < 0.001). Tukey HSD *posthoc* found all hot water spray treatments were statistically different to the control cold water treatments (p < 0.05 for all). Thus, the control data was removed from the data set to allow for the assessment of the effect of the components of treatment, distance and duration, on the maximum temperature. There was a significant effect of treatment on the maximum temperature (ANOVA, $F_{5,66}$ = 83.4, p < 0.001). Tukey HSD *posthoc* analysis identified all treatments applied from 10 cm were significantly different from all treatments applied from 30 cm (Figure 4a); there was no statistical significance between treatments applied from the same distance (p = > 0.97 for all). This was further confirmed with the analysis of the effect of distance and duration of spray on the maximum temperature. The interaction term of distance and duration was non-significant (ANOVA, $F_{2,66}$ = 0.286, p = 0.752) which was removed, thus improving the model (model 1 v model 2, AIC = 373.61 v 370.23). Distance was found to have a significant effect upon maximum temperature (ANOVA, $F_{1,66}$ = 425.08, p < 0.001) whilst duration of spray was found to be non-significant (ANOVA, $F_{2,66}$ = 0.133, p = 0.875). A similar trend in the thermal exposure data was found in the further hot water spray treatments applied to *C. helmsii*; as the duration increased and/or the distance decreased, the thermal exposure

increased (Figure 4b). The highest thermal exposure was 2028.74 °C seconds (± 96.42 SE) from hot water spray treatment applied from 10 cm for 90 seconds. There was a significant effect of treatment on thermal exposure (data log transformed, ANOVA, $F_{5, 66} = 109.3$, $p < 0.001$). Tukey HSD *posthoc* analysis identified all treatments were significantly different from each other ($p < 0.01$, for all). The interaction term of distance and duration was non-significant (ANOVA, $F_{2,66} = 0.156$, $p = 0.856$), which was removed, thus improving the model (model 1 v model 2, AIC = −1005.19 v AIC = −1008.85) Distance had a significant effect on thermal exposure (ANOVA, $F_{1,66} = 415.47$, $p < 0.001$) as did duration (ANOVA, $F_{2,66} = 72.21$, $p < 0.001$).

Discussion

High pressure hot water spray is successful in achieving complete mortality in the two tested invasive invertebrates when applied from a close distance of 10 cm for 15 seconds. In contrast hot water spray was ineffective against *C. helmsii*, even when applied for 90 seconds. Despite the highest machine temperature being used, the cooling of the water between exiting the heating component and the contact point was apparent as maximum water temperature did not exceed 50 °C (Stebbing and Rimmer 2013). Survival of IAS in the control treatments demonstrated the pressure of the spray does not induce mortality in the majority of IAS tested here. However, in field usage, high pressure sprays may, however dislodge IAS. This highlights the importance of containment and safe disposal of treatment water as it may contain living IAS. Longer durations of spray were successful in achieving mortality, of the animals tested. However, there may be a limited application for this in the field; spray applications for 15 seconds to a focal point of 10 cm² area, would require a total of 25 minutes to apply thermal treatment to a 1 m² area. As practitioners have identified the importance of time when considering ease of biosecurity (Sutcliffe et al. 2018), more efficient as well as effective practices may be required.

This is the first study to assess the effectiveness of hot water spray to induce mortality in the invasive plant *C. helmsii* in field conditions. Thermal immersion studies have identified that *C. helmsii* is particularly resilient to thermal treatments compared to other IAS (Anderson et al. 2015; Shannon et al. 2018). High survival has been documented when immersed in 45 °C for up to 10 minutes, and complete mortality was only achieved with immersion in ≥ 55 °C water for ≥ 1 minute (Shannon et al. 2018). Similar maximum temperatures were achieved with hot water spray treatments, however immersion exposures were both of considerably longer durations, at uniform temperatures. A combination of higher temperatures and longer durations of spray exposure (> 90 seconds) would be required to achieve *C. helmsii* mortality which would be of limited feasibility and practical

application. The other invasive plant species tested here, *H. ranunculoides*, showed fragmentation and subsequent complete mortality when exposed to both hot and cold water spray treatment. Therefore high pressure sprays are a suitable treatment for this IAS, although this mortality cannot be associated with thermal exposure and was likely due to the pressure of the spray.

Our findings are consistent with previous hot water spray studies that found 100% mortality occurred in *D. polymorpha* when exposed to hot water spray in laboratory conditions (Morse 2009) and in field conditions (Watters 2014). Similarly, mortality of *D. villosus* was consistent with that of Stebbing and Rimmer (2013), however we found no mortality in cold water spray treatments whilst their study reported up to 30% mortality. The assessment of *D. villosus* and *D. polymorpha* also provides evidence for the potential effectiveness of hot water spray against other IAS of high concern, including *Dreissena rostriformis bugensis* and *Dikerogammarus haemobaphes*. The hot water spray treatments seen to be effective here are likely also effective against these other closely related species, however direct assessment should be made (Peyer et al. 2009; Sebire et al. 2018). Furthermore, for biofouling species such as *Dreissena* spp.*, further assessment of effectiveness to induce mortality in groups of self-adhered individuals is needed, as likely when grouped certain individuals may not receive direct thermal spray during treatments in such scenarios. A wider range of freshwater IAS need to be assessed, across different taxonomic groups to identify the effectiveness of hot water spray machines to induce mortality in field conditions and therefore reduce the risk of accidental spread on pieces of large equipment.

This study highlights the importance of safe containment and disposal of treatment water. The incomplete mortality of IAS reported here highlights that when using hot water spray machines, propagules of IAS may be removed during cleaning yet are still viable, especially in the case of *C. helmsii*. Thus, biosecurity wash-down stations must consider this potential for inadvertent spread and either be enclosed with an interceptor to remove IAS from treatment water or at the exit point of a waterbody, therefore water run-off will enter the same waterbody from which the equipment originated. Therefore, biosecurity guidance should prioritise equipment having been cleaned prior to arrival at a location, such as a lake. Cleaning upon arrival to a location would not be sufficient to reduce the risk of invasive plant invasion as it is likely that hot water spray will not cause complete mortality, rather just dislodge fragments which may then enter the water course. A combination of thermal exposure and pressure to remove fragments in a controlled setting should reduce the risk of IAS spread.

Hot water spray machine operator behaviour must be considered when outlining biosecurity guidelines. Furthermore, personal observations have highlighted that the majority of operators use a sweeping motion when cleaning equipment; this may result in a specific area being directly sprayed

for a set time duration, however this exposure is not consistently applied. We found that the initial seconds of exposure show a dramatic increase in temperature, however ≥ 35 °C was not achieved until after ~ 3 seconds of hot water spray application. Furthermore, the maximum temperature achieved was significantly affected by both the duration and the distance the spray was applied from. These higher temperatures are needed to induce IAS mortality, therefore continuous spray to an area is needed. Furthermore, it is key to identify treatments appropriate in accordance with health and safety requirements, with likely differences in requirements dependent on commercial or recreational use. Recreational applications need to consider the potential for children and the elderly to be present and therefore the potential for scalding, known to occur at temperatures greater than 52.0 °C (Feldman et al. 1998). Training and awareness delivery to stakeholders must be designed for the use of these hot water spray machines for effective cleaning to remove IAS, including specifics concerning spray application methods and ongoing monitoring.

Hot water spray machines are widely used for the cleaning of large equipment including vehicles and boats, both in commercial and recreational industries. Desirable attributes to this biosecurity method include being a widely available product that is already widely used, requires minimal training and has less health and safety issues to that of steam methods. To reduce the risk of accidental spread of IAS, hot water spray applications must be applied continuously at a close distance for a long duration. Furthermore, given the incomplete mortality reported here, care must be taken to consider that any dislodged invasive organisms within water run-off may still be a viable propagule and therefore pose a risk of IAS spread. The use of thermal shock in combination with manual removal and desiccation should be recommended in biosecurity protocols to prevent introductions and further spread of IAS.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Percentage mortality of (a) *Dikerogammarus villosus*, (b) *Dreissena polymorpha*, 72 hours after exposure to high-pressurised hot water spray (n = 12).

Table S2. Tukey HSD *posthoc* outputs comparing average maximum temperature between hot water spray treatments applied to *Dreissena polymorpha, Dikerogammarus villosus* and *Crassula helmsii*.

Table S3. Tukey HSD *posthoc* outputs comparing average thermal exposure (area under the curve) between hot water spray treatments applied to *Dreissena polymorpha, Dikerogammarus villosus* and *Crassula helmsii*.

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