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SHORT COMMUNICATION

Touch too much: aquatic disinfectant and steam exposure treatments can inhibit further spread of invasive bloody-red mysid shrimp *Hemimysis anomala*

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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. However, spread-prevention of invasive peracarids is especially difficult due to ineffectiveness of detection and treatment options. For instance, bloody-red mysid shrimp, *Hemimysis anomala*, is a high impact ecosystem-destabilising invader, which continues to spread in both Europe and North America. Here, we examine the effectiveness of two commonly used aquatic disinfectants (Virasure®/Virkon® Aquatic), and steam treatments (≥ 100 °C) to kill *H. anomala*. Specimens were exposed to 1% disinfectant solutions for complete immersion or mist-spray treatments, both lasting 60 s. Steam exposures lasted for 10 or 30 s. All treatments caused 100% mortality of *H. anomala*. Accordingly, it appears that relatively brief exposures to disinfectant and steam treatments can curtail further *H. anomala* spread. Therefore, these treatments should be used to decontaminate all equipment, from wetsuits to boats. In particular, steam and disinfectant spray treatments may be useful for decontamination of large, complex equipment, such as vehicles, trailers, outboard motors, or live wells on fishing boats.

Keywords Biosecurity · Decontaminate · Disinfectant spray · Immersion · Invasive alien species · Steam spray

Introduction

Biological invasions often disrupt ecosystem structure and functioning, with adverse ecological and socioeconomic impacts (Ricciardi and MacIsaac 2011). Due to their exposure to multiple natural and anthropogenic transport pathways and a plethora of possible vectors (Rothlisberger et al. 2010; Coughlan et al. 2017), freshwater ecosystems are considered to be especially vulnerable to the introduction of invasive alien species (IAS) (Ricciardi and MacIsaac 2011; Piria et al. 2017). Unlike terrestrial habitats, submerged aquatic environments are particularly difficult to monitor and, as a result, invasions are often well advanced before they become apparent (Beric and MacIsaac 2015; Caffrey et al. 2016). Furthermore,

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management options for effective population suppression, or eradication of established invader populations, are often complex, expensive and damaging to non-target species (Piria et al. 2017; Coughlan et al. 2018). Accordingly, biosecurity protocols designed to prevent the introduction and spread of IAS are now considered to be an essential component of management strategies (Joyce et al. 2019; Bradbeer et al. 2020). However, despite the implementation of decontamination protocols, the species-specific efficacy of many proposed biosecurity applications is often unclear or unknown (Coughlan et al. 2019; De Stasio et al. 2019). As such, validation of the effectiveness of many existing techniques is required (De Stasio et al. 2019; Piria et al. 2017; Crane et al. 2020).

In recent years, oxidising-agent based disinfectants, such as Virasure® Aquatic and Virkon® Aquatic, have been increasingly used by various stakeholder groups to facilitate decontamination of equipment. Frequently, this trend appears to be based on perceived rather than proven effectiveness of these products as decontamination agents for IAS. For example, recent studies have only described partial effectiveness of these disinfectants in killing fragmentary propagules of invasive aquatic plants (Cuthbert et al. 2018, 2019; Crane et al. 2020) or invertebrates with a protective shell (Coughlan et al. 2019, 2020). However, crustaceans such as the killer shrimp, *Dikerogammarus villosus* Sowinsky, 1894, appear to be highly susceptible to brief disinfectant immersion and mist-spray exposure (Bradbeer et al. 2020). Equally, thermal shock treatments, such as rapid applications of steam, have also been proposed as a mechanism to enable improved decontamination of equipment that vector IAS (Joyce et al. 2019). In particular, applications of steam lasting 10–30 s can effectively kill invasive macrophytes (Crane et al. 2019) and invertebrates (Coughlan et al. 2019, 2020; Joyce et al. 2019; Bradbeer et al. 2020).

The bloody-red mysid shrimp, *Hemimysis anomala* Sars, 1907, is a highly invasive, eurytolerant and ecosystem-destabilizing crustacean species of Ponto–Caspian origin, which has invaded European waterways and the Laurentian Great Lakes (Iacarella et al. 2015; Sinclair et al. 2016). Notably, *H. anomala* has been observed to form self-sustaining populations in wetland ecosystems, such as within the Rhine and Danube rivers deltas, which have acted as major source pools for its further spread (Audzijonyte et al. 2008). *Hemimysis anomala* can negatively affect populations of native zooplankton and mysid species, resulting in both bottom-up and top-down impacts in nearshore food webs (Iacarella et al. 2015; Sinclair et al. 2016). Here, we assessed the effectiveness of disinfectant immersion and mist-spray treatments for two commonly used aquatic disinfectants: Virasure® and Virkon® Aquatic. In addition, we examined the effectiveness of rapid exposure to direct steam spray. As these treatments can effectively cause mortality of invasive invertebrates that lack a protective shell, such as *D. villosus*, we hypothesise that all examined treatments will cause mortality of *H. anomala* specimens.

Methods

Specimen collection and maintenance

Hemimysis anomala specimens were collected from Garrykennedy Harbour, Lough Derg, Republic of Ireland (52° 54′ 16.8″ N, 8° 20′ 39.1″ W). Specimens were transported in source water to the Queen's University Marine Laboratory, Northern Ireland, UK. Specimens were then maintained in aerated aquaria containing dechlorinated tap water at a constant temperature of 13 °C, under a 12:12 h photoperiod regime. Fresh tap water was dechlorinated through aeration for a 24–48 h period prior to use. Specimens were acclimated to laboratory conditions for 1 week, and only active individuals that responded to tactile stimuli were selected for assessment. Approximately one quarter of the collected species expired prior to experimental assessment. For all experiments, individual adult specimens were used (total length: 9–14 mm).

Immersion in disinfectant solutions

The efficacy of Virasure® Aquatic (Fish Vet Group) and Virkon® Aquatic (Antec Int. DuPont) was examined using 1% (10 g L^{-1}) disinfectant solutions, and a 0% (0 g L^{-1}) control. A concentration of 1% was selected as this is the minimum recommended by the manufacturers for general disinfection through immersion, with 1% solutions also having been shown to successfully cause mortality of *D. villosus*

(Bradbeer et al. 2020) and mosquito larvae (Cuthbert et al. 2020). All solutions were made using tap water that had been aerated in advance for > 24 h. All treatment groups were replicated three times (i.e. $n = 3$), and each replicate contained five mysids. Prior to experimentation, replicate groups of five individual *H. anomala* were briefly maintained (< 10 min) in aerated tap water. Using fine-meshed 100 µm sieves, groups were completely immersed into disinfectant solutions for 60 s. Control groups were likewise immersed in aerated tap water (i.e. 0% solution) for 60 s. Following experimental exposure, to prevent an extended exposure time due to lingering chemical effects, all groups of *H. anomala* were removed from the experimental solution and re-immersed into aerated tap water for a 2-min period to remove excess disinfectant. All groups were then immediately rinsed for second time in fresh aerated tap water, for a further 2-min. Following this, specimen groups were returned to 250 mL of aerated tap water for an arbitrarily-chosen standardised recovery period of 6 h (13 °C, i.e. pre-experimental maintenance conditions) after which mortality was formally assessed. Specimens were considered dead if they did not respond to tactile stimuli.

Disinfectant mist-spray

Mist-spray applications for both disinfectants were examined using 1% solutions, and a 0% control. All solutions were made using dechlorinated tap water that had been aerated in advance for > 24 h. As before, separate groups of five individual *H. anomala* were briefly maintained in aerated tap water ($n = 3$ per treatment group). Using fine-meshed 100 µm sieves, the groups were then directly exposed to ~ 4 mL of solution via an application of mist-spray, delivered using a hand-held mist-spray bottle at a distance of 3–4 cm from the bottle exit-point. Groups were then left air-exposed for a 1-min period (~ 20 °C), before being immersed in aerated tap water to removed excess disinfectant. As before, the two-minute washing process to remove excess disinfectant was repeated twice. Following this, specimen groups were returned to 250 mL of aerated tap water and mortality was assessed following a six hour recovery period (13 °C; as above). Control groups were exposed to the same methodological process, but were sprayed with aerated tap water.

Steam spray

Specimens were directly exposed to a continuous jet of steam for 10 or 30 s (≥ 100 °C; 350 kPa; Karcher® SC3). Groups of five individual *H. anomala* were briefly maintained in aerated tap water ($n = 3$ per treatment group). Using fine-meshed 100 µm sieves, steam was directly applied to groups held within sieves at a distance of 6–8 cm from the spout of the lance. Following exposure, to avoid a secondary thermal shock, groups were air-exposed for a 5-min period (~ 20 °C) before being re-immersed in tap water. Control groups were air-exposed for 5-min. Mortality was assessed following a 6-h recovery period (13 °C; as above).

Statistical analysis

Separate binomial generalised linear models with logit links were used to assess the efficacy of treatments (i.e. (1) disinfectant soak, (2) disinfectant mist-spray, and (3) steam spray) in driving mortality of *H. anomala*. Bias reductions were used to account for complete separation. Analysis of deviance with Type II sums of squares were used to test for the main treatment effect in each model. *Post-hoc* pairwise comparisons of treatments were undertaken using estimated marginal means with Tukey-style adjustments for multiplicity. Significance was considered at the 95% confidence level. All statistical analyses were performed in the R software environment (version 3.4.2: 2018), using the ‘car’, ‘emmeans’ and ‘brglm2’ packages.

Results

Immersion in disinfectant solutions

Immersion in disinfectant had a significant effect on mysid mortality (GLM: $\chi^2 = 54.43$, df = 2, $P < 0.001$). Both Virkon® Aquatic and Virasure® Aquatic caused total mortality of treated specimens, with no mortality observed for control groups (both $P = 0.003$ compared to controls; Table 1). Both disinfectant products had similar efficacies (both $P > 0.05$). As an observation, although mortality was formally assessed following a 6-h recovery period, it appeared that all *H. anomala* were

Table 1 Mean (± SE) raw percentage mortality of *Hemimysis anomala* at 6 h following exposure to disinfectant and steam treatments

Treatment	Concentration	Exposure	
Immersion in disinfectants		60 s	
Control	0%	0%	
Virasure® Aquatic	1%	100%	
Virkon® Aquatic	1%	100%	
Disinfectant mist-spray ~ 4 mL		60 s	
Control	0%	0%	
Virasure® Aquatic	1%	100%	
Virkon® Aquatic	1%	100%	
Steam spray	Control	10 s	30 s
Steam ≥ 100 °C	6.7 ± 6.7%	100%	100%

All treatments were replicated three times, i.e. $n = 3$

immediately killed following immersion within 1% disinfectant solutions.

Disinfectant mist-spray treatments

Disinfectant mist-spray treatment with disinfectants significantly affected mortality rates in *H. anomala* (GLM: $\chi^2 = 54.43$, df = 2, $P < 0.001$). Both mist-spray treatments with Virkon® Aquatic and Virasure® Aquatic caused total mortality of mysids, while no mortality was shown by control groups (both $P = 0.003$ compared to controls; Table 1). In turn, differences in efficacy between the two products were not statistically clear (both $P > 0.05$). Although mortality was formally assessed following a 6 h recovery period, it appeared that ~ 40–60% of *H. anomala* specimens were immediately killed following contact with the 1% disinfectant solutions.

Steam spray

Steam spray treatments had a significant effect on *H. anomala* mortality rates (GLM: $\chi^2 = 46.40$, df = 2, $P < 0.001$). Both 10 and 30 s exposures caused complete mortality of *H. anomala* (both $P = 0.003$ compared to controls; Table 1). There was no statistically clear difference between the two steam exposure durations ($P > 0.05$). Although mortality was formally assessed following a 6-h recovery period, it appeared that all *H. anomala* were immediately killed following exposure to direct steam spray treatments. A single control specimen died, apparently the result of a cannibalistic interaction.

Discussion

Immersion and mist-spray treatments using 1% disinfectant solutions were highly efficacious in driving total *H. anomala* mortality. Similarly, steam spray applications lasting ≥ 10 s were also highly effective. These results are consistent with the high levels of efficacy reported for juvenile bivalves immersed in aquatic disinfectants (Barbour et al. 2013), as well as for adult bivalve and macrophyte species exposed to steam spray treatments (Coughlan et al. 2019, 2020; Joyce et al. 2019; Crane et al. 2019). Further, Bradbeer et al. (2020) have shown that 1% disinfectant solutions (Virasure®/Virkon® Aquatic) and direct steam exposure can consistently achieve 100% mortality of *D. villosus* following ≥ 2 min immersion, 7.5 mL mist-spray with 5 min air exposure, and ≥ 10 s steam spray applications. Moreover, De Stasio et al. (2019) documented 100% mortality for faucet snail, *Bithynia tentaculata* Linnaeus, 1758, spiny water flea, *Bythotrephes longimanus* Leydig, 1860, and *H. anomala*, following 20 min immersion, or ~ 5.4 mL mist spray with 20 min of air exposure, using a 2% solution of Virkon® Aquatic. Similarly, it appears that immersion and mist-spray treatments using 2% Virkon® Aquatic can also reliably kill New Zealand mud snail, *Potamopyrgus antipodarum* Gray, 1843, following an exposure duration of ≥ 20 min (Stockton and Moffitt 2013; De Stasio et al. 2019). Overall, although 1% solutions can reliably kill *H. anomala*, the use of 2% concentrations will likely bolster spread-prevention protocols for multiple species.

Biosecurity practices utilising disinfectants may be especially beneficial for decontamination of small equipment. For example, wetsuits, waders and nets could be completely immersed within disinfection baths (Barbour et al. 2013). Equally, disinfectant mist-spray applications may aid decontamination of larger equipment such as canoes and paddles, while niche water reservoirs could be flushed with disinfectant solutions to kill *H. anomala*, e.g. water cooling systems for outboard motors. Although the risk of toxicity to non-target organisms through disinfectant residues and spills is considered low with careful disposal and the rapid onset of reagent deactivation following exposure to organic materials (Stockton and Moffitt 2013; Stockton-Fiti and Moffitt 2017), legal issues concerning the use of broad-spectrum disinfectants as biosecurity agents for non-microscopic organisms also need to be addressed (Sebire et al. 2018). Despite possible concerns, oxidising agent-based disinfectants, such as the products examined here, are already commonly used in aquaculture for the control of a wide range of bacteria, viruses and fungi (Stockton and Moffitt 2013).

Pressurised jets of steam may bolster existing biosecurity protocols (Crane et al. 2019; Joyce et al. 2019). In particular, steam treatments may be especially useful for decontamination of large equipment that is otherwise problematic to manually clean (Joyce et al. 2019). Notably, in-field steam cleaning facilities could be established as biosecurity stations at points of waterway ingress and egress, such as boat ramps (Coughlan et al. 2019; Crane et al. 2019). With guidance, these could be operated by all water users or by a trained attendant. However, protocols that target niche areas, blind-spots and high risk zones, such as intake grates, internal surfaces of pipework and baitwells, will need to be developed to achieve adequate decontamination.

Overall, the results presented herein indicate that aquatic disinfectants and steam spray applications could be used to prevent further spread of *H. anomala*. Especially as brief applications times could increase compliance by water users, as the length of time required to decontaminate large and complex equipment can frustrate users and reduce participation (Sutcliffe et al. 2018). Moreover, a greater focus on pre-entry biosecurity would be beneficial, i.e. decontamination prior to site entry, such as a 'No Dip, No Draw' policy enforced by some angling clubs, whereby equipment has to immersed in a disinfectant solution immediately prior to entering the site. Ultimately, despite uncertainties surrounding the upscaling and in-field application of these techniques, disinfectant and thermal shock treatments potentially represent suitable biosecurity measures to prevent further IAS spread.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest, and do not have a financial interest in the products tested.

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