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The effectiveness of Virkon® S disinfectant against an invasive insect and implications for Antarctic biosecurity practices

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Abstract: The flightless midge *Eretmoptera murphyi* is thought to be continuing its invasion of Signy Island via the treads of personnel boots. Current boot-wash biosecurity protocols in the Antarctic region rely on microbial biocides, primarily Virkon® S. As pesticides have limited approval for use in the Antarctic Treaty area, we investigated the efficacy of Virkon® S in controlling the spread of *E. murphyi* using boot-wash simulations and maximum threshold exposures. We found that *E. murphyi* tolerates over 8 h of submergence in 1% Virkon® S. Higher concentrations increased effectiveness, but larvae still exhibited > 50% survival after 5 h in 10% Virkon® S. Salt and hot water treatments (without Virkon® S) were explored as possible alternatives. Salt water proved ineffective, with mortality only in first-instar larvae across multi-day exposures. Larvae experienced 100% mortality when exposed for 10 s to 50°C water, but they showed complete survival at 45°C. Given that current boot-wash protocols alone are an ineffective control of this invasive insect, we advocate hot water (> 50°C) to remove soil, followed by Virkon® S as a microbial biocide on 'clean' boots. Implications for the spread of invasive invertebrates as a result of increased human activity in the Antarctic region are discussed.

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Key words: biosecurity, Chironomidae, invertebrate control, Signy Island, species management

Introduction

Throughout history, humans have acted as agents of change in ecological systems through the deliberate or unintentional introduction of species to various areas. Antarctica's geographical isolation and challenging environmental conditions have, to date, acted as barriers to non-native species dispersal and establishment, thereby minimizing non-native species impacts on the continent itself (Frenot *et al.* 2005, Hughes *et al.* 2015). In 1959, the Antarctic Treaty was signed, coming into force in 1961 and establishing the Antarctic Treaty area as all land, ice shelves and surrounding ocean south of 60°S latitude. From its inception, the Antarctic Treaty placed a high priority on the preservation of Antarctic ecosystems, although this has been achieved by different mechanisms over time, currently by the Protocol on Environmental Protection to the Antarctic Treaty (e.g. the Committee for Environmental Protection Non-native Species Manual; CEP 2016). The remote, lower-latitude sub-Antarctic islands are closely linked to the Antarctic Treaty area in biological terms, and similarly are of high conservation value, but they are instead regulated under national sovereignty. In recent decades, increasing levels of human activity are progressively breaking down the geographical barriers between Antarctica and the sub-Antarctic region, as well as the rest of the world, thereby increasing the risk of species introductions.

To date, most non-native species occurrences in the Antarctic and sub-Antarctic regions have been the result of historical intentional introductions, but with human activity in the region rapidly rising, the risk of unintentional introductions is becoming an increasing threat to Antarctic ecosystems (Frenot *et al.* 2005, Hughes *et al.* 2015). Human activity has already led to > 200 species of non-native animals and plants successfully establishing in the Antarctic and sub-Antarctic regions, the majority of these being in the sub-Antarctic, but with increasing numbers recorded from the maritime Antarctic (Frenot *et al.* 2005, Hughes *et al.* 2015). These include introductions of Acari, Collembola, Diptera, Coleoptera and Araneae (Pugh, 1994, 2004, Ernsting *et al.* 1995, Greenslade & Convey 2012). Furthermore, the transfer of pathogens may risk disease in local wildlife populations that may be

'immunologically naïve' due to evolution in microbial isolation (Grimaldi *et al.* 2014).

Through increased liquid water availability and extent of ice-free habitat, reduced numbers of extreme cold events and extending growing seasons, areas of Antarctica previously unsuitable for colonization are becoming available to both native and non-native species alike (Lee *et al.* 2017). Species introductions can have significant impacts within the simple terrestrial ecosystems of the Antarctic regions. For example, the introduction of a single detritivore to the maritime Antarctic, the midge *Eretmoptera murphyi* (Schaeffer, 1914), has been found to increase litter turnover within the local environment where it is established by almost an order of magnitude (Hughes *et al.* 2013). In the sub-Antarctic, a new non-native predatory ground beetle has led to significant declines in native terrestrial invertebrate species (Lebouvier *et al.* 2012). All Parties to the Antarctic Treaty are therefore responsible for developing and enacting measures to prevent or minimize the introduction of non-native species, to control and, if feasible, to eradicate any that have established (Hughes & Pertierra 2016). Available practical response measures are limited, however, by the requirement to keep collateral damage to native habitats and species to a minimum and by associated costs and practicability, as well as by sometimes contradictory existing legislation. For instance, Article 7 of Annex III Waste Disposal and Management bans the use of pesticides within Antarctica, unless under certain necessary circumstances (Hughes *et al.* 2015). Thus, the traditional and most widely used insecticides applied elsewhere (pyrethroids, neonicotinoids and insect growth regulators) may not be options for use in Antarctica. Disinfectants, in contrast, are permitted and are routinely deployed to destroy microbial pathogens and to prevent their spread (Curry *et al.* 2002).

The Virkon® S range of disinfectants is currently recommended by the Council of Managers of National Antarctic Programs (COMNAP) and the International Association of Antarctica Tour Operators (IAATO) as an approved biocide (IAATO 2018, COMNAP 2019). These products are also marketed in the UK as Department for Environment, Food and Rural Affairs-approved virucides for farms (http://disinfectants.defra.gov.uk/DisinfectantsExternal/Default.aspx?Module=ApprovalsList_SI), and they claim effectiveness through oxidation against bacteria, viruses and certain strains of fungi at temperatures as low as 4°C (Herńandez *et al.* 2000). Virkon® S powder is easy to transport and has low dermal toxicity, does not give off toxic vapour and, should it end up in an aqueous environment, will decompose over time into a harmless mixture of non-toxic salts (Curry *et al.* 2005, see also manufacturer declaration https://syndel.com/wp-content/uploads/2019/01/Information-Virkon-Aquatic-degradability-in-the-environment.pdf). The efficacy and low-toxicity of Virkon® S has led to its application in Antarctica, where it has proven effective at preventing the spread of microbial pathogens under ambient conditions when used to wash equipment or footwear (Curry *et al.* 2005).

The convenience of Virkon® S products has prompted toxicity testing against higher-order organisms beyond its intended use against microbial pathogens, in particular against invasive marine invertebrate species within aquatic environments that are more vulnerable to the off-target effects of harsher chemicals (Stockton-Fiti & Moffitt 2017). Tests on the New Zealand mud snail, *Potamopyrgus antipodarum*, found that 20 min exposure to 2% Virkon® S solution resulted in 100% mortality at 15°C and 22°C, but that a 1% solution only achieved total mortality at the lower temperature (Stockton-Fiti & Moffitt 2017). In the same study, 2% Virkon® S solution was highly effective against quagga mussels, *Dreissena rostriformis bugensis*. An invasive tunicate that affects mussel farming in Canada, *Ciona intestinalis*, has also been found to be vulnerable to Virkon® S at 1% concentration (Paetzold & Davidson 2011), whilst the faucet snail, *Bithynia tentaculata*, proved to be resistant to dilutions of 1% and 2% at 20–23°C over 1–24 h (Mitchell & Cole 2008). Its efficacy against insects remains largely untested, although soaking eggs of the yellow mealworm, *Tenebrio molitor*, in 1% Virkon® S for 10 min did not prevent hatching (Li *et al.* 2016), and mixing it with certain insecticides reduced its efficacy against the house fly, *Musca domestica* (Watson *et al.* 2008).

The flightless chironomid midge *E. murphyi* is endemic to the sub-Antarctic island of South Georgia (54°S, 36°W) (Fig. 1), but it was discovered in 1980 in the maritime Antarctic on Signy Island (South Orkney Islands, 60°S, 45°W) (Fig. 1) at the site of a previous plant transplantation experiment (Burn, 1982). Originally reported to be restricted to a 1 m^2 introduction site, the midge has since colonized an area of ~85 000 m^2 and can be found along footpaths regularly used by staff and visitors at the research station. It is now on the verge of entering into new valley systems (Bartlett *et al.* 2020).

At present, anthropogenic transfer of *E. murphyi* is the greatest known introduction risk in Antarctica. In 2005, a British Antarctic Survey (BAS) vessel carried construction vehicles contaminated with soil containing various invertebrate species, including *E. murphyi*, from South Georgia to Rothera Research Station on Adelaide Island, off the Antarctic Peninsula (68°S), where they were alive when discovered after arrival (Hughes *et al.* 2010). In this instance, no establishment has been detected, probably due to a lack of suitable habitat

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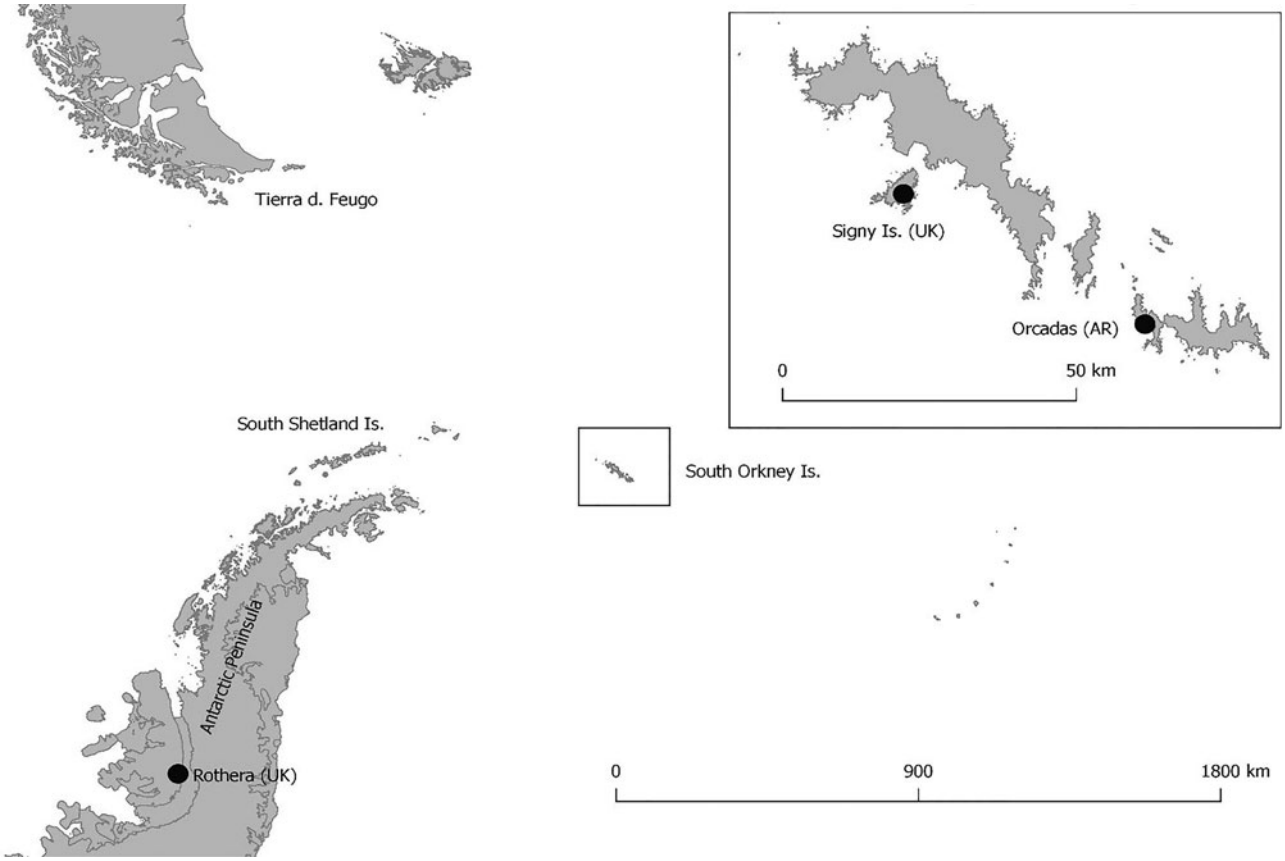

Fig. 1. Location of the South Orkney Islands and Signy Island in the Southern Ocean. Created using *Arc-Map*® 10.4.1 software by Esri. Copyright © Esri.

immediately adjacent to the offloading site, but many suitable locations across the maritime Antarctic are at risk, with the South Shetland Islands being a particularly suitable candidate region and a major logistical hub for the northern Antarctic Peninsula (Pertierra *et al.* 2019). Current biosecurity measures employed by BAS encompass the whole supply chain and include cleaning of containers and cargo, where pyrethrum-based insecticides may be used to fumigate shipping containers prior to transportation to Antarctica. Relevant to Signy Island and *E. murphyi*, BAS biosecurity regulations require the cleaning of soil from equipment, boots and clothing, and the use of Virkon® S products at a 1% dilution in boot-wash baths prior to entry and exit from the island (BAS 2019). However, Virkon® S is primarily an antimicrobial agent, and even then its effectiveness is limited without physical removal of any soils/organic loads from contaminated surfaces (Guan *et al.* 2013). The efficacy of Virkon® S to potentially control the spread of any Antarctic invertebrate remains untested.

Against this background, this study investigates whether current Virkon® S boot-wash protocols are effective biosecurity measures against the midge. We also examine *E. murphyi*'s tolerance to seawater and hot water immersion as possible alternatives to chemical control.

Materials and methods

Sample collection

Eretmoptera murphyi larvae were collected in soil on Signy Island (Fig. 1) close to the BAS's Signy Research Station during the 2016–17 summer. Samples were maintained on soil substrate from the site of collection, which is both the species' natural habitat on the island and source of food. Samples were returned to the UK by ship (4°C, constant darkness for 10 weeks) and then maintained under the same control conditions at the University of Birmingham. Soil containing larvae was kept moist and larvae hydrated using field water (water from a 3:1 mix of deionized water and Signy soil). Individual larvae were extracted by breaking apart soil substrate with a fine brush and tweezers or by washing through stacked 2.0 mm and 0.5 mm mesh sieves. In the latter instance, all larvae were rested in control conditions for 48 h to ensure that the extraction process was not an additional

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Table I. Summary of all treatments and methods explored in this study. Concentration refers to either salinity dilutions with a soil control or Virkon® dilutions. See 'Materials and methods' section for full details.

Treatment type	Life stage	Condition/concentration (%)	Temperature (°C)	Exposure duration	Survival assessment	*n*
Virkon® boot-wash simulation	Larvae	0.1	~20	10 s	72 h post-exposure	24
		1.0	~20	10 s	72 h post-exposure	24
		10	~20	10 s	72 h post-exposure	24
Virkon® thresholds	Larvae	0	4	18 h	Hourly	30
		1.0	4	18 h	Hourly	30
		4.0	4	18 h	Hourly	30
		10	4	18 h	Hourly	30
		0	20	8 h	Hourly	30
		1.0	20	8 h	Hourly	30
		4.0	20	8 h	Hourly	30
		10	20	8 h	Hourly	30
Hot water boot wash	Larvae	0	40	10 s	72 h post-exposure	15
		0	45	10 s	72 h post-exposure	15
		0	50	10 s	72 h post-exposure	15
Salinity thresholds	Larvae	Soil	4	7 days	72 h post-exposure	30
		0	4	7 days	72 h post-exposure	30
		25	4	7 days	72 h post-exposure	30
		50	4	7 days	72 h post-exposure	30
		75	4	7 days	72 h post-exposure	30
		100	4	7 days	72 h post-exposure	30
	Eggs	Soil	4	35 days	35 days	30
		0	4	35 days	35 days	30
		25	4	35 days	35 days	30
		50	4	35 days	35 days	30
		75	4	35 days	35 days	30
		100	4	35 days	35 days	30

stressor prior to treatment. All larvae were subsequently assigned to instars based on size (Bartlett *et al.* 2018a). Experiments using eggs were conducted in laboratories at Signy Research Station during January 2017, using recently laid egg sacs collected from moss banks surrounding the research station. Egg sacs were removed from the substrates as described in Bartlett *et al.* (2018b). As egg sacs are only available in quantity from the field, these were not included in the later Virkon® S experiments conducted in the UK. It has previously been shown that *E. murphyi* larvae can respire underwater (freshwater) for up to 28 days, so the effect of submersion itself is not considered a stressor within the timeframe of these experiments (Everatt *et al.* 2014b). A summary of all treatments and associated methods is presented in Table I.

Preparation of Virkon® S solutions

Correspondence with the manufacturers of Virkon® S (Lanxess, Germany, sourced from Fisher Scientific UK Ltd) indicated that Virkon® S begins to degrade at temperatures > 40°C and that, while a 10% Virkon® S solution can be prepared under laboratory conditions, the maximum recommended concentration for practical use is 5% at room temperature (~20°C). Therefore, all Virkon® S treatments took place at room temperature or below. Dilutions were measured using a colorimeter, and it was found that we were able to mix a 10% dilution that showed no re-granulation during the course of any treatments. Virkon® S solutions were thus made up in concentrations of 0% (control), 0.1%, 1.0% and 10% with deionized water and stored at 4°C.

Short-term exposures

Different life stages of insects can have various levels of pesticide tolerance (Athanassiou *et al.* 2012). It was therefore important to measure any difference in the boot-wash effects between the different larval instars of *E. murphyi*. Volumes of 20 ml of 0.1%, 1.0% and 10% Virkon® S were measured out using a graduated syringe and deposited into separate 100 ml beakers. Three replicates ($n = 8$) of either L4, L3 or L2 larvae were placed on a 250 µm nylon net, which was folded and gathered together so that the larvae were together at the base. Larvae were then completely submerged in the different Virkon® S dilutions for 10 s (to simulate a typical boot-wash period). Upon removal, the net was blotted on tissue paper to remove excess Virkon® S, and the larvae were quickly returned to control conditions. Survival was assessed after 72 h by visual monitoring of larvae movement, either spontaneously or with gentle stimulation with a brush. Independent peristalsis of the

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gut and/or movement of the mandibles were registered as live movement.

Long-term exposures

To assess the efficacy of warming Virkon® S and/or increasing exposure times, Virkon® S solutions of 0% (control), 1%, 4% and 10% were prepared and stored at either 4°C or 20°C. Three groups of $n = 10$ mixed L3/L4 larvae were placed in a Petri dish with 2 ml of each dilution at each temperature (no soil). The Petri dishes were kept at either 4°C or 20°C and survival was assessed every hour for 8 h. The time taken to reach 50% (lethal time, LT_{50}) or 100% (lethal time, LT_{100}) mortality was noted. Based on the results from the 8 h experiments, hourly assessments were repeated at only 4°C for all dilutions for a duration of 18 h, then left overnight and assessed again at 27 h, in order to assess the LT_{100} for each dilution.

High-temperature exposures

In order to establish the potential for hot water boot washes to act as an alternative biosecurity measure against *E. murphyi*, the above 'net and dip' method was used on three groups of $n = 5$ L4 larvae. A 28 ml test tube containing ~15 ml of field water was placed in an alcohol bath (Haake Phoenix II C50P) and heated to 40°C, 45°C or 50°C. A minimum of 40°C was chosen as *E. murphyi* larvae are known to survive short exposures to temperatures up to 39°C (Everatt *et al.* 2014a). Larvae were submerged in the heated water for 10 s, removed to control conditions and survival assessed immediately after exposure and then again at 24 and 72 h.

Salinity exposures

To assess the ability of *E. murphyi* to withstand immersion in seawater, we exposed both larvae and egg sacs to a range of salinities. For experiments on eggs, conducted on Signy Island, seawater was collected locally. All eggs within the egg sacs were confirmed to be at the first (opal) developmental stage prior to the start of experiments and were then used for the entire gestation period of 35 days (Bartlett *et al.* 2018a). If any eggs showed signs of yellowing or embryonic development, the whole egg sac was discarded and not used in this study. Experiments on larvae, conducted at the University of Birmingham, used Antarctic seawater obtained from stocks at the BAS. In all instances and for all dilutions, pH and salinity (µS) were measured using a Hanna HI-98129 Combimeter.

Three groups of $n = 10$ egg sacs were submerged for 35 days at 4°C in either a soil control, 0% (field/fresh water), 25%, 50%, 75% or 100% seawater. Development was noted weekly and, at the end of the gestation period (35 days), the egg sacs were carefully dissected and the percentage of eggs that had hatched recorded. For comparison with larvae, the same dilution experiment was conducted on three groups of $n = 10$ L4 larvae that were kept submerged for 7 days. After treatment, the larvae were returned to soil control conditions and survival assessed after 72 h, as described previously.

Results

Efficacy of the disinfectant Virkon® S and use of boot-wash protocols

Short (10 s) exposure to all concentrations of Virkon® S resulted in 0% mortality in both L4 and L2 larvae. Only one death was observed among L3 larvae. In the long-term experiments, immersion of larvae in water (control) over 18 h resulted in 0% mortality at both 4°C and 20°C (Fig. 2a). Exposure to the 1% Virkon® S resulted in some mortality after 4 h, but with no significant difference between 4°C and 20°C after 8 h (Mann-Whitney $U = 3$, $P = 0.7$), and with survival remaining > 50% even after 18 h at 4°C (Fig. 2b). There was a marked decline in survival in 4% Virkon® S, with LT_{50} observed after ~5 h at 20°C and after ~9 h at 4°C (Fig. 2c). LT_{100} was reached after 8 h at 20°C and after 14 h at 4°C. In 10% Virkon® S, mortality occurred after 3 h at 20°C, reaching LT_{50} at 5 h. Survival at 4°C also declined more rapidly at this concentration, with the LT_{50} being reached after ~7 h and LT_{100} being reached after 13 h (Fig. 2d). Overall, mortality after 8 h of exposure for all dilutions was significantly higher than controls at both 20°C (Kruskal-Wallis $H = 9.9$, $P < 0.0001$) and 4°C (Kruskal-Wallis $H = 8.2$, $P = 0.01$).

High-temperature treatments

At 40°C, there was no effect on survival after a 10 s exposure, whilst at 45°C, immediately post-exposure, all larvae were in a heat coma, but fully recovered to 100% survival within 24 h. Exposure to 50°C water resulted in 100% mortality of L4 larvae with no recovery over the post-exposure period of up to 72 h (Fig. 3).

Salinity exposure

The pH of field-collected *vs* laboratory-stored seawater (means of 6.7 ± 0.4 SEM and 6.2 ± 0.4, respectively) were not significantly different (Mann-Whitney $U = 7$, $P = 0.3$). Salinity values (means of 25 400 µS ± 9030 SEM and 27 385 µS ± 8312 SEM, respectively) were also not significantly different (Mann-Whitney $U = 12$, $P > 0.99$).

Survival of L4 larvae in 0%, 25%, 50%, 75% or 100% seawater over a period of 7 days was not significantly different from that in the non-submerged soil control

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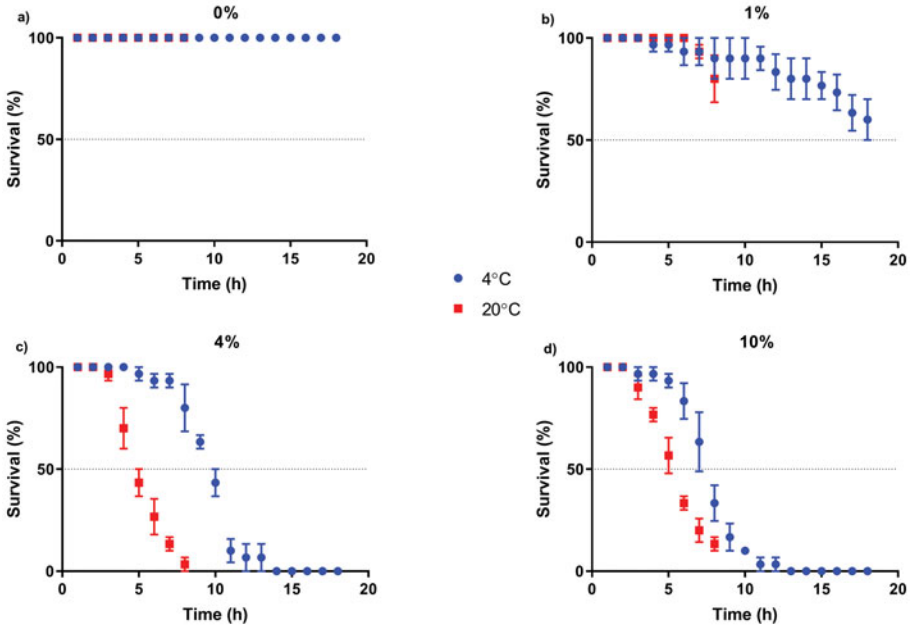

Fig. 2. Virkon® S exposures of **a.** control (0%), **b.** 1%, **c.** 4% and **d.** 10% dilutions at either 4°C (blue) or 20°C (red), with three groups of $n = 10$ larvae exposed for 1–18 h (4°C) or 1–8 h (20°C). Shown as mean survival at each time point ± SEM.

(Kruskal-Wallis $H = 7$, $P = 0.17$) (Fig. 4a), although there was a slight trend of declining survival with increasing salinity. In contrast, in egg sacs exposed to the same salinity range for their gestation of 35 days, the proportion of eggs hatching was greatly reduced even at low salinity (amongst all treatments: Kruskal-Wallis $H = 50.5$, $P < 0.001$; multiple comparisons between treatments: 100%, 75% and 50% *vs* soil, $P < 0.0001$; 25% and 0% *vs* soil, $P < 0.001$) (Fig. 4b). No eggs hatched under 50%, 75% and 100% seawater treatments. Exposure to 25% seawater or to field/fresh water led to hatching success rates of 4.5% ± 2.1% SEM and 9.1% ± 5.8% SEM, respectively, while hatching success rate in the soil control was 59% ± 7.7% SEM. Observations made throughout the 35 day exposure period confirmed that the eggs developed within the egg sacs as described in previous studies, but that in all submergence exposures development slowed at maturation and, of the few eggs that did hatch under saline treatments, the L1 hatchlings did not survive and often did not fully escape from the egg casings within the egg sac, although they did survive freshwater treatments.

Discussion

Given the increasing number of non-native species found in Antarctica, improvements in biosecurity practice will be essential to ensure the ongoing protection of marine and terrestrial ecosystems from biological invasion.

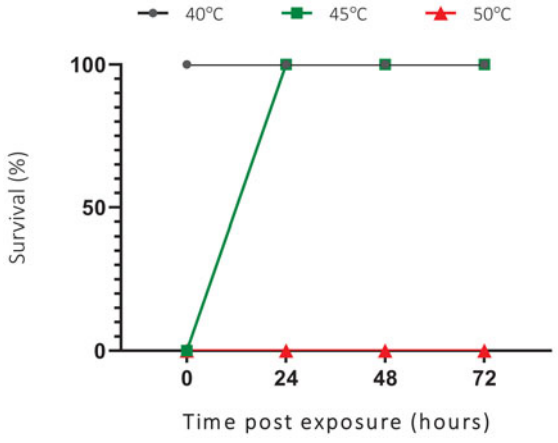

Fig. 3. Mean ± SEM tolerance of larvae to 10 s exposures to hot water temperatures of 40°C, 45°C and 50°C and recovery over 72 h.

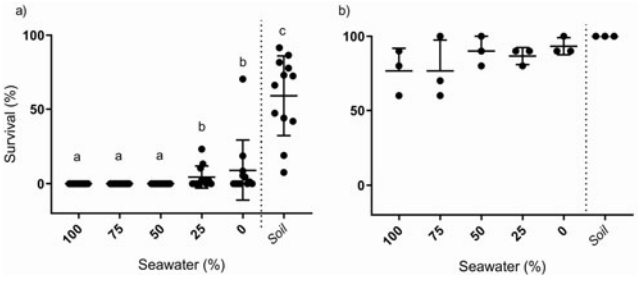

Fig. 4. a. Mean ± SEM tolerance of eggs to seawater dilutions and a soil control after exposure for the whole gestation period (35 days). Three groups of $n = 10$ egg sacs, with ~70 eggs in each sac. Dilutions with the same letter are not significantly different. **b.** Mean ± SEM tolerance of L4 larvae to seawater dilutions after 7 days of continuous exposure with a soil control.

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Current biosecurity protocols concerning the cleaning of footwear primarily focus on reducing the risk of microbial transfer, with the standard practice consisting of dipping footwear into baths containing a 1% Virkon® S solution for a few seconds and boot scrubbing to directly eliminate any visible soil and macro-biology (IAATO 2018, COMNAP 2019). While the consistency of implementation of this procedure varies across different operators in the region, both scrubbing and boot-wash dips are mandatory on arrival at research stations and deployment to field sites under the current BAS biosecurity regulations (BAS 2019). BAS operates a research station located on Signy Island (South Orkney Islands, maritime Antarctic). Here, a principal biosecurity threat is the transfer of two known non-native invertebrate species to locations beyond their current distribution on the island or to various islands and the Antarctic Peninsula: the flightless midge *E. murphyi* and the enchytraeid worm *Christensenidrilus blocki*. Both are thought to have been introduced to Signy Island in the 1960s during plant transfer experiments involving material from South Georgia and the Falkland Islands (Burn 1982). The physiological capacity of *E. murphyi* to survive conditions further south (Everatt *et al.* 2012), as well as to alter soil processes (Hughes *et al.* 2013), makes further transfer of this species to other sites in the region a particular concern. Currently, BAS regulations specify boot washing and scrubbing as a method to restrict the transfer of these species from Signy Island itself to other locations. However, recent evidence indicates that human footfall is also a primary mechanism extending the range of *E. murphyi* on Signy Island (Bartlett *et al.* 2020), and thus assessing the efficacy of boot-wash protocols in limiting the spread of this (and potentially other) invertebrate species on Signy Island is very timely.

There is clear evidence that Virkon® S can be lethal to aquatic invertebrates and mud snails (Stockton-Fiti & Moffitt 2017), but it has been ineffective in the only studies in which it has been applied to terrestrial insects to date: eggs of the yellow mealworm *T. molitor* (Li *et al.* 2016) and the house fly *M. domestica* (Watson *et al.* 2008). We present evidence that larvae of this invasive midge experienced 0% mortality in concentrations of up to 10% Virkon® S over periods of well over 1 h. Indeed, LT_{50} values at this highest concentration were only reached after 8 h at field temperatures (4°C), or after ~5 h at elevated temperatures (20°C). Importantly, these experiments were conducted with zero soil load (i.e. assuming 100% removal of soil from footwear, but with a chance that some larvae remained attached). This means Virkon® S boot-wash protocols alone are totally ineffective biosecurity measures for controlling the spread of *E. murphyi*, and only meticulous boot scrubbing under current protocols could prevent transfer of this species from Signy Island to other locations or limit its spread on the island.

Everatt *et al.* (2014a) showed that *E. murphyi* larvae enter heat coma at 31°C, and a few individuals can survive air temperatures up to 39°C for 1 h. Consequently, we assessed temperatures > 40°C in the absence of Virkon® S (which degrades at this temperature). We found that very short exposures (10 s) to 40°C or 45°C water, whilst inducing heat coma, were not lethal. Only 50°C water proved to be effective at killing *E. murphyi* larvae during typical/short boot-wash exposure times. Saltwater exposures also proved ineffective as a biosecurity measure for mature (L4) larvae, which experienced very little mortality even after 7 days of submersion in 100% seawater (Fig. 3b). First-instar larvae were highly susceptible to even dilute saltwater exposure, with very low survival from egg batches hatching under these conditions (Fig. 3a).

Based on the data obtained in this study, we suggest that the use of hot water (> 50°C) to scrub soil containing invertebrates off contaminated items, followed by a Virkon® S wash on the clean boots, would provide the most effective control measures currently available against *E. murphyi* whilst not sacrificing the benefits of Virkon® S as a microbicide/virucide. This could be implemented at existing boot-wash stations both prior to arrival and on departure from islands. To mitigate the further spread of *E. murphyi* around Signy Island, ideal scenarios would also include new scrub stations adjacent to trails at the edge of the known *E. murphyi* distribution (see Bartlett *et al.* 2020), although this raises issues of practicality related to sourcing/heating water and possible health and safety issues.

Whilst the focus of this study has been on the invasive midge *E. murphyi* on Signy Island, the findings and suggested additions to the existing protocols may be relevant to all areas of Antarctica that are vulnerable to invasive invertebrates or that have already been colonized. *Eretmoptera murphyi* is not a unique example in the Antarctic region, but as a flightless species, it is reliant on mechanical, or potentially oceanic, methods of dispersal to increase its range. Within the maritime Antarctic, another dipteran species, *Trichocera maculipennis*, was recently introduced to King George Island (South Shetland Islands) (Volonterio *et al.* 2013, Potocka & Krzemińska 2018). Although most attention has been given to observations of this species having colonized research station sewage systems, it is thought that it may be established in the local natural environment (Volonterio *et al.* 2013, Potocka & Krzemińska 2018). As adults of this species can fly, it is capable of greater natural dispersal than *E. murphyi*, but soil- or substrate-dwelling life stages could be dispersed through similar mechanisms to those of *E. murphyi* (Volonterio

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et al. 2013). It is probable that all invertebrates will succumb to temperatures > 50°C (Heinrich 1981); what remains to be seen is the minimum exposure time necessary to test this as a viable biosecurity method. We therefore suggest that future work explore simple hot water treatments such as that presented in this study against other non-native invertebrates in the Antarctic region in an attempt to develop a method that could be universally applied throughout the region with comparatively little logistical effort.

Conclusions

The combination of increasing human activity and ongoing regional climate change will probably facilitate further establishment and colonization events of non-native species in continental, maritime and sub-Antarctic regions. Regular review and revision of established biosecurity protocols and the development of new procedures will be necessary if the risk of introductions is to be minimized. Preventing the transfer of soil, and the micro- and macro-organisms contained therein, needs to be a priority action for all stakeholders involved in the protection of Antarctica. Here, using *E. murphyi* as a model species, we have demonstrated important limitations in probably the most widely implemented biosecurity measures, and we suggest alternative actions that could potentially be used to reduce the spread of non-native invertebrate species that, if left unchecked, have the potential to disrupt Antarctica's fragile ecosystems.

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Author contributions

JCB, SALH and PC conceived the study. JCB, SALH and PC designed the methodological approach. JCB and RJR conducted the laboratory experiments. KAH and PC provided policy input. JCB and RJR drafted the manuscript. All authors edited and revised the manuscript.

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Details of data deposit

The experimental data from this study are available online through Mendeley Data as: Bartlett, Jesamine; Radcliffe, Richard James; Convey, Pete; Hughes, Kevin; Hayward, Scott (2020), 'The effectiveness of Virkon® S disinfectant against the invasive chironomid *Eretmoptera murphyi* and implications for Antarctic biosecurity practices', Mendeley Data, V4, doi: 10.17632/3686s39g9j.4 (available at http://dx.doi.org/10.17632/3686s39g9j.4).

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