NeoBiota 37: 37–49 (2018)
doi: 10.3897/neobiota.37.22088
http://neobiota.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Prevention of microbial species introductions to the Arctic: The efficacy of footwear disinfection measures on cruise ships

Sabine B. Rumpf[1], Inger Greve Alsos[2], Chris Ware[2,3]

1 *Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Vienna, Austria* **2** *Tromsø University Museum, Kvaløyvegen 30, 9037 Tromsø, Norway* **3** *Land and Water, Commonwealth Scientific Industrial Research Organisation (CSIRO), GPO box 1700 Canberra 2601, Australia*

Corresponding author: *Sabine B. Rumpf* (sabine.rumpf@univie.ac.at)

Academic editor: *M. von der Lippe* | Received 6 November 2017 | Accepted 15 February 2018 | Published 6 March 2018

Citation: Rumpf SB, Alsos IG, Ware C (2018) Prevention of microbial species introductions to the Arctic: The efficacy of footwear disinfection measures on cruise ships. NeoBiota 37: 37–49. https://doi.org/10.3897/neobiota.37.22088

Abstract

Biosecurity measures are commonly used to prevent the introduction of non-native species to natural environments globally, yet the efficacy of practices is rarely tested under operational conditions. A voluntary biosecurity measure was trialled in the Norwegian high Arctic following concern that non-native species might be transferred to the region on the footwear of travellers. Passengers aboard an expedition cruise ship disinfected their footwear with the broad spectrum disinfectant Virkon S prior to and in-between landing at sites around the remote Svalbard archipelago. The authors evaluated the efficacy of simply stepping through a disinfectant foot bath, which is the most common practice of footwear disinfection aboard expedition cruise ships in the Arctic. This was compared to a more time consuming and little-used method involving drying disinfected footwear, as proposed by other studies. The two practices were evaluated by measuring microbial growth on paired footwear samples before and after disinfection under both conditions. Step-through disinfection did not substantially reduce microbial growth on the footwear. Allowing disinfected footwear to dry, however, reduced the microbial burden significantly to lower levels. Thus, the currently adopted procedures used aboard ships are ineffective at removing microbial burden and are only effective when footwear is given more time to dry than currently granted under operational conditions. These findings underscore results from empirical research performed elsewhere and suggest the need to better relay this information to practitioners. It is suggested that footwear should minimally be wiped dry after step-through disinfection as a reasonable compromise between biosecurity and practicability.

Keywords

biosecurity, disinfection, invasive species, microorganisms, monitoring, tourism

Introduction

Increases in trade and tourism have facilitated the spread of non-native species across the globe (Seebens et al. 2017). While there are generally fewer invasive species in the Arctic and Antarctic than in more temperate regions (Frenot et al. 2005, Elven et al. 2011, Coulson et al. 2013, Alsos et al. 2015a), some sub-Arctic and sub-Antarctic environments are heavily invaded (Frenot et al. 2005, Carlson and Shephard 2007). Moreover, increasing human activity in the polar regions combined with the effects of ongoing climate change stands to promote the possibility of high-latitude invasions (Cowan et al. 2011, Gederaas et al. 2012, Ware et al. 2012, 2016). Concern exists that disease transmission to and between wildlife populations might occur at high latitudes (Curry et al. 2005, Kerry and Riddle 2009), as might the introduction of pathogens (Cowan et al. 2011, Hughes et al. 2011), invertebrates (Hughes et al. 2011) and invasive plants (Chown et al. 2012, Ware et al. 2012, Alsos et al. 2015a). The consequences of such introductions are as yet largely unknown, but are likely to impact on existing community structure and functioning (Litchman 2010) and may cause disease to both fauna and flora (Kerry and Riddle 2009, Hughes et al. 2011). Acknowledgement of the serious impacts caused by a proportion of these species and the difficulties associated with their eradication, has spurred the implementation of management interventions designed to prevent biological introductions.

Footwear has been demonstrated to be contaminated by a range of non-native species (McNeill et al. 2011; Ware et al. 2012). Soil-borne organisms found on footwear have caused substantial impacts to wildlife (Hernandez et al. 2007) and native vegetation (Cahill et al. 2008), while footwear has been directly identified as the likely vector leading to the establishment and spread of non-native plants (Lloyd et al. 2006), plant pathogens (Cahill et al. 2008) and the transmission of diseases (Phillott et al. 2010). Strategies for reducing the risk of footwear-mediated non-native species introductions are typically inexpensive and rapid and are designed to both clean and disinfect. Empirical evaluations have been undertaken in controlled settings to determine processes under which efficacious outcomes can be achieved (Amass et al. 2001, Amass et al. 2005, Curry et al. 2005). As a result, best-practice or evidence-based footwear cleaning strategies have been incorporated into public (PAWS 2013) or industry-based guidelines (IAATO 2013) and state-based regulations (USDA 2017) in an effort to minimise non-native species transmission. Monitoring the efficacy of such interventions under operational conditions is, however, fundamental to ensuring the ongoing effectiveness of biosecurity management.

Expedition cruising ships constitute a large proportion of tourism opportunities in polar regions and is still increasing. In the Antarctic, the International Association of Antarctica Tour Operators (**IAATO**) has introduced biosecurity guidelines aimed at reducing the transmission of non-native species via the footwear of ship passengers. The northern equivalent, the Association of Arctic Expedition Cruise Operators (**AECO**), has not yet formalised such biosecurity practices. Amongst other objectives, AECO is dedicated to managing respectable, environmentally-friendly and safe expeditions in the Arctic (http://www.aeco.no). In 2012, AECO trialled voluntary biosecurity measures aimed at reducing the risk of non-native species introduction mediated by tourists and ship crews. One of these measures aimed at preventing the transmission of microorganisms to the natural environment through footwear disinfection.

Here, the efficacy of procedures used in the AECO trials was evaluated by undertaking an evaluation on board a single AECO expedition cruise ship under operational conditions. Specifically, the effectiveness of reducing microbial loads on footwear was measured using two different current disinfection practices: i) simple step-through disinfectant footbaths representing the most easily implementable and most often applied measure; and ii) the addition of a drying period following footwear disinfection to prolong the contact time of the disinfectant and microorganisms as urged by Amass et al. (2005), a technique which is rarely practised. These tests were not aimed at testing the effectiveness of the disinfectant product as this has been done elsewhere (e.g. Amass et al. 2001), but to determine whether footwear disinfection as practised aboard expeditions ships was effective.

Methods

Svalbard and expedition tourism

The voluntary biosecurity measures trialled by AECO in 2012 were undertaken by ships operating around the remote Svalbard archipelago (74–81°N, 10–35°E), approximately 700 km north of mainland Norway (Fig. 1). Around one hundred non-native plants have been observed in Svalbard during irregular field surveys, about 40 of them during the last decade (year of first record: 1883; Elven and Elvebakk 1996, Gederaas et al. 2012, Alsos et al. 2015a). Also, a number of non-native invertebrates have been observed (Coulson et al. 2013) and ecto- and intestinal parasites are known to be associated with the introduced sibling vole *Microtus levis* (the vole's survival in Svalbard is likely synanthropic). Microorganism biogeography is poorly understood in the Arctic and, consequently, it is not known whether non-native microbes have been introduced to the region (Strøm 2004, Lovejoy 2013).

Cruise ship tourism constitutes a large part of the tourism sector on Svalbard, with currently more than 70,000 passengers aboard cruise ships visiting Svalbard between the months of June and September annually (Statistics Norway 2017). Landings are carried out multiple times per cruise at about 150 different sites (Statistics Norway 2017). Both numbers of visiting tourists and numbers of landing sites have been significantly increasing during the last decades (linear regression; tourists: df =15, $t = 9.90$, $p < 0.001$; sites: df = 15, $t = 6.60$, $p < 0.001$, Fig. 2). Tourists undertaking an expedition cruise typically first fly to Svalbard and board ships at the local port in Longyearbyen. Opportunities for non-native species dispersal via footwear may occur upon landing in Svalbard, during landings around the archipelago or through the translocation of locally non-native species between Svalbard locations.

Figure 1. Geographical location of the study site Svalbard (highlighted in black).

Disinfection methods

During the voluntary biosecurity measures trialled by AECO, participating expedition cruise ships used baths of Virkon S (DuPont) to disinfect footwear without cleaning them beforehand. Virkon S is a broad spectrum virucidal disinfectant, commonly used in farm and tourism biosecurity settings that has been proven effective (Amass et al. 2001, Curry et al. 2005, Morley et al. 2005, Cheah et al. 2009, Hornig et al. 2016). Disinfectant baths were typically placed at the gangway such that passengers would simply step through the bath prior to entering tender boats before a landing (hereafter step-through disinfection). Tender boat trips to shore vary in length between landings and are dependent on the weather (typically 3–10 minutes). Given this, little time is afforded to allow the disinfectant to take effect and dry and may be further compromised by water pooled on the floor of the tender boat, diluting or removing the

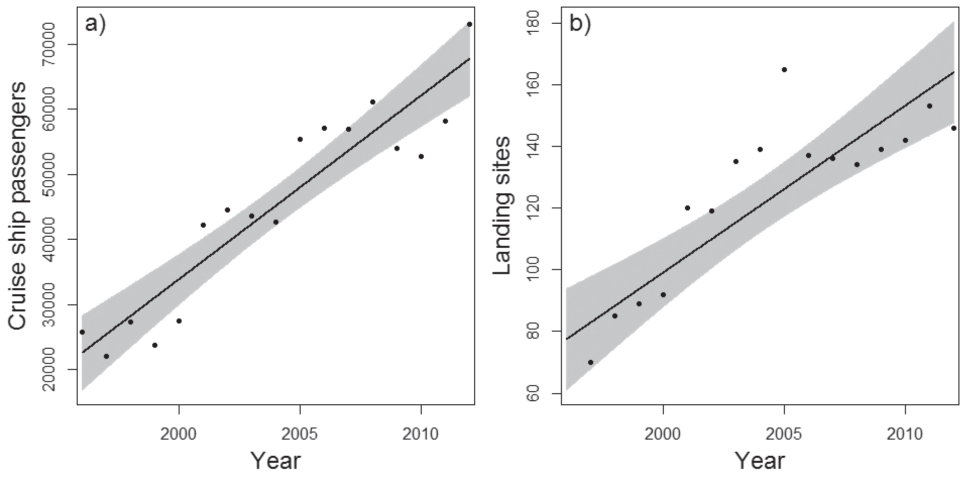

Figure 2. Temporal trend of cruise ship tourism on Svalbard: **a)** number of cruise ship passengers visiting Svalbard per year and **b)** number of different landing sites visited by cruise ships on Svalbard per year. Linear regressions and 95% confidence intervals are depicted as lines and shaded areas, respectively.

disinfectant. AECO reported that at least two ships additionally collected footwear of passengers in between landings and left them in a separate room near the gangway for a subsequent drying period following disinfection. As a second test, footwear of passengers was therefore collected upon return to the vessel after step-through disinfection and left to dry for one hour before samples were taken. One hour was selected as an appropriate drying period as this represented a reasonable duration over which the footwear was expected to dry and as it represented the shortest time between subsequent landings. Since few ships used an additional bath containing water and scrubbing brushes in which passengers could first clean their footwear before disinfection, this measure was not included in the study setup.

This study was carried out on board a single ship during the 2012 tourist season in conjunction with the biosecurity measures trialled by AECO. The study ship used a new solution of Virkon S for each voyage (four days' duration) to disinfect footwear. Used as a 1% solution, the agent is active for around five days, after which a loss of pink colour indicates the need to replace the solution (http://virkon.com/products-applications/disinfectants/virkon-s/how-to-use-virkon-s/disinfectant-foot-dips). Disinfection tubs, through which passengers stepped, were made of white plastic which allowed the colour of the solution to be monitored. Contact plates were used to sample the soles of footwear aboard the vessel since time and operational constraints imposed by the expedition-ship indicated that this would be the most effective sampling method. Following Amass et al. (2005), Columbia 5% sheep blood agar base was used since it enables the cultivation of a wide range of microorganisms (Ellner et al. 1966) and is specified by the manufacturer (Oxoid) as a ”multi-purpose medium suitable for the cultivation of fastidious organisms”. In all cases, contact plates (55 mm) were pressed lightly on a randomly selected flat area of the sole (typically hiking boots) and closed again immediately after. Sixty paired samples were taken for the step-through disinfection measure and 35 paired samples from different passengers for the disinfection measure including a subsequent drying period. In this way, the microbial load of the same tested boot was evaluated before (hereafter named control) and after the disinfection procedure. For both evaluated measures, procedures were tested aboard the cruise ship as practised under normal operational conditions. For step-through disinfection, control samples were taken while passengers waited to board tender boats prior to a landing and the paired disinfected samples were taken one minute after disinfection. This time period was the maximum afforded for passengers waiting to take a tender boat to shore. Furthermore, disinfectants designed for footbaths are required to be fast acting on microorganisms and Virkon S is advertised as being able to achieve disinfection within 30 seconds. Control samples for the set-up, including a drying period after step-through disinfection, were taken upon return to the vessel on the gangway following a landing. The footwear was then allowed to dry for one hour next to the passengers' doorway thereby prolonging the contact time of the disinfectant with the microorganisms before a subsequent paired sample was taken.

Contact plates were stored in a drying oven at 37 °C for 48 hours following sampling. Growth on the contact plates was scored after 24 and 48 hours by the same observer, following the method of Curry et al. (2005) using the categories in Table 1. Differences in microbial growth on control and disinfection contact plates were calculated using a one-sided Wilcoxon-Pratt signed rank test for paired samples in the programming environment R (R Core Team 2015) using the package *coin* (Hothorn et al. 2008) with the assumption that microbial burden would be reduced following disinfection. A restriction of this approach is the inability to distinguish between cases where the disinfection procedure had no effect and cases where the disinfection reduced, but did not substantially reduce or remove, microbial burden. Therefore, disinfection might reduce microbial burden, but contact plate samples still become carpeted by profuse growth of persisting microorganisms. In the use of contact plates, a method chosen for practicality, it was only possible to unequivocally identify a successful effect of disinfection where it results in the complete removal of microbial burden. However, the reasonable assumption was made that, if significant differences between the two disinfection practices exist, they are likely to be indicative of differences in procedural efficacy.

Table 1. Description of used growth scores on sample contact plates. CFUs = colony forming units.

Growth score	Growth description
1	No growth
2	Scanty growth (5–10 CFUs visible)
3	Moderate growth (>10 CFUs but none extending beyond a single grid square)
4	Heavy growth (CFUs extending beyond a single grid square)
5	Profuse growth (CFUs extending beyond two grid squares)

Data resources

The data underpinning the analysis reported in this paper are deposited in the Phaidra Data Repository at https://phaidra.univie.ac.at/detail_object/o:685247.

Results

No sample recorded a growth score of one, regardless of control or treatment level. Control samples produced scanty-to-profuse microbial growth on all 95 contact plates (growth score 2–5, Table 1) and microbial burden was hence present in all of the samples taken. Sample plates of the step-through disinfection measure without a subsequent drying period were already often carpeted with microbial colonies after 24 hours and appeared morphologically similar to those on the paired control plates. Microbial growth was only reduced on 17% after 24 hours and 30% after 48 hours of these samples (Fig. 3a, c, respectively) and the effect of this measure was not significant (24 hours: df = 59, $z = -2.37$, $p = 0.991$; 48 hours: df = 59, $z = -0.12$, $p = 0.547$). More than 95% of the samples had a considerable microbial burden as indicated by at least moderate growth (score 3), regardless of disinfection and time.

Footwear that was allowed to dry after disinfection showed reduced microbial growth in 47% after 24 hours and in 60% after 48 hours of the paired samples (Figs 3b, d, respectively), demonstrating a significant effect of reducing microbial load (24 hours: df = 34, $z = 3.20$, $p < 0.001$; 48 hours: df = 34, $z = 3.71$, $p < 0.001$). Only 10% of the control samples had a low microbial burden after 24 hours as indicated by scanty growth (score 2), compared to more than half of the paired disinfected and dried samples (51%, Fig. 3b). After 48 hours, microbial growth had increased on all contact plates and thus none of the control samples but still 17% of the paired samples showed scanty growth.

Discussion

Footwear disinfection is performed by tourism operators in the Arctic as a voluntary precautionary measure. Since there are no mandatory guidelines imposed as yet, disinfection procedures vary between operators and ships. Here, it is shown that the most common procedure, quick step-through disinfection prior to tender boat trips ashore, is ineffective at removing microbial load on footwear. This corroborates the findings of other studies making the same conclusions in different settings (Amass et al. 2001, 2005; Curry et al. 2002, 2005). Considering that this study was set up to monitor the efficacy of currently implemented measures aboard most ships, the practice of footwear disinfection is likely not effective across a wide section of the tourism sector. However, these results demonstrate that leaving disinfected footwear to dry completely in between landings, likely substantially reduces microbial loads transferred to and in-between landing sites. Thus, to improve disinfection outcomes, disinfecting passengers'

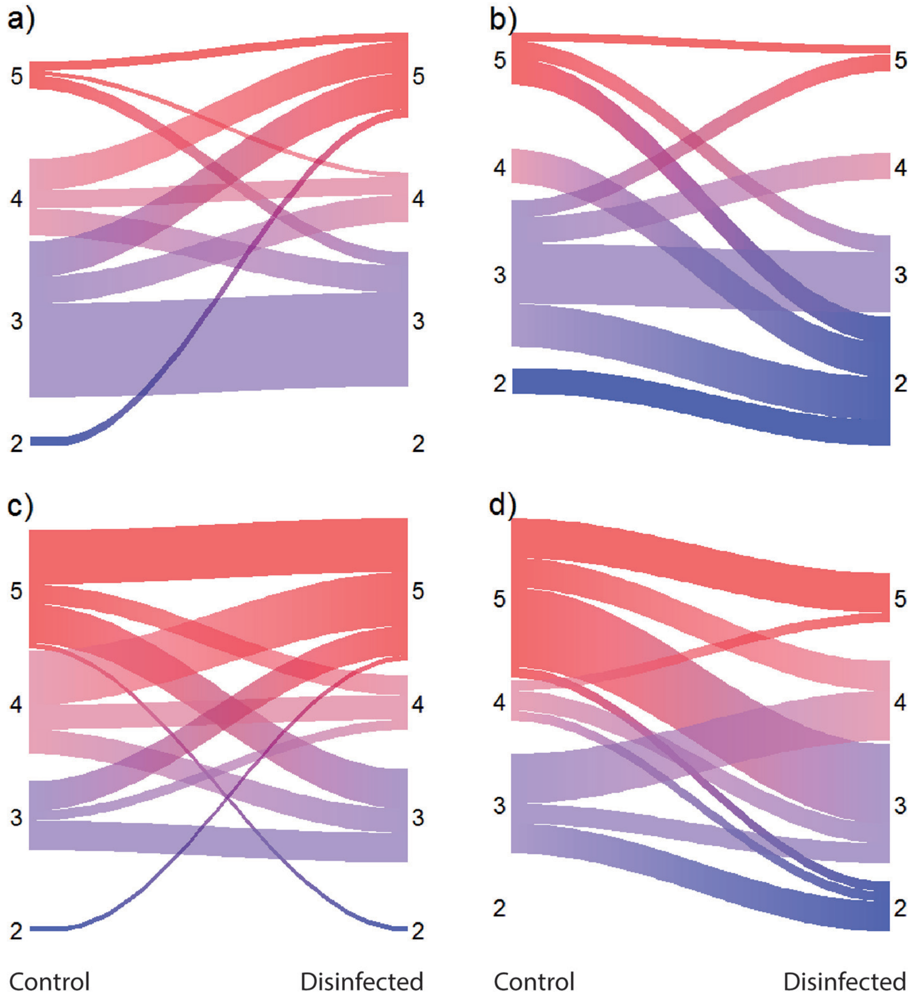

Figure 3. Flow diagram visualising the efficacy of footwear disinfection measures aboard a cruise ship on Svalbard. Numbers on the y-axes and colours represent growth scores (see Table 1), lines connect paired control and disinfected samples and the width of lines is proportional to the number of samples in each category. **a)** step-through disinfection after 24 hours **b)** step-through disinfection combined with a prolonged drying period after 24 hours **c)** step-through disinfection after 48 hours **d)** step-through disinfection combined with a prolonged drying period after 48 hours.

footwear as they board a ship and permitting them to dry, should be used in preference to step-through baths for disinfection prior to shore trips. Nonetheless, permitting disinfectant to dry on footwear may reduce, but not completely remove, microbial loading. Importantly, this study highlights the need to monitor biosecurity interventions to determine their efficacy under operational conditions.

However, prolonged drying periods preferably combined with a cleaning procedure might not be feasible for all cruise ships under operational conditions. Amass et al. (2005) showed that by additionally wiping the disinfected soles of footwear with paper towels, associated bacterial levels were significantly reduced. It is therefore suggested that, when prolonged drying periods are not practical, wiping shoes dry after step-through baths might serve as a reasonable compromise between biosecurity and practicability.

The present study was limited to one ship and to the testing of disinfection procedures under restricted, yet normal conditions aboard cruise ships. The potential for microbial growth was not tested under different temperatures, nor were organism groups determined. Furthermore, the use of growth scores does not allow for an exact quantification of microbial burden. However, the focus of the present study was evaluating the efficacy of practical biosecurity procedures to remove or decrease microbial burden on footwear, which are either already in use or readily implementable. Within this scope, the evaluation demonstrates that improvements could be made to these disinfection measures and suggests that other unevaluated biosecurity practices should be monitored under operational conditions to ensure that they are effective.

It is also important to note that other means of microbe introduction are likely active in transporting organisms to Svalbard, including both natural and anthropogenic means. Natural vectors of dispersal, such as sea-ice, birds or wind, may be effective transporters of microbes (Alsos et al. 2007, 2015b; Pearce et al. 2009). Anthropogenic transport and dissemination of microorganisms is an inevitable consequence of almost all forms of human presence: food, cargo, planes, vehicles and the human body itself may all carry and disseminate large numbers of microorganisms (Cowan et al. 2011). Given this, effective footwear disinfection can only prevent a fraction of the transferred microbial propagule load. Nonetheless, when considering the capacity of footwear to collect soil, guano and biological material that likely harbour microorganisms (McNeill et al. 2011), the pervasiveness of footwear as a species transport vector in Svalbard and the relative ease of managing footwear as a species transport vector (Amass et al. 2005), properly practised footwear disinfection presents an efficacious means for reducing non-native species threats to Svalbard.

While footwear disinfection was focused on removing associated microbial load, a biosecurity intervention would ideally also reduce the risk of introducing plant propagules and invertebrates. A range of plant (Alsos et al. 2015a, 2017) and invertebrate non-native species (Coulson et al. 2013) are already established around the archipelago, yet footwear disinfection alone is unlikely to prevent the further introduction of plant or invertebrate non-native species. While disinfectants are effective against bacteria, viruses and yeasts, they are not designed to render plant propagules or invertebrates non-viable and the act of stepping through a footbath does not reliably remove propagules (Curry et al. 2005). Requesting that passengers scrub footwear with brushes and water prior to stepping through a disinfection bath would reduce the transmission risk of a greater range of taxa and would also significantly improve disinfection rates (Curry et al. 2005).

Potential impacts caused by introduced microbial non-native species are not well indicated in Svalbard, though they are likely to be similar to those indicated elsewhere (e.g. Litchman 2010, Cowan et al. 2011). Impacts could include the transmission of disease to or between wildlife populations (particularly when visitors encounter landings where there is faecal material), genetic homogenisation and disruptions to ecosystem functions or impacts on native flora through the introduction of plant pathogens. Impacts from established plant and invertebrate on non-native species on Svalbard are presently highly localised (Gederaas et al. 2012, Coulson et al. 2013, Alsos et al. 2015a), though if they should colonise the floristically diverse and nutrient rich bird cliff environments characteristic of the high Arctic (Coulson et al. 2013), more substantial impacts to Svalbard's natural ecology would likely follow. Moreover, while the prevailing high-Arctic climate of Svalbard prevents the establishment of many non-native species, the establishment of non-native microbial species will likely be favoured under future moderating climatic conditions (Cowan et al. 2011).

Conclusion

This study underscores the need to monitor the efficacy of management interventions against the spread of non-native species. Footwear cleaning and disinfection protocols are underpinned by empirical research, yet, as evidenced through this study, details of best-practice have not filtered through to practitioners. Monitoring can uncover such deficiencies. Through this study, ways are highlighted in which this practice can be improved, consistent with other published research. Given the operational restrictions imposed by the expedition cruise tourism setting, it is suggested that best practice footwear disinfection consists of first brushing and cleaning footwear in a water footbath, followed by step-through disinfection. A drying step should then be incorporated. Minimally, the latter could be achieved by wiping disinfected footwear dry with paper towels (e.g. Amass et al. 2005). While the focus was on expedition ships operating around high Arctic Svalbard, these findings have relevance for ship and tour operators using similar footwear cleaning practices globally.

Acknowledgements

We are grateful to Mette Svenning and Anne Grethe Hestnes (University of Tromsø) for useful discussions about the experimental design and rationale and also for providing laboratory access. We thank Frigg Jørgensen and Ilja Lang (AECO) for liaising with expedition ships and also the operators and crew of expedition ships. Financial support for this project was received from the Svalbard Environmental Protection Fund (project 12/91 to Alsos).

References

Alsos IG, Ware C, Elven R (2015a) Past Arctic aliens have passed away, current ones may stay. Biological Invasions 17: 3113–3123. https://doi.org/10.1007/s10530-015-0937-9

Alsos IG, Arnesen G, Sanbdbakk BE, Elven R (2017) The flora of Svalbard. http://svalbardflora.no

Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY, Taberlet P, Brochmann C (2007) Frequent long-distance plant colonization in the changing Arctic. Science 316: 1606–1609. https://doi.org/10.1126/science.1139178

Alsos IG, Ehrich D, Eidesen PB, Solstad H, Westergaard KB, Schönswetter P, Tribsch A, Birkeland S, Elven R, Brochmann C (2015b) Long-distance plant dispersal to North Atlantic islands: colonization routes and founder effect. AoB PLANTS 7: plv036. https://doi.org/10.1093/aobpla/plv036

Amass SF, Ragland D, Spicer P (2001) Evaluation of the efficacy of a peroxygen compound, Virkon (R) S, as a boot bath disinfectant. Journal of Swine Health and Production 9: 121–123.

Amass SF, Schneider JL, Gaul AM (2005) Evaluation of current and novel protocols for disinfection of airplane passenger footwear under simulated conditions. Preventive Veterinary Medicine 71: 127–134. https://doi.org/10.1016/j.prevetmed.2005.05.006

Cahill DM, Rookes JE, Wilson BA, Gibson L, McDougall KL (2008) *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. Australian Journal of Botany 56: 279–310. https://doi.org/10.1071/bt07159

Carlson ML, Shephard M (2007) Is the spread of non-native plants in Alaska accelerating? Meeting the challenge: invasive plants in Pacific Northwest ecosystems. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Oregon, 111–127.

Cheah LH, Marsh AT, McNeill MR, Hedderley DI (2009) Evaluation of disinfectant products for microbial decontamination of imported footwear. New Zealand Plant Protection 62: 130–135.

Chown SL, Huiskes AHL, Gremmen NJM, Lee JE, Terauds A, Crosbie K, Frenot Y, Hughes KA, Imura S, Kiefer K, Lebouvier M, Raymond B, Tsujimoto M, Ware C, Van de Vijver B, Bergstrom DM (2012) Continent-wide risk assessment for the establishment of non-indigenous species in Antarctica. Proceedings of the National Academy of Sciences of the United States of America 109: 4938–4943. https://doi.org/10.1073/pnas.1119787109

Coulson SJ, Fjellberg A, Gwiazdowicz DJ, Lebedeva NV, Melekhina EN, Solhøy T, Erséus C, Maraldo K, Miko L, Schatz H, Schmelz RM, Søli G, Stur E (2013) Introduction of invertebrates into the High Arctic via imported soils: the case of Barentsburg in the Svalbard. Biological Invasions 15: 1–5. https://doi.org/10.1007/s10530-012-0277-y

Cowan DA, Chown SL, Convey P, Tuffin M, Elughes K, Pointing S, Vincent WF (2011) Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends in Microbiology 19: 540–548. https://doi.org/10.1016/j.tim.2011.07.008

Curry CH, McCarthy JS, Darragh HM, Wake RA, Todhunter R, Terris J (2002) Could tourist boots act as vectors for disease transmission in Antarctica? Journal of Travel Medicine 9: 190–193. https://doi.org/10.2310/7060.2002.24058

Curry CH, McCarthy JS, Darragh HM, Wake RA, Churchill SE, Robins AM, Lowen RJ (2005) Identification of an agent suitable for disinfecting boots of visitors to the Antarctic. Polar Record 41: 39–45. https://doi.org/10.1017/s0032247404003961

Ellner PD, Stoessel CJ, Drakeford E, Vasi F (1966) A new culture medium for medical bacteriology. American Journal of Clinical Pathology 45: 502–504. https://doi.org/10.1093/ajcp/45.4_ts.502

Elven R, Elvebakk A (1996) Vascular plants. In: Elvebakk A, Prestrud P (Eds) A catalogue of Svalbard plants, fungi, algae, and cyanobacteria. Norsk Polarinstitutt, Oslo, Norway, 9–55.

Elven R, Murray DF, Razzhivin VY, Yurtsev BA, Aiken SG, Kristinsson H, Nordal I (2011) Annotated Checklist of the Panarctic Flora (PAF). http://nhm2.uio.no/paf/

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biological invasions in the Antarctic: extent, impacts and implications. Biological Reviews 80: 45–72. https://doi.org/10.1017/s1464793104006542

Gederaas l, Moen T, Skjelseth S, Larsen L-K (2012) Fremmede arter i Norge – med norsk Svarteliste. Artsdatabanken, Trondheim, Norway. http://www.artsdatabanken.no/fremmedearter/fremmedearterinorge/2012

Hernandez J, Prado V, Torres D, Waldenström J, Haemig PD, Olsen B (2007) Enteropathogenic *Escherichia coli* (EPEC) in Antarctic fur seals *Arctocephalus gazella*. Polar Biology 30: 1227–1229. https://doi.org/10.1007/s00300-007-0282-2

Hornig KJ, Burgess BA, Saklou NT, Johnson V, Malmlov A, Van Metre DC, Morley PS, Byers SR (2016) Evaluation of the Efficacy of Disinfectant Footmats for the Reduction of Bacterial Contamination on Footwear in a Large Animal Veterinary Hospital. Journal of Veterinary Internal Medicine 30: 1882–1886. https://doi.org/10.1111/jvim.14576

Hothorn T, Hornik K, van de Wiel MAV, Zeileis A (2008) Implementing a Class of Permutation Tests: The coin Package. Journal of Statistical Software 28: 1–23. https://doi.org/10.18637/jss.v028.i08

Hughes KA, Lee JE, Tsujimoto M, Imura S, Bergstrom DM, Ware C, Lebouvier M, Huiskes AHL, Gremmen NJM, Frenot Y, Bridge PD, Chown SL (2011) Food for thought: Risks of non-native species transfer to the Antarctic region with fresh produce. Biological Conservation 144: 1682–1689. https://doi.org/10.1016/j.biocon.2011.03.001

IAATO (2013) Boot, clothing and equipment decontamination guidelines for small boat operators. Guidelines. International Association of Antarctica Tour Operators. http://www.iaato.org/guidelines.html

Kerry KR, Riddle M (2009) Health of Antarctic wildlife: a challenge for science and policy. Springer-Verlag, Berlin Heidelberg, 470 pp. https://doi.org/10.1007/978-3-540-93923-8

Litchman E (2010) Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecology Letters 13: 1560–1572. https://doi.org/10.1111/j.1461-0248.2010.01544.x

Lloyd KM, Lee WG, Walker S (2006) Takahe Valley Hut: a focal point for weed invasion in an isolated area of Fiordland National Park, New Zealand. New Zealand Journal of Ecology 30: 371–375.

Lovejoy C (2013) Microorganisms. In: Meltofte H (Ed) Arctic Biodiversity Assessment: Status and trends in Arctic Biodiversity. Conservation of Arctic Flora and Fauna (CAFF), Akureyri, 374–381.

McNeill M, Phillips C, Young S, Shah F, Aalders L, Bell N, Gerard E, Littlejohn R (2011) Transportation of nonindigenous species via soil on international aircraft passengers' footwear. Biological Invasions 13: 2799–2815. https://doi.org/10.1007/s10530-011-9964-3

Morley PS, Morris SN, Hyatt DR, Van Metre DC (2005) Evaluation of the efficacy of disinfectant footbaths as used in veterinary hospitals. Javma-Journal of the American Veterinary Medical Association 226: 2053–2058. https://doi.org/10.2460/javma.2005.226.2053

PAWS (2013) *Phytophthora* root rot: managing *Phytophthora*. Parks and Wildlife Service Tasmania. http://www.parks.tas.gov.au/index.aspx?base=12813

Pearce DA, Bridge PD, Hughes KA, Sattler B, Psenner R, Russell NJ (2009) Microorganisms in the atmosphere over Antarctica. Fems Microbiology Ecology 69: 143–157. https://doi.org/10.1111/j.1574-6941.2009.00706.x

Phillott AD, Speare R, Hines HB, Skerratt LF, Meyer E, McDonald KR, Cashins SD, Mendez D, Berger L (2010) Minimising exposure of amphibians to pathogens during field studies. Diseases of Aquatic Organisms 92: 175–185. https://doi.org/10.3354/dao02162

R Core Team (2015) R: A language and environment for statistical computing. 3.1.3 ed. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8: 14435. https://doi.org/10.1038/ncomms14435

Statistics Norway (2017) Cruise tourism. Sites for tourist going ashore outside settlements and Isfjorden on Svalbard. Tourists going ashore. 1996 – 2012. Statistics Norway. http://www.ssb.no

Strøm H (2004) A catalogue of the terrestrial and marine animals of Svalbard. Norwegian Polar Institute, Tromsø, 137 pp.

USDA (2017) Animal and Plant Health Inspection Service United States Department of Agriculture. http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

Ware C, Bergstrom DM, Müller E, Alsos IG (2012) Humans introduce viable seeds to the Arctic on footwear. Biological Invasions 14: 567–577. https://doi.org/10.1007/s10530-011-0098-4

Ware C, Berge J, Jelmert A, Olsen SM, Pellissier L, Wisz M, Kriticos D, Semenov G, Kwaśniewski S, Alsos IG (2016) Biological introduction risks from shipping in a warming Arctic. Journal of Applied Ecology 53: 340–349. https://doi.org/10.1111/1365-2664.12566