In for the kill: novel biosecurity approaches for invasive and medically important mosquito species

Cuthbert, R. N., Cunningham, E. M., Crane, K., Dick, J. T. A., Callaghan, A., & Coughlan, N. E. (2019). In for the kill: novel biosecurity approaches for invasive and medically important mosquito species. Management of Biological Invasions, 11. https://doi.org/10.3391/mbi.2020.11.1.02

Published in:
Management of Biological Invasions

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2020 the authors.
This is an open access article published under a Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/),
which permits unrestricted use, distribution and reproduction in any medium, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Research Article

In for the kill: novel biosecurity approaches for invasive and medically important mosquito species

Ross N. Cuthbert1,2,3,*, Eoghan M. Cunningham1,2, Kate Crane1,2, Jaimie T.A. Dick1,2, Amanda Callaghan3 and Neil E. Coughlan1,2

1Institute for Global Food Security, School of Biological Sciences, Queen’s University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, UK
2Queen’s Marine Laboratory, Queen’s University Belfast, 12-13 The Strand, Portaferry BT22 1PF, Northern Ireland, UK
3Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne Building, Reading RG6 6AS, England, UK

Author e-mails: rcuthbert03@qub.ac.uk (RNC), ecunningham18@qub.ac.uk (EMC), kcrane02@qub.ac.uk (KC), j.dick@qub.ac.uk (JTAD), a.callaghan@reading.ac.uk (AC), neil.coughlan.zoology@gmail.com (NEC)

*Corresponding author

Abstract

Invasive and medically important arthropods continue to spread and establish worldwide whilst adversely impacting ecosystems and public health. As the eradication and population suppression of these invaders and pests can be highly problematic and frequently unsuccessful, prevention of their further spread and establishment is imperative. Currently, however, there remains a lack of efficacious and cost-effective spread prevention techniques; particularly for species with complex life histories that span both aquatic and terrestrial habitats, such as mosquitoes. Here, we examine the use of steam exposure and disinfectant (Virkon® Aquatic) treatments to cause mortality of juvenile life stages of two invasive disease vector mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*. Steam treatments induced total mortality of *A. albopictus* and *C. quinquefasciatus* egg stages, following thirty second and five second exposures, respectively. Hatchability of *A. albopictus* eggs was substantially reduced following ten seconds of steam exposure. Total *A. albopictus* larval mortality was caused by steam exposures of one second or longer. Conversely, the aquatic disinfectant failed to impede hatchability of *A. albopictus* or *C. quinquefasciatus* egg stages, following thirty second and five second exposures, respectively. Hatchability of *A. albopictus* eggs was substantially reduced following ten seconds of steam exposure. Total *A. albopictus* larval mortality was caused by steam exposures of one second or longer. Conversely, the aquatic disinfectant failed to impede hatchability of *A. albopictus* or *C. quinquefasciatus* egg stages. However, disinfection with Virkon® Aquatic caused up to total mortality of mosquito larvae at exposure durations exceeding one minute at 4% concentrations, and five minutes at 1% concentrations. Our results suggest that steam treatments could be implemented as a biosecurity technique to prevent spread and establishment of invasive mosquitoes. Whilst the efficacy of chemical disinfections to cause mortality was not apparent towards mosquito eggs, applications could achieve significant mortality towards larvae in aquatic environments.

Key words: invasive alien species, disease vector mosquito, *Aedes albopictus*, *Culex quinquefasciatus*, steam, aquatic disinfectant

Introduction

The ecological and economic impacts driven by invasive species continue to detrimentally affect ecosystems worldwide (Early et al. 2016; Seebens et al. 2017; Doizy et al. 2018). Moreover, human-mediated dispersal of species continues to facilitate the circumvention of biogeographical
barriers by novel biota, which can result in the sudden emergence of new invaders (Seebens et al. 2018). Following establishment, secondary spread can be further intensified by natural and anthropogenic vectors (Hunt et al. 2018; Lovas-Kiss et al. 2019). In particular, for many invaders with partial or total aquatic life histories, tolerances to desiccation (Coughlan et al. 2017, 2018a) and abilities to enter diapause between hydroperiods (Hawley et al. 1987; Leisnham et al. 2011) can improve likelihood of survival during assisted overland dispersal. Accordingly, the efficacious suppression of organisms which tolerate both aquatic and terrestrial environments can present a distinct management challenge. In particular, invasive mosquito species can tolerate both aquatic and terrestrial environments in specific life stages, and medically important mosquitoes continue to spread and establish with profound public health implications (Yee 2016; Medlock et al. 2017). As eradication and population suppression of invaders is often complex and costly, the prevention of further species spread has been identified as the most economically-efficient means of mitigating invasive species impacts (Piria et al. 2017; Coughlan et al. 2018b, 2019). Indeed, examples of successful eradication of invasive species following establishment are relatively scarce (Beric and MacIsaac 2015; Hussner et al. 2017; but see Caffrey et al. 2018). Accordingly, the development of biosecurity protocols has become necessitated by multiple pieces of national and international legislation, which require member territories to actively take measures to prevent the spread of high impact invasive species (e.g. EU: Regulation 1143/2014; USA: Safeguarding the Nation from the Impacts of Invasive Species—amendment to Executive Order 13112).

Whilst a range of biosecurity protocols have been developed and proposed in recent years, knowledge of relative efficacies of different spread-prevention procedures among species and across life histories remains limited (Coughlan et al. 2019; Cuthbert et al. 2019; Crane et al. 2019). Indeed, biosecurity protocols designed to reduce secondary spread of invasive mosquitoes are scarce. For other taxa, thermal shock treatments derived through hot water (≥ 45 °C) submersion have been identified as a relatively environmentally-friendly means to induce mortality of invader propagule stages (Anderson et al. 2015; Shannon et al. 2018). However, practical difficulties associated with application of these treatments, alongside high costs for maintenance of water temperatures at sufficiently elevated levels to effectively decontaminate potential vectors, means it may not be a sustainable management option (Sebire et al. 2018). Recently, however, direct exposure to steam has been identified as an innovative and especially efficacious means for causing mortality of invasive species propagules through thermal shock (Coughlan et al. 2019; Crane et al. 2019). Steam applications could be aided by pre-existing industrial-scale devices which are practical for treating complex structures, and which minimise operating costs. Recently, Crane et al. (2019) found that steam
exposures for ten seconds or longer induced total degradation of multiple invasive aquatic macrophyte propagules, whilst Coughlan et al. (2019) observed total mortality of the Asian clam, *Corbicula fluminea*, following steam exposures for thirty seconds or longer. Furthermore, the use of chemical aquatic disinfectants has also been identified as an effective means of reducing invasive species spread (Barbour et al. 2013; Cuthbert et al. 2018a; but see also Coughlan et al. 2019 and Cuthbert et al. 2019). However, whilst the aforementioned biosecurity approaches have shown promise, applications have hitherto been limited to select groups of species. Accordingly, further validation of efficacy for the application of these treatments is still required for many other invaders, spanning multiple differential taxonomic groups.

Many invaders are also medically important, such as disease vector mosquitoes which have caused unparalleled morbidity and mortality in human populations through the efficient vectoring of pathogens and parasites (World Health Organisation 2019). Accordingly, the development of measures to counteract the spread of such species is of inherent public health importance (Golding et al. 2012; Yee 2016; Medlock et al. 2017). The spread of invasive mosquitoes can directly impact trophically analogous natives via resource competition (Juliano 2010; Yee and Skiff 2014), but may also drive human and wildlife disease outbreaks in recipient regions (Medlock et al. 2012; Schäffner et al. 2013). In particular, the Asian tiger mosquito *Aedes albopictus* (Skuse, 1895) can transmit pathogens which cause diseases such as chikungunya virus and dengue virus in human populations. Further, *A. albopictus* is known to be a superior resource competitor compared with native mosquitoes (e.g. Carrieri et al. 2003). *Aedes albopictus* is a notoriously invasive mosquito species; despite limited dispersal capabilities of adult stages, drought- and freeze-resistance eggs are easily spread by vectors such as the used tyre and ornamental plant trades (Schäffner et al. 2013). *Aedes albopictus* is native to temperate and tropical Asia, yet has successfully colonised the Americas, Africa and Europe (Juliano and Lounibos 2005). In Europe, this species has thus far spread into twenty-eight countries and is capable of establishment in temperate regions (Medlock et al. 2018). Furthermore, this species has been implicated in disease outbreaks within many countries (Medlock et al. 2015; Calba et al. 2017). *Culex quinquefasciatus* Say, 1823 is also considered a medically significant invasive mosquito species that is capable of spreading disease such as West Nile virus and Rift Valley fever virus (Juliano and Lounibos 2005; Turell 2012; Manimegalai and Sukanya 2014). Native to Africa, this species has colonised the Americas, Asia, New Zealand and southern Europe (Juliano and Lounibos 2005). Whilst eggs of *Culex* mosquitoes are largely intolerant to desiccation compared with *Aedes* species, *Culex* mosquitoes are also known to spread and establish
beyond their native range (Golding et al. 2012; Farnesi et al. 2015). However, there are currently limited biosecurity approaches to mitigate the spread of these invasive mosquito species, and therefore such mosquitoes continue to spread, establish and pose a severe risk to public health. The development of techniques to decontaminate poorly-regulated anthropogenic vectors, which have thus far facilitated unhindered overland dispersal of immature mosquito life stages, therefore warrants investigation (Medlock et al. 2012).

The present study thus tests and develops several novel biosecurity approaches to reduce the spread of immature stages of invasive and disease-spreading mosquitoes in a series of separate experiments. We experimentally assess the efficacy of direct steam and disinfectant treatments via spray or submergence to induce mortality of egg stages of *A. albopictus* and *C. quinquefasciatus*, across different exposure times and concentrations. Furthermore, we then test the use of steam and disinfectant to cause mortality of larval stages of *A. albopictus* and *C. quinquefasciatus*.

**Materials and methods**

*Animal collection and maintenance*

Eggs and larvae of *A. albopictus* were obtained through the Infravec2 project (https://infravec2.eu/) and originated from Montpellier, France. Eggs and larvae of *Culex quinquefasciatus* were obtained from a colony maintained at Queen’s University Marine Laboratory (QML), Portaferry, Northern Ireland, and reared as per Cuthbert et al. (2018b). Both mosquito species were maintained in a secure insectary facility within QML at 25 ± 2 °C and under a 16:8 light and dark photoperiod until experimentation.

*Efficacy of steam to cause mortality of *A. albopictus* and *C. quinquefasciatus* eggs*

Individual eggs of *A. albopictus* were transferred in replicate batches onto 100 μm mesh grids (2 cm²) for treatment. Each experimental group, i.e. batch, consisted of ten eggs. Batches of eggs were exposed directly to a continuous jet of steam (≥ 100 °C; Bissell® Steam Shot Handheld Steam Cleaner) at eight different exposure times: 1 s; 2 s; 5 s; 10 s; 15 s; 30 s; 1 m; and 2 m (n = 3 per experimental group). Similarly, individual egg rafts of *C. quinquefasciatus* were treated with steam within 20 mL arenas. All control groups of eggs were air dried for the maximum steam exposure duration of two minutes. For *A. albopictus* eggs, hatchability was quantified over a seven day observation period. A small amount of ascorbic acid was added to each replicate to promote egg hatching (as per Lacour et al. 2015). For *C. quinquefasciatus*, eggs were floated in dechlorinated tap water and numbers of hatched larvae were examined after two days.
Efficacy of Virkon® Aquatic spray applications to cause mortality of A. albopictus eggs

Batches of ten eggs were sprayed with Virkon® Aquatic disinfectant (DuPont) at 1% (10 g L⁻¹) and 4% (40 g L⁻¹) concentrations (0.9 mL spray⁻¹; 2 spray s⁻¹), mixed with tap water. Batches of eggs were, again, treated on 100 μm mesh grids, which minimised surface disinfectant accrual. Six different exposure times were examined: 5 s; 10 s; 30 s; 1 m; 2 m; and 5 m (n = 3 per experimental group). Following the allotted exposure times, to end any direct contact with the disinfectant, all replicates were washed by immediately spraying each batch of eggs ten times, consecutively, with tap water. Controls were sprayed with tap water and allowed to air dry for the maximum exposure time of five minutes. Hatchability was assessed over a seven day observation period.

Efficacy of Virkon® Aquatic submergence to cause mortality of A. albopictus and C. quinquefasciatus eggs

For A. albopictus, batches of ten eggs were submerged in either 1% or 4% concentrations of Virkon® Aquatic in 100 μm mesh bags (3 cm²) at one of eight exposure durations: 5 s; 10 s; 30 s; 1 m; 2 m; 5 m; 30 m; and 60 m (n = 3 per experimental group). Similarly, individual egg rafts of C. quinquefasciatus were likewise exposed to Virkon® Aquatic disinfectants at either 1% or 4% concentrations for three exposure times: 1 m; 2 m; and 5 m (n = 3 per experimental group). Following exposure, replicates were rinsed twice via 30 second submersions in tap water. All controls were submerged in tap water for the maximum exposure times of 60 minutes or up to five minutes for A. albopictus and C. quinquefasciatus, respectively. Following all experiments, batches of eggs were placed in 20 mL arenas (42 mm dia.) containing dechlorinated tap water for recovery in insectary conditions. As above, for A. albopictus eggs, hatchability was quantified over a seven day observation period, with the addition of ascorbic acid, while hatchability of C. quinquefasciatus was examined after two days.

Efficacy of steam to cause mortality of A. albopictus larvae

Batches of ten recently hatched 1st instar A. albopictus (0.1–0.2 mm) were steamed on 100 μm mesh grids for four different exposure times: 1 s; 2 s; 5 s; and 10 s (n = 3 per experimental group). Control larvae were allowed to air dry for the maximum exposure time of ten seconds. Larval mortality was examined immediately post-treatment, based on responses to mechanical stimuli; larvae which failed to respond to prodding were presumed to be dead.

Efficacy of aquatic disinfectant submergence applications to cause mortality of C. quinquefasciatus larvae

Batches of ten 4th instar larvae of C. quinquefasciatus (0.4–0.6 cm) were exposed to Virkon® Aquatic disinfectants at either 1% or 4% concentrations
for three exposure times: 1 m; 2 m; and 5 m (n = 3 per experimental group). Following exposure, replicates were rinsed twice via 30 second submersions in tap water. Controls consisted of three replicates at each exposure duration submerged in tap water alone. Larvae were then placed in 100 mL arenas (65 mm dia.) containing dechlorinated tap water in insectary conditions, with mortality rates being examined after two days.

**Data analyses**

Proportioned mosquito egg hatchability and larval mortality rates were analysed using generalised linear models assuming a binomial error distribution. Bias reductions were employed where complete separation occurred (Firth 1993), and quasi-binomial families were integrated where residual overdispersion was evidenced. Tukey comparisons were used where effects were significant at the 95% confidence level (Lenth 2018). For experiments on *C. quinquefasciatus* egg rafts, owing to the differences in the numbers of eggs within each raft, hatchability was assessed on a binary basis with respect to the presence of any recently hatched 1st instar larvae in each replicate. All statistical analyses were performed using R version 3.4.4 (R Core Development Team 2018).

**Results**

**Efficacy of steam to cause mortality of *A. albopictus* and *C. quinquefasciatus* eggs**

Overall, the effects of steam on *A. albopictus* hatchability were highly significant ($\chi^2 = 116.72$, $df = 8$, $p < 0.001$). Steam treatments of ten seconds or longer resulted in significantly greater mortality than control groups (all $p < 0.05$). No *A. albopictus* eggs hatched following steam treatments of thirty seconds and longer, whilst controls exhibited high hatchability (Supplementary material Table S1; Figure 1). In addition, substantial reductions in hatchability were evidenced for steam exposures of ten to fifteen seconds.

Overall, direct exposure to steam significantly induced mortality of *C. quinquefasciatus* egg rafts ($\chi^2 = 20.97$, $df = 8$, $p < 0.01$). Steam treatments of five seconds and longer caused total mortality in *C. quinquefasciatus*, whilst hatchability was observed across all control egg rafts (Table S1; Figure 2).

**Efficacy of Virkon*® Aquatic spray applications to cause mortality of *A. albopictus* eggs**

There was no significant effect of spray treatment on hatchability ($F_{12, 26} = 1.27$, $p = 0.29$). As such, *A. albopictus* eggs displayed hatchability following all exposure treatments to both 1% and 4% Virkon*® Aquatic via spraying (Table S1; Figure 3).
Figure 1. Mean (+ SE) proportioned hatchability rates of *Aedes albopictus* following different exposure durations to direct steam jet (*n* = 3 per experimental group).

Figure 2. Mean (+ SE) proportioned hatchability rates of *Culex quinquefasciatus* egg rafts following different exposure durations to direct steam jet (*n* = 3 per experimental group).
Efficacy of Virkon® Aquatic submergence to cause mortality of A. albopictus and C. quinquefasciatus eggs

There was no significant influence of disinfectant submersion treatments on hatchability ($F_{16,34} = 1.61, p = 0.12$). Accordingly, hatchability of A. albopictus eggs was observed following submersion in Virkon® Aquatic, across all exposure durations and concentrations (Table S1; Figure 4).
Similarly, there was no significant effect on hatchability by disinfection ($\chi^2 = 0.00, df = 2, p = 1.00$), exposure duration ($\chi^2 = 0.00, df = 2, p = 1.00$) or their interaction ($\chi^2 = 0.00, df = 4, p = 1.00$) on C. quinquefasciatus egg rafts. All examined C. quinquefasciatus egg rafts were observed to display hatchability across all disinfectant treatment groups (Table S1).

**Efficacy of steam to cause mortality of A. albopictus larvae**

Steam had a significant effect on mortality of mosquito larvae ($\chi^2 = 134.47, df = 3, p < 0.001$), with all steam treatments causing significant mortality in
comparison to controls (all $p < 0.001$). Total mortality was observed in
*A. albopictus* larvae following all steam exposures, whilst control groups
demonstrated consistent survival (Table S2; Figure 5).

**Efficacy of aquatic disinfectant submergence applications to cause mortality of C. quinquefasciatus larvae**

Treatment with Virkon® Aquatic *via* submersion had a significant direct
effect on larval mortality, whilst controls exhibited high survivability ($\chi^2 = 216.88$, $df = 2$, $p < 0.001$; Figure 6). All disinfectant treatments induced significantly greater mortality than controls (all $p < 0.001$). In turn, 4% treatments were significantly more efficacious than 1% treatments ($p < 0.01$). Exposure duration also significantly increased mortality ($\chi^2 = 33.59$, $df = 2$, $p < 0.001$), with mortality rates following five minute exposures significantly
greater than one or two minute exposures overall (both $p < 0.001$). One
and two minute exposures were more similar ($p > 0.05$). There was no
significant “treatment × duration” interaction term ($\chi^2 = 8.64$, $df = 4$, $p = 0.07$), however, total mortality was achieved at all exposure durations with
4% concentrations (Table S2; Figure 6).
Discussion

The present study demonstrates that a relatively brief exposure to a direct application of steam can cause substantial mortality to immature mosquito stages, whilst aquatic disinfectants are effective towards larval but not egg stages. Complete mortality of *A. albopictus* and *C. quinquefasciatus* eggs was achieved for steam treatment durations exceeding thirty seconds and five seconds, respectively. However, the application of an aquatic disinfectant, through either spray or submersion treatments, did not cause significant mortality in *A. albopictus* eggs, even following exposures of up to sixty minutes. In addition, exposure to aquatic disinfectants failed to impede hatchability, and accordingly the viability, of egg rafts of *C. quinquefasciatus*.

For recently hatched 1\textsuperscript{st} instar *A. albopictus* larvae, exposure to steam induced total mortality following one second treatments. Moreover, chemical disinfection caused significant mortality of 4\textsuperscript{th} instar *Culex* larval mosquito stages, particularly following exposure to a greater concentration and under longer exposure times. Overall, our results suggest that the use of steam should be considered for implementation in biosecurity strategies targeting immature mosquito stages. The use of chemical disinfectants,
such as Virkon® Aquatic, may be applied to induce mortality of larval mosquito stages within water in certain situations, but with limited effects on eggs.

To date, *A. albopictus* spread has been primarily driven by movement of desiccation-resistant eggs via human-mediated vectors associated with the trade in used tyres and, to a lesser extent, movement of ornamental plants (Reiter 1998; Medlock et al. 2012). Almost all invasive *A. albopictus* introductions in Europe have occurred through the trade of containers such as used tyres and wet-footed plants such as lucky bamboo, owing to the species’ ability to exploit various natural and artificial environments (Vaux and Medlock 2015). Currently, there is a distinct lack of biosecurity protocols to treat such surfaces prior to their translocation; however, the present study identifies the novel and innovative use of steam as a possible biosecurity technique to limit further spread of this damaging species. In particular, the application of steam treatments could be practically applicable to complex structures, such as tyres, yet would likely be inappropriate for treatment of traded ornamental plants. Further, whilst a potentially viable option for egg stages, the practical application of steam treatments for submerged larval mosquito stages is less clear. Nevertheless, our study identifies high efficacy of steam treatments across multiple mosquito life history stages. To facilitate the improved decontamination of large and structurally complex equipment, steam generators could be used to inject steam into niche areas at high pressures and temperatures, e.g., 10–12 Bar; ≥ 180 °C, which would otherwise be difficult to access or decontaminate (Crane et al. 2019). The use of industrial-scale steam cleaners may facilitate practical and cost-effective in-field applications, helping to reduce the spread of disease vector mosquitoes, which induce considerable medical costs. However, whilst the present laboratory-based study identifies steam as a potentially efficacious biosecurity approach for invasive vector mosquito species, further field-based trials are urgently required to test treatment efficacies prior to operationalisation by practitioners. Moreover, the operating costs of these practices would need to be quantified against the economic costs of inaction.

Although *Culex* egg rafts are characteristically not desiccation resistant (Farnesi et al. 2015), and are oviposited directly onto water (in contrast to *Aedes* mosquitoes), our results demonstrate the potential utility of steam exposures as highly efficacious towards multiple mosquito genera. Equally, larval stages of *A. albopictus* were also highly susceptible to mortality following steam applications, with total mortality evidenced after only one second of treatment. Whilst the efficacy of mosquito larvae translocation by natural and anthropogenic vectors between aquatic habitats requires investigation (Anderson et al. 2014; Coughlan et al. 2017), both egg and larval mosquito life stages are significantly impacted by steam. Our results
corroborate with the efficaciousness of direct steam applications towards other invasive species groups, such as aquatic macrophytes (Crane et al. 2019) and clams (Coughlan et al. 2019); accordingly, further exploration of this technique is warranted across taxa.

Although treatments via spray or submersion with aquatic disinfectants had little effect in reducing the viability of *A. albopictus* or *C. quinquefasciatus* egg stages, spray treatments for other taxa, such as invasive macrophytes, have previously been shown to be relatively less successful at inducing mortality than equivalent submersion treatments (Cuthbert et al. 2018a). Virkon® Aquatic contains potassium monopersulphate as an active ingredient, which is an oxidising agent that breaks down glycoproteins. This disinfectant is specifically designed for aquaculture given effectiveness against viral, bacterial and fungal disease; however, recently it has been suggested for use in invasive species biosecurity protocols (e.g. Barbour et al. 2013). In the present study, *A. albopictus* eggs remained viable even after 60 minutes of submersion in aquatic disinfectants at high concentrations. Therefore, it is unlikely that the use of chemical disinfectants is a practical solution to mitigating the spread of *A. albopictus* egg stages given this ineffectiveness at long exposure times, alongside potential environmental risks associated with such chemicals. The use of aquatic disinfectants has also shown limited efficiency towards fragmentary propagules of invasive aquatic macrophytes (Cuthbert et al. 2019). Indeed, the use of aquatic disinfectants is documented in other studies for routine treatment of mosquito eggs in order to avoid colony infections, with no clear impact on egg viability (e.g. Kamareddine et al. 2013). Physical features of mosquito eggs may impede the impact of aquatic disinfectants, such as their chitin content or surface density, which also naturally influences egg desiccation tolerances (Farnesi et al. 2015; Kreß et al. 2016). Nevertheless, exposure to aquatic disinfectants resulted in high levels of mortality in larval mosquito stages, which lack such protective structures. In solution, Virkon® Aquatic produces a low pH through organic acids, enabling high biocidal activity.

Our larval mortality results corroborate with the effectiveness of aquatic disinfectants towards other targeted aquatic invertebrate groups (e.g. Mitchell et al. 2007; Barbour et al. 2013; Moffitt et al. 2015). As a result, in some circumstances, the application of aquatic disinfectants may be beneficially used to kill larval stages inhabiting container environments.

In conclusion, this study has identified innovative and novel biosecurity protocols which could be implemented to help mitigate the spread of invasive and medically important mosquito species. Whilst a range of biosecurity protocols have been implemented for the control of invasive mosquitoes following initial arrival (e.g. Holder et al. 2010), there is a distinct lack of methodologies to prevent introductions. Given the efficacy of direct exposure to jets of steam in reducing viability of *A. albopictus* and
C. quinquefasciatus eggs shown here, as well as causing mortality of mosquito larvae, such treatments may provide for superior decontamination of equipment and structures known to vector or harbour mosquito eggs. Equally, as chemical disinfection achieved high levels of mortality towards larval mosquito stages of C. quinquefasciatus, chemical disinfection will likely have similar effects on other larval mosquito species. Despite little apparent efficacy in impeding hatchability, longer-term effects of aquatic disinfectants on mosquito development, survival and fecundity should be examined, such as through the loss of Rickettsia symbionts (Raoult and Roux 1997). Further, as there continues to be a proliferation of invasive mosquito species worldwide which are of public health importance, additional in-field assessment of the proposed biosecurity techniques should be undertaken to better-link laboratory-based efficacies to real-world situations. Nevertheless, our results require urgent consideration by management bodies and stakeholders involved in preventing the spread of such medically significant invasive species.

Acknowledgements

We acknowledge stimulating discussion from Marie Russell. We also thank two anonymous reviewers for helpful comments.

Funding Declaration

RNC acknowledges support from the Department for the Economy (DfE), Northern Ireland. JTAD and NEC are supported by the Irish Environmental Protection Agency (EPA) research grant 2015-NC-MS-4. EC acknowledges funding through the Department of Agriculture, Environment and Rural Affairs (DAERA), Northern Ireland. KC is supported through contributions from Queen’s University Belfast, the University of Windsor, McGill University and Waterways Ireland. This publication was supported by the project, Research Infrastructures for the control of vector-borne diseases (Infravec2), which has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 731060. JTAD acknowledges funding from the Natural Environment Research Council (NERC).

Author contributions

RNC and NEC proposed the study; RNC and NEC designed the experiments; RNC, EMC, KC and NEC conducted the experiments; RNC performed data analysis; all authors contributed to writing the manuscript, which was led by RNC.

References

Anderson LG, White PCL, Stebbing PD, Stentiford GD, Dunn AM (2014) Biosecurity and vector behaviour: evaluating the potential threat posed by anglers and canoeists as pathways for the spread of invasive non-native species and pathogens. PLoS ONE 9: e92788, https://doi.org/10.1371/journal.pone.0092788

Anderson LG, Dunn AM, Rosewarne PJ, Stebbing PD (2015) Invaders in hot water: a simple decontamination method to prevent the accidental spread of aquatic invasive non-native species. Biological Invasions 17: 2287–2297, https://doi.org/10.1007/s10530-015-0875-6

Barbour JH, McMenamin S, Dick JTA, Alexander ME, Caffrey JM (2013) Biosecurity measures to reduce the spread of the invasive freshwater Asian clam, Corbicula fluminea (Müller, 1774). Management of Biological Invasions 4: 219–230, https://doi.org/10.3391/mbi.2013.4.3.04

Beric B, Maclsaac HJ (2015) Determinants of rapid response success for alien invasive species in aquatic ecosystems. Biological Invasions 17: 3327–3335, https://doi.org/10.1007/s10530-015-0959-3

Caffrey JM, Gallagher K, Broughan D, Dick JTA (2018) Rapid response achieves eradication - chub in Ireland. Management of Biological Invasions 9: 475–482, https://doi.org/10.3391/mbi.2018.9.4.10
Calba C, Guerbois-Galla M, Franke F, Jeannin C, Auzet-Caillau M, Grard G, Pigaglio L, Decoppet A, Weicherdjing J, Savaill MC, Munoz-Riviero M, Chaud P, Cardiou B, Ramalli L, Fournier P, Noël H, De Lamballerie X, Paty M-C, Lepark-Goffart I (2017) Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Eurosurveillance 22: 00647, https://doi.org/10.2807/1560-7917.ES.2017.22.39.17-00647

Carrière M, Bacchi M, Bellini R, Maini S (2003) On the competition occurring between Aedes albopictus and Culex pipiens (Diptera: Culicidae) in Italy. Environmental Entomology 32: 1313–1321, https://doi.org/10.1603/0046-225X-32.6.1313

Coughlan NE, Kelly TC, Dove potv J, Jansen MAK (2017) Up, up and away: bird-mediated zooochorous dispersal between aquatic environments. Freshwater Biology 62: 631–648, https://doi.org/10.1111/fwb.12894

Coughlan NE, Cuthbert RN, Kelly TC, Jansen MAK (2018a) Parched plants: survival and viability of invasive aquatic macrophytes following exposure to various desiccation regimes. Aquatic Botany 150: 9–15, https://doi.org/10.1016/j.aquabot.2018.06.001

Coughlan NE, Walsh DA, Caffrey JM, Davis E, Lucy FE, Cuthbert RN, Dick JTA (2018b) Cold as ice: a novel eradication and control method for invasive Asian clam, Corbicula fluminea, using pelleted dry ice. Management of Biological Invasions 9: 463–474, https://doi.org/10.3391/mbi.2018.9.4.09

Coughlan NE, Cuthbert RN, Dickey JWE, Crane K, Caffrey JM, Lucy FE, Davis E, Dick JTA (2019) Better biosecurity: spread-prevention of the invasive Asian clam, Corbicula fluminea (Müller, 1774). Management of Biological Invasions 10: 111–126, https://doi.org/10.3391/mbi.2019.10.1.07

Cuthbert RN, Coughlan NE, Crane K, Caffrey JM, Maelsaas HJ, Dick JTA (2018a) A dip or a dab: assessing the efficacy of Viruses aquatic disinfectant to reduce secondary spread of the invasive curly waterweed Lagarosiphon major. Management of Biological Invasions 9: 259–265, https://doi.org/10.3391/mbi.2018.9.3.08

Cuthbert RN, Dick JTA, Callaghan A (2018b) Interspecific variation, habitat complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control. Biological Control 121: 80–87, https://doi.org/10.1016/j.biocontrol.2018.02.012

Cuthbert RN, Crane K, Dick JTA, Caffrey JM, Maelsaas HJ, Coughlan NE (2019) Die hard: impact of aquatic disinfectants on the survival and viability of invasive Elodea nuttallii. Aquatic Botany 154: 11–17, https://doi.org/10.1016/j.aquabot.2018.12.003

Crane K, Cuthbert RN, Dick JTA, Kregting J, Maelsaas HJ, Coughlan NE (2019) Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. Biological Invasions 121: 1311–1321, https://doi.org/10.1007/s10530-018-1901-2

Doisy A, Butler E, Mennott J, Varnham K, Gross T (2018) Impact of cyber-invasive species on a large ecological network. Scientific Reports 8: 13245, https://doi.org/10.1038/s41598-018-31423-4

Early R, Bradley BA, Dukes JS, Lawler JI, Olden JD, Blumenthal DM, Gonzalez P, Groszholz ED, Ibáñez I, Miller LP, Sorte CJB, Tatem AJ (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications 7: 12485, https://doi.org/10.1038/ncomms12485

Farnesi LC, Menna-Barreto RFS, Martins AJ, Valle D, Rezende GL (2015) Physical features and chitin content of eggs from the mosquito vectors Aedes aegypti, Anopheles aquasalis and Culex quinquefasciatus: Connection with distinct levels of resistance to desiccation. Journal of Insect Physiology 83: 43–52, https://doi.org/10.1016/j.jinsphys.2015.10.006

Firth D (1993) Bias Reduction of Maximum Likelihood Estimates. Biometrika 80: 27–38, https://doi.org/10.1093/biomet/80.1.27

Golding N, Nunn M, Medlock JM, Purse BV, Vaux AGC, Schäfer SM (2012) West Nile virus vector Culex modestus established in southern England. Parasites and Vectors 5: 1–5, https://doi.org/10.1186/1756-3305-5-32

Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig Jr GB (1987) Aedes albopictus in North America: probable introduction in used tires from northern Asia. Science 236: 1114–1116, https://doi.org/10.1126/science.3576225

Holder P, George S, Disbury M, Singe M, Kean JM, McFadden A (2010) A biosecurity response to Aedes albopictus (Diptera: Culicidae) in Auckland, New Zealand. Journal of Medical Entomology 47: 600–609, https://doi.org/10.1093/jmedent/47.4.600

Hunt R, Thomas JR, James J, Cable J (2018) Transmission and terrestrial dispersal of non-native ectosymbionts on invasive crayfish. Hydrobiologia 820: 135–144, https://doi.org/10.1007/s10750-018-3647-3

Hussner A, Stiers I, Verhofstadt MJM, Bakker ES, Grutters BMC, Haury J, van Valkenburg JLCH, Brundu G, Newman J, Clayton JS, Anderson LWJ, Hofstra D (2017) Management and control methods of invasive alien aquatic plants: a review. Aquatic Botany 136: 112–137, https://doi.org/10.1016/j.aquabot.2016.08.002

Juliano SA (2010) Coexistence, exclusion, or neutrality? A meta-analysis of competition between Aedes albopictus and resident mosquitoes. Israel Journal of Ecology and Evolution 56: 325–351, https://doi.org/10.1560/IJEE.55.3-4.325
Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. Ecology Letters 8: 558–574, https://doi.org/10.1111/j.1461-0248.2005.00755.x

Kamarededine L, Fan Y, Osta MA, Keyhani NO (2013) Expression of trypsin modulating oosac factor (TMOF) in an entomopathogenic fungus increases its virulence towards Anopheles gambiae and reduces fecundity in the target mosquito. Parasites and Vectors 6: 22, https://doi.org/10.1186/1756-3305-6-22

Krell A, Kuch U, Oehlmann J, Müller R (2016) Effects of diapause and cold acclimation on egg ultrastructure: new insights into the cold hardiness mechanisms of the Asian tiger mosquito Aedes (Stegomyia) albopictus. Journal of Vector Ecology 41: 142–150, https://doi.org/10.1111/jvec.12206

Lacour G, Chanaud L, L’Ambert G, Hance G (2015) Seasonal synchronization of diapause phases in Aedes albopictus (Diptera: Culicidae) in North America. Annals of the Entomological Society of America 108: 1309–1318, https://doi.org/10.1603/AN11032

Lenth R (2018) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.1.3. https://cran.r-project.org/web/packages/emmeans/index.html

Lovás-Kiss Á, Sánchez MI, Wilkinson DM, Coughlan NE, Alves JA, Green AJ (2019) Shorebirds as important vectors for plant dispersal in Europe. Ecography 42: 956–967, https://doi.org/10.1111/ecog.04065

Manimekalai K, Sukanya S (2014) Biology of the filarary vector, Culex quinquefasciatus (Diptera: Culicidae). International Journal of Current Microbiology and Applied Sciences 3: 718–724

Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, Van Bortel W (2012) A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-Borne Zoonotic Diseases 12: 435–447, https://doi.org/10.1089/vbz.2011.0814

Medlock JM, Hansford KM, Versteirt V, Cull B, Kampen H, Fontenille D, Hendrickx G, Zeller H, Van Bortel W, Schaffner F (2015) An entomological review of invasive mosquitoes in Europe. Bulletin of Entomological Research 105: 637–663, https://doi.org/10.1017/S0007485315001036

Medlock JM, Vaux AGC, Cull B, Schaffner F, Gillingham E, Pfluger V, Leach SA (2017) Detection of the invasive mosquito species Aedes albopictus in southern England. The Lancet Infectious Diseases 17: 140, https://doi.org/10.1016/S1473-3099(17)30024-5

Medlock JM, Hansford KM, Vaux AGC, Cull B, Gillingham E, Leech S (2018) Assessment of the public health threats posed by vector-borne disease in the United Kingdom (UK). International Journal of Environmental Research and Public Health 15: 2145, https://doi.org/10.3390/ijerph15102145

Mitchell AJ, Hobbs MS, Brandt TM (2007) The effect of chemical treatments on red-rim melania Melanoides tuberculata, an exotic aquatic snail that serves as a vector of trematodes to fish and other species in the USA. North American Journal of Fish Management 27: 1287–1293, https://doi.org/10.1577/M06-252.1

Moffitt CM, Barenberg A, Stockton KA, Watten BJ (2015) Efficacy of two approaches for disinfecting surfaces and water infested with quagga mussel veligers. In: Wong WH, Gertsenberger S (eds), Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States, CRC Press, USA, pp 467–477, https://doi.org/10.1201/b18447-38

Piria M, Copp GH, Dick JTA, Dupliči D, Groom Q, Jelić D, Lucy HE, Roy HE, Sarat E, Simonović P, Tomljanović T, Tricarico E, Weinlander M, Adámek Z, Bedolf S, Caffrey JM (2017) Tackling invasive alien species in Europe II: threats and opportunities until 2020. Management of Biological Invasions 8: 273–286, https://doi.org/10.3391/mbi.2017.8.3.02

Rooy C, Core Development Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.r-project.org/

Raoult D, Roux V (1997) Rickettsioses as paradigms of new or emerging infectious diseases. Clinical Microbiology Reviews 10: 694, https://doi.org/10.1128/CMR.10.4.694

Reiter P (1998) Aedes albopictus and the world trade in used tires, 1988-1995: the shape of things to come? Journal of the American Mosquito Control Association 14: 83–94

Schaffner F, Medlock JM, Van Bortel W (2013) Public health significance of invasive mosquitoes in Europe. Clinical Microbiology and Infection 19: 685–692, https://doi.org/10.1111/1469-0691.12189

Sebire M, Rimmer G, Hicks R, Parker SJ, Stebbing PD (2018) A preliminary investigation into biosecurity treatments to manage the invasive killer shrimp (Okierogammarus villosus). Management of Biological Invasions 9: 101–113, https://doi.org/10.3391/mbi.2018.9.2.04

Cuthbert et al. (2020), Management of Biological Invasions 11(1): 9–25, https://doi.org/10.3391/mbi.2020.11.1.02
Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Shyama Pagad, 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 A, 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

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke J, Pagad S, Pyšek P, van Kleunen M, Winter M, Ansong M, Arianoutsou M, Bacher S, Blasius B, Brockerhoff EG, Brundu G, Capinha C, Causton CE, Celesti-Grapow L, Dawson W, Dullinger S, Economo EP, Fuentes N, Guénard B, Jäger H, Kartesz J, Kenis M, Kühn I, Lenzner B, Liebhold AM, Mosena A, Moser D, Nentwig W, 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, Walker K, Ward DF, Yamanaka T, Essl F (2018) Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences* 115: E2264–E2273, https://doi.org/10.1073/pnas.1719429115

Shannon C, Quinn CH, Stebbing PD, Hassall C, Dunn AM (2018) The practical application of hot water to reduce the introduction and spread of aquatic invasive alien species. *Management of Biological Invasions* 9: 417–423, https://doi.org/10.3391/mbi.2018.9.4.05

Turell MJ (2012) Members of the *Culex pipiens* complex as vectors of viruses. *Journal of the American Mosquito Control Association* 28: 123–126, https://doi.org/10.2987/8756-971X-28.4.123

Vaux AGC, Medlock JM (2015) Current status of invasive mosquito surveillance in the UK. *Parasites and Vectors* 8: 351, https://doi.org/10.1186/s13071-015-0936-9

World Health Organisation (2019) Mosquito-borne diseases. https://www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases/en/ (accessed 02/01/2019)

Yee D (2016) What can larval ecology tell us about the success of *Aedes albopictus* (Diptera: Culicidae) within the United States? *Journal of Medical Entomology* 53: 1002–1012, https://doi.org/10.1093/jme/tjw046

Yee DA, Skiff JF (2014) Interspecific competition of a new invasive mosquito, *Culex coronator*, and two container mosquitoes, *Aedes albopictus* and *Cx. quinquefasciatus* (Diptera: Culicidae), across different detritus environments. *Journal of Medical Entomology* 51: 89–96, https://doi.org/10.1603/ME13182

### Supplementary material

The following supplementary material is available for this article:

- **Table S1.** Raw mean percentage mortality (± SE) of *A. albopictus* eggs and *C. quinquefasciatus* egg rafts following exposure to steam spray, disinfectant spray and disinfectant submergence treatments.

- **Table S2.** Raw mean percentage mortality (± SE) of *A. albopictus* larvae (1st instar) and *C. quinquefasciatus* larvae (4th instar) following exposure to steam spray or disinfectant submergence treatments.

This material is available as part of online article from: http://www.reabic.net/journals/mbi/2020/Supplements/MBI_2020_Cuthbert_etal_SupplementaryMaterials.pdf