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Research Article

Better biosecurity: spread-prevention of the invasive Asian clam, Corbicula fluminea (Müller, 1774)

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Abstract

Aquatic invasive species (AIS) negatively impact freshwater ecosystems on a global scale. As management options for control and eradication of established AIS populations are often complex, costly and resource-intensive, spread-prevention protocols are considered essential. The Asian clam, *Corbicula fluminea* (Müller, 1774), is considered a high-impact successful invader that can adversely alter freshwater habitats, community dynamics and ecosystem function. Accordingly, we examine the efficacy of a range of biosecurity techniques, including recommended (aquatic disinfectants, bleach and salt solutions) and more novel (hot water and direct steam) approaches, to induce adult *C. fluminea* mortality. In separate experiments, *C. fluminea* were submerged at 12 °C for up to 80 minutes in: 1) 2% and 4% solutions of Virasure® Aquatic and Virkon® Aquatic; 2) warm (30 °C) 2% and 4% solutions of these disinfectants; and 3) 10% and 20% bleach solutions. Furthermore, specimens were exposed to: 4) 30% and 70% salt solutions (NaCl) for up to 72 hrs; 5) hot water (35, 40 and 45 °C) for up to 20 minutes; and 6), direct steam exposure for up to 10 minutes. Adult *C. fluminea* were found to be largely resistant to aquatic disinfectants, bleach and salt solutions, with ≤ 58% mortality achieved at the maximum exposure times. However, immersion in hot water (≥ 45 °C) and direct steam exposure for five minutes and 30 seconds, respectively, rapidly caused mortality. Accordingly, simple biosecurity protocols that cause thermal shock appear highly effective. We discuss the need for further examination of biosecurity protocols across all life stages of current, emerging and potential AIS, and provide guidance for improving biosecurity practices.

Key words: angling equipment, bleach, hot water, invasive species control, steam clean, Virasure® Aquatic, Virkon® Aquatic, watersports equipment

Introduction

Aquatic invasive species (AIS) are a serious threat to the biodiversity, ecological functioning, economic and social value of freshwater ecosystems worldwide (Sala et al. 2000; Simberloff et al. 2013; Sousa et al. 2014). Currently, management options for effective control and eradication of

established AIS populations are often complex, resource-intensive, costly, and damaging to non-target species (Caffrey et al. 2011b, 2014; Piria et al. 2017; Coughlan et al. 2018b). Therefore, spread-prevention is considered key to mitigating further invader impacts (Barbour et al. 2013; Anderson et al. 2015; Coughlan et al. 2018a; Cuthbert et al. 2018). However, freshwater systems remain highly vulnerable to AIS introductions due to their exposure to multiple natural and anthropogenic transport pathways and a plethora of possible vectors, e.g. footwear, angling equipment, boats, vehicles and trailers (Rothlisberger et al. 2010; Banha et al. 2017; Coughlan et al. 2017a). Accordingly, new and innovative biosecurity protocols that maximise prevention of invader spread are urgently required. In essence, biosecurity measures relate to all activities enacted to prevent the introduction and spread of invaders (Caffrey et al. 2014; Shannon et al. 2018). Ideally, biosecurity protocols should utilise materials that are readily available, relatively easy to apply, be non-time-consuming, and environmentally friendly (Anderson et al. 2015; Sutcliffe et al. 2018; Coughlan et al. 2018b; Crane et al. 2018).

Although various biosecurity campaigns such as "Check, Clean, Dry" have attempted to promote public awareness and reduce AIS spread, there is limited information detailing the relative efficacies of recommended biosecurity measures (Caffrey 2010; Barbour et al. 2013; Anderson et al. 2015; Piria et al. 2017; Coughlan et al. 2018a). For example, broad-spectrum aquatic disinfectants (Barbour et al. 2013; Cuthbert et al. 2018), desiccation (Coughlan et al. 2017b; 2018a), hot water (Anderson et al. 2015; Shannon et al. 2018), and steam applications (Crane et al. 2018) have been suggested as suitable mechanisms to control AIS spread. Although not all invader life stages have been thoroughly assessed for susceptibility to chemical based treatments, these methods have proven effective against a variety of invasive Mollusca and macrophyte species (e.g. Barbour et al. 2013; Stockton-Fiti and Moffitt 2017; Cuthbert et al. 2018), and risks of toxicity to fish and other aquatic organism *via* residues and spills is low with good practice (see Stockton-Fiti and Moffitt 2017).

The Asian clam, *Corbicula fluminea* (Müller, 1774), is considered a high impact invader that can dominate macroinvertebrate communities, physically alter benthic habitats, and disrupt ecosystem regulating services (McMahon 1982; Karatayev et al. 2007; Sousa et al. 2008, 2014). Once established, populations of *C. fluminea* are notoriously difficult to eradicate or control (Caffrey et al. 2011a; Sheehan et al. 2014; Coughlan et al. 2018b). Moreover, *C. fluminea* has displayed a remarkable capacity for human-mediated passive dispersal (Belz et al. 2012; Lucy et al. 2012; Coughlan et al. 2017b). Despite repeated management efforts to reduce invader spread, *C. fluminea* continues to spread across hydrologically unconnected freshwater systems (Caffrey et al. 2016; Colwell et al. 2017). Moreover,

recent distribution models suggest that suitable habitat availability will increase under the current rate of climate change, favouring the further expansion of *C. fluminea* populations (Gama et al. 2017).

Effective biosecurity measures have been developed to prevent the spread of juvenile *C. fluminea* (≤ 10 mm; Barbour et al. 2013). The efficacy of these biosecurity protocols on larger adult specimens, which can become entangled in equipment such as fyke nets, is currently unknown (Caffrey et al. 2016). Here, we examine the efficacy of a variety of biosecurity techniques such as aquatic disinfectants, bleach and salt solutions, and more novel approaches of hot water and steam applications, to cause adult *C. fluminea* mortality.

Methods

Specimen collection and maintenance

Adult *Corbicula fluminea* specimens were collected from the River Barrow in the Republic of Ireland (52º29′15.11″N; 6º55′42.20″W), and transported in source water (11–14 °C) to Queen's Marine Laboratory (QML), Portaferry, Northern Ireland. Specimens were maintained in aerated aquaria, within locally sourced lake water, in a controlled temperature (CT) room (12 °C) on a 12:12 hr light and dark schedule. The clams were observed to display normal feeding behaviour and a high rate of survival (*c.* 95%). Specimens were allowed to acclimatise for at least one week prior to experimentation. All experiments were completed within a two week period, and performed within the CT room. Only specimens that were obviously alive and feeding were selected for experimental work, i.e. selected specimens were observed opening and/or extending the foot. Adult clams were selected based on shell height (SH), i.e. the maximum posterior to anterior axis, "umbo to gape". After completion of the experiments, ≥ 1000 clams were maintained within the aquaria for over a 3 month period. Greater than 95% survival of these specimens was observed.

Experiment 1: Efficacy of Virasure® and Virkon® solutions

Further developing the study performed by Barbour et al. (2013), the efficacy of Virasure® Aquatic (Fish Vet Group) and Virkon® Aquatic (Antec Int. DuPont) was examined using 2% (20 g L⁻¹) and 4% (40 g L⁻¹) concentrations. A pilot study indicated that 1% (10 g L⁻¹) would be ineffective, therefore, higher concentrations were chosen. Groups of ten medium (SH = 15–20.9 mm; with a mean ± SE of 17.1 ± 0.3 mm) and large (21–36 mm; 26.7 ± 0.8 mm) specimens were immersed in solutions (dechlorinated tap water) at 12 °C of either chemical for 10, 20, 40 and 80 minutes (*n* = 5 replicates). Exposure times were chosen to incrementally increase to access if complete mortality could be achieved within a reasonably prompt soaking time. Control groups were submersed in

dechlorinated tap water for the same time periods. Immediately after submersion for the defined periods, specimens were washed with tap water for *c.* two minutes. Controls were likewise washed. All specimens were returned to 600 ml of dechlorinated bubbled water at 12 °C for a 24 hr recovery period, after which mortality was assessed. Specimens were considered dead if they were gaping, or if they did not offer any resistance to being teased apart with tweezers and did not reclose (see Matthews and McMahon 1999).

Experiment 2: Efficacy of warm water solutions of Virasure® and Virkon®

The synergistic efficacy of warm water (30 °C) and solutions of Virasure® Aquatic and Virkon® Aquatic was examined using 2 and 4% concentrations. Adult clams had previously been observed to become more active (i.e. open and/or feed) in water baths maintained at 30 °C. Groups of ten medium to large sized specimens (SH = 15–26 mm; 20.9 ± 0.8 mm) were immersed in solutions of either chemical for 10, 20, 40 and 80 minutes (*n* = 3 replicates). Control groups were submerged in warm (30 °C) dechlorinated tap water for the same time periods. All solutions were maintained at a constant temperature using water-baths. As before, after the desired exposure time was obtained, specimens were immediately removed from the chemical solutions. All specimens were allowed to air cool for a five minute period, to prevent additional thermal shock from contact with cooler water, and were then washed with tap water for *c.* two minutes. All specimens were allowed to recover for a 24 hr period, after which mortality was assessed.

Experiment 3: Efficacy of bleach solutions

Groups of ten specimens (SH = 15–26 mm; 19.6 ± 0.9 mm) were immersed in 5 (50 ml L^{-1}), 10 (100 ml L^{-1}) or 20% (200 ml L^{-1}) bleach solutions (Parazone® Original) for 10, 20, 40 and 80 minutes (*n* = 3 replicates). Control groups were submersed in dechlorinated tap water. As before, after exposure, specimens were washed and given a 24 hr recovery period, after which mortality was assessed.

Experiment 4: Efficacy of salt (NaCl) solutions

Groups of ten specimens (SH = 15–26 mm; 20.1 ± 0.7 mm) were immersed in 35 (35 g L^{-1}) or 70% (70 g L^{-1}) aerated salt (NaCl) solutions (regular table salt) for 1, 6, 24, 48 and 72 hrs (*n* = 5 replicates). Control groups were submersed in aerated, dechlorinated tap water. After exposure, specimens were washed and allowed to recover for a 24 hr period before mortality was assessed.

Experiment 5: Efficacy of hot water

Groups of ten medium (SH = 15–20.9 mm; 16.8 ± 0.5 mm) and large (21–36 mm; 26.4 ± 0.8 mm) specimens were immersed in water at 35, 40 and 45 °C for 5, 10 and 20 minutes (n = 5 replicates). Constant water temperature was maintained using water-baths. Control groups were submersed in dechlorinated tap water at 12 °C. Immediately after submersion, all specimens were allowed to air cool for a 5 minute period, and were then washed. As before, specimens were given a 24 hr recovery period, after which mortality was assessed.

Experiment 6: Efficacy of steam

Groups of ten specimens (SH = 15–26 mm; 21 ± 0.3 mm) were directly exposed to a continuous jet of steam (≥ 100 °C; Bissell Steam Shot Handheld Steam Cleaner), at a distance of 2–3 cm from the source, for 10 sec, 30 sec, 1, 2, 5 and 10 minutes (n = 5 replicates). Control groups were taken out of water and allowed to air dry for the same time periods. After exposure, all specimens were allowed to cool for a 5 minute period, were washed, and then allowed to recover for a 24 hr period, after which mortality was assessed.

Statistical analysis

All data were analysed in R version 3.3.3 (R Core Team 2017). The number of dead *C. fluminea* in each experiment was converted to proportional mortality rates and further transformed to reduce extremes (0 s, 1 s) before analyses to meet model assumptions (Eqn. 1; Smithson and Verkuilen 2006):

$$y_t = (y(n-1) + 0.5)/n \qquad (1)$$

where y_t is the transformed output and n is the sample size. Beta regression using the "betareg" package in R (Cribari-Neto and Zeileis 2010) was used to analyse mortality rates in each experiment. Analysis of deviance was then applied to derive appropriate models, with χ^2 used to report the relevance of effects to the dependent variable. We employed Tukey's HSD method for specific pairwise comparisons, where required. In all cases α = 0.05.

Results

Experiment 1: Efficacy of Virasure® and Virkon® solutions

There was between 86–100% survival of control clams across both size classes. Mortality of medium and large clams exposed to aquatic disinfectants was up to 31 and 58 %, respectively (Figure 1). Significantly higher clam mortality was observed at greater disinfectant concentrations, for larger clams, and at longer exposure times (all $P < 0.001$; Figure 1,

Table 1). Mortality levels for clams submersed in aquatic disinfectants were significantly higher than control treatments, across all concentrations (all $P < 0.001$). However, no significant differences between the efficacy of the different concentrations of Virasure® and Virkon® solutions was observed (all $P > 0.05$). Further, there were no significant differences between 10 and 20 minute exposures, nor 20 and 40 minute exposures (all $P > 0.05$). Significant interactions between the treatment, clam size and exposure time effects reflect greater efficacies of Virkon® towards small clams and Virasure® towards large clams, alongside greater relative efficacies at longer exposure times (Figure 1).
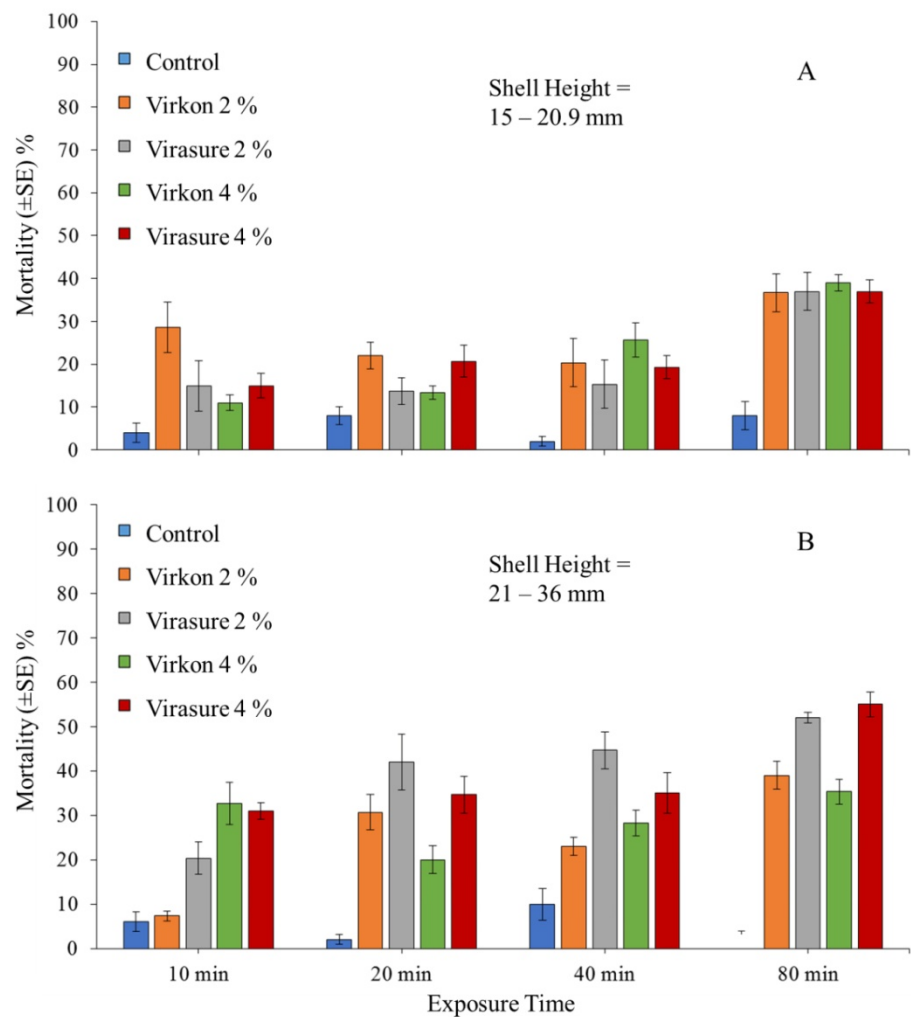
Figure 1. Mean mortality (± SE) of medium (A: SH = 15–20.9 mm) and large (B: SH = 21–36 mm) adult *Corbicula fluminea* specimens 24 hrs post-exposure to aquatic disinfectants Virasure® Aquatic and Virkon® Aquatic, at both 2% (20 g L^{-1}) and 4 % (40 g L^{-1}) concentrations. Experimental groups, each consisting of ten clams, were immersed in solutions (dechlorinated tap water) of either chemical for 10, 20, 40 and 80 minutes ($n = 5$, with 400 clams per n).

Table 1. Chi-squared test (χ^2) performed on the number of dead *Corbicula fluminea* specimens for Experiment 1: Efficacy of Virasure® and Virkon® solutions (VV). VV = Control, 2% concentration (20 g L^{-1}) or 4 % concentration (40 g L^{-1}). Exposure time = 10, 20, 40 or 80 minutes. Size ranges = medium (15–20.9 mm; maximum posterior to anterior axis) or large (21–36 mm) sized clams. NS = non-significant; α = 0.05.

Source of variation	χ^2	P
Virkon® and Virasure® (VV)	$\chi^2 (4) = 133.3994$	$P < 0.001$
Exposure Time (Time)	$\chi^2 (3) = 40.2124$	$P < 0.001$
Size	$\chi^2 (1) = 17.5215$	$P < 0.001$
VV × Time	$\chi^2 (12) = 25.0286$	$P < 0.05$
VV × Size	$\chi^2 (4) = 12.4574$	$P < 0.05$
Time × Size	$\chi^2 (3) = 5.5494$	NS
VV × Time × Size	$\chi^2 (12) = 23.7752$	$P < 0.05$

Experiment 2: Efficacy of warm water solutions of Virasure® and Virkon®

While between 87–100% of control clams survived, up to 54% mortality was recorded for clams exposed to aquatic disinfectants at 30 °C. There was significantly higher clam mortality with higher disinfectant concentrations, and at longer exposure times (both P < 0.001; Figure 2, Table 2). Mortality of clams treated with either aquatic disinfectant was significantly higher than controls (all P < 0.01), yet no significant differences between the efficacy of Virasure® and Virkon® solutions maintained at 30 °C were observed across any concentration (all P > 0.05). There was no significant difference in mortality rates amongst exposure times of 10, 20 or 40 minutes (all P > 0.05). Further, there was no significant interaction effect between treatment and exposure time (P > 0.05; Table 2).
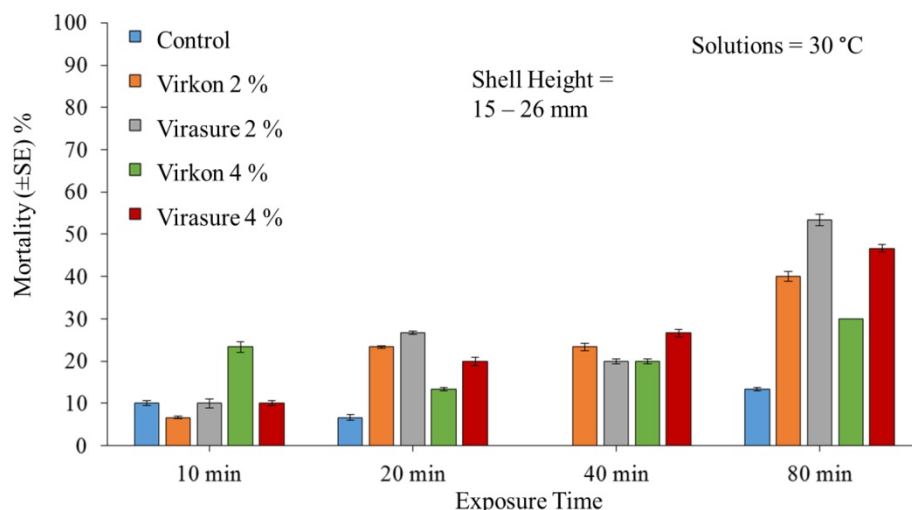
Figure 2. Mean mortality (± SE) of adult *Corbicula fluminea* specimens (SH = 15–26 mm) 24 hrs post-exposure to warm water (30 °C) solutions of aquatic disinfectants Virasure® Aquatic and Virkon® Aquatic, at both 2% (20 g L^{-1}) and 4% (40 g L^{-1}) concentrations. Experimental groups, each consisting of ten adult clams, were immersed in solutions of either chemical for 10, 20, 40 and 80 minutes (*n* = 3, with 200 clams per *n*).

Table 2. Chi-squared test (χ^2) performed on the number of dead *Corbicula fluminea* specimens for Experiment 2: Efficacy of warm water solutions (30 °C) of Virasure® and Virkon® (VVW). VVW = Control, 2 (20 g L⁻¹) or 4% (40 g L⁻¹) concentrations. Exposure time = 10, 20, 40 or 80 minutes. Medium to large sizes of *C. fluminea* (15–26 mm) were examined. Experimental *n* = 3. NS = non-significant; α = 0.05.

Source of variation	χ^2	P
Virkon® and Virasure® w/hot water (VVW)	χ^2 (4) = 24.840	$P < 0.001$
Exposure Time (Time)	χ^2 (3) = 44.598	$P < 0.001$
VVW × Time	χ^2 (12) = 19.354	NS

Experiment 3: Efficacy of bleach solutions

There was 89% survival of control clams and up to 21% mortality of clams exposed to bleach solutions. However, no significant treatment or exposure time effects were detected on clam mortalities (Figure 3, Table 3).
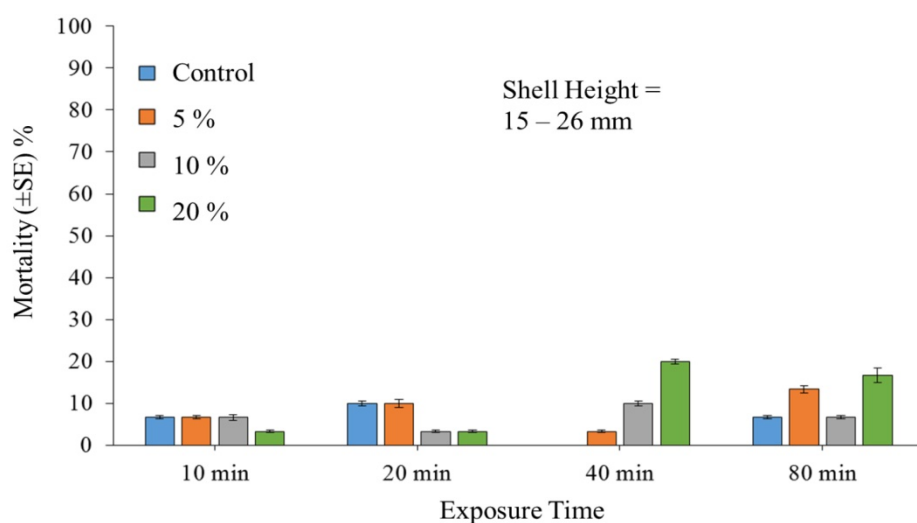

Figure 3. Mean mortality (± SE) of adult *Corbicula fluminea* specimens (SH = 15–26 mm) 24 hrs post-exposure to 5 (50 ml L⁻¹), 10 (100 ml L⁻¹) or 20% (200 ml L⁻¹) bleach solutions. Experimental groups, each consisting of ten clams, were immersed in solutions of either chemical for 10, 20, 40 and 80 minutes (*n* = 3, with 160 clams per *n*).

Table 3. Chi-squared test (χ^2) performed on the number of dead *Corbicula fluminea* specimens for Experiment 3: Efficacy of bleach solutions (B). B = Control, 5 (50 ml L⁻¹), 10 (100 ml L⁻¹) or 20% (200 ml L⁻¹). Exposure time = 10, 20, 40 or 80 minutes. Medium to large sizes of *C. fluminea* (15–26 mm) were examined. Experimental *n* = 3. NS = non-significant; α = 0.05.

Source of variation	χ^2	P
Bleach (B)	χ^2 (3) = 1.6879	NS
Exposure Time (Time)	χ^2 (3) = 1.6668	NS
B × Time	χ^2 (9) = 9.9118	NS

Experiment 4: Efficacy of salt (NaCl) solutions

While there was 89% survival of control clams, up to 48% mortality was recorded for clams exposed to salt solutions. Significantly greater clam mortality was observed at higher salt concentrations and for longer exposure times (both $P < 0.001$; Figure 4, Table 4). However, there was no significant difference in clam mortality detected between the 35 and 70 g

treatments ($P > 0.05$). The significant interaction effect between treatment and exposure time reflects greater differences between 35 and 70 g treatments at intermediate exposure times (Figure 4, Table 4).
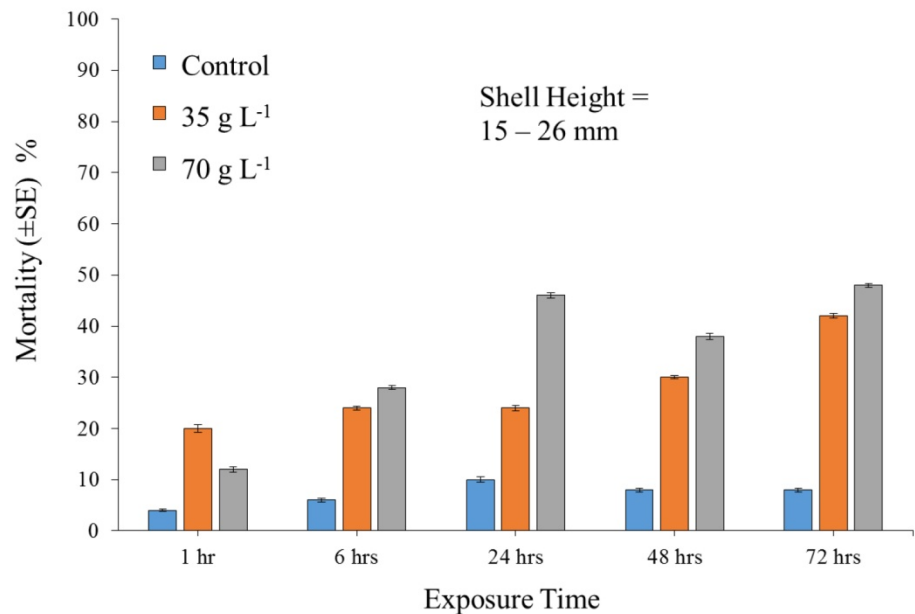

Figure 4. Mean mortality (± SE) for groups of ten adult *Corbicula fluminea* specimens (SH = 15–26 mm) 24 hrs post-exposure to 35 (35 g L⁻¹) or 70% (70 g L⁻¹) aerated salt solutions (regular table salt) for 1, 6, 24, 48 and 72 hrs ($n = 5$, with 150 clams per n).

Table 4. Chi-squared test (χ^2) performed on the number of dead *Corbicula fluminea* specimens for Experiment 4: Efficacy of salt solutions (S). S = Control, 35 (35 g L⁻¹) or 70 % (70 g L⁻¹) aerated salt solutions. Exposure time = 1, 6, 24, 48 or 72 hours. Medium to large sizes of *C. fluminea* (15–26 mm) were examined. Experimental $n = 5$. NS = non-significant; $\alpha = 0.05$.

Source of variation	χ^2	P
Salt (S)	$\chi^2 (2) = 107.410$	$P < 0.001$
Exposure Time (Time)	$\chi^2 (4) = 41.597$	$P < 0.001$
S × Time	$\chi^2 (8) = 17.902$	$P < 0.01$

Experiment 5: Efficacy of hot water

There was between 93–98% survival of control clams, and up to 100% mortality of hot water-treated clams across both size classes. Overall, there was significantly greater clam mortality with increasing water temperature ($P < 0.001$). However, there was no significant difference between the control and 35 °C treatment ($P > 0.05$). Mortality was not significantly affected by either clam size or exposure time, and there were no interactions between the treatment, clam size and exposure time effects (all $P > 0.05$; Figure 5, Table 5).
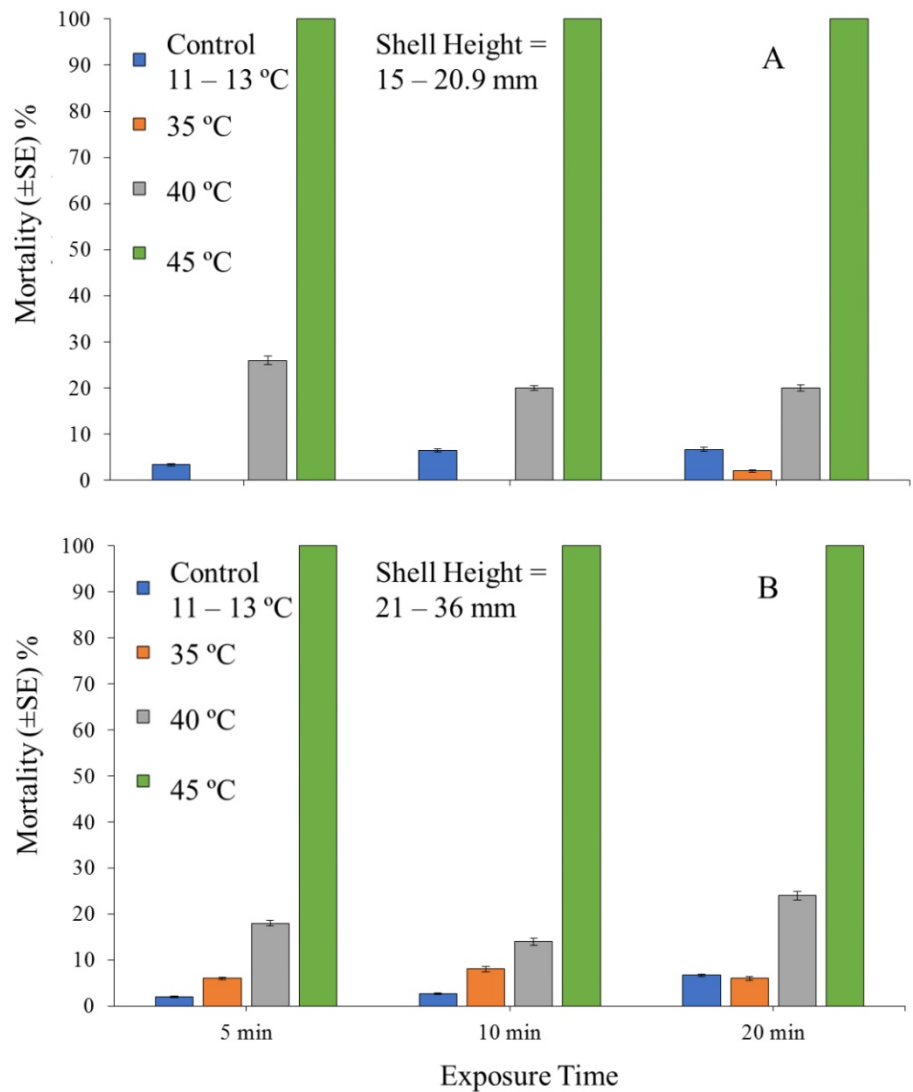
Figure 5. Mean mortality (± SE) for groups of ten medium (A: SH = 15–20.9 mm) and ten large (B: SH = 21–36 mm) adult *Corbicula fluminea* specimens 24 hrs post-exposure to hot water temperatures of 35, 40 and 45 °C for 5, 10 and 20 minutes (*n* = 5, with 120 clams per *n*).

Table 5. Chi-squared test (χ^2) performed on the number of dead *Corbicula fluminea* specimens for Experiment 5: Efficacy of hot water (HW). HW = Control (11–13 °C), 35, 40 or 45 °C temperatures. Exposure time = 5, 10 or 20 minutes. Size ranges = medium (15–20.9 mm) or large (21–36 mm) sized clams. Experimental *n* = 5. NS = non-significant; α = 0.05.

Source of variation	χ^2	P
Hot water (HW)	$\chi^2(3) = 1156.8740$	$P < 0.001$
Exposure Time (Time)	$\chi^2(2) = 0.1883$	NS
Size	$\chi^2(1) = 0.6019$	NS
DI × Time	$\chi^2(6) = 9.4194$	NS
DI × Size	$\chi^2(3) = 4.9317$	NS
Time × Size	$\chi^2(2) = 1.3423$	NS
HW × Time × Size	$\chi^2(6) = 1.0313$	NS

Experiment 6: Efficacy of steam

Although 94% survival of control clams was observed, up to 100% mortality was recorded for steam-treated clams. The exposure to direct steam applications significantly increased clam mortality (χ^2 (6) = 1684.4, $P < 0.001$; Figure 6), wherein maximum mortality was caused at, and beyond, an exposure time of 30 seconds.
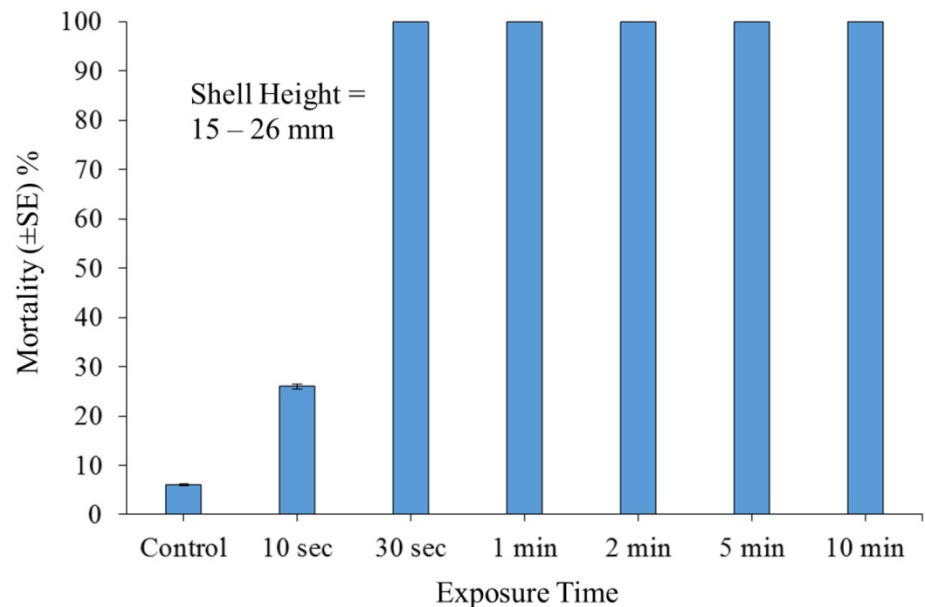

Figure 6. Mean mortality (± SE) of adult *Corbicula fluminea* specimens (SH = 15–26 mm) 24 hrs post-exposure to a direct steam application, at a distance of 2–3 cm from the source, for 10 sec, 30 sec, 1, 2, 5 and 10 minutes (*n* = 5, with 70 clams per *n*).

Discussion

Globally, AIS continue to spread at an escalated rate, reducing biodiversity and altering ecosystem function (Seebens et al. 2017, 2018). Spread-prevention through effective and efficient biosecurity protocols has become integral to AIS management strategies. As a damaging invader that has shown a high degree of physiological and ecological plasticity, further spread and expansion of C. fluminea populations is of high ecological concern (Sousa et al. 2008; Caffrey et al. 2016). Previously, Barbour et al. (2013) observed that a 2% solution of Virkon˚ Aquatic, at a 5 minute exposure, could induce c. 93% mortality of juvenile C. fluminea specimens (5.1–10 mm). Moreover, a variety of other studies have reported the efficacy of both Virasure˚ and Virkon˚ to cause substantial AIS mortality for species such as Melanoides tuberculate (Müller, 1774: Mitchell et al. 2007), Dreissena bugensis (Andrusov, 1897: Stockton-Fiti and Moffitt 2017) and Lagarosiphon major ((Ridley) Moss, 1928: Cuthbert et al. 2018). We found that the examined aquatic disinfectants Virasure˚ and Virkon˚ do not cause substantive mortality of adult C. fluminea, even when specimens are retained in a 4% solution for up to 80 minutes. In addition, Experiment

2 further highlighted the ineffectiveness of both disinfectants to cause substantial mortality of adult C. fluminea specimens. Solutions at 30 °C yielded no noticeable increase in C. fluminea mortality by the examined aquatic disinfectants.

Our results detailing the ineffectiveness of salt and bleach solutions to cause *C. fluminea* mortality concurred with those of Barbour et al. (2013). In particular, longer exposure times and a higher bleach concentration (20%) than those examined by Barbour et al. (2013) failed to induce substantial adult *C. fluminea* mortality. Previously, Anderson et al. (2015) found that exposure to hot water at 45 °C for 15 minutes can cause 100% mortality for a variety of AIS, including zebra mussels (*Dreissena polymorpha*) and killer shrimp (*Dikerogammarus villosus*). Here, Experiment 5 demonstrated the efficacy of hot water (45 °C) to cause 100% mortality of *C. fluminea* specimens at a 5 minute exposure. Furthermore, Experiment 6 has shown that our novel method of direct steam application can cause 100% mortality of adult *C. fluminea* specimens at a 30 second exposure.

When compared to results already described within the literature, our data suggest that adult *C. fluminea* are less susceptible than juveniles to previously proposed chemical biosecurity protocols. While the use of disinfection baths, cleaning protocols, and extended drying times are undoubtedly beneficial against AIS spread, we show that simple non-chemical methods such as immersion in hot water (≥ 45 °C) and direct steam exposure will rapidly cause mortality. Such methods appear to cause a substantial thermal shock and, therefore, will likely successfully kill many other AIS that are unable to tolerate brief, but rapid exposure to an environmentally extreme temperature (Coughlan et al. 2018b). In particular, hot water at 45 °C represents a safe, simple, cost effective, user- and environmentally-friendly biosecurity application (Anderson et al. 2015); when not discharged or cooled inappropriately.

Many conventional biosecurity techniques can be difficult to incorporate into daily working practices, e.g. extended drying times (Sutcliffe et al. 2018; Shannon et al. 2018). However, hot water and steam applications may likely encourage high compliance amongst stakeholders such as angling and sporting groups, practitioners whose employment roles place them in freshwater environments, and more general recreational water users. In particular, hot water and steam applications represent readily adoptable *in situ* biosecurity measures that are fast and easy to apply and produce rapid results. Moreover, when hot water immersion may not be feasible, such as for large nets, watercraft, trailers and vehicles, steam applications could be an effective alternative (Crane et al. 2018). Accordingly, the apparent excellent potential of hot water and steam applications must be further explored. However, possible adverse effects of

hot water and steam on carbon fibre fishing rods, fishing line, and other equipment items will need to be examined. In addition, the effect of applying steam at varying distances, i.e. a longer or shorter steam jet, will require further examination. Here, we applied steam at distance of 2–3 cm from its source point, however, greater distances between the source of a steam jet and the targeted organisms may reduce the efficacy of this treatment. Nevertheless, installation of basic steam cleaning devices and hot water facilities at frequently visited areas and points of entry (e.g. angling stations and boat ramps), may promote utilisation of these simple and environmentally-friendly biosecurity protocol (Crane et al. 2018).

Application of multiple differential treatments within biosecurity protocols may also enhance overall inhibition of invader spread. The synergistic effects of various applications could provide for greater efficacy. For example, immersion of equipment into a hot water bath, followed by 30 second exposure to steam, may improve biosecurity for a range of AIS across various life stages. Equally, most protocols aim to enforce biosecurity procedures as visitors leave a site, or off-site prior to visiting a new aquatic area. However, biosecurity measures could be substantially improved by performing simple but efficacious procedures prior to entering a freshwater site and repeating this process prior to leaving the site. Such pre-entrance and post-departure biosecurity protocols may be particularly beneficial when adopted by water-users who frequently travel between freshwater sites (e.g. anglers, canoeists and kayakers). Moreover, a stronger emphasis on pre-entrance biosecurity alone may be particularly beneficial for sites of conservation or economic importance. Further, the development of biosecurity related infrastructure should now be a priority of stakeholder groups and policy makers. Installation of hot water and steam cleaning stations at frequently visited sites and points of entry, such as angling stations, harbours and boat ramps, would likely facilitate increased uptake of these simple but efficacious treatments by those working or undertaking recreational activities in the freshwater environment (Crane et al. 2018; Shannon et al. 2018). Decontamination facilities could mimic the design of car wash stations.

As only mortality rates 24 hrs after treatment have been recorded in the present study, future research should investigate for possible sub-lethal effects (e.g. acute or chronic morbidity) upon *C. fluminea*, other invaders and non-target organisms. Further, greater consideration will need to be given to the susceptibility of all transportable life stages of current, emerging and potential AIS to biosecurity treatments. However, if a treatment can cause complete invader mortality at its most robust life stage, it will also likely do so at more vulnerable growth phases. Despite the apparent success of previously proposed biosecurity protocols, such as aquatic disinfectants, additional examination may be required to ascertain

the totality of treatment efficacy. Equally, determination of the efficacy of biosecurity treatments, other than Virkon® products, to prevent the spread of damaging aquatic pathogens such as the salmon fluke, *Gyrodactlylus salaris*, is required. However, until the complete efficacy of various biosecurity techniques are fully understood, at sites where damaging aquatic parasite and pathogens may be present, continued decontamination of equipment utilising chemical disinfectants such as Virasure® and Virkon® is recommended (Anderson et al. 2015).

The results presented here demonstrate that hot water (≥ 45 °C) and direct steam applications could be used for effective, efficient and environmentally-friendly biosecurity protocols to prevent further anthropogenic-mediated spread of adult *C. fluminea*. Accordingly, promotion and adoption of these techniques by biosecurity campaigns, stakeholder groups, and practitioners should be encouraged and incorporated into relevant Codes of Practice, with subsequent enforcement in relation to all water users.

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References

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

Banha F, Diniz A, Anastácio PM (2017) The role of anglers' perceptions and habits in biological invasions: perspectives from the Iberian Peninsula. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27: 51–64, https://doi.org/10.1002/aqc.2677

Barbour JH, McMenamin S, Dick JTA, Alexander ME, Caffrey JM (2013) Biosecurity measures to reduce secondary 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

Belz CE, Darrigran G, Mäder Netto OS, Boeger WA, Ribeiro Junior PJ (2012) Analysis of four dispersion vectors in inland waters: the case of the invading bivalves in South America. *Journal of Shellfish Research* 31: 777–784, https://doi.org/10.2983/035.031.0322

Caffrey JM (2010) Biosecurity Protocol for Field Survey Work. Inland Fisheries Ireland. http://www.fisheriesireland.ie/Research/invasive-species.html (accessed 02 December 2017)

Caffrey JM, Evers S, Millane M, Moran H (2011a) Current status of Ireland's newest invasive species – the Asian clam *Corbicula fluminea* (Müller, 1774). *Aquatic Invasions* 6: 291–299, https://doi.org/10.3391/ai.2011.6.3.06

Caffrey JM, Millane M, Evers S, Moran H (2011b) Management of *Lagarosiphon major* (Ridley) moss in Lough Corrib - a review. *Biology and Environment* 111B: 205–212, https://doi.org/10.3318/BIOE.2011.16

Caffrey JM, Baars J-R, Barbour JH, Boets P, Boon P, Davenport K, Dick JTA, Early J, Edsman L, Gallagher C, Gross J, Heinimaa P, Horrill C, Hudin S, Hulme PE, Hynes S, MacIsaac HJ, McLoone P, Millane P, Moen TL Moore N, Newman J, O'Conchuir R, O'Farrell M, O'Flynn C, Oidtmann B, Renals T, Ricciardi A, Roy H, Shaw R, Weyl O, Williams F, Lucy FE (2014) Tackling invasive alien species in Europe: the top 20 issues. *Management of Biological Invasions* 5: 1–20, https://doi.org/10.3391/mbi.2014.5.1.01

Caffrey JM, Dick JTA, Lucy FE, Davis E, Niven A, Coughlan NE (2016) First record of the Asian clam *Corbicula fluminea* (Müller, 1774) (Bivalvia, Cyrenidae) in Northern Ireland. *BioInvasions Records* 5: 239–244, https://doi.org/10.3391/bir.2016.5.4.08

Colwell H, Ryder J, Nuzzo R, Reardon M, Holland R, Wong WH (2017) Invasive Asian clams (*Corbicula fluminea*) recorded from 2001 to 2016 in Massachusetts, USA. *Management of Biological Invasions* 8: 507–515, https://doi.org/10.3391/mbi.2017.8.4.05

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

Coughlan NE, Stevens AL, Kelly TC, Dick JTA, Jansen MAK (2017b) Zoochorous dispersal of freshwater bivalves: an overlooked vector in biological invasions? *Knowledge and Management of Aquatic Ecosystems* 418: 42, https://doi.org/10.1051/kmae/2017037

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 J, 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

Crane K, Cuthbert RN, Dick JTA, Kregting L, MacIsaac HJ, Coughlan NE (2018) Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. *Biological Invasions* 8: 259–265, https://doi.org/10.1007/s10530-018-1901-2

Cribari-Neto F, Zeileis A (2010) Beta regression in R. *Journal of Statistical Software* 34: 1–24, https://doi.org/10.18637/jss.v034.i02

Cuthbert RN, Coughlan NE, Crane K, Caffrey JM, MacIsaac HJ, Dick JTA (2018) A dip or a dab: assessing the efficacy of Virasure® Aquatic disinfectant to reduce secondary spread of the invasive curly waterweed *Lagarosiphon major*. *Management of Biological Invasions*. (*in press*), https://doi.org/10.3391/mbi.2018.9.3.08

Gama M, Crespo D, Dolbeth M, Anastácio PM (2017) Ensemble forecasting of *Corbicula fluminea* worldwide distribution: Projections of the impact of climate change. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27: 675–684, https://doi.org/10.1002/aqc.2767

Karatayev AY, Padilla DK, Minchin D, Boltovskoy D, Burlakova LE (2007) Changes in global economies and trade: the potential spread of exotic freshwater bivalves. *Biological Invasions* 9: 161–180, https://doi.org/10.1007/s10530-006-9013-9

Lucy FE, Karatayev AY, Burlakova LE (2012) Predictions for the spread, population density, and impacts of *Corbicula fluminea* in Ireland. *Aquatic Invasions* 7: 465–474, https://doi.org/10.3391/ai.2012.7.4.003

Matthews MA, McMahon RF (1999) Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia. *Journal of Molluscan Studies* 65: 317–325, https://doi.org/10.1093/mollus/65.3.317

McMahon R (1982) The occurrence and spread of the introduced Asiatic freshwater clam *Corbicula fluminea* (Müller), in North America: 1924–1982. *Nautilus* 96: 134–141

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

Piria M, Copp GH, Dick JTA, Duplić A, Groom Q, Jelić D, Lucy FE, Roy HE, Sarat E, Simonović P, Tomljanović T, Tricarico E, Weinlander M, Adámek Z, Bedolfe S, Coughlan NE, Davis E, Dobrzycka-Krahel A, Grgić Z, Kırankaya ŞG, Ekmekçi FG, Lajtner J, Lukas JAY, Koutsikos N, Mennen GJ, Mitić B, Pastorino P, Ruokonen TJ, Skóra ME, Smith ERC, Šprem N, Tarkan AS, Treer T, Vardakas L, Vehanen T, Vilizzi L, Zanella D, 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

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rothlisberger JD, Chadderton WL, McNulty J, Lodge DM (2010) Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. *Fisheries* 35: 121–132, https://doi.org/10.1577/1548-8446-35.3.121

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A (2000) Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774, https://doi.org/10.1126/science.287.5459.1770

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke J, 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

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

Sheehan R, Caffrey JM, Millane M, McLoone P, Moran H, Lucy FE (2014) An investigation into the effectiveness of mechanical dredging to remove *Corbicula fluminea* (Müller, 1774) from test plots in an Irish river system. *Management of Biological Invasions* 5: 407–418, https://doi.org/10.3391/mbi.2014.5.4.11

Simberloff D, Martin J, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28: 58–66, https://doi.org/10.1016/j.tree.2012.07.013

Smithson M, Verkuilen J (2006) A Better Lemon Squeezer? Maximum-Likelihood Regression with Beta-Distributed Dependent Variables. *Psychological Methods* 11: 54–71, https://doi.org/10.1037/1082-989X.11.1.54

Sousa R, Antunes C, Guilhermino L (2008) Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: An overview. *Annales de Limnologie - International Journal of Limnology* 44: 85–94, https://doi.org/10.1051/limn:2008017

Sousa R, A Novais, R Costa, DL Strayer (2014) Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia* 735: 233–251, https://doi.org/10.1007/s10750-012-1409-1

Stockton-Fiti KA, Moffitt CA (2017) Safety and efficacy of Virkon® aquatic as a control tool for invasive Molluscs in aquaculture. *Aquaculture* 480: 71–76, https://doi.org/10.1016/j.aquaculture.2017.08.005

Sutcliffe C, Quinn CH, Shannon C, Glover A, Dunn AM (2018) Exploring the attitudes to and uptake of biosecurity practices for invasive non-native species: views amongst stakeholder organisations working in UK natural environments. *Biological Invasions* 20: 399–411, https://doi.org/10.1007/s10530-017-1541-y

Millikin MR, Williams AB (1984) Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. National Oceanic and Atmospheric Administration (NOAA). NOAA Technical Report NMFS 1, FAO Fisheries Synopsis 138, 39 pp

Nehring S (2011) Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. In: Galil BS, Clark PF, Carlton JT (eds), In the Wrong Place – alien marine crustaceans: distribution, biology and impacts. Springer, Netherlands, pp 607–624, https://doi.org/10.1007/978-94-007-0591-3_21

Perdikaris C, Konstantinidis E, Gouva E, Ergolavou A, Klaoudatos D, Nathanailides C, Paschos I (2016) Occurrence of the Invasive Crab Species *Callinectes sapidus* Rathbun, 1896 in NW Greece. *Walailak Journal of Science and Technology* 13(7): 503–510

Ribeiro F, Veríssimo A (2014) A new record of *Callinectes sapidus* in a western European estuary (Portuguese coast). *Marine Biodiversity Records* 7: 1–3, https://doi.org/10.1017/S1755267214000384

Streftaris N, Zenetos A (2006) Alien Marine Species in the Mediterranean - the 100 'Worst Invasives' and their Impact. *Mediterranean Marine Science* 7: 87–118, https://doi.org/10.12681/mms.180

Suaria G, Pierucci A, Zanello P, Fanelli E, Chiesa S, Azzurro E (2017) *Percnon gibbesi* (H. Milne Edwards, 1853) and *Callinectes sapidus* (Rathbun, 1896) in the Ligurian Sea: two additional invasive species detections made in collaboration with local fishermen. *BioInvasions Records* 6: 147–151, https://doi.org/10.3391/bir.2017.6.2.10

Williams AB (1974) The swimming crabs of the genus *Callinectes* (Decapoda: Portunidae). *Fishery Bulletin* 72(3): 685–798