Management of Biological Invasions (2013) Volume 4, Issue 3: 219–230
doi: http://dx.doi.org/10.3391/mbi.2013.4.3.04

Open Access

Research Article

Biosecurity measures to reduce secondary spread of the invasive freshwater Asian clam, *Corbicula fluminea* (Müller, 1774)

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Received: 2 July 2013 / Accepted: 2 September 2013 / Published online: 18 September 2013

Handling editor: Marnie Campbell

Abstract

Invasive species are currently considered one of the most important threats to global biodiversity. Established invaders are difficult to eradicate and management requires biosecurity measures to prevent secondary spread. *Corbicula fluminea* (Müller, 1774; Asian clam) has a worldwide invasive distribution and, as an ecosystem engineer, can have dramatic impacts on recipient systems, displacing native species and altering trophic structure. This study tested the efficacy of biosecurity methods for cleansing angling equipment (such as nets and waders) and other gear used in freshwater that may harbour *C. fluminea* and contribute to their secondary spread. *C. fluminea* of varying size were treated either with Virkon® Aquatic, common household bleach or salt at a variety of concentrations for a range of immersion times. Virkon® emerged as the most effective of the three treatment types and achieved 93.3% mortality when used at 2% for 5 minutes. With the bleach trials, there was no significant effect of immersion time on clam mortality, but 10% bleach for 60 minutes induced an average mortality of 76.7%. Further, maximum mortality in saltwater was only 13.3% with 60 minutes immersion. There was no significant difference in mortality among clam sizes. Virkon® is, therefore, recommended as the most effective product for treating angling and other gear that could potentially harbour and spread *C. fluminea*. We also assessed the effect of 'drying times' post-treatment and this was demonstrated to have no significant effect on clam mortality. Virkon® again gave the highest mortality of the three treatments, with 68.9% mortality achieved at 2% for 10 minutes. Overall, Virkon® emerges as an effective biosecurity measure, but further research is required to attain 100% mortality. Bleach may be useful when Virkon® is not available, but salt is relatively ineffective.

Key words: angling; Asian clam; biosecurity; bleach; *Corbicula fluminea*; saltwater; Virkon® Aquatic

Introduction

Biological invasions are a major threat to global biodiversity, second only to habitat loss and fragmentation (Vitousek et al. 1996; Walker and Steffen 1997; Sala et al. 2000; Allendorf and Lundquist 2003; Simberloff et al. 2013). It is has been suggested that freshwater ecosystems experience an increased threat to biodiversity from invaders in comparison to terrestrial systems as a result of a range of anthropogenic pressures, such as transport, trade and hydromorphological alterations (Sala et al. 2000). This is in addition to natural factors characteristic of these systems that increase this vulnerability, such as the innate dispersal abilities of aquatic organisms as well as the connectivity of river systems, both natural and man-made (Bij de Vaate et al. 2002; Muirhead and MacIsaac 2004; Minchin 2007; Gherardi et al. 2008). In addition to their negative ecological impacts, aquatic invasive species (AIS) are an economic burden, with estimates that the United States spends $7 billion per annum on managing AIS impacts (Pimentel et al. 2005), whilst within the United Kingdom, £26 million per annum has been attributed to their control (Oreska and Aldridge 2011). With the increasing rate of invasive species introductions (Ruiz et al. 1997), there is a growing requirement for mitigation of these ecological and economic costs (Hayes 2003; Pyšek and Richardson 2010; Keller et al. 2011).

Corbicula fluminea (Müller, 1774), the Asian clam, is one of the most widespread invasive freshwater bivalves in the Northern hemisphere (Karatayev et al. 2005). Native to Southeast Asia, the species has spread rapidly to North and South America, Africa, the Pacific Islands and also Europe, including the British Isles (Bij de Vaate 1991; Phelps 1994; Darrigran 2002). Although the actual vectors of this invasion are unknown, it has likely been facilitated by the use of *C. fluminea* as fish bait, in Asian cuisine, accidental release from aquaria and the transportation of juveniles attached to boats *via* their byssal threads (Sousa et al. 2008). In Ireland, *C. fluminea* was first reported in April 2010 in the freshwater tidal section of the River Barrow in Co. Carlow (Sweeney 2009) and in June 2010 in the adjoining River Nore (Caffrey et al. 2011). Smaller populations have subsequently been reported in the River Shannon at Carrick-on-Shannon (Hayden and Caffrey, in press) and in Lough Derg on the lower reaches of the River Shannon system (National Biodiversity Data Centre 2010). As these rivers experience a significant angling pressure and steady boat traffic, the further spread of *C. fluminea* within the country is likely (Ricciardi 2001; Lucy et al. 2012).

Although the precise pathways of invasion by *C. fluminea* are unknown, its success in invaded ranges may, in part, be attributed to a number of biological characteristics. The small size of juveniles facilitates transport by water currents for long distances downstream (McMahon 1999; McMahon 2002) and the attachment to floating surfaces and vegetation *via* a strong mucilaginous byssal thread (Sousa et al. 2008). *C. fluminea* is also highly tolerant to long emersion periods (Byrne et al. 1988), permitting possible transportation out of water over long distances. The capability of self-fertilisation (Williams and McMahon 1989; Sousa et al. 2008) and life history traits favouring rapid growth (McMahon 1999; Sakai et al. 2001) are further attributes that are likely to have contributed to its invasion success, leading to significant impacts of *C. fluminea* in its invaded ranges. Such impacts include the reduction in levels of phytoplankton (Cohen et al. 1984; McMahon 1999; Lucas et al. 2002; Lopez et al. 2006), increased submerged aquatic vegetation (Phelps 1994) and altered levels of available nutrients (Lauritsen and Mozley 1989; Vaughn and Hakenkamp 2008). *C. fluminea* can also impact native invertebrate communities by competing for food and habitat resources (Devick 1991; McMahon 1991; Strayer 1999; Sousa et al. 2008). Furthermore, the species is a known biofouler of industrial and power plant water systems, causing significant impacts to infrastructure (Darrigran 2002).

The detrimental effects of *C. fluminea* are such that they require the development of effective and efficient biosecurity measures. These measures can prevent the introduction, impacts and spread of new invaders from hubs to surrounding water bodies (Muirhead and MacIsaac 2005; Perrings et al. 2005; Minchin 2007). Research to control *C. fluminea* has considered options such as hypoxia (e.g. Matthews and McMahon 1995), fish predation (Robinson and Wellborn 1988), temperature (e.g. Johnson and McMahon 1998; Matthews and McMahon 1999), physical harvesting by suction and dredging (e.g. Wittmann et al. 2011) and benthic barriers, such as polyethylene and rubber (e.g. Wittmann et al. 2011). Although various non-chemical methods are currently employed to control the spread of AIS, disinfection is the most commonly used method for treatment (McMahon et al. 1993; Bidwell et al. 1995; Cairns and Bidwell 1996). A range of disinfection techniques and products are currently applied, ranging from hot water to proprietary aquatic disinfectants (Caffrey 2010).

A number of studies have considered the efficacy of common household disinfectants as biosecurity agents for inducing mortality in pest species. These include bleach (e.g. Johnson et al. 2003; Hosea and Finlayson 2005; Stebbing et al. 2011), salt (Hosea and Finlayson 2005; Matheson et al. 2007; Stebbing et al. 2011), Formula 409® (Hosea and Finlayson 2005; Schisler et al. 2008), Dettol (©2010 Reckitt Benckiser) and various dishwashing fluids (e.g. Dunmore et al. 2011). These products are readily accessible and can easily be obtained by stakeholders, including anglers. Household disinfectants are, however, not always the ideal option for biosecurity since they have been shown to damage gear (Hosea and Finlayson 2005) and are not always effective at inducing mortality in pest species (e.g. Matheson et al. 2007; Dunmore et al. 2011).

In this study, we explore the use of chemical products that have been previously trialled against other aquatic invaders such as *Dikerogammarus villosus* (Stebbing et al. 2011). Specifically, we compare the efficacies of household products (salt and household bleach) with that of the more specialised aquatic disinfectant, Virkon® Aquatic as biosecurity measures for disinfecting angling and other gear. The active ingredient of Virkon® Aquatic is potassium monopersulphate which acts

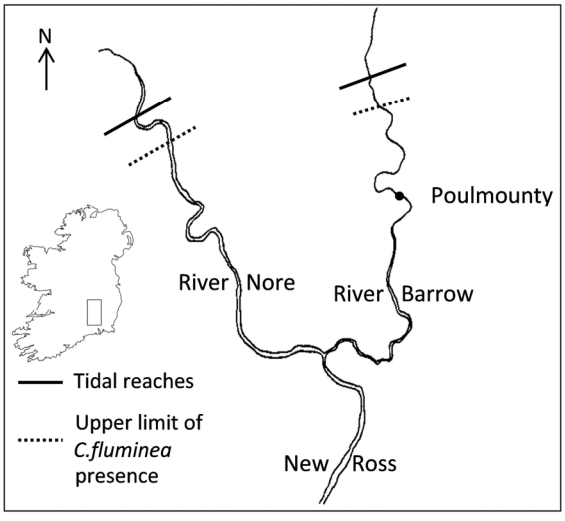

Figure 1. Distribution of *Corbicula fluminea* in the Rivers Barrow and Nore in 2010 (Caffrey et al. 2011) showing the current study site at Poulmounty.

as an oxidising agent to break down glycoproteins. The product was developed specifically for the disinfection requirements of aquaculture and is effective against viral, bacterial and fungal disease (Western Chemical, Inc. 2008). The present study aimed to: (1) identify an effective disinfectant for the purpose of treating angling gear (e.g. landing and keep nets, stink bags, unhooking mats, waders) in the field; (2) determine differences in efficacy of the chemical treatments listed in killing *C. fluminea*; (3) determine the optimal concentration of each treatment; (4) investigate whether immersion time and/or drying time affects *C. fluminea* mortality; and (5) determine whether different sized *C. fluminea* have different susceptibility to these chemical treatments. This research aims to inform public interest groups and legislators regarding the biosecurity protocols necessary to minimise the spread of *C. fluminea* by anthropogenic sources.

Methods

Efficacy of Virkon®, bleach and salt in killing Corbicula fluminea

Specimens of *C. fluminea* were collected randomly by hand, at low tide, from the River Barrow at Poulmounty, County Wexford, Ireland (Figure 1) in July 2011 (52°46.35′N, 6°91.7′W) and allowed to acclimatise in the laboratory for one week prior to experimentation. Small juvenile *C. fluminea* (5.1–10 mm) were used in this study as these are thought the most likely to be transported from one water body to another by anglers or other water users in nets, protective clothing, boots and other equipment, and they are not easily spotted by visual inspection. Solutions of three types of disinfectants, Virkon® Aquatic, bleach and salt, were prepared at a range of concentrations using de-chlorinated tap water, which also served as control water. Ranging studies were undertaken to discern appropriate timings for experimentation and the feasibility of use in the field was taken into account. All tests were carried out at temperatures of between 11–13 °C on a 12:12 hour light:dark schedule. Specimens of *C. fluminea* were confirmed to be alive prior to conducting any tests by observation of activity (e.g. clam opening and/or extension of the clam's muscular foot). Results were analysed using R statistical software (version 3.0.1, R Development Core Team 2013) by multi factor ANOVA with Tukey's *post hoc* tests.

Experiment 1: Virkon® Aquatic

Three replicates of 10 *C. fluminea* individuals were immersed in either 200 ml of control water, 200 ml of 1% Virkon® Aquatic (1 g per 100 ml) or 2% Virkon® Aquatic (2 g per 100 ml) for four immersion time periods of 30 seconds, 1 minute, 2 minutes or 5 minutes. Two size ranges of *C. fluminea* were used: 5.1–7 mm and 7.6–10 mm. Following immersion, specimens were removed and placed on a dry surface (plastic tray) for 15 minutes, then transferred into plastic containers (8.5 cm diameter) filled with 100 ml of dechlorinated tap water and the number of live and dead *C. fluminea* recorded after 24 hours. *C. fluminea* were considered dead if they were gaping, or if they offered no resistance to being teased apart with tweezers and did not reclose (see Matthews and McMahon 1999). *C. fluminea* mortality was measured as % dead in each replicate after 24 hours and the data were then arcsine transformed for analysis (Sokal and Rohlf 1995). However, we show raw % means in all Figures for clarity. Mean mortality was examined with respect to 'Virkon® treatment' (control, 1%, 2% Virkon®), 'Immersion time' (30 s, 1 min, 2 min, 5 min) and 'Clam size' (5.1–7 mm, 7.6–10 mm) in a three-factor ANOVA with Tukey's *post hoc* tests.

Experiment 2: Bleach

Three replicates of 10 *C. fluminea* individuals were immersed in either 200 ml of control water or Domestos (© Unilever 2011) household bleach (4.8% sodium hypochlorite) at 0.5% (0.5 ml per 100 ml), 5% (5 ml per 100 ml) or 10% (10 ml per 100 ml), with one size range of *C. fluminea* (7–10 mm) for three immersion times (2 minutes, 10 minutes and 60 minutes). *C. fluminea* mortality was assessed after 24 hours. Mean mortality (% dead, arcsine transformed) was examined with respect to 'Bleach treatment' (control, 0.5%, 5%, 10% bleach) and 'Immersion time' (2, 10, 60 min) in a two-factor ANOVA with Tukey's *post hoc* tests.

Experiment 3: Salt

Three replicates of 10 *C. fluminea* individuals were immersed in either 200 ml of control water or 70 g/L salt water (regular table salt, 7 g per 100 ml) for three immersion periods of 20 minutes, 40 minutes or 60 minutes. Two size ranges of *C. fluminea* were used (5.1–7.5 mm and 7.6–10 mm) and *C. fluminea* mortality was assessed at 24 hours. Mean mortality (% dead, arcsine transformed) was examined with respect to 'Salt treatment' (control, 70 g/L salt), 'Immersion time' (20, 40, 60 min) and 'Clam size' (5.1–7.5 mm and 7.6–10 mm) in a three-factor ANOVA with Tukey's *post hoc* tests.

Virkon® Aquatic with extended immersion times

To examine the practice of drying gear for a period of time after disinfection (currently employed in field biosecurity protocols), we conducted a fourth experiment with lengthened immersion times, included a drying time and assessed *C. fluminea* mortality over a longer time period (240 hours). *C. fluminea* specimens were collected from the same site on the River Barrow at Poulmounty in April 2012 and were transported to the lab in continuously aerated de-chlorinated tap water. An acclimatisation period of one week was allocated prior to experimentation. Experiments were performed on *C. fluminea* individuals 7–10 mm in length at temperatures of between 11–13°C.

Experiment 4: Virkon® Aquatic (extended immersion)

For each treatment, 3 replicates of 10 *C. fluminea* individuals, all of one size range (7–10 mm), were used. Individuals were immersed in either 200 ml of control water (de-chlorinated tap water), 200 ml of a 1% Virkon® Aquatic solution or 200 ml of a 2% Virkon® Aquatic solution for four immersion times of 1 minute, 5 minutes, 10 minutes and 15 minutes. Following immersion the specimens were transferred to plastic trays and left to dry for three time intervals or 'drying times' of 5, 15 and 25 minutes. After the appropriate 'drying time', *C. fluminea* specimens were put into small plastic dishes (dimensions as before), which were placed in a larger plastic container (13×31×48 cm) filled with 13.5 litres of continuously aerated de-chlorinated tap water. *C. fluminea* individual mortality was then assessed after 240 hours using the same methods as described above. Mean mortality (% dead, arcsine trans-formed) was examined with respect to 'Virkon® treatment' (control, 1%, 2% Virkon®), 'Immersion time' (1 min, 5 min, 10 min, 15 min) and 'Drying time' (5 min, 15 min, 25 min) in a three-factor ANOVA with Tukey's *post hoc* tests.

Results

As specimens of *C. fluminea* immersed in control water exhibited high survival after 24 hours monitoring time (> 99%), experimental deaths were attributed to treatment application.

Experiment 1: Virkon® Aquatic

There was significantly higher mortality in Virkon® treatments compared to controls and in Virkon® treatments of 2% concentration compared to 1% (Table 1; Figure 2; all Tukey's HSD tests $P < 0.001$). There was a significant effect of 'Immersion time' (Table 1) and this was driven by significantly higher mortality when *C. fluminea* specimens were immersed for 5 min as compared to 30 s and 1 min (Tukey's HSD; $P < 0.01$). There was no effect of clam size (Table 1) and there were no significant statistical interactions. Virkon® at 2% for 5 minutes yielded the most positive results in respect to *C. fluminea* mortality, at 93.3%.

Experiment 2: Bleach

There was significantly higher mortality in bleach treatments compared to controls and between all different treatment concentrations (Table 1; Figure 3; all Tukey's HSD tests $P < 0.01$). There was no significant overall effect of immersion time on clam mortality (Table 1), although there was a strong trend (Table 1; Figure 3). There was a significant

Table 1. Multi factor ANOVAs on number of dead *C. fluminea* in experiments. Experiment 1: Virkon® Aquatic with the factors 'Virkon® treatment' (control, 1%, 2% Virkon®), 'Immersion time' (30 sec, 1, 2, 5 min) and 'Clam size' (5.1-7 mm and 7.6-10 mm). Experiment 2: Bleach with the factors 'Bleach treatment' (control, 0.5, 5, 10% bleach) and 'Immersion time' (2, 10, 60 min). Experiment 3: Salt with the factors 'Salt treatment' (control or 70 g/L salt), 'Clam size' (5.1-7.5 and 7.6-10 mm) and 'Immersion time' (20, 40, 60 min). Experiment 4: Virkon® Aquatic (extended exposure) with the factors 'Virkon® treatment' (control, 1, 2% Virkon), 'Immersion time' (1, 5, 10, 15 min) and 'Drying time' (5, 15, 25 min). Significant P-values in bold, $\alpha = 0.05$.

Source of variation	*df*	MS	*F*	*P*
Experiment 1: Virkon® Aquatic				
Virkon® treatment	2	30193.5	513.8	<**0.001**
Immersion time	3	225.8	3.8	<**0.05**
Clam size	1	59.2	1.0	NS
Virkon® treatment × Immersion time	6	104.7	1.8	NS
Virkon® treatment × Clam size	2	36.5	0.6	NS
Immersion time × Clam size	3	59.7	1.0	NS
Virkon® treatment × Immersion time × Clam size	6	80.7	1.4	NS
Error	48	58.8		
Experiment 2: Bleach				
Bleach treatment	3	4161.7	103.9	<**0.001**
Immersion time	2	133.7	3.3	0.053
Bleach treatment × Immersion time	6	158.6	4.0	<**0.05**
Error	24	40.1		
Experiment 3: Salt				
Salt treatment	1	2229.3	33.6	<**0.001**
Immersion time	2	143.9	2.2	NS
Clam size	1	189.9	2.9	NS
Salt treatment × Immersion time	2	143.9	2.2	NS
Salt treatment × Clam size	1	189.9	2.9	NS
Immersion time × Clam size	2	14.8	0.2	NS
Salt treatment × Immersion time × Clam size	2	14.8	0.2	NS
Error	24	66.5		
Experiment 4: Virkon® Aquatic (extended exposure)				
Virkon® treatment	2	15883.0	65.7	<**0.001**
Immersion time	3	344.6	1.4	NS
Drying time	2	71.3	0.3	NS
Virkon® treatment × Immersion time	6	176.0	0.7	NS
Virkon® treatment × Drying time	4	99.4	0.4	NS
Immersion time × Drying time	6	121.2	0.5	NS
Virkon® treatment × Immersion time × Drying time	12	42.3	0.2	NS
Error	72	241.8		

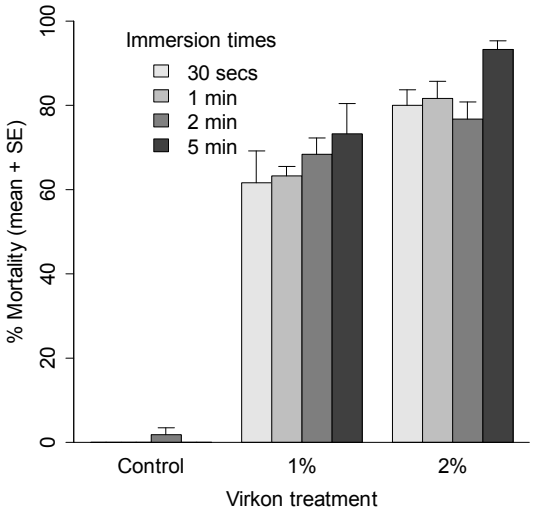

Figure 2. Mean mortality (+ SE) of *Corbicula fluminea* specimens immersed in two Virkon® solutions (1% and 2%) and control water (dechlorinated tap water) for four immersion times (30 seconds, 1 minutes, 2 minutes and 5 minutes) after 24 hours.

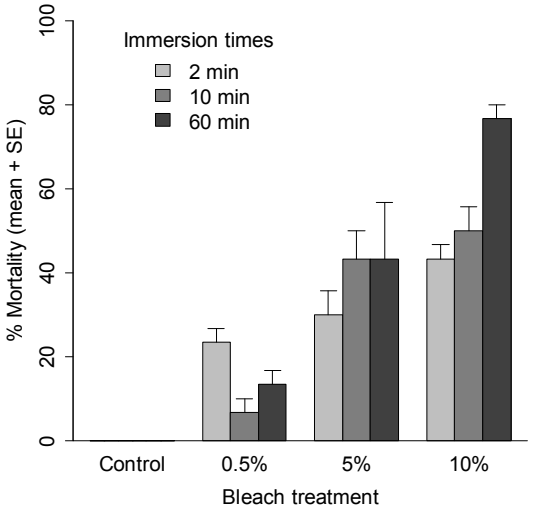

Figure 3. Mean mortality (+ SE) of *Corbicula fluminea* specimens immersed in three bleach solutions (0.5%, 5% and 10%) and control water (dechlorinated tap water) for three immersion times (2 minutes, 10 minutes, 60 minutes) after 24 hours.

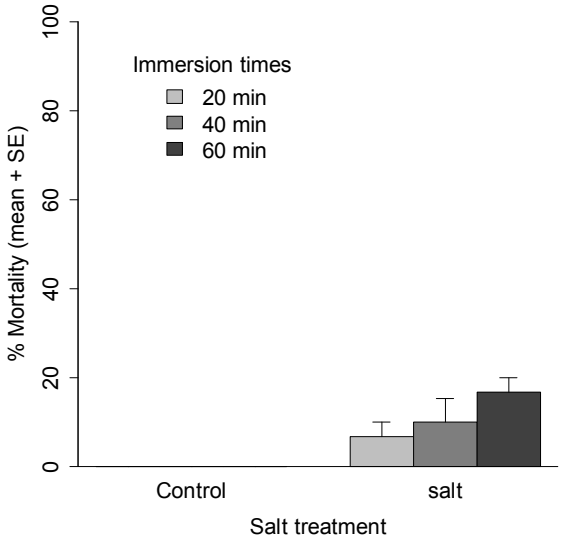

Figure 4. Mean mortality (+ SE) of *Corbicula fluminea* specimens immersed in 70g/L salt solution and control water (dechlorinated tap water) for three immersion times (20 minutes, 40 minutes, 60 minutes) after 24 hours.

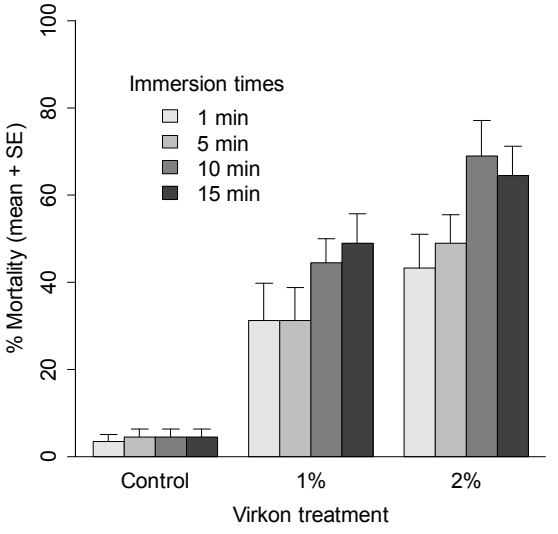

Figure 5. Mean mortality (+SE) of *Corbicula fluminea* specimens immersed in two Virkon® solutions (1% and 2%) and control water (dechlorinated tap water) for four immersion times (1 minute, 5 minutes, 10 minutes, 15 minutes) after 240 hours.

'Bleach treatment × Immersion time' interaction (Table 1), reflecting an increasing disparity in mortality over immersion time at the four treatments levels. Bleach at 10% for 60 minutes killed 76.7% of the *C. fluminea* specimens tested.

Experiment 3: Salt

C. fluminea mortality was significantly higher in the saltwater solution than in the control group (Table 1; Figure 4). There were no significant effects of 'immersion time' (Table 1) or 'clam size' (Table 1) and no significant statistical interactions. Salt immersion for 60 minutes only resulted in a 13.3% *C. fluminea* mortality.

Experiment 4: Virkon® Aquatic (extended immersion)

There was significantly higher mortality in Virkon® treatments compared to controls and in Virkon® treatments of 2% compared to 1% (Table 1; Figure 5; all Tukey's HSD tests $P < 0.03$). There was no significant effect of 'Immersion time' (Table 1) or 'drying time' (Table 1) and there were no significant statistical interactions. Virkon® at 2% for 10 minutes killed 68.9% of the specimens of *C. fluminea* tested.

Discussion

Biosecurity is increasingly required as an integral part of invasive species management and mitigation (Meyerson and Reaser 2002; Simberloff et al. 2013). One of the main aims of biosecurity is to reduce the introduction and spread of non-native invasive species (Meyerson and Reaser 2002; Vander Zanden and Olden 2008). In particular, chemical control has been highlighted as one of the most efficient methods for biosecurity, useable by a number of stakeholder groups (Wittenberg and Cock 2001).

The angling community has an understanding of the impacts that aquatic invasive species can have on their fishery watercourses and directly on the fish that they pursue as part of their sport or hobby (Granek et al. 2008). As such, they are generally keen to embrace any biosecurity measures that will provide protection for their waters, providing they are not excessively time consuming or costly (Granek et al. 2008). Thus, if biosecurity measures for anglers are to be widely used and encouraged, they must be effective, relatively inexpensive and simple to apply. In addition, the disinfection/cleaning process must not substantially interfere with the limited amount of time that anglers have for fishing and, hence, should be reasonably quick to apply. Ultimately, it will be important to have the support of the angling community for any biosecurity measures that are recommended.

The results of this study show that, of the three disinfection methods tested against specimens of *C. fluminea*, Virkon® Aquatic was the most effective. The highest average mortality achieved with Virkon® treatments was 93.3%. Bleach achieved a maximum average mortality of 76.7% while salt treatments recorded a maximum average mortality of only 13.3%. Similar mortality rates have been recorded in a study by Stebbing et al. (2011) who tested 12 different treatments against the 'killer shrimp', *Dikerogammarus villosus* (Sowinsky, 1894). These included Virkon® S, sodium hypochlorite (the active substance in household bleach) and salt water. Virkon® S contains the same active ingredients as Virkon® Aquatic, but the latter includes inert ingredients to make it suitable for aquatic applications (Syndel Laboratories Ltd. 2011). Of the 13 treatments tested by Stebbing et al. (2011), Virkon® S, sodium hypochlorite (the active component of bleach) and FAM30® (a disinfectant with an active component of iodine) were among the most effective chemicals tested, achieving a maximum mortality (100%) of *D. villosus* at 1 hour with a 1% solution. Crustaceans may, however, be more sensitive to chemical treatments than bivalve molluscs, since the latter can close their valves for long periods of time and thereby reduce exposure. *C. fluminea* specimens did, however, suffer high mortality rates with Virkon®, and one avenue of further research would be to examine methods to increase this mortality rate, possibly by exploring the reasons and stimuli for *C. fluminea* valve opening/closing (see below).

Virkon® Aquatic is labelled for use against fungal, viral and bacterial pathogens (DuPont 2010). Virkon® Aquatic comprises potassium monopersulphate (oxidizing agent), two organic acids (Sulphamic acid and Malic acid), a buffer, a surfactant and sodium chloride (DuPont 2010). It works by oxidising proteins and other parts of the cell protoplasm, which inhibits enzyme systems and degrades the integrity of the cell wall (Western Chemical, Inc. 2008; Stockton 2011). The organic acids in Virkon® Aquatic produce a low pH without corrosive effects and the inorganic buffer maintains a low pH, which optimizes biocidal activity (Syndel Laboratories Ltd. 2011). The effect of reduced pH on dreissenid mussels

has been shown to be multi-faceted (Claudi et al. 2013), and it is assumed that the same would be true of *C. fluminea*. The lower pH limit of *C. fluminea* has been reported as 5.6 (McMahon 2002), and Virkon® Aquatic has a low pH range of 2.4–2.7 (DuPont 2010).

Of the Virkon® concentrations and immersion times tested, specimens of *C. fluminea* immersed in a 2% solution for 5 minutes showed the greatest average mortality (93.3%). The 1% concentration showed a maximum average mortality of 66.7% with the same immersion time. Stockton (2011) reported similar findings when testing the efficacy of 1% and 2% Virkon® Aquatic on New Zealand mud snails, *Potamopyrgus antipodarum* (Gray, 1843), and quagga mussels, *Dreissena rostriformis bugensis* (Andrusov, 1897). In this instance, immersion in a solution of 1% Virkon® Aquatic was reportedly ineffective at killing both species. O' Connor et al. (2008) showed that immersion in 0.05% (500mg/L) of Virkon® Aquatic lead to abnormal embryo development in Sydney rock oysters, *Saccostrea glomerata* (Gould, 1850). In contrast, Mitchell et al. (2007) recommended 1.6% (1,600 mg/L) Virkon® to induce mortality in red-rim melania, *Melanoides tuberculatus* (Müller, 1774), with an immersion time of 24 hours. Further, Mitchell and Cole (2008) report that faucet snails, *Bithynia tentaculata* (Linnaeus, 1758), were highly resistant to Virkon® at concentrations of 0.5–1% (5–10 g/L), with one hour of immersion having no effect on mortality.

These examples suggest that high concentrations of Virkon® Aquatic are required to induce mortality in adult life stages of bivalve and gastropod molluscs, likely due to the presence of a shell that allows enclosure of body tissues. During the present study, specimens of *C. fluminea* were observed exhibiting valve closure in response to immersion in Virkon® Aquatic. When specimens of *C. fluminea* were assessed for mortality, those in control water were noticeably more active (open valves with siphons and muscular foot visible) than *C. fluminea* individuals treated with Virkon® Aquatic, and the *C. fluminea* individuals treated with 2% Virkon® Aquatic were generally less active than those treated with 1% Virkon® Aquatic. Furthermore, in the few instances of unexplained death in control *C. fluminea* individuals, valve gape was recorded. This trait was less common in dead *C. fluminea* from the Virkon® Aquatic treatments, whose shell valves remained closed despite mortality. This is similar to the findings of Doherty et al. (1987) who reported valve closure responses of the Asian clam to increased levels of cadmium and zinc. In comparison, Stockton (2011) states that the potassium in a Virkon® Aquatic solution inhibits New Zealand mud snails from closing their opercula, allowing the biocide to have a lethal effect on the snail (such as through the oxidising system or low pH of Virkon® Aquatic).

Of the three bleach concentrations tested, the strongest (10%) was found to be most effective and induced an average mortality of 67.7% in *C. fluminea* specimens immersed for 60 minutes. In other studies, different species were susceptible to lower concentrations of bleach. Cope et al. (2003) recommend rinsing gear with a 0.5% solution of bleach (6% sodium hypochlorite) to induce mortality of zebra mussels and quagga mussels. This difference may be explained by the concentration of active ingredient in the bleach used. The present study used bleach with 4.8% sodium hypochlorite, which may account for the lower level of mortality of *C. fluminea*. In contrast, Hosea and Finlayson (2005) concluded that bleach solution (at a concentration of 5%) was not an appropriate disinfectant for the New Zealand mud snail (*Potamopyrgus antipodarum*). Their study observed increased mortality in individuals with their opercula open rather than closed (Hosea and Finlayson 2005). The ability of *C. fluminea* to remain closed may explain the reduced mortality recorded in lower concentrations of bleach (Ortmann and Grieshaber 2003). In order to achieve higher mortalities, bleach would have to be used in higher concentrations, which have been shown to cause damage to angling gear (Hosea and Finlayson 2005).

The saltwater solution of 70 g/L was the least effective disinfectant tested, achieving only a maximum average mortality of 13.3% when *C. fluminea* individuals were immersed for 60 minutes. This is therefore an unsuitable disinfectant for *C. fluminea*, which again may be explained by physiological advantages of the bivalve shell. Other freshwater invasive species have been shown to be more susceptible to 70 g/L saltwater. Matheson et al. (2007) demonstrated that immersing nets in a saltwater solution of 70 g/L for one hour was a successful sterilising method against invasive fish and plant species including perch (*Perca fluviatilis* Linnaeus, 1758), koi carp (*Cyprinus carpio carpio* Linnaeus, 1758), tench [*Tinca tinca* (Linnaeus, 1758)], curly-leaf pondweed (*Potamogeton crispus* Linnaeus, 1753), Canadian pondweed (Elodea Canadensis Michx.) and curly-leaved waterweed *(Lagarosiphon major* (Ridley) Moss). In contrast, Stebbing et al.

(2011) tested 8 concentrations of saltwater, ranging from 5.29 g/L to 133.6 g/L, against *Dikerogammarus villosus* for up to 15 minutes. Mortality was assessed up to 120 minutes after the immersion period. No mortality was observed in any of the saltwater concentrations and saltwater was considered to be an unsuitable disinfectant against the 'killer shrimp', *D. villosus*. This is not surprising given its origin in the highly diverse salinity regimes of the Ponto-Caspian basin (see Dick and Platvoet 2000).

In the second Virkon® Aquatic experiment, a 2% solution of Virkon® Aquatic achieved greatest average mortalities (68.9%) when *C. fluminea* individuals were immersed for 10 minutes. Longer immersion times were examined in this experiment (10 and 15 minutes) and these did not significantly improve the results. Furthermore, highest average mortality (68.9%) was achieved in the 10 minute immersion group (in 2% concentration) and not the longer 15 minute immersion. It is possible that at longer monitoring times (e.g. after 240 hours) the effect of immersion time is diminished because the longer monitoring time allows long-term assessment of *C. fluminea* individuals, showing mortality is not instantaneous. If this is the case, it may be more important to focus on the concentration of the chemical than the length of time equipment is immersed in it. This discrepancy may also be due to the use of different *C. fluminea* cohorts in the first and second Virkon® experiments. The results from the two Virkon® Aquatic tests are not directly comparable as they used two different cohorts of *C. fluminea* and experiments were carried out at different times. A considerably lower proportion of *C. fluminea* individuals died in the second Virkon® Aquatic experiment than in the first experiment and there was higher *C. fluminea* mortality in the controls of the second experiment than the first, likely owing to unknown differences between the two cohorts. It is possible that one cohort was more resistant to Virkon® Aquatic than the other, or that physiological advantages were present in one cohort and not the other, such as metabolic acclimation to water temperature (Byrne et al. 1988).

In the field, it is recommended that a drying period should be implemented after treatment of equipment or footwear with the Virkon® Aquatic, prior to re-immersion in freshwater (Caffrey 2010; DuPont 2010). The effect of such drying time was tested in both Virkon® Aquatic experiments but was not found to be a significant factor in *C. fluminea* mortality. The elimination of a period where equipment and footwear were dried after being immersed in a disinfectant solution would reduce the time needed to carry out biosecurity protocols and more time could then be afforded to the immersion of gear in the disinfectant solution.

In the absence of Virkon®, our results suggest that bleach could be used to disinfect against *C. fluminea,* but would need to be used in a strong solution (10%) for an immersion time of one hour or more. This increased concentration and immersion time may incur damage to angling gear as well as incur long waiting times for anglers. Further research should consider other chemicals and combinations of chemicals for synergistic effects. In addition, we only considered the end point of mortality in this study and we recommend addressing other end points, such as growth and reproductive effects of chemical treatments, as well as developmental defects, for example, those that occur in oyster embryos when exposed to chemicals such as Virkon® (e.g. Dove and O'Connor 2007). The results of the present experiment recommend at least a 5 minute immersion in a 2% Virkon® Aquatic solution would be most effective at inducing mortality in *C. fluminea*.

Acknowledgements

We would like to thank Inland Fisheries Ireland for funding, assistance in field sampling and invaluable advice. This study was completed as part of a PhD, funded by the Department for Education and Learning, Northern Ireland. We would also like to thank our reviewers who provided helpful comments and recommendations.

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