

## Research Article

## The practical application of hot water to reduce the introduction and spread of aquatic invasive alien species

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**Co-Editors' Note:**

This study was contributed in relation to the 20th International Conference on Aquatic Invasive Species held in Fort Lauderdale, Florida, USA, October 22–26, 2017 (<http://www.icaais.org/html/previous20.html>). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

**Abstract**

Methods to reduce the introduction and spread of Invasive Alien Species (IAS) are key to reducing the economic, environmental and social impacts of IAS. IAS propagules can be moved around accidentally on clothing and equipment used in agriculture, transport, trade and recreation. Campaigns to slow the spread of aquatic IAS encourage water users to check, clean and dry their equipment and clothes, using hot water during the cleaning process where feasible. The UK *Check Clean Dry* biosecurity campaign recommends immersion in hot water (45 °C) for 15 minutes, however, implementation time may be a barrier to adoption of biosecurity practices. Here we aim to refine the advice given and test the efficacy of hot water treatment in actual conditions, with a view to reduce the recommended time to clean equipment while still preventing spread. The effectiveness was tested for a range of temperature (40, 45, 50, 55 and 60 °C) and time (10 seconds, 1, 5, 10, 15 minutes) treatments in killing IAS propagules of two invasive aquatic animal species (*Dreissena polymorpha*, *Dikerogammarus villosus*) and two invasive aquatic plant species (*Myriophyllum aquaticum*, *Crassula helmsii*), which are of global/national importance. For both invasive animal species, 100% mortality was achieved at all temperature and time treatments. However, plant mortality was affected by both temperature and time, with higher mortality resulting from higher temperatures and exposure times. Immersion for the recommended 15 mins at 45 °C caused complete mortality of *C. helmsii*, but only 40% mortality of *M. aquaticum*. Immersion in water at 50 °C or higher led to 100% mortality for *C. helmsii* and 90% mortality for *M. aquaticum* at shorter treatment durations of 5 or 10 mins. In addition, immersion in water at 60 °C caused 100% mortality after only 1 minute exposure. To ensure adoption and application of biosecurity practices, guidance should be simple, consistent and safe. For practical application in field we recommend, where feasible, immersion of equipment in water at 50 °C for a minimum of 5 minutes to achieve high mortality of IAS propagules.

**Key words:** biosecurity, invasive alien species, invasive non-native species, check, clean, dry, prevention**Introduction**

The rate of biological invasions is increasing as species are being moved (intentionally or unintentionally) through human activity outside their natural

distribution into novel terrestrial, marine and freshwater environments (Lockwood et al. 2005; Zieritz et al. 2016). Non-native species that have the capacity to spread and have negative ecological, economic or social impacts in their novel range are termed

Invasive Alien Species (IAS). Freshwater ecosystems are disproportionately affected by IAS (Dudgeon et al. 2006; Ricciardi and MacIsaac 2010) due to high anthropogenic activity, including trade and transport, recreation and environmental management. For example, recent research has indicated that almost 40% of aquatic species introductions into Europe are a result of aquaculture, boating, fishing and leisure activities (Gallardo and Aldridge 2013). IAS in the freshwater environment can be dispersed on footwear and motor vehicles (Waterkeyn et al. 2010) and on equipment such as netting and wetsuits (Anderson et al. 2015b). Once introduced and established, IAS can be extremely difficult and expensive to eradicate, particularly within aquatic environments (Barbour et al. 2013; Tidbury et al. 2016). After establishment has occurred, preventing secondary spread becomes paramount for slowing the spread of IAS (Vander Zanden and Olden 2008; Beyer et al. 2011).

Methods to prevent the introduction and spread of IAS are increasingly being recognised as the most cost effective means of reducing the impacts of IAS and are central to the Convention on Biological Diversity (CBD), to EU Regulation 1143/2014 on the Prevention and Management of Invasive Alien Species, and to the Invasive Non-Native Species Strategy for Great Britain (Perrings et al. 2009; Dunn and Hatcher 2015). The 20<sup>th</sup> International Conference on Aquatic Invasive Species, Florida, USA, 2017, widely recognised that prevention was one of the most cost effective methods to prevent and reduce the risk of new introductions (<https://www.icaiss.org>), as well as focusing on engaging the public to encourage prevention of new introductions. Biosecurity measures cover all activities aimed at preventing the introduction and/or spread of IAS (Caffrey et al. 2014). A key aspect of biosecurity is practices which reduce the risk of introduction and/or spread of IAS on fomites (e.g. clothing or equipment). As only a few individuals or plant fragments may be required to establish a new population, it is critical to establish simple, cost effective biosecurity messages and methods particularly when focused on engaging the public to encourage prevention of new introductions. Effective biosecurity practices can and have been adopted globally. For example, in the United States, the *Clean Drain Dry* campaign is a call to action that empowers recreational users of aquatic resources (<http://stopaquaticinvasive.org>). In addition to this, the New Zealand *Check Clean Dry* campaign, launched in 2004, has been effective in slowing the spread of an invasive diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt, 1899 (Branson 2006). A similar campaign was launched by the UK Department for Environment, Food and

Rural Affairs (Defra) in 2010 in response to the first reports of the invasive alien killer shrimp *Dikergammarus villosus* (Sowinsky, 1894). The UK *Check Clean Dry* campaign is aimed at recreational and other water users and promotes biosecurity best practice to reduce the risk of accidental introduction and spread of aquatic IAS. The campaign encourages people to check, clean and dry all equipment and clothing thoroughly to kill or remove any organisms that have the potential to survive while attached to equipment and be transported to a new location. The “Clean” recommendations advised by the Great Britain Non-Native Species Secretariat involve washing all equipment, footwear and clothes thoroughly.

The use of hot water has been identified as a technique globally to support the “Clean” process within the *Check, Clean, Dry* campaigns (Beyer et al. 2011; Stebbing et al. 2011; Anderson et al. 2015a) including the *Check Clean Dry* in the UK (<http://www.nonnativespecies.org/checkcleandry/index.cfm>), and the United States’ *Clean Drain Dry* (<http://www.stopaquaticinvasive.org>). Previous studies have found that aquatic plant fragments and animals are able to survive for at least 16 days in damp conditions, and that, although drying killed IAS propagules, several days drying time were required to reach high mortality (Anderson et al. 2015a). The effectiveness and rate of mortality was increased by using hot water to clean equipment and clothing: Anderson et al. (2015a) found that immersion for 15 minutes in 45 °C water caused 99% mortality among seven high-impact aquatic IAS in the UK. Anderson et al. (2015a) tested mortality using controlled water baths in laboratory conditions, however, in domestic/field settings, biosecurity is likely to be carried out using buckets in which hot water will cool naturally during treatment. Furthermore, through interviews with stakeholders from a range of environmental organisations (including business, leisure, conservation, education and public organisations), it has been argued that 15 minutes may be too long to ask people to wait for their equipment to soak and may be difficult to incorporate into their working practices (Sutcliffe et al. 2017). Reducing the time taken to undertake biosecurity may increase the adoption of good biosecurity practices.

This study aimed to reduce the time taken to perform key biosecurity activities (cleaning of equipment using hot water) and test the effectiveness of those activities. The objectives were: (a) to determine whether a shorter immersion time can result in high mortality of IAS propagules at the recommended 45 °C and (b) to investigate whether higher temperatures can be combined with shorter treatment times to induce high mortality of IAS propagules.

Experiments were carried out using hot water in buckets as is likely to occur in field or domestic settings, rather than laboratory water baths.

## Materials and methods

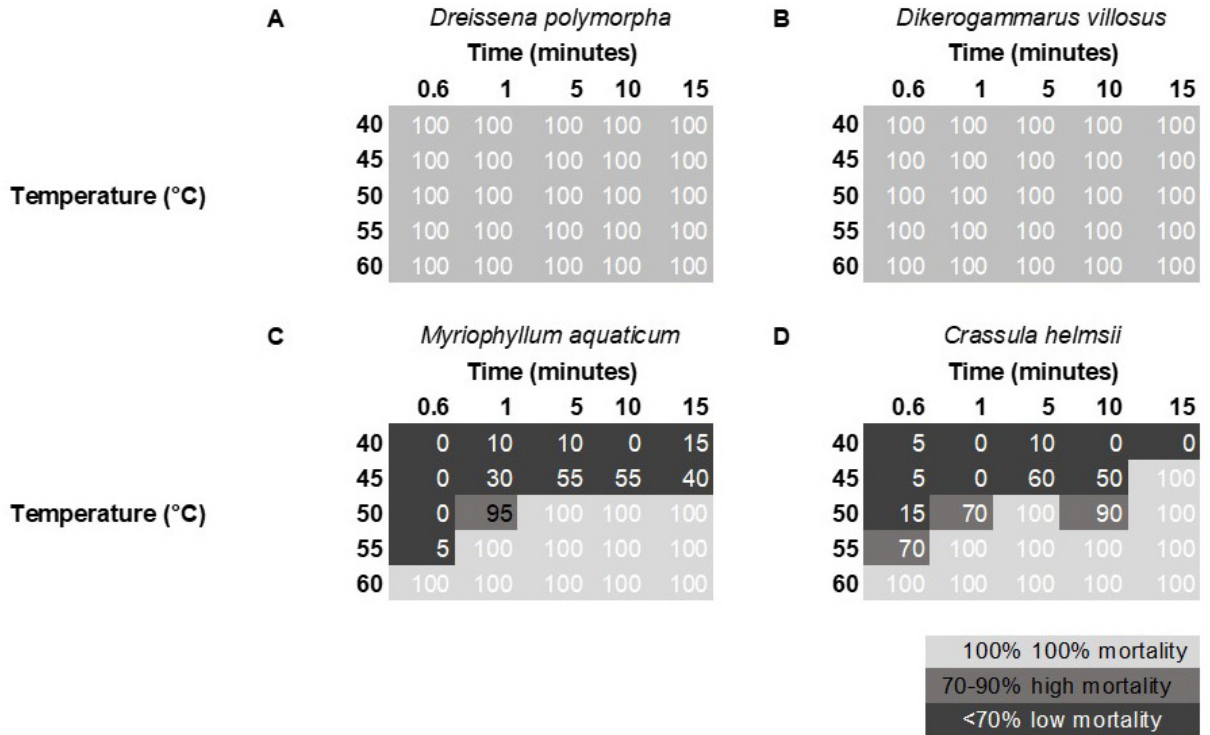
Experiments were conducted between 2016 and 2017 and focused on four representative species. Zebra mussels, *Dreissena polymorpha* (Pallas, 1771), are of global concern, potentially being transported through shipping from the Ponto Caspian region to Western Europe and North America. Killer shrimp (*Dikerogammarus villosus*), New Zealand pigmyweed, *Crassula helmsii* (Kirk) Cockayne, and Parrot's feather, *Myriophyllum aquaticum* (Vell.) Verd. (1753), are of EU concern (<http://www.europe-aliens.org/speciesTheWorst>). IAS were hand-collected from various sites within the UK. *D. polymorpha* were collected from Grafham Water, Peterborough (52.303°N; -0.321°E) in September 2016. *D. villosus* were collected from the same site in January 2017. In March 2016, emergent *C. helmsii* was collected from Potteric Carr Nature Reserve, Doncaster (53.499°N; -1.114°E) and in June 2016, *M. aquaticum* was collected from Stocks Moor Common, Wakefield (53.631°N; -1.588°E). Plants and animals were brought back after collection immediately and stored in separate tanks of aerated freshwater (tap water that had been allowed to stand for > 24 h before use at 14 °C) for 48 hours before the experiment to allow them to acclimatise prior to experimentation. Tanks were stored within a constant temperature room (14 ± 1 °C, light: dark cycle 12:12 h). During field collection, laboratory storage and experimentation, good biosecurity practices were observed at all times.

The health status of the animals and plant fragments was checked before use in experiments to ensure that only healthy individuals were used, and again at the end of the experiment to measure mortality after treatment. To determine whether the plants were healthy before use in the experiments, a FluorPen (FP 100, Photon Systems Instruments) was used. The FluorPen recorded two parameters: the equivalent variable fluorescence and the maximal fluorescence ( $F_v:F_m$ ) which is a measurement of the chlorophyll fluorescence, commonly used as an indicator of plant stress (Hetherington and Smillie 1982; Willits and Peet 2001). Plants with scores of 0.7 or above were considered healthy and were to be used for the experiment (Willits and Peet 2001). Healthy *D. villosus* and *D. polymorpha* were identified as those that responded to mechanical stimuli (swimming or siphoning, respectively) which involved gently touching animals with a probe. At the end of the

experiment, plants with  $F_v:F_m$  values of 0.3 or below were considered to be dead (Dan et al. 2000). A previous study using  $F_v:F_m$  to estimate plant mortality found that those plants recorded as dead 24 h after hot water immersion showed no evidence of recovery after a further 16 days (Anderson et al. 2015a). Hence, although we cannot discount the possibility of some plant recovery this method provides a simple means to compare mortality. *D. villosus* were considered dead if they failed to respond to stimuli or had decomposed, and *D. polymorpha* were assumed dead if their shells gaped and they did not respond to stimuli (Beyer et al. 2011).

For experimentation, plants fragments were taken and cut into fragments of 60 mm length, making sure the reproductive part of the plant was not removed. *D. polymorpha* and *D. villosus* were randomly selected from the stock tanks (Beyer et al. 2011). *D. polymorpha* ranged in total length from 11 mm to 33 mm (median 23 mm), and *D. villosus* ranged in total length from 3 mm to 13 mm (median 8 mm). Individual bags made from nylon mesh at 100 mm<sup>2</sup> contained ten replicates of each species and were sealed with staples. The netting aimed to replicate anglers' keep nets or sampling nets on which fragments or animals could be found/trapped. The bags were then submerged in a flexi bucket containing 16 litres of fresh water (tap water that had been allowed to stand for > 24 h before use) at 14 ± 1 °C for an hour before the experiment in order to simulate an angling trip or other water activities.

Nets containing the animals/plant fragments were subject to one of 5 different starting immersion temperatures of tap water to account for both domestic and commercial hot water temperatures (40, 45, 50, 55, 60 °C) and one of 5 treatment times (15, 10, 5, 1 minute, 10 seconds). Initially we conducted 10 replicates per treatment. As mortality was 100% across all treatments for the two animal species, but < 100% for the plants, we conducted a further 10 replicates for each plant treatment. As the *Check Clean Dry* campaign is aimed at recreational users, we did not use a water bath but used large (19 litre) buckets to simulate domestic or field depot setting. For example, water temperature decreased from 60 °C to 56.5 °C and 40 °C to 38.7 °C in 15 minutes. Exposure times ranged from 15 minutes down to 10 seconds, in order to cover all realistic times that might be applied in the field (Stebbing et al. 2011; Anderson et al. 2015a). Once exposure time was completed, bags were removed and placed back into fresh water at 14 ± 1 °C for 15 minutes. Plants and animals were returned to a constant temperature room in fresh water (14 ± 1 °C, light: dark cycle 12: 12 h) for 24 hours before being recorded as dead or alive.



**Figure 1.** Heat map illustrating percentage mortality of both animal (A–B) and plant (C–D) species after immersion in hot water at different temperatures and treatment durations.

An additional experiment was undertaken to confirm that propagule death was a result of the immersion in hot water, and not a result of the rapid return to water at 14 °C post hot water treatment. Propagules were exposed to hot water (10 replicates of each species at 40 °C, 10 of each species at 50 °C for 10 mins) as above. Post-immersion, the water was then allowed to cool naturally to 14 °C with mortality recorded at 24 h as above.

#### Data analysis

All statistical analyses were undertaken in R version 3.3.2 and RStudio version 1.0.136 (R Core Team 2016; RStudio Team 2016). To test the effectiveness of treatment (temperature and time), generalised linear models were initially used with binomial errors to account for the binary nature of the survival response variable. However, standard application of logistic regression produced perfect separation in some cases. Therefore, we employed Firth's bias-reduced penalised-likelihood logistic regression (Firth 1993) using the logistf package (Heinze and Ploner 2016) in R. Traditional post-hoc tests are not available for penalised-likelihood logistic regression, and so diffe-

rences between treatment levels were evaluated using tests of proportions, corrected for multiple tests using false discovery rates in R. The Exact-CI function within the PropCIs package (Scherer 2018, using the Clopper Pearson exact method) was used to compute a 95% confidence interval for each of the proportions being calculated for each parameter estimate.

#### Results

There was 100% mortality of both *D. villosus* and *D. polymorpha* animal species for all time and temperature treatments (Figure 1). In contrast, mortality in the two plant species was significantly affected by temperature and by treatment duration (Figure 1, Table 1 and 2).

While the time spent immersed at 45 °C had a significant effect on survival in *C. helmsii* ( $\chi^2_1 = 54.519$ ,  $P < 0.001$ ) and *M. aquaticum* ( $\chi^2_1 = 6.803$ ,  $P = 0.009$ ), there was a significant reduction in mortality of *C. helmsii* from 100% to 50% when treatment duration at 45 °C was reduced from 15 minutes to 10 minutes (Figure 1;  $\chi^2_1 = 7.135$ ,  $P = 0.007$ ). Mortality was only 40% for *M. aquaticum* with a treatment duration of 15 mins, and shorter treatment

times were less effective for both plant species (Figure 1, Table 1).

We therefore went on to explore the effectiveness of higher temperature treatments at time durations shorter than 15 minutes in killing IAS propagules. Immersion for as little as 10 seconds caused 100% mortality at all temperature treatments for *D. villosus* and *D. polymorpha* (Figure 1). In contrast, plant mortality was affected by both temperature and duration of treatment with higher temperatures and longer durations leading to greater mortality (Figure 1). Immersion in water at 50 °C and 55 °C for 5 minutes caused high mortality (90–100%) for all plant and animal species and immersion at 60 °C for a shorter time of 10 secs caused 100% mortality.

Mortality was also high (100% for all species) when propagules were immersed for 10 mins at 40 °C and 50 °C and then allowed to gradually return to 14 °C.

## Discussion

Soaking equipment and footwear in hot water represents a safe, practical cleaning protocol to improve the rapidity and effectiveness of the *Check Clean Dry* biosecurity practice (Stebbing et al. 2012; Anderson et al. 2015a). Stakeholder interviews have identified a need to minimise time spent on biosecurity, particularly for organisations where staff time spent on biosecurity imposes an economic cost and who want to optimise the work patterns of staff in the field (Sutcliffe et al. 2017). Therefore, any reduction in the time required to undertake the “Clean” phase of *Check Clean Dry* is likely to improve uptake of biosecurity practices. The effectiveness of hot water treatments in killing propagules of four aquatic IAS species was examined, and the treatments were applied using hot water in large buckets to simulate probable domestic or field depot situations. Our results indicate that hot water caused mortality in all four species, but with some variation in temperature required to cause total mortality, likely reflecting different thermal tolerance. Immersion in hot water of 45 °C for 15 minutes caused 100% mortality of both *D. polymorpha* and *D. villosus*, in accord with previous studies conducted by Stebbing et al. (2011) and Anderson et al. (2015a), and mortality was 100% even with shorter treatment times, however, temperatures of 50 °C or above for 15 mins were required to cause high mortality (90–100%) of the IAS plant propagules. We conclude that treatment of 45 °C for periods shorter than 15 minutes is less effective than treatment for the recommended 15 minutes. Therefore, any reduction in treatment duration at the suggested 45 °C would not cause consistent and high mortality across IAS.

**Table 1.** Penalised likelihood logistic regression for the effect of temperature and immersion duration on mortality of *Crassula helmsii*.

	Coefficient	X <sup>2</sup>	P
(Intercept)	-14.683	53.380	< 0.001
Temperature	0.299	49.872	< 0.001
Time	-2.754	27.729	< 0.001
Temperature × Time	0.068	33.035	< 0.001

**Table 2.** Penalised likelihood logistic regression for the effect of temperature and immersion duration on *Myriophyllum aquaticum*.

	Coefficient	X <sup>2</sup>	P
(Intercept)	-10.43	61.701	< 0.001
Temperature	0.209	59.114	< 0.001
Time	-1.074	13.119	< 0.001
Temperature × Time	0.028	18.336	< 0.001

Mortality was higher at higher temperatures and, in fact, high (90–100%) mortality was achieved for all species following immersion in water at 55 °C or 60 °C for 5 minutes. Temperatures of 55 °C or 60 °C could potentially be applied in a depot or laboratory setting where facilities and training are provided for safe working practices, and these temperatures would lead to high mortality of IAS propagules. In particular, the findings that, at temperatures above 50 °C, shorter immersion times lead to high mortality, may be important in increasing the uptake of biosecurity practices when time is a constraint.

However, temperatures above 50 °C are unlikely to be met in domestic settings (e.g., to treat recreational equipment or personal clothing) or in the field. Recommendations for the temperature of water from a hot tap are based on safety. Temperatures exceeding 51.66 °C can pose a serious risk of severe burn to adults and children (Feldman et al. 1998), whilst the World Health Organisation recommends water should be no less than 50 °C to minimise the risk of *Legionella* bacteria in water. The temperature of hot water in domestic settings is variable. To ensure uptake and application of biosecurity procedures, practices need to be easy to apply, and it is important that guidance is simple, consistent and safe (Sutcliffe et al. 2017). Therefore, for practical application in field or domestic conditions, we recommend that a minimum temperature of 50 °C is used, where feasible, for biosecurity with a minimum treatment time of 5 minutes, and with longer immersion times if practical. Although this treatment may not cause 100% mortality, it represents a safe compromise between ease of use, safety and effectiveness.

The availability of facilities for *Check Clean Dry* has also been identified as a barrier to good



biosecurity (Sutcliffe et al. 2017). We recommend investment in cleaning stations that include hot water facilities to enable those working or undertaking recreational activities in the environment to clean their equipment. In the absence of any specialist biosecurity facilities, using hot tap water as part of the *Check Clean Dry* protocol will reduce the risk of IAS transmission, even if the water temperature does not reach 50 °C. Immersion in hot water is a simple treatment for small equipment. However, there is also a need for practical biosecurity treatments that can be applied to large equipment such as boats and machinery. High pressure sprays are used to clean fouling organisms from boats, with hot water high pressure sprays reported to kill fouling animals *D. polymorpha*, and *D. bugensis* (Morse 2009; Comeau et al. 2011; Stebbing and Rimmer 2014). We recommend that further research is carried out into the effectiveness of cold and hot water sprays in dislodging propagules and in causing mortality of high-impact plant as well as animal IAS, particularly as not all propagules may be removed.

*Clean Drain Dry* and *Check Clean Dry* campaigns aim to raise biosecurity awareness and practice among water users, to reduce the risk of IAS spread. Awareness of these campaigns has been shown to lead to people being more likely to carry out good biosecurity measures than those who are not aware of campaigns (Anderson et al. 2014; GBNNSS 2015). Time constraints have been identified as a barrier to good biosecurity especially in large organisations with financial constraints (Sutcliffe et al. 2017). This study indicates that high mortality of IAS plants and animals can be achieved with shorter treatment times of 5 minutes, if temperatures above 50 °C are applied. Furthermore, even lower temperature treatments of 45 °C caused > 40% mortality and will therefore substantially reduce the risk of IAS introduction and spread. Environmental organisations are under a range of pressures to decrease costs whilst also undertaking and demonstrating good environmental stewardship. The development of time efficient and effective biosecurity practices will make an important contribution to uptake of biosecurity practices and to slowing the introduction and spread of IAS.

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