Management of Biological Invasions (2025) Volume, Issue 4: 1075–1089

Research Article

The effectiveness of pH in causing mortality of aquatic invasive alien species

Zoe K. Cole[1,5], Martin R. Tillotson[2,5], Polina Nikova[1,3], Alexander Donovan[1], Stephanie J. Bradbeer[1,4], Rachel Naden[4], Ben Aston[4] and Alison M. Dunn[1,5]

[1]*Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK*
[2]*School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, UK*
[3]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Boulevard "Tsar Osvoboditel" 1, Sofia 1000, Bulgaria*
[4]*Yorkshire Water, Western House, Bradford, BD6 2SZ, UK*
[5]*Water@Leeds, University of Leeds, Leeds, LS2 9JT, UK*

Corresponding author: Zoe K. Cole (zkc.cole@gmail.com)

Citation: Cole ZK, Tillotson MR, Nikova P, Donovan A, Bradbeer SJ, Naden R, Aston B, Dunn AM (2025) The effectiveness of pH in causing mortality of aquatic invasive alien species. *Management of Biological Invasions* 16(4): 1075–1089, https://doi.org/10.3391/mbi.2025.16.4.10

Received: 12 July 2024
Accepted: 26 May 2025
Published: 27 October 2025

Handling editor: David Wong
Thematic editor: Matthew A. Barnes

Abstract

Raw water transfers between waterbodies are crucial to water utility companies' abilities to balance water supply and demand. However, raw water transfers have been highlighted as a high-risk pathway for the spread of invasive alien species (IAS). Implementing biosecurity at the scale of raw water transfers is a considerable undertaking and the impact on water resources and the environment need to be considered when researching methods to biosecure raw water transfers. We explored the effectiveness of pH changes in causing mortality in aquatic IAS. We used two invasive invertebrate species; *Dreissena polymorpha, Dikerogammarus villosus* and two invasive plant species; *Hydrocotyle ranunculoides, Crassula helmsii.* pH was adjusted using chemicals already widely utilised within the water treatment process (ferric sulfate-acid and calcium hydroxide-alkali) to produce treatments of acid (pHs 3, 4, 5, 6) and alkali (pHs 9, 10, 11, 12) pH. For *D. villosus, c*omplete mortality was only seen following immersion at pH 12 for ≥ 1 hour. For *D. polymorpha*, 100% mortality was not achieved in any treatment. Mortality was highest in more extreme treatments of pH4 (44%) and pH12 (40%) following immersion for 8 hours. Incomplete mortality of *H. ranunculoides* was seen for all pH treatments, with the highest mortality (50%) at pH4 and pH 10. *Crassula helmsii* experienced no mortality following immersion in any of the pH treatments. These results indicate that immersion in water with altered pH is unlikely to be suitable as a biosecurity treatment to slow the spread of aquatic invasive invertebrates and macrophytes in raw water transfers.

Key words: biosecurity, bulk raw water transfer, *Dreissena polymorpha, Dikerogammarus villosus, Hydrocotyle ranunculoides, Crassula helmsii*

Introduction

Globalisation has played a key part in increasing global transport networks and movements of commodities around the world. With this greater connectivity and diversity of transport methods such as by sea, air and land, the spread of IAS has accelerated (Hulme 2009; Amano et al. 2016; Bonnamour et al. 2021). Freshwater environments such as rivers, lakes, ponds and wetlands are extremely important ecosystems. Whilst they make up less than 1% of the world's water, they constitute almost the entire supply of the worlds' drinking water (Shikloanov 1993; Dudgeon et al. 2006). Freshwater ecosystems are one of the worlds most threatened habitats and are particularly vulnerable to the introduction of IAS as a result of human activity (Dudgeon et al. 2006; Olden et al. 2010). The global cost of aquatic IAS has increased rapidly over the last 50 years and was estimated to be at least US$23 billion in 2020 (Cuthbert et al. 2021). IAS in freshwater systems are associated with loss of native species, introduction of diseases and alteration of ecosystem function (Pimentel et al. 2005; Hejda et al. 2009; Hulme 2014; Roy et al. 2023). Social and economic impacts of IAS. include increased flood risk, risk to human health, and risk to food and water security (Hulme 2014; Gallardo and Aldridge 2020; Roy et al. 2023). Aquatic IAS can be introduced or spread through trade, transport, and recreational activities. The risk of IAS spread through recreational activities such as boating and angling (Ricciardi 2001; Gollasch 2007; Anderson et al. 2014; Hulme 2015) is of particular concern to water utility companies. Furthermore, research has also highlighted raw water transfers (RWT, the movement of untreated water between waterbodies) as a potential pathway for the spread of IAS (Gallardo and Aldridge 2018, 2020; Waine et al. 2023).

Raw water transfer or RWT refers to the movement of untreated water between waterbodies, such as between different reservoirs or between a river and a reservoir. Changing climate and demography is placing increasing need on water utility companies, which can be both publicly and/or privately owned, to balance water supply and demand and safeguard water resources (Ludwig et al. 2014; Gasbarro et al. 2016; Gosling and Arnell 2016). Interbasin transfers of raw water are essential to ensuring water supplies to industry and domestic users (Das 2006; Cosgrove et al. 2015; Khadem et al. 2021). Current and future water transfer megaprojects move thousands of megalitres water across catchments, river basins and geopolitical borders to help elevate water scarcity under projected climate change and demographic scenarios. For example China's South-North Water Transfer Project will ultimately transport up to 4.5 billion litres of water p.a. within its 4300 km of canals and pipelines which is estimated to cost between US$20 to US$81 billion to complete; and the Central Arizona Project diverts water from the Colorado River 336 miles away to Tuscon, Arizona and is the most expensive water transfer project in US history, costing US$5 billion to construct (Hulme 2015; Gosling and Arnell 2016; Rogers et al. 2016, 2020; Sternberg 2016; Gallardo and Aldridge 2018; Shumilova et al. 2018). Such RWT pose a serious risk of spreading IAS due to the connectivity they provide between water bodies and the resulting risk of accidental translocation of animals or plant fragments during water transfers (Waine et al. 2023; Zhu et al. 2023).

In Great Britain freshwater IAS management costs £26.5 million annually (Oreska and Aldridge 2011), with water utility companies paying at least £4.6 million of that (Williams et al. 2010). Alongside the Water Framework Directive (WFD) which requires industries using river basins to protect, restore and prevent deterioration, water utility companies and other water authorities who use RWT to move millions of litres of raw water around Great Britain daily are highly driven to reduce the risk of the introduction and further spread of IAS through RWT to limit the resultant impacts on their assets. Therefore, it is crucial to develop and test methods to reduce the risk of IAS spread through RWT whilst allowing the movement of water that is essential to ensure water security. Preventing the introduction and spread of IAS is considered the most cost-efficient and effective method to manage IAS (Simberloff 2021). Biosecurity refers to actions taken to reduce the risk of introducing or spreading IAS.

There are various campaigns to raise awareness and encourage biosecure practices amongst the general public and practitioners, such as “Check, Clean, Dry” in the UK, Ireland and New Zealand (Ministries for Primary Industries Manatū Ahu Matua 2023; GBNNSS ND), “Clean, Drain, Dry” in the USA (U.S. Fish & Wildlife Service ND) or “Play, Clean, Go” in Canada (Invasive Species Council of BC ND) These campaigns promote simple practices to remove/kill of IAS propagules and decontaminate equipment and clothing using protocols including cold-water spray, disinfectants and immersion in hot water (Shannon et al. 2018; Crane et al. 2019; Bradbeer et al. 2020a, b; Smith et al. 2023). To date, the development and testing of biosecurity protocols has focused on decontamination of clothing, equipment and vehicles, with little work addressing biosecurity of RWT. Biosecurity to slow the spread of IAS in RWT presents a number of challenges owing to the volume of water, its flow rates and the complex structure of transfer networks. The UK based regional water utility company Yorkshire Water abstracts, treats and supplies around 1.3 billion litres daily from the environment (Yorkshire Water 2022). In addition to biosecurity effectiveness, any biosecurity protocols applied to RWT must be environmentally safe and appropriate for use in drinking water supplies.

Mechanical and chemical biosecurity treatment options for RWTs need to be environmentally safe. A potential biosecurity treatment for use in RWT is temporary alteration of water pH. Previous studies have shown that altered pH can induce mortality in *Dikerogammarus villosus* (Sowinsky, 1894) (Sebire et al. 2018) and *Dreissena polymorpha* (Pallas, 1771) (Martin et al. 1993; Bowman and Bailey 1998; Fisher et al. 1999; Claudi et al. 2012). However, these studies involved extended periods of exposure (20 days to 10 Weeks) (Bowman and Bailey 1998; Fisher et al. 1999; Claudi et al. 2012) and used a variety of chemical treatments (Fisher et al. 1999; Claudi et al. 2012; Barenberg and Moffitt 2017; Sebire et al. 2018) that have not been approved for use in raw water intended for drinking water supply. Furthermore, no studies to date have explored the effectiveness of pH in inducing mortality of IAS aquatic macrophytes.

Here, we used calcium hydroxide and ferric sulphate to achieve temporary changes to the pH of raw water. These chemicals are commonly used as flocculants for the removal of particulate material within the process of treating raw water for drinking water supply. The pH levels used were outside that of optimal flocculation pH in the water treatment process (6.5 to 8.5) (Zeng et al. 2011) and short treatment durations (60 minutes to 480 minutes) were explored to reflect the need for rapid biosecurity treatments due to potentially high flow rates of raw water transfers. Thus, we explored their application as short-term biosecurity treatments in inducing mortality in four aquatic IAS of high concern for water utility companies within the UK; two invertebrates *D. villosus* and *D. polymorpha*, and two macrophytes *Hydrocotyle helmsii* (Kirk) Cockayne and *Hydrocotyle ranunculoides* (Linnaeus) (Gallardo and Aldridge 2020).

Materials and methods

Study species

Dikerogammarus villosus (killer shrimp) is listed in the top 100 worst IAS within Europe (Nentwig et al. 2018) as it negatively impacts invaded ecosystems (DAISIE 2009). *Dikerogammarus villosus* spread from its native Ponto-Caspian region across Europe in less than three decades and is of concerns for future invasion of the great lakes in North America (Ricciardi and MacIsaac 2000; Rewicz et al. 2014).

Dreissena polymorpha (zebra mussel) is included with the IUCN top 100 worst invasive alien species, with a total estimated associated cost of US$3.4 billion globally between the years 1960–2020 (Cuthbert et al. 2022). Water treatment and electricity generation companies in the US have spent an estimated overall US$267 million between 1989 and 2004 on *D. polymorpha* management with US$87 million going into preventative measures (Connelly et al. 2007). These economic costs arise as a result of the biofouling ability of *D. polymorpha*, which allows them to attach to the inside of pipes, causing blockages, damaging infrastructure and affecting water quality through filter feeding (Gallardo and Aldridge 2018, 2020).

Crassula helmssi (swamp stone-crop) and *H. ranunculoides* (floating pennywort) are aquatic invasive plants. Specific concerns for water utility companies are the plants' ability to form dense mats in waterbodies. They can outcompete native plants, alter water quality, cause operational issues and increase flood risk (Leach and Dawson 1999; Stiers et al. 2011; Gallardo and Aldridge 2020).

Collection of experimental organisms

Dikerogammarus villosus and *D. polymorpha* were collected from Grafham Water, Cambridgeshire, UK (Latitude 52.291806, Longitude −0.323163) between October 2020 and March 2022. *Dikerogammarus villosus* and *D. polymorpha* were collected by hand from ropes and hard surfaces submerged in the water. *Hydrocotyle ranunculoides* were obtained from Water Haigh Woodland Park, Yorkshire, UK (Latitude 53.751144, Longitude −1.428923) between April 2022 and August 2022. *Crassula helmsii* were taken from Potteric Carr Nature Reserve, Yorkshire, UK (Latitude 53.498876, Longitude −1.113194) in October 2022. Samples of *H. ranunculoides* and *C. helmsii* were collected by hand. Organisms were transported to the University of Leeds and stored in aerated tanks in a controlled temperature room (~ 14 °C) under a 12 hour light:dark cycle. *Dikerogammarus villosus* were provided with *Alnus glutinosa* leaves as food source, and *D. polymorpha* with *D. villosus* faeces. All organisms were held for at least 72 hours prior to use in order to minimise the potential stress caused as a result of collection and transport.

For the invertebrates, only healthy adults which were seen either actively swimming (*D. villosus*) or actively filter feeding (*D. polymorpha*) were selected. Adult individuals are less vulnerable than juvenile/immature stages to environmental stressors (Sebire et al. 2018; Cuthbert et al. 2019; Coughlan et al. 2020; Crane et al. 2020). Mass was recorded prior to each experiment.

From the macrophytes (*H. rannunculoides* and *C. helmsii*) chlorophyll content readings were taken prior to start of each experiment using a chlorophyll meter (Konica Minolta SPAD 502-Plus). The chlorophyll content in plant leaves are used as a proxy for plant health (Dan et al. 2000; Bradbeer et al. 2020b) Only mature plants which showed positive phototropism, chlorophyll content of > 4.9 and a visual inspection score of 5 (no degradation present other than at fragment site) (Dan et al. 2000; Shannon et al. 2018; Crane et al. 2019; Bradbeer et al. 2020b) were selected for the experiment. Stem length section of 10 cm for *H. rannunculoides* and 5 cm sections for *C. helmsii* were used.

Prior to each experiment, individuals were maintained in ~ 250 ml dechlorinated tap water for < 1 hr. For *D. villosus,* a sterilized glass pebble was placed in the containers to avoid excessive swimming behaviour, which could increase mortality.

Experimental design

Solutions of ferric sulphate and calcium hydroxide were made to desired pH 3, 4, 5, 6 and pH 9, 10, 11, 12, respectively. Acid pH solutions were made by slowly adding small amounts of ferric sulphate powder to dechlorinated tap water under continuous stirring. The pH reading was taken using a pH meter (Hanna Combo Tester HI98129), with ferric sulphate addition continuing until the desired desired pH was achieved. A similar process was adopted for alkali pH solutions, this time using a premade calcium hydroxide solution. Experimental solutions of pH were replaced at least once an hour.

Individual IAS were exposed to one of eight pH treatments (pH 3, 4, 5, 6, 9, 10, 11, 12) or the control groups were immersed in dechlorinated tap water for an allotted time period. *Hydrocotyle ranunculoides* were exposed to one time treatment of 2 hours. The other three species were exposed to

Table 1. Shows the time durations (hours) used to test pH treatments (pH 3, 4, 5, 6, 9, 10, 11, 12 and Control) against four species (*Dreissena polymorpha, Dikerogammarus villosus, Crassula helmsii* and *Hydrocotyle ranunculoides*).

Species	Duration (Hours)		
	1	2	8
Dreissena polymorpha		▨	▨
Dikerogammarus villosus	▨	▨	
Crassula helmsii	▨	▨	
Hydrocotyle ranunculoides		▨	

two different time treatments; *D. villosus* and *C. helmsii* were immersed in one of the treatments for either 1 or 2 hours and *D. polymorpha* immersed for either 2 or 8 hours (Table 1). 30 replicates of each species per treatment for each time duration was run. Previous studies which have exposed *D. polymorpha* to various chemicals have shown low mortality following short duration treatments (Van Benschoten et al. 1993; Verween et al. 2009). Therefore, this species was immersed for treatments of 2 and 8 hours durations. To limit pH change over time, solutions were replaced at least once per hour. During the treatments a fine-mesh sieve was used to keep *H. ranunculoides* and *C. helmsii* fully immersed.

Following immersion in the treatment solutions, specimens were re-immersed in dechlorinated tap water for two-minutes, twice to aid in the removal of the treatment solution (Bradbeer et al. 2020a; Crane et al. 2020). Specimens were then returned to ~ 250 ml storage dechlorinated tap water and placed in the controlled temperature room (14 °C; 12:12 h light–dark) (Figure 1). If any individuals died, or if *D. villosus* moulted before the end of the experiment they were discounted.

Mortality assessment

To assess the effectiveness of the different treatments, IAS invertebrate mortality was measured after 1 hour, 1 day and 7 days after exposure. As macrophytes may show recovery from treatments (Crane et al. 2020) mortality was measured at 1 hour, 1 day, 7 days, 14 days and 21 days following the methods outlined in previous studies (Anderson et al. 2015; Bradbeer et al. 2020a; Crane et al. 2020; Shannon et al. 2018). This allows any residual impact on individual mortality to be assessed.

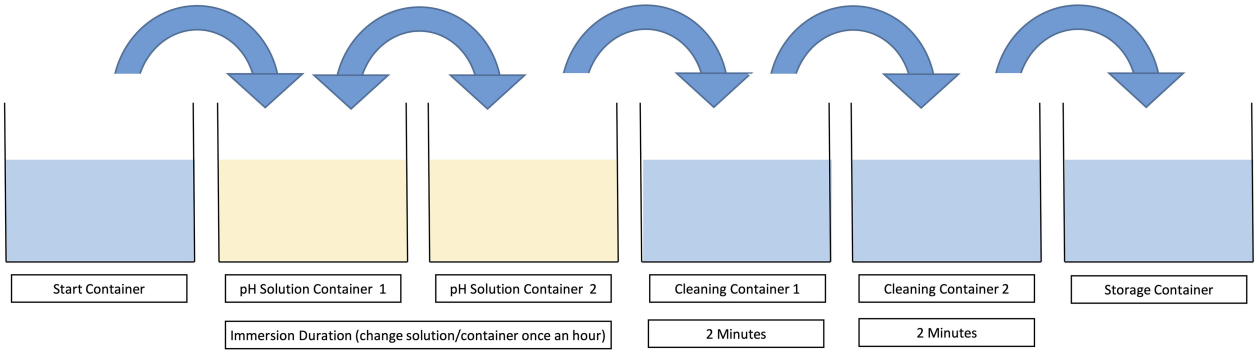

Figure 1. Experimental set up for immersing invasive alien species (*Dreissena polymorpha* adult is used as an example individual) in pH solutions and post-exposure cleaning (dechlorinated oxygenated tap water) stations.

Dreissena polymorpha were considered dead if they were gaping and not actively feeding, or if when tweezed apart they offered no resistance and did not reclose their shell. *Dikerogammarus villosus* were considered dead if they did not actively swim in response to mechanical stimulation, showed discolouration and pereopods were not actively moving (Bradbeer et al. 2020a).

To measure macrophyte mortality the chlorophyll content and a visual score were used. The visual score followed the method of Crane et al. (2019); in which plant fragments were assessed based on stages of degradation and regrowth on a scale of 0 to 10. A plant fragment with a score of 5 showed no shoot/root growth to be present, with only degradation at the fragmentation site. A score > 5 indicated more degradation along with no shoot/root growth; as the score increased so did the extent of degradation until a score of 10 represented complete degradation. A score of < 5 indicated there was shoot/root growth along with potentially degradation. Macrophytes were considered as not viable (dead) if they had a chlorophyll reading of ≤ 0.8 and had a visual score of 10 (Dan et al. 2000; Crane et al. 2019; Bradbeer et al. 2020b). Chlorophyll content measurements for *C. helmsii* were taken using a pair of leaves removed from the plant section; chlorophyll content has been shown not to differ between lead pairs within a plant (Bradbeer et al. 2020b). Chlorophyll content measurements for *H. ranunculoides* were taken from the same location on the leaf for all individuals.

Statistical analysis

All statistical analyses were conducted in R (Version 4.2.2). Mass measurements were compared between treatments for *D. villosus* and *D. polymorpha* using an Analysis of Variance (ANOVA). Pre-exposure chlorophyll contents for both macrophytes (*H. ranunculoides* and *C. helmsii*) were compared between treatments using an ANOVA. If residuals did not meet homoscedasticity assumptions (Levene's test, $p < 0.05$) or normality (Shapiro-Wilk, $p > 0.05$), the data was transformed by log and the ANOVA repeated. If assumptions were still not met a Kruskall Wallis test was undertaken.

For each species, mortality at the final time point (day 7 – *D. polymorpha* and *D.* villosus) or (day 21 – *C. helmsii* and *H. ranunculoides*) was compared across treatments. A generalised linear model (GLM) was used, which assumed a logistical error distribution. Components of treatments for invertebrates were "pH", "duration" and "mass" or "masslog". For *H. ranunculoides*, the components were "pH" and "starting chlorophyll content". Initially five models were created using these terms for each species, apart from *H. ranunculoides* were two models were created (Supplementary material Tables S1–S3). The models were then assessed using Akaike Information

Table 2. Percentage mortality of *Dreissena polymorpha*, *Dikerogammarus villosus* seven days post exposure; and *Hydrocotyle ranunculoides, Crasssula helmsii* twenty one days post- exposure to a range of pH treatments (pH 3–6 and pH 9–12) and control treatment for set durations. Colours: red (0–9.9% mortality), amber (10–49.9%), yellow (50–99.9% mortality) and green (100% mortality).

Species	Duration (Hours)	pH								
		3	4	5	6	9	10	11	12	Control
Dreissena polymorpha	2	3.85%	3.33%	20%	13.8%	0%	7.3%	9.7%	0%	5.1%
	8	3.1%	43.8%	25%	0%	0%	3.3%	0%	40%	0%
Dikerogammarus villosus	1	44.8%	20.7%	17.2%	7.7%	7.4%	8%	10%	100%	11.1%
	2	33.3%	28.6%	17.4%	21.4%	14.3%	10%	51.4%	100%	3.1%
Crassula helmsii	1	0%	0%	0%	0%	0%	0%	0%	0%	0%
	2	0%	0%	0%	0%	0%	0%	0%	0%	0%
Hydrocotyle ranunculoides	2	33.3%	50%	30%	40%	33.3%	50%	16.7%	36.7%	24.1%

Criterion (AIC) and the most appropriate model was selected. Post-hoc pairwise comparison (Tukey) was run on significant factors within the selected model.

Results

Mortality of *D. polymorpha* was significantly affected by pH, duration and the interaction of pH and duration (χ^2 = 51.63, df = 8, p < 0.001; χ^2 = 5.01, df = 1, p < 0.05; χ^2 = 48.24, df = 8, p < 0.001). Mass did differ between treatment groups (Kruskal Wallis: $\chi^2(17)$ = 178.23, p < 0.001) (*D. polymorpha* mean mass = 0.91g ± 0.01), and mass had no significant effect on mortality (χ^2 = 1.56, df = 1, p = 0.21). The best-fit model components were pH*duration + mass (AIC = 307.43 and AIC Weight = 75%). Mortality was highest in more extreme treatments of pH4 (44%) and pH12 (40%) following immersion for 8 hours. Interestingly, mortality did not appear to increase with the lower acid/higher alkali pHs. Pairwise comparison showed significant differences only between control (2 hour duration) and pH 4 (8 hours duration).

Dikerogammarus villosus mortality was significantly affected by pH (χ^2 = 214.69, df = 8, p < 0.001) but not by treatment duration or mass (χ^2 = 2.98, df = 1, p = 0.084, χ^2 = 0.44, df = 1, p = 0.51). Mass did differ between treatments (Kruskal Wallis: $\chi^2(17)$ = 92.89, p < 0.001) (*D. villosus* mean mass = 0.0798 g ± 0.0014). The best-fit model, which included all three factors was used (pH + duration + mass) (AIC = 419.83 and AIC Weight = 30%). Complete mortality for *D. villosus* was seen at pH 12 exposure for both 1 hour and 2 hours immersion. Mortality increased with increasing acidity/alkalinity and was greater following longer exposure (Figure 2B; Table 2).

Mortality of *H. ranunculoides* was not significantly affected by pH or by the starting chlorophyll (χ^2 = 13.03, df = 8, p = 0.11; χ^2 = 0.23, df = 1, p = 0.63). Starting chlorophyll did not differ between treatments (Shapiro Wilks: W = 0.99, p = 0.09; Levene's Test: F = 2.04, d.f. = 8, p = 0.04) (mean

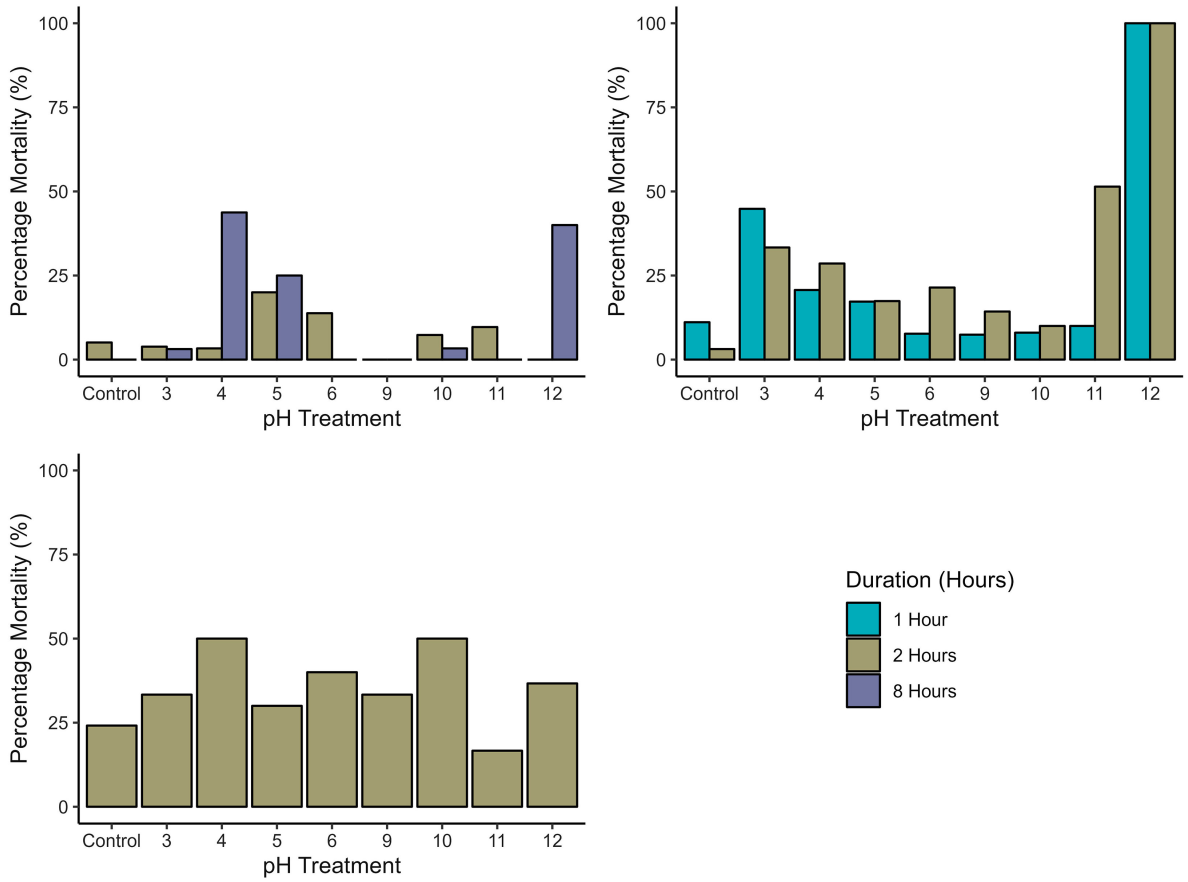

Figure 2. Percentage mortality of (A) *Dreissena polymorpha*, (B) *Dikerogammarus villosus* seven days post exposure; and (C) *Hydrocotyle ranunculoides* 21 days post exposure to pH treatments (pH 3–6 and pH 9–12) and control treatment for set durations.

chlorophyll of *H. rannunculoides* = 41.08 ± 0.24 SPAD value). The best-fit model was pH + start chlorophyll (AIC = 355.73, AIC Weight = 1.00).

No mortality of *C. helmsii* was seen in any of the treatments (Table 2).

Discussion

Raw water transfers pose a risk of accidental spread of IAS, and biosecurity implementation is key to reducing this risk (Waine et al. 2023; Zhu et al. 2023). This is the first study to explore the variance of pH as a biosecurity treatment for invasive aquatic macrophytes. The treatments tested however, had limited effectiveness against the four different study organisms. Hence, altering pH is not considered to be an effective biosecurity method for RWT.

Invertebrates

Complete mortality of *D. villosus* was achieved following exposure for ≥ 1 hour to pH 12 which is a pH higher than usually range found within the raw water treatment process. However, other pH treatments caused incomplete mortality ranging from 10%–50%. A previous study using hydrochloric acid reported 20% mortality following 15 minute immersion in pH 3 and 5, but no mortality at other pHs tested (4, 6, 7 and 8) (Sebire et al. 2018). The higher mortality achieved in the current study may reflect the choice of chemical used to alter pH and/or longer exposure times.

pH alteration was not effective in inducing complete mortality in *Dreissena polymorpha*. Mortality was low in the shorter exposure durations (2 hours) treatment, with the highest mortality of 44% at pH 4 exposure for 8 hours (Table 2, Figure 2A). Interestingly, lower mortality was observed at more extreme pH values (Table 2) This could reflect a defensive behavioural response to the pH exposure; *D. polymorpha* may close their shells in response to external stimuli. This shell-closing ability has been seen in response to chemicals, predators and desiccation, with individuals remaining closed for up to three weeks (Borcherding and Wolf 2001; Aldridge et al. 2006; Borcherding 2006; Dzierżyńska-Białończyk et al. 2019). The low mortality observed at pH 3 may reflect this avoidance behaviour, and may explain the limited effectiveness of 2 or 8 hour exposure to altered pH in inducing mortality in this species.

Although previous studies have achieved high levels of mortality in *D. polymorpha*, they required very long (> 24 hours) exposure times. For example, using phosphoric acid Claudi et al. (2012) reported 38% mortality in adult *D. polymorpha* exposed for 31 days to pH 6.9. Bowman and Bailey (1998) continually exposed *D. polymorpha* adults for 31 days and the highest observed mortality was 84% at pH 9.5. Similar experiments exposing *D. bugensis* adults to PolyDADMAC for 9 day exposure resulted in 87% adult mussel mortality (Takeguchi and Yates 2012). RWT require the movement of very large volumes of water over a relatively short time period, meaning long exposure periods suitable to kill IAS are infeasible. The low mortality achieved in the current study indicates that pH does not appear to offer a suitable treatment option for biosecurity in this situation.

Macrophytes

No mortality of *C. helmsii* was observed in any of the pH treatments. Previous biosecurity investigations have similarly found *C. helmsii* to be resistant to a range of treatments. For example, no mortality was observed following 60 minutes immersion in 4% Virkon Aquatic (Bradbeer et al. 2020a), or following 15 second exposure to 90 °C pressurised steam (Bradbeer et al. 2020b). Complete mortality of *C. helmsii* was reported following immersion in 45 °C water for 15 minutes and direct steam exposure for 10 seconds (Anderson et al. 2015; Shannon et al. 2018; Crane et al. 2019). Whilst useful in application of biosecurity for equipment, such thermal treatments are not suitable for RWT.

Altering pH resulted in no significant increase in mortality for *H. ranunculoides* compared to the control which also showed mortality. Previous research has shown *H. ranunculoides* is more vulnerable and less structurally rigid than *C. helmsii* to a variety of biosecurity methods such as high pressure sprays and disinfectants (Anderson et al. 2015; Bradbeer et al. 2020b; Crane et al. 2020). The physical structure of these two invasive macrophytes is notably different and may affect their vulnerability to biosecurity treatments. Mortality of *H. ranunculoides* in these studies may partly reflect structural damage. For example, full mortality has been seen for this species when exposed to 18 °C or 90 °C for 5 second durations of pressurised water spray (Bradbeer et al. 2020b). In the current study, the physical damage during treatment may have contributed to observed mortality of 24.1% in the control treatment. However, the highest mortality achieved in pH exposure was 50%, indicating the pH treatment would be only partially effective as biosecurity in RWT.

Conclusions

RWT poses a significant risk of accidental transport of IAS propagules, and presents a considerable challenge owing to the large volumes of water involved. Any interventions must consider downstream requirements for treatments that are environmentally safe and potable water supply. The aim of this study was to investigate whether altering pH, using chemicals utilised within drinking water treatment processes, was effective at causing mortality in aquatic IAS. Immersion in water of altered pH treatment had very limited effectiveness against invertebrate and macrophyte IAS suggesting that it is unlikely to be suitable as a biosecurity treatment in RWT. Whilst demonstrating this treatment is not suitable for considerations in biosecurity implementation in RWT by itself it highlights the opportunity to test already utilised technologies and treatments within the water industry operations to understand their potential for RWT biosecurity. Future research could investigate other chemicals used within drinking water treatment such as chlorine or could focus on potential mechanical methods such as filter screens. Furthermore, there may not be one type of treatment that achieves mortality of all IAS of concern and so furthering understanding of partially effective treatments remains of use to inform RWT biosecurity going forward.

Authors' contribution

Conceptualised this study: AMD, PN, ZKC, RN, BA, MRT; methodology: PN, ZKC, SJB, AMD; data collection: ZKC, PN, AD; data analysis: ZKC, SJB; writing – original draft: ZKC; writing – review and editing: AMD, SJB, MRT, BA, RN, PN.

Authors' ORCIDs

Zoe K. Cole: 0009-0007-8189-4972; Martin R. Tillotson: 0000-0002-3362-021X; Polina Nikova: 0000-0002-5701-0926; Stephanie J. Bradbeer: 0000-0002-6844-6194; Rachel Naden: 0009-0006-5691-0646; Ben Aston: 0000-0001-8340-7022; Alison M. Dunn: 0000-0002-4855-1077.

Acknowledgements

Thanks to Yorkshire Water Services Limited, Anglian Water Services Limited and Yorkshire Wildlife Trust for access to fieldsites for collection of specimens. Also, thanks to Animal Plants Health Agency for a Floating Pennywort Permit. The authors would also like to thank the reviewers for their contributions.

Funding declaration

The research was funded by Yorkshire Water Services Limited and a University of Leeds John Henry Garner Scholarship.

Ethics and permits

Invasive Alien Species (Permitting and Enforcement) Order 2019 Permit, Animal and Plant Health Agency, 79.

References

Aldridge DC, Elliott P, Moggridge GD (2006) Microencapsulated BioBullets for the control of biofouling zebra mussels. *Environmental Science & Technology* 40: 975–910, https://doi.org/10.1021/es050614+

Amano T, Coverdale R, Peh KS-H (2016) The importance of globalisation in driving the introduction and establishment of alien species in Europe. *Ecography* 39: 1118–1128, https://doi.org/10.1111/ecog.01893

Anderson LG, White PCL, Stebbing PD, Stentiford GD, Dunn AM (2014) Biosecurity and vector behaviour: evaluating the potential threat posed by anglers and canoeists as pathways for the spread of invasive non-native species and pathogens. *PLoS ONE* 9: e9278810, https://doi.org/10.1371/journal.pone.0092788

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

Barenberg A, Moffitt C (2017) Toxicity of aqueous alkaline solutions to New Zealand mudsnails, Asian clams, and quagga mussels. *Journal of Fish and Wildlife Management* 9: 14–24, https://doi.org/10.3996/022017-JFWM-013

Bonnamour A, Gippet JMW, Bertelsmeier C (2021) Insect and plant invasions follow two waves of globalisation. *Ecology Letters* 24: 2418–2426, https://doi.org/10.1111/ele.13863

Borcherding J (2006) Ten years of practical experience with the Dreissena-monitor, a biological early warning system for continuous water quality monitoring. *Hydrobiologia* 556: 417–426, https://doi.org/10.1007/s10750-005-1203-4

Borcherding J, Wolf J (2001) The influence of suspended particles on the acute toxicity of 2-chloro-4-nitro-aniline, cadmium, and pentachlorophenol on the valve movement response of the zebra mussel (*Dreissena polymorpha*). *Archives of Environmental Contamination and Toxicology* 40: 497–504, https://doi.org/10.1007/s002440010202

Bowman MF, Bailey RC (1998) Upper pH tolerance limit of the zebra mussel (*Dreissena polymorpha*). *Canadian Journal of Zoology* 76: 2119–2123, https://doi.org/10.1139/z98-155

Bradbeer S, Coughlan NE, Cuthbert RN, Crane K, Dick JTA, Caffrey JM, Lucy FE, Renals T, Davis E, Warren DA, Pile B, Quinn C, Dunn AM (2020a) The effectiveness of disinfectant and steam exposure treatments to prevent the spread of the highly invasive killer shrimp, *Dikerogammarus villosus*. *Scientific Reports* 10: 1919, https://doi.org/10.1038/s41598-020-58058-8

Bradbeer S, Renals T, Quinn C, Warren DA, Pile B, Hills K, Dunn (2020b) The effectiveness of hot water pressurized spray machines in field conditions to slow the spread of invasive alien species. *Management of Biological Invasions* 12: 125–147, https://doi.org/10.3391/mbi.2021.12.1.09

Claudi R, Graves A, Taraborelli A, Prescott RJ, Mastitsky SE (2012) Impact of pH on survival and settlement of dreissenid mussels. *Aquatic Invasions* 7: 21–28, https://doi.org/10.3391/ai.2012.7.1.003

Connelly NA, O'Niell CR, Knuth BA, Brown TL (2007) Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. *Environmental Management* 40: 105–112, https://doi.org/10.1007/s00267-006-0296-5

Cosgrove WJ, Loucks DP (2015) Water management: Current and future challenges and research directions. *Water Resources Research* 51: 4823–4839, https://doi.org/10.1002/2014WR016869

Coughlan NE, Bradbeer SJ, Cuthbert RN, Cunningham EM, Crane K, Potts S, Caffrey JM, Lucy FE, Dunn AM, Davis E, Renals T, Quinn C, Dick JTA (2020) Better off dead: assessment of aquatic disinfectants and thermal shock treatments to prevent the spread of invasive freshwater bivalves. *Wetlands Ecology and Management* 28: 285–295, https://doi.org/10.1007/s11273-020-09713-4

Crane K, Cuthbert RN, Dick JTA, Kregting L, MacIsaac HJ, Coughlan NE (2019) Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. *Biological Invasions* 21: 1311–1321, https://doi.org/10.1007/s10530-018-1901-2

Crane K, Cuthbert R, Cunningham M, Bradbeer SJ, Eagling L, Kregting L, Dick JTA, Dunn AM, Smith ERC, Shannon C, Caffrey JM, Lucy FE, Davis E, Coughlan NE (2020) Tomorrow Never Dies: biodegradation and subsequent viability of invasive macrophytes following exposure to aquatic disinfectants. *Management of Biological Invasions* 11: 26–43, https://doi.org/10.3391/mbi.2020.11.1.03

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

Cuthbert RN, Pattison Z, Taylor NG, Verbrugge L, Diagne C, Ahmed DA, Leroy B, Angulo E, Briski E, Capinha C, Catford JA, Dalu T, Essl F, Gozlan RE, Haubrock PJ, Kourantidou M, Kramer AM, Renault D, Wasserman RJ, Courchamp F (2021) Global economic costs of aquatic invasive alien species. *Science of The Total Environment* 775: 145238, https://doi.org/10.1016/j.scitotenv.2021.145238

Cuthbert RN, Diagne C, Haubrock PJ, Turbelin AJ, Courchamp F (2022) Are the "100 of the world's worst" invasive species also the costliest? *Biological Invasions* 24: 1895–1904, https://doi.org/10.1007/s10530-021-02568-7

DAISIE (2009) Handbook of Alien Species in Europe. Springer, Dordrecht, 399 pp, https://doi.org/10.1007/978-1-4020-8280-1

Dan TV, KrishnaRaj S, Saxena PK (2000) Metal tolerance of scented geranium (*Pelargonium* sp. "*Frensham*"): effects of cadmium and nickel on chlorophyll fluorescence kinetics. *International Journal of Phytoremediation* 2: 91–104, https://doi.org/10.1080/15226510008500032

Das DK (2006) Environmental impact of inter-basin water transfer projects: some evidence from Canada. *Economic and Political Weekly*: 1703–1707, https://doi.org/10.1007/s11356-016-6854-3

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81: 163–182, https://doi.org/10.1017/S1464793105006950

Dzierżyńska-Białończyk A, Jermacz Ł, Zielska J, Kobak J (2019) What scares a mussel? Changes in valve movement pattern as an immediate response of a byssate bivalve to biotic factors. *Hydrobiologia* 841: 65–77, https://doi.org/10.1007/s10750-019-04007-0

Fisher SW, Hwang H, Atanasoff M, Landrum PF (1999a) Lethal body residues for pentachlorophenol in zebra mussels (*Dreissena polymorpha*) under varying conditions of temperature and pH. *Ecotoxicology and Environmental Safety* 43: 274–283, https://doi.org/10.1006/eesa.1999.1789

Gallardo B, Aldridge DC (2018) Inter-basin water transfers and the expansion of aquatic invasive species. *Water Research* 143: 282–291, https://doi.org/10.1016/j.watres.2018.06.056

Gallardo B, Aldridge DC (2020) Priority setting for invasive species management by the water industry. *Water Research* 178: 115771, https://doi.org/10.1016/j.watres.2020.115771

Gasbarro F, Rizzi F, Frey M (2016) Adaptation measures of energy and utility companies to cope with water scarcity induced by climate change. *Business Strategy and the Environment* 25: 54–72, https://doi.org/10.1002/bse.1857

Gollasch S (2007) Marine vs freshwater invaders: is shipping the key vector for species introductions to Europe? In: Gherardi F (ed), Biological Invaders in Inland Waters: Profiles, Distribution and Threats. Springe, Dordrecht, pp 339–345, https://doi.org/10.1007/978-1-4020-6029-8_17

Gosling SN, Arnell NW (2016) A global assessment of the impact of climate change on water scarcity. *Climatic Change* 134: 371–385, https://doi.org/10.1007/s10584-013-0853-x

Hejda M, Pyšek P, Jarošík V (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology* 97: 393–403, https://doi.org/10.1111/j.1365-2745.2009.01480.x

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18, https://doi.org/10.1111/j.1365-2664.2008.01600.x

Hulme PE (2014) Invasive species challenge the global response to emerging diseases. *Trends in Parasitology* 30: 267–270, https://doi.org/10.1016/j.pt.2014.03.005

Hulme PE (2015) Invasion pathways at a crossroad: policy and research challenges for managing alien species introductions. *Journal of Applied Ecology* 52: 1418–1424, https://doi.org/10.1111/1365-2664.12470

Khadem M, Dawson RJ, Walsh CL (2021) The feasibility of inter-basin water transfers to manage climate risk in England. *Climate Risk Management* 33: 100322, https://doi.org/10.1016/j.crm.2021.100322

Leach J, Dawson F (1999) *Crassula helmsii* in the British Isles - An unwelcome invader. *British Wildlife* 10: 234–239

Ludwig F, van Slobbe E, Cofino W (2014) Climate change adaptation and Integrated Water Resource Management in the water sector. *Journal of Hydrology* 518: 235–242, https://doi.org/10.1016/j.jhydrol.2013.08.010

Martin M, Mackie G, Baker M (1993) Control of the biofouling mollusc, *Dreissena polymorpha* (Bivalvia: Dreissenidae), with sodium hypochlorite and with polyquaternary ammonia and benzothiazole compounds. *Archives of Environmental Contamination and Toxicology* 24: 381–388, https://doi.org/10.1007/BF01128738

Nentwig W, Bacher S, Kumschick S, Pyšek P, Vilà M (2018) More than “100 worst” alien species in Europe. *Biological Invasions* 20: 1611–1621, https://doi.org/10.1007/s10530-017-1651-6

Olden JD, Kennard MJ, Leprieur F, Tedesco PA, Winemiller KO, García-Berthou E (2010) Conservation biogeography of freshwater fishes: recent progress and future challenges. *Diversity and Distributions* 16: 496–513, https://doi.org/10.1111/j.1472-4642.2010.00655.x

Oreska M, Aldridge D (2011) Estimating the financial costs of freshwater invasive species in Great Britain: A standardized approach to invasive species costing. *Biological Invasions* 13: 305–319, https://doi.org/10.1007/s10530-010-9807-7

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288, https://doi.org/10.1016/j.ecolecon.2004.10.002

Rewicz T, Grabowski M, MacNeil C, Bącela-Spychalska K (2014) The profile of a “perfect”invader - the case of killer shrimp, *Dikerogammarus villosus*. *Aquatic Invasions* 9: 267–288, https://doi.org/10.3391/ai.2014.9.3.04

Ricciardi A (2001) Facilitative interactions amongst aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2513–2525, https://doi.org/10.1139/f01-178

Ricciardi A, MacIsaac HJ (2000) Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology & Evolution* 15: 62–65, https://doi.org/10.1016/S0169-5347(99)01745-0

Rogers S, Barnett J, Webber M, Finlayson B, Wang M (2016) Governmentality and the conduct of water: China’s South-North Water Transfer Project. *Transactions of the Institute of British Geographers* 41: 429–441, https://doi.org/10.1111/tran.12141

Rogers S, Chen D, Jiang H, Rutherfurd I, Wang M, Webber M, Crow-Miller B, Barnett J, Finlayson B, Jiang M, Shi C, Zhang W (2020) An integrated assessment of China’s South-North Water Transfer Project. *Geographical Research* 58: 49–63, https://doi.org/10.1111/1745-5871.12361

Roy HE, Tricarico E, Hassall R, Johns CA, Roy KA, Scalera R, Smith KG, Purse BV (2023) The role of invasive alien species in the emergence and spread of zoonoses. *Biological Invasions* 25: 1249–1264, https://doi.org/10.1007/s10530-022-02978-1

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

Simbberloff D (2021) Maintenance management and eradication of established aquatic invaders. *Hydrobiologia* 484: 2399–2420, https://doi.org/10.1007/s10750-020-04352-5

Shannon C, Quinn CH, Stebbing P, 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

Shikloanov IA (1993) World fresh water resources. In: Gleick PH (ed), Water in Crisis - A Guide to the World’s Fresh Water Resources. Oxford University Press, Oxford, pp 13–24

Shumilova O, Tockner K, Thieme M, Koska A, Zarfl C (2018) Global Water Transfer Megaprojects: A Potential Solution for the Water-Food-Energy Nexus? *Frontiers in Environmental Science* 6: 150, https://doi.org/10.3389/fenvs.2018.00150

Smith ERC, Heal R, Wood LE (2023) Understanding and improving biosecurity among recreational anglers in Great Britain. *Journal of Fish Biology* 102: 1177–1190, https://doi.org/10.1111/jfb.15345

Sternberg T (2016) Water megaprojects in deserts and drylands. *International Journal of Water Resources Development* 32: 301–320, https://doi.org/10.1080/07900627.2015.1012660

Stiers I, Crohain N, Josens G, Triest L (2011) Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds. *Biological Invasions* 13: 2715–2726, https://doi.org/10.1007/s10530-011-9942-9

Takeguchi W, Yates R (2012) Evaluating potential quagga mussel control measures in Colorado River water. *American Water Works Association* 104: E162–E172, https://doi.org/10.5942/jawwa.2012.104.0040

Van Benschoten JE, Jensen JN, Brady JT, Lewis DP, Sferrazza J, Neuhauser EF (1993) Response of zebra mussel veligers to chemical oxidants. *Water Research* 27: 575–582, https://doi.org/10.1016/0043-1354(93)90166-F

Verween A, Vincx M, Degraer S (2009) Comparative toxicity of chlorine and peracetic acid in the biofouling control of *Mytilopsis leucophaeata* and *Dreissena polymorpha embryos* (Mollusca, Bivalvia). *International Biodeterioration & Biodegradation* 63: 523–528, https://doi.org/10.1016/j.ibiod.2009.03.002

Waine A, Robertson P, Pattison Z (2023) Raw water transfers: why a global freshwater invasion pathway has been overlooked. *Hydrobiologia* 851: 1091–1094, https://doi.org/10.1007/s10750-023-05373-6

Williams F, Eschen R, Harris A, Djeddour D, Pratt C, Shaw RS, Varia S, Lamontagne-Godwin J, Thomas SE, Murphy ST (2010) The economic cost of invasive non-native species on Great Britain. CABI for The Scottish Government Department for Environment Food and Rural

Affairs UK, Government and Department for Economy and Transport Welsh Assembly Government, Wallingford, 198 pp

Yorkshire Water (2022) Annual impact and public value report our contribution to Yorkshire. London, UK, 53 pp

Zeng D, Hu D, Cheng J (2011) Preparation and study of a composite flocculant for papermaking wastewater treatment. *Journal of Environmental Protection* 2: 1370–1374, https://doi.org/10.4236/jep.2011.210159

Zhu K, Cheng Y, Zhou Q (2023) China's water diversion carries invasive species. *Science* 380: 1230–1230, https://doi.org/10.1126/science.adi6022

Web sites, online databases and software

GBNNSS (ND) Check Clean Dry. In: GBNNSS. https://www.nonnativespecies.org/what-can-i-do/check-clean-dry/ (accessed 17 January 2024)

Invasive Species Council of BC (ND) Play Clean Go Play Your Part. In: isc. https://bcinvasives.ca/play-your-part/play-clean-go/ (accessed 17 January 2024)

Ministries for Primary Industries Manatū Ahu Matua (2023) Check, Clean, Dry: preventing didymo and other pests. In: MPI. https://www.mpi.govt.nz/outdoor-activities/boating-and-watersports-tips-to-prevent-spread-of-pests/check-clean-dry (accessed 17 January 2024)

U.S. Fish & Wildlife Service (ND) Clean, Drain, Dry. In: U.S. Fish & Wildlife Service. https://www.fws.gov/story/clean-drain-dry (accessed 17 January 2024)

Supplementary material

The following supplementary material is available for this article:

Table S1. Model selection for *Dreissena polymorpha* mortality.
Table S2. Model selection for *Dikerogammarus villosus* mortality.
Table S3. Model selection for *Hydrocotyle ranunculoides* mortality.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Cole_etal_SupplementaryMaterial.pdf