

## Research Article

## Management of the invasive Nuttall's pondweed (*Elodea nuttallii*) in Lough Arrow, a Natura 2000 designated lake in Western Ireland

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### Abstract

A benthic geotextile was used to trial the management of an invasive aquatic macrophyte Nuttall's pondweed (*Elodea nuttallii* (Planch.) H. St. John, 1920) in Lough Arrow, a premier trout angling lake in the north-west of Ireland, designated as a Natura 2000 site (Special Area of Conservation (Annex I habitat, "Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.") and Special Protection Area for birdlife). The aim of this study was to manage *E. nuttallii*, a relatively recent arrival to this lake, while simultaneously promoting rehabilitation of native charophytes. The trial was carried out in Loughbrick bay, one of the lough's primary boating launch sites, an area determined as highly infested with *E. nuttallii*. Two experimental areas covering a total of 800 m<sup>2</sup> were treated by covering the invasive weed and substrate with jute textile, a single and double layer respectively. The trial was successful in controlling *E. nuttallii* for both single ( $P = 0$ ) and double layer treatments ( $P = 0.002$ ). The treatments applied resulted in a reduction of the mean percentage cover of the invasive species by > 60% for both treatments. Post-treatment mean percentage cover of *E. nuttallii* did not exceed 6% for either treatment. Furthermore, the mean percentage cover of indigenous charophyte flora present pre-treatment was not significantly impacted by the application of jute in either the single ( $P = 0.165$ ) or double treatment ( $P = 0.353$ ). For biosecurity purposes, the treatment areas were strategically positioned in close proximity to the bays pier and slipway. The treated transects were marked with navigational buoys to provide a corridor for boats entering and exiting the lake, which help to contain the invasive within the Natura 2000 site while reducing the spread risk *via* this pathway to other sites vulnerable to infestation.

**Key words:** macrophytes, jute matting, aquatic invasive species, biosecurity, conservation, EU Habitats Directive, charophytes

### Introduction

Invasive alien species are recognised as one of the leading causes of biodiversity loss worldwide, posing a significant threat to aquatic ecosystems (Maguire et al. 2011; Mollot et al. 2017). Aquatic invasive alien plant species (IAPS) are of particular concern due to their capacity to outcompete and displace native flora (Caffrey et al. 2011; Wang et al. 2016).

*Elodea nuttallii* (Planch.) H. St. John, 1920, an aquatic macrophyte native to North America, introduced to Europe in 1914 (Steen et al. 2019). It has

become the fourth most widespread alien aquatic plant in Europe, occurring in 20 countries causing serious problems in several (Hussner 2012). Contemporarily, the species is still spreading in Europe (Thiebaut et al. 2008) and the European Union has listed it as a “Species of Union Concern” under EU Regulation 1143/2014, due to its adverse impacts to native habitats (Zehnsdorf et al. 2015).

The species was first discovered in Ireland in 1984 (Josefsson 2011). The spread of *Elodea nuttallii* within Europe is likely due to both its reproductive mechanism and resistance to desiccation. *Elodea nuttallii* can reproduce asexually *via* fragmentation, which exacerbates local spread (Xie et al. 2010; Josefsson 2011). Furthermore, vegetative fragments can survive for extended periods in terrestrial environments, thus facilitating dispersal to new areas (Barrat-Segretain and Cellot 2007).

*Elodea nuttallii* commonly invades eutrophic, oligo or mesotrophic waters (Josefsson 2011). Once established, this plant has the capacity to form canopies at the water surface inhibiting the development of other aquatic flora (Barrat-Segretain et al. 2002), thus altering the ecosystem beneath its canopy (European Commission 2017).

In an Irish context, the ecological adaptations of this invasive species poses a significant threat to certain Natura 2000 sites under the European Union’s Habitats Directive (92/43/EEC). Most notably, the Annex I habitat “Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140)”.

One of the main features characterising this Annex I habitat is the presence of charophytes (O’Connor 2015), a benthic macroalgae community (Torn et al. 2010) that form dense perennial beds under favourable extrinsic conditions (Stewart and Church 1992; Kufel et al. 2016).

Charophytes thrive in the euphotic zone (Wang et al. 2008) and their distribution is influenced by species specific light requirements (Andrews et al. 1984; Kuster et al. 2004). Unfortunately, where light cannot penetrate the water column, their ecological niche is compromised (Lambert and Davy 2011). Due to this, charophytes are not immune to the negative impacts of canopy-forming invasive alien species (Caffrey et al. 2011) as they are prone to perishing where solar irradiance is inhibited (de Winton et al. 2004). This can potentially facilitate major ecological shifts in aquatic environments (Kelly and Hawes 2005). Notably, changes in the community composition of flora within the biotope (Hussner et al. 2017).

Ireland supports some of the best examples of the “Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140)” Annex I habitat typology, with a significant proportion of the total European habitat occurring in this geographical region. As a result, Ireland is considered a stronghold for this habitat in a European context, with a particular responsibility in ensuring its favourable conservation (National Parks and Wildlife Service 2019). Currently, this Annex I habitats conservation status is impaired,

with IAPS impacts constituting a significant component of the unfavourable conservation evaluation (National Parks and Wildlife Service 2019). Negative ecological impacts, such as those caused by IAPS, can potentially trigger an onus under the Habitats Directive, for member states to introduce “Conservation Measures” to “Restore” the “Favourable Conservation Condition” of an Annex I habitat.

*Elodea nuttallii* has been found to occur in this Annex I habitat throughout Ireland with significant infestations occurring at certain sites, such as Lough Arrow. Currently, there are no developed *in situ* management options for this invasive species that take into consideration the aforementioned Annex I habitats “Features of Interest”.

Invasive macrophytes are primarily managed in aquatic habitats through mechanical methods, as biological and chemical control options are generally restricted (Hoffmann et al. 2013). *Elodea nuttallii* has previously been successfully controlled using jute matting and it is considered a highly efficient method (Zehnsdorf et al. 2015). This is an environmentally friendly, biodegradable geotextile, which acts as a benthic barrier that excludes light inhibiting plant growth (Caffrey et al. 2010; Hoffmann et al. 2013). In addition to controlling invasive macrophytes, this geotextile has been shown to permit the regeneration of charophytes in areas where treatment occurs as they can grow through the small apertures in this benthic barrier (Caffrey et al. 2010, 2011).

This study aimed to trial jute matting as a potential *in situ* management option to control *E. nuttallii* and potentially facilitate the regeneration of *Chara* spp. in a designated Annex I habitat “Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140)”. The study also trialed multiple jute matting set ups *in situ* in order to ascertain which configuration was most suitable in suppressing the invasive while promoting the regeneration indigenous flora. In addition, the applicability of jute matting to aid in the maintenance or restoration of the conservation condition of this Annex I habitat was investigated.

## Materials and methods

### *Study site*

Treatment of *E. nuttallii* was conducted in Lough Arrow, a lake situated in the west of Ireland (54°03'36.7"N and 8°19'39.1"W). The lake area is 14.58 km<sup>2</sup> with an average depth of 9 metres (maximum 33 metres) and a secchi depth 3.9 metres (Environmental protection Agency 2020, internal report ) (National Parks and Wildlife Service 2015). A Natura 2000 site, it is both a designated Special Area of Conservation (SAC) under the EU Habitats Directive (92/43/EEC) (site code: 001673) and a Special Protection Area under the EU Birds Directive (2009/147/EC) (site code: 004050).

Lough Arrow's SAC designation is based on the presence of a representative example of the "Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140)" occurring within the lake (National Parks and Wildlife Service 2013). The current conservation condition of the lake is impaired with a classification of "Unfavourable-Poor" under Ireland's Article 17 assessment criteria (Roden and Murphy 2020). While it is not known when *E. nuttallii* was first recorded in the lake, it was not present when last surveyed for macrophytes in 2002 (Central Fisheries Board, internal report). Based on its current distribution in the lake, it was probably introduced about ten years before the current trials commenced.

Localised treatment of *E. nuttallii* with biodegradable jute matting was implemented in Loughbrick Bay (54°03'46"N and 8°18'18.16"W), on the eastern side of Lough Arrow. This bay has a pier and slipway, which is utilised for recreational boating. Due to the significant infestation of *E. nuttallii* and the intermittent but high intensity utilisation of the site for brown trout (*Salmo trutta*) angling, it was selected for *in situ* treatment trials.

#### *Pre-treatment survey*

Three 5 × 80 m long transects perpendicular to the shoreline in Loughbrick Bay were selected for the study. These transects extended through the depth zones where *E. nuttallii* was growing and buoys were utilised to mark the transects prior to surveying. All three transects were similar in terms of depth (max 2.4 metre), slope, benthic texture and macrophyte composition.

The pre-treatment survey (September 2018) utilised ten randomly selected 1 m<sup>2</sup> quadrats per transect. The percentage cover of both *E. nuttallii* and indigenous *Chara* spp. was visually estimated and recorded by divers. Additionally, each sampled quadrat was photographed utilising a GoPro (Hero 4+) for validating *in situ* estimates of percentage cover. Quadrats were placed on the lakebed by scuba divers at each sampling point and GPS positioning was recorded with a Garmin® GPSMAP® 64s. Depth was also recorded at using a Garmin® echoTM101.

#### *Treatment using jute matting*

Of the three transects used for the study, one acted as a control while the other two were utilised for treatment. One treatment involved the application of a single layer of jute matting while the other used a double jute layer. The jute matting weighed 0.203 kg/m<sup>2</sup> for a single layer with an aperture size of 2 mm. The double layer of jute matting was created by tying two layers of matting together at their outer edges with wire (1.6 mm diameter, 16 gauge). Treated transects were positioned perpendicular to the shoreline at both the launch point and the entry point of the pier at Loughbrick Bay. The control transect was positioned adjacent to the treated transects perpendicular to the shoreline (Figure 1).



**Figure 1.** Lough Arrow, location, aerial map and treatment location (Loughbrick Bay). Photo credit: Darren Garland.



**Figure 2.** Jute mat application in Loughbrick Bay. Photo credit: Darren Garland.

The sections of jute for both the treatment transects were cut from a roll. Additionally, weights were used to anchor the jute to the substrate. 0.5 m<sup>2</sup> sections of jute were cut from a roll and each filled with 2 kilograms of locally sourced pea gravel. The gravel was secured in the jute envelope utilising tying wire. A 30 cm piece of wire was left exposed on each weight to enable divers to stitch weights to the matting *in situ* on the lakebed.

The jute sections were placed perpendicularly to the shoreline at the location of each treatment transect (Figure 1). A 5-metre section of decking



**Figure 3.** Aerial view of jute mat application in Loughbrick Bay. Photo credit: William Earle.

timber (150 × 50 mm) was wrapped into the first one metre of each jute section. Another 5 metre section of scaffolding timber was secured to the wrapped section of scaffolding timber using carpentry screws. A hole was drilled at the ends of the 5 metre sections of scaffolding timber and rope was tied to each end. This rope was then tied to the stern of an angling boat and the jute was dragged from the shoreline into place (Figure 3). The jute sections were then allowed to saturate and sink to the lake bed. The scaffolding timber was then removed.

Divers ensured the jute was correctly placed and stitched the anchoring weights to the jute at intervals of 5 metres. In the double jute treatment divers stitched both sections of jute together with tying wire as the jute was being drawn from the shoreline.

#### *Post treatment survey*

A post-treatment survey of the site was conducted 13 months after the initial treatments took place (October 2019).

The post-treatment survey recorded the percentage cover of *E. nuttallii* and indigenous *Chara* spp. in ten randomly selected 1 m<sup>2</sup> quadrats per transect. Percentage cover estimates were recorded visually by divers. Quadrats were positioned on the lakebed with both the depth (Garmin® echoTM101) and GPS location (Garmin® GPSMAP® 64s) recorded for each sampling point. Additionally, each sampled quadrat was photographed utilising a GoPro (Hero 4+) for validating *in situ* estimates of percentage cover.

#### *Data treatment and analysis*

Statistical analysis was conducted using IBM SPSS 26 statistical software. Data collected from each treatment was tested for homoscedasticity and normality to meet the assumptions of the tests applied. A value of  $\alpha = 0.05$  was used to denote significance for all tests.

**Table 1.** Mean % cover ( $\pm$  SE) of *E. nuttallii* for control, single, and double treatments both pre- (September 2018) and post- (October 2019) treatment.

Treatment	Pre-treatment	Post-treatment	<i>p</i> -value (* < 0.05)
Control	60.0 $\pm$ 10.9	63.5 $\pm$ 9.2	1
Single	68.0 $\pm$ 12.9	6.0 $\pm$ 1.9	< 0.001*
Double	64.0 $\pm$ 13.1	3.8 $\pm$ 2.9	0.002*

Analysis of the percentage cover data collected utilised a Mann Whitney U test to compare individual treatments effectiveness in both controlling *E. nuttallii* and facilitating *Chara* spp. regeneration. Additionally, the significant differences between the control, single- and double-layer jute matting treatments were determined through the utilisation of Kruskal Wallis tests for both *Chara* spp. and *E. nuttallii* pre- and post-treatment. Post hoc Mann-Whitney pairwise multiple comparisons adjusted by Bonferroni correction were utilised to determine differences between each individual treatment pair, where a significant difference was observed.

## Results

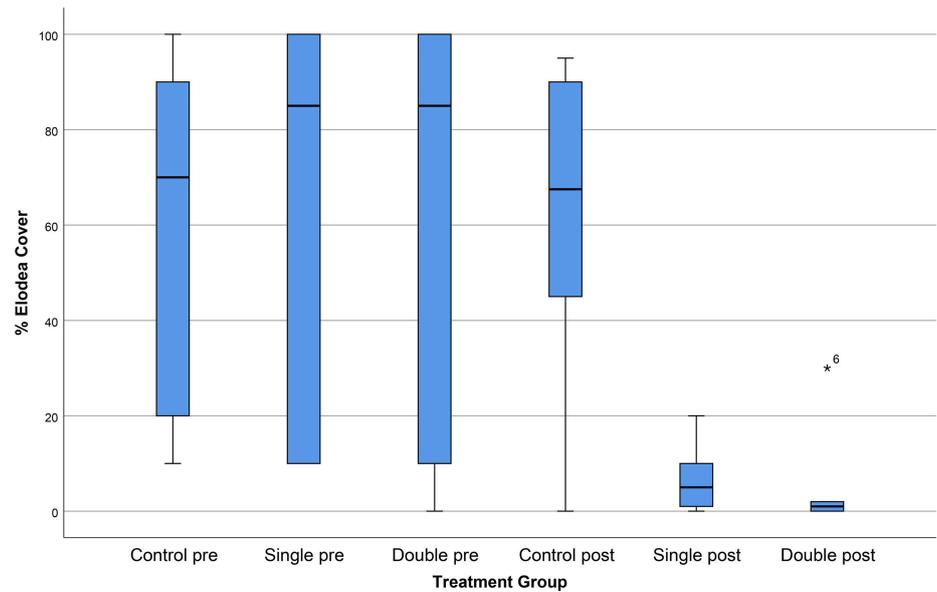
Analysis of the percentage cover of *E. nuttallii* showed no significant difference in the control transect between pre- and post-treatment ( $P = 1$ ). The treated transects, however, both showed a significant difference in *E. nuttallii* percentage cover between pre- and post-treatment. The mean percentage cover from the single jute layer treatment fell significantly from 68% to 6% pre- and post-treatment ( $P = < 0.001$ ), while that for the double layer was significantly reduced, from 64% and 3.8% ( $P = 0.002$ ) (Table 1).

The cross comparison of each individual treatment for *E. nuttallii* cover determined there was no significant difference between the control and both jute transects pre-treatment ( $P = 0.654$ ). Post-treatment analysis determined the control was significantly different from both the single and double layer of jute ( $P = < 0.001$ ), while the double and the single layer treatments showed no significant difference in *E. nuttallii* cover post-treatment ( $P = 0.603$ ) (Figure 4, Table 2).

*Chara* spp. percentage cover was not significantly different for the control, single and double jute matting transects between pre- and post-treatment, with  $P$  values of 0.912, 0.165 and 0.353, respectively (Figure 5) (Table 3). Furthermore, no significant difference was observed between the control, single and double jute matting treatment comparison in both the pre ( $P = 0.394$ ) and post ( $P = 0.219$ ) treatment surveys.

## Discussion

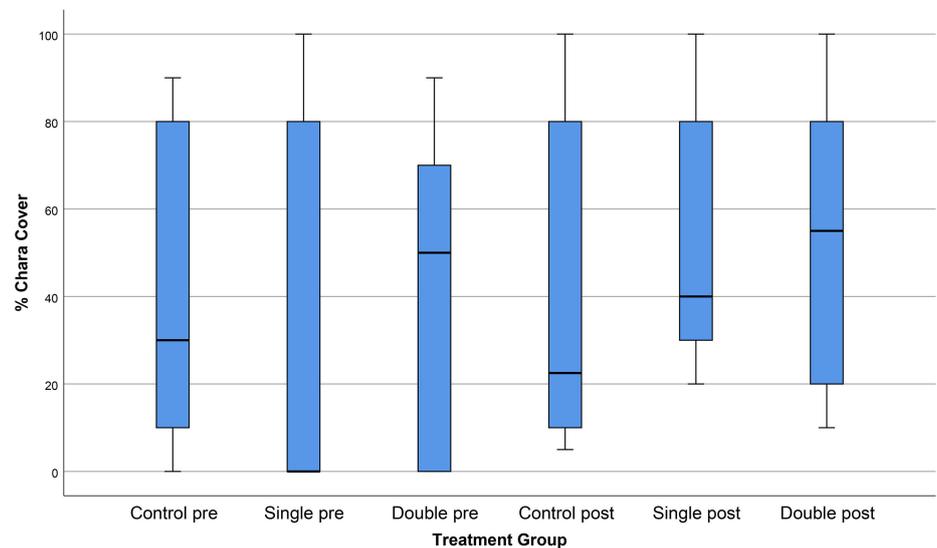
Natura 2000 sites represent some of Europe's most important natural areas. The overarching objective of this ecological network is to protect Europe's vulnerable habitats and species (Guerra et al. 2018). However, invasive alien plant species are of increasing concern as they are a frequently recognised pressure on ecosystems in Europe with the potential to impair



**Figure 4.** Boxplot of *E. nuttallii* % cover from control, single and double jute matting treatments pre and post application.

**Table 2.** Comparison of *E. nuttallii* % cover across the different post-treatment groups. P values (\* < 0.05) were obtained by Mann-Whitney pairwise multiple comparisons test, adjusted by Bonferroni correction.

Groups	Post-Treatment		
	Control	Single	Double
Control	–	0.037*	< 0.001*
Single	0.037*	–	0.603



**Figure 5.** Boxplot of *Chara* spp. % cover from control, single and double jute matting treatments pre and post application.

**Table 3.** Mean % cover ( $\pm$  SE) of *Chara* spp. for control, single, and double treatments both pre- (September 2018) and post- (October 2019) treatment.

Treatment	Pre-treatment	Post-treatment	p-value (* < 0.05)
Control	39.0 $\pm$ 10.8	40.5 $\pm$ 12.4	0.912
Single	35.0 $\pm$ 14.4	55.0 $\pm$ 10.0	0.165
Double	41.0 $\pm$ 11.8	54.5 $\pm$ 10.1	0.353

the conservation status of Annex I habitats (Dimitrakopoulos et al. 2017; Perzanowska et al. 2019). Furthermore, they have been noted to be impacting the designated Annex I habitat utilised in this study at a national level (National Parks and Wildlife Service 2019).

Lough Arrow's conservation condition is impaired due to a number of pressures impacting its features of interest which include IAPS, primarily *E. nuttallii* (Roden and Murphy 2020). Due to the obligations of the Habitats Directive, member states are required to adopt and implement conservation measures to ensure the favourable conservation status of Natura 2000 sites. In the case of *E. nuttallii*, it is also listed in the IAS list of Union concern and as such, EU member states are obliged to prevent its spread (Steen et al. 2019).

This trial effectively suppressed the growth of *E. nuttallii* with both treatments providing similar and significant control, with negligible differences between single and double layer treatments. This indicates that a single layer of geotextile can suppress *E. nuttallii*. The results of this trial present a *circa.* 60% reduction in mean *E. nuttallii* cover for both treatments, similar to those observed in a study conducted by Hoffmann et al. (2013) (50%–75% reduction). Hoffmann et al. (2013) utilised a heavier textile (weight – 0.3 kg/m<sup>2</sup>, mesh size – 0.5 mm) for field trials, while the current treatment successfully controlled *E. nuttallii* with a lighter textile (weight – 0.203 kg/m<sup>2</sup>, mesh size – 2 mm).

Hoffmann et al. (2013) conducted trials to compare 2, 1 and 0.5 mm aperture sizes and found the heavier jute with a smaller aperture to be more effective in controlling the IAPS in a laboratory setting. The laboratory study formed the basis for the selection of textile composition for *in situ* trials. In comparison, the lighter geotextile with a larger aperture size utilised in this study successfully suppressed *E. nuttallii*, furthermore, it suppressed the IAPS for a duration similar to that observed by Hoffmann et al. (2013). The post-treatment surveys were conducted 13 months after the application of the benthic geotextile and the matting was still intact, with greatly reduced cover of *E. nuttallii*. Hoffmann et al. (2013) observed intact textile suppressing *E. nuttallii* 15 months after the placement in one of their treatment sites. Due to the similar *in situ* effects of both geotextiles, the implementation of lighter jute would reduce the costs incurred in implementing control measures utilising jute matting. In addition, a lighter geotextile is easier to prepare, transport and manoeuvre in the water column by personnel carrying out *in situ* control measures.

Charophytes, the feature of interest for the Annex I habitat where *in situ* trials occurred, are considered pioneers, as their oospore bank facilitates their prompt recolonisation of rehabilitated habitats (Harwell and Havens 2003; Schneider et al. 2015). Despite this trait of the taxonomic group, in conjunction with the trials overall success in controlling *E. nuttallii*, a significant increase in *Chara* spp. cover was not observed in the post-

treatment surveys in comparison to pre-treatment surveys. Furthermore, neither treatment differed in relation to *Chara* spp. regeneration. However, *Chara* spp. were observed growing through both the single and double layers of jute and their cover was not negatively impacted due to the application of jute.

In comparison to other studies, Hoffmann et al. (2013) observed the regeneration of indigenous *Chara* spp. after the suppression of *E. nuttallii* in a German lake. Caffrey et al. (2010) also noted the regeneration capacity of *Chara* spp. under jute matting on Lough Corrib, a lake situated in the same geographical region as L. Arrow. Caffrey et al. (2010) observed significant regeneration in certain transects where *Chara* spp. cover exceeded 70% post-treatment. However, in some treated areas no *Chara* spp. cover was observed post-application. It must be noted that there was no *Chara* spp. cover data collected prior to application by Caffrey et al. (2010), so areas where regeneration may not have occurred may be due to other factors instead of the impacts of an invasive macrophyte. As this study utilised a geotextile with the same specifications as applied by Caffrey et al. (2010), it can be assumed that *Chara* spp. regeneration is not restricted by the geotextile but rather facilitated due to its suppression of IAPS. Invasive species are one of a number of factors negatively impacting this Annex I habitat, with water quality often cited as a main driver for degradation (National Parks and Wildlife Service 2019). A condition assessment of L. Arrow conducted by Roden and Murphy (2020) suggested water quality in conjunction with other factors such as IAPS may be impairing the ecological condition. This indicates that other extrinsic factors may explain why there was not a significant increase in *Chara* spp. cover in post-treatment surveys but rather a recolonisation of similar cover levels observed prior to treatment. Furthermore, the significant infestation of *E. nuttallii* prior to treatment is indicative of habitat degradation as these species are known for their ability to prosper in disturbed habitats (Dimitrakopoulos et al. 2017).

Multiple management methods can be implemented to control aquatic plants. However, the approach taken to control an invasive plant should be appropriate for the infestation occurring and the respective water body where the problem occurs (Thiebaut et al. 2008). Jute matting provides an ideal control measure for *E. nuttallii* in the Annex I habitat “Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140)”. This study observed no impacts on indigenous flora of conservation importance in this SAC while a significant reduction in *E. nuttallii* was achieved. Other methods commonly applied to control *E. nuttallii* such as herbicides, sediment dredging, cutting and water level drawdown are generally “not species specific” and they all have their ecological disadvantages (Zehnsdorf et al. 2015), some of which may negatively impact the Annex I habitats features of interest. Furthermore, the geotextile utilised in this trial is considered a cost-effective control measure in comparison to other control options available for invasive macrophytes (Caffrey et al. 2010). However,

cost effectiveness is dependent on scale and resources. *Elodea nuttallii* control has been noted to be a costly exercise, primarily due to their capacity to heavily infest vast waterbodies. Thus, the prevention of its colonisation to new areas is considered paramount (Steen et al. 2019).

Ordinarily, containment measures have been implemented to control IAS from spreading to new areas (Hussner et al. 2017). Anthropogenic activities such as boating have been highlighted to aid the spread of IAPS, both within an infested site and between waterbodies via fragments created and/or subsequently attached to boats (Cole et al. 2018; Steen et al. 2019). This recreational activity has been cited as an important dispersal pathway for *E. nuttallii* (Josefsson 2011) primarily due to its ease of dispersal and its capacity to form canopies at the water surface where interactions with boats are highly likely.

Due to the success of the trials and the strategic placement of the control measures, navigational markers were placed either side of the treated transects. This provided a pathway for boats entering and exiting the lake which potentially mitigates the spread of *E. nuttallii in situ* and minimises the potential spread of this invasive to new sites. The success of these containment measures indicates that jute matting could potentially be applied to other SACs with infestations of *E. nuttallii* as a containment measure where full lake control is not a feasible option. Furthermore, where intensive control is applied at a site wide level, jute matting is an effective control option which does not negatively impact this Annex I habitat types features of interest and is cost effective in comparison to other weed management methods.

The success of this trial in controlling *E. nuttallii* and insignificantly altering *Chara* spp. cover warrants further research to fully understand the dynamics of *Chara* spp. regeneration capacity and *E. nuttallii* suppression under different jute matting configurations.

This trial did not take into consideration the *Chara* spp. cover in treatment transects prior to the initial introduction of *E. nuttallii*. Furthermore, this study did not fully delineate other factors determining *Chara* spp. prevalence in the biotope, in particular water quality.

Further studies which utilise infested sites where there is historical data on *Chara* spp. cover and where water quality parameters have not changed significantly in the interim since establishment of the invasive would be beneficial in trialling further jute matting configurations. This could help to determine which type of jute matting provides the most cost-effective and manageable balance between suppression of the invasive and regeneration of indigenous *Chara* spp. If delineated, this control mechanism may prove to be a crucial management tool in this Annex I habitats restoration where conservation condition impairment has occurred due to *E. nuttallii* infestation. This research can be used as an IAPS management case study to provide both scientific and financial information for risk assessments of existing and proposed invasive alien freshwater aquatic plants of EU concern.

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## Authors' contribution

The research was conceptualised by CERIS, IT Sligo (Frances Lucy, Sara Meehan and Darren Garland) who obtained funding for the purposes of this research study. The experiment was designed by INVAS biosecurity (Joe Caffrey and William Earle) and IT Sligo (Sara Meehan, Frances Lucy and Darren Garland). Experimental set up was carried out by IT Sligo (Darren Garland) and INVAS biosecurity (William Earle and Joe Caffrey) who also conducted *in situ* data collection. Data analysis and interpretation was carried out by IT Sligo (Darren Garland and Cian Taylor). Figures for the purposes of this manuscript were obtained by INVAS Biosecurity and IT Sligo (William Earle, Darren Garland and Cian Taylor). The original draft manuscript was prepared by Darren Garland and William Earle. Reviewing authors included Sara Meehan, Joe Caffrey, Cian Taylor, Nicolas Touzet, Frances Lucy. Further editing was conducted by William Earle, Darren Garland, Frances Lucy and Joe Caffrey. All authors contributed to Final submission.

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