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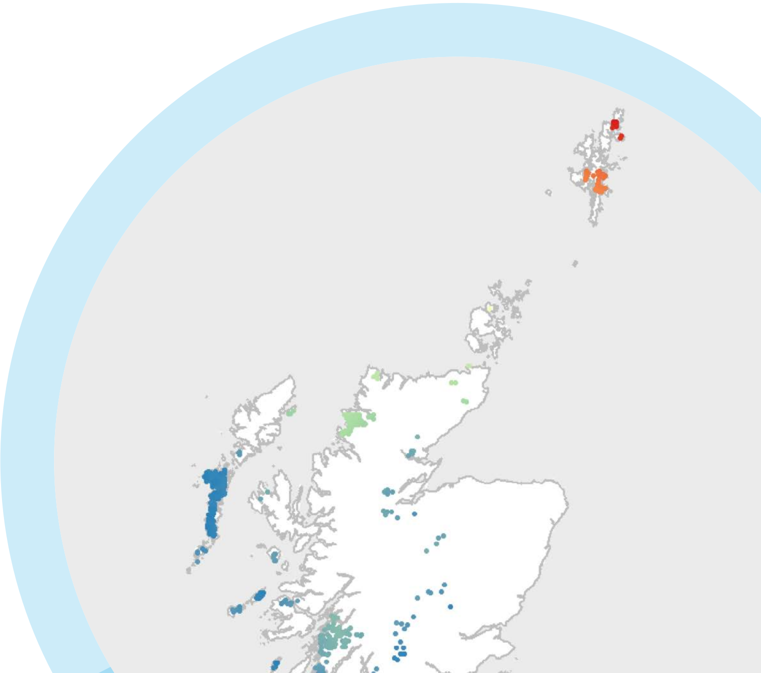
Slender Naiad (*Najas flexilis*) Habitat Quality Assessment – Site Prioritisation



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Slender Naiad (*Najas flexilis*) Habitat Quality Assessment – Site Prioritisation

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Executive Summary

Background

The Slender Naiad (*Najas flexilis* (Willd.) Rostk. & Schmidt) is a submerged rooted aquatic plant (macrophyte) occurring in lakes. It is a Red Data Book species and is listed in Annexes II and IV of the EC Habitats Directive 92/43/EEC. There are five Special Areas of Conservation where it is a qualifying interest. It is listed in Appendix 1 of the Bern Convention. It is also protected under domestic legislation, being listed in Schedule 8 of The Wildlife and Countryside Act, 1981, and Schedule 4 of The Conservation (Natural Habitats & c.) Regulations. It is a UK BAP species and is on the Scottish Biodiversity List. All of the UK sites are in Scotland, where it is known locally as "Aibhneag" in Gaelic. It is considered extinct in Esthwaite Water, its only English locality, but is found in sites in the Republic of Ireland. The species is believed to be under increasing threat in its Scottish stronghold, particularly in its mainland sites. In the first phase of this project, Gunn and Carvalho (2020) reviewed the existing knowledge and available information on the habitat requirements of *N. flexilis* from Scotland and other countries where the species is native. This review identified what data are already available, where they are, and how to access them. On the basis of the results of this review, Gunn and Carvalho (2020) made recommendations for an analysis of the habitat suitability of Scottish lochs, in order to identify potential sites where *N. flexilis* may be present but remains undiscovered, or where it could be suitable habitat for a species re-introduction programme.

Research questions

This report is the result of the second phase of the Slender Naiad (*Najas flexilis*) habitat quality assessment project. It arises directly from the recommendations of the first phase of the project (Gunn and Carvalho, 2020) to identify suitable lochs in Scotland for further investigation of the species' presence and to highlight potential sites for re-introduction. This second phase of the project specifically addressed the following objectives:

- To develop and apply a methodology to identify Scottish lochs, where *N. flexilis* is currently unrecorded, which provide suitable habitats for the species;
- To develop a prioritisation protocol and apply it to produce a ranked list of the most suitable sites for further field investigations as undiscovered or re-introduction sites for *N. flexilis*. Suitable sites on mainland Scotland, where populations are most threatened, should be prioritised.

Research undertaken

This research was undertaken in four component parts:

- Identifying threshold environmental values for *N. flexilis* habitat suitability;
- Identifying habitat suitability for *N. flexilis* based on aquatic macrophyte species data;
- Applying environmental thresholds and habitat suitability scores to produce a list of suitable sites;
- Developing and applying a prioritisation protocol for ranking and short-listing potential *N. flexilis* sites for further conservation action.

Main findings

The analysis carried out in this project highlights that there is high potential for discovering further *N. flexilis* sites on mainland Scotland using the targeted approach, described below. This approach can also be used to identify suitable sites for a potential re-introduction programme.

- Identifying threshold environmental values for *N. flexilis* habitat suitability.

Alkalinity and nutrient data were collated from a number of measured or modelled environmental data sources identified in Phase 1 to be used to identify suitable environmental conditions for *N. flexilis.* Application of a combination of thresholds for alkalinity and nutrient concentrations produced a large list of potential suitable loch sites (4092 sites) across Scotland where *N. flexilis* may potentially either occur unrecorded, or, be suitable for its re-introduction.

- Identifying habitat suitability for *N. flexilis* based on aquatic macrophyte species data.

To provide more certainty in habitat suitability, macrophyte data at two scales, individual waterbody and 10 km^2 grid (hectad), were used to refine a more targeted short-list of suitable sites. This was carried out by evaluating a set of 80 "associated" macrophyte species that have previously been recorded at known *N. flexilis* sites. For each of these 80 species we calculated an "Indicator Value" score based on their occupancy and fidelity at sites with, and without, *N. flexilis*. For example, a species that was only ever found in association with *N. flexilis* and occupied 100% of known *N. flexilis* sites would be considered as 100% fidelity and 100% occupancy. Two other measures of habitat suitability, used by SEPA to assess ecological status for the Water Framework Directive, were combined with the Indicator Value score. The first was a measure of the ecological status of the lake based on the community of aquatic macrophytes present in relation to their tolerance to nutrient pollution (Lake Macrophyte Nutrient Index (LMNI). The second was a measure of macrophyte species richness, specifically the number of macrophyte species present used to calculate the LMNI Index score (LMNI scoring taxa).

- Applying habitat suitability scores to produce a short-list list of suitable sites.

Application of the highest rated macrophyte habitat suitability scores provided a short-list of 867 potentially suitable lochs. Further prioritisation of sites on the mainland and sites within 60 km of an existing *N. flexilis* sites produced a final short-list of 156 lochs as priority sites for further investigation.

- Developing and applying a prioritisation protocol for ranking potential *N. flexilis* sites for further conservation action.

A prioritisation protocol was applied to rank the 156 priority lochs with the highest suitability scores across the three macrophyte measures, and then sort these in relation to their proximity to existing *N. flexilis* sites. Of the top 20 ranked sites in the short-list, the majority occur in Argyll and Bute, Dumfries and Galloway and Stirlingshire. All are relatively close to known existing *N. flexilis* sites. These would, therefore, be strong candidates for reintroduction, if it were confirmed through site survey that they meet the environmental requirements of *N. flexilis.*

Recommendations

We make the following recommendations:

- The use of a combination of environmental and macrophyte data has been used to provide a robust short list of 156 lochs that have suitable habitat for *N. flexilis*;
- We recommend using the measures of macrophyte habitat suitability developed in this project, alongside proximity to existing *N. flexilis* sites to rank these 156 lochs for prioritising further conservation actions;
- Additional criteria, such as connectivity to other lochs or absence of invasive non-native species such as *Elodea* spp. in the area, could also be used to prioritise sites for further investigations;
- Further field investigations should be carried out at these lochs on the suitability of environmental conditions and to survey the current macrophyte community;
- It is quite possible that *N. flexilis* may already be present at some of these sites but may have been missed previously if the site was not surveyed sufficiently, or was previously surveyed too early in the seasons;
- Any site prioritised on the shortlist should first be surveyed to check for the presence of *N. flexilis*, including sampling loch surface sediments to check if seeds are present. It may also be worthwhile additionally using eDNA techniques in future surveys.

1 Introduction

1.1 Background and scope

The Slender Naiad (*Najas flexilis* (Willd.) Rostk. & Schmidt) is a small, annual, permanently submerged and rooted aquatic plant found in lakes. It rarely grows above 30 cm in height and, typically, occurs in clear-water, lowland lakes, often with base-rich substrates (Preston and Croft, 1997). In large lakes the species is usually found in lakes growing in sheltered bays and behind islands (Ruth Hall, *pers. comm*.). *N. flexilis* is an easily overlooked species that reproduces only from seeds, with seedlings appearing around June before dying back after September/October, once the mature plants have set seed. Population sizes of *N. flexilis* can fluctuate widely from year-to-year owing to annual variations in seed production and germination (Preston and Croft, 1997).

Najas flexilis is a Red Data Book species and is listed in Annexes II and IV of the EC Habitats Directive 92/43/EEC. There are five Special Areas of Conservation where it is a qualifying interest. It is listed on Appendix 1 of the Bern Convention. It is also protected under domestic legislation, being listed in Schedule 8 of The Wildlife and Countryside Act, 1981, and Schedule 4 of The Conservation (Natural Habitats & c.) Regulations. It is a UK BAP species and is on the Scottish Biodiversity List. In the UK, *N. flexilis* is considered extinct in Esthwaite Water, its only English locality (Maberly et al., 2011), and is now found exclusively in Scotland where it is believed to be under increasing threat, particularly on the mainland. In the Republic of Ireland, *N. flexilis* is considered to be extant in 52 loughs, mainly in coastal locations (Gunn and Carvalho, 2020). Scotland's special responsibility to protect *N. flexilis* was given fresh impetus by the most recent six-yearly Article 17 country submission to the European Commission (a requirement under the EU Habitats Directive) (JNCC 2019). Although this report indicated that the overall number of *N. flexilis* sites was currently relatively stable in Scotland, *N. flexilis* has not been found for some time in a number of previously occupied sites, notably on the mainland. Of the fourteen Scottish mainland loch sites, *N. flexilis* is thought likely to be still present in only six, although it has only been recorded in three of these sites in the last ten years (JNCC, 2019; Table 1; Nick Stewart, *pers. comm.*). Of these three mainland sites, the population of *N. flexilis* in Loch Kindar appears to be particularly vulnerable as it was only re-recorded in 2018 despite not being found there in other recent intensive targeted snorkel surveys (Inger et al., 2018, Nick Stewart, *pers. comm*.). *N. flexilis* has not been recorded in the last decade or so in the five lochs that comprise the Dunkeld Blairgowrie Special Area of Conservation (SAC), despite them all being the subject of recent intensive targeted snorkel surveys (JNCC, 2019; Mackenzie et al, 2018; Table 1). Recent surveys have also been unable to find *N. flexilis* in Fingask Loch and White Loch in Perthshire (Ewan Lawrie, *pers. comm*.) This contraction in the number of Scottish *N. flexilis* sites has been masked to some extent by recent new records from lochs located in the Hebrides (Figure 1). Therefore, given *N. flexilis*' special status in Scotland as an European Protected species (under Annex IV of the EU Habitats Directive), actions to improve the species' conservation status in Scotland have assumed a pressing importance.

In the first phase of this Slender Naiad (*Najas flexilis*) habitat quality assessment project, Gunn and Carvalho (2020) reviewed the existing knowledge and available information on the habitat requirements of *N. flexilis* from Scotland and other countries where the species is native (https://www.crew.ac.uk/publication/slender-naiad-najas-flexilis-habitat-quality-assessment).

This review identified what data are already available, where they are, and how to access them. On the basis of the results of this review, Gunn and Carvalho (2020) made recommendations for an analysis of the habitat suitability of Scottish lochs, in order to identify potential sites where

Table 1. Current status of *Najas flexilis* in Scottish mainland loch sites.

Mainland loch sites where *N. flexilis* is thought to be extant	Mainland loch sites where *N. flexilis* is not considered present
Lake of Menteith (Stirlingshire)	Fingask Loch (Perthshire)
Loch a' Bhada Dharaich (Lochaber)	Loch of Butterstone (Perthshire)
Loch Kindar (Dumfries and Galloway)*	Loch of Clunie (Perthshire)
Loch Monzievaird (Perthshire)	Loch of Craiglush (Perthshire)
Loch nan Gad (Kintyre)*	Loch Flemington (near Inverness)
Loch Tangy (Kintyre)*	Loch of Lowes (Perthshire)
	Marlee Loch (or Loch of Drumellie) (Perthshire)
	White Loch (Perthshire)

*sites where *N. flexilis* has been recorded since 2008

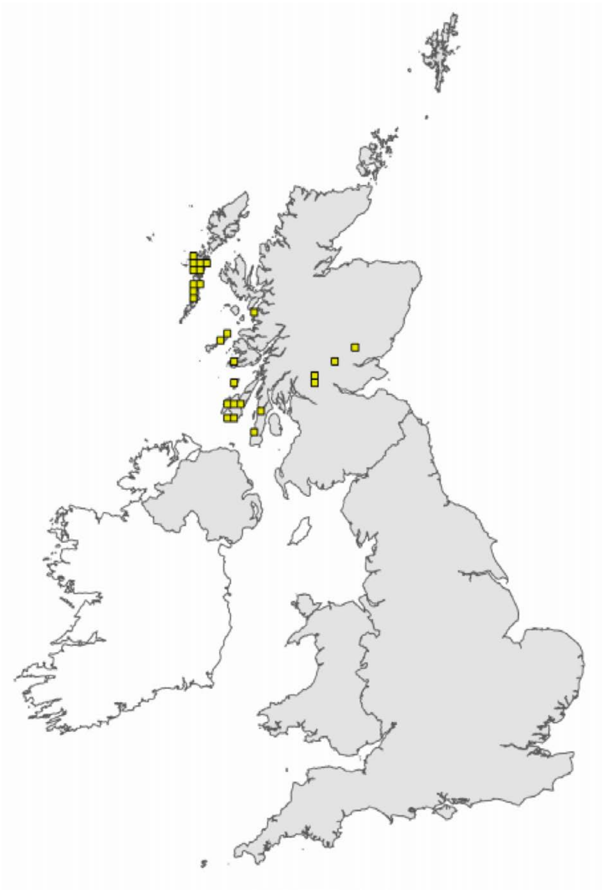

Figure 1. *Najas flexilis* distribution in the United Kingdom, showing hectads with recorded presence – presented as published (JNCC, 2019). [N.B. Loch Kindar, in Dumfries and Galloway, not shown on map].

N. flexilis may be present but remains undiscovered, or where it could be suitable for a species re-introduction programme.

Gunn and Carvalho (2020) identified a number of environmental factors that are key to the growth and reproduction and, ultimately, for sustaining healthy populations of *N. flexilis* in Scotland. Their literature review highlighted that *N. flexilis* is particularly sensitive to the threats of eutrophication (nutrient enrichment), competition with other plants and the mild acidification of circumneutral lakes[1], primarily because of its physiology as an obligate user of carbon dioxide (CO_2); *N. flexilis* plants being unable to metabolise bicarbonate for photosynthesis. This explains why *N. flexilis* is typically found in circumneutral lakes with a combination of impacts on seed production and carbon (C) - limitation of growth resulting in an ideal pH range from 6.5 to 8. Eutrophication also has the potential to lead to C-limitation of photosynthesis during daytime for obligate CO_2 users, such as *N. flexilis*, if pH levels rise above 8. Liming of agricultural land could also lead to alkalisation of loch waters, affecting CO_2 availability. The result of both eutrophication and alkalisation is a strong competitive advantage for aquatic plants that can use bicarbonate. This is especially true for plant species that can tolerate and survive the combination of low light and increased ratio of bicarbonate to CO_2, such as the invasive non-native *Elodea* species. Gunn and Carvalho (2020) also covered the limited measures that have been taken, up to now, to pre-empt, counter and subsequently restore habitats for the benefit of *N. flexilis* and the few examples of where *N. flexilis* species re-introduction programmes have been considered. Gunn and Carvalho (2020) also identified a number of large-scale datasets that could potentially provide information on some of these environmental criteria, listed above, to help evaluate the suitability of Scottish lochs as potential habitats to support *N. flexilis* populations.

Given that the restricted distribution of *N. flexilis* is based on this unusual combination of environmental factors, Gunn and Carvalho (2020) recommended that an analysis of the habitat suitability of Scottish lochs for *N. flexilis* should be carried out based on the optimal environmental conditions identified in Phase 1 of this project. However, given it would not be practical to carry out a detailed fieldwork study on the large number of lochs in Scotland to assess their suitability, it is necessary to identify, if possible, a much smaller group of priority lochs that could be practical for further study as potential conservation sites. Thus, the aim of this second phase of the project is to identify loch sites where *N. flexilis* is not currently known to be present but which might be suitable for further fieldwork study as potential recovery or introduction sites.

1.2 Project objectives

The overall aim of the project is to build upon the outputs of the Phase 1 of this project to match the parameters identified in the literature review and expert workshop against the environmental data from the sources listed by Gunn and Carvalho (2020). This will be used to identify loch sites and suggest methods for further investigation for the potential recovery or conservation introduction of *Najas flexilis* in mainland Scotland.

Specific project objectives are as follows:

1. Develop a methodology using the data sets identified by Gunn and Carvalho (2020) to identify a list of Scottish lochs, particularly those on the mainland, which potentially could provide suitable habitats for *N. flexilis,* where it is not currently known to be present;
2. Develop a prioritisation method and apply it to the list of potential sites to identify a short-list of the most suitable sites for further field investigations as recovery or introduction sites for *N. flexilis*;
3. Provide a summary of potential future more detailed work to confirm their suitability.

1 Circumneutral lakes have a pH that, typically, fluctuates around 7 (i.e. a neutral pH)

2 Methodology

2.1 Threshold environmental values for habitat suitability

The factors and data sources needed for evaluating habitat suitability were outlined in the Phase 1 report (Table 2, taken from Gunn and Carvalho, 2020). To carry out an analysis of the whole population of Scottish lochs, phytoplankton chlorophyll-*a* was excluded, as these data are only available for 1% of lochs. All the other determinands could be modelled, if measured data were unavailable.

Based on the literature review and the expert workshop in Phase 1, environmental thresholds were outlined for the key factors, which included free-CO_2 availability (alkalinity), total phosphorus (TP) and water colour (Table 3). These threshold values were reviewed by the Project Steering Group. Originally, we proposed to use Water Framework Directive (WFD) lake type-specific TP thresholds for the High/Good boundary and Good/Moderate boundary as the "Ideal" and "Range" thresholds for nutrients, but these were found to be too strict, excluding several existing *N. flexilis* sites. Instead, based on the data from existing *N. flexilis* sites, which indicate the species is often found where nutrient concentrations are raised a little, we raised the "Range" thresholds slightly to be 20 and 30 µg/L TP for medium and high alkalinity lake types, respectively.

2.2 Habitat suitability based on macrophyte community data

Another suitable indicator of suitable habitat for *N. flexilis* is using the presence or absence of macrophyte species. The Phase 1 report highlighted two potential indicators of suitable habitat:

1. The absence of competitive Invasive Non-Native Species (INNS), specifically *Elodea* spp. and *Crassula helmsii*, which the Phase 1 report identified as potentially strong competitors;

Table 2. Data needs and sources for assessing habitat suitability for *Najas flexilis*

Factor	Data Needs	Data source
pH, temperature and alkalinity (to calculate CO_2 availability)	Minimum monthly monitoring in summer Or use proxy data for CO_2 / bicarbonate availability	Scottish Environment Protection Agency (SEPA) UK Lakes Portal alkalinity estimates from soil and geology. See also Iversen et al. (2019) global bicarbonate map
Nutrients (phosphorus and nitrogen)	Minimum seasonal (quarterly) monitoring of total nitrogen (TN) and total phosphorus (TP) to estimate annual means Or use proxy estimates based on land-use models	SEPA UK Lakes Portal for all Scottish lochs SEPA/James Hutton Institute (JHI) for estimates using PLUS+ model
Phytoplankton chlorophyll-a (Chl-*a*)	Monthly monitoring April to October or satellite Earth Observation (EO)	SEPA, University of Stirling for satellite EO-derived Chl-*a* estimates
Presence of invasive plants (especially *Elodea* spp.)	Depth transects with objective frequency data	NatureScot (Common Standards Monitoring (CSM) and SEPA (Water Framework Directive (WFD) & Mesotrophic Loch Action Plan)
Presence of associated aquatic plant species *Isoetes lacustris* and *Potamogeton perfoliatus*	Aquatic plant species records for all surveyed Scottish lochs	Joint Nature Conservation Committee (JNCC) aquatic plant + Botanical Society of Botanical Society of Britain and Ireland (BSBI) databases

Table 3. Environmental thresholds used for assessing habitat suitability for *Najas flexilis*

Factor	Determinand	Ideal Value	Range	UK Lakes Proxy
Free-CO_2 availability	Alkalinity	120-600 µequiv./L or 6-30 mg/L $CaCO_3$	120-1400 µeqiv./L or 6-70 mg/L $CaCO_3$	Moderate Alkalinity (MA) lake type with 50-90% siliceous solid geology in catchment
Nutrients	TP as P (µg/L)	<8 (MAD), <=11 (MAS), <=15 (MAVS) <=30 (HA)	<=20 (MA) <=30 (HA)	Modelled TP from NUPHAR project (in UK Lakes database)
Water colour	Colour (mg Pt/L)	<=30	<=60	Clear, Humic or Unknown

2. The presence of species commonly associated in lochs where *N. flexilis* is known to be present. Specifically, based on suggestions from the expert workshop in Phase 1, we planned to use the presence of two indicator associated species, *Isoetes lacustris* and *Potamogeton perfoliatus,* where these two species are found growing together in the same loch.

The original approach was to apply these two indicators to all 1939 loch sites in Scotland, which had aquatic macrophyte survey data from NatureScot (Site Condition Monitoring and Scottish Lochs Survey data, and specific surveys carried out at *N. flexilis* sites). The approach was, however, also repeated on data available from UKCEH's Biological Records Centre, from the Botanical Society of the British Isles (BSBI), at the hectad (10 x 10 km grid) level. This allowed an assessment of the whole of Scotland, giving an indication of the habitat suitability of 7999 lochs. It is important to note that presence or absence at the hectad scale does not provide an indication of the suitability of any specific loch, but indicates the potential for any lochs within the same hectad. Where specific site survey data have been used, confidence is greatly increased.

The first indicator, the absence of INNS, had to be adapted at the hectad scale as it was found that *Elodea* spp. are very widely distributed across Scotland and this criterion would exclude most hectads where *N. flexilis* was currently recorded. For this reason, the presence of INNS in the hectad were not used, but their presence in the hectad is recorded for potential use in further prioritisation.

For the second indicator, the presence of commonly associated species, we refined this approach from the recommendation in Phase 1. Rather than just rely on the presence of two associated indicator species, we evaluated a broader set of 80 "associated" macrophyte species that have previously been recorded at known *N. flexilis* sites. For each of these 80 species we calculated an "Indicator Value" score based on their occupancy and fidelity at sites with, and without, *N. flexilis*. For example, a species that was only ever found in association with *N. flexilis* (i.e., complete fidelity) and also occurred at all sites where *N. flexilis* occurred (i.e., 100% occupancy of all known *N. flexilis* sites) would have a maximum Indicator Value score of 1. An indicator Value score of zero would reflect species that have never been recorded at a *N. flexilis* site (0% fidelity and 0% occupancy). This approach provides a much more robust assessment of the suitability of the habitat based on 80, rather than two, indicator species.

Two other measures of habitat suitability were combined with the "Indicator Value" score. The first was a measure of the ecological status of the lake based on the community of aquatic macrophytes present in relation to their tolerance to nutrient pollution (Lake Macrophyte Nutrient Index (LMNI). The second was a measure of macrophyte species richness, specifically the number of macrophyte species present used to calculate the LMNI Index score ("LMNI scoring taxa")) Full details for calculating "Habitat Suitability" using these three measures are provided in *Appendix 1: Analysis of macrophyte data to identify sites suitable for Najas flexilis*.

2.3 Prioritisation protocol for short-listing and ranking sites

To create a priority list of suitable sites, we first short-listed sites using the following criteria:

1. Environmental quality parameters outlined in Section 2.1;
2. Lochs that had a total habitat suitability score of 2.5 or 3 (see Section 2.2 and Appendix 1). Sites where this was based on a specific loch survey were prioritised over lochs where the habitat suitability was based on the BSBI hectad records, detailed in Section 2.2;
3. Sites on the mainland;
4. Sites relatively near existing *N. flexilis* sites. On this basis lochs at a distance >60km were filtered out due to their long-distance from existing *N. flexilis* sites. This distance was selected, as there is a big jump between suitable sites from 60 to 90 km away in the Highlands.

The resulting priority set of lochs were then sorted to give a priority ranking of sites, using the following sort rules:

- Sort level 1: Surveyed *N. flexilis* suitability score (3>2.5);
- Sort level 2: Hectad *N. flexilis* suitability scores (3>2.5);
- Sort level 3: Distance to nearest *N. flexilis* site (m) (shortest to longest).

Sites with no surveyed data were not omitted, as their specific suitability is unknown; the hectad suitability score could help guide potential site visits to assess for *N. flexilis* suitability.

A number of additional criteria, not used in the prioritisation process, were collated in the output dataset, for potential further use by NatureScot in selecting sites for further investigation. These criteria were those highlighted in the draft protocol, originally discussed with the Project Steering Group:

- Complexity of shoreline habitat;
- Shoreline perimeter– shoreline development index

(SDI), which indicates the length of shoreline habitat present, the greater the better;

- Number of islands; a further indicator of shoreline habitat;
- Connectivity of lochs – sites that are upstream or downstream of existing *N. flexilis* sites may be considered higher priority for investigation as natural dispersal between sites is easier, enhancing resilience of the populations. Additionally, sites with INNS present in upstream lochs may be given lower priority over more isolated loch sites, which are less exposed to the spread of invasive species;
- Absence of previous surveys. Previous macrophyte surveys, such as the Scottish Loch Survey, were thorough, so if the aim is to identify new sites for re-introduction it is better to prioritise sites with no previous survey.

3 Results

The following outputs detail the outcome of applying the habitat / loch suitability criteria to produce a list of 867 potential priority sites with a habitat suitable for *N. flexilis*. Further prioritisation based on mainland sites that were relatively near existing *N. flexilis* sites (criteria 3 and 4 outlined in Section 2.3) reduced the list down to 156 sites. These were ranked using the three sorting criteria and from this a 'Top 20' ranked table is presented in this report (Table 5). The output dataset for these 156 sites has all the information needed to further prioritise this list based on additional criteria (loch data, typologies, nutrients and biological records), and the processing code and all outputs are available at the following website: https://shiny-apps.ceh.ac.uk/slender_naiad/ [contact authors for access].

3.1 Habitat suitability

The number of lochs considered suitable based on alkalinity and nutrient thresholds is given in Table 4.

Taking the sites that are deemed to have a suitable habitat from Table 4, there are 4092 in the dataset, with 4056 not known to have *N. flexilis* present. These represent the number of sites after the first short-listing criteria in section 2.3 has been applied.

To apply the second criteria (habitat suitability based on macrophyte community), first only sites in hectads with IndVal/LMNI suitability >= 2.5 were selected, giving a dataset of 1151 (including 33 *N. flexilis* sites). Then this dataset was further filtered for sites with existing macrophyte surveys with IndVal/LMNI suitability >= 2.5 as well (or no data), resulting in a dataset of 895 (including 28 *N. flexilis* sites).

After removing known existing *N. flexilis* sites, the resultant filtered selection is a dataset of 867 potentially suitable loch sites. The distribution of these and their proximity to the nearest *N. flexilis* site is shown in Figure 2. This helps to identify geographic clusters where *N. flexilis* may be present, or sites suitable for re-introduction. It also shows geographic areas with clusters of potential sites, where survey effort could be reduced by targeted campaigns.

Table 4: Number of lochs considered suitable based on alkalinity and nutrient thresholds

Loch Type	Number of Lochs	Number of lochs where *N. flexilis* known to be present
All lochs	7999	54
Moderate Alkalinity lochs (MA)	1638	27
MA + Low Alkalinity lochs (LA) (alk> 120 μeqiv./L)	4315	32
LA + MA + High Alkalinity lochs (HA) (alk<1400 eqiv./L)	5411	45
LA + MA + HA sites meeting TP standards	4092	36

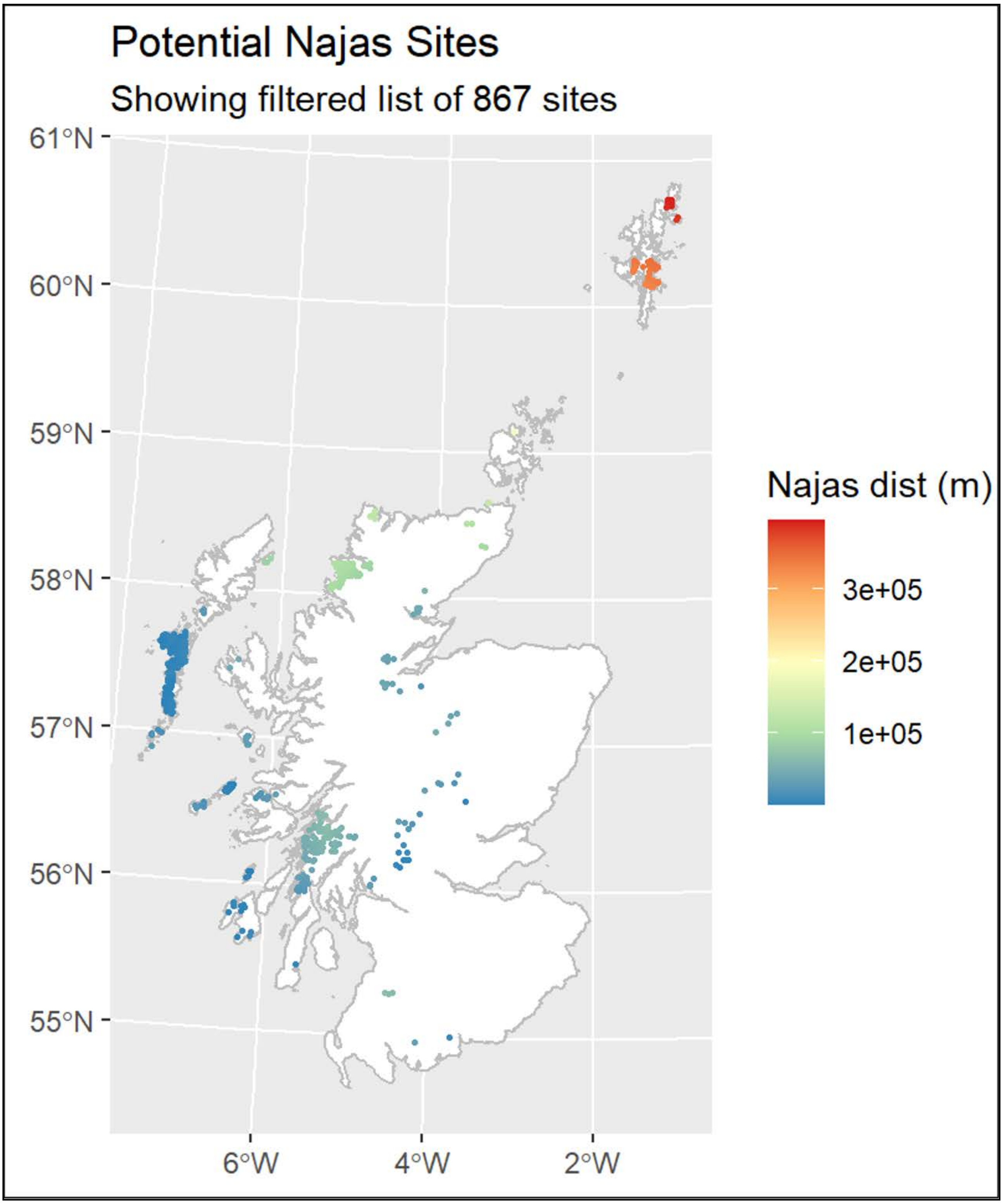

Figure 2 Map showing distribution of the 867 potentially suitable sites based on environmental and macrophyte suitability. Colour signifies distance to an existing *N. flexilis* site, with the distance scale ranging from 0m to 400km (4e+05m).

3.2 Priority list of sites

In order to better prioritise sites, the remaining short-listing criteria (3 and 4) from section 2.3 were applied to the dataset, reducing the 867 potential sites down to 156 sites. This list was sorted using the three specified sort levels and a top 20 list of sites was produced (Table 5). This is a first indicator of the lochs and locations that might be particularly suitable for N. *flexilis*, although the full set of 156 sites can be re-ordered on a number of other criteria to best suit the intention.

Of these top 20 ranked sites, all the identified Highland sites should be treated with a degree of caution as their selection is partly based on their proximity to Loch Flemington where *N. flexilis* has only been recorded paleoecologically, albeit with the record showing recent growth based on a core taken on 29/01/06 (Bennion et al., 2006). However, there are no known survey records of it growing at this site despite it being relatively intensively studied. All the other listed sites occur in Argyll and Bute, Dumfries and Galloway, Perthshire or Stirlingshire where *N. flexilis* is currently known to occur and would therefore be potential candidates for reintroduction if they can be shown to meet other requirements of *N. flexilis*. The seven Argyll listed sites are more promising as they are adjacent to existing *N. flexilis* sites at Loch Tangy and Loch nan Gad, have high site survey suitability scores and have low numbers of records of non-native invasive species of *Elodea nuttallii* and *Crassula helmsii* in their hectads. The two highlighted sites in Dumfries and Galloway, Loch Arthur and Lochenbreck Loch, also have high site survey suitability scores with Loch Arthur situated only c. 7 km to a known *N. flexilis* site at Loch Kindar, with no records of invasive species of *Elodea nuttallii* and *Crassula helmsii* in its hectad. The six listed Stirlingshire sites are very near to the only known *N. flexilis* site in the county, the Lake of Menteith, and all have high hectad suitability scores but have no site survey suitability scores. The only highlighted site in Perthshire is Loch Tay, which is relatively near the Dunkeld lochs where *N. flexilis* has historically been recorded. It is worth noting that all of the top ranked 20 sites have all got records of the non-native species *Elodea canadensis* in their hectads.

This final short list of 156 sites could be seen as strong candidates for *N. flexilis* reintroduction, but equally as a list of sites where *N. flexilis* may already be present but remains undetected. Therefore, we recommend that action should first be taken to establish whether *N. flexilis* is present, or not, in this shortlist of potential sites, before any consideration of transplanting. This is because *N. flexilis* is a hard plant to detect, especially under sub-optimal conditions or if previous surveys occurred too early in the season. We suggest that any identified potential site be surveyed in mid to late summer for growing populations of *N. flexilis* with complementary sampling of loch sediments in order to identify if *N. flexilis* seeds are present. Sites that formerly held *N. flexilis* may have been revealed, by the above analysis, to no longer be suitable for this species, given their current vegetation. This applies to a number of the Perthshire sites. In such cases, in the interests of long-term success, it would be better to target re-introduction at new and suitable sites than pre-existing but unsuitable sites. In this context, it is probably worth considering which potential sites have already been subject in the past to an in-depth macrophyte survey by professional botanists in the appropriate *N. flexilis* survey time window of July-end September, such as carried out by the Scottish Lochs Survey. Hence, if you are looking for new *N. flexilis* sites you should prioritise looking at sites, for instance, which had not been previously surveyed by the Scottish Loch Survey teams (R. Hall, *pers. comm*.).

The output .csv files include a number of additional fields for filtering or sorting further prioritisation steps if necessary. These fields are listed in Appendix 2.

Table 5: The Top 20 ranked sites following prioritisation protocol

Rank	WBID	NAME	UKCOUNTY	WB Latitude	WB Longitude	Distance to nearest Najas site	Elodea canadensis records in hectad	Elodea nuttallii records in hectad	Crassula helmsii records in hectad	Hectad suitability score - total	Site survey suitability score - total
1	20481	Loch Pityoulish	Highland	57.2006	-3.7885	39.5	42	NA	3	3	3
2	20742	Loch an Eilein	Highland	57.1469	-3.8242	44.5	36	NA	NA	3	3
3	28189	Loch Arthur	Dumfries and Galloway	55.0027	-3.7135	7.4	26	NA	NA	2.5	3
4	12995	Loch Migdale	Highland	57.8882	-4.3022	41.5	2	NA	NA	2.5	3
5	24770	Loch na Beiste	Argyll and Bute	56.1955	-5.5255	44.2	38	NA	2	2.5	3
6	24025	Loch Awe	Argyll and Bute	56.3077	-5.2265	47.7	30	12	1	2.5	3
7	24293	Dubh Loch	Argyll and Bute	56.3273	-5.5591	48.5	20	NA	2	2.5	3
8	23515	Loch Tay	Perth and Kinross	56.5067	-4.1644	21.0	8	NA	1	3	2.5
9	24509	Loch a Phearsain	Argyll and Bute	56.2682	-5.4657	50.3	24	NA	NA	3	2.5
10	25179	Loch Coille Bharr	Argyll and Bute	56.0522	-5.563	32.2	24	NA	NA	2.5	2.5
11	28271	Lochenbreck Loch	Dumfries and Galloway	54.9665	-4.1217	32.3	16	12	NA	2.5	2.5
12	12578	Loch an Lagain	Highland	57.9294	-4.2674	45.6	2	NA	NA	2.5	2.5
13	24852	Lochan Fearphorm	Argyll and Bute	56.176	-5.4886	45.8	38	NA	2	2.5	2.5
14	24399	Loch na Sreinge	Argyll and Bute	56.3002	-5.3528	58.0	12	NA	NA	2.5	2.5
15	24830	unnamed	Stirling	56.2076	-4.3036	3.6	104	2	1	3	NA
16	24820	Lochan Balloch	Stirling	56.2099	-4.2719	4.0	104	2	1	3	NA
17	24822	unnamed	Stirling	56.2094	-4.3253	4.3	104	2	1	3	NA
18	24965	unnamed	Stirling	56.1528	-4.3663	5.1	32	NA	NA	3	NA
19	24934	Lochan Spling	Stirling	56.173	-4.4129	7.2	104	2	1	3	NA
20	24623	Glen Finglas Reservoir	Stirling	56.2529	-4.3838	8.9	104	2	1	3	NA

4 Conclusions

A combination of measured or modelled environmental data produced a large list of potential suitable loch sites (4092 sites) across Scotland where *N. flexilis* may either occur unrecorded or be suitable for its re-introduction. To provide more certainty in habitat suitability, macrophyte data were used to refine a more targeted short-list of suitable sites. Development and application of "Indicator Value" scores for 80 macrophyte species associated with *N. flexilis* may provide the most robust measure of habitat suitability. These were combined with two other macrophyte measures to give a habitat suitability score. Screening of the long list of potential suitable sites, with sites that had high macrophyte habitat suitability scores, provided a final short-list of 867 potentially suitable lochs.

A prioritisation protocol was then applied to rank sites with the highest suitability scores across the three macrophyte measures, and target mainland sites relatively close (within 60 km) to existing *N. flexilis* sites. This reduced the short-list to 156 lochs which are considered the highest priority for further investigation. Additional criteria, such as connectivity to other lochs (especially upstream *N. flexilis* sites) and shoreline length and complexity could be used to refine this list further to provide a more manageable shortlist for further site investigations of the presence of *N. flexilis* and the suitability of environmental conditions for a re-introduction programme. In particular, we suggest that any identified potential site be surveyed for *N. flexilis* with complementary sampling of loch surface sediments in order to identify if *N. flexilis* seeds are present. Survey techniques using eDNA may also be possible in future.

The analysis highlights there is high potential for discovering further *N. flexilis* sites on mainland Scotland using this targeted approach. It can also be used to identify suitable sites for a re-introduction programme to help protect this rare and threatened species of European conservation importance.

5 Recommendations

We make the following recommendations:

- The use of a combination of measured or modelled environmental data can be used to provide a large list of potential suitable *N. flexilis* sites across Scotland;
- To provide more certainty in habitat suitability, existing macrophyte data should be used to refine a more targeted short-list of suitable *N. flexilis* sites in Scotland. This is based on the recognition that the existing macrophyte community of a site (or hectad) will integrate long- term information on nutrients, alkalinity and other unknown or unmeasured key factors for species associated with *N. flexilis*. We recommend use of Indicator Value scores of 80 macrophyte species associated with *N. flexilis* at the waterbody or hectad scale to produce *N. flexilis* suitability scores for lochs. We recommend using Indicator Value scores in combination with two other macrophyte measures used routinely by SEPA in their assessment of ecological status for the Water Framework Directive;
- Prioritise potential sites/hectads that are relatively close (within 60 km) to known existing *N. flexilis* sites, particularly those sites on the mainland (e.g., in Argyll) where *N. flexile* populations are most threatened;
- Additional criteria, such as connectivity or absence of invasive species in the area, could be used to provide a more targeted prioritised shortlist for further site investigations of the presence of *N. flexilis.*
- Further investigations should be carried out at these lochs on the suitability of environmental conditions and current macrophyte composition for planning a future re-introduction programme;
- As part of this, action should first be taken to establish whether *N. flexilis* is present or not in this shortlist of potential sites, before any consideration of transplanting. It is quite possible that the species may already be present but may have been missed previously if the site was not surveyed thoroughly, or if it was surveyed too early in the season in past surveys;
- As well as re-surveying for *N. flexilis*, complementary sampling of lake surface sediments should be undertaken in order to identify if *N. flexilis* seeds are present;
- Sites that formerly held *N. flexilis* may be revealed by this analysis as no longer being suitable for this species given their current vegetation. This applies to a number of the Perthshire sites. In such cases, in the interests of long-term success it would be better to target reintroduction at new and currently suitable sites than pre-existing but now unsuitable ones.

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Appendix 1: Analysis of macrophyte data to identify sites suitable for *Najas flexilis*

This approach provides a simple analysis of site suitability for *Najas flexilis* (Nf) based on characteristics of the aquatic vegetation. The basic assumption is that sites without Nf that otherwise share the floristic characteristics of sites with Nf would be potentially suitable for this plant. The basis of the analysis was to use an Nf master set of data (40 surveys, 32 unique WBIDs) and to then compare floristic attributes of those sites with other non Nf lochs in Scotland.

The survey data used here is taken 'as is' with the exception of correction of Nf records to *Nitella flexilis* at a handful of sites where there are no previous records of Nf and which are distant from other known locations for this species. The primary data source was the SNH Loch survey database, supplemented by more recent data from a small number of lochs when available. Note:

1. Some of these surveys are now very dated and in some cases it is 20-30 years since a site was last surveyed. Suitability, as defined below, may therefore have changed;
2. Some surveys are of sites that previously did or have subsequently been found to support Nf and these sites therefore appear both in the Nf master set and in the set of surveys where Nf was not recorded;
3. There are some multiple surveys of the same water body in different years, or different parts of the same water body in the same year that generate different levels of suitability for Nf, depending on the composition and richness of the species recorded. In such cases it would be reasonable to take the most optimistic view of that water body.

The suitability score draws on 3 components:

Najas_IndVal – this looks at the occupancy and fidelity of species in sites with and without Nf, using MA and HA lakes as a simple filter to exclude lake types that would not generally be considered suitable for Nf, to define an indicator value for each species. The IndVal approach is an accepted and widely used method for identifying species that are indicative of a particular group (in this case the grouping being based on the presence or absence of Nf). The IndVal score is calculated as:

IndVal (group 1) = occupancy of group 1 * fidelity for group 1

A species that was only ever found in association with Nf (i.e., complete fidelity for the *Najas flexilis* sites group), and also occurred at all sites where Nf occurred (i.e., occupancy of all those sites with *Najas flexilis*) would thus acquire a score of 1. In practice, of course, this scenario never arises in nature and species display varying levels of fidelity and occupancy.

We calculated IndVal values for all 80 macrophytes in the survey data set from *Najas flexilis* sites to indicate their association with *Najas* or non-*Najas* sites.

For ease, and to distinguish easily between association with Nf sites versus non-Nf sites, we then standardised these scores by subtracting the Nf group indicator score for each species from its non Nf group indicator score. This revealed that *Potamogeton perfoliatus*, *Lobelia dortmanna*, *Potamogeton gramineus*, *Potamogeton berchtoldii* and *Isoetes lacustris* were the strongest indicators of Nf sites. By contrast *Callitriche brutia var. hamulata* and *C. stagnalis* were weakly indicative of non-*Najas flexilis* sites.

A site score was then calculated for each sites, across all water body types, based on the average standardised IndVal values of the species present. High values for site scores indicate that a high share of the vegetation is associated with species with which Nf most characteristically co-occurs.

LMNI – this comes from the LEAFPACS WFD method for assessing ecological status of lakes based on aquatic vegetation. Lake Macrophyte Nutrient Index (LMNI) scores for each species are derived from empirical TP optima based on median TP values for the sites at which each species was recorded, the TP data being drawn from a UK-wide dataset. These values were subsequently rescaled to range from 1-10. The LMNI site score is recorded, as above, based on the average scores of the species present. High scores are associated with more fertile environments.

LMNI scoring taxa – this is the number of species that contribute to the LEAFPACS LMNI score. Using the number excludes the small number of inconsistently recorded taxa or those not recorded to a sufficiently high resolution but is otherwise v similar to a count of the total number of aquatic taxa recorded in a survey. Generally, it is advisable to include some measure of richness alongside compositional metrics to counteract the risk that extreme scores from compositional metrics can arise when only very few species are present.

Table A1 provides a list of the 80 macrophyte species present in *Najas flexilis* sites and their associated IndVal and LMNI scores.

One point to note here is that Nf tends to occur at sites that are significantly botanically richer than what is observed for MA and HA lakes more generally.

This could be a genuine environmental effect (e.g. if the requirements of multiple species are met then the requirements of Nf are also likely to be met, or, alternatively, a small degree of disturbance that enhances richness may also benefit Nf, or sites with a larger species pool have high connectivity, including increased density of dispersal vectors, that might also increase the chance of colonization by Nf). Conversely, it could be an artefact of the fact that sites with high richness were better surveyed (e.g., by more experienced botanists and/or under better survey conditions) and Nf was therefore more likely to be detected.

Nf suitability is based on a 7-point scoring system from 0 to 3, increasing at intervals of 0.5, as detailed below, based on summation of the three components described above.

For Najas_indVal and LMNI scoring taxa the higher the value the better. In these cases a site scores 1 if the value for that component is above the 25th percentile of the range of values observed at sites that contained Nf (0.16 for IndVal and 14 taxa for richness) and scores 0.5 if it lies above the 5th percentile (as an indication of the lower end of the range but discounting outliers; 0.14 for IndVal and 9 taxa for richness). If it falls below the 5th percentile it scores 0 (i.e., it lies out with the range of vegetation characteristics that Nf is associated with). In the case of LMNI, values that are too high are indicative of higher fertility than that at which Nf typically occurs, while values too low are indicative of fertility below which Nf typically occurs. Therefore, in this case a site scores 1 if the LMNI value is within the interquartile range of the values for that component in those sites that contained Nf (3.84 to 4.62), and 0.5 if it lies between the 5th and 95th percentiles (i.e., is basically within the observed range but not the core part of it; 3.41 to 5.02). A value of 0 is assigned if a site lies outside this range.

Based on this approach sites/surveys have been ranked from the most to least suitable based on summing the scores for these three components. Highly suitable sites (score of 3, i.e., scores of 1 for each of the three components) share all the attributes of sites known to contain Nf, in the sense that values of Najas_IndVal, LMNI and LMNI scoring taxa all fall within the core range of these components at those sites. Effectively, based on what we know about Nf sites, other than the fact that it is rare it would be unsurprising to find Nf growing in these additional lakes (indeed it could be present but undetected). There are 24 rows (23 unique WBIDs) covering such sites. These are predominantly MA lakes. These include sites within the core range of Nf, some of which have been found to contain Nf in other surveys or which lie very close to sites with current records of Nf. However, they also include lakes in areas such as Shetland and Highland that are distant from other Nf sites.

Sites scoring 2.5 provide an additional 87 rows (82 unique WBIDs). These fall outside the core range for one component but are otherwise highly suitable. Sites scoring 2, but with a minimum score of 0.5 for any one component, add a further 103 rows (100 unique WBIDs). Using a minimum score ensures that these sites always fall within the observed range for all three components of suitability.

In total across sites scoring 2 (min 0.5), 2.5 or 3 for Nf suitability there are 194 unique WBIDs (some sites appear under multiple different scores), of which 4 also appear in the Nf master set, confirming that they or part of the site held Nf at some other point over the recording period. This leaves 190 unique WBIDs where Nf has not been recorded (at least not within this dataset) but which are floristically suitable for Nf. For comparison, surveys with the same range of scores account for 68% of those in the Nf master set (40 rows). This suggests that using this range would be conservative enough to give a useful site shortlist but equally that across the Nf master set itself there are some sites of marginal suitability (and where Nf might be considered vulnerable).

Hectad method

As above for lochs, except the species were matched to the 10km BNG ('hectads') then all taxa were counted for each hectad for records post-1950, as well as a total count of unique taxa to give a species richness score. For each hectad, IndVal and LMNI scores were applied from a separate analysis (using surveyed flora in Scotland) to create suitability scores for *Najas flexilis*. These record counts and suitability scores were saved as .csv and .gpkg, then are used to create presence rasters (.tif) for each taxa group individually.

Table A 1. Macrophyte species present in *N. flexilis* sites and their associated IndVal and LMNI scores.

Species	IndVal Score	LMNI Score
Apium inundatum	0.189	4.32
Baldellia ranunculoides	0.302	3.97
Butomus umbellatus	-0.011	7.97
Callitriche brutia subsp. *hamulata*	-0.143	4.08
Callitriche hermaphroditica	0.139	8.08
Callitriche stagnalis s.s.	-0.106	6.38
Ceratophyllum demersum	-0.015	7.99
Chara aspera	0.301	4.19
Chara globularis	-0.033	6.86
Chara hispida	-0.016	3.95
Chara virgata	0.210	4.29
Chara virgata var. *annulata*	-0.028	4.07
Chara vulgaris	-0.030	5.56
Elatine hexandra	0.226	3.81
Elatine hydropiper	0.014	5.34
Eleocharis acicularis	0.076	8.68
Eleocharis multicaulis	0.086	3.03
Eleogiton fluitans	0.120	2.03
Elodea canadensis	-0.043	7.45
Elodea nuttallii	0.024	6.19
Fontinalis antipyretica	0.193	4.19
Fontinalis squamosa	0.015	3.09
Hippuris vulgaris	-0.039	5.23
Isoetes echinospora	0.097	2.47
Isoetes lacustris	0.315	2.22
Juncus bulbosus	0.236	2.42
Lemna minor	-0.004	8.52
Lemna trisulca	-0.053	7.96
Littorella uniflora	0.195	3.73
Lobelia dortmanna	0.373	2.16
Lythrum portula	0.054	4.31
Menyanthes trifoliata	0.083	5.17
Myriophyllum alterniflorum	0.247	2.66
Myriophyllum spicatum	0.095	6.23
Nitella flexilis agg.	0.101	5.19
Nitella opaca	0.056	2.36
Nitella translucens	0.304	2.73
Nuphar lutea	0.068	7.47
Nuphar pumila	0.030	4.82
Nuphar x spenneriana	-0.015	3.65
Nymphaea alba	0.078	6.84
Persicaria amphibia	0.087	8.25
Pilularia globulifera	0.015	3.59
Potamogeton alpinus	0.029	4.48
Potamogeton berchtoldii	0.330	6.58
Potamogeton crispus	-0.062	7.5
Potamogeton filiformis	0.046	3.68
Potamogeton friesii	-0.028	4.71
Potamogeton gramineus	0.348	2.85
Potamogeton lucens	0.002	4.37
Potamogeton natans	-0.007	4.71
Potamogeton obtusifolius	-0.013	6.97
Potamogeton pectinatus	0.205	7.19
Potamogeton perfoliatus	0.535	4.42
Potamogeton polygonifolius	-0.027	2.39
Potamogeton praelongus	0.079	3.92
Potamogeton pusillus	0.008	7.54
Potamogeton rutilus	0.134	5.49
Potamogeton x nitens	0.128	3.48
Potamogeton x zizii	0.017	4.04
Ranunculus (sub sect. *Batrachian*) sp.	-0.011	5.31
Ranunculus aquatilis var. *aquatilis*	0.057	5.81
Ranunculus aquatilis var. *diffusus*	-0.020	4.2
Ranunculus circinatus	-0.013	8.7
Ranunculus hederaceus	0.002	8.33
Ranunculus lingua	-0.028	6.79
*Ranunculus peltatus**	-0.045	6.485
*Scorpidium scorpioides***	0.007	N/A
Sparganium angustifolium	0.264	2.52
Sparganium emersum	0.084	6.06
Sparganium natans	-0.026	2.79
Subularia aquatica	0.022	1.8
Tolypella glomerata	-0.018	5.32
Utricularia australis	-0.023	2.87
Utricularia intermedia s. l.	-0.031	1.61
Utricularia minor	-0.029	2.36
Utricularia ochroleuca	-0.001	1.04
Utricularia stygia	0.022	1.3
Utricularia vulgaris	0.036	4.24
Zannichellia palustris	0.018	8.69

*IndVal is mean of subsp. *baudotii* and subsp. *peltatus*, although they have the same score. LMNI is mean of subsp. *baudotii* (6.48) and subsp. *peltatus* (6.49)

**Too rare for LMNI score

Appendix 2: Additional fields of potential use in further prioritisation

Parameter Name	Potential Use in Prioritisation
Rank	
WBID	
NAME	
UKCOUNTY	Regional representation
10km Grid	
WB Latitude	
WB Longitude	
WB Area (ha)	Larger lochs prioritised
WB Perimeter (Km)	Larger shorelines prioritised
Shoreline Development Index	Larger shorelines prioritised
Island Count	Complex shorelines prioritised
Altitude Type	Lowland prioritised
Depth Type	
Alkalinity Type	
Humic Type	
Lake area in catchment (%)	Connectivity for dispersal
Lake count per 100ha in catchment	Connectivity for dispersal
Distance to nearest *N. flexilis* site (km)	Already used in ranking
Elodea canadensis	Absence prioritised
Elodea nuttallii	Absence prioritised
Crassula helmsii	Absence prioritised
Elodea spp.	Absence prioritised
suitability_score_taxa	
suitability_score_indval	
suitability_score_lmni	
Hectad suitability score - total	Already used in ranking
surveyed_suitability_score_taxa	
surveyed_suitability_score_indval	
surveyed_suitability_score_lmni	
Site survey suitability score - total	Already used in ranking

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