CREW CENTRE OF EXPERTISE FOR WATERS

Slender Naiad (*Najas flexilis*) Habitat Quality Assessment

CENTRE OF EXPERTISE FOR WATERS

Slender Naiad (*Najas flexilis*) Habitat Quality Assessment

Iain D.M. Gunn & Laurence Carvalho, UK Centre for Ecology & Hydrology

Hydro Nation International Centre
The James Hutton Institute
Scottish Government
Riaghaltas na h-Alba
gov.scot

CENTRE OF EXPERTISE FOR WATERS

Published by CREW – Scotland's Centre of Expertise for Waters. CREW connects research and policy, delivering objective and robust research and expert opinion to support the development and implementation of water policy in Scotland. CREW is a partnership between the James Hutton Institute and all Scottish Higher Education Institutes and Research Institutes supported by MASTS. The Centre is funded by the Scottish Government.

Authors: Iain D.M. Gunn & Laurence Carvalho, UK Centre for Ecology & Hydrology

Project Manager: Sophie Beier, The James Hutton Institute

Please reference this report as follows: I. D. M. Gunn & L. Carvalho (2020). Slender Naiad (*Najas flexilis*) Habitat Quality Assessment. CRW2018_27. Scotland's Centre of Expertise for Waters (CREW).Available online at: crew.ac.uk/publications

ISBN: 978-0-902701-80-9

Dissemination status: Unrestricted

Acknowledgements: We thank the Project Steering Group (Sophie Beier (CREW - Project Manager), Alison Bell (SEPA), Ewan Lawrie (SNH) and Ruth Hall (Natural England)) for all their invaluable help and advice throughout this project. We are also very grateful for all the inputs and comments, which greatly improved this report, that we received from the following aquatic plant experts, from both the UK and overseas: Sue Bell (UK), Helen Bennion (UK), Izzy Bishop (UK), Seppo Hellsten (Finland), Marit Mjelde (Norway), Áine O'Connor (Ireland), Cilian Roden (Ireland), Carl Sayer (UK), Iain Sime (UK), Bryan Spears (UK), Nick Stewart (UK), Kate Waters-Hart (UK) and Nigel Willby (UK).

Cover photographs courtesy of: Iain Gunn, UKCEH

Contents

Executive Summary

Research questions

- To summarise the existing literature on the Slender Naiad (*Najas flexilis*), within both Scotland and the UK, as well as internationally. It specifically addressed the following questions:
    - o What are the habitat requirements for *N. flexilis*?
    - o What changes in environmental/habitat conditions have led to the decline of *N. flexilis*?
    - o What measures have been taken elsewhere in order to pre-empt, counter, or subsequently restore habitats for *N. flexilis*?
    - o What, if any, are the experiences with species reintroduction?
- To identify the data needed for the (future) assessment of the suitability of Scottish lochs as a habitat to support *N. flexilis* populations;
- To Identify what data are already available, where, and how to access it.

Key findings

- What are the habitat requirements for *N. flexilis*? What changes in environmental/habitat conditions have led to the decline of N. flexilis?

This assessment of the habitat requirements of *N. flexilis* identified a number of factors that are key to its growth and reproduction and, ultimately, for sustaining healthy populations in Scotland. The 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.

- What measures have been taken elsewhere in order to pre-empt, counter, or subsequently restore habitats for *N. flexilis*?

Very few measures have been taken, up to now, to pre-empt, counter and subsequently restore habitats for the benefit of *N. flexilis*. A few examples are described of where *N. flexilis* species re-introduction programmes have been considered, although none have been implemented.

- Data needs for site selection of habitat suitability, data availability and access

Data are listed on the factors that would help evaluate the suitability of Scottish loch sites as potential habitats to support *N. flexilis* populations. Possible data sources and data availability (mainly from the UK Lakes Portal, Scottish Environment Protection Agency (SEPA) and Scottish Natural Heritage (SNH)), are also indicated.

Background

N. flexilis is a rare aquatic plant species of European conservation importance. The species is believed to be under increasing threat in its Scottish stronghold. However, the factors that affect the health of *N. flexilis* populations in Scotland are not fully understood, such as, why does the species disappear, and where and why it fares well in some sites. In addition, more needs to be known about what actions can be taken to ensure that the habitat quality needed to support populations of *N. flexilis* is either maintained or restored. Thus, this project was set-up to review the existing knowledge and available information on the habitat requirements of *N. flexilis* from Scotland and other countries where the species is native. The aim is also to identify what data are already available, where they are, and how to access them. This is further outlined in the recommendations below.

Research undertaken

This project was undertaken in three component parts:

- International expert interviews
- Literature review
- Expert workshop

Recommendations

Based on the review conducted, and comments from the expert workshop, consideration should be given to an analysis of the habitat suitability of Scottish lochs, in

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

order to identify potential sites where *N. flexilis* is present, but as yet unrecorded, or where it could be suitable for a species re-introduction programme. As well as available aquatic plant and water quality data for sites from SEPA and SNH, national data sources, such as those available through the UK Lakes Portal, on geology, catchment soils and agricultural land-cover, will be needed to assess potential existing sites and suitable sites for restoration programmes. Further monitoring of the seasonal changes in free-CO_2 availability in current sites may be useful to identify current sites at risk. Similarly, detailed spatial surveys of pH and alkalinity within and outside existing patches of *N. flexilis* may help confirm whether localised hotspots within lochs are associated with CO_2-rich groundwater springs.

1 Introduction

1.1 Background and scope

Slender Naiad (*Najas flexilis* (Willd.) Rostik. & W.L.E. Schmidt) is a small, annual, permanently submerged, rooted aquatic plant. 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). It is commonly found in lakes growing beyond shoreweed (*Littorella uniflora* (L) Asch.) and lily zones, 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). *N. flexilis* is a rare and a globally declining aquatic plant species (Figure 1). Its core European range is in Ireland and Scotland, but it also occurs in a number of other countries across northern and central Europe, including Finland, Latvia, Norway, Russia and Sweden. However, it is now believed to be extinct in Germany, Poland (Galka et al., 2012) and Switzerland (European Commission, 2009). Its European extent is declining by ≤1% every year across its 44 recorded sites (European Commission, 2019). *N. flexilis* does occur more frequently in Canada and the USA (Preston and Croft, 1997; Figure 1) although these populations are much more genetically variable than Irish and Scottish populations, with '*N. flexilis*' an aggregate of several species types or races (Wingfield et al., 2004). Because of its European conservation importance, *N. flexilis* is protected as a Priority Species under both the EU Habitats Directive and the Bern Convention[2]. In addition, in the UK, *N. flexilis* is legally protected under Schedule 8 of the 1981 Wildlife and Countryside Act, has a UK Action Plan (BAP) for its preservation and of its habitat (HMSO, 1994), and is included on the Scottish Biodiversity List[3] .

In the UK, *N. flexilis* was previously found in Esthwaite Water, in the English Lake District (Maberly et al., 2011). It is now found exclusively in Scotland and is believed to be under increasing threat here. 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). 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 eight, although it has only been recorded in three of these sites in the last ten years (JNCC, 2019b; Table 1; Figure 2; Nick Stewart, *pes comm.*). Of these three mainland sites, the population of *N. flexilis* in Loch Kindar appears to be particularly vulnerable as

2 Annex II (i.e. species requiring the designation of Special Areas of Conservation) and IV (i.e. species needing strict protection) of the EU Habitats Directive (Council of the European Union, 1992) and Appendix I of the Bern Convention (Council of Europe, 1979).

3 https://www.nature.scot/scotlands-biodiversity/scottish-biodiversity-strategy/scottish-biodiversity-list; accessed 26/03/2020.

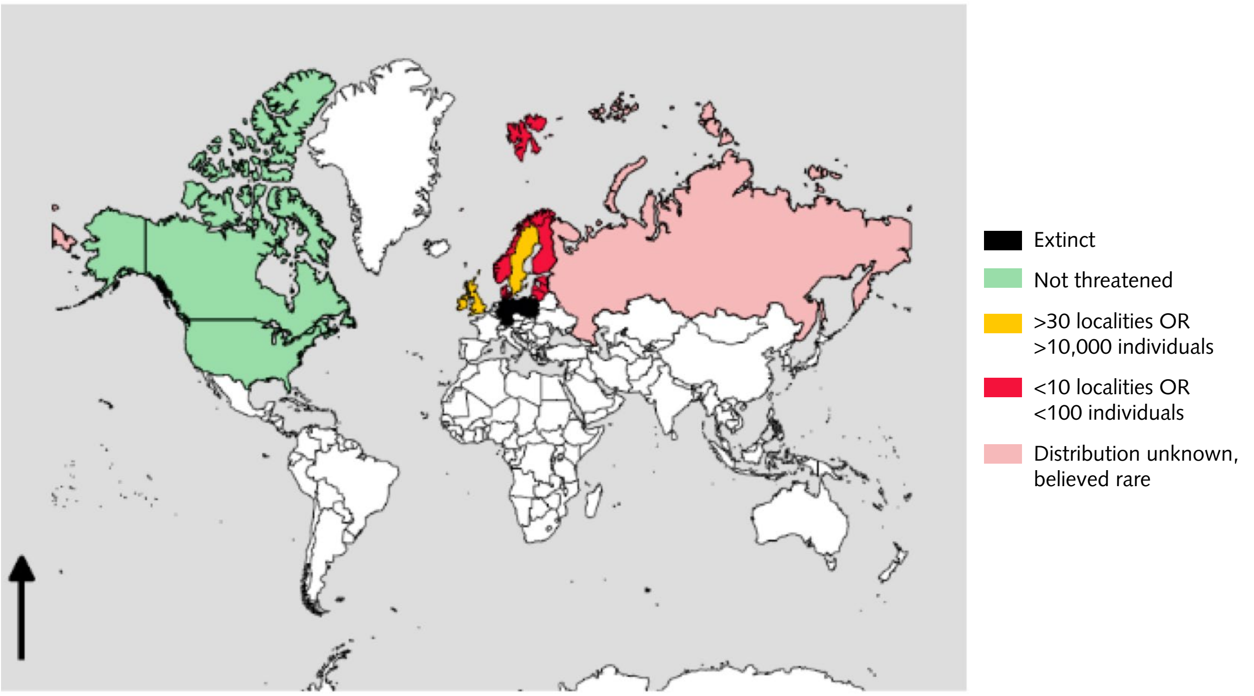

Figure 1. Global distribution of *Najas flexilis* and status (European Commission, 2009).

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 thought to be extinct**
Fingask Loch (Perthshire)	Loch of Butterstone (Perthshire)
Lake of Menteith (Stirlingshire)	Loch of Clunie (Perthshire)
Loch a' Bhada Dharaich (Lochaber)	Loch of Craiglush (Perthshire)
Loch Kindar (Dumfries and Galloway)*	Loch Flemington (near Inverness)
Loch Monzievaird (Perthshire)	Loch of Lowes (Perthshire)
Loch nan Gad (Kintyre)*	Marlee Loch (or Loch of Drumellie) (Perthshire)
Loch Tangy (Kintyre)*	
White Loch (Perthshire)	

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

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, 2019b; Mackenzie et al, 2018; Table 1).

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 2). Therefore, given *N. flexilis*' special status in Scotland as a 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.

It is not fully understood what factors affect the health of *N. flexilis* populations in Scotland. Eutrophication (nutrient enrichment) and the spread of invasive non-native species are considered important (Wingfield, 2002; Bishop, 2019). The reasons why the species disappears, and where and why it fares well in some sites needs to be better understood. In addition, more needs to be known about what actions can be taken to ensure that the habitat quality needed to support populations of *N. flexilis* is either maintained or restored. Thus, this project was commissioned to review the existing knowledge and available information on the habitat requirements of *N. flexilis* from Scotland and other countries where the species is native. The aim is also to identify what data are already available, where they are, and how to access them.

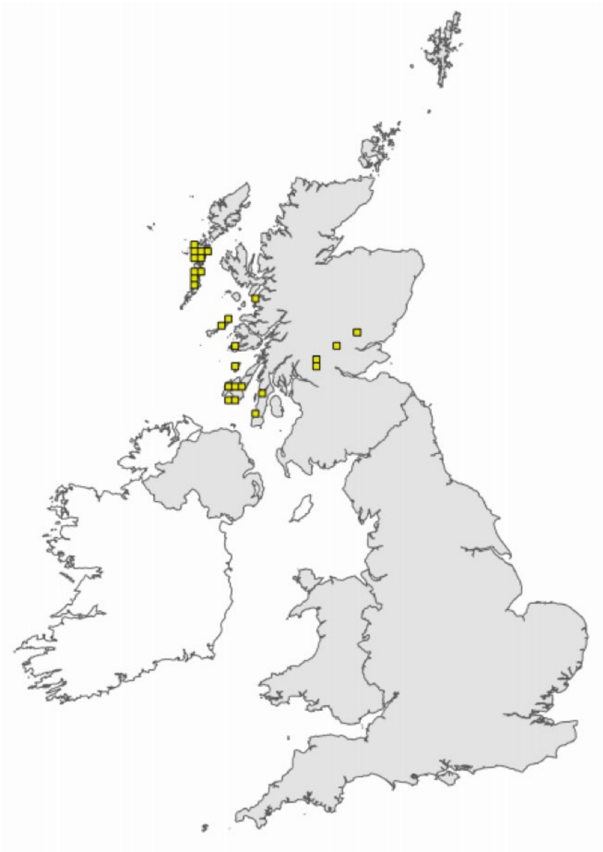

Figure 2. *Najas flexilis* distribution in the United Kingdom (European Commission, 2019). [N.B. Loch Kindar, in Dumfries and Galloway, not shown on map].

1.2 Project objectives

1. Summarize the existing literature on Slender Naiad (*Najas flexilis*), within both Scotland and the UK, as well as internationally. Specifically answering the following questions:
    a. What are the habitat requirements for *N. flexilis*?
    b. What changes in environmental/habitat conditions have led to the decline of *N. flexilis*?
    c. What measures have been taken elsewhere in order to pre-empt, counter, or subsequently restore habitats for *N. flexilis*?
    d. What, if any, are the experiences with species reintroduction?
2. Identify the data needed for the (future) assessment of the suitability of Scottish lochs as a habitat to support *N. flexilis* populations;
3. Identify what data are already available, where, and how to access it.

2 Methodology

Based on the above background, scope and project objectives, this project was undertaken in three component parts:

1. International expert interviews;
2. Literature review;
3. Expert workshop.

2.1 International expert interviews

In January 2020, we carried out structured interviews, by email, Skype or telephone, with a number of international aquatic plant experts to gather their opinion and experiences of *N. flexilis*. These interviews were structured around questions on:

- Current status of *N. flexilis* in their country;
- Habitat requirements of *N. flexilis* in their country;
- Availability of data on *N. flexilis* autecology from their country;
- Availability of any relevant grey literature pertaining to *N. flexilis.*

Formal interviews were carried out with the following international aquatic plant experts: Seppo Hellsten (Finland); Áine O'Connor (Ireland); Marit Mjelde (Norway); and Ruth Hall (UK). In addition, we had email communications with Patricia Chambers (Canada) and Jo Latimore (USA) about *N. flexilis* in their respective countries.

2.2 Literature review

The aim of the literature review was to collate and synthesise all the available existing information on the species requirements of *N. flexilis* and to ascertain, if possible, what actions may harm or benefit the species. To achieve this aim, we carried out an evidence review using standard literature review techniques. Following established best practice (e.g. Collins et al., 2015), there were three main stages in the evidence review:

- Setting clear review questions and developing a protocol (setting scope and methods);
- Evidence search;
- Extracting, appraising and synthesising evidence.

Given the project time constraints, a quick scoping review was used to identify and summarise the available evidence rather than conduct a full systematic review. The formal rapid evidence review approach was supplemented by the knowledge derived from the international expert interviews. Priority was given to studies conducted in the UK but the wider European and international literature in English was also consulted. We developed the search protocols and methods, detailing key search terms, review database structure, key questions and primary evidence sources (grey literature, peer-reviewed literature (using web-based sources such as Web of Science and Google Scholar) and expert knowledge).

Web of Science (WoS)

Evidence of peer-reviewed publications were gathered using three separate Web of Science (WoS) literature searches. The original search was conducted on the 16th January 2020, using the following search terms (covering the period 1970 to 2020 with no geographical restrictions):

(Najas flexilis OR Slender Naiad) AND (ecolog OR habitat* OR water quality OR stressor*).*

This initial search yielded 19 potentially useful references related to the habitat requirements of *N. flexilis.* 14 references were selected from this list after removing irrelevant studies (by checking the titles and abstracts of these studies to assess whether or not they were pertinent to assessing the habitat requirements of *N. flexilis*).

The results of this initial search were then augmented by a second WoS search: *(Najas flexilis OR Slender Naiad) AND (restore OR restoration* OR reintroduction*).*

This second search only produced seven references, of which only two were new references (the other references had already been selected or were omitted for being irrelevant).

A third and final WoS search was carried out just using the following simple search terms: *(Najas flexilis or slender naiad).*

This supplementary search was carried out in order to try to capture any remaining relevant peer-reviewed references that may have been missed by the search terms used in the first two searches. This third search yielded a total of 48 potentially useful peer-reviewed references of which 14 were new references (the other references had already been selected or were omitted for being irrelevant). After the removal of irrelevant studies, 30 separate WoS references remained for further consideration in relation to the ecology of *N. flexilis*.

Google Scholar

An additional literature search was carried out on Google Scholar using the simple search term 'Najas flexilis' to check for any peer-reviewed articles not picked up in the above Web of Science searches, as well as any relevant 'grey literature' that is not covered by Web of Science. This search, which had no time constraints put on it, yielded a total of 3,230 results that referred to 'Najas flexilis' somewhere in each listed publication.

All these records were then manually checked for any useful additional sources of information relevant to the habitat quality assessment of *N. flexilis*. Nearly all the already selected relevant references identified in the above WoS searches were also picked up by Google Scholar plus several, potentially useful, new peer-reviewed publications, as well as some key published reports (i.e. 'grey literature').

Grey literature

A list of relevant non peer-reviewed 'grey' literature on the habitat quality assessment of *N. flexilis* was compiled from the above Google Scholar search. This initial list of grey literature was supplemented by the following additional sources of information: from our (overseas) expert consultations; from citations within both the already selected peer-reviewed and 'grey' literature; and from our own knowledge of relevant published and non-published reports in this particular field of aquatic plant research.

The evidence from the above literature review was then compiled, coupled with the information distilled from the structured interviews with our panel of international aquatic plant experts, to provide a framework for:

1. Assessing the environmental/habitat requirements of *N. flexilis* (e.g. climate, growing depth, lake types, sediment and water quality);
2. Outlining any changes in environmental/habitat conditions that have led to the decline of *N. flexilis;*
3. Reviewing what measures have been taken elsewhere to pre-empt or counter the decline of *N. flexilis,* and/or, subsequently, restore habitats for *N. flexilis;*
4. Reviewing experiences, if any, of species reintroduction of *N. flexilis.*

This literature review provided the evidence base for this report with the aim of identifying what data needs to be analysed, collected and modelled in order to underpin future actions to counter and, if possible, reverse the decline of this species of national and international conservation importance.

2.3 Expert workshop

The collated draft evidence review provided the basis for a one-day expert workshop, held as a web meeting on Friday 24th April 2020, to review the draft report, prior to finalising the project recommendations.

The following aquatic plant experts and agency staff attended the workshop:

- Sophie Beier (Centre of Expertise for Waters (CREW), Project Manager and member of Project Steering Group)
- Alison Bell (Scottish Environment Protection Agency (SEPA), Senior Specialist Ecologist and member of Project Steering Group)
- Sue Bell (Sue Bell Ecology, UK aquatic plant expert)
- Helen Bennion (University College London, Professor and former PhD co-supervisor of Izzy Bishop)
- Izzy Bishop (Earthwatch Institute and former PhD student studying the habitat requirements and reasons for decline of *N. flexilis*)
- Laurence Carvalho (United Kingdom Centre for Ecology & Hydrology (UKCEH), Professor and Freshwater Ecologist, organiser of meeting and co-author of Slender Naiad (*N. flexilis*) Habitat Quality Assessment report)
- Iain Gunn (UKCEH, Freshwater Ecologist, co-author of Slender Naiad (*N. flexilis*) Habitat Quality Assessment report)
- Ruth Hall (née Wingfield) (Natural England and former PhD student studying the functional ecology of *N. flexilis*)
- Ewan Lawrie (Scottish Natural Heritage (SNH), Freshwater Advisory Officer, instigator of project and member of Project Steering Group)
- Áine O'Connor (Freshwater Ecologist, Department of Culture, Heritage and the Gaeltacht, the Republic of Ireland)
- Cilian Roden (Irish *N. flexilis* expert)
- Carl Sayer (University College London, Reader and former PhD co-supervisor of Izzy Bishop)
- Iain Sime (SNH, Freshwater and Wetlands Group Manager)
- Bryan Spears (UKCEH, Freshwater Ecologist, project leader on joint SNH/UKCEH joint project on *N. flexilis* propagation project and PhD co-supervisor of Kate Waters-Hart)
- Nick Stewart (Independent Consultant, UK aquatic plant expert)
- Kate Waters-Hart (UKCEH, PhD student, studying, as part of PhD, responses of *N. flexilis* to lanthanum-modified bentonite (Phoslock®))
- Nigel Willby (University of Stirling, Professor, UK aquatic plant expert and Kate Waters-Hart PhD co-supervisor)

3 Results

3.1 What are the habitat requirements for *Najas flexilis*?

Water depth and exposure

In general, the water depth at which *N. flexilis* will grow will depend on a number of factors, including available light (affected by water clarity) and how sheltered or exposed a site is (Wingfield et al., 2004). In Ireland, Áine O'Connor reports that *N. flexilis* is, generally, associated with high water clarity/transparency and deep euphotic zones, growing at depths > 2 m (Cilian Roden, *pers comm*.). Preston and Croft (1997) also state that in the UK *N. flexilis* is seldom found in water less than 1 m deep, being usually found at depths of 1.5 m or more. This reported favoured growth of *N. flexilis* in deeper water in the British Isles may not be its optimal habitat. It may be that the apparent loss of *N. flexilis* from shallower habitats is an artefact of lower detection rates in shallower, more densely vegetated situations containing a range of similar species. Alternatively, it could be the realised niche of the species, a consequence of being restricted to deeper water by faster growing competitors that are favoured by eutrophication (Nigel Willby, *pers comm*.). In Norway, according to aquatic plant expert, Marit Mjelde, *N. flexilis* is normally found growing in 2-3 m water depth, or deeper (e.g. in Steinsfjorden), however, it is also present in shallower water (e.g. in the Vest-Agder lakes). In Finland, Seppo Hellsten reports that *N. flexilis* is, generally, found in the 70-90 cm water depth range but can also occur in water as shallow as 20 cm and as deep as 2 m in the five lakes where it is currently extant. The shallower depth of where *N. flexilis* is found in Finnish sites could be related to the higher humic content of many of their lakes. In Scotland, *N, flexilis* can also be found in a few, very shallow, humic and acidic sites, such as Tangy Loch (Izzy Bishop, *pers comm.*). Here *N. flexilis* occurs in water depths of <1 m (Nigel Willby, *pers comm*.). Given that the distribution of *N. flexilis* can be very variable in relation to water depth, it should be regarded as an 'unpredictable' variable for describing the habitat requirements of *N. flexilis* (Cillian Roden, *pers comm*.).

Exposure will also have a direct physical effect on where in a lake *N. flexilis* plants can become established and an indirect effect, by affecting the physical characteristics of lake sediments. Bill (2020) found that moderate water level fluctuations might be a stimulatory factor for *N. flexilis* in Scotland. In slightly more productive lochs (split by morpho-edaphic index) *N. flexilis* (along with *Littorella uniflora*, *Juncus bulbosus* L., *Potamogeton gramineus* L., and *P perforfoliatus* L.) was an indicator species of lochs with moderate water level fluctuations (ranging from 1.5 m to 3.5 m) compared with lochs with stable water levels (ranging between 0.2 m to 1 m) (Bill, 2020). Studies in the USA have also indicated that water level drawdown (for recreational management) can have a positive, stimulatory effect on *N. flexilis*. This type of disturbance would appear to favour an annual plant, such as *N. flexilis*, with a weak competitive ability that could be penalised by artificially stabilised water levels (Nichols, 1975; Siver et al., 1986). Fluctuating water levels could potentially be beneficial here in terms of disturbance of competitor plants, but also through an increase of $CO_{2,}$ following the decomposition of desiccated vegetation when re-submerged (Nigel Willby, *pers comm*.). However, one needs to be careful in extrapolating this American experience to the European *N. flexilis* picture, as American *N. flexilis* populations have much more genetic variation than the European *N. flexilis* populations (Ruth Hall, *pers comm*.). In the USA, where lakes freeze over during winter, *N. flexilis* plants are much sturdier (e.g. they can withstand ice exposure), so there are few littoral plants to compete with *N. flexilis.* Nevertheless, disturbance is likely to be a factor affecting *N. flexilis* populations in Scotland where moderate drawdown in lochs may potentially help create open space *for N. flexilis,* with reduced competition from other aquatic plants. However, it should be stressed that *N. flexilis* populations can be impacted in lakes with a big water drawdown, as has been the case in Kiltooris Lough, Donegal, Ireland, where there has been a significant decline in the population, most notably a contraction in its depth distribution (Áine O'Connor, *pers comm*.).

Sediment structure

Sediment composition is another important factor in determining where *N. flexilis* is found in lakes. Wingfield et al. (2004) comment that *N. flexilis* appears to favour growing on a certain type of silty, organic and flocculent sediment. This has also been reported by James and Barclay (1986) in the Butterstone, Craiglush and Lowes lochs in Perthshire. Preston and Croft (1997) also note that *N. flexilis* grows over 'soft, silty substrates'. Wingfield et al. (2004) concluded that the establishment of *N. flexilis* populations in particular locations in a lake will rely on the presence of such suitable fine, silty sediments. Norwegian aquatic plant expert, Marit Mjelde, supports the claim that *N. flexilis* populations are associated with fine sediment structures. In Finland, Seppo Hellsten explains, *N. flexilis* populations are also associated with fine sediments (i.e. sand and clay), with a thin layer of organic material. In Finnish lake sites, *N. flexilis* has been observed by divers to be influenced by groundwater, occurring in areas of 'fluid sediments' where groundwater (often visible by precipitated iron oxides) enters lakes via sandy bottoms, thereby, helping to keep sediments away. The formation of these ideal sediments, which may occur at different water depths, will depend on the level of wind exposure affecting sediment resuspension and stability, which in turn, will be a consequence of the particular setting and physical characteristics of an individual standing water.

It is likely that the interactions between sediment structure, the level of exposure and water clarity will primarily dictate the physical location of *N. flexilis* populations within a lake. There is evidence that *N. flexilis* is able to exploit disturbed conditions where there is often erosion and deposition of sediment in lakes (Wingfield et al., 2004).

Water quality – pH and alkalinity

Hough and Fornwall (1988), Hough and Wetzel (1978) and Keeley and Sandquist (1992) have all reported that *N. flexilis* is an obligate carbon dioxide user and cannot utilise bicarbonate. This restricts the plant's ability to photosynthesise in both very soft (pH<5) and very hard (pH>8) waters (Figure 3), thereby limiting its distribution. Wingfield et al. (2004) summarised the then known water quality conditions that favour *N. flexilis* growth. From a range of American studies, *N. flexilis* appears to occur in lakes with pH's between 6.0 and 9.0 and alkalinity values of between 6 and 307.7 mg/l. Titus and Hoover (1993) demonstrated that *N. flexilis'* reproductive capacity (i.e. seed production) was detrimentally affected by a decrease in pH to 5.0. As *N. flexilis* is an annual, it relies on seeds for establishing each year. It is not known, however, where within the pH range seed production begins to drop off. Another North American example of *N. flexilis* preferring more neutral pHs was provided in a study at Thrush Lake, Minnesota, in which the species flourished following an increase in pH to above 7, following liming, but which decreased once the original conditions returned (Hagley et al., 1996). Wingfield et al. (2004) also reported on physiological experimental research that showed that at calcium levels >30 mg/l and magnesium levels of >10 mg/l (levels greatly exceeded in hard water lakes) rates of carbon fixation declined. Nevertheless, Wingfield et al. (2004) did report that *N. flexilis* had, on occasion, been recorded at sites with pHs as high as 9 (where there is virtually no free carbon dioxide (CO_2); Figure 3), although whether the plant can survive prolonged exposure to such high pH's is unlikely. In Scotland, *N. flexilis* appears to particularly thrive in locations where it is subject to the influences of both base-rich sediments and water run-off from acidic peatlands/hard igneous rock. Ruth Hall commented that the evidence from former *N. flexilis* sites in Scotland, suggests that they became unfavourable once the pH fell below 6.5 and as a result, she concluded that you should not look for *N. flexilis* in acidic waters. Instead, *N. flexilis* sites require a circumneutral pH and an alkalinity that is not too low or too high (Ruth Hall, *pers comm*.). These conditions are, typically, found in machair type lochs where such base-poor and base-rich conditions commonly co-exist (Wingfield, et al., 2004) and may help maintain optimal circumneutral pH levels. Áine O'Connor, similarly, relates that in Ireland, *N. flexilis* occurs in moderate alkalinity lakes with base-rich influences in otherwise peatland dominated catchments, typically overlying coastal calcareous sands (machair) or sometimes limestone. These types of lakes are often characterised by high water clarity/transparency and deep euphotic zones. In Norway, according to Marit Mjelde, *N. flexilis* is also, usually, found in clear water lakes with a medium calcium content, i.e. mesotrophic (medium nutrient) standing waters. For example, Steinsfjorden, near Oslo, which has a very big catchment, has a moderate calcium content of c.

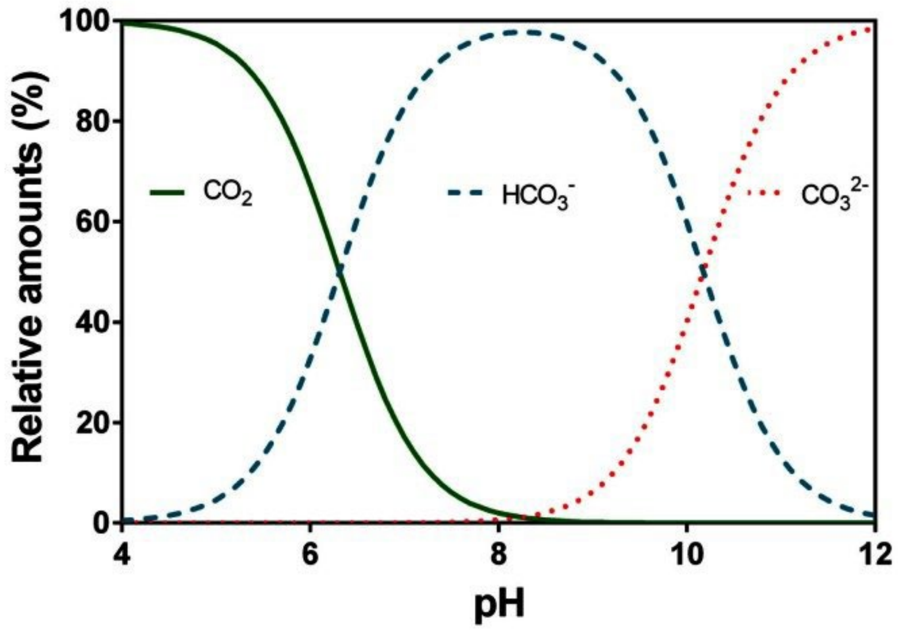

Figure 3. Relative speciation (%) of carbon dioxide (CO2), bicarbonate (HCO−3), and carbonate (CO2−3) in water as a function of pH. Source: Pedersen et al. (2013).

6-9 mg /l. , Although *N. flexilis* is, generally, regarded as a freshwater species in Norway, it does occur in some lakes where the water quality is slightly brackish (at altitudes of 1-3 m). According to Seppo Hellsten, in Finland, *N. flexilis* is found growing in naturally clear (without algae/humic substances), slightly enriched, marl type lakes that are rich in minerals and which have a pH c. 7-8. N.B. in the UK marl lakes are defined as highly alkaline, carbonate rich lakes that precipitate calcite and contain distinct aquatic plant communities. This definition may differ slightly in Finland where many lakes are acidic and humic.

Water quality – nutrients

In terms of nutrient uptake requirements, *N. flexilis* is dependent on phosphorus in the sediments rather than in water, although the nutrient status of the water is important as it can determine competition from other plants, including algae (Wingfield, 2004). Wingfield et al. (2004) provide data on nutrient concentrations from Scottish lochs where *N. flexilis* is recorded. Dissolved reactive phosphorus concentrations were often below detection limits (<1 µg/L) and Total Phosphorus (TP) concentrations ranged from <1 to 56 µg/L, but were typically <20 µg/L, indicating their association in Scotland with predominantly oligotrophic (low nutrient) waters. Similarly, Bishop (2019) summarised water quality data from 26 former and current *N. flexilis* sites in Scotland that had been surveyed since 2000. She showed that all current sites had low concentrations of both nitrate (<0.13 mg L^{-1}) and TP (<30 µg L^{-1}), although nitrate concentrations were slightly higher in the sites where it was currently present (averaging 0.08 mg L^{-1}), compared with sites where it had been lost (averaging 0.01 mg L^{-1}). These nutrient data may, however, be based on very limited spot sampling, just representing bioavailable forms of nitrogen during summer and not represent trophic status of these lochs more generally. The threats from nutrient enrichment are documented in Section 3.2 below.

Associated aquatic plant species

In the UK, *N. flexilis* is, typically, found to thrive in mesotrophic standing waters (Palmer, 1989), with low nutrient levels but which have moderate/medium alkalinity and a circumneutral pH (Wingfield et al., 2004). *N. flexilis* populations are not supported by more oligotrophic and eutrophic (high nutrient) lochs (Wingfield et al., 2004). Typical associated species in Scotland have been found to be, as follows: isoetid species (e.g. *Isoetes lacustris* L., *Littorella uniflora* and *Lobelia dortmanna* L.), bulbous rush (*Juncus bulbosus*), charophytes (i.e. macroscopic algae, e.g. *Chara* spp.) and pondweeds (*Potamogeton berchtoldii* Fieber, *P. gramineus* and *P. perfoliatus*) (Wingfield et al., 2004). In Ireland, *N. flexilis* lakes are nearly always typified by a combination of *Isoetes lacustris and Potamogeton perfoliatus* (*c*. 80-90% of lakes where species combined) and tend to support some *Potamogeton* species, but not too many (Cilian Roden, *pers comm*.). These core Irish *N. flexilis* lakes are also usually classed as "mesotrophic" lakes, characterised by medium/intermediate alkalinity (*c.* 20-70 mg l^{-1}), clear water with a low nutrient status (i.e. very low P) (Cillian Roden, *pers comm*.). *N. flexilis* can be found in peaty standing waters in Ireland but are regarded as a marginal and diminishing lake type for the species (Cilian Roden, *pers comm*.). In Norway, *N. flexilis* is, typically, found growing in association with charophytes (e.g. *Calltriche hermaphroditica* L., *Chara globularis* Thuiller and *Nitella opaca* (Bruz.) C. Agardh and pondweeds *(Potamogeton berchtoldii, P. crispus* L. and *P. pusillus* L.) (Marit Mjelde, *pers comm*.). According to Seppo Hellsten, in Finland, *N. flexilis* is also found in association with *Chara* and/or *Nitella* species, and *Najas tenuissima* (A. Bruaun ex Magnus) Magnus. Nick Stewart has also observed that the charophyte, *Nitella confervacea* (Brébisson) A. Braun ex Leonhardi, is sometimes associated with *N. flexilis*, particularly in Ireland. This may just indicate that both species need clear water and open habitats with few competitors.

Interspecific competition is another factor thought to determine locations of *N. flexilis* in lakes (Wingfield et al., 2004). As an obligate carbon dioxide user, *N. flexilis* will be outcompeted in hard waters by species that can utilise bicarbonate. However, Hough and Fornwall (1988) showed that *N. flexilis* could be competitive where light-limitation reduced the efficiency of a bicarbonate user species, e.g. *Potamogeton pectinatus* L., although this is only likely to occur when pH levels generally remain <8. There is some evidence that *N. flexilis* is an early colonising species that is able to exploit disturbed conditions where there is often erosion or deposition of sediment in lakes (Wingfield et al, 2004). The suggestion is that once such disturbance ceases *N. flexilis* will be out-competed, thereby, limiting its distribution. Interactions with competitive, invasive non-native species, such as *Elodea* spp., are described in more detail in Section 3.2 below. In Finland, Seppo Hellsten reports that *N. flexilis* competes with aquatic bryophytes or other thick bottom flora.

3.2 What changes in environmental/habitat conditions have led to the decline of *Najas flexilis*?

Country status of N. flexilis

In Scotland, *N. flexilis* is considered to be declining with the current number of localities estimated to have dropped from 46 (in the 2013 Article 17 report) to 44 in the most recent Article 17 reporting (JNCC, 2019b). Of the total of 54 locations with post-1999 records, *N. flexilis* has been found in eight new sites but assessed to be lost from a further 10 sites (JNCC, 2019b), although *N. flexilis* has since been recorded in the flotsam of Loch Kindar by a local botanist in 2018 (Nick Stewart, *pers comm*.). The new, additional, sites are all near existing *N. flexilis* localities in the Hebrides and so may reflect short-range dispersal or may simply be just new observations rather than new sites *per se*. The addition of these new *N. flexilis* records has masked the contraction in range of the species on the Scottish mainland. For example, despite intensive targeted snorkel surveys, *N. flexilis* has not been recorded in recent years in the five lochs in the Dunkeld Blairgowrie SAC (JNCC, 2019b; Mackenzie et al, 2018; Table 1) and Site Condition Monitoring (SCM) aquatic plant surveys have not found it growing in Loch Flemington, either (JNCC, 2019b; Table 1). In addition, a further five Scottish mainland sites (i.e. Loch a' Bhada Dharaich, Fingask Loch, Lake of Menteith, Loch Monzievaird and White Loch) are still considered to contain populations of *N. flexilis*, although have there have been no new records of the species recorded from these locations over the last decade (JNCC, 2019b; Table 1). New eDNA techniques may lead to the discovery of further new localities for *N. flexilis* (Crutchley, 2019) although the methods still need refining (Ewan Lawrie, *pers comm*.). In addition, it was suggested that searches for sites based on finding *N. flexilis* seeds in surface sediments (e.g. using simple Ekman grab samples) may also add new records, as there is evidence that *N. flexilis* seeds can be found in near surface sediment in sites where no *N. flexilis* plants appear to be present (Carl Sayer, *pers comm*.). Therefore, there could be many more *N. flexilis* sites in Scotland than the above reporting suggests

In England, *N. flexilis* has not been recorded at its only known site, Esthwaite Water, since c. 1982, despite regular aquatic plant surveys (JNCC, 2019a). The extinction of *N. flexilis* at Esthwaite Water is believed to have been caused by nutrient enrichment from a combination of fish farm and sewage treatment work discharges plus diffuse sources of nutrients (JNCC, 2019a). Wingfield et al. (2004) summarised the main hypotheses behind the decline of *N. flexilis* in the UK, as a whole, as being, eutrophication, competition from invasive non-native aquatic plant species and the mild acidification of circumneutral lakes.

In Ireland, following the recent EU Article 17 reporting, *N. flexilis* is currently considered extant in 52 loughs, mainly in coastal locations, in the western counties of Clare, Donegal, Galway, Kerry and Mayo, and is thought to be extinct in six loughs (NPWS, 2019a; NPWS, 2019b). Currently, the status of *N. flexilis* in Ireland is assessed as being, overall, as 'inadequate', with the trend described as deteriorating because of population extinctions, population decreases and declining habitat quality. Nine out of 30 sites were assessed as decreasing in quality, 61% of sites were assessed to be in 'good' habitat condition, 30% of sites in 'poor' condition and 9% of sites in 'bad' condition. The main threats to the survival of the species are thought to be acidification, eutrophication and peatland damage (Áine O'Connor, *pers comm*.). Due to peat (blanket bog) erosion, excess water colour may be becoming more of a problem in Ireland (Cilian Roden, *pers comm*.). However, many *N. flexilis* Irish lakes still remain 'secure'; the key factor is thought to be that they are on Dalradian limestone (Cilian Roden, *pers comm*.), although this may be more related to raising the vegetation diversity above the more mundane soft water flora rather than anything inherent in the limestone chemistry *per se* (Nick Stewart, *pers comm.).* Such limestone lakes can still hold wonderful populations of *N. flexilis (*Cilian Roden, *pers comm.*). As indicated above, in Ireland, *N. flexilis* is generally, associated with mesotrophic lakes that have clear water, medium alkalinity with both *Isoetes lacustris* and *Potamogeton perfoliatus* present. However, it is important to note that In Irish lakes the *N. flexilis* populations can be very variable between years, with localised distributions, sometimes present and sometimes not. For conservation planning in Ireland, it is crucial that the focus is on the lake, as a whole, rather than on separate populations within a lake (Cilian Roden, *pers comm.*).

The expert workshop highlighted the problem of *N. flexilis* appearing in some years and not others, especially in Scottish lochs where the aquatic flora are not stable due to eutrophication pressures. There is uncertainty as to what factors determine the presence or absence of *N. flexilis* in some years and why *N. flexilis* populations are so variable between years. One relevant issue is the problem of getting an accurate picture of the presence or absence of *N. flexilis* at individual sites because of the methods that are often used for surveying. Isabel Bishop pointed out, that snorkel surveys need to be carried out to search between plants as *N. flexilis* plants are often found underneath other plants and, thus, can be easily overlooked. Grapnels or rakes, commonly used in most macrophyte surveys, are regarded as poor tools for sampling *N. flexilis* populations (Cilian Roden, *pers comm*.). *N. flexilis* has a relatively late germination, so could struggle in competition with other species that get established earlier in the open habitats (Ruth Hall, *pers. comm.*). Surveys carried out early in the year may also miss *N. flexilis* (Nick Stewart, *pers. comm*.). Wingfield et al (2004) found in laboratory-based experiments *N. flexilis* germination was greatest in light, deoxygenated conditions in sediments at warm temperatures (16^{0}C threshold); germination could, however, occur in darker waters in cooler temperatures (greatest at 11^{0}C). These are conditions that, typically, can be found in sheltered areas of Scottish lochs in June. This suggested that, perhaps, space and secondary dormancy are important factors in germination (Ruth Hall, *pers comm*.).

Outwith the British Isles, Sweden is thought to have the largest European populations of *N. flexilis* (Bishop et al., 2019). According to Marit Mjelde, *N. flexilis* is currently considered an 'endangered' species in Norway, and had an action plan drafted in 2012, although it was not published. Existing *N. flexilis* populations are thought to be, relatively, stable in Norway although they do seem to have large fluctuations in numbers*. N. flexilis* was recorded in 13 lakes in 2012, although there now may be as many as 15-20 lakes with the addition of several new sites discovered since 2012. The distribution of *N. flexilis* is currently restricted to three southern counties: Rogaland (12-15 lakes); Vest-Agder (4 lakes) and Buskerud (1 lake). *N. flexilis* is thought to be extinct in three sites (all located in Rogland), due to eutrophication pressures. *N. flexilis* is also assessed as being presently an 'endangered' species in Finland and is considered to be declining with *N. flexilis* populations restricted to c. 20 sites in five lakes and is thought to be extinct in c. five sites in three lakes (Hyvärinen et al., 2019). In recent decades *N. flexilis* has declined particularly in Central Europe with the species believed to have become extinct in Germany, Poland and Switzerland (Bishop et al, 2019), although it was recently recorded for the first time in Austria, in the Millstätter See (Pall, 2011).

Eutrophication

Eutrophication (or nutrient enrichment) is caused by excessive amounts of nutrients (mainly nitrogen (N) and phosphorus (P)) entering lakes from their catchments due to increased levels of human impact, such as from agricultural runoff, and nutrient-laden discharges from industry and treated and untreated wastewater. Nutrient run-off from agricultural land has also been highlighted as a possible eutrophication pressure affecting *N. flexilis* loch sites, as feeding, grassland management and animal poaching of ground, may also give rise to nutrient enrichment (JNCC, 2019b). In the case of Loch Tangy in Argyll, forestry operations within the heavily forested catchment were listed as a potential threat to existing *N. flexilis* populations. Given *N. flexilis's* requirement for circumneutral pH levels, managing alkalinity (by reducing liming in catchments and lochs) could be an important additional management option, as well as reducing nutrient loading.

Bishop (2019) in her PhD thesis on *N. flexilis* found that in her study sites, although nutrient concentrations were low, total phosphorus concentrations were significantly higher in lochs where *N. flexilis* was absent. This was particularly true in mainland sites where *N. flexilis* was formerly present, such as the Dunkeld-Blairgowrie lochs. Recent SCM surveys in these lochs have found all the expected aquatic plant species, apart from *N. flexilis* (e.g. Mackenzie et al., 2016). Here phosphorus concentrations were relatively high with an aquatic plant community characteristic of more mesotrophic conditions. Wingfield (2002) also noted similar higher phosphorus concentrations in lochs without *N. flexilis.* The normal accepted mechanism for eutrophication to cause the decline of aquatic plant species is by reducing light availability, due to shading by phytoplankton or epiphytes. This is, however, normal in quite eutrophic waters and does not necessarily apply to *N. flexilis*. Bishop (2019) highlights that *N. flexilis* appears to flourish in low light conditions and in fine, silty sediments that are often a characteristic of more enriched systems. One reason that *N. flexilis* is thought to be unsuccessful in sites where eutrophication has occurred is due to the lack of available carbon dioxide required for photosynthesis. This is because *N. flexilis* is an obligate carbon dioxide user and cannot utilise bicarbonate (Hough and Wetzel, 1978). In eutrophic waters, daytime pH levels are often >8, at which point, free carbon dioxide would be largely unavailable to *N. flexilis* for aquatic photosynthesis (Figure 3; Falkowski and Raven, 2007: p.157). Bishop (2019) considered this as an important trait to explain the absence of *N. flexilis* in mesotrophic lochs with relatively higher phosphorus concentrations and a more mesotrophic flora. In circumneutral lakes, the dominant form of available carbon is CO_2, and it can compete equally with other aquatic plants and grow in a greater range of depths due to its tolerance of low light conditions. With increased eutrophication, water clarity decreases with increased phytoplankton productivity, thereby, preventing *N. flexilis* from growing in deeper zones away from other bicarbonate-utilising aquatic plants. Alkalinisation may often occur in tandem with eutrophication, due to liming of agricultural land and increased photosynthesis of competitor plants raising pH levels. Therefore, Bishop (2019) concluded that while *N. flexilis* could survive at relatively low abundances in naturally base-rich lochs, it was vulnerable to combined alkalinisation and eutrophication in mesotrophic lochs. In Norway, *N. flexilis* is thought to be extinct in three sites (all located in Rogland) due to eutrophication (Marit Mjelde, *pers comm*.).

A good case study of the likely impacts of eutrophication on a circumneutral and mildly alkaline lake is provided by the paleoecological study at Esthwaite Water in Cumbria (Bishop et al., 2019). Here *N. flexilis* seeds, in dated sediment cores, indicated that the species had been abundant in association with clear, oligo-mesotrophic, mildly alkaline conditions before eutrophication led to the disappearance of the species in the 1980s (Bishop et al., 2019). Typically, the aquatic plant community had been composed of shoreline communities of *Isoetes lacustris* and *Littorella uniflora* with *Chara* spp., *Nitella* spp., and *N. flexilis* in deeper water. In these mildly alkaline conditions, *N. flexilis* is not outcompeted by bicarbonate-utilising aquatic plant species, such as *Potamogeton perfoliatus*. However, with the onset of eutrophication, post-1915, increased photosynthetic rates would have led to a shift from available CO_2 to bicarbonate (Figure 3), leading to a decline in the abundance of *N. flexilis*. The intensification of eutrophication pressures in the 1980s are likely to have resulted in further reductions in available CO_2, which combined with reduced light penetration, probably led to the loss of *N. flexilis* in Esthwaite Water (Bishop, 2019). It is possible that *Elodea canadensis* Michx. was also a significant competitor to *N. flexilis* during this period of increased eutrophication, but there is a lack of concrete evidence to determine this direct effect.

The impact of the shift in CO_2 availability on aquatic plant community structure is well described in Iversen et al. (2019). Here they illustrate that plant communities, particularly in lakes, are very sensitive to reductions in CO_2 availability, which can occur through rapid photosynthesis under eutrophic conditions. As bicarbonate use is an energetically costly process, in circumneutral waters obligate CO_2 users may be at a competitive advantage, but the chance of observing a plant that can use bicarbonate increases greatly with increasing bicarbonate concentrations (Figure 4B). The effect of these shifts in CO_2 availability on the geographical distribution of obligate CO_2 users is illustrated by Iversen et al. (2019) for two contrasting pondweed species, one an obligate CO_2 user, the other a bicarbonate user (Figure 5).

Competition from invasive non-native species

Bishop (2019) reported that 12 of the 26 current and former UK *N. flexilis* sites regularly surveyed by SCM, contained the non-native species, *Elodea canadensis* and *E. nuttallii* (Planch.) H. St. John. These *Elodea* species were found in association with *N. flexilis* and other aquatic plant communities ranging from more acid-tolerant to base-rich conditions, with *E. nuttallii* apparently replacing *E. canadensis* in the more alkaline lochs. Given the reported effectiveness of *Elodea* spp. at utilising bicarbonate for photosynthesis, and its ability to tolerate low light conditions, *N. flexilis* is very vulnerable to competition from this species, particularly at the more base-rich sites where it usually thrives in low light conditions growing away from other plants (Bishop, 2019). In the case of Loch Kindar, the reported use of herbicide in the 1990s by the local angling club to control *Elodea,* may have led to the decline of *N. flexilis* there (Nigel Willby, *pers comm*.). In Steinsfjord in Norway, the mass spread of *Elodea canadensis,* since its appearance in the lake in 1978, is thought to be responsible for the dramatic decline in the *N. flexilis* population (Mjelde et al., 2012). Mjelde et al. (2012) also suggested that the reason behind this significant decrease in the *N. flexilis* population was from *Elodea canadensis* depleting free CO_2 in the water column and/or nutrients in the

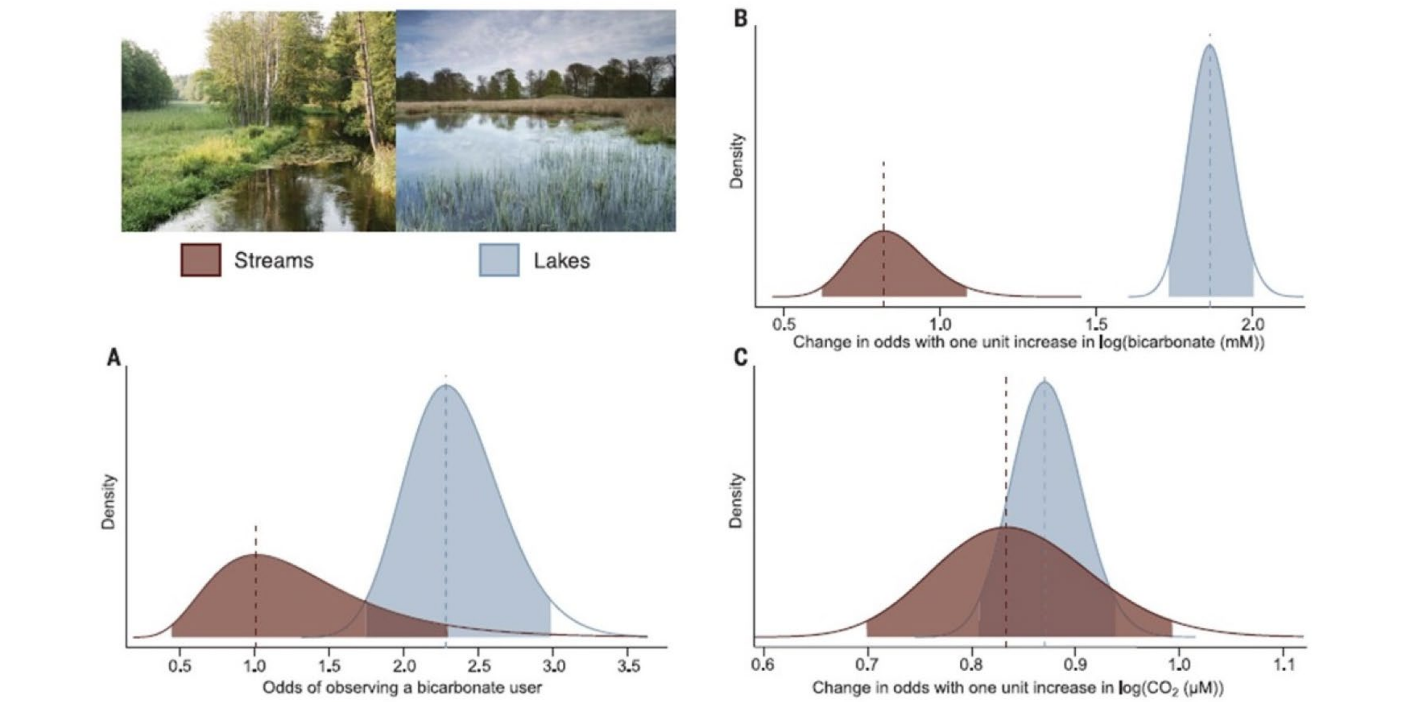

Fig. 1. Bicarbonate use in submerged freshwater plant communities. **(A)** Likelihood of observing a bicarbonate user versus a CO_2 user in streams (n = 172 samples; red) and lakes (n = 791 samples; blue). (**B** and **C**) Modeled odds of observing a bicarbonate user versus a CO_2 user as a function of bicarbonate (B) or CO_2 (C) concentration. Values >1 indicate a higher likelihood (A) or increase in likelihood (B and C) of observing a bicarbonate user versus a CO_2 user with a one-unit increase in bicarbonate (B) or CO_2 (C) concentration. The dotted vertical lines show mean estimates, and shaded areas show the 95% confidence limits around the mean.

Figure 4. Bicarbonate use in submerged freshwater plant communities (from Iversen et al., 2019).

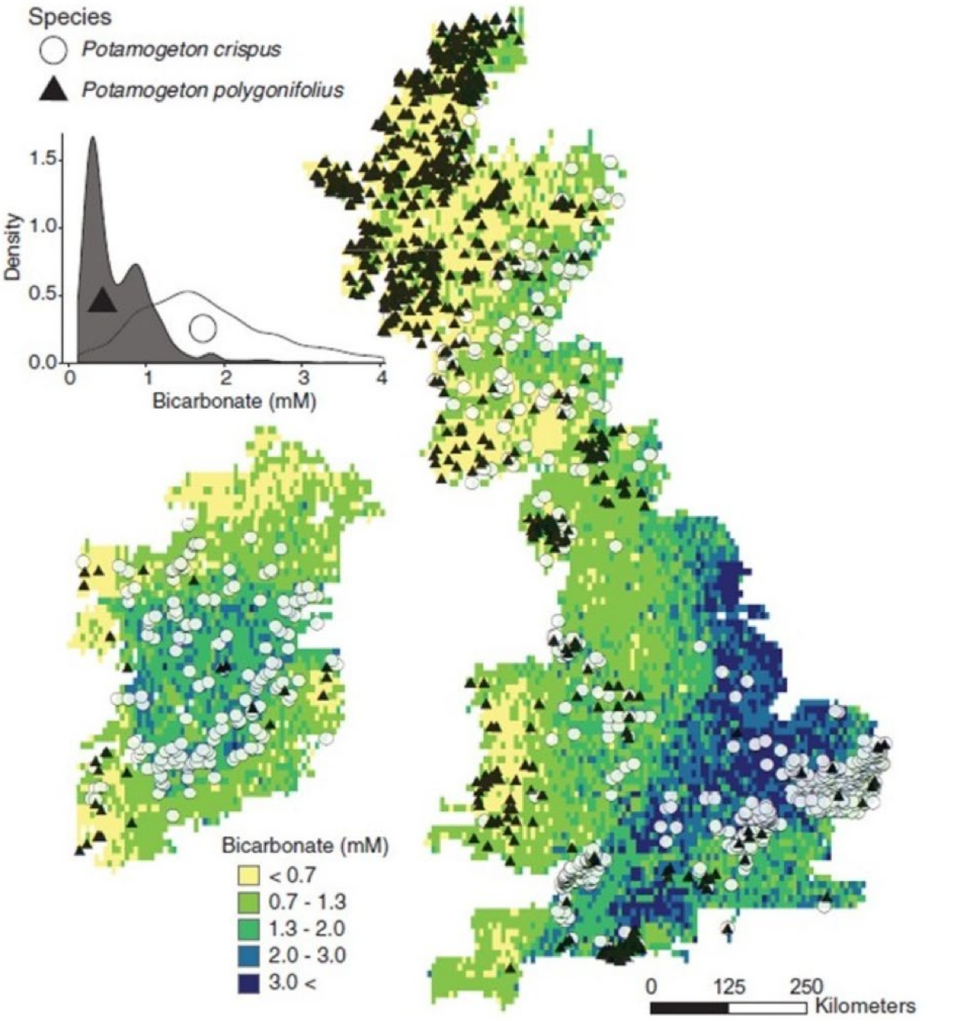

Fig. 3. Steep gradients in bicarbonate concentrations and spatial separation in species distribution in the British Isles. Distribution of two pondweed species with contrasting bicarbonate use in the British Isles. *Potamogeton polygonifolius* (obligate CO_2 user; black triangles) is found in areas with lower bicarbonate concentrations than are present where *Potamogeton crispus* (bicarbonate user; white circles) is found. The top left inset shows the density distribution of the two species across bicarbonate concentrations. Bicarbonate concentrations are from the global bicarbonate map (fig. S2), and species data were extracted from the geo-referenced plant occurrences (*15*).

Figure 5. Spatial separation in species distribution of two *Potamogeton* species in relation to bicarbonate concentrations (From Iversen et al., 2019).

sediment. Although it is not fully clear what caused the contemporary loss of *N. flexilis* from Loch of Craiglush in the early 2000s, populations of the invasive species *Elodea canadensis* and *E. nuttallii* apparently boomed in the loch in the mid-late 2000s, coinciding with the loss of *N. flexilis*. Bishop et al. (2019) recognised the overlapping timing of these ecological changes, but concluded that there was insufficient evidence to make a direct causal link between the two events, over and above the impacts of eutrophication (and associated lack of free CO_2 availability). In another Scottish example, in Loch Tangy, *N. flexilis* is now seemingly restricted to shallower depths than normally would be expected, while *Elodea canadensis* occupies the majority of sample points >1m depth (Bishop, 2019), although it should be noted that this is a very shallow site with maximum water depths c. 1.5 m (Scott Wilson, 2004). In the case of former *N. flexilis* sites, like Loch of Lowes, *Elode*a spp. were found growing abundantly with raised phosphorus concentrations. This evidence suggests that with nutrient enrichment *Elodea* spp. can tolerate and survive the combination of low light and increased ratios of bicarbonate to CO_2 while *N. flexilis* cannot (Bishop, 2019). In the Loch of Butterstone and the Loch of Lowes, the disappearance of *N. flexilis* occurred much more quickly than other sites suffering from nutrient enrichment, for example, Esthwaite Water. This extinction in these Dunkeld-Blairgowrie lochs was preceded by the retreat of *N. flexilis* to deeper waters, as expected with nutrient enrichment. However, the extinction *of N. flexilis* from these deeper water zones in the mid-late 2000s coincided with the rapid expansion of *Elodea* spp. in these lochs. Therefore, Bishop (2019) concluded from this that competition from *Elodea* spp. had possibly facilitated the loss of *N. flexilis* populations that were already made more vulnerable from increased eutrophication pressures. Áine O'Connor also suggested that competition with *Elodea* spp. and nutrient enrichment important factors affecting *N. flexilis* populations in Irish loughs.

In terms of what can be potentially done to eradicate *Elodea* spp., in order to help restore *N. flexilis* sites, the general opinion of the expert workshop was that it is very difficult or impossible to control *Elodea* spp., once they are established in a lake. Sue Bell studied 13 mesotrophic Scottish loch sites, which supported (or had supported) either *N. flexilis* or *Potamogeton rutilus* Wolfg. and where *Elodea* spp. were present (Scott Wilson, 2009). She looked at the suitability of these sites for applying different control measures, including potentially introducing measures to encourage or re-establish *N. flexilis* populations. Subsequently, Sue Bell trialled using hessian to control *Elodea canadensis* in Loch Libo, where it proved effective in supressing the growth of *Elodea* but its ability to achieve long-term control of this species, or promote the regrowth of native aquatic plant species was unproven (Bell, 2013). *N. flexilis* can co-exist with *Elodea* in some sites, although it is not clear why *Elodea* influences *N. flexilis* in some sites and not others (Izzy Bishop, *pers comm*.). It was suggested at the expert workshop that *Elodea canadensis* can 'settle down' over a decade or so in absence of any other eutrophication pressures. However, it was thought that *Elodea nuttallii* is more of a concern, as in the example of Loch Scaraidh and Loch Grogary on North Uist, where it has become over-dominant and overwhelmed the native *N. flexilis* populations (Nick Stewart *pers comm*.).

Mild acidification of circumneutral lakes

Bishop (2019) in her PhD thesis on *N. flexilis,* found that the species, when recorded in SCM surveys of Scottish standing waters (e.g. Gunn, 2011a; Gunn 2011b) contrary to expectations, were generally more abundant at sites with relatively low pHs. Yet none of her study sites had a measured pH <6.5. pH levels less than 6 are thought to be at the lower end of the plant's tolerance range, based on laboratory experiments carried out by Titus and Hoover (1993). However, at some sites, *N. flexilis*, was either absent or, for example at Loch an t-Sagairt on Coll, *N. flexilis* was present in very low levels of abundance where the pH was <7, and where the aquatic plant community was characterised by an acid-tolerant flora. Bishop (2019) concluded that at such sites with upland, moorland catchments, *N. flexilis* is probably just surviving outwith its preferred ecological conditions, thus, making it particularly susceptible to any future increases in acid deposition. Bishop (2019) could not find any empirical evidence for attributing acidification for causing the decline of *N. flexilis* in the UK. She presented plant macrofossil paleoecological evidence for indicating relatively acidic conditions in Loch of Craiglush (part of the Dunkeld and Blairgowrie SAC), a site where *N. flexilis* has gone extinct (c. 2004). In contrast, to the neighbouring Loch of Butterstone and Loch of Lowes, seeds of *N. flexilis* were far less abundant in the dated sediment cores from Loch of Craiglush, where contemporary pHs fluctuate between 7 and 7.5. These are a little lower than pH levels recorded in previous decades, although not considered a level to impact greatly on the photosynthetic or reproductive performance of *N. flexilis*. In addition, Bishop (2019) discovered from the plant macrofossil record that the current period of *N. flexilis* absence was one of several over the last 100 years, although it was not possible to determine whether this was the result of short-term fluctuations in pH. The species represented in the sediment cores in the Loch of Craiglush were indicative of relatively nutrient-poor, slightly more acidic conditions with an aquatic plant community indicative of base-poor conditions. Given *N. flexilis'* stated preference for more circumneutral to alkaline conditions, it may be that Loch of Craiglush was never a particularly favourable location for the species to flourish in, being at the lower end of its pH tolerance range (Bishop et al., 2019). Although there is no contemporary evidence for relating any site extinction of *N. flexilis* in the UK directly due to acidification, palaeobotanical research from Poland suggests that natural decreases in pH, due to historical climate change, led to its disappearance in two lakes (Galka et al., 2012).

Climate change

Climate change has been highlighted as a potential risk to *N. flexilis* populations in Scotland by, for instance, causing increases in nutrient and sediment run-off from lake catchments with a predicted rise in the number and severity of storm events, particularly in summer months when seedlings are very vulnerable to disturbance (Alison Bell, *pers comm*.; JNCC, 2019). Bishop (2019) recognised that it is difficult to separate the possible impacts of climate change on the UK distribution *of N. flexilis* from other possible deleterious influences, discussed above, i.e. eutrophication, invasive non-native plants and the mild acidification of circumneutral lakes. However, Bishop (2019) does note the recurring pattern of *N. flexilis* being lost from many of its locations at its southern distributional limit in the British Isles, in continental Europe and in the USA. Although no direct connection has been made between the decline of *N. flexilis* in Central Europe and climatic warming, the movement of other aquatic species northwards have been linked (e.g. Gallo et al., 2017). With climate change, it is likely that primary productivity of freshwater ecosystems will increase with warming, leading to an increase in the negative effects of alkalinisation and eutrophication, as well as resulting in more extreme rainfall events. The latter changes in rainfall patterns could potentially adversely affect the balance between the acid-run-off from upland catchments and underlying base-rich input from underlying geology that characterises many of the *N. flexilis* current strongholds, for example in the Outer Hebrides machair loch sites (Bishop, 2019).

3.3 What measures have been taken elsewhere in order to pre-empt, counter, or subsequently restore habitats for *Najas flexilis*?

Given the observed contraction in the range of *N. flexilis* sites on the Scottish mainland, described above, efforts have become increasingly focussed on water quality measures to try and restore its loch habitats, with the aim of facilitating the species re-establishing in its, formerly, occupied sites. General water quality improvement measures, such as the Scottish Environment Protection Agency (SEPA)'s Priority Catchments initiative and the Agri-Environment Climate Scheme (AECS) have been adopted, as well raising public awareness of the dangers of spreading non-native aquatic plant species. In the case of the five lochs (i.e. Butterstone, Clunie, Craiglush, Lowes and Marlee) that comprise the Dunkeld and Blairgowrie Lochs SAC in Perthshire, several specific catchment measures have also been undertaken. These include the introduction of a SNH Natural Care Scheme, the removal of fish cages from the Loch of Butterstone and the control of inputs from the Loch of the Lowes visitor centre, all with the aim of improving water quality (JNCC, 2019b). This is because, despite an increased survey effort in recent years, including snorkel surveys in 2016 (Mackenzie et al., (2018), no *N. flexilis* plants have been recorded in any of the five lochs in the Dunkeld and Blairgowrie SAC, since 2007. As outlined above, the disappearance of *N. flexilis* from these lochs has been primarily attributed to nutrient enrichment. However, it is likely, despite all these restoration measures that a complete recovery in water quality will be delayed due to the legacy of internal nutrient loading from the loch sediments, which, coupled with competition from non-native *Elodea* spp., will continue to make it difficult to produce the suitable habitat conditions for *N. flexilis* to become re-established here (JNCC, 2019b).

In England, no specific measures have been undertaken in Esthwaite Water to restore its habitats for the benefit of *N. flexilis*. However, given that the extinction of *N. flexilis* at Esthwaite Water is believed to have been caused by eutrophication, restoration efforts have been focussed on improving water quality in the lake. This has led to the closure of the local fish farm, upgrading of the sewage treatment works (Maberly et al., 2011) and more recently, the cessation of fish stocking. This reduction in external nutrient loading has resulted in a decline in total phosphorus (TP) and a slight improvement in Secchi depth, although phytoplankton levels failed to show a decrease between 2010 and 2015, probably because of weather effects (Maberly et al., 2016). However, diffuse sources of nutrients continue to pose a particular threat to the long-term objective of improving water quality in Esthwaite Water. The legacy of nutrients that have accumulated in the lake sediments also remains a problem, as they can continue to be released from the sediments, under warm weather conditions, into the overlying open water (JNCC, 2019a). Recent, regular, SCM aquatic plant surveys of Esthwaite Water, have showed that these continuing nutrient-rich conditions have helped the abundant growth of the non-native aquatic plant, *Elodea nuttallii*, in the lake (JNCC, 2019a). Even if water quality was to improve to the mesotrophic or oligo-mesotrophic conditions, typically, favoured by *N. flexilis*, the ongoing presence of *Elodea nuttallii* could prevent its re-establishment in Esthwaite Water.

3.4 What, if any, are the experiences with reintroduction of *Najas flexilis*?

As part of her PhD research, Kate Waters-Hart collected *N. flexilis* specimens from Tangy Loch in Argyll in 2018, as part of a core experiment with Phoslock® (Waters-Hart, 2020). While acknowledging it was difficult to collect *N. flexilis* seeds from plant fragments, she successfully managed to grow some *N. flexilis* individuals from fragments held down in Loch Leven sediment and water in the greenhouses at UKCEH Edinburgh. Since then *N. flexilis* plants have germinated continuously (Waters-Hart, *pers comm.*). This is the first time that *N. flexilis* has been grown ex-situ, despite a number of previous attempts. As Ruth Hall commented at the expert workshop, she had previously tried to grow *N. flexilis* seeds from sites where it had become extinct, but without success. It is not clear what the water quality or temperatures were that favoured the *N. flexilis* growth in the UKCEH greenhouses, as these were not closely monitored at the time. Nevertheless, as a result of this unexpected bonus from Kate Waters-Hart's PhD studentship, a follow-up project has been set up between SNH, Phoslock GmBh and UKCEH. This project aims to better understand the factors needed for successful propagation of *N. flexilis* under controlled conditions. It will involve the expansion of the existing population of *N. flexilis* being cultivated in the UKCEH greenhouses, using standard propagation techniques, with the accompanying monitoring of water quality and the assessment of plant health through fluorescence measurements. Another element of the project will be the use of incubators to carry out germination trials to investigate the optimum germination requirements of *N. flexilis* seeds (using the stock grown in the UKCEH greenhouses), such as exploring what light, day-length and water temperature regimes are important. If the up-scaled propagation of *N. flexilis* is successful, then the plan is to translocate *N. flexilis* plants into a suitable refuge or a restored site within Scotland, e.g. Tangy Loch, where the original *N. flexilis* material collected. The successful propagation and translocation of *N. flexilis* plants would directly help address the contraction in the species' range, by providing an exciting new tool to help address the species' decline. Hopefully, the lessons learnt from these SNH funded germination and propagation trials, will, following on from this CREW funded literature review, feed into a proposed second phase of the project that aims to identify suitable site requirements and practical measures that are necessary to be met in order to reverse the decline in *N. flexilis*. A key question that might be answered by this work is: what do you need to do to break *N. flexilis* seed dormancy? For example, in Esthwaite water, there are *N. flexilis* seeds present that are not germinating, perhaps because of the ongoing *Elodea nuttallii* problem, despite water quality improvements (Ruth Hall, *pers comm*.). Do the conditions here exist for *N. flexilis* seeds to germinate (Ruth Hall, *pers comm*.)?

No specific *N. flexilis* restoration programmes have been established in Finland, Ireland or Norway. A re-establishment action plan was, however, set up for *N. flexilis* in Poland in the mid-2000s. *N. flexilis* is extinct in Poland but was formerly present in four lakes. The aim of the plan was to assess the potential for re-establishing *N. flexilis*, based on a comprehensive review of available information on its biology and ecology (Thiry et al., 2005). The Polish action plan for *N. flexilis* comprised separate components of research, assessment, measures, re-establishment and monitoring of the species and habitat. If seeds of *N. flexilis* were found at any of the Polish study sites, the plan was then to try and germinate them in plastic tanks based on their known favoured ecological conditions, i.e. sandy substrates and calcium-rich, circumneutral to alkaline, oligotrophic water. The plan outlined that if no *N. flexilis* seeds were found in any of the surveyed Polish sites then the option to obtain plant material or seeds from either Scandinavia or the UK sites would be explored for the reintroduction of *N. flexilis*. The aquatic plant communities of two potential *N. flexilis* re-introduction sites, Binowskie Lake and Glinna Lake, were surveyed in 2004 by scuba diving and samples collected for sediment analysis. However, these surveys found no evidence of *N. flexilis,* either as living plants or as seeds in the sediments. The results from these surveys indicated that both of these lakes were eutrophic and that remediation or improvement in their water quality (both of surface water and groundwater) was a necessary pre-requisite for these or any other potential reintroduction Polish lake sites before any attempt could made to reintroduce *N. flexilis.* As far as the authors of this review are aware, given the enormous practical difficulties in achieving these required improvements in water quality, this attempt at the reintroduction of *N. flexilis* in Poland, was halted at this point.

3.5 Data needed to assess the suitability of Scottish loch sites as a habitat to support *Najas flexilis* populations and data availability

Given the decline of *N. flexilis* in mainland Scottish lochs, SNH are keen to adopt a measured approach to its re-introduction. SNH want a 'backstop' for mainland sites with the aim of maintaining the genetic diversity of *N. flexilis* in mainland lochs (Ewan Lawrie, *pers comm*.) Key questions are:

- What conditions does *N. flexilis* need for it to be successfully re-introduced?
- How to identify potential lochs where it may be suitable for *N. flexilis* re-introduction?

This review has identified a number of key factors affecting the growth and reproductive performance of *N. flexilis*. Using this information, a modelling or data screening approach could be adopted to pinpoint where *N. flexilis* could be (for existing and potential sites) and identify potential re-introduction sites. This could be done by mapping potential *N. flexilis* sites by modelling habitat suitability and by screening on ecological conditions necessary for *N. flexilis* to grow in the current Scottish loch population.

Data on the factors that would help evaluate the suitability of sites for potential re-introduction of *N. flexilis* and possible data sources are highlighted in Table 2.

Other potential factors discussed in the expert workshop for identifying potential *N. flexilis* re-introduction sites included the following**:**

- Geology, i.e. sites with a mix of base-rich/base-poor lochs – this is available in the UK Lakes Portal estimates of alkalinity type
- Satellite-derived chlorophyll data being made operationally available to SEPA from 2020 at 60m resolution. Currently being processed for SEPA for WFD lakes, but potentially available for smaller lochs (Claire O'Neil & Peter Hunter (SEPA/University of Stirling), *pers comm*.).
- Landscape hydrological connectivity metrics now developed by UKCEH for the Hydroscape Project and available in the UK Lakes Portal. Could rank potential sites on connectivity/resilience. Connectivity important for *N. flexilis* reintroduction as high connectivity could enhance resilience and spread, but need to also consider presence/absence of invasive species in connected systems. So connectivity could be a positive attribute weighting if connections are with high quality sites (particularly absence of competitor species like *Elodea*), but given a negative weighting if connected to sites where invasive competitive plant species are present.

Table 2. Data needs and potential 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	SEPA
	Or use proxy data for CO_2 / bicarbonate availability	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 TN and TP to estimate annual mean	SEPA
	Or use proxy estimates based on land-use models	UK Lakes Portal for all Scottish lochs SEPA/JHI for estimates using PLUS+ model
Phytoplankton chlorophyll-*a*	Monthly monitoring April to Oct or Satellite 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	SNH (CSM) and SEPA (WFD & Mesotrophic Loch Action Plan)
Presence of associated species *Isoetes lacustris* and *Potamogeton perfoliatus*	Aquatic plant species records for all surveyed Scottish lochs	JNCC aquatic plant + BSBI databases

- Consider existence of islands in lakes as element of fetch reduction and creating favourable patches for *N. flexilis*. Numbers of islands are recorded in the UK Lakes database.
- The very variable *N. flexilis* sites may be those lochs where it is at the edge of its habitat suitability. It may be better to focus on improving other sites that are more favoured habitat.
- Bird connectivity could be quite important for the transfer of *N. flexilis* seeds. Potentially could relate a bird connectivity metric to parameters such as loch size and proximity to migration pathways. Such a bird connectivity metric is available through the NERC Hydroscape project.

It was suggested that rather than focus on re-introduction, the next phase of the project should be looking for other existing *N. flexilis* sites in Scotland (Cilian Roden, *pers comm*.) and recommended using *Isoetes lacustris/ Potamogeton perfoliatus* as an index to identify potential *N. flexilis* sites. As a follow on from the Phase 2 modelling/ecological screening approach, it was suggested that a randomised survey is carried out of identified suitable sites for *N. flexilis*. This could be carried out by taking Ekman grab samples of surface sediments to search for the presence of *N. flexilis* seeds (Carl Sayer, *pers. comm*.) and could be supplemented by eDNA sampling. The randomised sampling would give a better estimate of the true population of lochs where *N. flexilis* is present. Genetic variation in seeds is an important consideration and best conservation practice would use local seeds, rather than re-introduced seeds from other sites with different genetic material (Ruth Hall, *pers comm*.).

It was also recommended that a similar approach could be adopted to check for the presence of viable *N. flexilis* seeds from samples collected from the upper sediment layers of lochs where *N. flexilis* has not been recorded in recent years, for example, the Lake of Menteith (Alison Bell, *pers comm*.). Wingfield et al. (2004) listed 51 sites where there were historical records of *N. flexilis* in Scotland. Since then, *N. flexilis* has been found in a further 10 standing waters in Scotland, records derived from various aquatic plant surveys carried out by the BSBI (Botanical Society of the British Isles), SEPA and SNH. In the case of SNH, they have commissioned SCM surveys of *N. flexilis* in c. 19 Scottish standing waters of conservation importance (i.e. Sites of Special Scientific Interest (SSSIs), SAC and Ramsar sites) over three rounds of Common Standards Monitoring (CSM) from 2005 onwards. These CSM lochs include, as follows: Lake of Menteith, Loch Ballyhaugh, Loch of Butterstone, Loch of Clunie, Loch Cuithe Moire, Loch Druidibeg, Loch an Eilein, Loch Fada (Colonsay), Loch Flemington, Loch Grogary (Croghearraidh), Loch Hallan, Loch of Lowes, Loch Phurit-ruadidh, Marlee Loch, Loch Olaidh an Iar (West Loch Ollay), Loch Olaidh Meadhanach (Mid Loch Ollay), Loch an t-Sagairt, Loch Scaraidh (Sgaraigh) and Tangy Loch. At these SNH CSM survey sites, generally, only spot chemistry samples were collected. Further SCM habitat surveys, in addition to *N. flexilis* surveys, have been undertaken at a number of sites which were not included in previous cycles of CSM, for example, Loch of Craiglush (in 2016), Fingask Loch (in 2019), Loch Kindar (in 2017) and White Loch (in 2019) (Nick Stewart, *pers comm*.). SEPA have, since 2008, also carried out aquatic plant surveys on 21 lochs where there are historic *N. flexilis* records. The summary details of these SEPA sites

Table 3. Summary details of SEPA post-2008 surveys of lochs with historic *Najas flexilis* records.

Lake Name	Survey Years	*N. flexilis* present	Reason for survey
Loch Olabhat	2008, 2010	Yes	WFD
Loch Druidibeg (North)	2008, 2013	Yes	WFD
Loch Olaidh Meadhanach	2008	Yes	WFD
Loch Gorm	2008, 2014, 2019	Yes	WFD
Lake of Menteith	2008, 2010, 2016	No	WFD
Loch Kindar	2008	No	WFD
Loch Lossit	2009	Yes	Non-WFD
Loch Grogary (Croghearraidh)	2009	Yes	Non-WFD
White Loch (Perth)	2010	Yes	Non-WFD
Glenastle Loch	2008, 2011	Yes	Non-WFD
Loch Skerrols	2008	Yes	Non-WFD
Loch a Mhadaidh	2009	Yes	Non-WFD
Loch Ghearraidh Mhic Iain	2009	Yes	Non-WFD
Loch nan Cnamh	2009	Yes	Non-WFD
Loch Runabhat	2009	Yes	Non-WFD
Loch nan Gad	2009	Yes	Non-WFD
Tangy Loch	2009	Yes	Non-WFD
Lindores Loch	2008	No	Non-WFD
Lake of Menteith	2008, 2010, 2016	No	Non-WFD
Monk Myre	2010	No	Non-WFD
Loch Monzievaird	2009	No	Non-WFD
Loch Scaraidh	2009	No	Non-WFD

N.B. WFD – Water Framework Directive

are given in Table 3. Most of the non-WFD site surveys were carried out under the auspices of SEPA's Mesotrophic Loch Action Plan (Alison Bell, *pers comm*.). In addition, SEPA have regular water chemistry monitoring data available for 11 lochs with historic *N. flexilis* records: Lake of Menteith, Loch of Butterstone, Loch Ceann a Bhaigh, Loch of Clunie, Loch nan Cnamh, Loch Gorm, Loch of Lowes, Loch Kindar, Loch Olaidh Meadhanach (Mid Loch Ollay), Marlee Loch, and Tangy Loch.

4 Conclusions

The literature review highlights that much of the sensitivity of *N. flexilis* to the known threats of, eutrophication, competition with other plants and the mild acidification of circumneutral lakes can be related to its physiology as an obligate user of CO_2; *N. flexilis* plants being unable to metabolise bicarbonate for photosynthesis. This physiological restriction puts limits on its distribution, particularly with respect to the pH and alkalinity of the lake, and is the reason that *N. flexilis* is typically found in circumneutral waters, with C-limitation of growth likely to be present at pH <5.5 and pH>8. This physiological requirement may be the reason for the favourable habitat being associated with machair and with anecdotal evidence of populations in lakes around groundwater springs – which are normally high sources of free CO_2 (Falkowski and Raven, 2007). In terms of acidification, lower pH levels below 6.5 may be detrimental to reproductive performance of *N. flexilis*, before lower pH levels <5 start impacting growth rates through CO_2-limitation. In relation to eutrophication, nutrient enrichment leads to increases in phytoplankton, epiphyte and aquatic plant growth. This has the potential to lead to C-limitation for obligate CO_2 users during daytime if pH levels rise above 8. This is likely to be exacerbated by grassland or forestry improvements if liming of the land leads to increased pH of circumneutral lakes (but beneficial in acid lakes). The result of both eutrophication and alkalisation is a strong competitive advantage for aquatic plants that 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 *Elodea* spp. Whether or not invasive non-native species, such as *Elodea*, have impacted *N. flexilis* populations indirectly, through reducing CO_2 availability, or directly, through competition for deeper, low-light habitat is unknown; a combination of both direct and indirect impacts may be involved.

5 Recommendations

An increased understanding of the habitat requirements of *N. flexilis* has identified a number of factors that are key to its growth and reproduction and, ultimately, for sustaining healthy populations in Scotland. We recommend that this review be followed up with an analysis of the habitat suitability of Scottish lochs, in order to identify potential sites where it may be present or suitable sites for a species re-introduction programme. As well as available water quality and aquatic plant data for selected sites from SEPA and SNH, national data sources, such as those available through the UK Lakes Portal, on geology, catchment soils and agricultural land-cover, will be needed to assess potential sites for restoration programmes. This could be used in combination with aquatic plant databases, using the presence of associated species, such as *Isoetes lacustris* and *Potamogeton perfoliatus* to identify potential sites.

Further monitoring of the seasonal changes in free-CO_2 availability in current sites may also be useful to identify sites at risk. Similarly, detailed spatial surveys of pH and alkalinity within and outside existing patches of *N. flexilis* may help confirm whether localised hotspots within lochs are associated with CO_2-rich groundwater springs.

6 References

Peer-reviewed publications

Bishop, I. J., Bennion, H., Patmore, I. R. and Sayer, C. D. (2018). How effective are plant macrofossils as a proxy for macrophyte presence? The case of *Najas flexilis* in Scotland. *Journal of Paleolimnology*, 60, 153-165.

Bishop, I. J., Bennion, H., Sayer, C. D., Patmore, I. R. and Yang, H. (2019). Filling the "data gap": Using paleoecology to investigate the decline of *Najas flexilis* (a rare aquatic plant). *Geo-Geography and Environment*, e00081.

Falkowski, P. G. and Raven, J. A (2007). *Aquatic Photosynthesis*. Second edition, Princeton University Press, Princeton.

Galka, M., Tobolski, K. and Kolaczek, P. (2012). The Holocene decline of slender naiad (*Najas flexilis* (Willd.) Rostk. & W. L. E. Schmidt) in NE Poland in the light of new paleobotanical data. Acta Palaebotanica, 52, 127-138.

Hagley, C. A., Wright, D., Owen, C. J., Eller, P. and Danks, M. (1996). Changes in aquatic macrophytes after liming Thrush Lake, Minnesota. *Restoration Ecology,* 4, 307-312.

Hay, F .R. and Muir, J. S. K. (2000). Low temperature survival of slender naiad (*Najas flexilis*) seeds. *Cryoletters*, 21, 271-278.

Hough, R. A. and Fornwall, F. D. (1988). Interactions of inorganic carbon and light availability as controlling factors in aquatic macrophyte distribution and productivity. *Limnology and Oceanography*, 33, 1202-1208.

Hough, R. A. and Wetzel, R. G. (1978). Photorespiration and CO_2 compensation point in *Najas flexilis*. *Limnology and Oceanography*, 23, 719-724.

Iversen, L. L., Winkel, A., Baastrup-Spohr, L., Hinke, A. B., Alahuhta, J., Baattrup-Pedersen, A., Birk, S., Brodersen, P., Chambers, P. A. Ecke, F., Feldmann, T. Gebler, D., Heino, J., Jespersen, T. S., Moe, S. J., Riss, T., Sass, L., Vestergaard, O., Maberley, S. C., Sand-Jensen, K. and Pedersen, O. (2019). Catchment properties and the photosynthetic trait composition of freshwater plant communities. *Science*, 366, 878– 881.

Johnson, R. K. and Ostrovsky, M. L. (2004). Effects of sediment nutrients and depth on small-scale spatial heterogeneity of submersed macrophyte communities in Lake Pleasant, Pennsylvania. Canadian *Journal of Fisheries and Aquatic Sciences*, 61, 1493-1502.

Keeley, J. E. and Sandquist, D. R. (1992). Carbon: freshwater plants. *Plant Cell and Environment*, 115, 1021-1035.

Kupryjanowicz, M., Filoc, M. and Czerniawska, D. (2018). Occurrence of slender naiad (*Najas flexilis* (Willd.) Rostk. & WLE Schmidt) during the Eemian Interglacial - An example of a palaeolake from the Hieronimowo site, NE Poland. *Quaternary International*, 467, 117-130.

Mjelde, M., Lombardo, P., Berge, D. and Johansen, S. W. (2012). Mass invasion of non-native *Elodea canadensis* Michx. in a large, clear-water, species-rich Norwegian lake - impact on macrophyte biodiversity. *Annales De Limnologie-International Journal of Limnology*, 48, 225-240.

Moeller, R. E., Burkholder, J. M. and Wetzel, R. G. 1988). Significance of sedimentary phosphorus to a rooted submersed macrophyte (*Najas flexilis* (Willd) Rostk and Schmidt) and its algal epiphytes. *Aquatic Botany*, 32, 261-281.

Nichols, S. A. (1975). The use of overwinter drawdown for aquatic vegetation management. *Water Resources Bulletin,* 11, 1137–1148.

Pall, K. (2011). *Najas flexilis* (Najadaceae or Hydrocharitaceae) a Natura 2000 species – new for Austria. *Neilreichia*, 6, 11-26.

Pedersen, O., Colmer, T. and Sand-Jensen, K. (2013). Underwater Photosynthesis of Submerged Plants – Recent Advances and Methods. Frontiers in Plant Science, 4: 140.

Peredo, E. L., King, I. M. and Les, D. H. (2013). The Plastid Genome of *Najas flexilis*: Adaptation to Submersed Environments Is Accompanied by the Complete Loss of the NDH Complex in an Aquatic Angiosperm. *PloS ONE*, 8(7) e68591.

Preston, C. and Croft, J. (1997). *Aquatic plants in Britain and Ireland*. Colchester, Harley Books.

Siver, P. A., A. M. Coleman, G. A. Benson, and J. T. Simpson (1986). The effects of winter drawdown on macrophytes in Candlewood Lake, Connecticut. *Lake and Reservoir Management*, 2, 69–73.

Titus, J. E. and Hoover, D. T. (1993). Reproduction in two submersed macrophytes declines progressively at low pH. *Freshwater Biology*, 30, 63-72.

Wingfield, R., Murphy, K. and Gaywood, M. (2005). Lake habitat suitability for the rare European macrophyte *Najas flexilis* (Willd.) Rostk. & Schmidt. *Aquatic Conservation-Marine and Freshwater Ecosystems*, 15, 227-241.

Wingfield, R., Murphy, K. J. and Gaywood, M. (2006). Assessing and predicting the success of *Najas flexilis* (Willd.) Rostk. & Schmidt, a rare European aquatic macrophyte, in relation to lake environmental conditions. *Hydrobiologia*, 570, 79-86.

Grey literature

Bell, S. (2013). *Experimental shading to control/eradicate Canadian waterweed*. Scottish Natural Heritage Commissioned Report No.557.

Bennion, H., Clarke, G., Davidson, T., Morley, D., Rose, N., Turner, S., and Yang, H. (2008). *Paleaoecological study of seven mesotrophic lochs*. Report to SEPA and SNH. Environmental Change Research Centre, Research Report No. 121.

Bill, A. R. (2020). *Effects of lake water level fluctuations on macrophytes and littoral macroinvertebrates*. PhD thesis. University of Stirling.

Bishop, I. J. (2019). *Understanding the habitat and decline of Najas flexilis (Willd.) Rostk. & W. L. E. Schmidt in the UK using ecology and paleoecology*. PhD thesis, University College, London.

Bishop, I. J., Bennion, H. and Sayer, C. D. (2018). *Understanding the habitat and decline of Najas flexilis in the UK using ecology and paleoecology*. Scottish Natural Heritage Commissioned Report.

Collins, A. M., Coughlin, D., Miller, J. and Kirk, S. (2015). The Production of Quick Scoping Reviews and Rapid Evidence Assessments: A How to Guide.

Council of Europe (1979). Convention on the Conservation of European Wildlife and Natural Habitats. Bern, Switzerland.

Council of the European Union (1992). Council Directive 92/43/EC of 21 May 1992 on the Conservation of Natural Habitats and Wild Fauna and Flora.

Crutchley, N. B. (2019). *Development of eDNA techniques for Slender naiad survey and monitoring*. Scottish Natural Heritage Research Report.

European Commission (2009). EU Biodiversity strategy 2020. https://doi.org/10.2779/39229

European Commission (2019). European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora: Fourth Report by the United Kingdom under Article 17.

Gallo, B., Jackson, M. C., O'Gorman, E. and Woodward, G. (2017). *Adaption of freshwater species to climate change* . Grantham Institute Briefing Note, Imperial college, London.

Gunn, I D. M. (2011a). *Report on work carried out under SNH Plant Science, Research and Education Licence 10925 – Najas flexilis (Slender Naiad)*. Report to Scottish Natural Heritage, Centre for Ecology & Hydrology.

Gunn, I D. M. (2011b). *Report on work carried out under SNH Plant Conservation Licence 9952 - Najas flexilis (Slender Naiad)*. Report to Scottish Natural Heritage, Centre for Ecology & Hydrology.

HMSO (1994). The UK Biodiversity Action Plan.

Hyvärinen, E., Juslén, A., Kemppainen, E., Uddström, A. and Liukko, U.-M. (eds.) (2019). The 2019 Red List of Finnish Species. Ympäristöministeriö and Suomen ympäristökeskus. Helsinki. 704 pages. Finnish Environment Institute. The publication is available in the internet (in Finnish): http://hdl.handle.net/10138/299501.

Inger, S. D., Nisbet, C. and Birkinshaw, N. (2018). *Site Condition Monitoring Report 2018 Loch Kindar.* Report by Ecus Ltd. Scottish Natural Heritage Commissioned Report.

Issakainen, J., Kemppainen, E., Mäkelä, K., Hakalisto, S. and Koistinen, M. (2011). Hentonäkinruoho (*Najas tenuissima*) ja notkeanäkinruoho (*Najas flexilis):* Suomen uhanalaisia lajeja. Suomen ympäristö 13/2011. 223 pages. English Summary: *Najas tenuissima* and *Najas flexilis*, threatened species in Finland. The Finnish Environment 13 / 2011. The publication is available in the internet (in Finnish): http://hdl.handle.net/10138/37048.

James V. A. and Barclay, A. M. (1986). *The occurrence and distribution of Najas flexilis in Loch of Craiglush, Loch of the Lowes and Loch of Butterstone*. Report to Scottish Natural Heritage.

JNCC (2015). *Common Standards Monitoring Guidance for Freshwater Lakes*. Joint Nature Conservation Committee, Peterborough.

JNCC (2019a). *England supporting documentation for the conservation status assessment for the species: S1833 – Slender Naiad (Najas flexilis) as part of the Fourth Report by the United Kingdom under Article 17 of EU Habitats Directive*. Joint Nature Conservation Committee, Peterborough.

JNCC (2019b). *Scotland supporting documentation for the conservation status assessment for the species: S1833 – Slender Naiad (Najas flexilis) as part of the Fourth Report by the United Kingdom under Article 17 of EU Habitats Directive*. Joint Nature Conservation Committee, Peterborough.

JNCC (2019c). *UK conservation status assessment for the species: S1833 – Slender Naiad (Najas flexilis) as part of the Fourth Report by the United Kingdom under Article 17 of EU Habitats Directive*. Joint Nature Conservation Committee, Peterborough.

Kalliovirta, M. and Ahola, A. 2(017). Notkeanäkinruoho (*Najas flexilis* (Willd.) Rostk. & W. L. E. Schmidt). In: Nieminen, M. & Ahola, A. (eds.), Euroopan unionin luontodirektiivin liitteen IV lajien (pl. lepakot) esittelyt, pages 233-236. Suomen ympäristö 1/2017. English Summary: Presentation of the species (except for bats) in Annex IV of the European Union's Habitats Directive. The Finnish Environment 1/2017. The publication is available in the internet (in Finnish): http://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/79301/SY_1_2017.pdf.

Kemppainen, E., Mäkelä, K. and Uotila, P. (2012). Notkeanäkinruoho – *Najas flexilis*. In: Ryttäri, T., Kalliovirta, M. and Lampinen, R. (eds.), Suomen uhanalaiset kasvit (Threatened species in Finland), pages 243–245. Tammi, Helsinki [In Finnish].

Maberly, S.C., De Ville, M. M., Feuchtmayer, H., Jones, I. D., Mackay, E. B., May, L., Thackeray, S. J. and Winfield, I. J. (2011). *The limnology of Esthwaite Water: historical change and its causes, current state and prospects for the future*. Report to Natural England, Centre for Ecology & Hydrology.

Maberly, S.C., De Ville, M. M., Thackeray, S. J., Ciar, D., Clarke, M., Fletcher, J. M., James, J. B., Keenan, P., Mackay, E. B., Patel, M., Tanna, B., Winfield, I. J., Bell, K., Clark, R., Jackson, A., Muir, J., Ramsden, P., Thompson, J., Titterington, H. and Webb, P. (2016). *A survey of the status of the lakes of the English Lake District: the Lakes Tour 2015.* Report to United Utilities, Centre for Ecology & Hydrology.

McKenzie, S. W., Baxter, E., Korba, L., Stewart, N. and Birkinshaw, N. (2018). *Site Condition Monitoring Report 2016 Dunkeld and Blairgowrie Lochs SAC; Lochs Clunie and Marlee SSSI; and Lochs Butterstone, Craiglush, and Lowes SSSI*. Report by Ecus Ltd. Scottish Natural Heritage Commissioned Report.

NPWS (2019a). *The Status of EU Protected Habitats and Species in Ireland. Volume 1: Summary Overview.* Unpublished National Parks and Wildlife report.

NPWS (2019b). *The Status of EU Protected Habitats and Species in Ireland. Volume 3: Species Assessments.* Unpublished National Parks and Wildlife report.

O'Connor, A. (2013). *Article 17 assessment form and audit trail for Najas flexilis, the Slender naiad (species code 1833)*. Backing document. April 2013. National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Ireland.

Palmer, M. (1989). *A botanical classification of standing waters in Great Britain: and a method for the use of macrophyte flora in assessing changes in water quality*. Nature Conservancy Council, Peterborough.

Scott Wilson (2004). *Assessment of Najas flexilis in Tangy Loch, Kintyre*. Scott Wilson Ltd report to Scottish Natural Heritage.

Scott Wilson (2009)*. Control of invasive non-native species in priority mesotrophic lochs*. Scott Wilson Ltd report to Scottish Environment Protection Agency. Research reference R80014.

Stringer, A. P., Blake, D. and Gaywood, M. J. (2015). *A review of beaver (Castor spp.) impacts on biodiversity, and potential impacts following a reintroduction to Scotland*. Scottish Natural Heritage Commissioned Report No. 815.

Thiry, E., van de Weyer, K. and Abts, U. W. (2005). *Re-establishment Plan for the Natura 2000 species Najas flexilis in Poland.* Ministry of Environment report, Warsaw, Poland.

Waters-Hart, K. E (2020). *Assessing aquatic macrophyte recovery responses following lanthanum-modified bentonite applications*. PhD thesis, University of Stirling.

Wingfield, R. A. (2002). *The Functional Ecology of Najas flexilis*. PhD thesis, University of Glasgow.

Wingfield, R. A., Evers, S. and Murphy, K. J. (2002). *Survey of Najas flexilis, slender naiad in Loch Kindar*. Glasgow University report.

Wingfield, R. A., Murphy, K. J., Hollingsworth P. and Gaywood, M. (2004). *The Ecology of Najas flexilis.* Scottish Natural Heritage Commissioned Report No. 017.

CREW CENTRE OF EXPERTISE FOR WATERS

CREW Facilitation Team

Hydro Nation International Centre

James Hutton Institute

Craigiebuckler

Aberdeen AB15 8QH

Scotland UK

Tel: +44 (0)1224 395 395

Email: enquiries@crew.ac.uk

www.crew.ac.uk

Hydro Nation International Centre

The James Hutton Institute

Scottish Government
Riaghaltas na h-Alba
gov.scot

CREW is a partnership between the James Hutton Institute and all Scottish Higher Education Institutes and Research Institutes. The Centre is funded by the Scottish Government.