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The Ecology of *Najas Flexilis*. Scottish Natural Heritage Commissioned Report No. 017

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COMMISSIONED REPORT

Commissioned Report No. 017 The Ecology of *Najas flexilis*

(ROAME No. F98PA02)

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The Ecology of *Najas Flexilis*

Commissioned Report No. 017 (ROAME No. F98PA02)

Contractor: Glasgow University

Background

Najas flexilis, slender naiad, is a submerged rooted macrophyte occurring in lochs. It is protected under domestic and international legislation. In order to protect this species information is required on the ecological tolerances of the plant and the main threats to the species need to be elucidated. In addition, effective monitoring strategies are required to identify when and where the plant is endangered. The UK Biodiversity Action Plan for this species suggests reintroduction of *N. flexilis* to certain sites. If this is to occur information is required on when to reintroduce the plant, in what form (seed or plant) and from where. This study attempts to address some of these gaps in our knowledge of the ecology of *N. flexilis*. This report is a summarised version of the PhD thesis of Wingfield (2002).

Main findings

- *N. flexilis* fitness can be assessed using the trait measurements (leaf area/shoot length) x (reproductive number/shoot length). This can be predicted for Scottish and Irish sites.
- The two main threats to *N. flexilis* appear to be eutrophication (in eutrophic, high pH, conditions plants cannot photosynthesise due to a lack of CO₂ for this obligate dissolved CO₂ – user species); and acidification, when plants appear unable to produce seeds in low pH conditions.
- *N. flexilis* “present” (recorded within the past 4 years) and “absent” (recorded >4 years ago but not subsequently) sites differ significantly in pH, conductivity, alkalinity, calcium, magnesium, potassium and total phosphate levels in the water; and iron and phosphate levels in the sediment.
- *N. flexilis* was found to grow in National Vegetation Classification (NVC) (Rodwell, 1995) communities A13, A24a and A23. It was also found to grow in Palmer’s (1989) Standing Water Types (SWT) 3, 4 and 5. The SWT whole-loch plant community description was a better indication of the ability of the loch to support *N. flexilis* than the NVC micro-habitat plant community description. *N. flexilis* appeared always to thrive in type 4 lochs, but the most oligotrophic type 3 and most eutrophic type 5 lochs could not support *N. flexilis*.
- The presence of invasive *Elodea canadensis* and/or *Elodea nuttallii* has been considered as threats to *N. flexilis*. Although both *Elodea* spp. can be found to grow with *N. flexilis* with no observed detrimental effects, there are also reports of *N. flexilis* being out-competed by *Elodea* spp.

- *N. flexilis* individuals appear to be genetically more similar to other individuals within the same loch than to those from other lochs. There does not appear to be a link between geography and genotype. No relationship was found between genotype and environment. A relationship was found between genetic variability within a population and exposure.
- *N. flexilis* was found to germinate best in light, deoxygenated conditions at 16°C and above; and in dark, deoxygenated conditions at 11°C. These conditions would typically prevail in late June in Scottish lochs.

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1 INTRODUCTION

1.1 Introduction

Najas flexilis (Willd.) Rostk. & Schmidt is a submerged rooted macrophyte, which occurs in lochs. It is listed in Annexes II and IV of the EC Habitats Directive 92/43/EEC. It is also protected under domestic legislation, being listed in Schedule 8 of The Wildlife and Countryside Act, 1981, and Schedule 4 of The Conservation (Natural Habitats &c.) Regulations, 1994. The Najadaceae family is classified as elodeid in form (Den Hartog & Segal, 1964). The vegetative structure of *N. flexilis* appears visually similar to other members of this functional group, but it has some unusual attributes for an elodeid plant (or indeed any aquatic plant). *N. flexilis* is relatively short, rarely reaching above 30cm tall. It does not grow to the water surface and lives its entire life cycle completely submerged. The Najadaceae is one of nine plant families that flower underwater. Hydrophily (water pollination) occurs in less than 5% of aquatic species overall and is present in both marine and freshwater angiosperms (Cox 1988; Les 1988; Philbrick & Les 1996). What makes this state more unusual is that *N. flexilis* cannot reproduce vegetatively. In this respect, it is unlike the majority of aquatic plant species, particularly those exhibiting hydrophily (Hutchinson, 1975). *N. flexilis* is also an annual. This reliance on seed production, annual state and completely submerged life style is unusual for an aquatic plant and has consequences for growth, dispersal and establishment.

1.2 Description of *N. flexilis*

N. flexilis is a glabrous plant with linear, sessile leaves opposite or whorled, with denticulate sheaths. Flowers are inconspicuous, 1–3 are found in each leaf axil. This species is a monoecious annual; the flowers are hypogynous and sessile. The male flowers are surrounded by scales and possess one sessile anther; female flowers have one ovule, one carpel and 2–4 elongated stigmas, and are scaleless. The fruit is a sessile drupe (Hellquist & Crow, 1980; Preston & Croft, 1997; Stace, 1997). The seeds are easily identifiable by their smooth shiny brown coat. The seeds are elliptical, oblong, with a persistent membranous pericarp. The testa is smooth with squarish to hexagonal areoles regularly arranged in longitudinal rows (Triest, 1988).

1.3 Taxonomy

The Najadaceae family contains one genus, *Najas*, with forty species, all of which are submerged aquatics. Although the genus is cosmopolitan, the majority of the species can be found in the tropical and subtropical regions (Cook, 1996; Preston & Croft, 1997). The species are either monoecious or dioecious and most are seed producing annuals. Vegetative reproduction in the entire genus appears to be rare (Triest, 1988). All the *Najas* species are hydrophilous and can grow in brackish or freshwater. Two subgenera are reported *Caulinia* and *Najas*. *N. flexilis* belongs to *Caulinia*, which includes species that are usually slender, monoecious and lack spines on their stems (Triest, 1988).

1.4 Distribution

1.4.1 World and European

N. flexilis can be found in North America, Europe and Asia. However, the frequency of its distribution is not even. The plant has a much denser frequency in North America (Haynes, 1979) compared to the sparse

disjunct European range (Godwin, 1975). This distribution is not uncommon in aquatic plants. *Eriocaulon aquaticum* and *Potamogeton epihydrus* have an even more unequal cross-Atlantic distribution (Preston, 1995; Preston & Croft, 1997). The unequal distribution of *N. flexilis* has been described by Hultén & Fries (1986) as amphi-atlantic. However, Preston & Hill (1997) describes the distribution as disjunctly circumpolar. The plant's range in Europe is not restricted to the west and its range in Asia is incompletely known, although it is found as far east as Mongolia (Hultén & Fries, 1986). This suggests that despite the denser frequency of sites in America the species is indeed circumpolar.

Within Eurasia, its climatic region is classified as Boreal-montane by Preston & Hill (1997). In western and central Europe, the Boreal-montane region is concentrated within the Alps, Scandinavia, Britain and Ireland. It is evenly spread across Britain, but concentrated in the north and west of Ireland. *N. flexilis* has a northerly distribution in Europe today and can be found between the latitudes of 57° and 62°N. The British distribution of *N. flexilis* is now limited to Scotland. Within Europe, the species is most densely frequent in Scotland and Ireland.

1.4.2 Scotland and Ireland

N. flexilis was first found in Cregduff Lough just outside Roundstone in Connemara, Ireland in 1850 by Daniel Oliver (Oliver, 1851). It was later found in Scotland in 1875 at Loch Clunie, Perthshire (Sturrock, 1875). *N. flexilis* was also found in England in 1914 by Pearsall (Pearsall, 1915). All records but one for *N. flexilis* in England are from Esthwaite Water, the other is recorded from Lake Windermere (but the grid reference is for the Ferry House on the shore). Since these early records a total of 51 sites in Scotland have been reported to support *N. flexilis* and 28 sites have been reported in Ireland (Table 1.1). The Irish sites are found predominantly on the west coast in three clusters one in the northwest in Donegal, one in Connemara and one in Kerry. In Scotland, the distribution of *N. flexilis* can again be grouped into three main clusters. The majority of the sites are found in the Outer Hebrides, on the west side of North and South Uist. Another cluster can be found in Kintyre and the Inner Hebridean Islands of Islay, Colonsay, Mull and Coll. The third cluster is in mainland Scotland in Perthshire. There are three further sites in mainland Scotland where the plant has been recorded, one in Dumfries and Galloway, one in Stirlingshire and one near Mallaig in northwest Scotland (Fig. 1.1).

1.4.3 Native?

When *N. flexilis* was first discovered in Scotland in 1875 it was known that *N. flexilis* was common in America and the status of *N. flexilis* as a native species was questioned. The native status of *N. flexilis* was particularly questionable as the Rev. Mr M. Ritchie, the minister of the parish of Cluny, and an enthusiastic botanist, was known to have introduced *Stratiotes* and *Butomus* to Clunie Loch. However, after Mr Duff, the 'intelligent and obliging' gardener, confirmed that these plants had all been introduced from Wales (where *N. flexilis* had not been found) it was considered by Abraham Sturrock that *N. flexilis* was a native rarity of Loch Clunie (Sturrock, 1975). Sturrock went on to find a further five new locations for the plant in the Perthshire region in the next ten years.

Despite the increasing number of sites where *N. flexilis* could be found in Scotland, the question of an American origin lingered due to its apparent amphi-atlantic distribution. Such questions were finally quashed by fossil evidence. Fossil evidence showed that *N. flexilis* had been part of the British and Irish flora in the

Hoxnian interglacial, the Ipswichian interglacial and then from the middle Weichselian through into the Flandrian where it was both more widespread and more abundant than it is today (Godwin, 1975). Godwin (1975) states that:

'In a nutshell the present exiguous and extreme westerly range of *N. flexilis* in this country is shown by the fossil evidence to be the consequence of withdrawal in late Flandrian time from a much larger and more easterly range, and an occupation of very long duration.' He also believed that *N. flexilis* is a species of circumpolar distribution whose range has been reduced and restricted in Europe and Asia.

This reduction in sites since the Flandrian time is possibly due to a decrease in temperature since the plant was at its most abundant at the time of the thermal maximum, in zone VI of the late Flandrian. The requirement for long warm summers can be extrapolated from the fossil records in the Boreal times. At such times, *N. flexilis* was found as far north as Lapland in Finland where it is no longer found today (Vasari, 1962). However, it can also withstand cold winter temperatures, as shown by its distribution today. An example of this is that *N. flexilis* can be found on the Mongolian highlands where the summer temperatures are high but the winter temperatures are as low as -20°C . *N. flexilis* would also have had to withstand more constantly cold temperatures when it grew in Britain in the Middle and Late Weichselian. However, Bell (1969) believes that even then the summer average temperatures would not have been so very cold, at approximately 16°C .

Figure 1.1 The distribution of *N. flexilis* in Britain and Ireland.

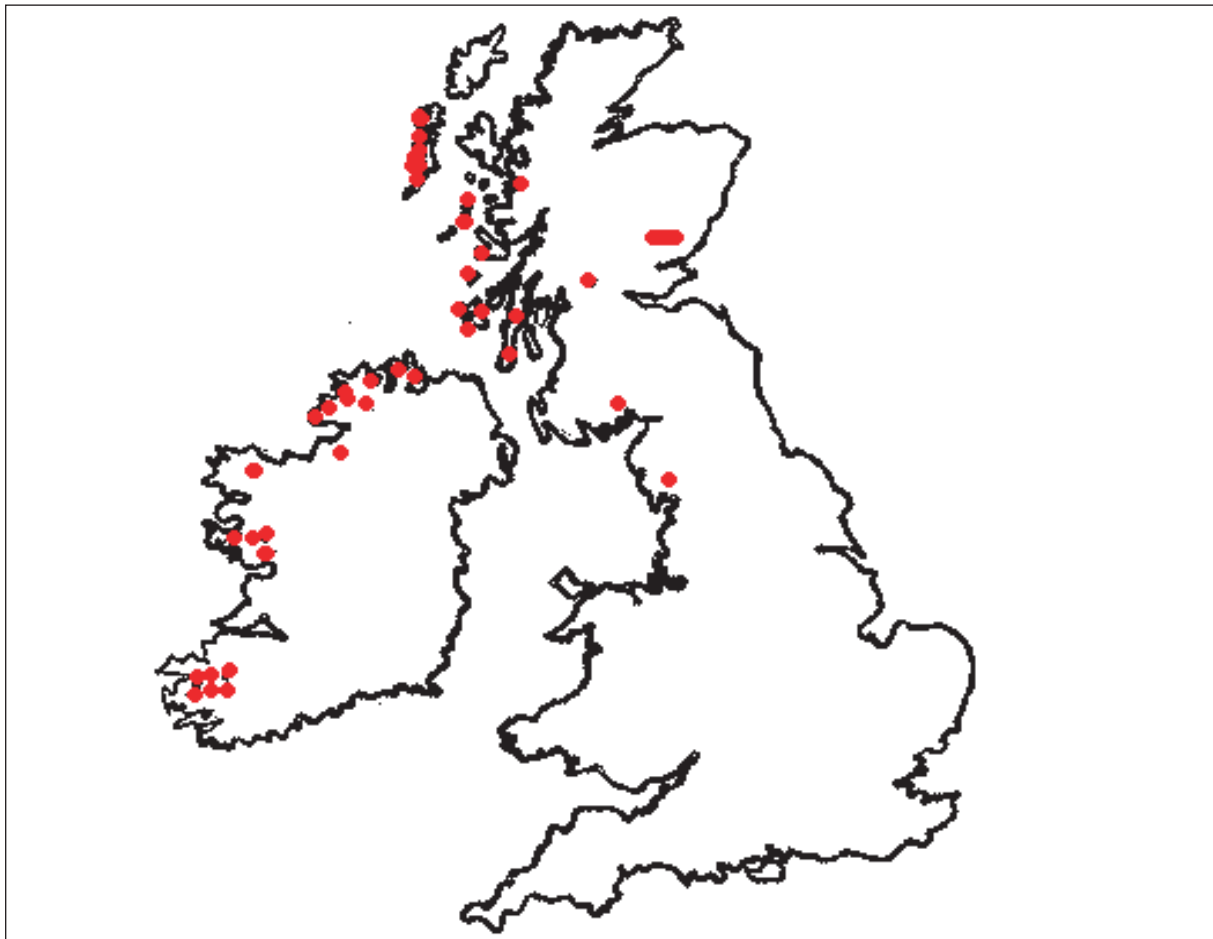


Table 1.1 The sites where *N. flexilis* has been recorded in the British Isles. (Six figure grid references are given if available.)

Site	Grid reference	Date first recorded	Date last recorded	Date last surveyed
Mainland				
Nr River Tay, Aberfeldy	NN85-49-	1929	1929	1929
Loch a' Bhada Dharach	NM695946	1977	2000	2000
Loch of Butterstone	NO062453	1986	1999	1999
Loch of Clunie	NO116444	1875	2000	2000
Loch of Craiglush	NO044446	1977	2000	2000
Fingask Loch	NO165431	1881	1999	1999
Loch Kindar	NX968640	1959	1996	1998
Lindores Loch	NO26-16-	1878	1878	2000
Loch of Lowes	NO055443	1883	2000	2000
Loch of Drumellie (Marlee)	NO14-44-	1881	1997	1999
Lake of Menteith	NN577002	1994	2000	2000
Monk Myre	NO20-42-	1883	1883	1999
Loch Monzievairst	NN84-23-	1884	1884	2000
White Loch	NO16-42-	1881	1882	1999
Inner Isles & Kintyre				
Loch Ballyhaugh, Coll	NM176582	1987	1999	1999
Loch Fada, Colonsay	NR383955	1902	1999	1999
Glenastle Loch, Islay	NR294450	1994	1999	1999
Loch Gorm, Islay	NR230655	1993	2000	2000
Loch Lossit, Islay	NR408652	1949	2000	2000
Loch Nan Gad, Kintyre	NR787575	1971	2000	2000
Loch Poit na h-I, Mull	NM314227	1921	1999	1999
Loch Skerrols, Islay	NR34-63-	1949	1950	1950
Tangy Loch, Kintyre	NR695280	1973	1998	1998
Loch an t-Sagairt, Coll	NM251609	1989	1999	1999
Outer Isles, North & South Uist				
Loch Altabrug	NF745340	1984	2000	2000
Benbecula Aerodrome	NF79-56-	1950	1950	1950
Loch Bun an Ligidh	NF769371	1994	2000	2000
Loch Ceann a Bhaigh	NF76-30-	1940	1940	1940
Loch nan Capull	NF754161	1983	2000	2000
Loch nan Cnamh	NF762355	1994	1999	1999
Loch Cuilc	NF772369	1994	1998	1998
Loch na Cuithe Moire	NF738234	1983	2000	2000
Loch Druidibeag	NF780388	1987	2000	2000
Loch Dun Mhurchaidh	NF79-54-	1940	1940	1940
Loch Eaval	NF724710	1977	1999	1999
Loch an Eilean (Drimsdale)	NF762372	1984	1999	1999
Loch Eilean a' Ghille-ruaidh	NF769366	1984	1999	1999
Loch an Eilean (South Boisdale)	NF748169	1983	1999	1999
Loch nam Faoileann	NF752210	1995	2000	2000
Loch Gearraidh Mhic Iain	NF763360	1994	1999	1999

Table 1.1 (continued)

Site	Grid reference	Date first recorded	Date last recorded	Date last surveyed
Loch Grogary	NF717710	1942	1999	1999
Loch Leodasay	NF80-63-	1982	1982	1982
Loch a'Mhadaidh	NF762353	1994	1999	1999
Loch a' Mhuilinn	NF74-33-	1947	1951	1951
Loch Olaidh Meadhanach	NF758314	1987	2000	2000
Loch Runavat	NF730693	1974	1974	1974
Loch Scarie	NF718705	1994	2000	2000
Loch an Taigh-sgoile (Schoolhouse)	NF763364	1984	1999	1999
Loch ? (no name)	NF766369	1994	2000	2000
Staoinebrig	NF74-33-	1939	1939	1939
South Uist, unnamed loch	NF737327	1994	1994	1994
England				
Esthwaite Water	SD360965	1914	1982	1994
Lake Windermere	SD390956	1977	1977	1977
Ireland				
Lough Adoolig	00645740	1985	1985	1985
Lough Akibbin	24068183	1977	1991	1991
Anaserd Lough	026-4-	1936	1936	1936
Ard Bay Lough (probably Nageeron)	0275-31-	1991	1991	1991
Barnahallia Lough	0259-55-	1975	1975	1975
Carragh Lough	00717905	1877	2000	2000
Cloonee Loughs	008-6-	1957	1957	1957
Clooney Lough	137-9-	1939	1939	1939
Cregduff Lough (Roundstone)	02718392	1850	2000	2000
Lough Dahybaan	13000200	1978	1978	1978
Fin Lough	02840660	1937	1937	2000
Fort Lake	2400-34-	1989	1989	1989
Glenade Lough	13828461	1978	1978	1978
Kiltorris Lough	1368-96-	1989	1989	1989
Lough Kindrum	25193429	1916	2000	2000
Kylemore Lough	02770583	1977	1977	1977
Leane Lough	00932890	1885	2000	2000
Lehanagh Lough	028-5-	1975	1975	1975
Mullaghderg Lough	14767203	1919	2000	2000
Lough Nafeakle	02683475	1977	1977	2000
Lough Nageeron	027-3-	1974	1974	1974
Lough Nagreary	2414-41-	1990	1990	1990
Lough Namanwaun	0266-39-	1881	1881	1881
Pollacapul Lough	02755620	1977	2000	2000
Renvyle Lough (Rusheenduff Lough)	02673640	1957	2000	2000
Sessiagh Lough	24040360	1981	1989	1989
Lough Shennagh	24213452	1989	2000	2000
Sheskinmore Lough	13700960	1981	1981	1981
Tully Lough	02692620	1978	1978	1978

As well as a reduction in temperature the other reason suggested for the restriction of the distribution of *N. flexilis* since Boreal times is the lack of appropriate habitats. In particular, the number of calcareous or basic loch basins unaffected by drainage and other human interference such as eutrophication has been reduced (Godwin, 1975).

This lack of lochs unaffected by human interference is emphasized by the fact that not all the *N. flexilis* sites that have been discovered still support *N. flexilis*. Eight lochs in Britain and Ireland where *N. flexilis* had previously been found were searched specifically for the plant in this study (and other recent surveys), yet the plant has not been found (Table 1.1). It is this decrease in site numbers that has led to concern over the status of this species in Britain and Ireland. However, it is not only in Britain where the species is thought to be in decline. *N. flexilis* is also reported to be on the decline in Ohio possibly due to increased turbidity of the lochs (Wentz & Stuckey, 1971).

1.5 Conservation and *N. flexilis*

The protection of *N. flexilis* by the European Habitats Directive requires that Special Areas of Conservation (SAC's) be designated where the species occurs, in order to protect it. The statutory body then undertakes surveillance of the species at these sites. Five such areas are proposed for *N. flexilis* in Tayside, Coll, South Uist, North Uist and Colonsay. In order that these protected sites are managed adequately, information is required on exactly what conditions are suitable for *N. flexilis* growth, so that these conditions can be maintained. The European Habitats Directive requirements overlap with the targets and objectives for *N. flexilis* according to the Biodiversity Action Plan (BAP) for this species. The three main objectives and targets of the BAP are to:

1. Clarify the status of the species in the UK.
2. Safeguard the remaining populations.
3. If feasible restore to former sites.

The majority of the proposed actions in the BAP (proposed actions 5.1.1, 5.2.1–5.2.3) relate to safeguarding the species via maintaining or creating suitable conditions for the plant to grow. However, at the advent of this study it was unknown precisely what these conditions were and what the threats to the species might be, although the restriction of light penetration due to weed growth or nutrient enrichment were suggested as factors causing loss or decline. Three further issues are also dealt with by the BAP proposed actions:

1. Establish a strategy for monitoring and maintaining the natural distribution of this species in Scotland (proposed action 5.1.2).
2. Collect seed from a range of sites for preservation at RBG Kew's seed bank (proposed action 5.3.1).
3. If *N. flexilis* is found to be absent, but conditions are suitable, consider restoring to former sites (proposed action 5.3.2).

As the issue of seed collection is addressed as part of the work by the millennium seed bank, the three remaining primary conservation objectives for *N. flexilis* addressed in this project were to determine:

- the conditions in which *N. flexilis* thrives; thereby also determining the main threats to the species.
- how to monitor the species.
- the best way to reintroduce the species to extinct sites.

1.6 Aims

This study provided information to help meet the conservation objectives by fulfilling the following aims:

- To investigate the environmental conditions determining the distribution of *N. flexilis* both within and between lochs.
- To investigate the ability of loch community descriptions to describe the loch environment where *N. flexilis* grows.
- To investigate the effect of macrophyte competition on *N. flexilis*.
- To investigate the population genetic structure of *N. flexilis*.
- To investigate the environmental factors controlling *N. flexilis* germination.

This study was carried out as a PhD project and a full thesis has been produced, this report is a distillation of the PhD thesis (Wingfield, 2002). Further information can be found in the thesis, a copy of which is held by Scottish Natural Heritage.

2 FITNESS OF *N. FLEXILIS* POPULATIONS IN RELATION TO LOCH ENVIRONMENTAL CONDITIONS

2.1 Introduction

2.1.1 Aims

- Assess the current status of *N. flexilis* in all post 1980 recorded sites in Scotland.
- Survey sites with older records where the plant is thought to be extinct, to determine the environmental habitat range of *N. flexilis*.
- Determine the relationships between the target plant, and environmental factors, concentrating on plastic phenotypic traits of the species.
- Develop a predictive model of *N. flexilis* success in relation to environmental controls.
- Use the model to assess what the threats to the present populations are.
- Determine the factors which control the distribution of the plant within lochs, so that *N. flexilis* can be reintroduced to a position where it can thrive.

2.1.2 Background

N. flexilis has its European stronghold in Scotland, with 35 sites at which there have been positive identifications during 1998–2000. There are also a number of sites where the plant is believed to be extinct. There are eight sites in Scotland and Ireland with previous records for the plant, but where searches during 1998–2000 failed to find it. *N. flexilis* is also believed to be extinct at the only post-1980 site in England (Esthwaite Water) after a specific search for the plant in 1994 failed to locate it (Wade, 1994). Due to the high conservation status of the plant and this apparent trend of extinctions, knowledge of the environmental conditions controlling plant success is required in order to conserve it. An effective method of assessing plant success within a site is also required, so that sites at risk can be identified and appropriate action taken to prevent further extinctions.

2.1.3 Loch chemistry

Most of the information on the environmental conditions in which *N. flexilis* grows is from American populations of the species. Studies on macrophyte community composition, in relation to environmental parameters in American lakes have elucidated a range of pH and alkalinity values at which *N. flexilis* can grow (Table 2.1).

Table 2.1 The pH and conductivity range of sites where *N. flexilis* can grow according to the literature on its distribution in North American lakes.

Environmental variable	Range	Reference
pH	6.0–9.0	(Jackson & Charles, 1998; Moyle, 1945; Roberts <i>et al.</i> , 1985)
Alkalinity (mg/l)	6–307.7	(Jackson & Charles, 1998; Moyle, 1945; Pip, 1988; Roberts <i>et al.</i> , 1985)

Table 2.1 shows that a circum-neutral pH appears to be important in the distribution of *N. flexilis*. Experiments by Titus & Hoover (1991, 1993) indicate that at a low pH the reproductive capacity of *N. flexilis* is limited. They found that seed production per *N. flexilis* plant dropped from 95.5 to 0.25 seeds/plant, when water pH was decreased from 7.5 to 5. *N. flexilis* is an annual and therefore relies on seed for perennation, as a result at pH 5 *N. flexilis* populations would quickly become extinct. It is not known where within the pH range of 7.5–5 seed production would begin to fall or whether in natural systems pH is the only factor controlling reproductive success.

Another example of *N. flexilis* preferring a more neutral pH was shown after the liming of Thrush Lake in Minnesota. This was carried out to increase pH from 6.46 to above 7 in an attempt to improve fishing. The increased pH first led to a decrease in *Nitella* spp., then *N. flexilis* and *Potamogeton pusillus* appeared for the first time the following year. As conditions returned to their state prior to liming, the *Nitella* spp. grew back, the competitive advantage of *N. flexilis* was lost and it concurrently decreased (Hagley *et al.*, 1996).

Physiological experiments have also illustrated some of the relationships between *N. flexilis* and its environment. Wetzel (1969) and Wetzel & McGregor (1968) show that calcium levels of up to 10mg/l and magnesium levels of up to 5mg/l appear to have a positive effect on the rates of carbon fixation. However, calcium levels of >30mg/l and magnesium levels of >10mg/l, both greatly exceeded in hard water lochs, lead to a decline in rates of carbon fixation and altered the secretion rates of dissolved organic matter in *N. flexilis*. Increasing the level of sodium had the contrary effect of increasing both of the above. Wetzel & McGregor (1968) found that high concentrations of Ca^{2+} and Mg^{2+} , resulting in low monovalent:divalent cation (M:D) ratios, inhibit carbon fixation; that Mg^{2+} can offset to a limited extent the inhibitory effects of Ca^{2+} and that M:D ratios $\gg 1$ provide optimal conditions for growth. However, the mechanisms resulting in these effects were not explained.

Hough & Fornwall (1988) and Hough & Wetzel (1978) have illustrated that, unlike hard water plants, *N. flexilis* is an obligate carbon dioxide (CO_2) user and cannot utilise bicarbonate (HCO_3^-). This reduces the photosynthetic capacity of *N. flexilis* in hard waters and therefore restricts its distribution. However, *N. flexilis* has been reported to occur at pH 9.0 where there can be virtually no CO_2 . This appears to be the upper limit of the plant's range and the plant has not been reported frequently at such a high pH (Table 2.1). All the pH measures from the studies referred to in Table 2.1 are snapshots of a loch's pH, and whilst *N. flexilis* can obviously survive exposure to such a high pH for a short time, whether it can survive prolonged exposure to a pH as high as this must be considered.

This might help explain the intriguing UK distribution of *N. flexilis* (see section 1.4.2). In mainland Scotland it grows mostly in lochs along the Highland Boundary Fault. In the Hebrides it grows on the border of the division between machair and moorland and formerly on the border of hard Borrowdale volcanic rock and sedimentary Silurian rocks in the Lake District. These locations mean that, almost without exception, the lochs are influenced both by the base-rich input of sedimentary rocks and sands, and the water run off from the catchments of acidic peatlands or hard igneous rock, thus supplying the very particular conditions in which *N. flexilis* appears to thrive.

The phosphorus requirement of the plant comes overwhelmingly from the sediment (Carignan and Kalff, 1987; Moeller *et al.*, 1988). This illustrates that for *N. flexilis* the nutrient status of the sediment rather than the water is what is important for nutrient uptake. However, the nutrient status of the water is still important

because it determines competition, especially from algae. Moeller *et al.* (1988) also showed that *N. flexilis* does not work as a pump for phosphorus from sediment to the water body as very little phosphorus is released from the leaves to the water.

2.1.4 Physical factors; depth, light, exposure and sediment structure

Preston & Croft (1997) state that, in the British Isles, *N. flexilis* can be found in water less than 1m deep, but is usually found at depths of 1.5m or more. The depth at which macrophytes grow is a result of a number of factors: these include light and exposure. As depth increases the amount of light available to the plant decreases. This will be affected by the water clarity, which is altered due to suspended solids, algal growth and the properties of the catchment. *N. flexilis* has been found by Pip & Simmons (1986) in Shoal Lake, Manitoba–Ontario at a depth of 12–14m. This is an extreme depth for any macrophyte and thought to be a result of exceptionally clear water, with Zen (zenith extinction coefficient) at 0.5–1% of the surface light, warm summer temperatures in the deep waters and the presence of oxygen in the sediments during the ice-free season.

Exposure affects the physical characteristics of the sediment, as well as having a direct effect on the plant. On exposed shores, elutriation takes place resulting in coarse particles and compacted soils, whereas on sheltered shores or at depth (where the effect of exposure is less) the finer soils develop (Spence, 1972). *N. flexilis* appears to grow in a particular silty, organic, flocculent sediment. This has been noted by McKenna (1984) in Kahle Lake, US, and in Scotland, in the lochs in Perthshire by James and Barclay (1996) and as a general habitat requirement of the plant in the British Isles by Preston & Croft (1997). Pearsall (1920) also noted that in Esthwaite Water, English Lake District, *N. flexilis* grows on fine semi-liquid muds with an organic content of 5–10 % and that it is the first colonist and takes the place often occupied elsewhere by *Nitella*. The intensity of exposure depends on the shape, depth and size of the loch as well as wind direction (Keddy, 1982; Weisner, 1987). Therefore, depending on the nature of the loch, suitable fine silty soils may have developed at a number of different depths. It is most probable that it will be an interaction between exposure and water clarity, which will primarily determine the locality of *N. flexilis* within a loch.

2.1.5 Competition

Another factor determining the locality of *N. flexilis* within a loch is interspecific competition. The competitive ability of *N. flexilis* will vary depending on environmental conditions such as light and carbon dioxide concentration. It could be presumed that in hard water lochs bicarbonate utilising species would have a competitive advantage over an obligate carbon dioxide utiliser such as *N. flexilis*. However, bicarbonate utilisation requires more energy and Hough & Fornwall (1988) observed *N. flexilis* to be significantly competitive when light limitation reduced the efficiency of bicarbonate user *Potamogeton pectinatus*.

It has already been discussed (section 2.1.3) how increasing pH from 6.46→7.0 increased the competitive ability of *N. flexilis* over *Nitella* spp. However, it is also suggested that *N. flexilis* thrived, in this situation, due to the disturbance during the liming of the loch and grew in trenches and holes presumed to be created by anchors and nets (Hagley *et al.*, 1996). This suggests that *N. flexilis* is a disturbance tolerator, which might be expected due to its annual life cycle (Grime, 1988) and Pearsall's (1920) observation that it is often the first colonist. The plant's preference for the more liquid muds where there is often erosion or deposition of sediment, i.e. disturbance, agrees with this life strategy. Kahle Lake, US, is an artificial lake,

which consists of an impounded creek. Two years after the lake's creation *N. flexilis* was reported to be a problem species (Tazik *et al.*, 1982). This supports the view of *N. flexilis* as an early colonist taking advantage of freshly disturbed environments. Further indication that disturbance is acceptable for *N. flexilis* has been the failed attempts to control *N. flexilis* growth by winter draw down in Kahle Lake, which is actually thought to have enhanced its growth (Tazik *et al.*, 1982). This pattern of events was also seen after the dredging of Lily Lake, Wisconsin, US. Previous to dredging *N. flexilis* was not present in any of the studied areas, but the growth season after the disturbance *N. flexilis* had replaced the less disturbance-tolerant species such as *Potamogeton robbinsii*. However, at one site within the lake it had already been out competed by the following growth season (Nichols, 1984). The decline of *N. flexilis* after the cessation of disturbance suggests that *N. flexilis* is more likely to be a poor competitor in stable conditions, and better suited to disturbed conditions, which could limit its distribution within lochs.

2.2 Methods

2.2.1 *N. flexilis* presence and absence

During the course of the study, 42 lochs were investigated in Scotland, (July–September, 1998–2000), together with 10 loughs in Ireland (August 2000) see Table 3.1. Only sites where *N. flexilis* had previously been recorded on at least one occasion were included. A set of environmental factors likely to be relevant to the ecology of *N. flexilis* in Scotland, was included in a field survey of lochs from which *N. flexilis* had been recorded (post 1980). Mainland sites where the plant had been recorded before 1980 were also surveyed, this included sites where the plant is thought to be extinct. Only mainland pre-1980 sites were surveyed in this study because of logistical reasons. At each site *N. flexilis* presence or absence was recorded. Measurements of environmental factors could then be compared between sites where *N. flexilis* was present or absent.

The location of *N. flexilis* populations was determined within a loch from past records, where available, especially the Scottish Loch Survey data (held by Scottish Natural Heritage) and the results of M. Gaywood's (personal communication 1998) previous surveys for the plant. Other sources of information used were the surveys carried out on the lochs in Perthshire by James & Barclay (1996) and Howson *et al.* (1997) and the survey of South Uist lochs by staff of the Royal Botanic Garden, Edinburgh (McKean *et al.*, 1983). Three basic survey methods were used to locate *N. flexilis* within the loch; boat, shoreline and snorkel surveys. Snorkel surveys were considered the most effective for finding *N. flexilis*, agreeing with comments in the literature (Capers, 2000), and were adopted as the standard approach wherever feasible. Boats were used when the lochs were particularly large and there was no knowledge of exactly where the plant could be found; this enabled a larger area to be surveyed. When boats were not available, a shoreline search was carried out. A shoreline search included searching the drift, wading into the water as far as safety would allow, and throwing grapnels into the deeper water (Table 3.1).

2.2.2 Quantitatively assessing *N. flexilis* status within a loch

It was also important to see whether there was a relationship between the environmental factors, which appeared to differ between present and absent sites, and the success of *N. flexilis* in sites where it still grew. A quantitative relationship between these parameters would give further evidence to indicate which environmental factors were causing the decline of *N. flexilis* in Scotland. In order to determine the success

of a plant at a particular site, plant abundance is often used. This was not possible for *N. flexilis* due to a number of methodological reasons. Preliminary surveys showed that *N. flexilis* could not be seen at all sites by snorkelling at the water surface. This was due to the deep water typically inhabited by *N. flexilis* and the coloration of the water (often peat stained). Tests in clear water showed that values of abundance attained by grapnelling did not correspond to the abundance of *N. flexilis* observed growing in the loch. *N. flexilis* was consistently under recorded by this method, agreeing with comments in the literature (Capers, 2000). Scuba diving or the use of underwater video cameras could have been an option, but resources prevented the use of these two approaches.

Instead, measures of the plant attributes were used. In clear water, the plants required to gain these measures could be collected by duck diving or careful use of a grapnel, where entire plants could be dislodged from the sediment and scooped up. In low visibility lochs, populations of *N. flexilis* were located with the use of grapnels, and then the plants were collected by use of an Ekman grab, which can collect entire plants for trait measurement.

2.2.2.1 Traits

Assessment of performance or success of a species involves the measurement of some relevant part of a plant to provide an index of growth rate or vigour. Typical measurements are leaf size, length and shape, plant height and flower or fruit characteristics, the latter providing information about reproductive allocation (Kent & Coker, 1995). Specific plant traits or combinations of traits are often used as indicators of a species' fitness. This has often been used to look at the genotypic effect on plant fitness (Dudash, 1990; Fischer & Matthies, 1998a; Fischer & Matthies, 1998b; Oostermeijer *et al.*, 1994; Oostermeijer *et al.*, 1995; Ouborg & Van Treuren, 1995; Ouborg *et al.*, 1991). Plant traits have also been used to compare the fitness of species within the same genus under different environmental regimes (Sultan, 2001). The ideal fitness measure would include; seed production, probability of seed germination, probability of seedling survival to adulthood and reproductive number of those individuals that survived to adulthood, as used by Dudash (1990). However complete experiments following the success of progeny are not always viable. Instead, probability of the seed reaching maturity and reproducing is substituted by reproductive weight or vegetative success of the reproducing adult, as this represents the resources allocated or resources available to the offspring. Such measures are used by Sultan (2001).

Such fitness traits are phenotypically plastic within genotypic constraints, this plasticity will be determined by the way in which the genotype of the organism interacts with its environment. It is this environmentally driven plasticity which can be used to determine the realised niche of *N. flexilis* in Scotland, as the patterns of fitness response (along with the growth traits that underlie them) determine the range of conditions in which species may survive and successfully reproduce and hence their ecological breadth (Sultan, 2001). Phenotypic plasticity has successfully been related to environmental variables in the field such as specific leaf area and depth as a function of light (Spence *et al.*, 1973).

Three whole plant samples were randomly collected from each population, and trait analysis undertaken on the fresh plants. The plant traits listed in Table 2.2 were measured for each plant. Leaf and root area was measured using a flatbed optical scanner downloading images to a PC. Customised Delta-T scan plant analysis software was then used to analyse the images to produce the required data (Ali *et al.*, 1999). The problem with relating wild population plant traits to the environment is that they can be affected by the age of the individual, which is often unknown in wild perennial populations. However, this problem is avoided

when measuring plant traits of an annual such as *N. flexilis*. To account for the differences found in plants collected at the beginning, to those at the end of the field season all traits were divided by shoot length, a function of age.

Table 2.2 Traits measured for each *N. flexilis* plant collected.

Leaf area
Leaf weight
Root weight
Root length
Root area
Stem weight
Number of reproductive structures
Weight of reproductive structures
Shoot length
Number of branches

2.2.3 Environmental measures

In order to assess the relationship between *N. flexilis* fitness and the environment a number of environmental measures were taken at each site. Conductivity and pH were measured with Hanna portable meters, and photosynthetically active radiation (PAR) was measured with a Skye SKP210 twin-sensor meter, on site. The readings were taken at the water surface and 0.5m beneath the water surface. From these readings, the light extinction coefficient was calculated using the standard formula (Wetzel, 1983). The extinction of PAR was used rather than PAR itself in all the following analyses due to the methodological difficulties in measuring light in shallow waters. One water sample per loch was collected at a sub-sample of the sites (n = 35) and analysed by the Scottish Environmental Protection Agency for alkalinity, iron, calcium, magnesium, ammonia, total oxidised nitrogen (TON), dissolved reactive phosphate (DRP), total phosphate (TP) and potassium. All these measurements were made after filtration so as to exclude particulates. One sediment sample per loch was also taken where *N. flexilis* was found to grow. These were collected by Ekman grab due to the deep water in which *N. flexilis* grew. Therefore, all samples were collected from approximately the same depth in the sediment as the same apparatus was used at each site. The sediment was then dried in the oven at 80°C then ground with a pestle and mortar and sieved with a 2mm sieve to remove solid particulates. These were analysed by the Scottish Agricultural College for extractable phosphorus, calcium, and total iron and total nitrogen. A subset of sites (n = 33) were also analysed for extractable potassium, sodium and magnesium content

Exposure was calculated using a simplified version of the formula used by Keddy (1982) and Weisner (1987) where:

$$E = \sum_{i=1}^8 \text{exceedance}_{45i} \times \text{fetch}_{45i}$$

Fetch is the distance in km from the nearest shoreline and E = exposure. In Keddy (1982) exceedance is the number of times the wind speed was greater than 12mph. Since a number of geographically disparate lochs were used in this study, exceedance could not be measured at every site, and the method was simplified. All west-facing compass points were doubled, this is because the predominant wind direction for Scotland and Ireland is from the west. Therefore, exceedance was assumed to be 2 for westerly directions and 1 for the rest.

The method used does not take into account the differing wind force experienced by the different sites and assumes they are the same. This is obviously not true, some lochs occur right next to the Atlantic coast where wind forces are greater whilst others occur in sheltered hollows in the landscape. This method was used due to the geographic distance between sites preventing in-depth meteorological measurements to be made. A better estimate of exceedance would make the calculation of exposure more accurate.

2.2.4 Competition

An Ekman grab was used to take 20 x 20cm² samples of the vegetation, three such samples were taken within the *N. flexilis* population community. The mean dry weight for the three samples was used as the quantitative measure of biomass, for that site. Roots were included in these samples, as nearly all roots were collected within the Ekman grab. In contrast to the measurements of the *N. flexilis* plants it did not matter if the roots were detached from the rest of the plant when the plants were extracted from the sediment collected in the Ekman grab in the laboratory, as only a measure of biomass was required. This was calculated twice; once including *N. flexilis* plants and once excluding them. This measure was used as an indication of the total competition and the interspecific competition that the *N. flexilis* specimen plants were experiencing from other macrophytes.

2.2.5 Data analysis

The sites where *N. flexilis* was absent (i.e. not found in this study) are split into two groups. The first absent group contains the sites that appear to be the more oligotrophic absent sites and the second absent group contains the sites that appear to be the more eutrophic absent sites. The environmental variables of both the absent groups were compared to those where *N. flexilis* was present, using a Mann-Whitney U test. This test was chosen due to the small sample size in the more oligotrophic absent group, and the non-parametric nature of the data due to the detection limit imposed on many of the environmental variables (the concentration of iron, total phosphate, dissolved reactive phosphate, total oxidised nitrogen and ammonia in the water are all ordinal data because of the detection limit, the rest are continuous variables). The Mann-Whitney U tests are used to see if there is a significant difference between the environmental variables measured at present and absent sites. If an environmental variable is significantly different between present and absent sites, it is an indication that the environmental variable is involved in the processes leading to the decline of *N. flexilis*.

All data were tested for normality using Ryan Joiner test procedures and appropriate transformations applied to normalise individual data sets. Stepwise multiple regression procedures were used to relate *N. flexilis* fitness to environmental variables and biomass, residuals were tested for normality and randomness against fitted values on the completion of any modelling process. The multiple regression procedures will determine if there is a relationship between *N. flexilis* fitness and the environmental variables measured. This will determine whether the environmental variables found to be significantly different between present and absent sites have a relationship with *N. flexilis* growth and development.

Sites were grouped into three depth categories (<1m, 1–2m, >2m) depending on the depth at which *N. flexilis* was found to grow at that site. The light extinction coefficient and the surface exposure experienced by the sites within these three groups were then compared. The groups were compared using an one-way analysis of variance (ANOVA) and Tukey's post hoc test. An ANOVA was used to analyse these data as both the light extinction coefficients and the measures of exposure are parametric as they are continuous variables. This is different to the other environmental measures where the lower end of the scale is not continuous. This is because many of the environmental variables can only be detected to a certain point. The ANOVA compares the data to determine whether there is a significant difference between the three depth categories in the light extinction coefficient or surface exposure measure. Tukeys post hoc tests is used to determine which depth category is significantly different from which other depth categories. If there is a significant difference in these parameters at different depths it will suggest that these parameters i.e. light and exposure are affecting the depth at which *N. flexilis* can grow.

2.3 Results

2.3.1 *N. flexilis* presence and absence

The Mann-Whitney U tests show that there is a significant difference between the more oligotrophic absent group and the present group for pH, conductivity, alkalinity, calcium and potassium in the water and total iron in the sediment. There is a significant difference between the more eutrophic absent group and the present group for pH, alkalinity, calcium, magnesium and total phosphate in the water and available phosphate in the sediment (Table 2.3). The environmental variables for which a significant difference was found between either absent group and the present group may be involved in the decline of *N. flexilis*. However, the sample sizes in the absent groups are small. Therefore these results must be treated with some caution. It is also of note that water clarity was not significantly different between present and absent sites. Although algal growth was noted at some of the more eutrophic absent sites such as Loch Monzievaired, others such as White Loch were dominated by charophytes and had clear water, whilst present sites could have highly peat stained water, decreasing water clarity.

2.3.2 Quantitative assessment of *N. flexilis* status within lochs

2.3.2.1 Traits

The plant fitness traits used in the model were leaf area/shoot length and number of reproductive structures/shoot length. Leaf area and leaf weight appeared to explain the same variation in fitness, however the leaf area measurements had a more normal distribution, so were chosen for use in the model. Root measurements were not used as the roots of *N. flexilis* are very thin and brittle and may well have broken in collection, therefore there was not enough confidence in the root data for it to be used. Reproductive number was used instead of reproductive weight, as it was not affected by the maturity of these structures, which could be a problem as plants were collected throughout the *N. flexilis* growth season. The variation in number of branches was shown by the leaf area/shoot length measurement, so was not included.

In unfavourable conditions for *N. flexilis* growth, the two elements of fitness used, reproductive number and leaf area did not reduce uniformly together. In more oligotrophic lochs, leaf area was of an intermediate

level, however the number of reproductive structures reduced dramatically. At one site only one seed was found in three mature plants. This agrees with the results of the experiments by Titus & Hoover (1993) that low pH reduces reproductive fitness in *N. flexilis*. In other environmentally similar lochs, only one or two plants were found, so they could not be sampled for fitness. It is probable that this low frequency of plants and low seed frequency among plants is related. At the more eutrophic end of the range, plants appeared to have longer internodes, this reduced the total leaf area of the plants as there were fewer leaf nodes and therefore fewer leaves per shoot length and total shoot length was not any longer in these plants with extended internodes. The lack of nodes also reduced the number of reproductive structures as these develop in the leaf nodes. Both of these conditions could possibly lead to population collapse. This is illustrated in Fig. 2.1 and 2.2, where each fitness component is regressed against pH. Both of the plots are noisy as pH is not the only factor controlling fitness, but an indication of trophic state, however they illustrate the unequal decline in the two components of plant fitness. As a result of this situation (reproductive number/shoot length) x (leaf area/shoot length) is used as the measure of fitness to assess *N. flexilis* status in the lochs surveyed (Fig. 2.3). It is also the measure predicted by the resulting models. The number of reproductive structures describes the reproductive success of the adult plant and the leaf area indicates the resources available for allocation to the reproductive structures, which indicates the survival chances of that seed becoming a mature plant.

Table 2.3 The range of environmental variables measured at *N. flexilis* present and absent sites, (a) in the sediment, (b) in the water. A * denotes a significant difference between the present and absent group with the *, $p \leq 0.05$, using a Mann-Whitney U test.

(a) In the sediment

	Oligotrophic absent	Present	Eutrophic absent
Available P (mg/l) Median	0.9–2.9 (n = 2) 1.9	0.3–17.3 (n = 41) 2.6	5.3–72.9* (n = 7) 21.8
Available K (mg/l) Median	– –	4–166 (n = 31) 35.1	28.3–89.2 (n = 4) 77.1
Extractable Mg (mg/l) Median	– –	16.3–388 (n = 31) 87	54.1–189 (n = 4) 146
Extractable Ca (mg/l) Median	145–5250 (n = 2) 2697.5	198–331000 (n = 41) 950	805–25000 (n = 7) 3140
Total Fe (mg/kg) Median	27600–20600* (n = 2) 24100	94.4–168000 (n = 41) 1250	123–14500 (n = 7) 216
Total N (mg/kg) Median	320–6220 (n = 2) 3270	240–142000 (n = 41) 3320	600–11400 (n = 7) 1540
Extractable Na (mg/l) Median	– –	8.3–300 (n = 29) 52.4	20.5–72.5 (n = 4) 50.05

Table 2.3 (continued)

(b) In the water

	Oligotrophic absent	Present	Eutrophic absent
pH Median	6.46–6.98* (n = 2) 6.72	6.62–8.3 (n = 42) 7.46	7.45–9.01* (n = 7) 8.64
Conductivity (us/cm) Median	54.9–116* (n = 2) 85.45	55–447 (n = 42) 235	158–410 (n = 7) 215
Light extinction coefficient Median	1.35–4.4 (n = 2) 2.88	0.53–7.32 (n = 42) 1.91	0.91–5.18 (n = 7) 1.83
Alkalinity (mg/l) Median	4.91–5.7* (n = 2) 5.3	6.71–69.71 (n = 29) 23.45	41.5–98.66* (n = 3) 72.65
Ca (mg/l) Median	1.32–2.02* (n = 2) 1.67	2.06–33.4 (n = 30) 9.59	14.22–24.72* (n = 3) 21.7
Fe (mg/l) Median	0.2–0.16 (n = 2) 0.18	<0.03–1.69 (n = 30) 0.21	0.04–0.35 (n = 3) 0.18
Mg (mg/l) Median	1.54–2.12 (n = 2) 1.83	1.34–8.8 (n = 30) 4.8	6.4–12* (n = 3) 8.8
N-NH3 (mg/l) Median	< 0.04 (n = 2) <0.04	<0.04–0.1 (n = 33) <0.04	<0.04–0.28 (n = 4) 0.07
N-TON (mg/l) Median	<0.1 (n = 2) <0.1	<0.1–1.369 (n = 33) <0.1	<0.1–2.646 (n = 4) <0.1
P-DRP (PO4) (mg/l) Median	<0.003 (n = 2) <0.003	<0.003–0.045 (n = 33) <0.003	<0.003–0.074 (n = 4) 0.012
P-TP (mg/l) Median	0.012 (n = 2) 0.012	<0.003–0.056 (n = 30) 0.0095	0.014–0.16* (n = 3) 0.017
K (mg/l) Median	0.3–0.54* (n = 2) 0.42	0.16–2.82 (n = 33) 1.2	0.3–2 (n = 4) 1.4

Figure 2.1 The effect of pH on Log (leaf area/shoot length).

$$y = -0.3415\text{pH}^2 + 4.9015\text{pH} - 15.642 \quad r^2 = 0.181, \quad p < 0.05$$

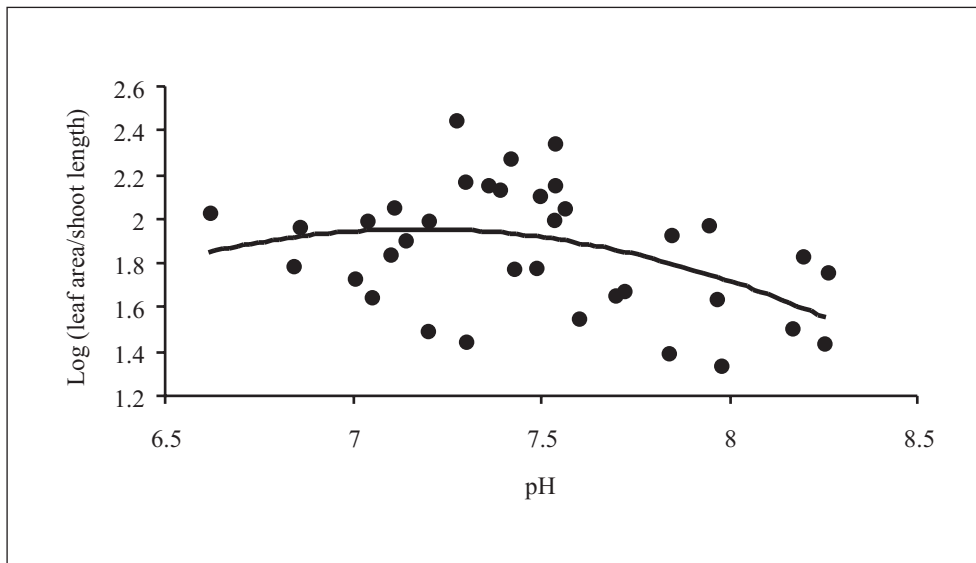


Figure 2.2 The effect of pH on Log (reproductive number/shoot length).

$$y = -0.2169\text{pH}^2 + 3.2823\text{pH} - 12.131 \quad r^2 = 0.165, \quad p = 0.052$$

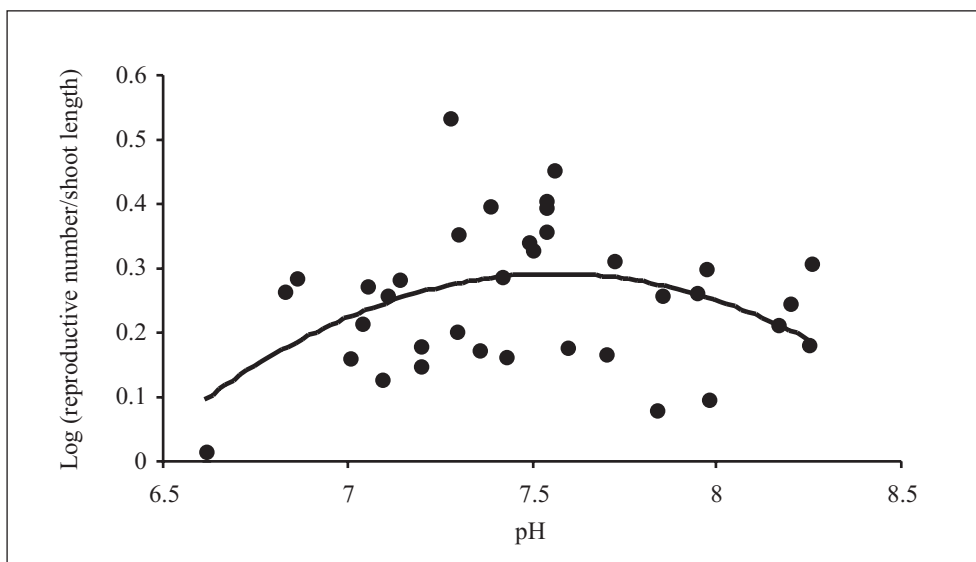


Figure 2.3 The observed mean log fitness (leaf area/shoot length x reproductive number/shoot length) at all the sites where sufficient material could be collected. Site codes and names are in Appendix 1.

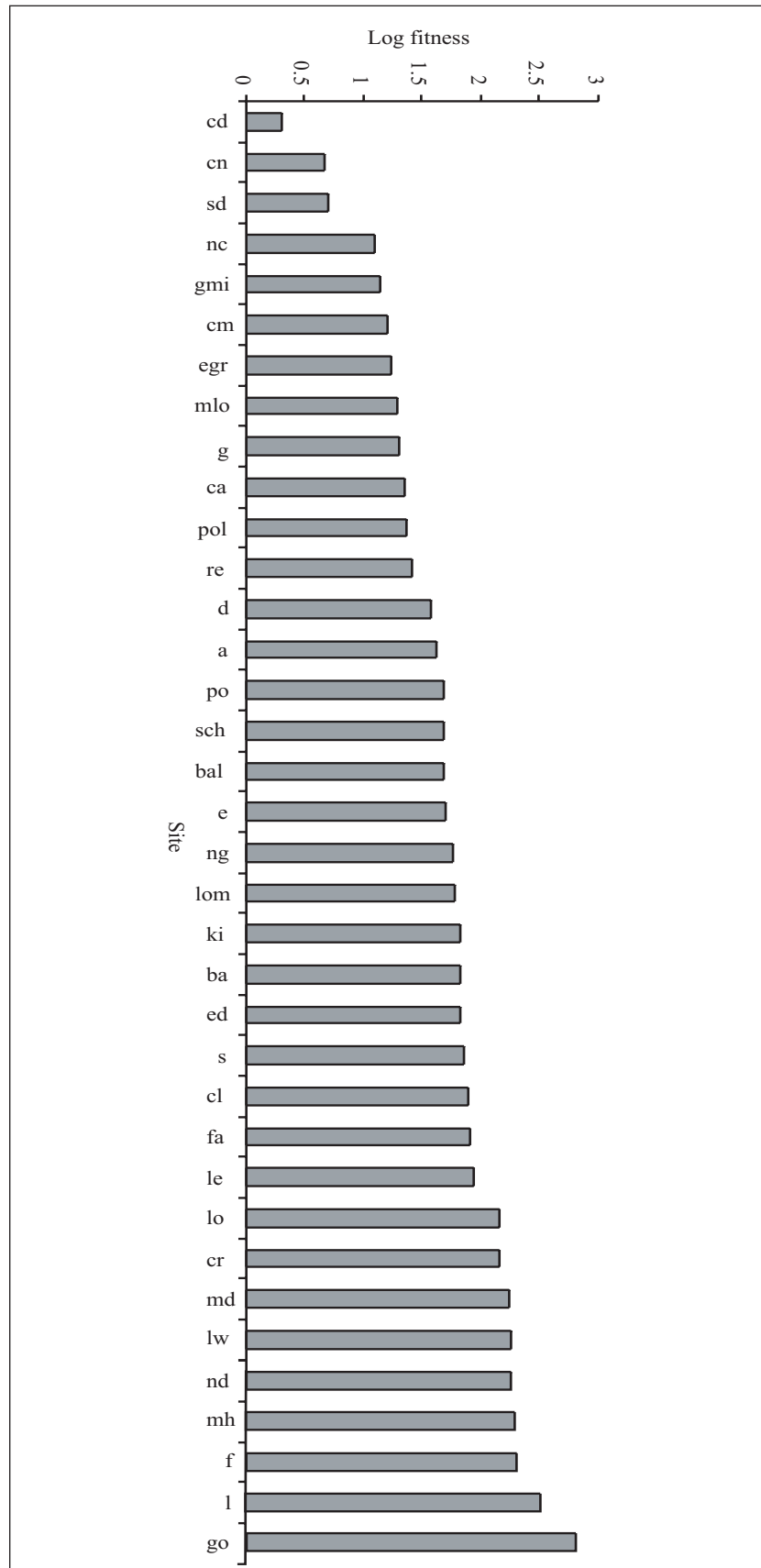


Fig. 2.3 shows the log fitness for each site surveyed where three entire plants could be collected. Three sites have a log fitness value below one, these sites are those perceived to be at risk. It was also observed that at all three at risk sites *N. flexilis* did not grow in as great an abundance as at other sites where the plants could be seen. Fig. 2.3 shows that there is a step increase between the three at risk sites and the rest of the *N. flexilis* populations. Cregduff Lough despite having relatively leafy plants only had one seed between three plants. This suggests that the population size will be smaller next year, this site had the lowest pH value of all sites surveyed. Loch nan Cnamh and Loch an Eilean, South Boisdale, had a pH of 7.84 and 7.98 and possessed an average of 3 and 2 seeds per plant respectively, both sites also possessed plants with low leaf area/shoot length measures suggesting lower levels of resource to be allocated to seeds. Loch nan Cnamh was the site with the highest total vegetation biomass and *N. flexilis* appeared to be struggling to survive amongst the great amount of *Myriophyllum alterniflorum* and *Chara* sp. In contrast *N. flexilis* in Loch an Eilean, South Boisdale had no competitors in the immediate vicinity, but grew in highly peat stained water, although the water was not of the lowest clarity out of the lochs visited. It can be assumed that both of these sites were suffering from a lack light due to either water coloration or macrophyte competition.

2.3.2.2 Predicting *N. flexilis* fitness

The models for leaf area/shoot length and reproductive number/shoot length are intrinsically similar; this could be expected as the leaves produce the resources for the production of reproductive structures. Light extinction coefficient is good at predicting photosynthetic success as leaf area/shoot length. However it is not so good for predicting the number of reproductive structures produced, instead the quantity of calcium in the sediment is a better indicator. This is probably because it will affect the pH of the water, which appears to affect reproductive success.

Model 1: $n = 17, r^2 = 0.886, p < 0.005$

$$\log La/sl = 0.515 + (1.276 \log Alk) - (1.012 \log Ps) + (50.775 TPw) - (0.0921 Mgw) - (0.599 \log Lec)$$

Model 2: $n = 17, r^2 = 0.672, p < 0.005$

$$Rep/sl = 0.993 + (2.154 \log Alk) - (1.179 \log Ps) + (44.801 TPw) - (0.132 Mgw) - (0.856 \log Cas)$$

Model 3: $n = 17, r^2 = 0.768, p < 0.005$

$$\log Fit = -0.628 + (2.263 \log Alk) - (1.810 \log Ps) + (84.067 TPw) - (0.156 Mgw) - (0.919 \log Lec)$$

Model 4: $n = 17, r^2 = 0.743, p < 0.005$

$$\log Fit = 0.642 + (2.738 \log Alk) - (1.392 \log Ps) + (59.509 TPw) - (0.199 Mgw) - (0.649 \log Cas)$$

Where:

La/sl = Leaf area/shoot length

Fit = (Leaf area/shoot length) × (Reproductive structure number/shoot length)

Rep/sl = Number of reproductive structures/shoot length

Alk = Alkalinity mg/l

Ps = Extractable phosphorus in the sediment mg/l

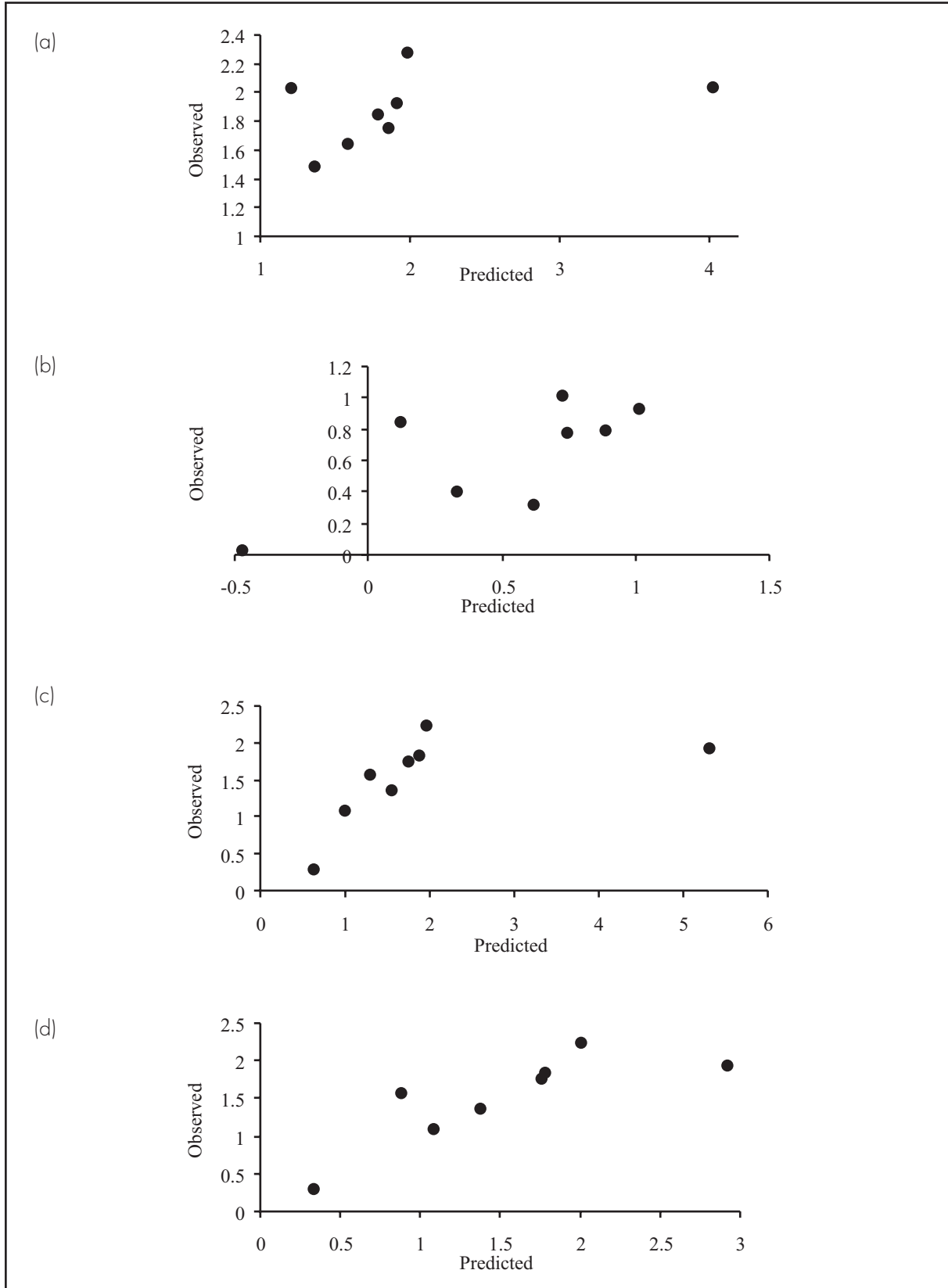
TPw = Total phosphate in the water mg/l

Mgw = Magnesium in the water mg/l

Cas = Calcium in the sediment mg/l

Lec = Light extinction coefficient

Figure 2.4 The observed and predicted fitness values of *N. flexilis* for 4 Irish sites and 4 additional Scottish sites, 3 in S. Uist and 1 in Kintyre, (a) = model 1, (b) = model 2, (c) = model 3, (d) = model 4. The site with a constantly higher prediction of fitness than that observed is Lough Leane.



The models created were tested on the four Irish sites, where sufficient plant material was obtained, and three further Scottish sites from Kintyre and South Uist. The predicted values were plotted against the observed values (Fig. 2.4). The model predicting reproductive success alone, works the least well. All the other models appear to predict the fitness of *N. flexilis* relatively well. The major exception from the test data is from Lough Leane where the fitness of the plant is consistently predicted to be greater than the observed fitness. Lough Leane is presently becoming increasingly eutrophic. This recent change may well be affecting the plants, but it is possible that phosphate will not yet have accumulated in the sediment, hence the over prediction of plant fitness. These results show that, with the exception of Lough Leane, the models can equally be applied to the Irish and Scottish *N. flexilis* populations.

2.3.3 Competition

Macrophyte biomass (both including and excluding *N. flexilis*) did not have a significant relationship with *N. flexilis* fitness on its own. This is because there are many other factors also affecting the fitness of *N. flexilis*. It did not contribute any further to the model either, as the nutrient levels in the water and sediment would be an indication of both algal and macrophyte growth as would the variables that indicate CO₂ concentration. Therefore although competition probably is playing a part in determining *N. flexilis* fitness the data set is too noisy to determine a significant relationship, and other parameters in the model are more effective indicators of *N. flexilis* fitness.

2.3.4 Depth

Fig. 2.5 shows that at sites where *N. flexilis* grows at a depth of less than 1m, the surface exposure is significantly less than that experienced at sites where *N. flexilis* grows at greater depths. At sites where *N. flexilis* grows at depths of 1–2m the surface exposure experienced is significantly greater than sites where *N. flexilis* grows at depths of less than 1m, but significantly less than sites where *N. flexilis* grows at depths greater than 2m. At sites where *N. flexilis* grows at depths greater than 2m the surface exposure is significantly greater than at sites where *N. flexilis* grows at shallower depths. Therefore, only when exposure is low were *N. flexilis* plants found in shallow water. This suggests that the the depth of water in which *N. flexilis* grows depends on exposure at that site. This does not show whether exposure affects *N. flexilis* by damaging the fragile *N. flexilis* plants or whether it is due to the effect exposure has on the sediment texture.

Figure 2.5 The mean surface exposure experienced by sites where *N. flexilis* can be found at depths of <1m, 1–2m and >2m. Different letters signify significantly different exposure values, $p \leq 0.05$ using Tukey post hoc test after a one way analysis of variance. The error bars show + or – 1 standard error.

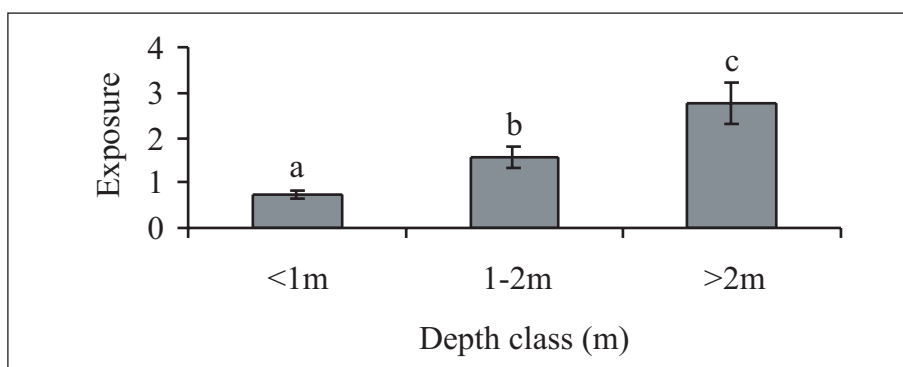
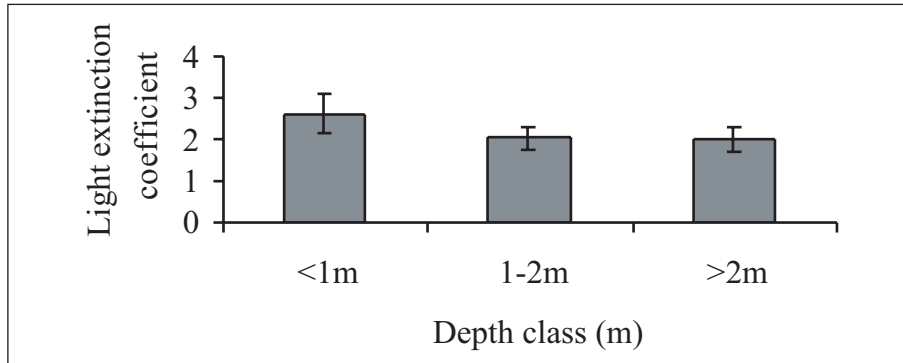


Figure 2.6 The mean light extinction coefficients at sites where *N. flexilis* can be found at depths of <1m, 1–2m and >2m. No significant difference was found between the depth classes using a one-way analysis of variance. The error bars show + or – 1 standard error.



Although there is no significant difference in water clarity between depth classes, Fig. 2.6 shows that there is a trend for sites with lower water clarity to have *N. flexilis* populations in shallower water. This lack of a significant difference is most probably due to the sampling method, the first population of *N. flexilis* encountered was usually the population surveyed and consequently this would be one of the shallowest populations. Whilst it will be the depth of the shallowest population that will be affected by exposure, it will be the depth of the deepest population that will be affected by light. This is because whilst plants in lochs with low water clarity can only grow in the shallows, in lochs with clear water and low exposure plants can grow from the shallows to the depths, thus merging the distinction between the three groups. The recording of plants at their maximum depth could have solved this. Therefore, Fig. 2.5 and Fig 2.6 only suggest that it will be an interaction between exposure and water clarity, which will determine the locality of *N. flexilis* within a loch.

2.4 Discussion

There is an overlap between the ranges of the chemical parameters of the present and absent site groups. This is because the population at the absent site could have become extinct at any time since the last record, in some cases a period of over 100 years. Since the time of extinction, the loch quality may have improved to a state where *N. flexilis* could theoretically grow, but recolonisation may have been hindered due to geographic distance from neighbouring populations or ineffective dispersal from these populations may have limited its re-establishment. Another possible reason why the ranges for the present and absent site groups are not discrete is that the environmental parameters do not affect *N. flexilis* independently and extinctions will be brought about by a number of contributing factors.

The water chemistry measurements are a snapshot of the loch water chemistry. The nutrient levels in the samples are low, as they are taken in summer, when most of the nutrients will be incorporated in the growing organisms. However, it is within this time period that *N. flexilis* grows, so this is when the measurements were taken. As a result many of the DRP, TON, and NH₃ levels are below the detection limit. Therefore, no significant difference was found between presence and absence groups and their effect on the fitness of *N. flexilis* could not be determined. This does not mean that the presence and absence groups do not have different quantities of these nutrients, or that they have no effect, just that it cannot be detected.

The more oligotrophic absent sites have significantly lower pH, conductivity, alkalinity, calcium and potassium levels in the water than the present sites. These low levels appear to affect the reproductive success of the plants, but not their photosynthetic capacity. Unless the plant is preferentially allocating resources to the photosynthetic tissue instead of the reproductive structures, which is an unlikely strategy for an annual plant, this suggests that these environmental conditions are inhibiting reproductive success. However, experiments on floral development and pollen viability in various environmental conditions are required to confirm this. These sites also have significantly higher iron sediment levels.

In contrast, the more eutrophic absent group has significantly higher pH, alkalinity, calcium, magnesium and total phosphate levels in the water and significantly higher phosphate levels in the sediment. These parameters do not fluctuate independently and are all integrally linked. The levels of these parameters found within a loch will be due to both the nutrients and minerals available from the catchment and the interactions within the loch.

Alkalinity, pH and calcium levels in the water all indicate the concentration of carbon dioxide in the water. Higher levels of these measures indicate that the main form of inorganic carbon in the water is bicarbonate. The pH, alkalinity and calcium levels and also the inorganic carbon equilibrium will be determined, in part by, the characteristics of the catchment, however they will also shift in accordance with the biological activity within the loch. High levels of photosynthesis by either algae or macrophytes within a loch will shift the equilibrium towards a bicarbonate system at least in the daytime. This is because photosynthesis will use up the available CO_2 . Plants characteristic of such hard water systems avoid this problem by utilising HCO_3^- , however this requires significantly more energy than CO_2 utilisation. *N. flexilis* is an obligate CO_2 user and can not utilise HCO_3^- (Hough & Fornwall, 1988). However it can still compete in marginal habitats by living in low light conditions, usually at greater depths, where there is not enough light to fuel bicarbonate utilisation, it is also in these depths where any available CO_2 may be found.

High levels of photosynthesis require high nutrient levels as well as light. As nitrogen and phosphorus often limit primary production in mesotrophic systems (where *N. flexilis* grows) an increase in these nutrients would increase photosynthetic production, which would decrease available CO_2 concentrations. This would seriously limit the growth of *N. flexilis* as in such an environment it would be incapable of fixing carbon. This would result in a situation where plants would produce fewer leaves, leaf nodes and therefore fewer reproductive structures, and this is seen in lochs with a high pH (which indicates low CO_2 levels), see Fig. 2.1. If this situation continued, fewer seeds would be produced each year and the population size would decline. The significantly higher levels of phosphate in the water and the sediment and the higher pH, alkalinity and calcium levels, in the more eutrophic absent sites, suggest that these may be the cause of extinction. The absent eutrophic sites in this study are all found in the more intensive agricultural areas in Perthshire, suggesting phosphate sources from agriculture may be a threat to the species. Another potential source of phosphate, referred to in the *N. flexilis* BAP plan, is fish farms although, at present, sites containing fish farms successfully support *N. flexilis*.

Models 1 and 3 show that light is also important for predicting plant success. The quantity of photosynthetically active radiation reaching *N. flexilis* plants will decrease as algal and macrophyte growth increases, as these will intercept the light before it reaches the relatively low growing *N. flexilis*. *N. flexilis*, like all plants, is limited in its distribution by light availability. However, it naturally grows at low light levels and can grow productively in unclear water, this is shown by the low light extinction coefficients in lochs

supporting *N. flexilis*. This suggests that photosynthetically active radiation would have to be severely limited to cause *N. flexilis* extinction. The fact that some of the absent *N. flexilis* lochs, dominated by charophytes, have exceedingly clear water now does not mean that they always have. Charophyte populations often boom and bust and with these cycles water clarity also improves and declines and *N. flexilis* could have become extinct at that site during a decrease in water clarity.

2.5 Conclusions

The fitness measures developed here can be used to monitor and assess the present *N. flexilis* populations and elucidate which populations are at risk. The models can be used to calculate the predicted fitness of *N. flexilis* at absent sites to determine if the loch quality is suitable for reintroduction of *N. flexilis*. Where there are no records of where *N. flexilis* previously grew within an absent loch, the relationship between depth and exposure can be used to determine where within a loch *N. flexilis* should be introduced to, in order that it can thrive. The models and analysis of present and absent sites have also suggested the major threats to the species in Scotland to be those related to eutrophication and acidification.

Summary

- *N. flexilis* present and eutrophic absent sites differ significantly in pH, alkalinity, calcium, magnesium and total phosphate levels in the water and phosphate levels in the sediment.
- *N. flexilis* present and oligotrophic absent sites differ significantly in pH, conductivity, alkalinity, calcium and potassium levels in the water and total iron in the sediment.
- *N. flexilis* fitness can be assessed using the trait measurements (leaf area/shoot length) x (reproductive number/shoot length).
- *N. flexilis* fitness in relation to the loch environmental conditions can be predicted with reasonable success for Scottish and Irish sites.
- The depth at which *N. flexilis* grows is significantly affected by exposure.
- There is a trend between the light extinction coefficient and the depth at which *N. flexilis* grows, this may be significant if the deepest population in a loch was studied instead of the shallowest population.

3 AQUATIC PLANT COMMUNITY DESCRIPTION AT DIFFERENT SCALES; APPROACHES TO DESCRIBE THE ENVIRONMENT IN WHICH *N. FLEXILIS* GROWS

3.1 Introduction

3.1.1 Aims

- Determine whether plant community descriptions are a suitable tool for assessing site condition.
- Compare and assess two approaches to macrophyte community classification; the whole loch qualitative approach and the micro-habitat quantitative approach for their ability to differentiate different freshwater environments.
- Assess the present British classification schemes for their applicability for this purpose.
- Assess the suitability of these schemes for assessing site condition for *N. flexilis*.

3.1.2 Background

N. flexilis is listed in Annex 2 of the European Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (The 'Habitats Directive'). This requires member states to identify sites for this species, which will be designated as Special Areas of Conservation (SACs). A number of *N. flexilis* sites in Scotland have already been put forward as SACs. SACs as well as Special Protection Areas (SPAs) and Special Sites of Scientific Interest (SSSIs) must undergo 'site condition monitoring' (SCM) which means that 'interest features' (e.g. *N. flexilis*) on these sites must be monitored once every six years. The results for SACs and SPAs (European designations) will be passed onto Brussels, as required under the Habitats Directive. The Habitats Directive does not state how the monitoring should be done, that is to be decided by the organisations concerned.

Common standards for SCM are being developed by the UK nature conservation agencies. One common standard agreed on is nature conservation objectives for each interest feature (e.g. *N. flexilis*) on each site, which define when the condition of an interest feature is favourable. Such objectives should include characteristics (attributes) for assessment of the interest feature against set targets. Targets can directly describe the interest feature, or be good indicators of the condition of the interest feature. The degree of acceptable change of the characters should be described and the characters should be comparable across all sites where the interest feature is found.

Submerged aquatic plants are intrinsically difficult to survey as they often grow in low-visibility water. Not only is locating the plant problematic, but determining the plant's condition once it has been found is also difficult. This is because obtaining measures of plant success such as abundance is not easy if the plant cannot be seen. Destructive techniques are also not ideal as *N. flexilis* is a protected species under the Wildlife and Countryside Act, 1981 and the Conservation Regulations, 1994, and therefore any destructive surveys require a licence. One solution is SCUBA diving, but this is expensive for the nature conservation agencies to carry out. As a consequence the use of indicators of the condition of the interest feature, i.e. *N. flexilis*, is appealing. Using plant community descriptions as one such indicator is presently being considered by English Nature.

A qualitative plant community description is much easier to obtain than a quantitative survey of plant abundance within a water body. If only qualitative information is required, non-quantitative survey methods, such as grappelling, can be used; this reduces both the cost and time involved. However, it is important to know how good an indicator such descriptions would be, and how variable they can be, before the interest feature (*N. flexilis*) is also impacted. There have been several classifications of British aquatic plant communities. It is important to determine whether these community descriptions could be applied as indicators to the monitoring of *N. flexilis*, or whether new community descriptions, solely for this purpose, are required.

3.1.3 Survey methods used for deriving plant community descriptions

Rodwell's (1995) National Vegetation Classification (NVC), is concerned with classifying community types within aquatic systems, i.e. there will usually be more than one community type per loch. Rodwell (1995) took a number of quadrats to devise his communities. The sizes of these quadrats were 2 x 2m² or 4 x 4m². This classification is concerned with individual communities within lochs, as well as among them. The different communities found within a single loch are most often assigned to different depths, which are related to sediment type (particularly particle size), and the light reaching the plants. This type of macrophyte survey and classification would elucidate exactly which plants *N. flexilis* grows in close association with and would indicate the zone within the loch where it would grow.

In contrast to the above classifications, the classification of standing water types (SWT) in Great Britain (Palmer, 1989) looks at all the macrophytes in the whole loch, and classifies a loch type rather than a community type. Due to this larger survey scale, a greater number of species, representative of a number of communities, would be recorded. This greater species number would give a better estimate of the environment of the loch, because there is a greater chance of including a species that is a good indicator of the environment. However, minor changes in sediment characteristics will not be detected as most lochs contain a range of sediment types. Both the whole loch and the micro-habitat scale approach are used in this study to determine which of these methods of classification would be best for indicating the differences in loch environment and the condition of *N. flexilis*.

3.1.4 British plant community descriptions and *N. flexilis*

The National Vegetation Classification and Standing Water Type (SWT) were assessed and compared for their suitability as a SCM tool to determine *N. flexilis* condition. This is because they represent the two different scale approaches to plant community description in lochs. In order for community classifications to be a suitable SCM tool for *N. flexilis*, the community classification must reflect the underlying environmental conditions that affect the ability of *N. flexilis* to grow. Ideally *N. flexilis* would grow in a number of communities, one or more where it could thrive, one or more where it was at risk, and the community would be different again if *N. flexilis* could not grow at all. Change in the community classification could then be used as an early warning that *N. flexilis* is at risk before it is lost from a site.

3.2 Methods

3.2.1 Sites

During the course of the study, a total of 52 lochs were investigated, 42 in Scotland (July–September, 1998–2000) and 10 in Ireland (August 2000). All sites with post 1980 records.

Table 3.1 The lochs surveyed in this study, the date of survey and the method used. bo denotes boat surveys, sn, snorkel surveys and sh, shoreline survey (grid references for the sites can be found in chapter 1).

Site	Survey method	1988	1999	2000
Mainland Scotland				
Loch a Bhada dharaich	sn & sh	–	–	11/09/00
Loch of Butterstone	bo & sn & sh	03/07/98	16/08/99	–
Loch Clunie	bo & sn & sh	14/07/98	18/08/99	05/08/00
Loch of Craiglush	sn & sh	–	17/08/99	05/08/00
Fingask Loch	sn & sh	–	08/07/99	–
Lindores Loch	sh	–	–	05/08/00
Loch of Lowes	sn & sh	–	16/08/99	05/08/00
Loch Marlee	bo & sn & sh	13/07/98	17/08/99	–
Lake of Menteith	bo & sn & sh	30/07/98 8/10/98	14/10/99	08/08/00
Monk Myre	sn & sh	–	07/07/99	–
Loch Monzievairst	sh	–	–	04/08/00
White Loch	bo & sn & sh	–	08/07/99	–
Inner Isles and Kintyre				
Loch Ballyhaugh	sn & sh	–	24/07/99	–
Loch Fada	sn & sh	–	21/07/99	–
Glenastle Loch	sn & sh	–	19/07/99	25/08/00
Loch Gorm	bo & sh	–	20/07/99	24/08/00
Loch Lossitt	sn & sh	–	20/07/99	25/08/00
Loch nan Gad	sn & sh	07/08/98	–	30/08/00
Loch Poit na h-I	sn & sh	–	22/07/99	–
Tangy Loch	bo & sn & sh	06/08/98	–	29/08/00
Loch an t-Sagairt	sn & sh	–	25/07/99	–
Outer Isles				
Loch Altabrug	sn & sh	–	02/09/99	14/09/00
Loch Bun an Ligidh	sn & sh	17/08/98	29/08/99	13/09/00
Loch nan Capull	sn & sh	21/08/98	01/09/99	13/09/00
Loch nan Cnamh	sn & sh	–	31/08/99	–
Loch Cuilc	sn & sh	17/08/98	–	–
Loch na Cuithe Moire	sn & sh	21/08/98	01/09/99	13/09/00
Loch Druidibeg	bo & sn & sh	18/08/98	31/08/99	13/09/00
North Loch Druidibeg	sn & sh	16/8/98 1/10/98	31/08/98	14/09/00
Loch Eaval	sn & sh	–	30/08/99	–
Loch an Eilean (Drimsdale)	sn & sh	–	02/09/99	–

Table 3.1 (continued)

Site	Survey method	1988	1999	2000
Loch Eilean a' Ghille-Ruaidh	sn & sh	–	02/09/99	–
Loch an Eilean (South Boisdale)	sn & sh	–	01/09/99	–
Loch nam Faileann	bo & sn & sh	19/8/98 1/10/98	01/09/00	–
Loch Gearraidh Mhic Iain	sn & sh	–	02/09/99	–
Loch Grogary	sn & sh	20/08/98	30/08/99	–
Loch a'Mhadaidh	sn & sh	–	31/08/99	–
Mid Loch Ollay	sn & sh	–	29/08/99	14/09/00
Loch Scarie	sn & sh	20/08/98	30/08/99	14/09/00
School House Loch	sn & sh	–	03/09/99	–
Loch with no name (?)	sn & sh	–	02/09/99	14/09/00
Ireland				
Carragh Lough	sn & sh	–	–	17/08/00
Cregduff Lough	sn & sh	–	–	17/08/00
Fin Lough	sh	–	–	16/08/00
Kindrum Lough	sn & sh	–	–	15/08/00
Lough Leane	bo & sh	–	–	18/08/00
Mullaghderg Lough	sn & sh	–	–	15/08/00
Nafeakle Lough	sn & sh	–	–	16/08/00
Pollacappul Lough	sh	–	–	16/08/00
Renvyle Lough	sn & sh	–	–	16/08/00
Shennagh Lough	sn & sh	–	–	14/08/00

for *N. flexilis* in Scotland were visited (36 sites). In addition 6 sites where no surveys had been carried out post 1980, but where *N. flexilis* had previously been reported were visited; when surveyed for this study 5 of these appeared now not to contain *N. flexilis*. All ten Irish loughs visited had previous records for *N. flexilis* (Table 3.1).

3.2.2 Survey methods

Three basic survey methods were used; boat, shoreline and snorkel surveys. Snorkel surveys were considered the most effective for finding *N. flexilis*, agreeing with comments in the literature (Capers, 2000), and were adopted as the standard approach wherever feasible. Boats were used when the lochs were particularly large and there was no knowledge of exactly where the plant could be found, this enabled a larger area to be surveyed. When boats were not available, a shoreline search was carried out. A shoreline search included searching the drift and wading into the water as far as safety would allow and throwing grapnels into the deeper water. This method was used by the Nature Conservancy Council when surveying macrophytes for the classification of SWT (Palmer, 1989). The location of *N. flexilis* communities was determined within a loch from past records where available, see section 2.2.1. It was important to locate *N. flexilis* within the loch, as the purpose was to describe the community in which it grew.

3.2.2.1 Loch community

In order to ascertain the loch communities in which *N. flexilis* grew all plants encountered within a loch were recorded on a presence/absence basis. This included submerged and floating leaved plants from shoreline to deep water. Emergent plants were excluded from the analysis for two reasons. Firstly, because of their emergent nature they are often subject to influences not experienced by the open-water vegetation and can therefore skew the results. Secondly, they were not used in the classification of SWT in Britain, to which the communities in this study are compared. Although a representative sample of each loch was surveyed, the entire loch was not surveyed in a detailed manner. Therefore, plants that were scarce within the loch or with a very patchy distribution, not occurring in the vicinity of the *N. flexilis* community surveyed, may have been missed by the survey. This should not hinder the classification of these communities on a loch basis using the SWT scheme as the classification relies on common and dominant species within a loch.

3.2.2.2 Micro-habitat

The micro-habitat includes only the plants growing in close proximity to *N. flexilis*. A single micro-habitat was identified and surveyed in each loch, this was at the same site within a loch as the environmental measures were taken. Due to the intrinsic difficulties of surveying deep water plants in low-visibility water, it was decided to collect quantitative samples at the micro-habitat scale by the use of an Ekman grab. This takes 20 x 20cm² samples of the vegetation; three such samples were taken within the micro-habitat. This sampling method does survey a much smaller area than the NVC, however only three samples were taken due to resource, effort and time limitations. In sites where the community was visible it was noted that most species present in the micro-habitat were included in the three Ekman grab samples. Three 20 x 20cm² samples would not be sufficient to represent the community in all ecosystems however macrophyte communities have been noted by Rodwell (1995) often to be mono-specific even at the larger scale of 2 x 2m². The three Ekman grab samples were sorted into species and dried. The mean dry weight of each species for the three samples was used as the quantitative measure of each species, for that site, at the micro-habitat level. These data are not appropriate for classification using the SWT method as even species that are common within a loch may be excluded at such a small scale. Neither is it the purpose of these data to classify loch type, but specific community type, therefore the NVC should be a more appropriate classification system to use. These samples were only collected from the Scottish lochs where *N. flexilis* was found. If *N. flexilis* was not found, the micro-habitat where it grew could not be assumed, thus collection of such samples would have been of no value for this exercise.

3.2.3 Environmental measures

In order to determine the underlying environment of the different communities a number of environmental measures were taken at each site. These measures are the same as those in section 2.2.3. Exposure was calculated using the same method as in section 2.2.3. This was only calculated for the micro-habitat sites as exposure can only be calculated for a fixed point not a whole loch.

3.2.4 Data analysis

TWINSpan (Hill, 1979) was used to group the sites according to their species composition. The resulting groups were classified according to SWT (Palmer, 1989) and the NVC (Rodwell, 1995) with the use of the

MATCH program (Malloch, 1996). MATCH calculates match coefficients; these reflect the goodness of fit of your community group to the NVC community group. A match coefficient of 100 is a perfect match and 0 shows no similarity. The quantitative environmental measures do not have homogeneous variance, this is because the lower limit of detectability is surpassed in some of these measures. Consequently, nonparametric tests were used to compare the medians of the environmental variables between groups. The two tests used were the multi-comparison Kruskal-Wallis test and the single comparison Mann-Whitney U test. Using the Mann-Whitney U test a separate test must be carried out for each 2 groups compared. As the number of tests being carried out increases, it becomes increasingly likely that the null hypothesis (that there is no significant difference) is rejected rather than accepted. The 95% confidence level used in this study would lead to this error being made once in every twenty tests performed. The Kruskal-Wallis test is a multiple comparison test; a single test compares all site groups thus reducing the chances of making the error described above. However, larger sample sizes are required for this test than the Mann-Whitney U test. As the TWINSPAN groups created from the micro-habitat scale survey contain sample sizes of only $n = 2$, Mann-Whitney U tests were carried out to compare the environmental conditions in these groups. The Kruskal-Wallis test was used to compare the environmental measures of each of the loch scale TWINSPAN groups. This is because of the increased number of site groups to be compared and the sample sizes being large enough to allow this test to be performed. The nonparametric multiple comparison test of Dunn (1964) was then used on the loch-scale data (Zar, 1999). This elucidates which of the loch-scale groups is significantly different from which other loch-scale group, thus performing the same function as Tukeys post hoc test, see section 2.2.5.

These comparisons of the environmental variables of the different site groups will indicate the environment that the plant communities typical of that site group reflect. If the plant communities can reflect differences in the environmental variables that are important for *N. flexilis* growth and development (see section 2) the plant community present at a site could be used to indicate habitat suitability for *N. flexilis* or even the state of *N. flexilis* itself.

3.3 Results

3.3.1 Loch communities

3.3.1.1 Community groupings

TWINSpan shows that *N. flexilis* can be found in a number of different whole loch communities. This is potentially useful for a SCM tool, if these communities correspond to conditions suitable for *N. flexilis* growth. TWINSpan produced 4 main site-groups (Table 3.2a). All of these groups contain sites from more than one of the geographical areas of mainland, Inner Isles, Outer Isles and Ireland. This suggests that the difference in floristic composition between the groups is not due to geographical reasons. The species occurring in the TWINSpan groups are shown in the floristic table (Table 3.3). These TWINSpan groups have been classified according to SWT, the classification of these groups is shown in Table 3.4.

Table 3.2 Sites contained in each of the TWINSPAN groups for the loch scale (a) and micro-habitat scale (b). Where E = Ireland, I = Inner Isles and Kintyre, M = Mainland Scotland and O = Outer Isles.

(a)

Group 1	Group 2	Group 3	Group 4
Loch nan Capull O	Loch na Cuithe Moire O	Loch Grogary O	Loch Clunie M
Loch an t-Sagairt I	Glenastle Loch I	Loch Scarie O	Lough Leane E
Nafeakle Loch E	Mid Loch Ollay O	Tangy Loch I	Loch Marlee M
Loch a Bhada dharaich M	Loch Eaval O	Loch Lossitt I	Monk Myre M
Loch Altabrug O	Loch Gearraidh Mhic Iain O	Loch nan Cnamh O	White Loch M
Loch Fada I	Loch of Craiglush M	Loch an Eileen (S. Boisdale) O	Lindores Loch M
Renvyle Lough E	Loch an Eilean (Drimsdale) O	Fingask Loch M	Loch Monzievaird M
Fin Lough E	Loch Poit na h-I I	Loch of Lowes M	
Carragh Lough E	Loch Bun an Ligidh O	Loch of Butterstone M	
Shennagh Lough E	Loch Druidibeg O	Lake of Menteith M	
Kindrum Lough E	Loch nan Gad I		
Cregduff Lough E	School House Loch O		
Loch nam Faileann O	Loch Gorm I		
Loch Kindar M	North loch Druidibeg O		
Mullaghderg Lough E	Loch with no name (?) O		
Pollacappul E	Loch Eileen a' Ghille-Ruaidh O		
	Loch Ballyhaugh I		
	Loch a'Mhadaidh O		

(b)

Group 1	Group 2	Group 3	
Loch Eaval O	Loch of Craiglush M	Loch Grogary O	
Loch Poit na h-I I	Loch Fada I	Loch a'Mhadaidh O	
	Loch an t-Sagairt I	Loch Gorm I	
	Loch Bun an Ligidh O	Loch nan Capull O	
	Loch na Cuithe Moire O	Loch Scarie O	
	Loch an Eilean (Drimsdale) O	Loch Ballyhaugh I	
	Glenastle Loch I	Loch Druidibeg O	
	Loch Gearraidh Mhic Iain O	School House Loch O	
	Loch Eileen a' Ghille-Ruaidh O	Loch nan Gad I	
	Mid Loch Ollay O	Loch Altabrug O	
	North loch Druidibeg O	Loch nam Faileann O	
		Loch Lossitt I	
		Loch of Butterstone M	
		Loch nan Cnamh O	
		Loch with no name (?) O	
		Loch of Lowes M	
		Lake of Menteith M	
		Loch Clunie M	

3.3.1.2 Species composition

The main split in the groupings created by TWINSPAN on the loch scale data is between groups 1 and 2, and groups 3 and 4. Groups 1 and 2 most frequently support species associated with a more oligotrophic environment, primarily the isoetids; *Littorella uniflora*, *Isoetes lacustris* and *Lobelia dortmanna* as well as other species such as *Juncus bulbosus* and *Sparganium angustifolium*. Groups 3 and 4 contain less of these species and more frequently contain species most commonly found in more eutrophic environments, such as *Callitriche hermaphroditica* and *Elodea* spp. (if the area has been invaded by the latter). These are also the sites where the *Potamogeton* spp. associated with higher nutrient concentrations can be found, such as *Potamogeton pectinatus*, *Potamogeton lucens* and *Potamogeton obtusifolius* (Palmer, 1989). Within these groups differences are also apparent, group 2 is more dominated by *Potamogeton perfoliatus* and *Potamogeton gramineus* than group 1, suggesting a slightly more eutrophic group 2. Group 4 contains *Persicaria amphibia*, which is not found in group 3, and less *N. flexilis*, suggesting group 4 is the most eutrophic. This is represented by the SWTs assigned to each group (Table 3.4).

3.3.1.3 Environmental conditions

The differences in water conditions between these community groupings are shown in Table 3.5a. Group 1 is constantly the most nutrient poor and group 4 is the most nutrient rich, this is shown by significantly different levels of total oxidised nitrogen and dissolved reactive phosphate. Other factors that are significantly different between the two groups are pH and alkalinity, both of which also tend to show higher values at higher trophic states. Groups 2 and 3 have a more intermediate nutrient status, this is as the species composition suggested. The underlying sediment chemistry is more confusing (Table 3.5b), group 4 is still consistently the most rich in phosphate followed by group 3, group 1 seems then to be more phosphate rich than group 2. However, the latter two groups are not significantly different ($p > 0.05$). Calcium levels are also highest in group 4, but group 3 has the lowest levels with groups 1 and 2 as intermediates. Iron is highest in group 1 and lowest in groups 2 and 3 with intermediate levels in group 4. These differences in the environmental conditions are potentially very useful for predicting *N. flexilis* growth as it is the nutrient levels and calcium concentration which appear to be important in predicting its success. However, group 4 often contains small sample sizes; results from these small samples must be used cautiously.

3.3.2 Micro-habitat

3.3.2.1 Community groupings

TWINSpan shows that more than one community at the micro-habitat scale contains *N. flexilis*, again suggesting that this approach could be a useful SCM tool. Three main TWINSpan site-groups were produced with this approach (Table 3.2b). Each group contains sites from more than one geographical area, suggesting geographical reasons do not determine floristic composition within a group. The species occurring in the TWINSpan groups are shown in the floristic table (Table 3.3b). These TWINSpan groups have been classified according to the NVC (Table 3.4b). The coefficients provided by the MATCH programme for the goodness of fit for these communities to NVC types are also shown in Table 3.4b. A match coefficient of 100 is a perfect match and 0 shows no similarity.

3.3.2.2 Species composition

The splits between the groups created on the micro-habitat species assemblages are more clear-cut than those for the whole loch species assemblages. The groups obtained from the micro-habitat surveys did not

all contain *N. flexilis*. When *N. flexilis* was dominant within the community it was always obtained within the samples, however due to the small size of the vegetation samples taken, this was not the case when it was an occasional within the community. This is because it was the community that was sampled and not *N. flexilis*. This illustrates that only the dominant species within the community would necessarily be found in the samples, and therefore these samples are classified based on the dominant species. Both group 2 and 3 did constantly contain *N. flexilis* (Table 3.3b). Group 2 most frequently contains the isoetids, *Isoetes lacustris*, *Lobelia dortmanna* and *Littorella uniflora* as well as *Juncus bulbosus*, whilst group 3 most frequently contains *Chara* spp., *Potamogeton berchtoldii* and *Potamogeton perfoliatus*. Group 1 is grouped on the lack of these otherwise constant species, the high frequency measurements for this group are artefacts of the small sample size (only two). These groupings are all classified as separate NVC types (Table 3.4b).

Table 3.3 Floristic table showing the frequency of plant occurrence within the groups created on (a) the loch scale and (b) the micro-habitat scale. V = 80–100%, IV = 60–80%, III = 40–60%, II = 20–40%, I = <10%.

(a)

	Group 1 n = 16	Group 2 n = 18	Group 3 n = 10	Group 4 n = 7
<i>Juncus bulbosus</i>	V	V	II	
<i>Littorella uniflora</i>	V	V	V	V
<i>N. flexilis</i>	V	V	V	II
<i>Potamogeton natans</i>	V	IV		
<i>Isoetes lacustris</i>	IV	IV	II	II
<i>Potamogeton berchtoldii</i>	IV	I	V	III
<i>Elatine hexandra</i>	III	I	I	I
<i>Fontinalis antipyretica</i>	III	I	II	
<i>Lobelia dortmanna</i>	III	IV		
<i>Menyanthes trifoliata</i>	III	II		II
<i>Miriophyllum alterniflorum</i>	III	IV	V	IV
<i>Nitella</i> spp.	III	I	I	I
<i>Nymphaea alba</i>	III	I	I	I
<i>Potamogeton perfoliatus</i>	III	V	V	III
<i>Sparganium angustifolium</i>	III	III	I	II
<i>Chara</i> spp.	II	IV	III	V
<i>Eleocharis palustris</i>	II	I		II
<i>Elodea canadensis</i>	II	I	II	V
<i>Potamogeton x nitens</i>	II	I	II	I
<i>Ranunculus aquatilis</i>	II			III
<i>Ranunculus flammula</i>	II			I
<i>Utricularia vulgaris</i>	II	II	I	
<i>Eriocaulon aquaticum</i>	II			
<i>Baldellia ranunculoides</i>	I	I		
<i>Callitriche hamulata</i>	I	I	I	

Table 3.3 (continued)

	Group 1 n = 16	Group 2 n = 18	Group 3 n = 10	Group 4 n = 7
<i>Callitriche hermaphroditica</i>	I	II	IV	V
<i>Callitriche stagnalis</i>	I			
<i>Eleocharis acicularis</i>	I	I	II	II
<i>Eleogiton fluitans</i>	I			
<i>Elodea nuttallii</i>	I		III	
<i>Isoetes setacea</i>	I			
<i>Lemna minor</i>	I			II
<i>Nuphar lutea</i>	I		I	III
<i>Potamogeton alpinus</i>	I			II
<i>Potamogeton crispus</i>	I		I	II
<i>Potamogeton gramineus</i>	I	IV	III	III
<i>Potamogeton polygonifolius</i>	I	I		
<i>Potamogeton praelongus</i>	I	I	I	
<i>Potamogeton pusillus</i>	I		I	II
<i>Potamogeton rutilus</i>	I	I	II	
<i>Subularia aquatica</i>	I			I
<i>Persicaria amphibia</i>	I			II
<i>Hydrilla verticillata</i>	I			
<i>Apium inundatum</i>		I		
<i>Myriophyllum spicatum</i>		I	I	
<i>Potamogeton lucens</i>		I		II
<i>Potamogeton obtusifolius</i>				III
<i>Potamogeton pectinatus</i>		I	III	
<i>Potamogeton x zizii</i>		I		II
<i>Sparganium erectum</i>		I		I
<i>Zannichellia palustris</i>			I	
<i>Ceratophyllum demersum</i>				I

Table 3.3 (continued)

(b)

	Group 1 n = 2	Group 2 n = 11	Group 3 n = 18
<i>Isoetes lacustris</i>		IV	I
<i>Juncus bulbosus</i>	III	IV	II
<i>N. flexilis</i>		IV	IV
<i>Littorella uniflora</i>		III	I
<i>Potamogeton gramineus</i>	V	III	II
<i>Lobelia dortmanna</i>		II	
<i>Myriophyllum alterniflorum</i>		II	I
<i>Callitriche hermaphroditica</i>		I	II
<i>Chara</i> spp.		I	III
<i>Elodea canadensis</i>		I	II
<i>Potamogeton berchtoldii</i>		I	IV
<i>Potamogeton perfoliatus</i>		I	IV
<i>Sparganium angustifolium</i>	III	I	II
<i>Elatine hexandra</i>			I
<i>Eleocharis acicularis</i>			I
<i>Elodea nuttallii</i>			I
<i>Fontinalis antipyretica</i>	V		I
<i>Nitella</i> spp.	III		I
<i>Potamogeton lucens</i>	III		
<i>Potamogeton pectinatus</i>			I
<i>Potamogeton rutilus</i>	III		I
<i>Utricularia vulgaris</i>	III		
<i>Potamogeton pusillus</i>			I

Table 3.4 (a) Standing water type classification of the groups created from the loch scale species composition data, (b) NVC of the groups created from the micro-habitat species composition data given with MATCH coefficient.

(a)

	Group 1	Group 2	Group 3	Group 4
Palmer	SWT 3	SWT 4	SWT 5a	SWT 5a

(b)

	Group 1	Group 2	Group 3
NVC	A24a	A23	A13
Match coefficient	45.4%	64.6%	66.3%

3.3.2.3 Environmental conditions

The only environmental variable that is significantly different ($p < 0.05$) between groups at the micro-habitat scale is the light extinction coefficient where group 3 communities inhabit clearer water than group 2 (Table 3.6).

Table 3.5 Environmental conditions of the loch scale plant community groups (a) water measurements, (b) sediment measurements. Values with different letters are significantly different ($p < 0.05$; Kruskal-Wallis test and Dunn multiple comparisons test).

(a)

	Group 1	Group 2	Group 3	Group 4
pH	6.46 – 7.97 ^a (n = 16)	6.84 – 8.26 ^{ab} (n = 18)	7.04 – 8.78 ^b (n = 10)	7.11 – 9.01 ^b (n = 7)
Median	7.15	7.44	7.77	7.95
Conductivity (us/cm)	54.9 – 447 (n = 16)	97 – 405 (n = 18)	77 – 370 (n = 10)	143 – 410 (n = 7)
Median	179.5	270.5	278	214
Light extinction coefficient	0.78 – 7.32 (n = 16)	1.11 – 6.23 (n = 18)	0.53 – 5.18 (n = 10)	0.91 – 4.32 (n = 7)
Median	2.608668	2.000661	0.940007	1.393594
Alkalinity (mg/l)	4.91 – 65.7 ^a (n = 12)	8.61 – 69.3 ^{ab} (n = 12)	9.7 – 69.71 ^{ab} (n = 7)	30.16 – 98.66 ^b (n = 3)
Median	16.28	22.01	32.4	72.65
Ca (mg/l)	1.32 – 22.4 (n = 13)	3.88 – 33.4 (n = 12)	8.26 – 25.8 (n = 7)	11.42 – 24.72 (n = 3)
Median	7.96	8.78	13.14	21.7
Fe (mg/l)	<0.03 – 0.99 (n = 13)	<0.003 – 1.69 (n = 12)	<0.03 – 1.51 (n = 7)	0.04 – 0.35 (n = 3)
Median	0.22	0.21	0.135	0.08
Mg (mg/l)	1.34 – 7.2 (n = 13)	2.8 – 6.2 (n = 12)	1.34 – 8.8 (n = 7)	2.08 – 12 (n = 3)
Median	4.4	4.8	6.4	8.8
N-NH ₃ (mg/l)	<0.03 – 0.1 (n = 13)	<0.03 – 0.08 (n = 12)	<0.03 – 0.1 (n = 9)	<0.03 – 0.28 (n = 5)
Median	0.04	<0.03	<0.03	0.056
N-TON (mg/l)	<0.01 – 0.03 ^a (n = 13)	<0.01 ^a (n = 12)	<0.01 – 0.11 ^a (n = 9)	<0.01 – 2.64 ^b (n = 5)
Median	<0.01	<0.01	<0.01	0.88
P-DRP (PO ₄) (mg/l)	<0.001 ^a (n = 13)	<0.001 – 0.005 ^{ab} (n = 12)	<0.001 – 0.004 ^{ab} (n = 9)	<0.001 – 0.074 ^b (n = 5)
Median	<0.001	<0.001	<0.001	0.023
P-TP (mg/l)	<0.001 – 0.021 (n = 13)	<0.001 – 0.02 (n = 12)	<0.001 – 0.026 (n = 7)	0.014 – 0.16 (n = 3)
Median	0.01	0.0085	0.013	0.056
K (mg/l)	0.3 – 2.82 (n = 13)	0.16 – 2.14 (n = 12)	0.3 – 1.9 (n = 9)	1.2 – 2 (n = 5)
Median	0.94	0.9	1.1	1.52

Table 3.5 (continued)

(b)

	Group 1	Group 2	Group 3	Group 4
Available P (mg/l)	0.3 – 17.3 ^{ab} (n = 15)	0.3 – 3.6 ^a (n = 17)	1.1 – 84.4 ^{bc} (n = 10)	3.6 – 72.9 ^c (n = 7)
Median	1.3	1.3	2.8	16.65
Available K (mg/l)	4 – 166 (n = 5)	4 – 136 (n = 17)	9.5 – 104 (n = 9)	28.3 – 95.5 (n = 4)
Median	15.7	37.5	29	77.1
Extractable Mg (mg/l)	50.9 – 344 (n = 5)	17.3 – 388 (n = 18)	16.3 – 302 (n = 9)	54.1 – 189 (n = 4)
Median	211	141	66.4	106.5
Extractable Ca (mg/l)	145 – 5720 ^{ab} (n = 15)	198 – 1930 ^{ab} (n = 18)	1.8 – 1550 ^a (n = 10)	805 – 331000 ^b (n = 7)
Median	816	896	544	3180
Total Fe (mg/kg)	516 – 168000 ^b (n = 15)	94.4 – 11900 ^a (n = 18)	105 – 14500 ^a (n = 10)	123 – 11300 ^{ab} (n = 7)
Median	890	786	570	187
Total N (mg/kg)	290 – 142000 (n = 15)	240 – 89200 (n = 18)	350 – 27100 (n = 10)	600 – 40800 (n = 7)
Median	15100	6220	2120	6255
Extractable Na (mg/l)	34.2 – 205 (n = 4)	17.4 – 300 (n = 16)	8.3 – 239 (n = 9)	20.5 – 72.5 (n = 4)
Median	63.25	76.85	35.6	33.8

Table 3.6 Environmental conditions of the micro-habitat scale plant community groups (a) water measurements, (b) sediment measurements. Values with * are significantly different (p<0.05; Mann-Whitney U test).

(a)

	Group 1	Group 2	Group 3
pH	7.2 – 8.2 (n = 2)	6.84 – 8.25 (n = 11)	7.04 – 8.3 (n = 18)
Median	7.7	7.3	7.54
Conductivity (us/cm)	269 – 345 (n = 2)	97 – 398 (n = 11)	77 – 447 (n = 18)
Median	307	235	232
Light extinction coefficient	1.11 – 3.21 (n = 2)	1.23 – 6.23 (n = 11)*	0.53 – 7.32 (n = 18)*
Median	2.169054	2.978303	1.767662
Alkalinity (mg/l)	69.3 (n = 1)	8.61 – 40.95 (n = 7)	9.7 – 69.71 (n = 12)
Median	69.3	19.15	25.06
Ca (mg/l)	33.4 (n = 1)	3.88 – 15.86 (n = 7)	5.72 – 25.8 (n = 12)
Median	33.4	7.98	9.82
Fe (mg/l)	0.13 (n = 1)	0.003 – 0.94 (n = 7)	0.003 – 1.69 (n = 12)
Median	0.13	0.21	0.15
Mg (mg/l)	6.2 (n = 1)	2.8 – 5.6 (n = 7)	1.34 – 8.8 (n = 12)
Median	6.2	5.2	4.8
N-NH ₃ (mg/l)	<0.03 (n = 1)	<0.03 – 0.08 (n = 7)	<0.03 – 0.1 (n = 15)
Median	<0.03	<0.03	<0.03
N-TON (mg/l)	<0.01 (n = 1)	<0.01 (n = 7)	<0.01 – 1.369 (n = 15)
Median	<0.01	<0.01	<0.01
P-DRP (PO ₄) (mg/l)	<0.001 (n = 1)	<0.001 – 0.004 (n = 7)	<0.001 – 0.005 (n = 15)
Median	<0.001	<0.001	<0.001
P-TP (mg/l)	<0.001 (n = 1)	<0.001 – 0.013 (n = 7)	<0.001 – 0.026 (n = 12)
Median	<0.001	0.003	0.0105
K (mg/l)	2.14 (n = 1)	0.52 – 2.02 (n = 7)	0.54 – 2.02 (n = 15)
Median	2.14	0.86	1.2

Table 3.6 (continued)

(b)

	Group 1	Group 2	Group 3
Available P (mg/l)	1.9 – 2.2 (n=2)	0.3 – 3.6 (n = 11)	0.5 – 9.4 (n = 18)
Median	2.05	1.3	2.6
Available K (mg/l)	38.2 – 74.5 (n = 2)	4 – 87.1 (n = 11)	8 – 166 (n = 17)
Median	56.35	37.5	28
Extractable Mg (mg/l)	151 – 92.4 (n = 2)	28.7 – 308 (n = 11)	17.3 – 388 (n = 17)
Median	121.7	77.6	87
Extractable Ca (mg/l)	600 – 1610 (n=2)	290 – 1800 (n = 11)	253 – 1930 (n = 18)
Median	1105	816	1019
Total Fe (mg/kg)	190 – 1630 (n = 2)	126 – 7780 (n = 11)	121 – 11900 (n = 18)
Median	910	578	875
Total N (mg/kg)	590 – 11400 (n = 2)	350 – 38100 (n = 11)	350 – 142000 (n = 18)
Median	5995	10200	8960
Extractable Na (mg/l)	41.6 – 145 (n = 2)	21.1 – 257 (n = 11)	13 – 300 (n = 15)
Median	93.3	72	39.7
Exposure	1.58 – 2.13 (n = 2)	0.58 – 2.73 (n = 11)	0.39 – 4.52 (n = 15)
Median	1.85	0.94	1.26

3.4 Discussion

3.4.1 The ability of the loch scale qualitative approach and the micro-habitat scale quantitative approach to illustrate the different loch conditions.

Both approaches produce multiple community groups that could be used as site condition monitoring tools. Both sets of communities are also found to reflect the environment where they grow, which is to be expected. However, the two approaches elucidate very different aspects of the environment. It could be expected that because the micro-habitat approach looks at a finer scale and includes information on the quantity of a species instead of its presence or absence it should produce a finer scale result, for example, a greater number of communities representing a number of different environments. Instead, in this case, this scale approach only elucidated a difference in light. As predicted, this approach appears to elucidate the different zonations within a loch, but surprisingly it failed to elucidate differences in any other environmental conditions.

The very small scale 20 x 20cm² plant collection for the micro-habitat community may have limited the effectiveness of this approach. Previous attempts at macrophyte classification schemes have used 2 x 2m² or 4 x 4m² quadrats. Instead, in this study we replicated the smaller samples three times to increase the chance of collecting more species. However, macrophytes often grow in monospecific stands or species-poor communities much greater in dimension than 4 x 4m² (Rodwell, 1995). Therefore, although increasing the quadrat size would increase the chances of collecting more species a very large quadrat would be required to collect many more species. It is of interest that the NVC, which used a quantitative quadrat-based approach, produced classifications of communities which existed in a number of the SWT types, which uses a semi-quantitative loch scale approach.

In contrast, the whole loch approach elucidated the differences in several environmental conditions including pH and phosphorus, which represent acidification and eutrophication, the greatest threats to vegetation in north European softwater lochs (Murphy, 2002). These are the kind of environmental changes that threaten *N. flexilis*, which site condition monitoring needs to detect (see sections 2.3 & 2.4). This greater ability to detect the environmental differences is due to the greater number of species encountered by surveying a greater area, thus increasing the chance of finding a species that is a good environmental indicator. The level of detail lost by using a qualitative rather than a quantitative technique does not appear to have hindered this technique. This is important, as accurate quantitative measures of deep water plants are difficult to acquire without snorkelling or SCUBA diving. This is because the quantity of a macrophyte captured on a rake or grapnel is only partly dependent on its abundance. It is also greatly affected by its structure; whether it is likely to be snared on the device and how firmly it is rooted in the sediment (Capers, 2000, James, personal communication 1999 & personal observation).

3.4.2 Standing Water Type and National Vegetation Classification. How well do these classifications fit the data collected?

Standing Water Type appears to fit the data collected in this study well, whilst the NVC fitted groups 2 and 3 reasonably well and group 1 not so well.

3.4.2.1 Standing Water Type

Groups 1–4 are generally very similar in species composition to the SWTs into which they have been classified, however there are exceptions. All groups contain *N. flexilis*, which is not included in the SWT classifications. This is not surprising, as due to the rarity of *N. flexilis* it would not frequently have been found in general loch surveys, compared to this study where lochs containing the plant were purposefully visited and the plant sought out. The other main differences between the groups and their assigned SWT are that group 1 contains a greater quantity of *Potamogeton berchtoldii* than SWT 3. The difference between group 2 and SWT 4 is a greater quantity of *Isoetes lacustris* and *Lobelia dortmanna* in group 2. Group 3 is a good match for SWT 5 with no great differences between them. Group 4 is different to SWT 5 in that it contains a greater amount of *Chara* spp.

The SWT study (Palmer, 1989) provides some indication of the differences in the chemistry of the lochs studied. This means that the environment found to be representative of the groups in this study can be compared to the environment found to be representative of the SWT, which the group has been classified as. Standing Water Type 3 is the most oligotrophic SWT in this study. Sites classified as SWT 3 would most likely have a pH between 6 and 7 and alkalinities of 2–30mg/l: lochs in this group are often on igneous and metamorphic rocks. When compared to the environmental measures in group 1 (Table 3.5) it can be seen that although the ranges include higher measurements than these, the medians are reasonably similar. The median alkalinity measurement is in the middle of the suggested alkalinity range for this type of vegetation, however pH is slightly higher, this may be because these measurements are based on a single pH reading in summertime.

Palmer (1989) states that SWT 4 is a wide ranging category which represents lochs with a variety of influences, for example machair lochs where the base poor waters run off the peatlands into a more base rich loch overlying shell sand or other calcareous material. This variety of influences on loch chemistry can lead to plants from both base rich and base poor associations coexisting within a loch. Many of the lochs

categorised as SWT 4 in this study are machair lochs from the Western Isles of Scotland. The environmental measures from the sites assigned to this SWT, group 2, do not seem to show a wider breadth of environmental conditions, but do show a slight increase in both pH and alkalinity from group 1 assigned to SWT 3.

Both groups 3 and 4 were classified as SWT 5. Standing Water Type 5 is considered typical of mesotrophic conditions. These SWTs typically have a higher pH and alkalinity than SWT 3 and sometimes SWT 4 (only sometimes due to the wide ranging underlying environment in SWT 4). Both Group 3 and 4 fit with this trend of increasing pH and alkalinity. The environmental conditions represented in group 3 seem to be those most frequently found to support SWT 5 whereas the environmental conditions found in group 4 sites appear to be at the extreme end of those typically found to support SWT 5. This can be seen by comparing the alkalinity measures. The SWT 5 sites most frequently have an alkalinity that ranges between 10–30mg/l. The median alkalinity for group 3 is 32.5mg/l. The highest alkalinity range found to support SWT 5 vegetation is 30–100mg/l. The median alkalinity for group 4 is 72.65 mg/l.

Standing water type appears to describe similar floristic and environmental groups to those found in this study. However SWT 5 has been split into two further groups in this study with group 3 being a typical mesotrophic loch and group 4 being at the most eutrophic extreme for the SWT 5 category.

3.4.2.2 National Vegetation Classification

Three NVC community types were found in association with *N. flexilis*: A13, *Potamogeton perfoliatus-Myriophyllum alterniflorum*; A23, the *Isoetes lacustris/setacea* community; A24, the *Juncus bulbosus* community. This illustrates that the plant can thrive in more than one community type contrary to Pearsall (1920) and Rodwell (1995). These classifications fit the data from this study to varying degrees.

Group 3, A13

Group 3 is most similar to NVC type A13, however it is not a perfect match. The dominants of community type A13 are constantly under recorded in group 3 with the exception of *Potamogeton perfoliatus* and *Potamogeton berchtoldii*. The following all have a much higher constancy score in A13 than group 3; *Myriophyllum alterniflorum*, *Littorella uniflora*, and *Potamogeton gramineus*. The under representation of these species is probably due to the smaller scale of sampling in this study and the domination of the communities in many cases by *N. flexilis*. *N. flexilis* is over represented in group 3 compared to A13, this can be expected due to the fact that it is a rare plant so would not have been encountered as many times in a general survey, as in a study focused on this species.

A13 is the only community within the NVC to be reported to contain *N. flexilis*. It is representative of a wide habitat covering shallow to quite deep, mesotrophic and rather base poor waters, which are still or gently flowing with fine to coarse mineral beds, but not peats. The community type is generally confined to the north and west of Britain and occurs in pools, lochs and the middle to lower reaches of rivers (Rodwell, 1995).

Group 2, A23

The Match programme provides a good match between the group 2 data and the NVC type A23. However, the sample data contain a greater abundance and frequency of *Juncus bulbosus* and also contain

Potamogeton gramineus as a dominant, this species is not mentioned in the NVC classification. This suggests that the community described by the sample data is not strictly the same as the NVC classification. The *Isoetes* community described in Rodwell (1995) is typically found on barren, stony substrates, in clear, deep waters of less fertile lochs through north west Britain. *N. flexilis* was never found on stony substrate in this study, but always on fine silt. The water was not necessarily clear either, particularly in those lochs where the water was stained by the peat. These lochs clearly represent the oligotrophic end of the range of *N. flexilis* communities, but with occasional species such as *Callitriche hermaphroditica* and *Elodea* spp. occurring in both the description by Rodwell and the equivalent *Isoetes* society described by Spence (1964) and such species being present in group 2, it suggests that this group may contain sites, which are not exceedingly oligotrophic.

Group 1, A24

The species found in group 1 are not a good match to NVC type A24. This is probably due to the low sample number making meaningful classification difficult. These two samples are obviously outliers from the other two communities, this seems to be due to the lack of constant species found in the other 2 groups, rather than the presence of different species within group 1. They are unlikely both to be members of a *Juncus bulbosus* community when only one site contains this species, the misleadingly high frequencies are due to the small sample size. Instead, these outliers emphasise that although the presence of specific species of macrophytes or their communities can indicate suitable conditions for *N. flexilis* growth, the absence of one or two of these species does not make the site necessarily unsuitable.

3.4.3 How well would SWT or the NVC predict *N. flexilis* success?

3.4.3.1 Standing Water Type

Standing Water Type communities vary across the sites where *N. flexilis* grows and this appears to coincide with trends in trophic state. This indicates that *N. flexilis* does not appear to be restricted to just one "ideal" plant community, but occurs across a range. Standing Water Type 3 is the most oligotrophic SWT found in this study, whilst SWT 5 is the most eutrophic SWT found in this study. As Standing Water Type 3 and SWT 5 are the only SWTs which contained sites where *N. flexilis* is thought to be extinct, it suggests that it is those sites at the most oligotrophic and eutrophic extremes of its range that are most at-risk. This would appear to agree with Murphy (2002) that the greatest threats to vegetation in north European softwater lochs are eutrophication and acidification.

Both groups 3 and 4 were identified as SWT 5, yet group 4 appeared to be at the eutrophic limit for this SWT and a reasonably unsuitable environment for *N. flexilis* growth. SWT 3 is obviously at the opposite end of the scale and group 1, assigned to this SWT, contains the 2 extinct oligotrophic sites. However many other sites assigned to this group contain healthy *N. flexilis* populations. A more sensitive scheme than SWT would be needed to indicate exactly which *N. flexilis* sites are most at risk. Therefore, it appears that although plant communities can indicate the groups that will contain at risk sites, many of the sites within such a group will also contain healthy *N. flexilis* populations. This is because many plants appear to have a wider niche than *N. flexilis*, so these species cannot be used as indicators for *N. flexilis* condition. Therefore, a loch scale approach to community classification could indicate a group of sites that were likely to contain "at risk" sites for *N. flexilis*. However it would be unable to pin-point exactly which sites were at risk.

3.4.3.2 NVC

The NVC communities in which *N. flexilis* is found illustrate which depth zones it occupies within the loch. The *Isoetes* community is often the deepest vegetated zone within the loch (Rodwell, 1995; Spence, 1964). The *Potamogeton perfoliatus-Myriophyllum alterniflorum* community is thought to represent an intermediate zone with most commonly a *Littorella-Lobelia* community between it and the shore. This occurs when the shoreline is exposed and therefore made up of coarse material rather than silt. When the first sediment type encountered after leaving the shore is silt, A13 can be the first community encountered. This is particularly the case in small lochs or sheltered bays. In deeper water, this community can be replaced by *Nitella* spp. as seen in the Lake of Menteith or *Chara* spp. as in Pollacapull Lough. It can also be replaced by *Isoetes* communities, containing abundant *N. flexilis* as seen in Loch Druidibeg. This explains the significant difference found in the light extinction coefficient between group 2, A23 and group 3, A13. However, *N. flexilis* appears to grow equally well in both these communities, but not the *Littorella-Lobelia* community, which grows on the gravelly substrate. This indicates that the positioning of *N. flexilis* within a loch is more often due to sediment type (and probably competition, this will be discussed in Chapter 4) than by light, within the vegetated region of the loch, as the plant occurs in communities in the shallows and the depths wherever there is fine sediment. Fine sediment will only occur where exposure is low (see section 1.4.4). Therefore, it is likely that both A23 and A13 are in areas with lower exposure than the *Littorella-Lobelia* community. Exposure may control *N. flexilis* growth directly through disturbance or indirectly through its effect on sediment type.

As an indicator of the condition of a loch and its favourability for *N. flexilis* growth, use of the NVC at the micro-habitat scale does not appear to be the best method. This is because the species that can grow in association with *N. flexilis* appear to have wider ranges in which they can grow than *N. flexilis*. As a result, no differences in the environmental conditions that appear to control *N. flexilis* growth are elucidated by looking at the vegetation at the micro-habitat scale. However, it does elucidate the zones within a loch that can support *N. flexilis*. NVC type A13 is thought to be represented in lochs, which are SWT 2, 3, 5, and occasionally 4 and NVC type A23 represented in SWT 2 and 3. This illustrates the wide range of environments incorporated into a single NVC community. This wide environmental range makes the NVC a poor tool for indicating *N. flexilis* condition.

3.5 Conclusion

SWT is a much better predictor of the condition of a loch than NVC due to the greater number of species included. This means it can detect finer scale differences in the underlying environment. However, due to the narrow niche that *N. flexilis* inhabits a further division in SWT 5 is required. These SWTs could indicate the "at risk" sites for *N. flexilis* and would include sites at the most eutrophic and oligotrophic ends of the *N. flexilis* range. These would be lochs that contain SWT 3, and the more eutrophic SWT 5 communities. If SWT were used as a preliminary monitoring regime, perhaps in conjunction with water and sediment chemistry information (see Chapter 2), more in-depth monitoring efforts concentrating on *N. flexilis* itself could be targeted at "at risk" lochs supporting SWT 5 and 3. This would save time and resources even though some of these lochs would contain healthy populations.

Summary

- The loch scale qualitative approach elucidated significant differences in pH, alkalinity and the concentration of total oxidised nitrogen and dissolved reactive phosphate in the water as well as differences in phosphate, calcium and iron in the sediment.
- The small-scale quantitative approach only elucidated a difference in light extinction coefficient between community groups.
- The community groups derived from the small-scale quantitative survey were roughly comparable to NVC communities A13, A24a and A23.
- The loch scale qualitative approach produced community groups comparable to standing water types 3, 4 and 5, however, this study produced two type 5 sub-groups.
- Extinct *N. flexilis* sites were classified as type 3 and the most eutrophic type 5, as were other sites that still supported *N. flexilis*.

4 A TRAIT-BASED METHODOLOGY TO PREDICT COMPETITION BETWEEN NATIVE AND INVASIVE SPECIES: *N. FLEXILIS* AND *ELODEA* SPP.

4.1 Introduction

4.1.1 Aims

- To determine whether the incidence of individual, or sets of, morphological, physiological and/or life-history traits exhibited by the associated species which form loch *N. flexilis* communities can act as an indicator of the degree of probable competitive impact on *N. flexilis* fitness in such habitats.
- To use this information to assess the likely competitive threat posed by *Elodea canadensis* and *Elodea nuttallii* to *N. flexilis* survival in Scottish lochs.

4.1.2 Background

The previous chapters have concentrated on the relationships between *N. flexilis* growth and the physical and chemical environment of the loch habitat. The composition of macrophyte communities has been considered as a method to describe this environment, and assess the suitability of the environment for *N. flexilis* growth. However, associated aquatic plant species may have a more direct impact on *N. flexilis*. Competitive interactions, coexistence and coevolution are all factors that may play an important role in determining the distribution and abundance of plant species (Rosch *et al.*, 1997).

4.1.2.1 *Elodea* spp. in Scotland

Elodea canadensis and *Elodea nuttallii* are native to North America, yet behave as invasive aquatic weeds throughout Europe. They have also caused problems in Japan, Australia and New Zealand (Murphy *et al.*, 1990; Preston & Croft, 1997). *Elodea* spp. appear to exhibit a series of successional growth phases after invasion of a new site. After introduction to a new habitat, *Elodea canadensis* often grows in abundance to nuisance levels, over a period varying from a few months to 4 years (Phase 1). The plant may then persist at high abundance for as many as 5 years during the second phase, and then wanes either gradually or quickly during the third phase. In the fourth phase *Elodea canadensis* persists at non-nuisance levels (Sculthorpe, 1967). If a disturbance such as cutting interrupts these phases, the process can start again. *Elodea nuttallii* biomass has also been reported to fluctuate naturally and after an initial period of infestation has been seen to decline of its own accord (Hamabata, 1997; Simpson, 1990).

Table 4.1 Sites in Scotland where *N. flexilis* and *Elodea* spp. occurred together during 1998–2000.

Site	Grid reference	Area	<i>Elodea canadensis</i>	<i>Elodea nuttallii</i>
Loch of Butterstone	NO 062453	Perthshire	✓	✓
Loch of Craiglush	NO 044446	Perthshire	✓	✓
Loch of Lowes	NO 055443	Perthshire	✓	✓
Loch Clunie	NO 116444	Perthshire	✓	✓
Lake of Menteith	NS 577002	Stirling	✓	✓
Tangy Loch	NR 695280	Kintyre	✓	✗
Loch Nan Gad	NR 783573	Kintyre	✓	✗
Loch Fada	NR 383955	Colonsay	✓	✗
Loch Scarie	NF 718705	North Uist	✗	✓
Loch Grogary	NF 717710	North Uist	✗	✓
Loch nam Faoleann	NF 752210	South Uist	✓	✗

The majority of the mainland lochs in Scotland containing *N. flexilis* also contain one or both of the *Elodea* spp. (Table 4.1). The invasion of *Elodea* spp. into these sites does not appear to have been detrimental to *N. flexilis*, and the *Elodea* spp. exist in small quantities within limited areas of the lochs concerned. It could be that *Elodea* has already invaded, had its initial abundance phase and now exists in a small quantities or it could be that it has not reached its abundance phase or it may never happen. With one exception, these mainland lochs are at the larger end of the scale of those inhabited by *N. flexilis* in Scotland and as such could be environmentally more heterogeneous than the smaller, shallow lochs of Uist and Colonsay. During the initial *Elodea* spp. invasion, this environmental heterogeneity may have ensured *N. flexilis* survival, due to the occurrence of refugia where *N. flexilis* could successfully compete with the *Elodea* spp. or where *Elodea* spp. could not grow. Tanga Loch (Kintyre) is the exception among the mainland sites. This is a smaller loch, where *Elodea canadensis* is a recent invasive. In 1994, *Elodea canadensis* was extremely abundant and a specific diver search for *N. flexilis* at this time only found two plants within the loch (Martin Gaywood, Scottish Natural Heritage, pers. comm). Whether the invasion of *Elodea* spp. caused the extinction of *N. flexilis* in sites where *N. flexilis* can no longer be found is unknown. Both *Elodea* spp. can be found in some, but not all of these are 'absent' sites. However, the environment at these absent sites also appears unfavourable for *N. flexilis* growth (Chapter 2).

One of the major threats to the *N. flexilis* stronghold in the Hebrides is weed competition, specifically from *Elodea canadensis* and *Elodea nuttallii*. In Colonsay, and North and South Uist the threat of pollution and eutrophication is small, due to the lack of intensive agriculture or any other kind of industry. However, there have been a series of introductions of alien species, including the two aforementioned aquatic weed species. The Scottish Natural Heritage (SNH) Fresh Water Loch Survey Team first reported *Elodea canadensis* in Loch nam Faoileann in S. Uist, near Daliburgh, in August 1995 (Scott, 1996). *Elodea nuttallii* is also now present in North Uist in Lochs Grogary and Scarie. This is a recent introduction, probably occurring in the early 1990's although no hard data are available. This invasion in Loch Grogary had reached a state where it was restricting boat use by anglers in 1999. These recent invasions are a cause for concern for the conservation agencies because as well as being the stronghold for *N. flexilis* in Britain, the Uist lochs support rare *Potamogetons* such as *Potamogeton rutilus* and *Potamogeton epihydrus*, as well as other uncommon plants such as *Callitriche hermaphroditica*. The N. Uist lochs where *Elodea nuttallii* has invaded are two of only 12 known current sites for *Potamogeton rutilus* in the British Isles (G. Wallace: unpublished 2002 survey data).

North and South Uist have many lochs often connected by small streams and drainage ditches (Waterston *et al.*, 1979). This intricate network of freshwater bodies is ideal for the spread of *Elodea* spp., which rely on the movement of vegetative fragments and turions for dispersal in the UK. The popularity of angling in Uist and the movement of fishermen from loch to loch is a vector for *Elodea* spp. dispersal, a single viable fragment transferred in fishing tackle could establish a new population in another loch. This fear was to some extent realised when SNH commissioned a survey of Loch nam Faoileann and the surrounding lochs for *Elodea canadensis* in October 1996. This found that *Elodea canadensis* was now in four lochs in the vicinity rather than one, as previously thought (Scott, 1996).

The concern of the conservation agencies is that as the *Elodea* spp. spread and invade new lochs and lochans in Uist, either of the *Elodea* spp. at the height of their abundance could outcompete *N. flexilis*. This is only a threat if competition actually occurs between these species (and no habitats exist in which *N. flexilis* can successfully withstand competition from *Elodea* spp.) and if *N. flexilis* does not create a persistent seedbank. There is little knowledge on *N. flexilis* seedbank persistence and the length of time required to carry out experiments into this exceeds the duration of this study.

When the species all occur in the same loch, they can occur side by side. The macrophyte surveys described in section 3.2.2.2 found *Elodea canadensis* in the *N. flexilis* micro-habitat in 6 out of 7 lochs where both occurred, whilst *Elodea nuttallii* was found in the *N. flexilis* micro-habitat in 2 out of 5 lochs where both occurred. This illustrates that *Elodea* spp. and *N. flexilis* can and do cohabit the same habitats. This niche overlap, suggests there is a strong likelihood of competition. *Elodea* spp. also inhabits environments where *N. flexilis* does not grow i.e. in eutrophic and slow flowing systems. However, whether *N. flexilis* grows in environments uninhabitable to the *Elodea* spp. is unclear; if this is the case, it appears that they would most likely be in low light and nutrient poor conditions.

A comparison of life history strategies suggests that *Elodea* spp. may well have the competitive advantage over *N. flexilis* due to its ability to grow earlier in the growth season and to produce canopies, suggesting competition for light may be a problem for *N. flexilis*. The ability of both *Elodea* spp. to utilise bicarbonate also suggests they would have a competitive advantage in conditions of sufficient light and low dissolved carbon dioxide concentration. Whether these traits will actually confer a competitive advantage over *N. flexilis* in the environments where *N. flexilis* grows has not hitherto been determined.

4.2 Methods

The effect different plant traits had on the plant fitness measure leaf area/shoot length was investigated. In order to obtain quantitative measures of plant traits for the species growing in association with *N. flexilis*, quantitative abundance measures of the plant species growing in association with *N. flexilis* were obtained. These were taken using Ekman grabs (three per site), the mean dry weight of the plant species collected in the Ekman grab was then used as the quantitative abundance measure (see section 3.2.2.2). This data was then used to produce a site x species matrix that was quantitative.

The matrix of the attributes possessed by European hydrophytes produced by Willby *et al.* (2000) was used as the attribute matrix in this study. This matrix includes traits on leaf morphology, growth form and reproduction and perennation characteristics. A trait such as leaf area is split into four attribute groups; small, medium, large and extra large. Each species is then scored 0 if the attribute is absent, 2 if present and 1 indicating occasionally, but not generally exhibited. This species x attribute matrix was produced by Willby *et al.* (2000) from the literature and extensive fieldwork experience with European hydrophytes and was therefore not specific to particular environmental conditions, and it contained all the species found in association with *Najas flexilis*. It was therefore deemed possible to use the matrix directly in this study.

The species abundance x site matrix was then multiplied by the species x attribute matrix so that a quantitative measure of attribute abundance at each site was produced. For example, the abundance of species 1 at site 1 was multiplied by the score given to species 1 for attribute 1. The abundance of species 2 at site 1 multiplied by the score given to species 2 for attribute 1 was added to this number and so forth, until all the species abundances for site one had been multiplied by their attribute scores for attribute 1 and summed together. If attribute 1 was medium leaf area, you would then gain the total abundance of medium sized leaves at site 1. This is then repeated for each site and each attribute until an attribute abundance x site matrix is produced.

Leaf area/shoot length was regressed against the abundance of individual plant traits found in the *N. flexilis* micro-habitat. This regression analysis was carried out using the same method as in section 2.2.5. The plant traits used were the same as those used by Willby (2000). This would elucidate which plant traits had an effect on *N. flexilis* fitness. These in turn can be compared to the traits possessed by *Elodea* spp. and any other plant species considered as a possible threat to *N. flexilis* to determine if they have the traits required to act as a competitor with *N. flexilis*.

4.3 Results

The following traits were found to have the most significant relationship with *N. flexilis* fitness:

- perennial habit
- anchored submerged leaves
- seed production
- wind pollination
- June–July flowering

Table 4.2 European hydrophyte species investigated in Willby *et al.*, (2000) which regularly exhibit all the attributes with the most significant relationship with *N. flexilis* fitness (where $p < 0.1$) and also grow in habitats with permanent standing water with a depth of over 0.5m.

Species
<i>Callitriche hamulata</i>
<i>Juncus bulbosus</i>
<i>Littorella uniflora</i>
<i>Myriophyllum alterniflorum</i>
<i>Potamogeton alpinus</i>
<i>Potamogeton gramineus</i>
<i>Potamogeton lucens</i>
<i>Potamogeton natans</i>
<i>Potamogeton perfoliatus</i>
<i>Potamogeton praelongus</i>
<i>Schoenoplectus lacustris</i>
<i>Sparganium emersum</i>

The British aquatic species that inhabit a similar environment as *N. flexilis* i.e. Permanent standing water of a depth greater than 0.5m and possess all of these key traits, are listed in Table 4.2. The traits which the *Elodea* spp. possess in comparison to those found to have an effect on *N. flexilis* leaf area/shoot length are listed in Table 4.3.

Table 4.3 A comparison of the attributes possessed by both *Elodea* spp. and those with the most significant ($p < 0.1$) relationship with *N. flexilis* fitness. ✓ = possession of a trait, ✗ = absence of the trait.

Attribute	<i>Elodea canadensis</i>	<i>Elodea nuttallii</i>
Perennial	✓	✓
Anchored submerged leaves	✓	✓
Seed production	✗	✗
Wind pollination	✗	✗
Flowering June–July	✓	✓

A multiple regression model incorporating the traits, wind pollination, June–July flowering and being a perennial can explain 68% of the variation seen in leaf area/shoot length in *N. flexilis*.

Model 1: $r^2 = 0.686$ $p = 0.031$ $n = 27$

$$la/sl = 0.187 + 2.987FJJ - 12.267FJJ^2 + 1.582WP - 0.769WP^2 - 3.656P + 10.99P^2$$

Where:

la/sl = Leaf area/shoot length

FJJ = Flowers in June–July

WP = Wind pollinated

P = Perennial

4.4 Discussion

4.4.1 Hypotheses as to why individual traits are important

Flowering in June–July is probably important as it represents early growing species that could potentially have used resources and be shading *N. flexilis* before it germinates. Perennial plants probably have the advantage as they have more resources in their over-wintering organs than *N. flexilis* does in its seeds. Wind pollination may be advantageous as there are accounts that it can be more reliable than water pollination. However it is more likely to be advantageous because wind pollinated plants have to grow to the surface to place their flowers above the water. This tall growth would then shade *N. flexilis*.

4.4.2 *Elodea* spp. as a competitor with *N. flexilis*

Elodea spp. possess some but not all of the traits discussed above as having a relationship with *N. flexilis* fitness. They are perennials, have anchored submerged leaves, and flower early in the growth season, they do not produce seeds in Britain and are not wind pollinated. *Elodea* spp. are water pollinated, but this occurs on the water surface, rather than completely submerged like pollination in *N. flexilis*, so that *Elodea* spp. must still grow to the surface. If the hypothesis relating to the shading effect of flowering parts and their support structure is correct, pollination by water at the water surface would be just as important as wind pollination and *Elodea* spp. would possess another key trait. However, if the reason wind pollination is a trait which has an impact on *N. flexilis* fitness is due to its effect on production and dispersal of seed, and not shading, then *Elodea* spp. are lacking these traits as no seed is produced in Britain.

4.5 Conclusion

This approach does not explain the mechanisms by which different traits interact with *N. flexilis* fitness. Experiments would be required to elucidate these mechanisms, now that at least some of the traits that do have a relationship with *N. flexilis* fitness are known. This approach illustrates which traits are important within the environment that *N. flexilis* grows. *Elodea* spp. do appear to possess many of the traits which do have a relationship with *N. flexilis* fitness, but not all of them. Before deciding on the extent of the threat of *Elodea* to *N. flexilis* it is important to understand further the importance of pollination mechanism and reproduction via seed in macrophyte communities.

Summary

- A generic trait-based methodology can explain some of the variation in the growth of the target species.
- The abundance of wind pollinated, June–July flowering perennials in the *N. flexilis* micro-habitat can explain 68% of the variation seen in *N. flexilis* leaf area/shoot length.
- *Elodea* spp. in Britain possess the perennial and June–July flowering traits, but are epiphytous.

5 GENETIC VARIATION IN *N. FLEXILIS*: A HYDROPHILOUS MACROPHYTE SPECIES

5.1 Introduction

5.1.1 Aims

As Scotland is the European stronghold for *N. flexilis*, it is important for conservation purposes to have some understanding of its population genetic structure. This can be addressed by asking two questions:

- I. Is the genetic variation held mostly within or between populations?
- II. Does the variation correlate with demographic or ecological factors?

This is particularly important if reintroduction of *N. flexilis* is to be carried out at sites where the plant has become extinct, providing water quality is suitable or can be improved.

5.1.2 Background

N. flexilis occurs as both a diploid and a tetraploid with chromosome numbers of $2n = 12$ or $2n = 24$ respectively (Chase, 1947). Chase (1947) also noted that tetraploids were more robust than diploids. In Europe only the tetraploid has been found and this is also the most common form in Eastern North America. The diploid is found in both America and Canada (Love & Love, 1958). Both ploidy races appear to be fully fertile (Chase, 1947).

5.1.3 Breeding system

Reproduction in *N. flexilis* is obligately sexual, with seed produced from monoecious flowers (single sex flowers, where both sexes can be found on the same plant). Monoecy does not necessarily prevent self-pollination unless it is coupled with temporal or spatial separation of male and female flowers. There have been no specific studies on the pollination of *N. flexilis*, although there have been reports of temporal and spatial separation of male and female flowers, which if correct might be expected to favour outcrossing. Preston & Croft (1997) state that the male flowers are to be found in the upper nodes of the plant with the female flowers below. Triest (1988) states that *N. flexilis* have male and female flowers predominantly in different nodes with the males positioned more to the top. However, occasionally the male and female flowers can be in the same nodes on the top of the plant. However, Polsluszny and Sattler (1976) found male and female flowers with no preference for positioning on the plant. They also stated that only one flower was to be found in each leaf axil. However, Stace (1997) states that one to three flowers could be found in a single node. Such conflicting reports makes drawing any conclusions impossible without further studies.

The pollen in *Najas* species has been reported to begin to germinate on liberation, the elongate shape of the pollen tube increases the surface area and thus increases the chance of it coming into contact with the long forked stigmas (Hutchinson, 1975; Triest, 1988). *N. flexilis* is a prolific seed producer with a seed found in almost every node of the plant; an average of all plants studied showed that there is approximately one seed every 1.25cm of plant length. This indicates that reproduction is not generally pollen limited. This level of reproductive success shows that regardless of whether *N. flexilis* is selfing or out-crossing the pollination mechanism can be successful.

5.1.4 Levels of between population gene flow

The dispersal of pond and loch species between habitats is intrinsically difficult because standing water bodies are effectively aquatic islands in a terrestrial sea. *N. flexilis* is no exception. The dispersal unit for *N. flexilis* are its seeds. External attachment dispersal (e.g. fishermen, birds feet) or wind dispersal are unlikely, because the seeds are intolerant to desiccation (Hay & Muir, 2000), this trait is not shared with all aquatics. *N. flexilis* seeds are reported to be eaten by wildfowl (Martin *et al.*, 1961) and wigeon (Knappton & Pauls, 1994), these accounts are both from the North American continent. Following ingestion by a bird, seeds have to pass through the digestive system in a viable state and be deposited in a suitable habitat. If the seeds are dispersed a long distance they may lack the necessary local adaptations to become established. Instead, more localised movement through interconnected water bodies by streams and rivers could be an efficient means of dispersal.

5.1.5 History and ecology of *N. flexilis* in Britain

The plant's history in Britain is also an important factor to consider regarding genetic variation in *N. flexilis*. *N. flexilis* has a circumboreal range, however the majority of its populations occur in Northern America. The sparse disjunct European range of *N. flexilis* is thought to be a recent Flandrian product, possibly due to a decrease in temperature since the plant was at its most abundant at the time of the thermal maximum in zone VI of the late Flandrian. With the exception of sites in Perthshire, it is now restricted in the British Isles to the Atlantic fringe (Godwin, 1975).

The other reason suggested by Godwin (1975) for the restriction of *N. flexilis* distribution since boreal times is the lack of appropriate habitats. In particular, the number of calcareous or basic loch basins unaffected by drainage and other human interference (such as eutrophication), has been reduced. This has also been documented by fieldwork at former *N. flexilis* sites in Perthshire, which have suffered from eutrophication (see Chapter 2). The occurrence of eutrophication could represent bottlenecks for the surviving populations. The annual nature of the plant makes it particularly sensitive to environmental change, and year to year fluctuations in number could promote genetic drift and loss of variability. Genetic depauperacy at the edge of species' ranges in geographically peripheral populations is a common phenomenon (Levin, 2000). Water depth might also influence patterns of genetic variability in *N. flexilis*. It is in the shallow depths where outcrossing is most likely to occur for two reasons. The first is the greater turbulence experienced by the plant in shallow waters, thus increasing pollen and seed movement, increasing the chance of mating with non-self or non-sibling plants, the second is the increased density of pollen (Les, 1988), generally increasing the chances of pollination. This would lead to a possible scenario, where plants would most likely be selfing or mating with siblings in the still depths and sheltered areas of the loch, and outcrossing and mating with less related plants in the more turbulent shallows and exposed parts of the loch. In America the plant can be found at depths as diverse as 0.2m–14m (Sheldon & Boylen, 1975). It is noteworthy that in Britain *N. flexilis* is rarely found in the immediate littoral zone and instead occurs in sheltered deeper water usually greater than 1.5m (Preston & Croft, 1997). If the speculation about deeper water and shelter leading to increased selfing is correct, this scenario of selfing might be typical of the sheltered waters in which *N. flexilis* grows in the British sites.

5.2 Methods

The original intention was to collect five plants from a range of Scottish and Irish sites and 20 plants from a subset (at least five) of these sites. With this aim, samples were collected from the sites surveyed in 1999.

However, the DNA did not amplify successfully and a number of methods and new samples were attempted. RAPD markers produced the best results in preliminary tests and were therefore used in this study to elucidate the genetic variation in the species.

Only sixty-three samples were successfully amplified from 13 sites covering the geographic range of *N. flexilis* within Britain and Ireland; this represents 5 samples from all but two sites where only four samples could be amplified. Five samples were also amplified from one site in Lake George, New York State for comparative purposes (Table 5.1). This is a big reduction from the original sampling strategy, especially with regard to the number of samples from a site, reducing the ability to answer the question of within-population diversity.

Table 5.1 Sites from which *N. flexilis* was collected.

Sites	Abbreviation	Grid reference
Mainland		
Lake of Menteith	lom	27577002
Loch Clunie	Cl	37116444
Loch of Craiglush	Cr	37044446
Outer Isles		
Loch Druidibeg	Dr	08780388
Loch nan Capull	Nc	08754161
Loch with no name	Lo	08766369
Loch na Cuithe Moire	cm	08738234
Inner Isles and Kintyre		
Loch Gorm	Go	16230655
Loch Lossitt	los	16408652
Loch Nan Gad	Ng	16783573
Ireland		
Lough Leane	Le	14768203
Cregduff Lough	Cd	02718392
Mullaghderg Lough	md	00932890
USA		
Lake George	Us	NY state

5.2.1 Data analysis

The presence/absence data matrix generated using the 9 RAPD primers was converted to a similarity matrix using Jaccard's similarity index: $S_j = c/(a + b + c)$, where c is the number of bands shared by the two samples and a and b are the numbers of bands unique to each of the samples (Jaccard, 1908). In calculating this measure of similarity between pairs of samples, only presence of RAPD bands is taken into account. The absence of a RAPD fragment may be caused by a number of factors, thus using shared absences to represent similarity may misrepresent relationships (Coleman *et al.*, 2000; Weising *et al.*, 1995). The relationships of inter-individual similarity in multidimensional space were examined by principal co-ordinates (PCO) analysis on the Jaccard's similarity matrix using the 'R' package, provided by Phillippe Casgrain and Pierre Legendre. Neighbour joining trees, based on Nei and Li's (1979) distance, (again not

using shared absence to indicate similarity) were produced in PAUP (Swofford, 2001). To assess levels of intra-population variation, the proportion of bands that were polymorphic was calculated. Both Neighbour joining trees and PCO plots show how similar the genotypes of individuals are. Those samples that are genetically most similar appear closest to each other in the neighbour joining tree or in the PCO plot.

Multivariate analysis was used to determine whether there was a relationship between the genotype observed as RAPD bands and the environment where the plant was grown or between the genotype observed as RAPD bands and the morphometric traits exhibited by the plant. The morphometric data used was the trait data (see section 2.2.2.1), and the environmental data used was the water and sediment chemistry data (see section 2.2.3). A preliminary Detrended Correspondence Analysis (DCA) (Hill, 1980) was carried out on the RAPD data set. This yielded axis lengths greater than 4 S.D. so it was decided to proceed with a Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). As the environmental data and morphometric data were not directly related to an individual, but to the loch population as a whole, treating the RAPD data as phenotypic characters it was converted to the frequency of band occurrence within a population. This is an oversimplification, as the band frequencies on the gel do not reflect the allele frequency in the population. However, it is extremely difficult to determine actual allele frequencies in the population, with no data on the breeding behaviour, such as a F_{IS} value, coupled with the tetraploid state and no evidence as to whether it is an auto or allopolyploid. This band frequency data was used in the same way as community composition data are normally used in CCA, i.e. this is the primary data set. The environmental and morphometric data were used as the external variables (the secondary data set), which constrains the RAPD data in CCA. These are normally habitat factors but can equally be phenotypic characters (Prentice & Cramer, 1990). A Monte Carlo permutation test was then used to test the extent to which variation in the RAPD data was significantly related to variation in the external variables, in this case environmental variables or morphometric variables.

In order to investigate whether the variability of a population is related to its position in a loch, the percentage of polymorphic loci per population was regressed with exposure. Exposure was calculated as in section 2.2.3. This was then divided by log of the depth of the plants plus one, to take into account the fact that, with depth, the exposure experienced by the plant will decrease.

The results from this regression will be limited by the lack of in-depth meteorological measurements of wind speed at each site (see section 2.2.3). An improvement in the measurement of exposure would make the analysis more accurate. An increase in the number of samples per site would also increase the accuracy of the assessment of genetic polymorphism.

5.3 Results

5.3.1 Genetic diversity

A total of 63 reproducible and polymorphic bands were resolved, this is reduced to 49 if the American samples are excluded. All but two individuals, from loch with no name in S. Uist, could be distinguished as an individual genotype. The number of polymorphisms within the populations is shown in Table 5.2. Neighbour joining analysis and Principle Coordinate analysis (PCO) were used to look at the genetic distance between individuals. Two separate neighbour joining analyses were carried out, one including and one excluding the American site. The greatest genetic distance in the neighbour joining tree for all sites was that between the American and the Scottish and Irish sites. Little other separation of samples can be elucidated from Fig. 5.1, because the other sites are all clustered in comparison to the large genetic distance between the sites on either side of the Atlantic.

For the analysis excluding the American samples both the PCO (Fig. 5.3) and neighbour joining tree (Fig. 5.2) show similar results. The greatest genetic distance is between the two sites in Perthshire, particularly Loch Clunie and the remaining sites. It is also apparent that partitioning of genetic variation is mostly between populations rather than within them. This is illustrated clearly by the neighbour joining tree (Fig. 5.2), where all samples within a site are connected to the same branch. However, the genetic distance between these populations is not large. The PCO analysis (Fig 5.3) shows that although samples are most similar to samples from the same population, the populations are not greatly different from one another.

Table 5.2 Intra population RAPD polymorphism.

Sites	Number of polymorphic bands	% of polymorphic bands present in Scotland and Ireland	% of polymorphic bands present in Scotland, Ireland and USA
Mainland			
Lake of Menteith	9	18.4	14.3
Loch Clunie	11	22.4	17.5
Loch of Craiglush	14	28.6	22.2
Outer Isles			
Loch Druidibeg	7	14.3	11.1
Loch nan Capull	4	8.2	6.3
Loch with no name	4	8.2	6.3
Loch na Cuithe Moire	13	26.5	20.6
Inner Isles and Kintyre			
Loch Gorm	5	10.2	7.9
Loch Lossitt	6	12.2	9.5
Loch Nan Gad	5	10.2	7.9
Ireland			
Lough Leane	7	14.3	11.1
Cregduff Lough	8	16.3	12.7
Mullaghderg Lough	4	8.2	6.3
USA			
Lake George	13		20.6

5.3.2 Relationships between the genetic properties of a population and the environment and morphological traits of the population

Canonical correspondence analysis (CCA) was carried out to investigate the relationship between the frequency of RAPD bands and the environment and observed morphometric traits. The Monte Carlo permutation tests showed that the relationship between the environment or morphometric variables and the gradients of change in the RAPD data extracted by CCA was not significant. Therefore, there is no reason to reject the null hypothesis; that there is no correlation between variation in RAPD data and the environment or morphometric measurements. However, this does not mean there is no relationship between genotype and the environment, just that it was not observed in the RAPD data.

Figure 5.1 Neighbour joining tree showing the relationship between the RAPD genotypes for samples from USA, Britain and Ireland (also see Fig. 5.2). Abbreviations are for sites are in Appendix 1 with the additional prefixes of ma = mainland, pe = Perthshire, ik = Inner Isles and Kintyre, ou = Outer Isles, ir = Ireland and us = USA.

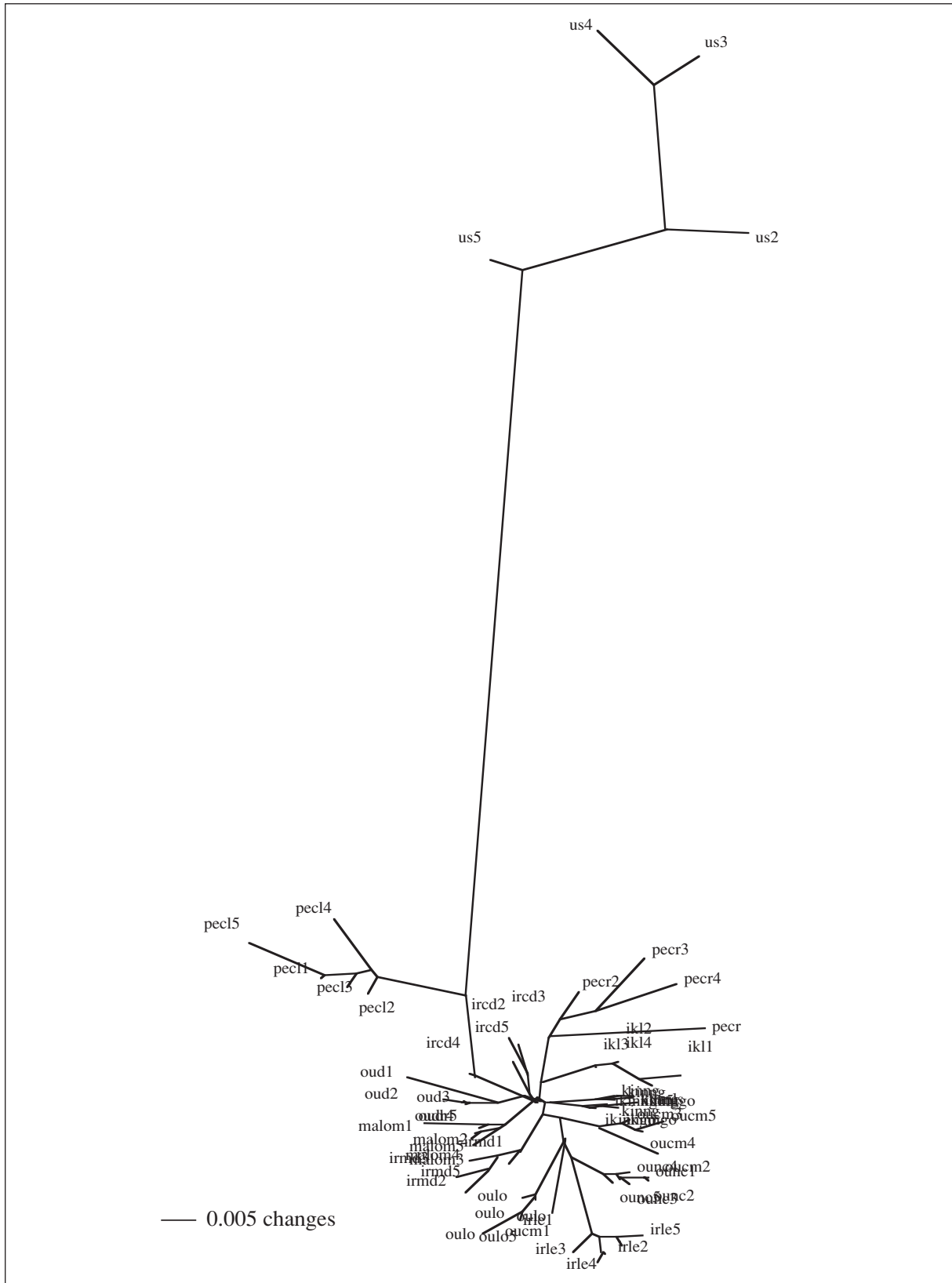


Figure 5.2 Neighbour joining tree showing the relationship between the RAPD genotypes for samples from Britain and Ireland. Abbreviations are for sites are in Appendix 1 with the additional prefixes of ma = mainland, pe = Perthshire, ik = Inner Isles and Kintyre, ou = Outer Isles and ir = Ireland.

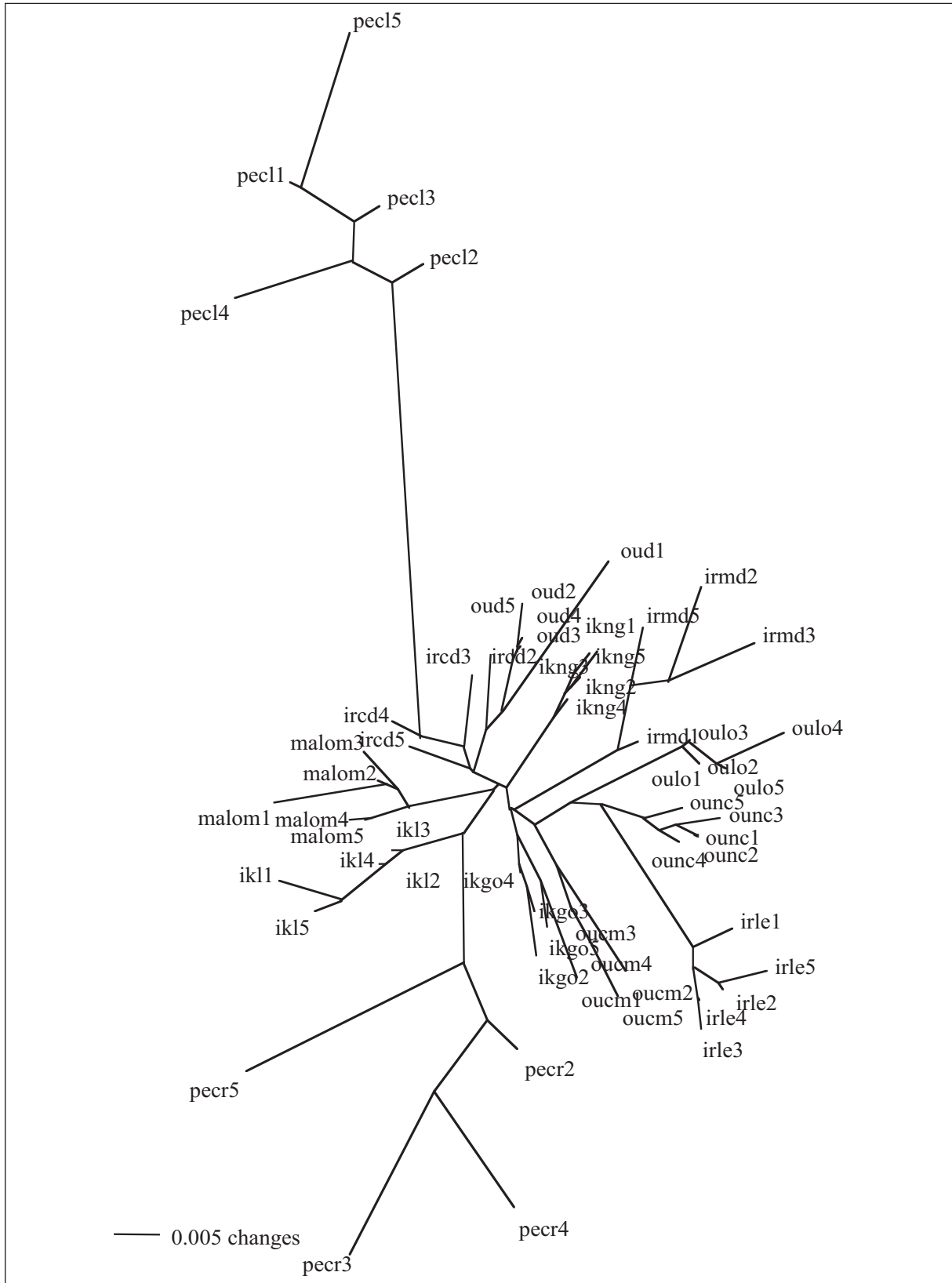
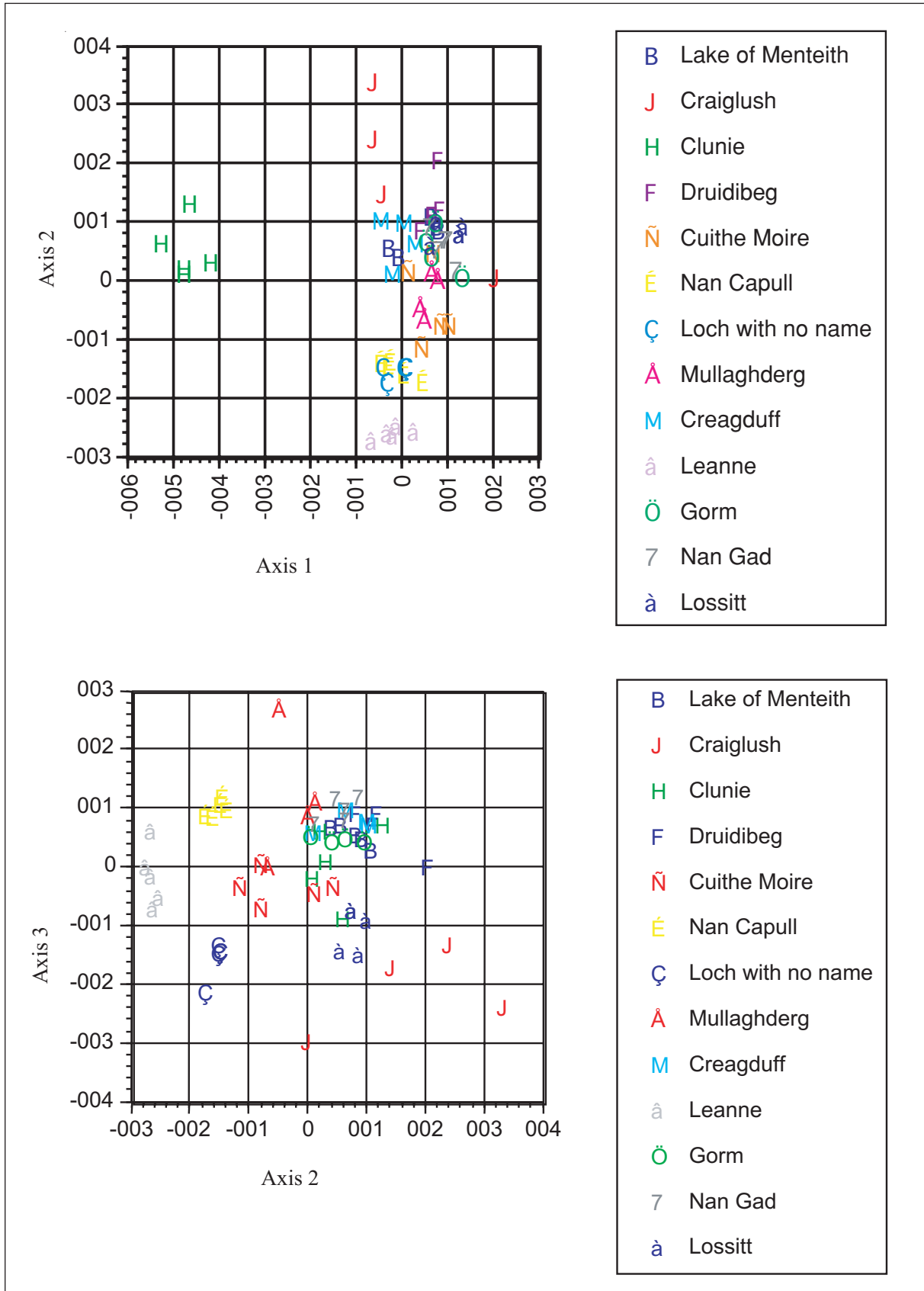


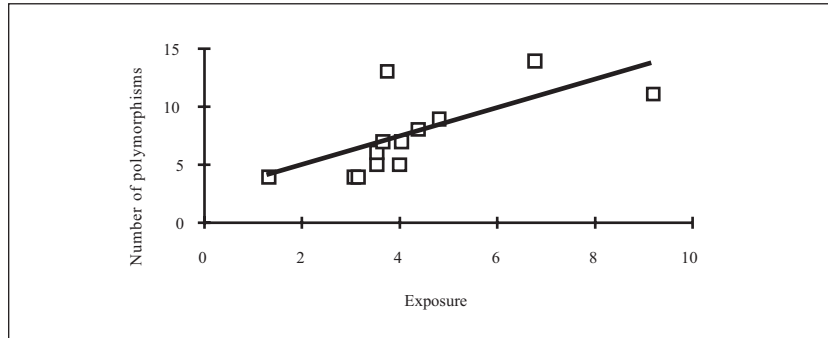
Figure 5.3 PCO plot showing the relationship between the RAPD genotypes for samples from Britain and Ireland.



5.3.3 The relationship between the genetic variability of a population and its environment

The regression of exposure against number of polymorphism per population shows there is a direct relationship between the two (Fig 5.4). With increased exposure, an increase in the number of polymorphic bands is seen. However there is still a relatively large amount of noise in the data presented, this is probably due in part to the exposure calculation method used and the small sample sizes.

Figure 5.4 The relationship between number of polymorphisms per population and exposure. $r^2 = 0.476$ $p < 0.01$ where Number of polymorphisms = $1.2 \text{ Exposure} + 2.3$.



5.4 Discussion

Technical difficulties experienced while working on the population genetics of *N. flexilis* have resulted in a small sample size being available for the final analysis. This naturally limits the ability to answer the original aims of this study from these results, and hence all conclusions are tentative.

5.4.1 Partitioning between lochs

The fact that samples within a loch are most closely related to each other, rather than those from other populations, implies that geographic isolation is having an effect. This is in common with many water-pollinated plants (Barrett *et al.*, 1993; Les *et al.*, 1997). It is however quite surprising that the populations in South Uist are not more similar to each other. This island contains at least 20 extant *N. flexilis* lochs all of which are in close proximity in a habitat mosaic with many potential dispersal corridors. The obvious genetic separation of Loch Clunie and Craiglush in Perthshire is also surprising. These two lochs are part of a chain of five lochs interconnected by the Lunan Burn. All five lochs in the chain have been reported in the past to contain *N. flexilis*, however the last loch in the chain appears to be too eutrophic to support a *N. flexilis* population now. Craiglush is the first loch in the chain and Clunie the fourth, a distance of less than 9km. This obvious genetic difference between two *N. flexilis* populations in these two lochs (see Fig. 5.2) implies that a single loch is the population unit for this species in the geographical area studied. There does not appear to be any relationship between geographical distance and genetic distance. It is then likely that each population has become distinctive due to genetic drift. This would explain the overall low genetic distance between populations, and the distinct population units.

5.4.1.2 Genetic variability within populations

While it is not possible to make any strong inferences about reproductive biology from a small sample of individuals and a dominant marker data set, it is interesting to note that each individual of *N. flexilis* (except for one pair of samples) could be identified as a separate genotype by the RAPD primers used. This implies

that populations do not consist of completely homozygous and uniform selfing lines. This could be attributable to out-crossing, although without information on mutation rates of the variable loci and long term estimates of population sizes and migration rates, selfing (or partial selfing) cannot be excluded.

5.4.2 Relationships between genotype and the environment and morphological traits

The lack of a significant relationship between the environment and the RAPD data does not imply that there is no relationship between the genotype and the environment. RAPDs are neutral markers and unless they are closely linked to an area of the genome that has an adaptation to the environment, they will not show a significant relationship. The same is true for the lack of relationship between the RAPD data and the morphometric data.

5.4.3 Relationships between population variability and the environment

The fact that the number of polymorphisms per population is related to the exposure experienced by the plants is interesting. The greater exposure the plant experiences, the more likely it is to break up in the autumn gales and spread its seed amongst the population. *N. flexilis* plants do not float, so unless water movements pick them up and carry them around the loch the plant and its seed will sink where it grew and there will be no dispersal even within the loch. Lochs with a relatively high level of exposure often have *N. flexilis* plants found in the drift in the Autumn, whereas those with low exposure often do not (personal observation from fieldwork 1998–2000). Other factors as well as exposure also will affect this, such as substrate type (allowing plants to be uprooted) and the topography of the lochbed (plants rarely appear to reach the drift line if the loch bed suddenly shelves).

Pollen movement is also going to be affected; without exposure or waves, the pollen will sink and geitonogamous selfing will be the most likely result. However, if the water is moving, the pollen has a greater chance of finding a non-self or sibling stigma. The formula used for calculating exposure contains a depth function, so those plants occurring in shallow depths have a greater exposure measure. Plants occurring in shallow water are not only benefitting from the greater exposure, but also because pollen will be denser than in deep water and therefore the chances of pollination will increase. A simple test of this last hypothesis would be to compare the proportion of flowers that set fruit in marginal versus deep water zones.

5.5 Conclusions

The primary step in any reintroduction programmes for *N. flexilis* is to ensure environmental conditions are suitable for *N. flexilis* growth. This is a fundamental prerequisite to translocation; otherwise, the introduced individuals are likely to suffer the same fate as their extirpated predecessors. When the environmental conditions are suitable, it is then necessary to consider which populations to use for donor propagules. Populations that are geographically proximal and ecologically similar would be the primary candidates. Donor populations should be of a large size to be able to withstand seed removal. Sampling large populations should also decrease the likelihood of reintroducing plants suffering from inbreeding depression. Selection is less efficient in small populations in weeding out less-fit individuals.

In terms of using the molecular data generated during this study to identify donor populations, inferences are hampered by the small final sample sizes and the lack of clear geographical structure detected. At present, the most appropriate criteria for reintroduction programmes should be based on ecological data.

Summary

- RAPD primers were used to look at the genetic diversity of *N. flexilis*, specifically in Scotland and Ireland.
- All but two individuals could be identified as different genotypes.
- Individuals were generally more similar to other individuals within the same loch than individuals outwith the loch, suggesting that the loch populations are relatively isolated with little movement of pollen or seeds between them.
- Geographic distance between sites did not appear to be related to the genetic distance between the Scottish and Irish populations.
- The genetic difference among Scottish and Irish populations was much less than the genetic difference between the American population and the Scottish and Irish populations.
- No significant relationship was found between the water and sediment chemistry of the loch or the *N. flexilis* trait data and the frequency of RAPD bands within a population.
- A direct relationship was found between the number of polymorphisms within a population and the exposure experienced by that population. This is most likely to be due to an increase in the movement of seeds and pollen due to an increase water movement. This would result in a greater amount of cross pollination instead of self pollination and a greater chance of outcrossing with a non sibling plant.

6 THE GERMINATION ECOLOGY OF *N. FLEXILIS*

6.1 Introduction

6.1.1 Aims

To determine the environmental factors controlling *N. flexilis* germination using laboratory based experiments.

6.1.2 Background

As an annual, *N. flexilis* is dependent for population survival on seed production, and the consequent successful germination of these seeds. The ripe fruits of *N. flexilis* are shiny and dark brown-black in colour. The embryo makes up most of the fruit with a very thin translucent testa and a thin brittle epicarp (Hay & Muir, 2000). The shape of the seeds is described as elliptical, oblong (Triest, 1988). Surveys of plants across Scotland and Ireland have found that seed production varies, with 0–43 seeds found on a single individual. It has been found that seed number is dependent on a number of environmental factors including pH (Titus & Hoover, 1991; Titus & Hoover, 1993; and section 2.3.2.1).

Little has hitherto been known about the conditions required for germination. The seeds are produced from late summer to early autumn (August–October) and the seedlings begin to appear in early summer (June) in Scotland (personal observation). This phenology suggests a sensitivity of seeds to climatic conditions, which prevents immediate germination in autumn and stimulates germination in the summer. However, whether the seeds form a persistent seedbank beyond this short time period is unknown. What is known about *N. flexilis* germination is summarised below.

Muenscher (1936) found that *N. flexilis* seeds had a stratification requirement. No germination was obtained from seeds stored in water at room temperature. However, seeds that had been stored in chilled water for one month germinated gradually over the following six months. Seeds that had been stored in chilled water for the past 7 months germinated uniformly and quickly (87% in six days). Wetzel & McGregor (1968) also found that germination of *N. flexilis* was easily induced after a dormancy period of 15–30 days at 4°C. A stratification requirement for germination in *N. flexilis* would delay germination from autumn, immediately after seed production, until the following summer. Haas (1998) has suggested that germination occurs at a temperature of around 19°C in the second half of June, in the Swiss Alps. *N. flexilis* does not appear to withstand desiccation well (Hay & Muir, 2000; Muenscher, 1936). Hay & Muir (2000) classify *N. flexilis* seeds as partially desiccation tolerant, with seeds more likely to withstand some drying before a cold treatment rather than post cold treatment. A desiccation treatment has not been reported to enhance germination.

6.2 Methods

Brown ripe seeds were collected from Loch na Cuithe Moire in September 1998, by Dr. Fiona Hay of Wakehurst Botanic Gardens. These seeds were then stored at 6°C until November 1998. A selection of 10 seeds were tested for viability and all were found to be viable. Therefore, any seed not germinating in the experiment would be assumed not to be germinating due to the experimental treatment rather than a lack of viability.

In order to determine the effects environmental factors have on *N. flexilis* germination, a factorial experiment was conducted (experiment 1). The treatments applied to these seeds were; +/- oxygenation and +/- light at 4 different temperature regimes: 11°C, 16°C and, in order to replicate a diurnal temperature fluctuation, 11°C/21°C and 6°C/16°C.

These results are put into perspective by comparing them with actual temperatures recorded in lochs known to support *N. flexilis*. The water temperature data were collected repeatedly over four years. The mean temperature for each month was calculated and this along with the standard error is shown in Fig. 6.1. These data are supplied by the Scottish Environmental Protection Agency (SEPA). They were not collected from December to March, and never more than once a month. This timing did not coincide with observations on *N. flexilis*. The temperature data was collected from 1993–1996 and this study began in 1998. These data therefore can only be used to suggest realistic temperatures at different *N. flexilis* growth stages that have been observed on separate occasions in these lochs and should be viewed cautiously. Experiment 2 investigated the effect of burial by sedimentation on the germination of seeds.

6.3 Results

6.3.1 Experiment 1

High levels of germination were observed at all temperatures in light deoxygenated conditions. In these conditions the seeds exposed to the warmer temperature germinated fastest. However, high levels of germination were also achieved at 11°C in dark, deoxygenated conditions. Dark, deoxygenated conditions did not promote high levels of germination at all the other temperature regimes used in this experiment. Oxygenated conditions did not promote high levels of germination at any temperature and fluctuating temperature did not appear to promote higher levels of germination than stable temperatures.

A variety of stratification treatments had been planned as part of this experiment. However, any stratification requirements by the seeds appear to have been satisfied by the 2-month period at 6°C, which all seeds in this experiment were kept at for storage before experimentation. Therefore, all seeds in this experiment were subject to a single stratification treatment of 2 months at 6°C. The high levels of germination success also suggested that there was no physical dormancy to be broken by scarification as in *Najas marina* (Bone & Probert, 1994).

In Scotland, germinating *N. flexilis* plants have been observed in June, they grow throughout July and August and by September ripe brown seeds can be seen on the plants (personal observation & Valerie James pers comm. 1999). When this phenology is compared to the temperatures recorded on separate occasions in lochs supporting *N. flexilis* (Fig. 6.1) it can be suggested that germination would occur when loch temperatures are approximately between 11 and 16°C. Growth would continue as the temperature rises. Ripe seeds are produced in late summer and early autumn when temperatures are approximately 10–14°C. This temperature range does not differ much from the observed temperature range at which germination occurs. However, seed germination in *N. flexilis* has never been observed until spring. This suggests that some form of mechanism is preventing immediate germination, this could be a stratification requirement. November and April temperatures do not always drop below 6°C, the stratification temperature used in this experiment, however these are not the coldest months of the year in Scotland. Unfortunately, there are no consistent data for the colder months of the year. It can be presumed that temperatures would consistently reach below 6°C in the winter, as the temperature is almost that low in the months preceding and following the colder months. Although temperatures rise to approximately 8°C in April, germination does not occur until June when temperatures are above 10°C. These temperatures correspond to those seen to promote germination in experiment one.

6.3.2 Experiment 2

The greatest quantity of seeds germinated at the sediment-water surface, however seeds also germinated consistently regardless of depth. These results can be seen in Fig. 6.2. A χ^2 test carried out on frequency data rather than percentage data shows that the observed frequencies are homogeneous and the departure is merely due to sampling error or scatter. This shows that burial alone does not affect seed germination. However germination only reached a maximum of 50% after two weeks, this is less than in the previous experiment suggesting that some other factor may also be inhibiting germination in this experimental set up. The sediment was left in pots for only 2 weeks before the experiment began. In this time it is unlikely that the environmental gradients usually found with depth in the sediment would have established, for example decreasing oxygen concentration with depth. However, the previous experiments have already indicated that deoxygenated conditions promote *N. flexilis* germination. It is also of note that a similar experiment on *Myriophyllum spicatum* found a significant decrease in germination with depth when again the sediment had not been left for a prolonged time to establish such environmental gradients. It is difficult to draw conclusions from this experiment because the depth at which the seed is found in the sediment in the loch environment may have a different effect to that observed in this experiment due to established environmental gradients in the loch sediment.

Figure 6.1 Average monthly water temperatures in (a) Loch Butterstone and (b) Loch Craighull both lochs containing *N. flexilis*. Average temperature is calculated from once monthly measurements from the 4 years 1993–1996 inclusive (Data supplied by the Scottish Environmental Protection Agency). The error bars represent + or – 1 standard error.

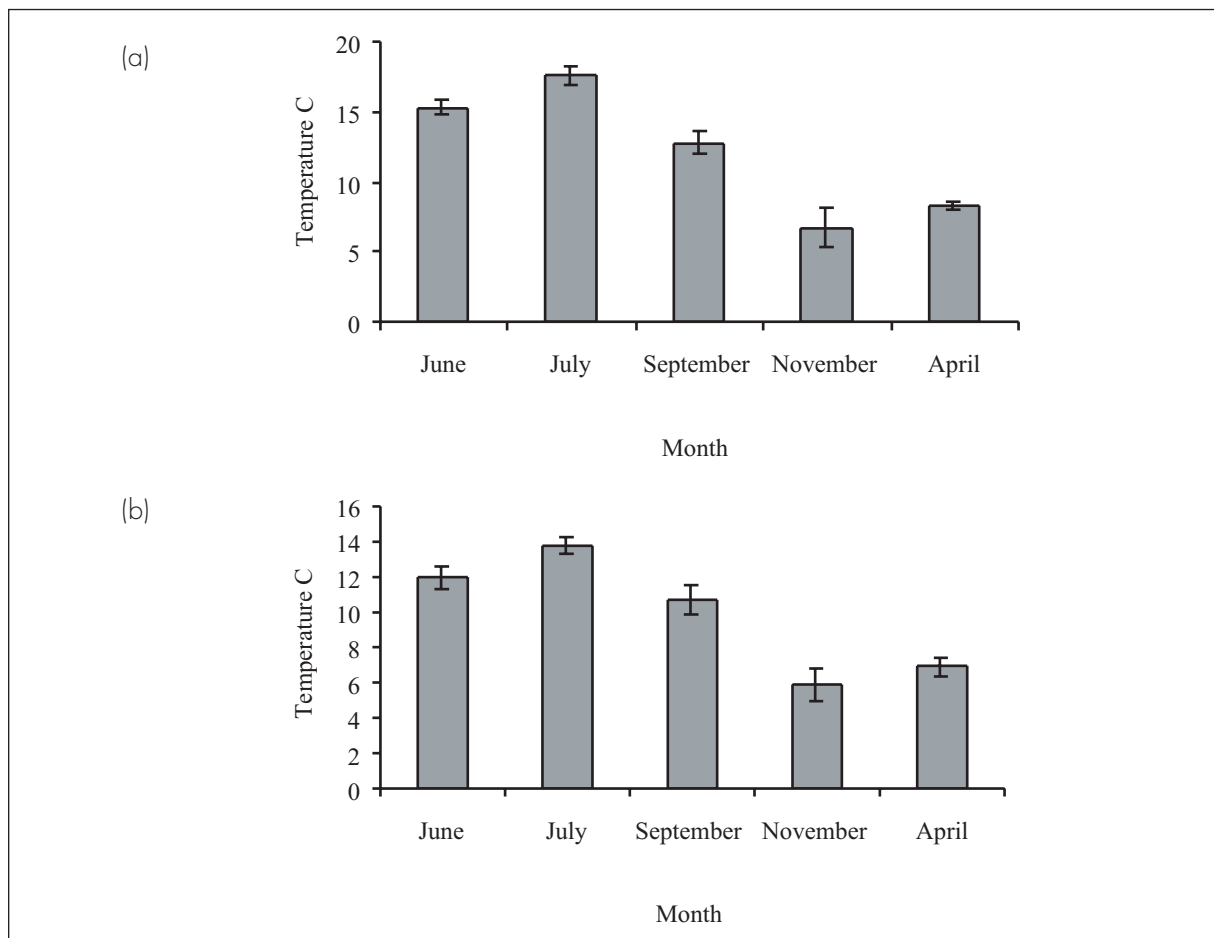
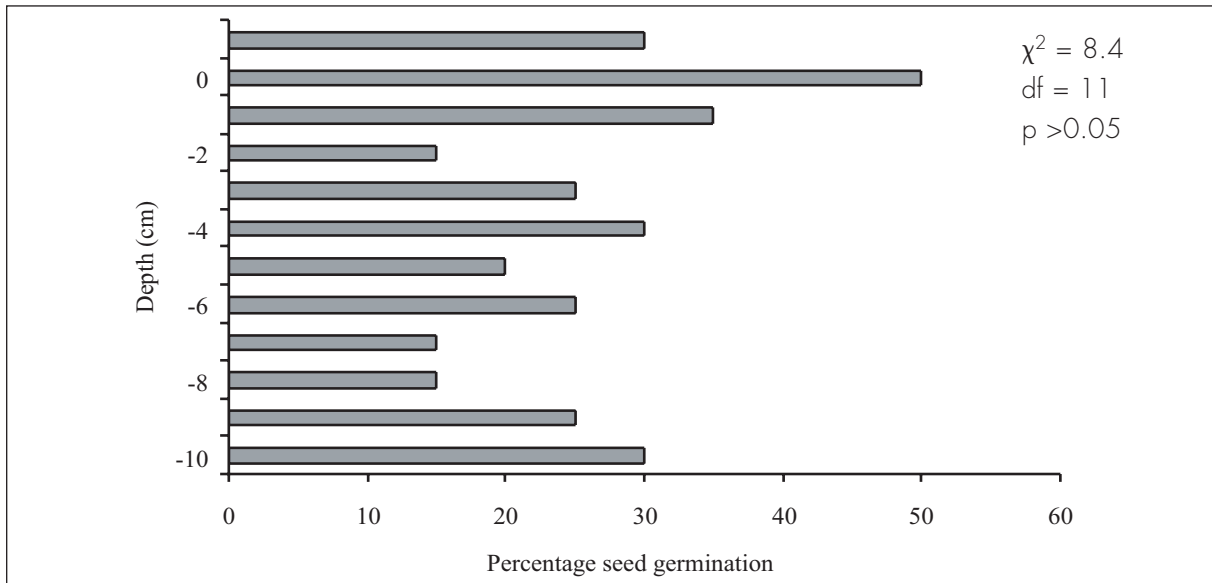


Figure 6.2 Percent germination of *N. flexilis* seeds at varying depth in the sediment. χ^2 is calculated from the total number of seeds germinated after 2 weeks.



6.4 Discussion

6.4.1 Temperature

Germination does not occur at 6°C, this was observed when seeds were stored at this temperature before germination. Therefore, warmer temperatures are required to stimulate germination. 11°C appears to be sufficient to stimulate germination. Although warmer temperatures appear to stimulate greater quantities of germination in light, deoxygenated conditions, there is no significant difference between the responses to these temperatures in these conditions. It is probable that in the shallow waters where *N. flexilis* grows it will germinate in response to the warmer light conditions. Where *N. flexilis* grows in deeper water, it is most likely that germination will occur in response to the cooler temperature of 11°C and darker conditions. A fluctuating temperature did not appear to be required. As *N. flexilis* can grow at depths of up to 14m this is not surprising as the water will act as a buffer to temperature fluctuations.

6.4.2 Light/dark

Fieldwork measurements and observations (see Chapter 2) have shown that *N. flexilis* can grow in waters of varying clarities, at varying depths, which would suggest that a tolerance of both light and darker conditions would be advantageous. The ability of *N. flexilis* seeds to germinate in the dark was further emphasised by the consistent germination of seeds at depth in the sediment. The ability to germinate after burial under a limited amount of sediment could be seen as an advantage, yet germinating under 10cm of sediment would appear a waste if seedlings could not reach the surface. The greatest depth of sediment through which *N. flexilis* can germinate, grow and survive is unknown and requires further experimentation.

6.4.3 Deoxygenation

A preference for deoxygenated conditions in constantly aquatic systems has been suggested to ensure that seedlings grow in stable environments. High oxygen levels in the water or sediment will be predominantly due to mixing, which is a form of disturbance to seedlings. Seedlings germinating where oxygen levels are

higher, in the water column, on the sediment surface or in the top oxygenated microzone of the sediment, are also those that are most likely to be disturbed by wave action before a sufficient anchorage system can be grown. Germination in the deoxygenated sediment therefore permits the establishment of an effective root anchorage system and an immediate access to a supply of nutrients, which would promote successful seedling establishment (Moore *et al.*, 1993).

6.5 Conclusion

Knowledge of the germination requirements of *N. flexilis* helps elucidate the phenology of *N. flexilis* establishment and growth in Scotland. This illustrates that the timing of any seed reintroduction programme would be important, especially if the loch water clarity was low. This is because the temperature window for optimal germination is lower in dark conditions. A cold pre-treatment for seeds before reintroduction would be advisable. This could be gained by introducing seeds to the loch in autumn so that they experience the natural winter conditions. However, a greater time spent in the loch before germination increases the chance of predation or burial. Therefore, it is probably safer to keep seeds in water at 6°C before reintroduction in the spring, as 6°C appears to satisfy any stratification requirement of the seeds. The preference for deoxygenated conditions is most likely to be met in relatively stable substrates. This agrees with the findings of Chapter 2, which illustrates that *N. flexilis* often grows in the sheltered parts of lochs. If the previous situation of the plant within a loch was unknown, these findings further suggest that a sheltered area would appear to be an appropriate site for reintroduction.

Summary

- *N. flexilis* germination is greatest in light, deoxygenated conditions at warm temperatures (a temperature of 16°C is sufficient).
- If dark conditions prevail germination is greater at cooler temperatures (11°C).
- These conditions can be found in Scottish lochs in sheltered areas in June.
- Burial of seeds in loch sediment to a depth of 10cm did not appear to affect germination, although the high percentages of germination observed in the previous experiments were not repeated in this experiment.

7 DISCUSSION

7.1 Summary of the results

7.1.1 Chapter 2

Chapter 2 considers the relationship between *N. flexilis* and the environment. It shows that measurement of specific plant traits; leaf area and reproductive number, from a representative sample of the population is a possible method for assessing the condition of the population in response to the environment. This is important because accurately measuring plant abundance to assess the condition of the population in deep water plant communities is extremely difficult.

This chapter also found that the pH, conductivity, alkalinity, calcium, magnesium, total phosphate and potassium concentrations in the water were significantly different in sites where *N. flexilis* is extinct compared to sites where it thrives today. It also found that the available phosphate and iron in the sediment are significantly different at present and absent sites. Models were produced which found that the concentration of phosphate in the sediment and water and magnesium in the water, along with the alkalinity and either light or calcium in the sediment could predict 67–88% of the variation in the plant traits of *N. flexilis*, depending on the traits and the environmental variables included. Chapter 2 also shows how exposure affects the distribution of *N. flexilis* within a loch. It also shows that when *N. flexilis* grows in shallow water the water clarity can be less than when it grows in deep-water, although the difference in light extinction coefficient is not significant. However, improved methodologies might find this effect significant. There was no significant relationship between macrophyte biomass and *N. flexilis* plant traits.

7.1.2 Chapter 3

Chapter 3 compares a small-scale quantitative approach to a loch-scale qualitative approach of plant community classification, to determine their ability to describe the loch environment. The loch scale qualitative approach found significant differences in pH, alkalinity and the concentration of total oxidised nitrogen and dissolved reactive phosphate in the water. It also found differences in phosphate, calcium and iron in the sediment. In contrast, the small-scale quantitative approach only found a difference in light. The community groups attained from the small-scale quantitative survey were roughly comparable to NVC communities A13, A24a and A23. The loch scale qualitative approach produced community groups comparable to standing water type 3, 4 and 5. However, this study produced two type 5 sub-groups. Extinct *N. flexilis* sites were classified as type 3 and the most eutrophic type 5, as were some other sites that could still support *N. flexilis*. No extinct sites were classified as type 4. This shows that although standing water type can detect which sites may be more at risk and allow resources to be concentrated on those sites, it can not pinpoint extinct and present sites.

7.1.3 Chapter 4

Chapter 4 looks at the plant attributes present within a macrophyte community, which may confer a competitive advantage over *N. flexilis*. This is compared to the possible threat from the alien invasive *Elodea* spp. and the attributes these species possess. A method for calculating the abundance of plant attributes within a macrophyte community is described and the relationship these attributes have with *N. flexilis* is calculated. The resultant model, which includes the abundance of plants in the community possessing the following attributes; wind pollination, June–July flowering and being a perennial, explains 68% of the variation in *N. flexilis* leaf area/shoot length. Both *Elodea* spp. are perennials and flower in June–July, but are epiphytic and not wind pollinated.

The possible reasons for the importance of these traits are that early flowering plants not only flower before *N. flexilis*, but also begin to grow before *N. flexilis* thus obtaining resources before *N. flexilis* can compete for them and then shading *N. flexilis* with its early growth. The perennial plants will have a greater quantity of stored resources to facilitate growth than *N. flexilis* seeds, hence providing perennial plants with a head start in any competition with the annual *N. flexilis*. The benefit of being a wind pollinated plant in competition with the hydrophilous *N. flexilis* may be the reliability of pollination, but is also possibly due to the fact that wind pollinated plants must grow to the water surface to support flowers above the water surface. These taller plants will then potentially shade *N. flexilis*.

This work indicates that a proliferation of invasive or native plants with these traits within the *N. flexilis* micro-habitat can potentially have a deleterious effect on the *N. flexilis* populations. However, a proliferation of a native species is most likely to be due to an environmental change such as the increase in a previously limiting resource such as phosphate. Therefore the cause of any weed problem must be considered as well as the weed itself. Monitoring invasive plants would indicate when or if invasive species cause a threat to *N. flexilis*. To protect the species from invasive species such as *Elodea* spp. would require action such as the removal of the invasive species or saving the genotype until conditions are more favourable for *N. flexilis* growth (possibly in the Millennium Seedbank).

7.1.4 Chapter 5

Chapter 5 looks at the genetic diversity within the Scottish and Irish populations of *N. flexilis* using RAPDs. The division of diversity within and between populations is compared to what would be expected according to current theory relating genetic diversity to life history characteristics. The data suggest that there is no great division of diversity between Scottish and Irish populations. The individuals appear to be more similar to other individuals within the same waterbody, rather than individuals in other waterbodies. All but one pair of individuals appear to be genetically unique, suggesting that selfing is not the predominant form of pollination and that each population is relatively isolated with little movement of seed or pollen between lochs.

The frequency of RAPD bands within a population is compared to the environment of that site and the traits of *N. flexilis* at that site and no significant relationship is identified. A significant relationship is identified between exposure and diversity within a population. This is most likely to be because increased water movement caused by exposure also moves pollen and seed making it less likely that a plant will be self-pollinated or will cross-pollinate with a sibling plant. This is because seeds from the same parent plant are dispersed within the loch so that a plant's nearest neighbour is less likely to be a sibling.

7.1.5 Chapter 6

Chapter 6 looks at the germination requirements for *N. flexilis*. It was found that germination can occur at temperatures above 11°C, which is comparable with reports of seeds germinating in June in Scotland. The best germination results were achieved in warm (16°C), deoxygenated, light conditions. However, if dark conditions prevailed, greater germination occurred at 11°C. Further experiments found that there was no significant relationship between seed depth within the sediment and germination response.

7.2 What are the implications for the conservation of *N. flexilis*?

7.2.1 Threats to the species

It appears from this research that *N. flexilis* occupies a relatively small realised niche in Britain and Ireland. This is illustrated by the fact that a single standing water type can describe communities that do and do not support *N. flexilis*. Therefore, whilst other macrophytes can exist over a wider variety of environmental conditions, the environment that can support *N. flexilis* is more specific. If a DOME score (Palmer, 1989) were to be assigned to this species it would be 'om' as it is present in some, but not all oligotrophic sites (SWT 2 & 3) and some but not all mesotrophic sites (SWT 5). The only other species with this DOME score assigned to it is *Pilularia globulifera*, another rare aquatic species. This narrow realised niche means that compared to other aquatic plants which are more ubiquitous, there are fewer environments to support *N. flexilis* and the environments are more likely to become unsuitable for *N. flexilis* growth as small changes in the environment can have a detrimental impact. There may be more sites where this species grows than is presently recorded, as the plant grows in deep unclear water. In sites where it occurs it is not necessarily widespread throughout the loch. It is not easily caught on grapnels, and has a relatively short growth season, so if the site was not surveyed in August or September it is unlikely to be found.

It is also likely that this plant is under-recorded in Britain and Ireland along with other aquatic plants of a similar habit such as *Elatine hexandra*. Both these plants can grow in deep water never reaching the surface, thus keeping them from the view of the more terrestrial botanist. Neither of these plants float, this means that they are less likely to be found in the strandline. *N. flexilis* can be found in the strandline, but only in lochs where the slope of the loch bed is not steeply sloping or rocky as the plant then gets trapped and does not make it to the shore. *N. flexilis* is never found in the shoreline as abundantly as other floating plants such as *Elodea* spp. or *Littorella uniflora*. This may also be related to the fact that it is rarely as abundant as these two species within a loch. This patchy distribution within a loch may also be a factor in its under-recording. Even a keen aquatic botanist searching from a boat or swimming in the water could believe they have recorded all the plant species within a loch when they are yet to find the small single patch of *N. flexilis*. The other two factors which lead to the under-recording of *N. flexilis* are its short life cycle and the fact that the standard tool for finding aquatic plants, the grapnel, does not work particularly well at sampling *N. flexilis*. This is due to its slender form, which allows it to escape the grapnel and the fact that it can easily break and not be entangled in the hooks. However, in deep unclear waters this is often the only tool available to the aquatic botanist to find the plant without expensive scuba diving or video recording. Furthermore, the success of identifying plant species from videotape can be questionable.

7.2.1.1 Eutrophication

As *N. flexilis* thrives only in mesotrophic conditions, it is of no surprise that eutrophication is a threat to the species. However, light is not always the limiting factor and clear water is not a prerequisite for *N. flexilis* growth. It has been reported here (Chapter 2 & 3) and elsewhere in the literature (e.g. Hough & Fornwall, 1988) that *N. flexilis* can grow in low light conditions and often grows in deep water, occupying the same area of a loch as *Nitella* spp. (Pearsall, 1920). Instead, the reason for the lack of success of *N. flexilis* in sites where eutrophication has occurred appears to be its inability to photosynthesise, due to a lack of available CO₂. Indicators of such conditions are high pH, alkalinity and phosphorus. These can all be seen in Loch Marlee in Perthshire from which *N. flexilis* recently appears to have become extinct.

7.2.1.2 Acidification

Acidification is another threat to *N. flexilis*. The effect of this on *N. flexilis* was much harder to quantify. This is because in lakes with a pH of less than seven the plant becomes even more elusive. This is probably due to decreasing reproductive success in such conditions. In Lough Pollacapull and Lough Shennagh, a long time was spent searching for the plant using both snorkelling and shoreline approaches. In these situations after a frustrating search, fragments were found floating in the water with no seeds attached. The other more acidic sites surveyed, where whole plants were found, also had low seed numbers, particularly the plants found at Roundstone Lough. The mechanism limiting seed production is unknown, and this would require further studies to elucidate. However, these observations of reduced reproductive capacity in more acidic conditions agree with experimental results in the literature (Titus & Hoover, 1991; Titus & Hoover, 1993).

7.2.1.3 Weed growth and competition

The effect of competition from other macrophytes, particularly the alien invasives *Elodea canadensis* and *Elodea nuttallii*, was difficult to determine. This is because the competitive effect of the *Elodea* spp. would depend on the time since introduction and the environmental condition of the loch. It would appear from the literature that *Elodea* spp. are most likely to be a threat in situations where the lochs are small and shallow (Pearsall, 1920; Sheldon & Boylen, 1975; Pip & Simmons, 1986; Hough & Fornwall, 1988; Simpson, 1988; Spicer & Catling, 1988; Scott, 1996). This is because there are no deep areas in the loch, where light levels may be too low for *Elodea* spp. to grow, but *N. flexilis* can survive. This situation will be exacerbated by higher nutrient levels, as the literature (Grime *et al.*, 1988; Simpson 1990; Best *et al.*, 1996; Preston & Croft, 1997) suggests that the niches of *Elodea* spp. and *N. flexilis* overlap in the more nutrient poor sites of the *Elodea* spp. ranges, which are the more nutrient rich sites of *N. flexilis*' range. This information from the literature agrees with observational data from this study. The site where *N. flexilis* has been reported to be limited by *Elodea canadensis* growth is Tangy Loch. This loch is small and shallow in comparison to the large lochs in Perthshire and Stirlingshire where both *N. flexilis* and *Elodea* spp. grow without obvious detrimental effects. However, this observational evidence is confused by the fact that these mainland sites were invaded by *Elodea* spp. before the smaller Tangy Loch. It may be that time since invasion is the factor that affects the ability for *Elodea* spp. and *N. flexilis* to coexist, and that loch size is coincidental.

It is worth reiterating that *Elodea canadensis* was reported to be detrimentally affecting *N. flexilis* growth only at Tangy Loch. The only site surveyed in this study where excessive weed growth appeared to be detrimentally affecting *N. flexilis* was Loch nam Cnamh. In this case, the plant species in question were *Myriophyllum alterniflorum* and *Chara* spp. The biomass of these two species combined from Ekman samples was greatly in excess of any other sample collected. We were told by the crofter that this site was 'Loch of the Bones' and that people who could not be buried in the nearby churchyard, had in the past been put into the loch. Therefore, the excessive weed growth may well have been due to nutrient enrichment from this source although further studies would be required to confirm where the nutrients were coming from. Chapter 4 suggests which plant traits may make another macrophyte species an aggressive competitor for *N. flexilis*. Chapter 4 also provides a list of species, which possess these traits and grow in the same environment as *N. flexilis*. *Elodea* spp. are not included in this list, but *Myriophyllum alterniflorum* is included.

The relationship between *N. flexilis* and other macrophytes is not clear. Observational evidence suggests that competition can be a problem, but is not always so. The situations where aquatic weed growth could

be a threat are likely to be in sites where there are sufficient nutrients and light to enable excessive plant growth. It is suggested that the *Elodea* situation in the Outer Hebrides and its effect on *N. flexilis* should be monitored over the forthcoming years to help clarify the situation. However, in order to ensure the success of *N. flexilis*, action would need to be taken if monitoring found *N. flexilis* to be threatened. This may take the form of removing competing macrophytes or conserving the genotype of the loch as seed until it can be reintroduced when conditions are improved.

The distribution of *N. flexilis* may appear to be increasing, but the recent, increased intensity of survey is likely to be a key reason for this. However there are also a number of sites where the plant is believed to be extinct. These extinctions appear to be due to acidification and eutrophication. The reversal of this state is a question for the statutory government agencies. Before reintroduction is considered the sites must first be suitable for *N. flexilis* growth. Due to logistical reasons only the mainland sites with pre-1980 records were visited. Those where *N. flexilis* was not found were in Perthshire and Fife, the more intensively agricultural areas where *N. flexilis* occurs. All these sites appear to be more eutrophic than those that currently support *N. flexilis*. It is likely that the eutrophication has occurred through pollution from diffuse agricultural sources in the catchment. Fish farms are mentioned in the Biodiversity Action Plan as a possible threat to *N. flexilis*, yet sites with fish farms (Loch Butterstone and Lake of Menteith) successfully support *N. flexilis* populations of reasonable fitness. Therefore if *N. flexilis* is to be reintroduced to extinct Scottish sites the source of nutrients into the lochs need to be confirmed and reduced first. These reductions are most likely to be achieved through agri-environmental schemes if the main source of nutrients is from agriculture.

7.2.2 Monitoring

As discussed in Chapter 3, EC member states have to report on the implementation of measures taken under the Habitats Directive every six years. That includes the 'surveillance' of the conservation status of certain species, including *N. flexilis*. The UK conservation agencies are also currently drawing up 'site condition monitoring' protocols for SSSIs, which will be applicable to many *N. flexilis* sites. Monitoring is important both for sites where the plant is present and sites where the aim is to reintroduce the species. Monitoring the present sites should provide a warning system if sites become unfavourable for *N. flexilis* growth. Monitoring of potential sites for reintroduction should provide information on when a site is in a condition where reintroduction stands a chance of success. As discussed in Chapter 3, the factors being monitored do not have to be *N. flexilis* itself, but indicators of its status. This study has produced three possible monitoring methods; monitoring *N. flexilis* itself using plant traits such as leaf area and shoot length, monitoring the plant community present, monitoring water and sediment chemistry.

7.2.2.1 Approach 1; *N. flexilis* plant traits

Chapter 2 describes the use of traits to assess plant fitness. This is a useful alternative approach to abundance measures in aquatic systems, where water clarity can cause problems for assessing abundance accurately. This would be a suitable monitoring method for present sites. The variation in the plant fitness value: $\log(\text{leaf area}/\text{shoot length}) \times (\text{number of reproductive structures}/\text{shoot length})$ for *N. flexilis* is shown in Fig. 2.3. It can be seen that there is a step increase between those sites with a value of less than one, and the rest of the sites. If sites have a score of less than one it would seem sensible to be concerned about the future of these sites. The sites found to have scores of less than one, in this study, had so few seeds that the future of the population would be uncertain unless the environment improved and there was recruitment from the seedbank. It was also these sites where few individuals were found. This monitoring method would

involve snorkelling or boat surveys, as plants cannot always be reached from the shore. It would be advisable to undertake such a monitoring programme in August or September in order to find mature plants. By October, the plants are dead and in July, they are small, harder to find via grapnel surveys and have fewer mature seeds.

7.2.2.2 Approach 2; water and sediment chemistry

Chapter 2 describes the sediment and water chemistry at present and extinct sites. If the sediment and water chemistry is to be analysed for monitoring habitat condition for *N. flexilis*, it is suggested that samples are taken at the time of *N. flexilis* growth i.e. August or September. This is because this is when the data in this study were collected, and these are the data to which new samples would be compared. Many of the water and sediment chemistry results overlap both the present and absent groups. This is because the factors that will affect *N. flexilis* will interact and not affect *N. flexilis* growth in isolation. In addition, the measurements taken at the extinct sites were not taken at the time of extinction, so environmental conditions may have changed since that time. Monitoring the water and sediment chemistry would give an indication of the site condition at both present and extinct sites, but could not be used to pinpoint when a site is suitable for *N. flexilis* growth. The models developed in Chapter 2 would give a more precise estimate of site condition suitability for *N. flexilis* growth. This is because these models take into account some of the interactions between the chemical factors. These models would be useful tools to predict when a site is in a suitable condition for reintroduction.

7.2.2.3 Approach 3; macrophyte community classification

Plant community type could also be used to monitor *N. flexilis* present and absent sites. Qualitative surveys for plant communities may well be easier, less expensive and less time consuming than locating *N. flexilis* within a loch and assessing its fitness. This is a broad scale approach and would not pinpoint "at risk" sites. However, it could indicate the group of lochs most likely to include "at risk" sites. This is because extinct sites were only classified as standing water type 3 and the eutrophic standing water type 5. All sites classified as these standing water types, could then be surveyed for *N. flexilis* specifically. Alternatively, a change in standing water type classification since the previous survey would also merit investigation.

The problem with using macrophytes for monitoring (approach 1 & 3) is that it is not known how quickly macrophytes respond to a change in the environment. It is possible that, by the time a change is shown in the macrophytes, the environment will have been unsuitable for a number of years. Whilst using water and sediment chemistry methods is more sensitive, it may be too sensitive. Measurements are unlikely to be constant over the years, as pH fluctuates daily with light availability and photosynthesis. A change in this measure between one year's sampling and the next may have little consequence for *N. flexilis*. Therefore, it is important either to compare such chemical measures with the presence and absence chemical data (Table 2.3 a & b), or apply them to the models in Chapter 2, to ascertain if this fluctuation in water chemistry is likely to have any consequences for *N. flexilis*.

Results of any monitoring will show sites to have a range of conditions and a range of *N. flexilis* fitness measures. Those with lower fitness measure or conditions less than optimal for its growth will not necessarily require management action, as that is their normal state. However, if sites are changing towards an unsuitable state for *N. flexilis*, action may be required. Since the start of this study, work has been carried out at Wakehurst Botanic Gardens as part of the Millennium Seedbank project on methods for storing

N. flexilis seeds (Hay & Muir, 2000). If sites are deteriorating, or *Elodea* has newly invaded a site, it may be a precautionary measure to conserve the loch's genotype by storing these seeds appropriately. Seeds are not tolerant to desiccation.

7.2.3 Reintroduction

Before *N. flexilis* can be reintroduced to any site, the loch environment has to be in a suitable condition. This can be assessed by the above monitoring schemes. Selection of a suitable, specific area within a loch where *N. flexilis* should be reintroduced is an important issue. There are not always records of the distribution of *N. flexilis* within lochs before extinction. This is certainly the case for the extinct Perthshire sites with the exception of Loch Marlee. The relationship between *N. flexilis*, light, depth and exposure described in Chapter 2 would help to determine possible reintroduction sites within a loch. *N. flexilis* was only ever found in this study on fine silty sediment, which agrees with comments in the literature (Pearsall, 1920; James & Barclay, 1996; Howson *et al.*, 1997). This explains in part the relationship between *N. flexilis* and exposure. *N. flexilis* is often found behind islands or peninsulas or in sheltered bays. If the loch fetch is large, *N. flexilis* plants are most often found in deep water. However, the depth at which the plant can occur is likely to be limited by light (see chapter 2). The range of exposure and light where *N. flexilis* was observed to grow at different depths is shown in Fig. 2.5 & 2.6. This can be used as an indication as to where to reintroduce *N. flexilis* within a loch. The NVC community type may also be a guide as *N. flexilis* was never observed in the *Littorella-Lobelia* community, which is usually abundant in the littoral zone, but in the NVC communities A13, A24a and A23 described in Chapter 3.

As *N. flexilis* does not reproduce from vegetative fragments and is an annual with fragile above- and below-ground structures, introduction of seed rather than plants would be the advisable method of reintroduction. The results of the germination experiments (Chapter 6) show that any seed should be introduced by May to allow germination in June. It would be advisable to keep seed in water at temperatures of 6°C or below before introduction. This would satisfy any stratification requirements and would prevent loss of viability due to the seeds' intolerance to desiccation.

Where any seeds should be introduced from is an important issue and the genetic study described in Chapter 5 does not provide any easy answers. Therefore, the best approach could well be one of matching sites environmentally to find a donor site. This also obviously depends on the fitness of the population at the matched sites. Collecting seeds from populations which produce few seeds may be detrimental to the donor population, and it is also possible that the donor population may be suffering from inbreeding depression, thus making it an unsuitable donor site.

This study has provided information for the conservation of *N. flexilis* in Britain and Ireland; elucidating the threats to the species, proposing possible methods for monitoring and advice on reintroduction. It was beyond the scope of this study to investigate the *N. flexilis* seedbank and the longevity of *N. flexilis* seeds. If viable seeds could be found in the seedbank it would be worth considering it as an alternative source for reintroduction. The growth of *Elodea* spp. in North and South Uist is an issue worthy of further study. As these species invade new lochs, they should be monitored throughout their establishment phases, in different environmental conditions, so that the risk they pose to the aquatic macrophytes of this area, including *N. flexilis*, can be understood more clearly.

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APPENDIX 1 – Site abbreviations

Site	Abbreviation
Mainland	
Loch a Bhada dharaich	Bh
Loch of Butterstone	But
Loch Clunie	Cl
Loch of Craiglush	Cr
Fingask Loch	Fin
Loch Kindar	K
Lindores Loch	Li
Loch of Lowes	Lw
Loch Marlee	M
Lake of Menteith	Lom
Monk Myre	Mm
Monzieward	Mo
White Loch	W
Inner Isles and Kintyre	
Loch Ballyhaugh	Ba
Loch Fada	Fa
Glenastle Loch	Gl
Loch Gorm	Go
Loch Lossitt	L
Loch nan Gad	Ng
Loch Poit na h-l	Po
Tangy Loch	T
Loch an t-Sagairt	Ats

Site	Abbreviation
Outer Isles	
Loch Altabrug	A
Loch Bun an Ligidh	Bal
Loch nan Capull	Nc
Loch nan Cnamh	Cn
Loch Cuilc	Cu
Loch na Cuithe Moire	Cm
Loch Druidibeg	D
North Loch Druidibeg	Nd
Loch Eaval	E
Loch an Eilean (Drimsdale)	Ed
Loch Eileen a' Ghille-Ruaidh	Egr
Loch an Eileen (South Boisdale)	Sb
Loch nam Faicileann	F
Loch Gearraidh Mhic Iain	Gmi
Loch Grogary	G
Loch a'Mhadaidh	Mh
Mid Loch Ollay	Mlo
Loch Scarie	S
School House Loch	Sch
Loch with no name (?)	Lo
Ireland	
Carragh Lough	Ca
Cregduff Lough	Cd
Fin Lough	Fi
Kindrum Lough	Ki
Lough Leane	Le
Mullaghderg Lough	Md
Nafeakle Lough	Nf
Pollacappul Lough	Pol
Renvyle Lough	Re
Shennagh Lough	Sh

APPENDIX 2 – Site water chemistry data

Site	pH	Conductivity	Light extinction coefficient	Alkalinity (mg/l)
Carragh Lough	7.1	63	1.60	6.71
Cregduff Lough	6.62	196	1.77	16.66
Fin Lough	6.98	54.9	1.36	5.7
Fingask Loch	8.64	370	0.67	
Glenastle Loch	7.26	272	2.35	26.35
Lake of Menteith	7.04	77	0.62	9.7
Leane Lough	7.11	143	2.35	30.16
Lindores Loch	7.45	215	3.47	98.66
Loch a' Mhadaidh	7.39	289	1.77	
Loch a'Bhada Dharaich	6.97	55	1.68	8.21
Loch Altabrug	7.97	447	2.17	25.61
Loch an Eilean (Drimsdale)	7.49	235	1.96	
Loch an Eilean (South Boisdale)	7.98	291	2.64	17.55
Loch an t-Sagairt	6.87	163	3.05	
Loch Ballyhaugh	7.36	284	2.85	
Loch Bun an Ligidh	6.84	97	6.24	8.61
Loch Cuilc	6.88	100	2.26	
Loch Drudibeag	7.05	142	1.78	12.56
Loch Eaval	8.2	345	1.12	69.3
Loch Eilean a' Ghille-ruaidh	7.6	305	1.83	16.76
Loch Fada	6.86	293	3.19	
Loch Gearraidh Mhic Iain	8.25	305	1.96	
Loch Gorm	7.28	199	2.04	26.46
Loch Grogary	7.7	265	0.94	
Loch Kindar	7.49	160	2.12	
Loch Monzievaird	7.85	158	4.32	72.65
Loch na Cuiithe Moire	7.3	183	4.07	19.15
Loch nam Faoileann	7.56	197	0.78	31.86
Loch nan Capull	7.2	143	7.33	15.9
Loch nan Cnamh	7.84	316	1.65	32.4
Loch nan Gad	8.26	145	4.61	19.51
Loch no name	7.5	405	1.91	24.51
Loch of Butterstone	8.3	139	4.06	
Loch of Clunie	7.95	198	1.33	
Loch of Craiglush	7.54	127.4	2.98	16
Loch of Drumellie	9.01	214	1.46	
Loch of Lowes	7.54	126	1.76	23.45
Loch Olaidh Meadhanach	8.17	398	1.28	40.95
Loch Poit na h-I	7.2	269	3.22	
Loch Scarie	7.14	365	0.54	69.71
Lough Kindrum	7.85	311	1.36	65.7
Lough Nafeakle	6.46	116	4.40	4.91
Lough Shennagh	7.87	239	4.40	
Monk Myre	8.79	400	1.83	
Mullaghderg Lough	7.42	281	1.91	50.86
North Loch Duidibeag	7.3	134	3.30	30
Loch Lossit	7.54	370	0.82	52.76
Pollacapul Loch	7.01	77	1.76	9.51
Renvyle Lough	7.43	235	1.46	23
Schoolhouse Loch	7.72	369	1.29	
Tangy Loch	8.78	208	5.19	41.5
White Loch	7.96	410	0.91	

Appendix 2 (continued)

Site	Ca (mg/l)	Fe (mg/l)	Mg (mg/l)	N-NH3 (mg/l)	N-TON (mg/l)
Carragh Lough	2.06	0	1.4	<0.03	<0.01
Cregduff Lough	7.96	0	3.6	<0.03	<0.01
Fin Lough	1.32	0	1.54	<0.03	<0.01
Fingask Loch					
Glenastle Loch	8.84	0	5.2	0.08	<0.01
Lake of Menteith	8.26	0	1.34	<0.03	<0.01
Leane Lough	11.42	0	2.08	<0.03	0.88
Lindores Loch	24.72	0	12	0.28	<0.01
Loch a' Mhadaidh					
Loch a' Bhada Dharaidh	3.44	0	1.82	0.07	<0.01
Loch Altabrug	11.8	1	7.2	<0.03	<0.01
Loch an Eilean (Drimsdale)					
Loch an Eilean (South Boisdale)	9.68	2	6.4	<0.03	<0.01
Loch an t-Sagairt					
Loch Ballyhaugh					
Loch Bun an Ligidh	3.88	0	3.2	<0.03	<0.01
Loch Cuilc					
Loch Drudibeag	5.72	0	4.4	0.04	<0.01
Loch Eaval	33.4	0	6.2	<0.03	<0.01
Loch Eilean a' Ghille-ruidh	6.62	0	4.4	0.06	<0.01
Loch Fada					
Loch Gearraidh Mhic Iain					
Loch Gorm	10.14	0	5.2	<0.03	<0.01
Loch Grogary				0.036	<0.01
Loch Kindar					
Loch Monzievaire	21.7	0	8.8	<0.03	<0.01
Loch na Cuithe Moire	7.98	1	5.6	<0.03	<0.01
Loch nam Faoileann	13.12	0	4.8	<0.03	<0.01
Loch nan Capull	7.34	1	6	<0.03	<0.01
Loch nan Cnamh	13.14	0	4.8	0.05	<0.01
Loch nan Gad	8.72	0	3.2	<0.03	<0.01
Loch no name	9.5	2	4.4	0.06	<0.01
Loch of Butterstone				0.1	0.11
Loch of Clunie				0.056	1.369
Loch of Craiglush	7.48	0	2.8	<0.03	<0.01
Loch of Drumellie				0.116333	2.64666
Loch of Lowes	8.28	0	2.8	0.08	<0.01
Loch Olaidh Meadhanach	15.86	1	5.2	<0.03	<0.01
Loch Poit na h-I					
Loch Scarie	25.8	0	8.8	<0.03	<0.01
Lough Kindrum	22.4	<0.03	7.2	0.05	<0.01
Lough Nafeakle	2.02	0	2.12	0.04	<0.01
Lough Shennagh	12.32	0	4.4	0.1	0.03
Monk Myre					
Mullaghderg Lough	19.56	0	5.2	0.1	<0.01
North Loch Duidibeag	12.24	0	5.2	<0.03	<0.01
Loch Lossit	17.42	<0.03	6.4	<0.03	<0.01
Pollacapul Loch	3.58	0	1.34	0.04	<0.01
Renvyle Lough	10.46	1	5.2	0.09	<0.01
Schoolhouse Loch					
Tangy Loch	14.22	0	6.4	<0.03	<0.01
White Loch					

Appendix 2 (continued)

Site	P-DRP(PO4) (mg/l)	P-TP (mg/l)	K (mg/l)	Ortho- phosphate (mg/l)	N02-N (mg/l)
Carragh Lough	<0.001	0.021	0.46		
Cregduff Lough	<0.001	0.004	0.94		
Fin Lough	<0.001	0.012	0.3		
Fingask Loch					
Glenastle Loch	<0.001	0.011	0.7		
Lake of Menteith	<0.001	0.013	0.54	0.068	0.0055
Leane Lough	0.045	0.056	1.72		
Lindores Loch	0.074	0.16	2		
Loch a' Mhadaidh					
Loch a'Bhoda Dharaich	<0.001	0.009	0.74		
Loch Altabrug	<0.001	0.004	2.02		
Loch an Eilean (Drimsdale)					
Loch an Eilean (South Boisdale)	0.004	0.014	1.26		
Loch an t-Sagairt					
Loch Ballyhaugh					
Loch Bun an Ligidh	0.004	0.009	0.86	<0.01	0.016
Loch Cuilc					
Loch Drudibeag	0.005	0.008	1.32	<0.01	0.015
Loch Eaval	<0.001	<0.001	2.14		
Loch Eilean a' Ghille-ruaidh	<0.001	<0.001	0.94		
Loch Fada					
Loch Gearraidh Mhic Iain					
Loch Gorm	<0.001	0.015	1.54		
Loch Grogary	<0.001		1.2	<0.01	0.011
Loch Kindar					
Loch Monzievaireid	<0.001	0.014	1.52		
Loch na Cuithe Moire	<0.001	<0.001	2.02	<0.01	0.016
Loch nam Faoileann	<0.001	<0.001	1.48	<0.01	0.017
Loch nan Capull	<0.001	0.01	0.64	<0.01	0.034
Loch nan Cnamh	<0.001	0.004	1.9		
Loch nan Gad	<0.001	0.02	0.16	0.015	0.008
Loch no name	0.003	0.017	0.56		
Loch of Butterstone	<0.001		1.1	<0.01	0.007
Loch of Clunie	<0.001		1.2	<0.01	0.015
Loch of Craiglush	<0.001	0.013	0.52		
Loch of Drumellie	0.023		1.36667	0.023	0.015
Loch of Lowes	<0.001	0.026	1.1		
Loch Olaidh Meadhanach	<0.001	<0.001	1.44		
Loch Poit na hI					
Loch Scarie	<0.001	<0.001	1.78	<0.01	<0.01
Lough Kindrum	<0.001	0.01	2.74		
Lough Nafeakle	<0.001	0.012	0.54		
Lough Shennagh	<0.001	0.006	2.82		
Monk Myre					
Mullaghderg Lough	<0.001	0.013	1.22		
North Loch Duidibeag	<0.001	0.003	0.74	<0.01	0.016
Loch Lossit	<0.001	0.011	0.72		
Pollacapul Loch	<0.001	0.006	0.46		
Renvyle Lough	<0.001	0.01	1.66		
Schoolhouse Loch					
Tangy Loch	<0.001	0.017	0.3	<0.01	<0.01
White Loch					

APPENDIX 3 – Site sediment chemistry data

Site	Available P (mg/l)	Available K (mg/l)	Extractable Mg (mg/l)
Carragh Lough	2.9		
Cregduff Lough	2.8		
Fin Lough	0.9		
Fingask Loch	84.4	104	302
Glenastle Loch	1.8	37.5	66.8
Lake of Menteith	2.6	45.7	16.3
Leane Lough	5.3		
Lindores Loch	24.1		
Loch a' Mhadaidh	1	25.9	141
Loch a' Bhada Dharraich	17.3		
Loch Altabrug	1.1	8	87
Loch an Eilean (Drimsdale)	1.3	4.8	28.7
Loch an Eilean (South Boisdale)	2.8	9.5	66.4
Loch an t-Sagairt	3	50.4	246
Loch Ballyhaugh	2.7	16.9	141
Loch Bun an Ligidh	0.3	49	243
Loch Cuilc			
Loch Drudibeag	0.5	136	388
Loch Eaval	1.9	74.5	151
Loch Eilean a' Ghille-ruaidh	1	87.1	308
Loch Fada	1.6	4	211
Loch Gearraidh Mhic Iain	1.1	35.1	152
Loch Gorm	1.2	4	17.3
Loch Grogary	1.1	18.7	48.3
Loch Kindar			
Loch Monzievairst	21.8		
Loch na Cuithe Moire	1.2	32.5	77.6
Loch nam Faoileann	1.3	15.7	50.9
Loch nan Capull	0.3	166	344
Loch nan Cnamh	3.7	104	213
Loch nan Gad	3.1		
Loch no name	3.4	67.3	211
Loch of Butterstone	6.1	29	75
Loch of Clunie	3.6	95.5	110
Loch of Craighlush	2.3	29	65.1
Loch of Drumellie	21.8	89.2	103
Loch of Lowes	9.4	15.9	21.2
Loch Olaidh Meadhanach	3.6	38.1	47.1
Loch Poit na h-I	2.2	38.2	92.4
Loch Scarie	2.6	49.7	53.7
Lough Kindrum	3.7		
Lough Nafeakle	2.9		
Lough Shennagh	5.7		
Monk Myre	11.5	28.3	54.1
Mullaghderg Lough	4.2		
North Loch Duidibeag	0.4	37.5	58.3
Loch Lossit	2.8	21.1	85.1
Pollacapul Loch	3.9		
Renvyle Lough	7.2		
Schoolhouse Loch	1.7	28	340
Tangy Loch	5.3		
White Loch	72.9	65	189

Appendix 3 (continued)

Site	Extractable Ca (mg/l)	Total Fe (mg/kg)	Total N (mg/kg)	Extractable Na (mg/l)
Carragh Lough	383	9420	360	
Cregduff Lough	4370	168000	9700	
Fin Lough	145	20600	320	
Fingask Loch	1.8	105	4980	74.9
Glenastle Loch	809	578	1880	53.9
Lake of Menteith	319	139	670	13
Leane Lough	331000	2710	830	
Lindores Loch	3760	11300	3320	
Loch a' Mhadaidh	1230	1140	6220	135
Loch a' Bhada Dharaidh	5720	22000	16900	
Loch Altabrug	523	534	2230	52.4
Loch an Eilean (Drimsdale)	290	126	350	21.1
Loch an Eilean (South Boisdale)	544	254	670	36.3
Loch an t-Sagairt	1180	1250	14000	205
Loch Ballyhaugh	1130	679	3320	81.7
Loch Bun an Ligidh	1520	7780	38100	239
Loch Cuilc				
Loch Drudibeag	1930	11900	89200	
Loch Eaval	1610	1630	11400	145
Loch Eilean a' Ghille-ruaidh	1800	1510	10200	257
Loch Fada	816	516	15100	74.1
Loch Gearraidh Mhic Iain	896	184	1830	101
Loch Gorm	198	94.4	240	17.4
Loch Grogary	513	570	14600	35.6
Loch Kindar				
Loch Monzievairst	3140	11200	1540	
Loch na Cuiithe Moire	585	2870	20700	72
Loch nam Faoileann	482	890	21500	34.2
Loch nan Capull	1710	8680	142000	
Loch nan Cnamh	1550	2530	13600	239
Loch nan Gad	1830	8930	490	
Loch no name	1440	3950	11700	300
Loch of Butterstone	885	904	2120	23.4
Loch of Clunie	1440	7850	40800	40
Loch of Craiglush	642	424	6140	24.8
Loch of Drumellie	805	216	1110	27.6
Loch of Lowes	253	121	350	8.3
Loch Olaidh Meadhanach	872	183	1040	46.4
Loch Poit na h-I	600	190	590	41.6
Loch Scarie	908	860	27100	39.7
Lough Kindrum	1530	3760	1200	
Lough Nafeakle	5250	27600	6220	
Lough Shennagh	1420	4870	570	
Monk Myre	4920	158	600	20.5
Mullaghderg Lough	1440	7370	510	
North Loch Duidibeag	467	1290	16400	53.6
Loch Lossit	875	741	2000	26.4
Pollacapul Loch	2560	21400	830	
Renvyle Lough	950	9180	290	
Schoolhouse Loch	1790	786	11700	175
Tangy Loch	969	14500	1470	
White Loch	25000	123	11400	72.5

APPENDIX 4 – Species presence or absence data, site codes as in Appendix 1

	A	Ats	Ba	Bal	Bh	But	Ca	Cl	Cm	Cn	Cr	D	E	Ed	Egr	F	Fa	Fi	Fin	G	Gl	Gmi	Go	K	Ki	L
<i>Apium inondatum</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baldellia ranunculooides</i>	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Callitriche hamulata</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>Callitriche hermaphroditica</i>	0	0	0	0	0	1	0	1	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1
<i>Callitriche stagnalis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Ceratophyllum demersum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chara</i> spp.	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	1	0
<i>Elatine hexandra</i>	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0
<i>Eleocharis acicularis</i>	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
<i>Eleocharis palustris</i>	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Eleogiton fluitans</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Elodea canadensis</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
<i>Elodea nuttallii</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
<i>Eriocaulon aquaticum</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Fontinalis antipyretica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1
<i>Hydrilla verticillata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isoetes lacustris</i>	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	0
<i>Isoetes setacea</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Juncus bulbosus</i>	0	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1
<i>Junger mannia</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lemma minor</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Littorella uniflora</i>	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1
<i>Lobelia dortmanna</i>	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	1	1	1	1	0
<i>Menyanthes trifoliata</i>	0	1	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	1	0	0
<i>Myriophyllum alterniflorum</i>	0	1	1	0	0	1	0	1	1	1	0	0	1	1	0	1	0	1	0	1	1	1	1	1	0	1

Appendix 4 (continued)

	Le	Li	Lo	Lom	Lw	M	Md	Mh	Mlo	Mm	Mo	Nc	Nd	Nf	Ng	Po	Pol	Re	Cd	S	Sb	Sch	Sh	T	W	
<i>Apium inundatum</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baldellia ranunculoides</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Callitriche hamulata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Callitriche hermaphroditica</i>	1	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1
<i>Callitriche stagnalis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratophyllum demersum</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chara</i> spp.	1	1	1	0	0	1	0	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1
<i>Elatine hexandra</i>	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0
<i>Eleocharis acicularis</i>	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Eleocharis palustris</i>	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0
<i>Eleogiton fluitans</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Elodea canadensis</i>	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1
<i>Elodea nuttallii</i>	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Eriocaulon aquaticum</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
<i>Fontinalis antipyretica</i>	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0
<i>Hydrilla verticillata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Isoetes lacustris</i>	1	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	1	1	1	0	0	0	1	1	0	0
<i>Isoetes setacea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Juncus bulbosus</i>	0	0	1	1	0	0	1	1	1	0	0	1	1	1	0	1	1	0	1	0	0	0	1	1	0	0
<i>Junger mannia</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lenna minor</i>	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Littorella uniflora</i>	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1
<i>Lobelia dortmanna</i>	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0
<i>Menyanthes trifoliata</i>	0	0	1	0	0	1	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0
<i>Myriophyllum alterniflorum</i>	1	0	0	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	0	1	1	1	1	0	1

Appendix 4 (continued)

	A	Ats	Ba	Bal	Bh	But	Ca	Cl	Cm	Cn	Cr	D	E	Ed	Egr	F	Fa	Fi	Fin	G	Gl	Gmi	Go	K	Ki	L
<i>Myriophyllum spicatum</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Najas flexilis</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1
<i>Nitella</i> spp.	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
<i>Nuphar lutea</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Nymphaea alba</i>	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
<i>Pericarita amphibia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton alpinus</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton bertholdi</i>	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0
<i>Potamogeton crispus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
<i>Potamogeton ephedrus</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton gramineus</i>	0	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	1	1	0	0	1
<i>Potamogeton lucens</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton natans</i>	0	1	1	1	1	0	1	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0
<i>Potamogeton obtusifolius</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton pectinatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Potamogeton perfoliatus</i>	0	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Potamogeton polygonifolius</i>	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Potamogeton praelongus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
<i>Potamogeton pusillus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton rutilus</i>	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
<i>Potamogeton x nitens</i>	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
<i>Potamogeton x zizii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ranunculus aquatilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
<i>Ranunculus flamula</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sparganium angustifolium</i>	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	0	0
<i>Sparganium emersum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sparganium erectum</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Subularia aquatica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Utricularia vulgaris</i>	0	1	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0
<i>Zannichellia palustris</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Appendix 4 (continued)

	Le	Li	Lo	Lom	Lw	M	Md	Mh	Mlo	Mm	Mo	Nc	Nd	Nf	Ng	Po	Pol	Re	Cd	S	Sb	Sch	Sh	T	W
<i>Myriophyllum spicatum</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Najas flexilis</i>	1	0	1	1	0	1	0	1	1	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	0
<i>Nitella</i> spp.	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
<i>Nuphar lutea</i>	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Nymphaea alba</i>	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Pericarita amphibia</i>	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0
<i>Potamogeton alpinus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton bertholdi</i>	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1
<i>Potamogeton crispus</i>	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Potamogeton ephedrus</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton gramineus</i>	0	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0
<i>Potamogeton lucens</i>	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Potamogeton natans</i>	0	0	1	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	0	0
<i>Potamogeton obtusifolius</i>	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton pectinatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
<i>Potamogeton perfoliatus</i>	1	0	1	1	1	1	0	1	1	0	0	0	1	0	1	1	0	1	0	0	0	1	1	1	1
<i>Potamogeton polygonifolius</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamogeton praelongus</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Potamogeton pusillus</i>	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Potamogeton rutilus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Potamogeton x nitens</i>	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Potamogeton x zizii</i>	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Ranunculus aquatilis</i>	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Ranunculus flamula</i>	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0
<i>Sparganium angustifolium</i>	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0
<i>Sparganium emersum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sparganium erectum</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Subularia aquatica</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Utricularia vulgaris</i>	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Zannichellia palustris</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0