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RESEARCH ARTICLE

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Filling the "data gap": Using paleoecology to investigate the decline of *Najas flexilis* (a rare aquatic plant)

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In the absence of long-term monitoring records, paleoecology can be used to extend knowledge of species and community ecology into the past. The rare and declining aquatic plant *Najas flexilis* is a priority species for conservation across Europe, and is an ideal candidate for paleoecological study; not only are historical records of the plant sparse, but its seeds are commonly found and well preserved in lake sediment cores. In this study, we investigate the timing and causes of decline at two UK sites at which *N. flexilis* has recently become extinct: Esthwaite Water (England) and Loch of Craiglush (Scotland). For both sites, multiple paleoecological indicators and available historical biological records and monitoring data are compared to numbers of *N. flexilis* seeds enumerated in dated sediment cores representing the last 150 years. At Esthwaite Water, *N. flexilis* seeds were found in abundance in association with indicators of a clear, oligo-mesotrophic, mildly alkaline lake. Eutrophication led to the disappearance of *N. flexilis* in the 1980s. By contrast, far fewer *N. flexilis* seeds were found in a core from Loch of Craiglush, and the current period of *N. flexilis* absence was found to be one of several over the last 100 years. Species represented in cores taken from Loch of Craiglush were indicative of slightly more acidic conditions than Esthwaite Water. Given that *N. flexilis* favours circumneutral to alkaline conditions, it is possible that Loch of Craiglush has not always been favourable for the plant. These findings have important implications for future conservation efforts, particularly at Esthwaite Water where they suggest that recent failed attempts to reintroduce the species may have been premature. More generally, this study demonstrates the value of paleoecological techniques as a means to provide the long-term context that is often missing from conservation planning and management.

KEYWORDS
conservation, macrofossils, *Najas flexilis*, paleoecology, rare species

1 | INTRODUCTION

Consistent, reliable documentary records and long-term monitoring both play an important role in conservation. Documentary records and existing datasets are essential to understanding the population histories of target species and, in turn, changes to their habitats (Swetnam et al., 1999). However, long-term ecological records, particularly those concerning biota,

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are often lacking (Dudgeon et al., 2006). Museum records in particular tend to be sparse, and often suffer from spatial bias (Rondinini et al., 2006). Sutherland et al. (2004) argue that the paucity of historical data currently forces many conservation decisions to be based on anecdotal evidence, equating current environmental practices to those common in healthcare before the introduction of "evidence-based practice." In order to carry out effective, evidence-based conservation, this data gap must be urgently addressed.

Environmental studies spanning geological timescales routinely make use of fossil deposits to provide evidence for past ecological records. Lake sediments, in particular, can contain large numbers of fossils from a wide range of different biological groups that are easily extracted (using lake sediment coring), and identified to genus or species level by paleolimnologists (Birks, 2008). The field of "applied paleoecology" practices these geological techniques on much shorter timescales in order to address specific ecological questions. Typically, applied paleoecological research spans periods of around 100–200 years; the time scale most relevant to nature conservation. In recent years, a growing number of such studies have used paleolimnological approaches to try to fill in data gaps in contemporary documentary records (e.g., Bennion et al., 2018; Davidson et al., 2005; Salgado et al., 2010; Sayer et al., 2012). The combination of documentary evidence, monitoring data, and applied paleoecology has proved to be a powerful tool in assessing the causes and timing of major ecological changes in lake ecosystems, and has been used to help set appropriate lake management strategies. For example, Salgado et al. (2010) used macrofossils found in a sediment core to establish that the plant community of Loch Leven, Scotland, had undergone a shift from *Isoetes* spp. dominance towards a *Potamogeton* spp. and *Chara* spp. community long before regular systematic monitoring of plants began. These findings led to the reassessment of macrophyte conservation targets at the lake. Further, at Upton Great Broad, a shallow lake in Norfolk, UK, plans to dredge the lake to improve water quality were abandoned following analyses of sediment cores and historical plant records, which showed a strong association between the protected plant *Najas marina* and a fluid green upper sediment layer (Ayres et al., 2008).

One aquatic macrophyte species of high conservation interest whose fossils are regularly recorded in lake sediments is the aquatic macrophyte *Najas flexilis*. This species is listed as a "Priority Species" in the EU Habitats Directive (Council Directive 92/43/EEC, 1992). It has a global circum-polar distribution (Figure 1), but is found much more frequently in North America than in Europe (Godwin, 1975; Haynes, 1979). In Europe, the climatic region of *N. flexilis* is defined as

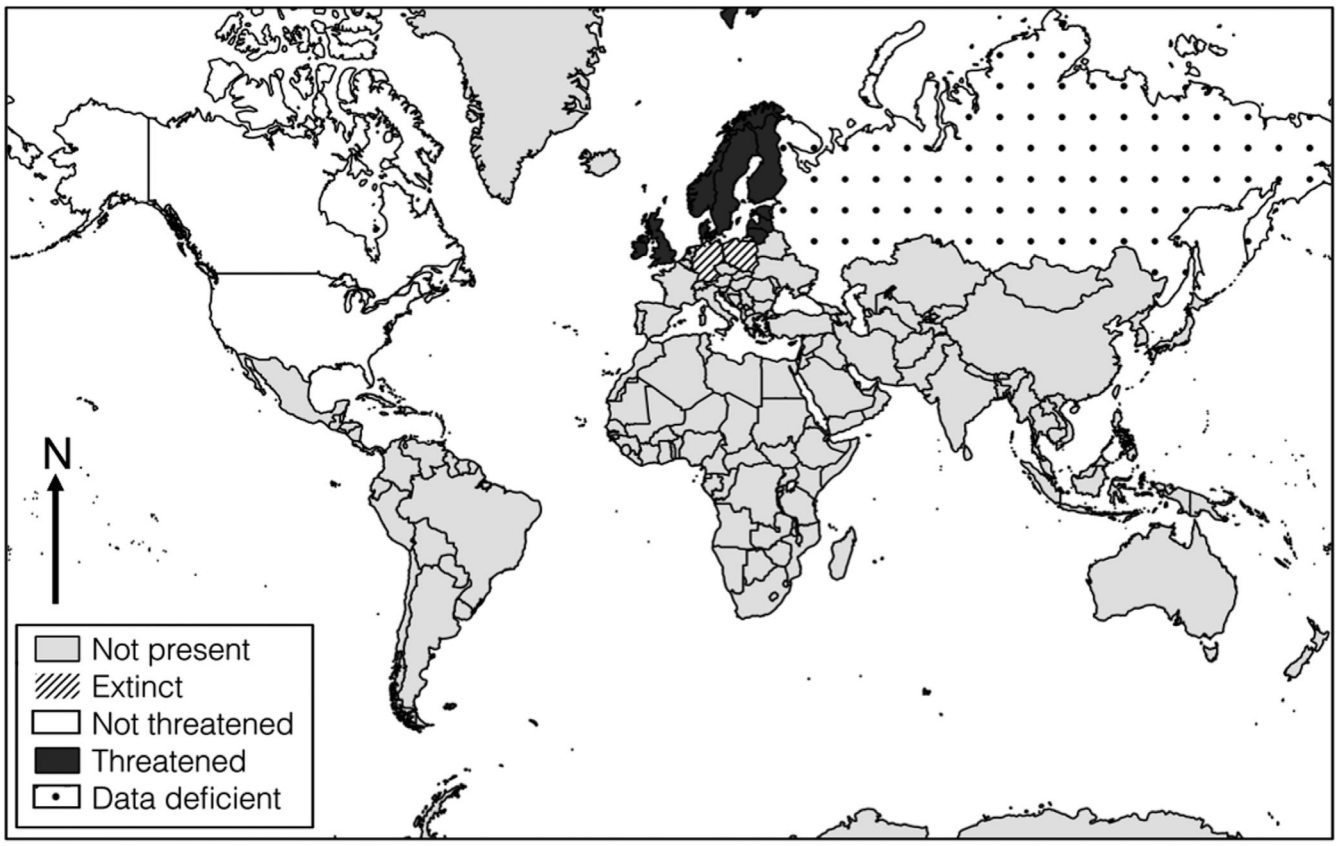


FIGURE 1 Global distribution of *N. flexilis*.

Boreal-montane, and the species is generally confined to altitudes of less than 300 m (Preston & Hill, 1997). The largest European populations of *N. flexilis* are in Sweden, where there are thought to be around 10,500 individuals, and in the British Isles, where records exist from 51 sites in Scotland and 28 in Ireland according to the most recent study (Gärdenfors, 2010; Wingfield et al., 2004). In recent decades, *N. flexilis* has undergone a decline in Europe, particularly in the south; the plant has not been seen in Germany since 1973, has declined in the Russian regions of Pskov, Novgorod, Tver, and Moscow, and is thought to be extinct in Poland and Switzerland (Commission of the European Communities, 2009; Kalinka & Nowak, 2004; Tzvelev, 2000). Commonly cited causes for these declines include climate change, eutrophication, acidification, and competition from invasive species (Wingfield et al., 2004, 2006). However, due to a lack of consistent historical observations of *N. flexilis* spanning further than approximately the past 20 years, it is not possible to relate changing *N. flexilis* populations to environmental trends based on existing data alone. Paleoecology allows comparison of long-term species population trends with changing environmental conditions, and therefore offers a means to explore these hypotheses for *N. flexilis* decline.

Although consistent historical records of *N. flexilis* are lacking, the species is well suited for paleoecological study. *N. flexilis* has distinctive elliptical seeds with a dark, shiny pericarp, which are easily identified and preserved in lake sediments. As an annual, it produces large numbers of seeds. These seeds remain attached to "rafts" of fragmented vegetation, which are carried by water currents and dispersed widely across lake basins, meaning that seeds are highly likely to be found in sediment cores taken from any location at a site where the plant is present (Bishop et al., 2018). Furthermore, many of the perceived threats to *N. flexilis* are readily explored through paleoecological proxies.

This applied paleoecological study investigates the history of occurrence and causes of decline of *N. flexilis* at two sites in the UK from which *N. flexilis* has been lost in the past 50 years or so: Esthwaite Water (English Lake District) and Loch of Craiglush (Perthshire, Scotland). *N. flexilis* was last recorded at Esthwaite Water in the 1980s, and is believed to have disappeared from the Dunkeld-Blairgowrie lochs in the 2000s (Wingfield et al., 2004). These lakes have also been subject to a variety of anthropogenic pressures, including eutrophication, aquaculture, and invasive species introduction (Bennion et al., 2010; Dong et al., 2011, 2012; Vernon & Hamilton, 2011). Our study had the following objectives:

1. To reconstruct changing *N. flexilis* populations over approximately the past 150 years using a combination of historical botanical records and macrofossil data.
2. To evaluate the causes of *N. flexilis* decline by inferring parallel lake environmental changes using macrofossil, diatom, and cladoceran remains.

2 | METHODOLOGY

2.1 | Study sites

Esthwaite Water (Figure 2a) is a relatively shallow lake (mean depth 6.4 m) in the English Lake District, located in a sheltered valley between the much larger lakes of Windermere and Coniston Water. It has a surface area of 96 ha, and was given formal legal protection as a Special Site of Scientific Interest (SSSI) in 1965 on the basis that it supports a diverse macrophyte community (including, at the time of designation, both *N. flexilis* and the similarly rare *Hydrilla verticillata*). Esthwaite Water is one of the most intensively monitored lakes in the world, with water chemistry monitoring data extending back to the 1940s (Talling & Heaney, 1988). Based on its nutrient levels in 1940, it is classified as naturally "moderate alkalinity/mesotrophic" in UK conservation legislature, and "oligotrophic to mesotrophic" under the EU Habitats Directive (Hughes et al., 2004). *N. flexilis* was recorded in Esthwaite Water on 15 separate occasions between 1914 and 1982, but has not been recorded at the site since 1982 (Darwell, 2000; National Biodiversity Network, 2014; Pearsall, 1920; Stokoe, 1983).

Loch of Craiglush (Figure 2b) is the uppermost lake of the Dunkeld-Blairgowrie Lochs, a chain of five shallow (<20 m), mesotrophic lochs in Perthshire, Scotland, hydrologically linked by the Lunan Burn. Together, the Dunkeld-Blairgowrie lochs form a Special Area of Conservation (SAC), designated because of their populations of *N. flexilis*. Loch of Craiglush has a maximum depth of 13.4 m and a surface area of 26 ha, and is located within 2 km of the next lochs in the chain – Loch of Butterstone and Loch of Lowes. *N. flexilis* was first recorded in Loch of Craiglush in 1967 (Scottish Natural Heritage, unpublished data). Following unconfirmed reports that the extent of *N. flexilis* cover was declining in the late 1980s, several SCUBA and snorkel surveys of the loch were commissioned by Scottish Natural Heritage. Collectively, these surveys suggest that the plant was present in Loch of Craiglush between 1987 and 2004 (Benthic Solutions, 2007;

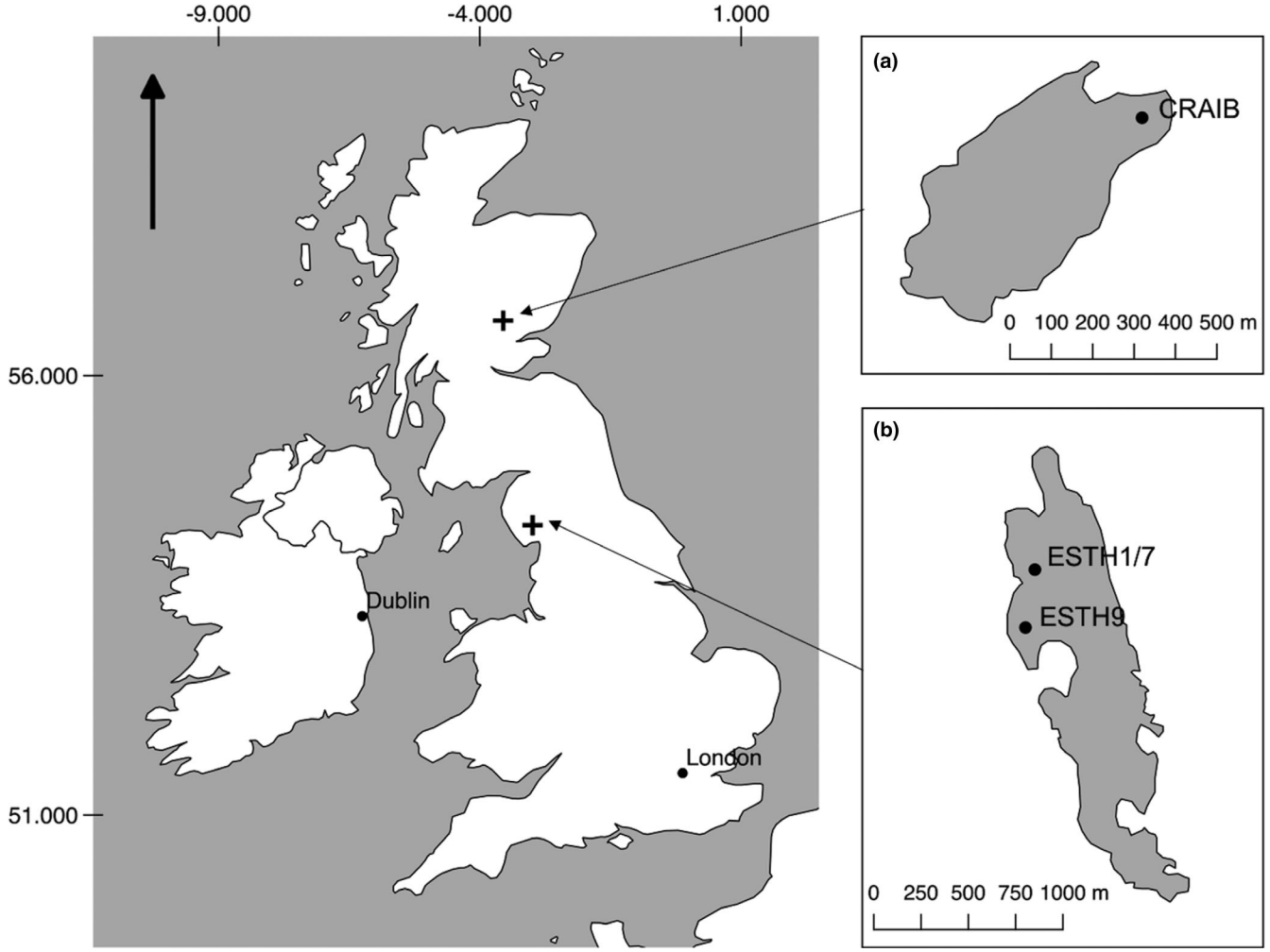


FIGURE 2 Map of study sites, with locations of sediment cores (described in section 3.1) labelled.

Dale et al., 1997; James & Barclay, 1996). *N. flexilis* has not been recorded at Loch of Craiglush since 2004, nor at any of the Dunkeld-Blairgowrie Lochs since 2007.

2.2 | Data collection

Large diameter piston cores were taken from the littoral zones of Esthwaite Water (ESTH9) and Loch of Craiglush (CRAIB) using a "Big Ben" wide-bore piston corer (Patmore et al., 2014) in 2013. Both cores were extruded in the field at 1 cm intervals, with notes made of any visual stratigraphic changes. Percentage loss on ignition and percentage carbonate were analysed following standard methods (Dean, 1974). Core details are given in Table 1, which also includes other cores taken from Esthwaite Water that are used for secondary data in this study. Core locations are marked in Figure 2.

TABLE 1 Core details

Core	Date	Latitude/longitude	Water depth (m)	Core type	Core length (m)	Sampling intervals (cm)
ESTH9	1/9/2013	54.3612/–2.9907	1.5	Big Ben	1.09	1
CRAIB	25/8/2014	56.5828/–3.5536	1.1	Big Ben	0.56	1
ESTH1[a]	7/6/1995	54.3639/–2.9900	15	Mackereth corer	0.86	1
ESTH7[a]	12/4/2006	54.3639/–2.9900	15	Percussion piston corer	0.80	0.5

[a]ESTH1 and ESTH7 were originally collected by Dong et al. (2011, 2012) and are used for secondary data in this study.

All cores were radiometrically dated in the Environmental Radiometric Facility at University College London, where they were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs, and ^{241}Am by direct gamma assay using ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. Lead-210 was determined via its gamma emissions at 46.5 keV, and ^{226}Ra by the 295 and 352 keV gamma rays emitted by its daughter isotope ^{214}Pb following three weeks of storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 and 59.5 keV (Appleby et al., 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby et al., 1992). In both cores, variations in unsupported ^{210}Pb were non-monotonic, which precluded the use of the constant initial concentration dating model (Appleby, 2001). The simple constant rate of ^{210}Pb supply (CRS) dating model was therefore used.

Sediment samples of approximately 50–75 cm^3 (determined by water displacement) were sieved at 350 and 125 μm for macrofossil analyses, following the methodology described by Birks (2001). Residues were examined at ×10–40 magnification under a Leica light microscope, and plant macrofossil remains (e.g., seeds, leaf spines, leaf tips) were identified and counted. Identification was made by comparison with reference material held at the Environmental Change Research Centre, University College London. The results for each species (or species group where identification to species level was not possible) were expressed as number of remains per 100 cm^3 wet sediment. In order to gain further insight into ecological changes in the lake ecosystem, analysis for chitinous cladoceran remains was carried out on every other sample (i.e., at 2 cm intervals), following Korhola and Rautio (2001). Sediments were air dried and samples were heated in 10% KOH (a deflocculating agent) for 20 minutes, before being magnetically stirred and passed through a 25 μm mesh sieve. A sub-sample was then stained using safranin and examined under a Leica light microscope. Remains were identified with reference to Szeroczyńska and Sarmaja-Korjonen (2007) and expressed in terms of percentage relative abundance.

In addition to ESTH9, data from previous cores were also used: ESTH1 and ESTH7 (Dong et al., 2011, 2012 described in Table 1). These cores have been previously dated radiometrically (as above) and analysed for diatoms following standard methods (Battarbee et al., 2001).

Long-term monitoring data (including alkalinity, pH, total phosphorus [TP], nitrate [NO^-_3] and Secchi depth) have been collected fortnightly from Esthwaite Water since 1945 by the Freshwater Biological Association and the Centre for Ecology and Hydrology (Maberly et al., 2017). Measurements were taken from a boat at the deepest point in the lake, using an integrated water sample from 0 to 5 m. Water quality data (alkalinity, pH, TP, and NO^-_3) from the inflow to Loch of Craiglush were available since 1987 (SEPA [Scottish Environmental Protection Agency], unpublished data used here with special permission).

2.3 | Data analysis

Bishop et al., 2018 used kernel density analysis to estimate the probable *N. flexilis* abundance represented by a sediment sample with a fixed number of *N. flexilis* seeds. Based on this analysis, supplemented by information derived from historical botanical records, sediment cores from Esthwaite Water and Loch of Craiglush were split into depth zones in relation to predicted *N. flexilis* abundance. Species most closely associated with different periods of *N. flexilis* abundance were identified using principal components analysis (PCA) conducted using the "vegan" R package (Oksanen et al., 2017). All other statistical analysis was carried out using the R "stats" package version 2.15.3 in the software RStudio (R Core Team, 2013; RStudio Team, 2016).

3 | RESULTS

3.1 | Esthwaite water

Radiometric dating (Figure 3a) showed that equilibrium of total ^{210}Pb activity with supported ^{210}Pb activity was reached in the core at around 26 cm. The maximum value of unsupported ^{210}Pb activity was at around 3.5 cm, suggesting increased sediment accumulation towards the top of the core. Between 9.5 and 15.5 cm, unsupported ^{210}Pb activities declined exponentially with depth, implying stable sedimentation rates within this section. However, a sharp dip at 17.5 cm suggests a possible sediment slumping event. The CRS model placed 1986 at 7.5–9.5 cm. This is in agreement with the ^{137}Cs peak at 9.5 cm, which likely represents the 1986 fallout from Chernobyl. A relatively slow and gradual increase in ^{137}Cs activity from the deep side of the ^{137}Cs peak, combined with the detectable ^{241}Am from 9.5 to 13.5 cm, suggests that the ^{137}Cs maximum expected from the 1963 peak testing of nuclear weapons is obscured by fallout from Chernobyl. The CRS model

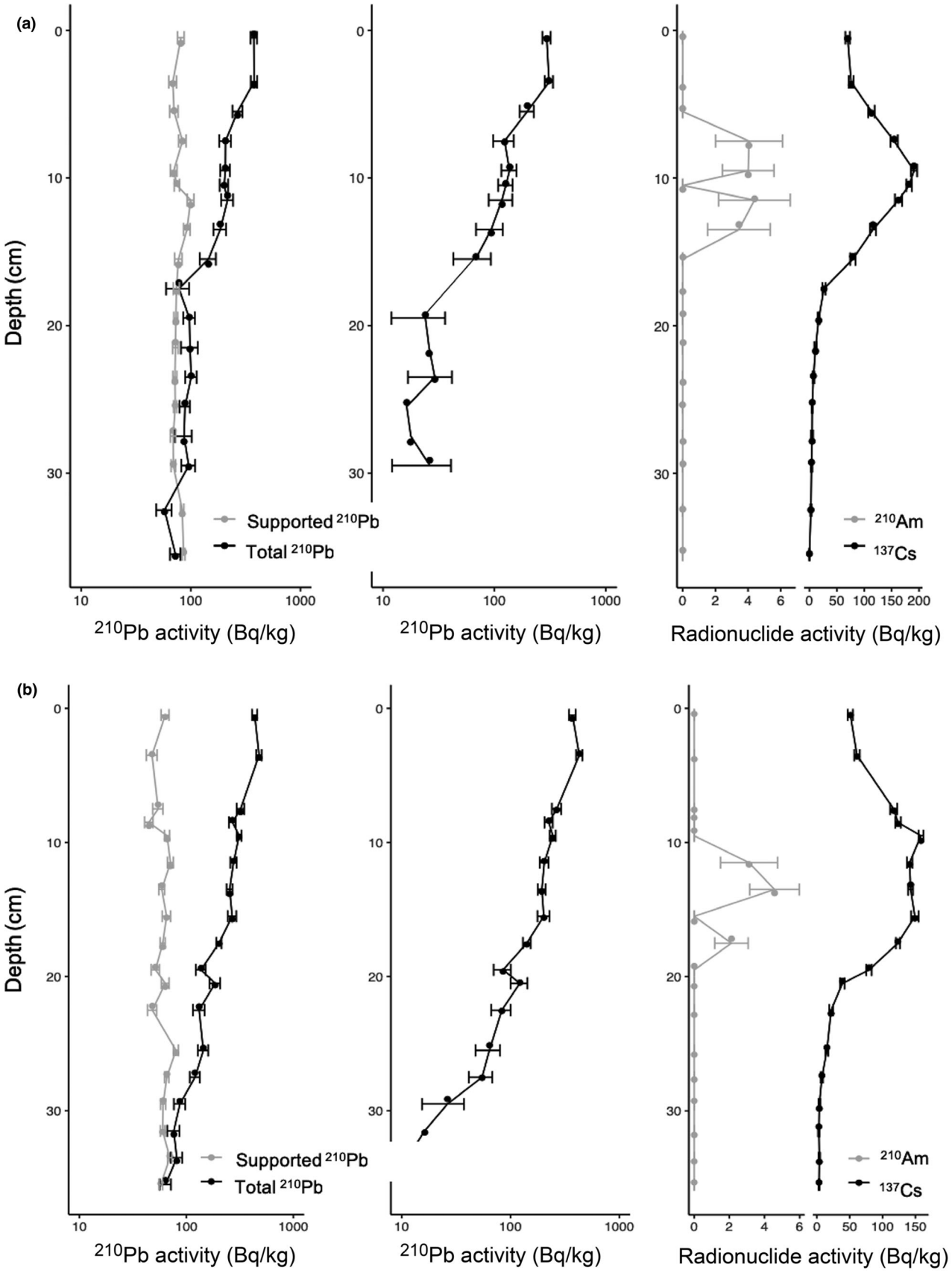


FIGURE 3 Fallout radionuclide concentrations (left to right: total ^{210}Pb; unsupported ^{210}Pb; ^{137}Cs, and ^{241}Am) in (a) ESTH9 and (b) CRAIB.

placed 1963 between 11.5 and 13.5 cm. Sedimentation rates before the 1920s were calculated at around 0.02–0.03 g cm^{-2} $year^{-1}$. In the 1930s, the sedimentation rate peaked at 0.06 g cm^{-2} $year^{-1}$, before recovering until another increase in the 1970s. Over the past 30 years, sedimentation rates fluctuated between 0.036 and 0.057 g cm^{-2} $year^{-1}$.

Key changes in sediment characteristics, water chemistry, and species composition at Esthwaite Water are summarised in Figure 4. According to the numbers of seeds found in ESTH9, *N. flexilis* was relatively abundant until around 1915. PCAs (Figure 5) showed samples with high numbers of *N. flexilis* seeds were closely associated with the presence of the plant macrofossils *Chara* spp. and *Nitella* spp., the cladocerans *Sida crystallina* and *Camptocercus rectorostri,* and the diatom *Achnanthes minutissima*. From 1915, the macrofossils suggest that *N. flexilis* steadily declined, with seeds only intermittently present between around 1938 and 1987. The numbers of *N. flexilis* leaf spines also reflect this decline in abundance, but indicate that the decline occurred later, from around 1936. Although *N. flexilis* has not been recorded at Esthwaite Water since 1982, *N. flexilis* seeds reappear in ESTH9 in small numbers at around 2002, suggesting possible presence in the lake at this time. On the other hand, *N. flexilis* spines are not present after around 1987.

Between around 1915 and 1978, macrofossils indicated increases in *Potamogeton* spp., particularly *Potamogeton perfoliatus* and *Potamogeton obtusifolius*. In the cladoceran record, *Alona affinis* and *Chydorus sphericus* were dominant during this period. In cores ESTH1/ESTH7, *Cyclotella comensis* abundance declined from around 1915 and *Fragilaria crotenensis* appeared for the first time. Water chemistry monitoring shows that Secchi depth decreased from approximately 4 to 3 m during this time.

Nymphaeaceae remains were closely associated with samples from after around 1978. *Daphnia* remains in the form of ephippia (identified during macrofossil analysis) also increased in ESTH9 after around 1968. In cores ESTH1/7, the diatoms *Stephanodiscus hantzschii* and *Fragilaria crotenensis* were associated with samples from this period, while *Tabellaria flocculosa*, which had an increased abundance from around 1915 to 1968, declined. TP and nitrate concentrations as well as alkalinity were at their highest in the late 1980s and early 1990s, while Secchi depth was at its lowest point of below 2 m in 1991.

Water chemistry monitoring shows that nutrient concentrations began to decrease in the lake after around 2000, and Secchi depth recovered to approximately 3 m. In the top 3 cm of ESTH9 there was evidence for a small increase in numbers of *Chara* and *Nitella* oospores, a decrease in *Chydorus sphericus,* and increases in the cladocerans *Bosmina longispina* and *Acroperus harpae*.

3.2 | Loch of Craiglush

In CRAIB, radiometric dating (Figure 3b) showed that equilibrium of total ^{210}Pb activity with supported ^{210}Pb activity was reached at around 36 cm. Like ESTH9, the maximum value of unsupported ^{210}Pb activity was below the surface sediments, suggesting recent increases in sedimentation rates. Gradients of decline in unsupported ^{210}Pb activity increased with depth, suggesting higher rates of sedimentation towards the top of the core. The ^{137}Cs activity versus depth profile showed peaks at 9.5 and 15.5 cm. These are likely derived from the 1986 Chernobyl fallout and the maximum fallout of atmospheric testing of nuclear weapons in 1963, respectively. The latter date is supported by ^{241}Am, which was detected between 11.5 and 17.5 cm. The CRS model placed 1986 at 12.5 cm and 1963 at 18 cm – deeper than suggested by ^{137}Cs and ^{241}Am. Chronologies and sedimentation rates were therefore corrected by referring sediments at 15.5 cm as formed in 1963. This puts 1986 at 9.5–11.5 cm, in agreement with the ^{137}Cs record. Mean sedimentation rates were calculated at 0.025 g cm^{-2} $year^{-1}$ before the 1930s, increasing to 0.067 g cm^{-2} $year^{-1}$ after this date.

Key changes in sediment characteristics, water chemistry, and species composition at Loch of Craiglush are summarised in Figure 6. The first historical botanical record of *N. flexilis* is from 1967 (National Biodiversity Network, 2014), however no indication of the abundance of *N. flexilis* at the site is given until 1994, when the plant was found growing at just one location within the loch, albeit fairly densely within that patch (James & Barclay, 1996). Similar observations of local abundance but lake-wide rarity were made in 1995, 1996, and 1997, but in 2004 only a few plants were found (Murphy, 2007, unpublished). *N. flexilis* seeds and spines were retrieved in small numbers throughout core CRAIB, suggesting species presence (but low abundance) since pre-1850. There was no clear evidence for any significant changes in the abundance of *N. flexilis* throughout the core, although there were short periods during which seeds were not present. Although botanical records indicate that *N. flexilis* was last recorded in Loch of Craiglush in 2004, seeds were found in sediments towards the top of CRAIB dating from around 2007. In line with the historical records derived from extensive snorkel and SCUBA surveys, *N. flexilis* was considered "absent" from 2004 onwards during analysis and interpretation of CRAIB.

Plant macrofossils found in CRAIB comprised *Isoetes lacustris, Potamogeton* spp., *Nuphar lutea, Nitella* spp., *Sphagnum* spp., and *Juncus bulbosus,* all of which are present throughout the core. From around 1895, there was a decline in

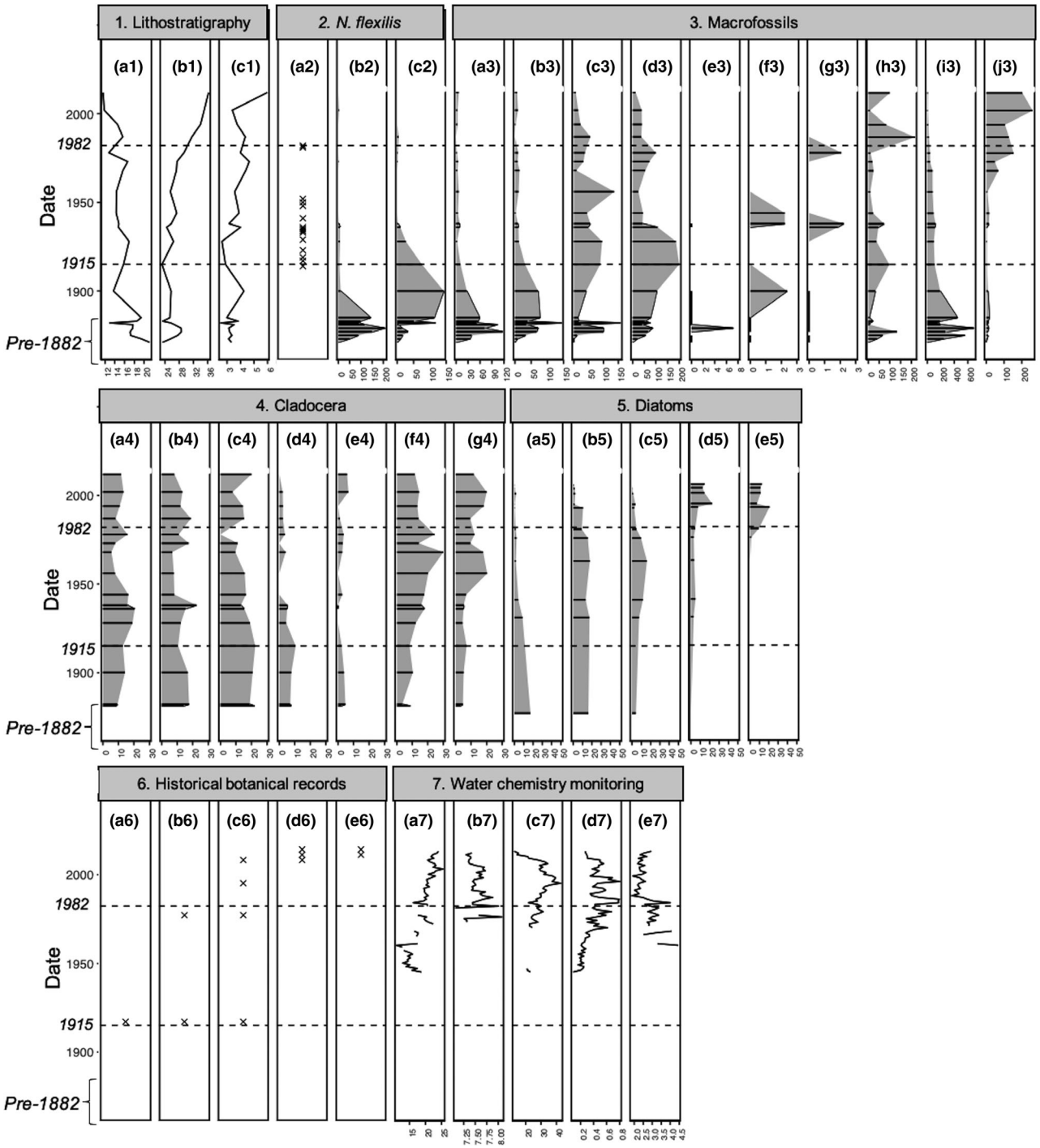


FIGURE 4 Summary stratigraph showing key downcore changes in ESTH9 and ESTH1/7, and associated historical data from Esthwaite Water. Dotted lines indicate timings of changes in likely *N. flexilis* abundance based on the analysis of Bishop et al. (2018). 1: Lithostratigraphy (ESTH9): (1a) % dry weight, (1b) % loss on ignition, (1c) % carbonate; 2: *N. flexilis* (historical records/ESTH9): (2a) historical records, (2b) seeds per 100 cm^3 wet sediment, (2c) leaf spines per 100 cm^3 wet sediment; 3: macrofossils per 100 cm^3 wet sediment (ESTH9): (3a) *Nitella* spp. oospores, (3b) *Chara* spp. Oospores, (3c) *Isoetes lacustris* megaspores, (3d) *Potamogeton* spp. fine-leaf fragments, (3e) *Potamogeton berchtoldii* agg. seeds, (3f) *Potamogeton perfoliatus* seeds, (3 g) *Potamogeton obtusifolius* seeds, (3 h) *Nymphaeaceae* trichlosclerids, (3i) *Trichoptera* spp., (3j) *Daphnia* spp. Ephippia; 4: cladocera % relative abundance (ESTH9): (4a) *Bosmina coregoni*, (4b) *Bosmina longirostris*, (4c) *Bosmina longispina*, (4d) *Camptocercus rectirostris*, (4e) *Acroperus harpae*, (4f) *Alona affinis*, (4 g) *Chydorus sphericus*; 5: diatom % relative abundance (ESTH1/ESTH7): (5a) *Cyclotella comensis*, (5b) *Achnanthes minutissima*, (5c) *Tabellaria flocculosa*, (5d) *Fragillaria crotonensis*, (5e) *Stephanodiscus hantzschii*; 6: historical botanical records: (6a) *Hydrilla verticallata*, (6b) *Potamogeton gramineus*, (6c) *Elodea canadensis*, (6d) *Elodea nuttallii*, (6e) *Lemna minor*; 7: water chemistry monitoring: (7a) alkalinity (mg $CaCO_3$ L^{-1}), (7b) pH, (7c) TP (mg/L), (7d) NO^{-}_3 (mg/L), (7e) Secchi depth (m).

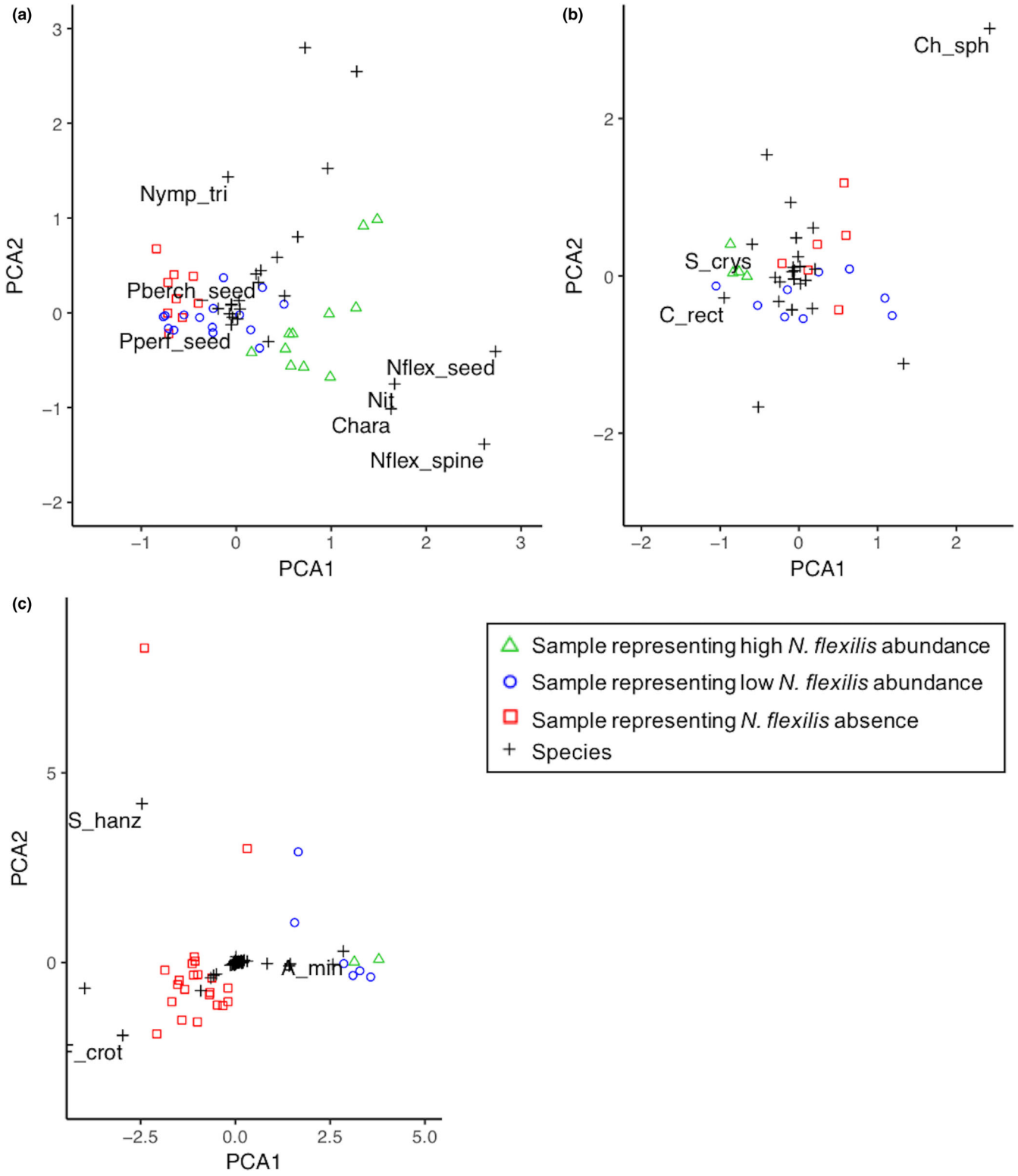


FIGURE 5 Ordination plots showing the first two axes of PCAs for (a) plant macrofossils, (b) cladocera, and (c) diatoms in samples from ESTH9 (macrofossils and cladocera) and ESTH1/7 (diatoms). Samples are grouped by likely *N. flexilis* abundance based on the analysis of Bishop et al. (2018). Key species only labelled. "Chara" = *Chara* spp. oospores; "Nflex_seed" = *N. flexilis* seeds; "Nflex_spine" = *N. flexilis* spines; "Nit" = *Nitella* spp. oospores; "Nymph_tri" = *Nymphaea* spp. trichloscereids; "Pberch_seed" = *Potamogeton berchtoldii* seeds; "Pperf_seed" = *Potamogeton perfoliatus* seeds; "C_rest" = *Camptocercus rectirostris*; "Ch_Sph" = *Chydorus sphericus*; "S_crys" = *Sida crystallina*; "A_min" = *Achnanthes minutissima* "F_crot" = *Fragillaria crotonensis*; "S_hanz" = *Stephanodiscus hantzschii*.

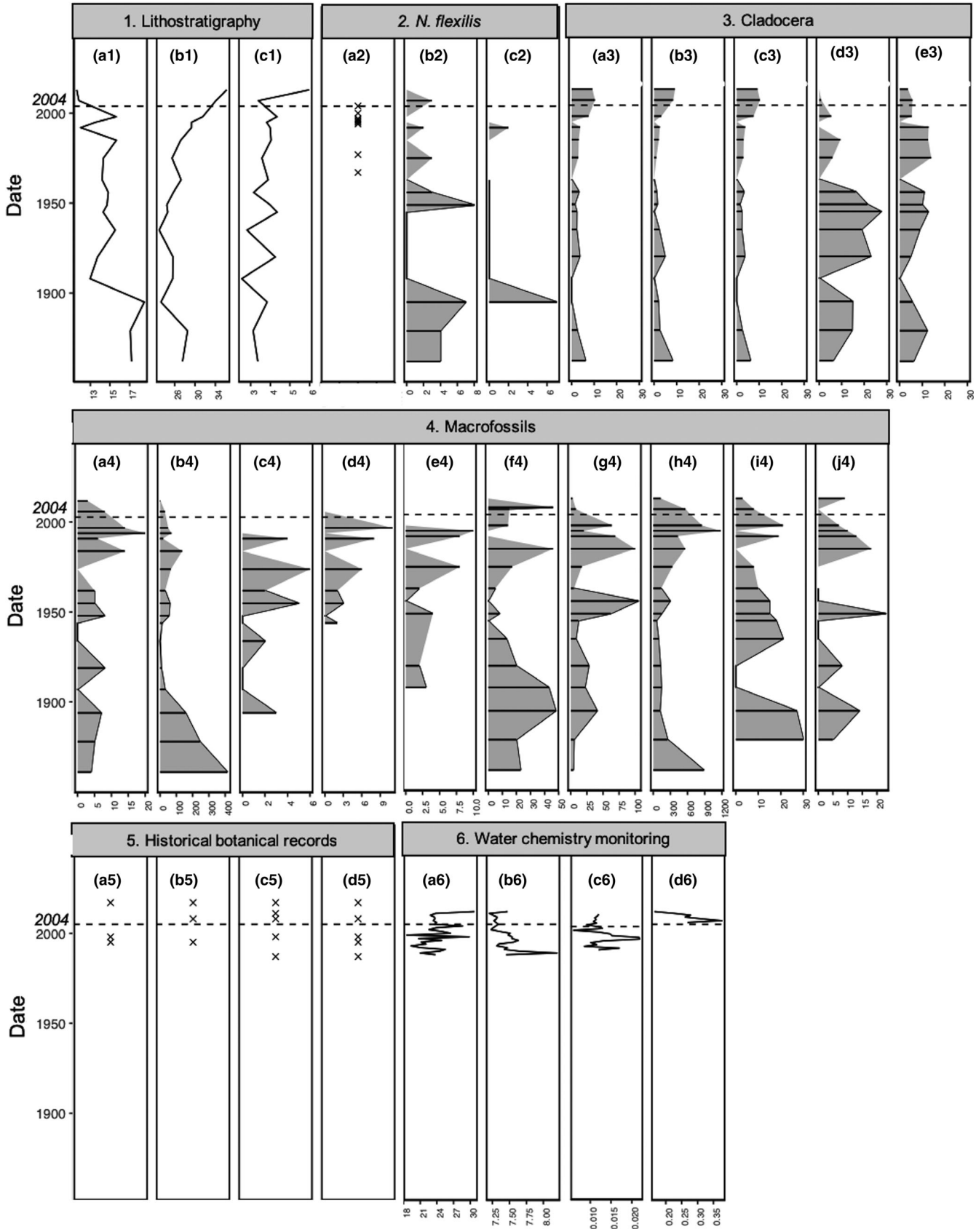

1. Lithostratigraphy
2. N. flexilis
3. Cladocera
4. Macrofossils
5. Historical botanical records
6. Water chemistry monitoring
Date
2004
2000
1950
1900
(a1)
(b1)
(c1)
(a2)
(b2)
(c2)
(a3)
(b3)
(c3)
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(c6)
(d6)

FIGURE 6 Summary stratigraph showing key downcore changes in CRAIB and associated historical data from Loch of Craiglush. Dotted lines indicate timings of changes in likely *N. flexilis* abundance based on the analysis of Bishop et al. (2018). 1: Lithostratigraphy: (1a) % dry weight, (1b) % loss on ignition, (1c) % carbonate; 2: *N. flexilis* (historical records/CRAIB): (2a) historical records, (2b) seeds per 100 cm^3 wet sediment, (2c) leaf spines per 100 cm^3 wet sediment; 3: cladocera % relative abundance (CRAIB): (3a) *Bosmina coregoni*, (3b) *Bosmina longirostris*, (3c) *Bosmina longispina*, (3d) *Chydorus piger*, (3e) *Chydorus sphericus*; 4: macrofossils per 100 cm^3 wet sediment (CRAIB): (4a) *Nitella* spp. oospores, (4b) *Potamogeton* spp. fine-leaf fragments, (4c) *Potamogeton perfoliatus* seeds, (4d) *Potamogeton berchtoldii* seeds, (4e) *Potamogeton gramineus* seeds, (4f) *Juncus bulbosus* seeds, (4g) *Isoetes lacustris* megaspores, (4h) *Sphagnum* spp., (4i) *Nuphar lutea* seed fragments, (4j) *Chydorid carapaces*; 5: historical botanical records: (5a) *Isoetes echinospera*, (5b) *Isoetes lacustris*, (5c) *Litorella uniflora*, (5d) *Lobelia dortmana*; 6: water chemistry monitoring: (6a) alkalinity (mg $CaCO_3$ L^{-1}), (6b) pH, (6c) TP (mg/L), (6d) NO^-_3 (mg/L).

fine-leaved *Potamogeton* spp. leaf fragments and increases in *Potamogeton perfoliatus* and *Potamogeton gramineus* seeds. This may reflect a change in *Potamogeton* species composition (specifically a shift from fine-leaved to broad-leaved species), but the numbers of *Potamogeton* seeds found in the core are too small to infer species-specific changes of abundance based on this evidence alone. Historical botanical records confirm the presence of both broad-leaved (*Potamogeton perfoliatus* and *Potamogeton gramineus*) and fine-leaved (*Potamogeton berchtoldii*) pondweeds as well as the *Isoetid* plants *Isoetes lacustris, Isoetes echinospera, Litorella uniflora*, and *Lobelia dortmana* throughout the period between 1986 (when records began) and 2016.

Before around 1985, the cladoceran remains are composed of a diverse variety of *Chydoridae,* including *Graptoloberis* spp., *Chydorus piger*, and *Alona guttata/rectangularis*. There is a notable shift from *Chydoridae* dominance to *Bosmina* spp. dominance after around 1985. This was accompanied by a simultaneous increase in numbers of *Chydorid* carapaces in the macrofossil record, but no other synchronous changes were observed for other macrofossils, including *N. flexilis,* and the causes of these changes in the zooplankton community are therefore beyond the scope of this study. Contemporary water chemistry monitoring of the inflow between 1987 and 2016 suggests that water entering Loch of Craiglush consistently had an annual mean pH of between 7 and 7.6 (except for 1988, when mean pH was 8.2), and that nutrient concentrations entering the lake from the inflow were low (<0.0025 mg/L TP and <0.5 mg/L NO^-_3).

4 | DISCUSSION

Similar to results of other paleoecological studies of *N. flexilis* (e.g., Gałka et al., 2012; Kupryjanowicz et al., 2018; Rasmussen & Anderson, 2005; Rūtiņa et al., 2012), seed preservation in the analysed sediments was good. Further, where data were available (e.g., the Dunkeld-Blairgowrie Lochs in the 1990s–2000s), the abundance of the plant inferred from macrofossil analysis broadly corresponded with the abundance of the plant observed historically. In the dated core ESTH9 and the top portion of CRAIB, sections of the cores in which *N. flexilis* seeds were absent aligned with the documented disappearances of *N. flexilis* within a reasonable margin of error. The exceptions are (1) a few seeds found in sediments dated from the 2000s in ESTH9, which could represent recent failed attempts to reintroduce *N. flexilis* to Esthwaite Water from seed; and (2) seeds found in sediments dating from around 2007 in CRAIB, which, when compared with the results of intensive SCUBA surveys, create an unexplained "false positive" effect similar to that found at Loch of Butterstone by Bishop et al. (2018). Despite these minor discrepancies, the evidence presented here strongly suggests that *N. flexilis* is ideally suited to paleoecological study at sites where historical macrophyte surveys are lacking.

4.1 | Baseline conditions and the decline of *N. flexilis* at Esthwaite Water

A timeline of *N. flexilis* decline and associated ecological events at Esthwaite Water can be found in Figure 7. Before around 1915, *N. flexilis* was relatively abundant at Esthwaite Water, and, according to both the macrofossil record and an early vegetation survey undertaken in 1914 (Pearsall, 1920), it co-occurred with a plant community typical of a mildly alkaline lake. In such lakes, shoreline communities are usually dominated by *Isoetes lacustris* and *Littorella uniflora,* with *N. flexilis* found alongside *Chara* spp., and *Nitella* spp. in deeper water. In these conditions, macrophytes like *Potamogeton perfoliatus* that use bicarbonate for photosynthesis are present, but because the energy required for active transport of bicarbonate requires higher photosynthetic rates, their abundance is likely limited by light availability, particularly towards the lower edge of the photic zone (Tracy et al., 2003). As an obligate carbon dioxide user, *N. flexilis* has a strong competitive advantage over bicarbonate-utilising macrophytes in the deeper waters where light levels are lower (Wingfield et al., 2006). Another example of this can be seen in Shoe Lake, Minnesota, where a drought event that reduced nutrient inflow into a nitrogen-limited system was associated with increased growth of all rooted macrophytes except for *N. flexilis* and

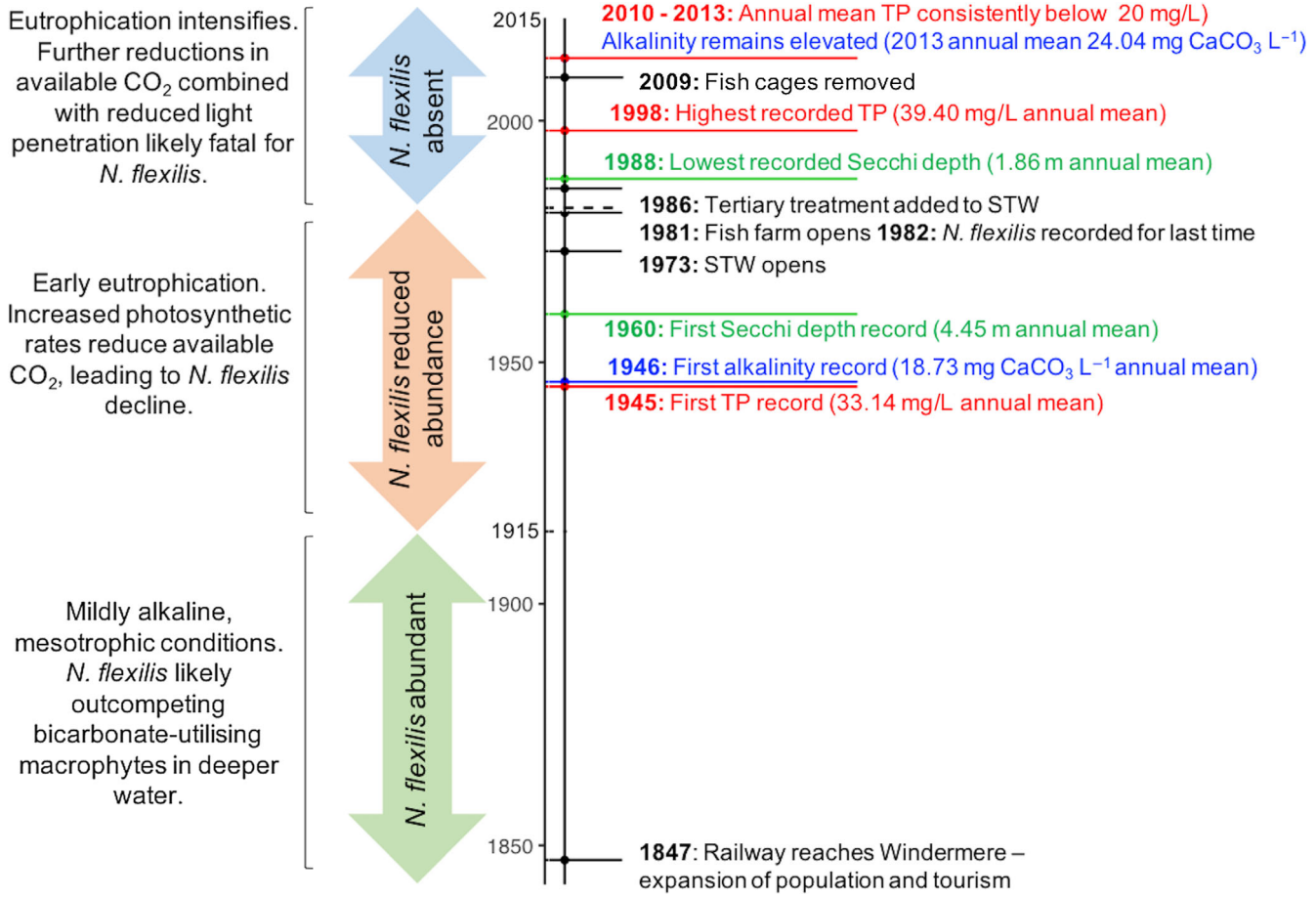


FIGURE 7 Timeline summarising *N. flexilis* decline and associated ecological changes at Esthwaite Water.

Myriophyllum verticallatum, whose abundances declined (Tracy et al., 2003). This was attributed to increased water clarity during a drought year, which favoured bicarbonate-using plants.

The eutrophication of Esthwaite Water in the 20th century has been well documented both by long-term monitoring and paleoecological studies (Dong et al., 2011, 2012; Maberly et al., 2011; Moorhouse et al., 2018). This study adds to the understanding of the ecological changes that have occurred at the lake and determines the potential eutrophication influence on *N. flexilis*. Between around 1915 and 1978, the ESTH9 core suggests a community shift towards vegetation more tolerant of eutrophic conditions with declines of *Characeae* and persistence of *Potamogeton perfoliatus* and *Potamogeton obtusifolius*. From around 1915, the oligotrophic-associated centric diatom *Cyclotella comensis* declined and *Fragilaria crotonensis* – a species typically seen in lakes during the early onset of eutrophication (e.g., Lotter, 1989, 1998) – appeared for the first time. Water chemistry monitoring confirms that Secchi depth decreased from around 4 m to 3 m during this period, which likely explains the replacement of shorter growing macrophytes like *Chara* spp. and *Nitella* spp. with canopy-forming *Potamogeton* spp. Similarly, declines in the clear-water associated cladoceran *Camptocercus rectirostris* and increases in *Alona affinis* and *Chydorus sphericus* are typical of declining water clarity and loss of vegetation cover (Davidson et al., 2010). Monitoring records also showed that nutrient concentrations in Esthwaite Water rose slowly but steadily. This reflects increased discharges from domestic sewerage and increased nutrient application to catchment livestock pastures, and strongly suggests eutrophication as a driver of change. The timing of the initial decline of *N. flexilis* from peak abundance after around 1915 implies that *N. flexilis* growth was negatively affected by this eutrophication. Reduced illumination of deeper depth zones in the lake likely reduced *N. flexilis* photosynthesis. In shallower waters where light availability was higher, increased growth of canopy-forming macrophytes may have led to more rapid consumption of available CO_2 (see Talling, 1976, 1985). This interplay between lake productivity, turbidity, and inorganic carbon speciation has been shown to have an important influence on macrophyte distribution in Danish lakes (Vestergaard & Sand-Jensen, 2000). It is likely that, at Esthwaite Water, bicarbonate-utilising plants had a competitive advantage over *N. flexilis* in the shallower depth zones as eutrophication occurred. It is possible that *E. canadensis* was a significant competitor of

N. flexilis at this time, although more evidence is needed to support this claim since there were no full macrophyte surveys at Esthwaite Water between 1917 and 1980 (Maberly et al., 2011) and *E. canadensis* tends not to leave macrofossil remains in UK lakes (Clarke et al., 2014).

In 1973 a sewage treatment plant was opened in the Esthwaite Water catchment, and fish cages were introduced in 1981 (Dong et al., 2012). Chemical and ecological changes in Esthwaite Water after around 1973 have been widely attributed to these anthropogenic sources of pollution (Bennion et al., 2000; Dong et al., 2011, 2012), and are further reflected in the ESTH9 macrofossil and Cladocera records. A shift to water-lily dominance, seen in ESTH9 after around 1978, has often been observed at sites undergoing eutrophication, with isoetids and elodeids gradually replaced by canopy-forming, emergent, and floating-leaved plants (e.g., Madgwick et al., 2011; Rasmussen & Anderson, 2005). The increase in *Daphnia* remains in ESTH9 after around 1968 also suggests phytoplankton development, as often evident in shallow lakes (Davidson et al., 2010). Dong et al. (2011) used the changing ratio of benthic to planktonic diatoms in ESTH7 to indicate nutrient enrichment from around 1976 onwards that particularly increased the dominance of *Stephanodiscus hantzschii* and *Fragilaria crotonensis*. It was not until this latter period that *N. flexilis,* whose abundance had been severely reduced during the earlier stages of eutrophication between around 1915 and 1978, finally disappeared. Monitoring records of pH, temperature, and alkalinity from 1993 confirm that HCO_{3-} was the dominant form of inorganic carbon in the lake during the *N. flexilis* growing season at this time (Maberly, 1996). It is likely that *N. flexilis* was unable to survive the combination of low CO_2 availability in the majority of the photic zone and low availability of light in the deeper waters away from bicarbonate-utilising species.

4.2 | Loch of Craiglush

Timings of *N. flexilis* loss at Loch of Craiglush and the events discussed below are summarised in Figure 8. Both the macrofossil record and monitoring records suggest that the abundance of *N. flexilis* at Loch of Craiglush has always been

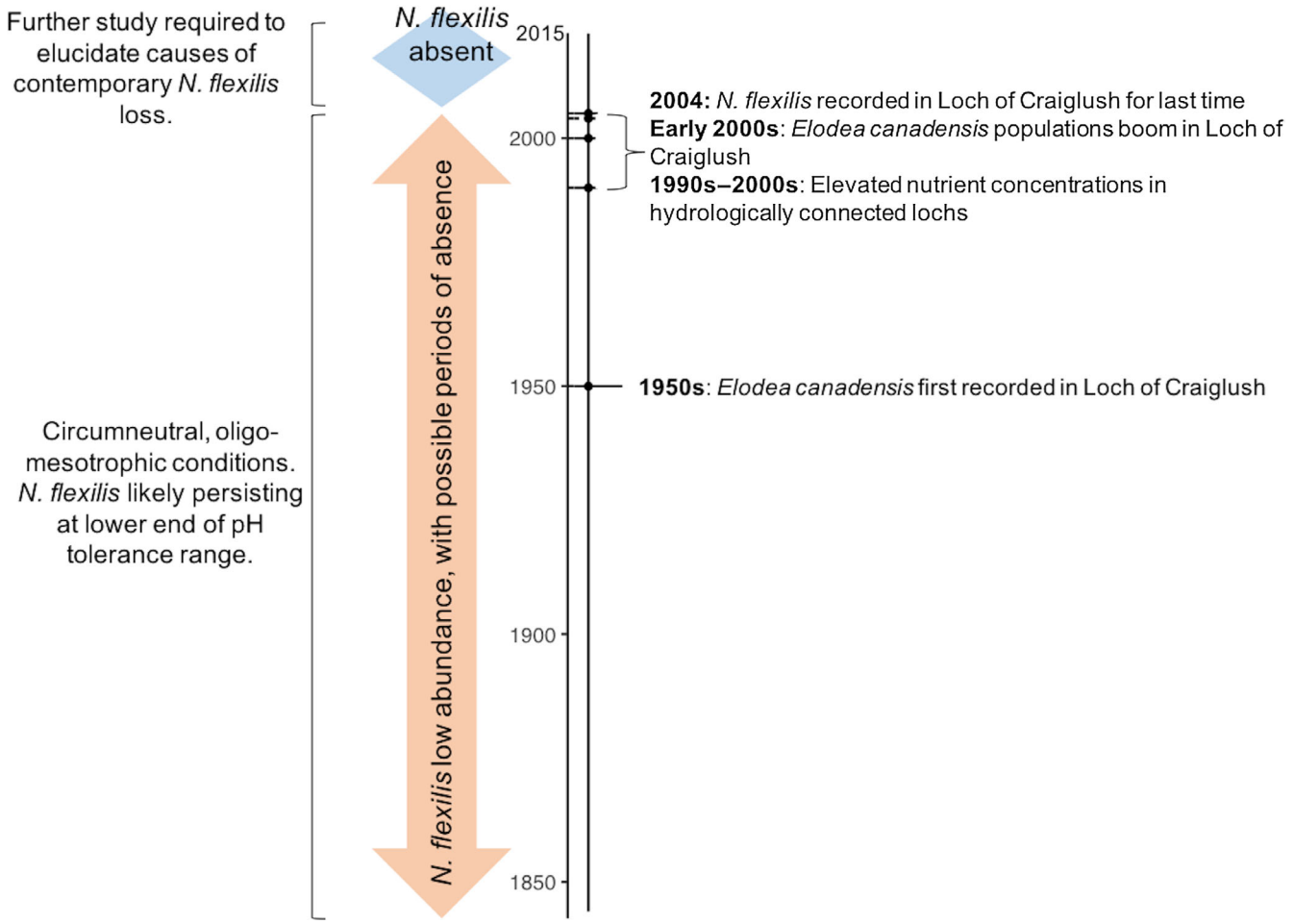


FIGURE 8 Timeline summarising *N. flexilis* decline and associated ecological changes at Esthwaite Water.

relatively low, and that the species grew alongside a plant community typical of relatively nutrient-poor, slightly acid conditions, including *Isoetes lacustris, Potamogeton* spp., *Nuphar lutea*, and *Nitella* spp. Remains of *Sphagnum* spp. and *Juncus bulbosus,* which typically indicate base-poor conditions, were present in relatively large quantities, while *Chara* spp., which prefer base-rich conditions (Duigan et al., 2006), were largely absent. The Cladoceran remains were dominated by macrophyte-associated taxa commonly found in acid/circumneutral lakes with low nutrient concentrations, such as *Graptoloberis* spp., *Chydorus piger*, and *Alona guttata/rectangularis* (Chen et al., 2010; Davidson et al., 2010). There were intervals during which *N. flexilis* was notably absent from the macrofossil record (e.g., around 1879–1920). It is possible that *N. flexilis* may have been present during this period, but that seeds did not disperse to the core site. Given the low tolerance of *N. flexilis* to acidic conditions it is also possible that these absences are linked to short-term fluctuations in pH, but there is no evidence to support this.

Extensive snorkel and SCUBA surveys were carried out at Loch of Craiglush between 1987 and 2007 (Benthic Solutions, 2007; Dale et al., 1997; James & Barclay, 1996; Murphy, 2007 (unpublished)), and indicate that *N. flexilis* disappeared from the lake in 2004. Despite this, seeds were found in sediments towards the top of CRAIB dating from around 2007. This is likely either a "false positive," as similarly identified by Bishop et al. (2018), or a result of standard errors associated with radiometric dating, which, at this point in the core, are ±3 years. Water chemistry monitoring at the inflow to Loch of Craiglush suggests that there was a small, slow downward trend in pH from around 7.5 to 7 over the 17-year period leading up to the disappearance of *N. flexilis* in around 2004. Since 2004, pH at this inflow has remained relatively stable and has only fallen below pH 7 for short periods. The minimum recorded pH was 6.5 in November 2005. Although this is towards the lower end of the pH tolerance range of *N. flexilis,* the plant reproduced at pH 6.5 in the laboratory (Titus & Hoover, 1991). Nevertheless, at these pH levels, the number of reproductive structures per *N. flexilis* plant is reduced and recruitment success is likely to be affected (Wingfield et al., 2004). It is unclear how long low pH levels need to be maintained for plant reproductive fitness to be affected or whether these records from the inflow reflect the pH in the lake itself, so the impact of this decline in pH on *N. flexilis* is difficult to elucidate. Importantly, there is no evidence in the fossil record to suggest any major ecological changes in the lake itself at the time of *N. flexilis* disappearance in around 2004. There are also no indications in monitoring data or from the sediment record for significant nutrient inputs or for ecological responses to eutrophication that may have affected water clarity or CO_2 availability to a large enough degree to remove *N. flexilis*.

The invasive species *Elodea canadensis* and *Elodea nutallii* were first recorded in the Dunkeld-Blairgowrie Lochs in the 1950s (National Biodiversity Network, 2014). Their populations have apparently boomed in all three of the upper lochs (Loch of Craiglush, Loch of Butterstone, and Loch of Lowes) since the mid–late 2000s, coinciding with the loss of *N. flexilis* (Benthic Solutions, 2007). However, care must be taken not to equate correlation with causation. In Loch of Butterstone and Loch of Lowes, the timing of the arrival of *Elodea* spp. in around 1950 coincided with mild eutrophication (evident from around 1940 [Bennion et al., 2010]). Further, the expansion of *Elodea* spp. in the 2000s coincided with peak nutrient concentrations. Since *Elodea* spp. thrive where nutrient concentrations are elevated (Eugelink, 1998; Melzer, 1999), it cannot be assumed that competition from *Elodea* spp. and eutrophication were independent actors on *N. flexilis* at Loch of Butterstone and Loch of Lowes. Although Loch of Craiglush was apparently relatively unaffected by eutrophication, increasing *Elodea* spp. populations in the neighbouring lochs would have increased the likelihood of *Elodea* spp. propagules reaching Loch of Craiglush (see Lockwood, 2006). However, since it is not possible to fully elucidate the relative timings and dynamics of *N. flexilis* decline and *Elodea* spp. expansion at Loch of Craiglush from either the paleoecological record or contemporary data, it cannot be concluded that *Elodea* spp. directly led to *N. flexilis* decline.

In the late 1990s and early 2000s, monitoring records from the Dunkeld-Blairgowrie lochs indicated elevated nutrient concentrations. These observations were based on data collected at the outflows of Loch of Lowes and Loch of Butterstone, both of which are downstream of Loch of Craiglush. Additionally, paleolimnological evidence from both Loch of Butterstone and Loch of Lowes clearly indicate eutrophication (Bennion et al., 2004, 2010). Prior to this study, nutrient enrichment seemed the most likely explanation for the decline of *N. flexilis* at Loch of Criaglush. The data presented here do not support this theory, nor do they provide conclusive evidence that acidification or competition from aquatic invasive plants led to the decline of *N. flexilis*. What importantly this study does show is that *N. flexilis* abundance has likely always been relatively low at Loch of Craiglush, possibly due to base-poor conditions. Although the cause of *N. flexilis* decline at Loch of Craiglush remains unclear, this study highlights the need to treat Loch of Craiglush as a unique ecosystem within the Dunkeld-Blairgowrie chain. Further research into the ecological interactions between the relatively base-poor Loch of Craiglush and the hydrologically connected Loch of Lowes and Loch of Butterstone is needed to better determine the causes of *N. flexilis* decline at Loch of Craiglush.

5 | CONCLUSION

Prior to this study, commonly cited causes for the decline of *N. flexilis* were derived from existing knowledge on the plant's ecological preferences and not from long-term data tracking real-time change. Analysis of the timing of *N. flexilis* decline at Esthwaite Water has revealed a potential mechanism for the impact of eutrophication on the species at the site. As an obligate CO_2 user, *N. flexilis* is vulnerable to changes in inorganic carbon speciation that result from increased photosynthesis following nutrient enrichment (Wingfield et al., 2006). Thus, the early stages of eutrophication may have drastically reduced the abundance of *N. flexilis* at the site some 70 years before the eventual disappearance of the plant. At Loch of Craiglush the mechanism for *N. flexilis* loss remains uncertain, but paleoecological data suggest that *N. flexilis* abundance in the past was likely lower than previously believed. This may have been linked to a lower pH, known to negatively affect the ability of the plant to reproduce (Titus & Hoover, 1991).

The historical abundance and timing of decline of *N. flexilis* are relevant for future conservation efforts in the UK. At Esthwaite Water, the addition of tertiary treatment to the sewage treatment works and the removal of fish cages have led to measured recoveries of total phosphorus, chlorophyll-a and Secchi depth since 2000 (Dong et al., 2012). With water quality now returning to pre-1980 levels, attempts have been made to reintroduce *N. flexilis* at the site from seed (JNCC, 2012). However, the paleoecological evidence presented here shows the decline of *N. flexilis* to be much earlier than indicated by historical records, with the plant historically associated with clearer, less nutrient rich, and less alkaline waters than currently exist. Continued increases in alkalinity at Esthwaite Water reflect the fact that plant biomass in the lake remains high, suggesting that further recovery is necessary before the species can be successfully reintroduced. At Loch of Craiglush, before restoration or reintroduction attempts are made, it is imperative that further research be conducted to assess the importance of competition from *Elodea* spp. and the impacts of close hydrological connections to neighbouring sites that are undergoing eutrophication. By extending existing knowledge on the history and ecology of *N. flexilis* at these important conservation sites in the UK, this study highlights the huge value of paleoecology for driving evidence-based conservation practices by providing insights that could not be gained from historical records alone.

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