Natural England Commissioned Report NECR074

Conservation of ribbon-leaved water-plantain

Current status, the species recovery programme and future outlook

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Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

Ribbon-leaved water-plantain (Alisma gramineum) is protected under Schedule 8 of the Wildlife and Countryside Act (1981), considered Critically Endangered in Britain following IUCN criteria, and is a Biodiversity Action Plan priority species. As such it is subject to a Species Recovery Programme (SRP), a partnership project aimed at understanding its ecology and carrying out conservation action. The ultimate aim of the SRP is to safeguard current populations but also to increase the size and resilience of its populations in England so that it is no longer Critically Endangered.

This report was commissioned by Natural England in June 2010 to document the work undertaken within the SRP over the last two years and put it into context of the SRP work carried out to date. It includes:

- A review of the past and current distribution of ribbon-leaved water-plantain in England.
- A report on surveys carried out in the Spalding area in 2009 and 2010.
- Documentation of the 2009 reintroduction attempt at Baston Fen SSSI and a review of previous introductions.
- Conclusions about its status in England, and recommendation for future management and conservation work.

This report is an important summary of what we have learnt about the conservation of ribbon-leaved water-plantain through the work of the SRP, and will be used to inform future work to conserve this interesting but vulnerable species.

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Further information
This report can be downloaded from the Natural England website: **www.naturalengland.org.uk**. For information on Natural England publications contact the Natural England Enquiry Service on 0845 600 3078 or e-mail **enquiries@naturalengland.org.uk**.

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Summary

This report brings together past information on ribbon-leaved water-plantain (*Alisma gramineum*) in the UK; it is presented in three main sections. The past, present and future of the four natural populations are discussed, and the results of surveys in the Spalding area in 2009 and 2010 are presented. An account is then given of all attempts to introduce or re-introduce ribbon-leaved water-plantain to sites in the UK. The final section discusses the current status of ribbon-leaved water-plantain in Britain, management and intervention opportunities, need for survey, monitoring and further research. A list of specimens in UK herbaria, the reintroduction protocol employed at Baston Fen and a report on DNA bar-coding are presented as Appendices.

Ribbon-leaved water-plantain has been recorded at Westwood Great Pool since 1920. In the early 20th century, large emergent annual populations were recorded, but such populations have not been recorded for many years. Conversely, a large, permanently submerged, perennial population was found in 2004. Ribbon-leaved water-plantain survives at the site but its population dynamics have dramatically changed; the only obvious causes of this change are stabilisation of water levels and eutrophication. Opportunities for intervention to restore the historic population dynamics of ribbon-leaved water-plantain at Westwood Great Pool are discussed and a monitoring protocol proposed that would both enable quantification of population trends and enable measurement against targets.

Ribbon-leaved water-plantain has been recorded from a total of four water bodies in the Spalding area since 1955. However, toward the end of the 20th century it was only recorded from the Blue Gowt and there, only sporadically. Surveys in 2009 and 2010 located submerged flowering plants in the Counter to Vernatt's Drain complex, indicating the survival of a persistent population in the area. These surveys also showed that the water bodies in the area support a remarkably rich range of relict fenland plant species and are of high conservation value, independent of the conservation requirements of ribbon-leaved water-plantain. The population of ribbon-leaved water-plantain in the Counter to Vernatt's Drain complex is small and at best stable, while that in the Blue Gowt apparently only grows and sets seed in the years immediately following de-silting. The possibility of modifying management to increase the population is discussed, together with requirements for monitoring the condition of the metapopulation in the Spalding area.

Ribbon-leaved water-plantain occurred for short periods at Vermuyden's Drain in the Ouse washes, Cambridgeshire and Langmere in the Brecks in Norfolk. It is possible that it persists at the former site or other water bodies in the Ouse Washes but the submerged vegetation of these has never been surveyed. It is unlikely that it persists at Langmere or other meres in the Brecks.

There have been three planned attempts to introduce ribbon-leaved water-plantain in the UK; to Kingfishers Bridge NR in Cambridgeshire and twice to Baston Fen NR in Lincolnshire. In addition, there is a report that plants were introduced to Hauxley NR in Northumberland. There is no evidence that ribbon-leaved water-plantain has persisted at any of these sites, although there has not been enough time to adequately assess the success or failure of the second attempt at Baston Fen. The discovery of a surviving ribbon-leaved water-plantain metapopulation in the Spalding area has weakened the justification for reintroduction and no further attempts are planned at present.

Small and stable or declining populations of ribbon-leaved water-plantain survive at Westwood Great Pool and two water bodies in the Spalding area, but it cannot be considered to be in Favourable Condition in the UK. There are opportunities for intervention at both these sites to restore and enhance the populations of ribbon-leaved water-plantain, including control of eutrophication and drawing down water levels at the former and modifying management at the latter. All populations must be monitored against realistic targets to quantify trends. Surveys are needed to search for submerged populations in the Ouse Washes. There are few outstanding research requirements, apart from a need to clarify aspects of population dynamics which may be fundamental to improving the condition of populations in the UK.

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The (re)introduction at Baston Fen would not have happened without Tim Pankhurst of Plantlife who provided the original seedlings, Pete Michna and Julie Clos of Cambridge Botanic Gardens who grew them, potted them and generally cared for them, Stan Pywell and Nick Morris of Welland and Deepings Internal Drainage Board who created suitable conditions for planting, and Rosie Glossop (Rosie Blackman at the time of the work) and Holly Tucker of the Environment Agency (EA) who planted most of the seedlings out. Chris Extence, Nick Riches and Gill Walters of the Environment Agency and Kate Fagan and Danny Newman of Natural England generally facilitated the project in various ways. Holly Tucker also collected and compiled the information on management of drains and the River Glen.

Administration, coordination and logistics for the various elements of the project have been dealt with by Gen Madgwick (NE), Stewart Clarke (NE) and Richard Chadd (EA), all have been extremely efficient and capable, and a pleasure to work with.

The distribution map presented as Figure 1 was generated using DMAP software developed by Alan Morton.

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1 Introduction

1.1 Since the early 1990s, ribbon-leaved water-plantain has been the subject of research and conservation action under the Natural England (formerly English Nature) Species Recovery Programme (SRP) (Wells *et al*. 1992, 1993, 1994; Wells 1998, 2000, Pankhurst and Lansdown 2004). Palmer (2006) reviewed the work carried out under the SRP and the UK Biodiversity Action Plan (BAP) process.

1.2 This report has been produced under the NE SRP. It presents a review of the history and status of ribbon-leaved water-plantain at each of the four sites where it has occurred naturally in the UK, this information is used to derive a prognosis for the species at each site, review potential for management intervention to enhance the size and or stability of population and means of establishment of condition targets, together with a proposed protocol for condition monitoring. It also presents the results of surveys of water bodies in the Spalding area in 2009 and 2010, as well as brief surveys of Vermuyden's Drain and Langmere in 2009 and Hauxley NR in 2010 both in terms of ribbon-leaved water-plantain and the nature and conservation value of other vegetation. This is followed by a description of available information on attempts to introduce ribbon-leaved water-plantain to three sites in the UK.

1.3 This information is then reviewed to summarise the current status and the prognosis for ribbon-leaved water-plantain in the UK, potential for management and other intervention to enhance the size and stability of populations and proposals for monitoring and research. A list of the specimens located to-date in UK herbaria, the reintroduction protocol employed at Baston Fen and a report on DNA bar-coding are presented as Appendices.

1.4 Ribbon-leaved water-plantain appears to be uncommon or rare throughout most of its range and may be subject to massive population fluctuations. However it is possible the perception of rarity may be exaggerated by under-recording, as it has recently been shown to be quite abundant on the Rhine floodplain in Germany (K. Van de Weyer pers comm.), the Netherlands (J. Bruinsma pers comm.), Hungary (Z. Hroudová pers. comm.) and Slovakia (Király, G. pers. comm.). The forthcoming IUCN Red List of European Aquatic Plants is likely to class it as Least Concern (analysis ongoing by the author), while it is considered Near Threatened in the Mediterranean (IUCN in preparation). In Britain, it is protected under Schedule 8 of the Wildlife and Countryside Act (1981), considered Critically Endangered and the subject of a Biodiversity Action Plan (BAP).

1.5 Two former subspecies of ribbon-leaved water-plantain are currently recognised as separate species (although they have, at times been treated as varieties or subsumed within *A. gramineum*): *A. wahlenbergii* is usually a very delicate plant, with the leaves 0.1-0.3 cm wide and up to 45 cm long, overtopping the inflorescence in submerged plants and of ± equal length in emergent plants (Björkqvist 1967); all other populations are assigned to *A. gramineum*, including all British plants. Material from Langmere in Norfolk was identified as *A. wahlenbergii*, but the voucher specimen has been lost.

1.6 Ribbon-leaved water-plantain appears to be a nitrophile, occurring in nutrient-rich drains and ditches, as well as eutrophic lakes, ephemeral pools on large eutrophic lowland rivers and even in seasonally inundated arable fields (Lansdown 2011). This is contrary to the statement by Wells *et al* (1993) that ribbon-leaved water-plantain "only occurs in open habitats and favours muddy/silty lakes, backwaters and ditches *not subjected to high levels of eutrophication*" (my emphasis).

1.7 Ribbon-leaved water-plantain appears to have a number of relatively discrete survival strategies (Lansdown 2011). It can survive as potentially long-lived deep-submerged perennial plants which flower and set seed under water. These perennial populations may be stable and less dynamic, and are extremely vulnerable to localised catastrophic events. It can also survive as predominantly annual, shallow water or temporarily terrestrial plants which flower and set seed out of the water. These populations usually function as dynamic metapopulations and are relatively robust, able to tolerate localised and small-scale events through propagules-flow from other populations within a metapopulation, but vulnerable to large-scale habitat modification. It will also occur as a combination of these two forms, such as at Westwood Great Pool. The relationship between the two growth forms at such sites is poorly understood; yet it may be critical for the conservation of the species in the UK.

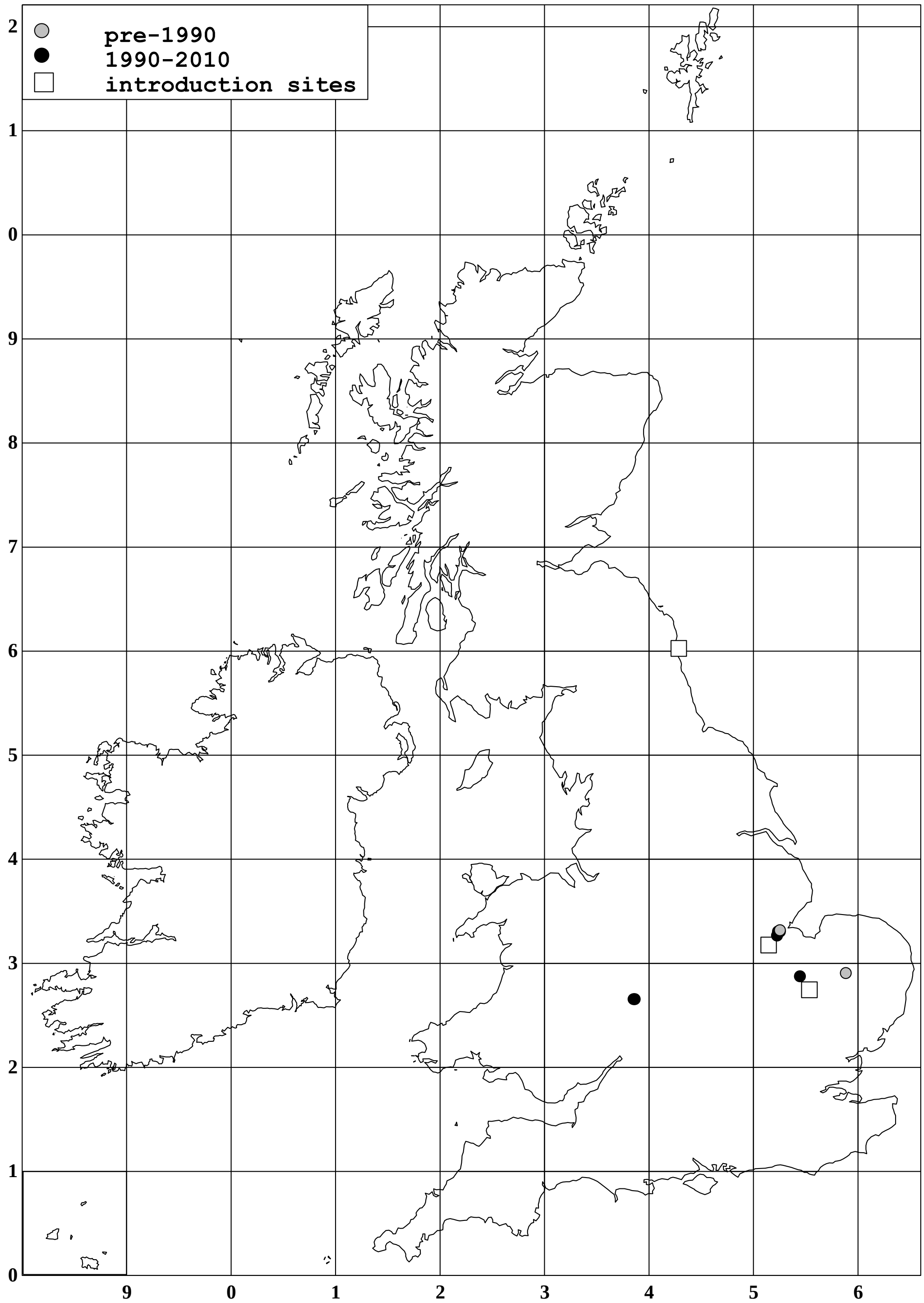

Figure 1 The distribution of records of ribbon-leaved water-plantain in the UK

1.8 Ribbon-leaved water-plantain has been recorded from a total of seven sites in Britain (Figure 1), two involve intentional introductions under the Natural England Species Recovery Programme, while one at Hauxley N.R. in Northumberland in 1987 is presumed to have been planted (Palmer 2006). Of the other sites, it has been recorded more or less continuously at Westwood Great Pool in Droitwich since 1920, intermittently in drains and the River Glen near Spalding in Lincolnshire and for a few years each at Langmere in Norfolk and the Ouse Washes in Cambridgeshire. It was planted in Baston Fen Nature Reserve (NR) in Lincolnshire in 1996 and Kingfisher Bridge (NR) in 1998 but did not persist at either site.

1.9 For the purposes of this document the following definitions are employed:

- Site – a named area, with a defined boundary, supporting one or more populations of ribbon-leaved water-plantain;
- Sub-population – a discrete group of ribbon-leaved water-plantain plants separated from other groups by an area of habitat-type similar to that supporting ribbon-leaved water-plantain;
- Population – a discrete group of ribbon-leaved water-plantain plants separated from other groups by an area of a habitat-type different to that supporting ribbon-leaved water-plantain;
- Metapopulation – a group of ribbon-leaved water-plantain populations connected by exchange of genetic material; and
- A dynamic metapopulation is one in which extinction and establishment of populations is normal and in which few, if any, populations are permanent.

1.10 In this document, scientific names follow Stace (2010) and common names follow the list of the Botanical Society of the British Isles (www.bsbi.org.uk/ resources.html) for vascular plants, Stewart and Church (1992) for charophytes and Hill *et al*. (2008) for bryophytes.

1.11 The names of water bodies are notoriously unreliable and often confusing, for example both the Ouse Washes and the Spalding areas have large ditches called the Counter Drain, named because they run alongside a large river. The names employed for water bodies in the Spalding area are those used by Welland and Deepings IDB, those relating to the Ouse Washes are used by the RSPB.

1.12 The term monitoring is used here to mean recording against a target, which complies with the basis for Condition Assessment as employed by the statutory agencies. For this to be effective there must be a baseline that has been used to define targets, against which replicable data-sets can be collected.

1.13 Acronyms employed are listed below. The acronyms of herbaria used in the text follow Index Herbariorum (online, hosted by the New York Botanical Garden).

BAP - Biodiversity Action Plan
BSBI - Botanical Society of the British Isles
BM - British Museum (Natural History) herbarium
CGE - University of Cambridge herbarium
EA - Environment Agency
IDB - Internal Drainage Board
K - Royal Botanic Gardens, Kew herbarium
LWT - Lincolnshire Wildlife Trust
MSB - Millennium Seed Bank
NE - Natural England
NNR - National Nature Reserve
NR - Nature Reserve
NWH - Norfolk Museums and Archaeology Service herbarium
NWT - Norfolk Wildlife Trust
RNG - University of Reading herbarium
RSPB - Royal Society for the Protection of Birds
SRP - Species Recovery Plan
SSSI - Site of Special Scientific Interest
STW - Sewage Treatment Works
WTNC - Worcestershire Trust for Nature Conservation

2 Westwood Great Pool, Droitwich

The history of Westwood Great Pool

2.1 Westwood Great Pool is a large artificial lake, totalling approximately 24 ha. of open water. It was created in about 1620 by Sir John Parkington, by construction of an earth dam approximately 8m high and 200m long across a small valley (WTNC 1987). A study in the 1990s states that "The only Superficial Deposit beneath the site is spoil resulting from the excavation of Westwood Great Pool into the underlying solid marl and mudstone" (Aspinwall and Co. 1995), suggesting that some excavation took place. However, according to (Aspinwall and Co. 1995) the lake is not linked to groundwater deriving all its water from surface run-off and drainage from fields, with a catchment of about 84 ha. It is almost certain that there was a marsh in the valley before construction of the dam (Jones *et al*. *in litt*.).

2.2 Information on the pool is, at best, anecdotal and it is difficult to establish a good account of its nature over time. It seems likely that water levels in the pool always fluctuated, apparently including occasions when the dam was intentionally breached both by the owner and by members of the public, in response to various disputes. Even into the 20th century, there were apparently significant fluctuations in water level, for example "in some years the pool is quite dry and in others there is 2ft of water up to the edge" and "there is an annual c5-10m marginal zone but one in five years it is flooded all summer" (R.C.L. Burgess 1955). It is likely that occasional emptying of the pool would have benefited the rare plant species known to occur at the site, as all (except *Chara aspera*) are associated more with bare mud and the drawdown areas of ponds than with permanent water. However a report that "In 1962 the pool was drained and took 3 years to refill" (WTNC 1987) suggests that even into the 20th century draining the pool was no light matter. It is equally clear that the pool has often had high water levels, with comments such as "In recent years the water level has been raised above previous normal levels flooding areas of shallow water.." and "the deep water also drowned extensive areas of the reed bed and marsh" (WTNC 1987). The presence of a marginal drawdown zone of at least a few metres wide is clear from images taken by J.J. Day in 1989 in the Natural England (NE) Worcester files. The variation in levels will have been exacerbated by leaks from the dam, reported up to 1988 when they are said to have been repaired (Walker 1988 *in litt*. in NE Worcester files, P. Holmes 1988 *in litt*. in NE Worcester files) although the leaks were again reported in 1997 (Wells 1998).

2.3 It has been suggested (C. Newbold *in litt*. 1985) that there have been "algal problems" in the lake since the 1960s. In October 1993, a sample analysed for the Environment Agency showed total phosphorous concentration of 105µg/$^{-1}$ which falls just within the hyper-eutrophic range on the OECD classification. Cyanobacteria recorded from the site include *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. There is now apparently a "bloom" of cyanobacteria (blue-green "algae") each spring or summer (Lansdown 2011), resulting in a collapse in dissolved oxygen which kills those parts of vascular plants (and possibly charophytes) in the water column.

2.4 A major change in the management of the lake apparently occurred in 1962 when a proposal was submitted to drain the lake and restock it with trout. According to C. Newbold (1985 *in litt*. in NE Worcester files), trout were apparently introduced in the 1960s (although this cannot be confirmed). At the same time, the observation was made that "fisheries people [were] netting the lake and removing all the reasonable sized coarse fish and taking them to restock the waters in the area" (E. Copeland Watts 1962 *in litt*. in NE Worcester files).

2.5 Throughout most of the last century, landowners have wanted to use the site for recreation and, at times, make a profit from recreation at the pool. Various reports in the NE files review proposals for increased recreational use, as well as an occasion when public windsurfing use was considered to breach the permission and control was apparently imposed on the owner at the time. Linked to this was a report of a decline in the avifauna and a number of bird deaths blamed on boat activity (P. Holmes 1994 *in litt*. in NE Worcester files). Currently there is low intensity use of the lake for water-sports.

Ribbon-leaved water-plantain and conservation at Westwood Great Pool

2.6 There are records of aquatic or wetland plants, including needle spike-rush (*Eleocharis acicularis*) and mudwort (*Limosella aquatica*) from Westwood Great Pool prior to the first record of ribbon-leaved water-plantain (Amphlett and Rea 1909). In the same publication, narrow-leaved water-plantain (*Alisma lanceolatum*) was sufficiently uncommon to merit a list of sites from which it had been recorded, although water-plantain (*A. plantago-aquatica*) was not. Narrow-leaved water-plantain is not listed as having been recorded at Westwood (even though ribbon-leaved water-plantain was initially recorded as this species). Ribbon-leaved water-plantain was described new to science in 1811 and it is of note that it was not recognised at Westwood Great Pool for more than 100 years. It must therefore be recognised that ribbon-leaved water-plantain may not have occurred at Westwood Great Pool before 1920.

2.7 Ribbon-leaved water-plantain was probably first found at Westwood Great Pool in 1920 and 1930 but recorded as follows "*Alisma lanceolatum* (the narrow-leaved water-plantain) is here the dominant species and flourishes almost to the exclusion of the common form" (Anon 1924 and 1932 in Lousley 1955). Lousley (1955) notes that the 1920 record is repeated by Rea in 1925 who subsequently recorded it correctly as *Alisma plantago-aquatica* var. *graminifolium* (which is a synonym of *A. gramineum*). Lousley goes on to say that R.C.L. Burges, who first distributed material through the Botanical Exchange Club, had plants of ribbon-leaved water-plantain from the site from 1939. In 1948, Burges recorded it again at the site, as follows: "muddy edge of Westwood Park pool, Droitwich in several inches of water [..], all of the plants found in the pool appear to belong to this form although some of the leaf shapes approach f. *lanceolatum*" (specimen in BM) and in 1955, he reported "well over a hundred plants scattered over the muddy edge of the pool along the north west margin" (R.C.L. Burges 1955 *in litt*. in NE Worcester files). It is clear that Lousley then visited the pool and collected a specimen which is dated 1960 (BM).

2.8 There are four specimens in the Royal Botanic Garden herbarium at Kew (those collected prior to 1967 confirmed by Ingmar Björkqvist in 1967), one collected in 1948 by R.C.L. Burgess (as f. *graminifolium*), one collected in 1953 by C.C. Townsend and another collected in 1957 by N.Y. Sandwith (as f. *arcuatum*). The fourth was collected in 1991 by T.E. Wells as a voucher for the Millennium Seed Bank (MSB) Project, although NE files in Worcester suggest that seed was submitted to the MSB in 1994. There are three specimens in the herbarium of the British Museum (Natural History), one collected by R.C.L. Burges in 1948, one by Lousely in 1960 and an undated specimen collected by D.P. Young (No. 4889), who lived from 1917-1972. All of these specimens are of terrestrial or emergent plants.

2.9 Until recently, all plants of ribbon-leaved water-plantain found at Westwood Great Pool were terrestrial or emergent and this was considered the only growth form produced at the site, to the extent that when Lousley (1957) saw deep-submerged plants in Lincolnshire, he commented that it was "the antithesis of the form previously known from shallow water on the margin of an artificial lake in Worcestershire". In 2000 and 2004, based on experience gained from study of populations in the Netherlands, T.J. Pankhurst surveyed the standing water of Westwood Great Pool and found more than 250 and 200 submerged plants respectively, many flowering on the latter occasion. It seems that most plants were initially recorded in more or less the same area, although descriptions differ, for example "The part where I have found *Alisma gramineum* growing was approximately in the NE corner but actually a little to the W of the corner itself and so on the N. shore" (F. Fincher in litt. to Dr. T. Prichard, 2 November 1962). However, in the early 1980s, plants were mainly along the southern margin (J.J. Day rare plant surveys).

Table 1 Timeline of information relevant to ribbon-leaved water-plantain at Westwood Great Pool

Year	No. plants	Notes
1620		the "Great Pool" created (WTNC 1987)
1920	dominant	no quantitative data (Anon 1924 in Lousley 1955)
1930	present	no quantitative data (Anon 1932 in Lousley 1955)
1932	present	no quantitative data (Anon 1932 in Lousley 1955)
1939	present	no quantitative data (R.C.L. Burges 1955 *in litt*. in NE Worcester files)
1948	present	no quantitative data (F. Gibbons), specimens R.C.L. Burges (K, BM)
1950	present	no quantitative data (F.M. Day)
1953	present	specimen by C.C. Townsend (K)
1955	>100	in some years the pool is quite dry and in others "there is 2ft of water up to the edge" (R.C.L. Burges 1955 *in litt*. in NE Worcester files)
1957	present	specimen by N.Y. Sandwith (K)
1960		specimen in BM, Lousley; (approximate date) algal problems noted
1962		pool drained and took three years to refill; (approximate date) trout introduced
1970	20+	P. Wilson
1980-84	2-8	(NE Worcester files)
pre-1984		lake entirely drained and piscicide applied prior to stocking (G. Walters pers. comm.)
1984		between February and June, >5,000 rainbow trout introduced (G. Walters pers. comm.)
1985	44	J.J. Day
1987		"recently the water level has been raised, flooding areas of shallow water and drowning extensive areas of the reed bed and marsh" (WTNC)
1988	4	leaks in dam walls repaired; levels "lowest since drought year of 1976" however later described as high due to wet summer
1989	59	photograph of wide marginal zone with diverse range of annuals; ITE & J.J. Day
1991	>250	ITE
1992	13	ITE
1993	21	water sample total phosphorous concentration of 105µg/$^{-1}$; ITE
1994		decline in avifauna and bird deaths from boats
1995	0	ITE
1996	0	two bays scraped, fenced and boom installed; ITE
1997	2	dam described as leaking ; T.E. Wells

Table continued...

Year	No. plants	Notes
1998	1	T.E. Wells
1999	0	T.E. Wells
2000	<250	all plants submerged, Pankhurst and Lansdown (2004); however none of the plants were flowering and so their identity cannot be confirmed
2003		Contour map of margins prepared (copies of map in EA and NE files); costed design for outfall prepared (copy in EA files); data-logger installed (G. Walters pers. comm.); survey by T.J. Pankhurst, no plants found
2004	200	Survey by T.J. Pankhurst, many plants flowering (*in litt*. to Environment Agency); scrub clearance by English Nature

2.10 Westwood Great Pool was designated as an SSSI in 1955 for the rare plants which occur; for wildfowl and; as an example of standing water habitat. Two plant species listed on the citation are ribbon-leaved water-plantain and eight-stamened waterwort (*Elatine hydropiper*). Avifaunal interest is cited as a combination of the wintering wildfowl as well as birds nesting in the reedbed, including reed warblers (*Acrocephalus scirpaceus*) and ducks. Subsequent information, deriving from reports by the Worcestershire Biological Records Centre (http://wrbc.org.uk), rare plant surveys by J.J. Day and other documentation in the NE files in Worcester lists the following taxa of note at the site:

- Hirundines (swallows and martins) roosting in the reedbed in the autumn;
- needle spike-rush (*Eleocharis acicularis*) and mudwort (*Limosella aquatica*) (R.C.L. Burges 1955 *in litt*. in NE Worcester files);
- orange foxtail (*Alopecurus aequalis*), golden dock (*Rumex maritimus*) and pale willowherb (*Epilobium roseum*) (J.J. Day rare plant survey 1989); and
- Rough stonewort (*Chara aspera*) (material collected by J. Day and determined by J. Moore).

2.11 Since a waste-disposal land-fill site was opened at Throckmorton, there has been a roost of 2,000 (BTO WeBS data) to 7,000 (Jones *et al*. *in litt*.) gulls on the lake every night throughout the winter, in addition to flocks of ducks and Canada geese. The high nutrient levels in the lake are often blamed on agricultural run-off, however it is hard to deny that the presence of the gull roost will influence nutrient levels. Clearly it is important to identify the precise cause of elevated nutrient levels if any attempt is to be made to reduce them. It would not be acceptable to dissuade birds from using the site because they are an important element of its conservation value. Therefore some compromise would be needed. Alternatively, if the cause is agricultural run-off, then there are established methods of reducing this.

Plate 1 Gill Walters standing in the western scrape at Westwood Great Pool (September 2009)

2.12 The only active, intrusive management for ribbon-leaved water-plantain to-date, has involved the scraping of two bays in reeds on the western margin in August 1996 (Plate 1). These were protected by fencing to prevent grazing by waterfowl and subsequently boomed to stop seedlings being swamped by mats of vegetation deposited along the strandline (Wells, 1998, Pankhurst and Lansdown *in litt*. 2004). However, it is possible that the boom actually actively prevented establishment of germinands from submerged populations, while the main seed-bank may have been missed by the scrapes (see below), and this may explain the lack of success of these works.

The current situation

2.13 The current situation at Westwood Great Pool can be summarised as follows:

- The last confirmed massive growth of an emergent or terrestrial population of ribbon-leaved water-plantain at Westwood Great Pool was in 1991 (Wells, Preston and Croft 1992) and this is presumably the last occasion on which the seed-bank was significantly restored unless by submerged plants. This element of the population must be considered technically extinct, although there is presumably a chance that it could recover from the seedbank or through dispersal of propagules from deep submerged plants. In 2000 a perennial, submerged population likely to involve ribbon-leaved water-plantain was found at the site but none of the plants was flowering and so their identity could not be confirmed. In 2004, a population of at least 200 deep-submerged plants was found (T.J. Pankhurst *in litt*. to Environment Agency) of which many were flowering. This represents the last record of the species at Westwood Great Pool to-date. Ribbon-leaved water-plantain has therefore not been recorded from Westwood Great Pool for seven years and cannot be considered to be in favourable condition;
- It appears likely that some parts of the lake bed are exposed or only very shallowly submerged in most years, but no major draw-down of the lake has been recorded since 1990 and it is not clear how long before this significant drawdown last occurred, but it certainly pre-dates the repair of the dam;

- There is a bloom of blue-green algae each year resulting in a peak in biological oxygen demand which kills those parts of most vascular plants (and possibly charophytes) in the water column (authors pers. obs.);
- Recreation at the site continues and apparently mainly involves jet skis or water skis;
- The site continues regularly to attract rare birds, but also supports a nightly roost of 2-7,000 gulls on winter nights;
- The site still supports most or all of the rare plants that have been recorded, although it is more than ten years since most of these have been confirmed at the site; and
- The site still supports reasonable numbers of breeding birds, although there are often comments about declines.

2.14 On a brief visit to the site in September 2009, there was a zone at least 20m wide to the lake side of the reed fringe in which the substrate was exposed or submerged to a depth of less than 1cm. The fact that this occurred and was not apparently unusual, without corresponding germination and growth of ribbon-leaved water-plantain suggests that simply lowering the water level may not be enough to restore the terrestrial/ emergent population and that it may be necessary to disturb the upper horizons of the substrate.

The future of ribbon-leaved water-plantain at Westwood Great Pool

Non-intervention or intervention toward ribbon-leaved water-plantain conservation

2.15 Without intervention, it appears unlikely that the marginal population will recover, leaving the submerged population (if it is ribbon-leaved water-plantain) vulnerable to localised catastrophic events. Potential to predict the possible future of these submerged plants is constrained by the limits to our knowledge of the ecology of the species. Thus, for example, if the lake was drained totally (e.g. through accidental breach of the dam) and remained dry for a summer season, it would probably result in the death of the entire perennial population, however it is equally possible a) that the perennial plants would flower and set seed, thus restoring the seed-bank and b) that there would be significant germination from the existing seed-bank, revitalising the perennial population and possibly even restoring the terrestrial/ emergent population. In essence, apart from the likely loss of the emergent / terrestrial population, the future of ribbon-leaved water-plantain at the site cannot be predicted, but is definitely uncertain. Therefore, to ensure the future conservation of ribbon-leaved water-plantain some action will have to be taken simply because ribbon-leaved water-plantain occurs at too few other sites for one to be considered expendable.

2.16 The options for intervention that will benefit ribbon-leaved water-plantain are fairly limited:

- It would be possible to establish the capacity to artificially draw-down the lake to relatively precise levels by installing a controlled outflow on the existing dam. However, given that there is some draw-down each year and the emergent / terrestrial population has still been in decline, simply drawing down the lake may not be enough. Both the sites at which populations greater than 1 million plants have been recorded (southern Holland and the Czech Republic) had been ploughed in the year preceding the record and similar occurrences have been reported from the Brenne in central France (F. Pinet pers. comm..) and Austria (G. Király pers. comm..). In some regions in continental Europe, as well as historically in parts of the UK, when lakes have been drawn down a crop has been raised and harvested to reduce nutrient levels in the substrate. Ideally, the potential to stimulate germination through substrate disturbance should be experimentally tested, for example by raking blocks 5m^2 to two depths every 20m along a line from the margin until the depth precluded work. This should be undertaken in late June to combine maximal draw-down with the best chance of any plants that germinate setting seed. This work would not be worth doing unless the response was measured, at least monthly through the growing season following work. A possible decision-making process for intervention is presented as Figure 2.

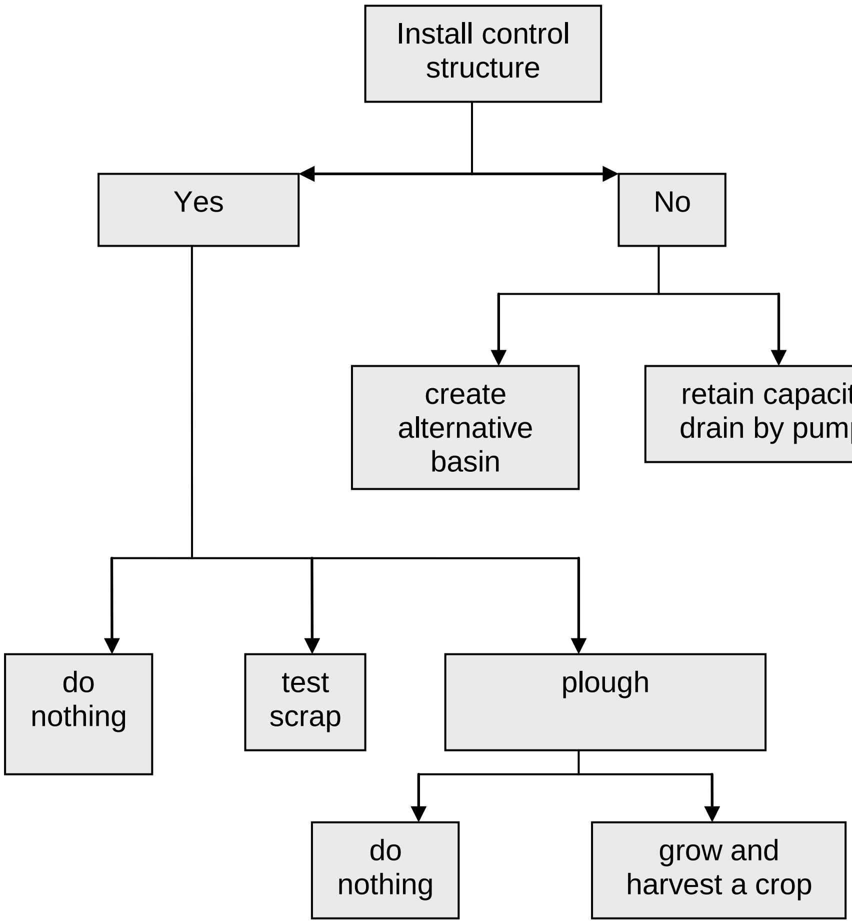

Figure 2 Decision tree for ribbon-leaved water-plantain management at Westwood Great Pool

- An alternative, which should not conflict with other uses of the site, except temporarily, would be to create a subsidiary basin on the northern or western side of the lake, which would be at a level to be flooded in winter, but dry in mid-summer. This secondary basin would only acquire seed naturally if this was brought in by the winter overflow. However, it should be possible to introduce growing plants to the basin and thereby establish a viable population.
- There is an urgent need to stop the blooms of blue-green algae, as well as a need to take some measure to restore the seed-bank, at least until some indication of the dormancy capacity of seed can be established. Whilst not a permanent solution, it is likely that the most effective method of controlling blooms of blue algae is through the use of barley straw bales. This will effectively mean addressing the symptoms, but not the cause of the problem, but may provide us with time to establish the precise cause of high nutrient levels and identify measures to address these.

2.17 Any decision to manipulate the site for the conservation of ribbon-leaved water-plantain will only be effective if designed not to conflict with the other “uses” of the site; other plant species, birds and recreation. Otherwise, it will always be subject to pressure from the other interests. The aim of conservation work for ribbon-leaved water-plantain at Westwood Great Pool must be to ensure the continued survival of a self-sustaining population at the pool. Conservation aimed at ribbon-leaved water-plantain can only be deemed a success if a population occurs within which seed is set, without intervention, within the dormancy capacity of the species and at the same time, there is a continuous perennial, submerged population of plants.

A monitoring protocol for ribbon-leaved water-plantain at Westwood Great Pool

2.18 In any monitoring protocol, the most important element of design is the basic question to be answered. In the case of ribbon-leaved water-plantain at many of the sites, a monitoring protocol is suggested here based on the premise that there are two indicators of success:

1) A viable perennial, submerged population of plants is present.
2) A population occurs within which adequate seed is set, without intervention, within the dormancy capacity of the species.

2.19 Monitoring against the first indicator simply requires an annual survey to assess whether a perennial, submerged population of plants is present. The only issue is whether or not the population is viable. To some extent any answer to this will be somewhat arbitrary as not only is the population viability of ribbon-leaved water-plantain very poorly understood, but it will be strongly influenced by environmental factors. It may be considered appropriate that if 50 plants are present and there is no algal bloom in the year, the target has been met. Alternatively, a slightly more demanding target might be that 50 plants flower each year, where flowering is considered indicative of the capacity of a plant to form turions and survive until the following year.

2.20 Monitoring against the second indicator must be able to demonstrate that significant seed-set has occurred within the seed dormancy capacity of the species. It is likely that ribbon-leaved water-plantain undergoes massive natural variation in growing populations and therefore the results of a single year of survey should not be taken as indicative of trends. Van den Berg *et al*. (2001) showed that ribbon-leaved water-plantain recurred at only 14% of locations from one year to the next, compared with *Chara* spp. which recurred at 65% of sites. So that a lack of growing plants in one or more years at a site should not be considered indicative of overall trends.

2.21 There are two critical questions that need to be answered:

1) What level of seed-set can be considered significant in this context?
2) What is the seed dormancy capacity of ribbon-leaved water-plantain?

2.22 It is not possible to quantify an answer to the first question based on available information and therefore, at least at first, the target must be set based on the best available data. Estimates of the number of flowering plants in the peak years appear to exceed 100 but not exceed 500. Estimates of the number of flowers produced by a plant under natural conditions vary, but an estimate of 100 (ranging from 5-308) flowers per plant (Lansdown 2011) seems reasonable. This would mean that the target would be for 10,000-50,000 flowers to be produced in a peak year. It must be recognised that the largest known populations of this species have involved 1,000,000-2,000,000 plants which presumably bore a total of 1-200,000,000 flowers, with an average of 15.5 seed per flower (Björkqvist 1967). This would mean that these populations were potentially setting 1.5-3 billion seeds in a single season. It is of note that both of the sites with these huge numbers of plants had been drained and ploughed in the season when the record was made. The most effective means of measuring against this target would be to record the number of flowers produced by a sub-set of the population of flowering plants. A count of the number of flowers produced by five groups of ten plants ± randomly selected throughout the flowering plant population should be adequate. **If the estimated total number of flowering plants multiplied by the average number of flowers produced by a sub-set of fifty plants is equal to or greater than 10,000 flowers then seed-set could be considered adequate**. A higher target would theoretically lead to greater seed-set, but may not be appropriate for Westwood Great Pool.

2.23 The issue of seed dormancy capacity is more complicated; if it is totally unknown, then it must be assumed that seed can last no more than one year. In this case there would be a need for the target for the number of flowers to be achieved each year; however it is unlikely from the historic record that this level of seed-set has ever occurred on an annual basis. What is not currently known is exactly how long seed can remain dormant and still be viable. The historic record suggests that a 10 year dormancy capacity is likely and a minimum of five years would be a reasonable working basis. Seed collected at Westwood Great Pool was apparently sent to the Millennium Seed Bank either in 1991 or 1994. Therefore, it should be possible to test whether seed dormant for either 18 or 15 years is still viable. Table 2 shows an example of the decision-making process for a monitoring protocol based on a) five and b) 10 year seed dormancy capacity, where the response to a failure to meet the target involves at least partly draining the lake to stimulate a major seed-set event.

Table 2 Proposal for monitoring emergent ribbon-leaved water-plantain populations

Five year seed dormancy capacity												
No. flowers	2000	800	25000	400	5900	8000	1300	2100	34000	0	200	100
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Response								*			*	

Ten year seed dormancy capacity												
No. flowers	2000	800	25000	400	5900	8000	1300	2100	3000	0	200	100
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Response												

2.24 Thus, for example if monitoring starts in year 1 and under a five-year seed dormancy capacity, because the seed-set target is met in year 3 (therefore three years into monitoring), it is not until there has been inadequate seed-set for a complete five-year monitoring cycle, that a response is provoked. Working with a ten year seed dormancy capacity, in the first twelve years of monitoring there is not a full ten-year monitoring cycle without adequate seed-set and so the lake is not drawn down. However if in year 13 the target was not met, then a response would be provoked.

2.25 Any monitoring protocol <u>must</u> be based on the best available information. It must therefore be possible to modify and adapt a protocol as new information becomes available. However, as with any scientific data collection exercise, any modification of an ongoing programme <u>must</u> ensure that data collection is replicable and the results comparable.

2.26 The fundamental considerations outlined above enable development of a basic monitoring protocol for ribbon-leaved water-plantain *at* Westwood Great Pool, using information available in 2009.

Perennial submerged populations

2.27 To be carried out each year:

1) Survey the open water by boat (due to the health risks of snorkel surveys from blue-green algae) to count submerged plants.
2) Derive information (most effectively through casual observations by bird watchers) on the occurrence of algal blooms and consequent aquatic plant die-back.
3) In years in which there is a continued population of 50 or more perennial submerged plants and no algal bloom the population may be considered to be in Good Condition.

2.28 The biggest problem is that the only response to the algal bloom situation which might be effective is to reduce nutrient levels in the lake. One way to achieve this is to draw the lake down and raise a crop from the mud. Currently this is impractical because of the water level control structures in place. Another option would be to carry out water quality sampling and establish a nutrient budget. This would identify the main sources of nutrients which could then be addressed.

Annual emergent or terrestrial populations

2.29 To be carried out each year:

1) Record the number of flowers produced by 5 groups of ten plants ± randomly selected throughout the flowering plant population (average number of flowers per plant A).
2) Estimate the total number of plants flowering (F).
3) Calculate the estimated seed-set for the year (A x F).
4) Review data for the preceding four years, if there has been an estimated seed-set greater than 10,000 then the population can be considered to be in Good Condition.

2.30 Again, available evidence suggests that the annual emergent or terrestrial populations have not been in Good Condition for many years. The biggest problem is that the only response to this situation which might be effective is to draw the lake down over at least three months of a summer season. Currently this is impossible because of the water level control structures in place. If the information presented here is accepted as realistic, it is not possible to improve the conservation condition of ribbon-leaved water-plantain at Westwood Great Pool without significant intervention.

3 The Spalding Area, Lincolnshire

Introduction

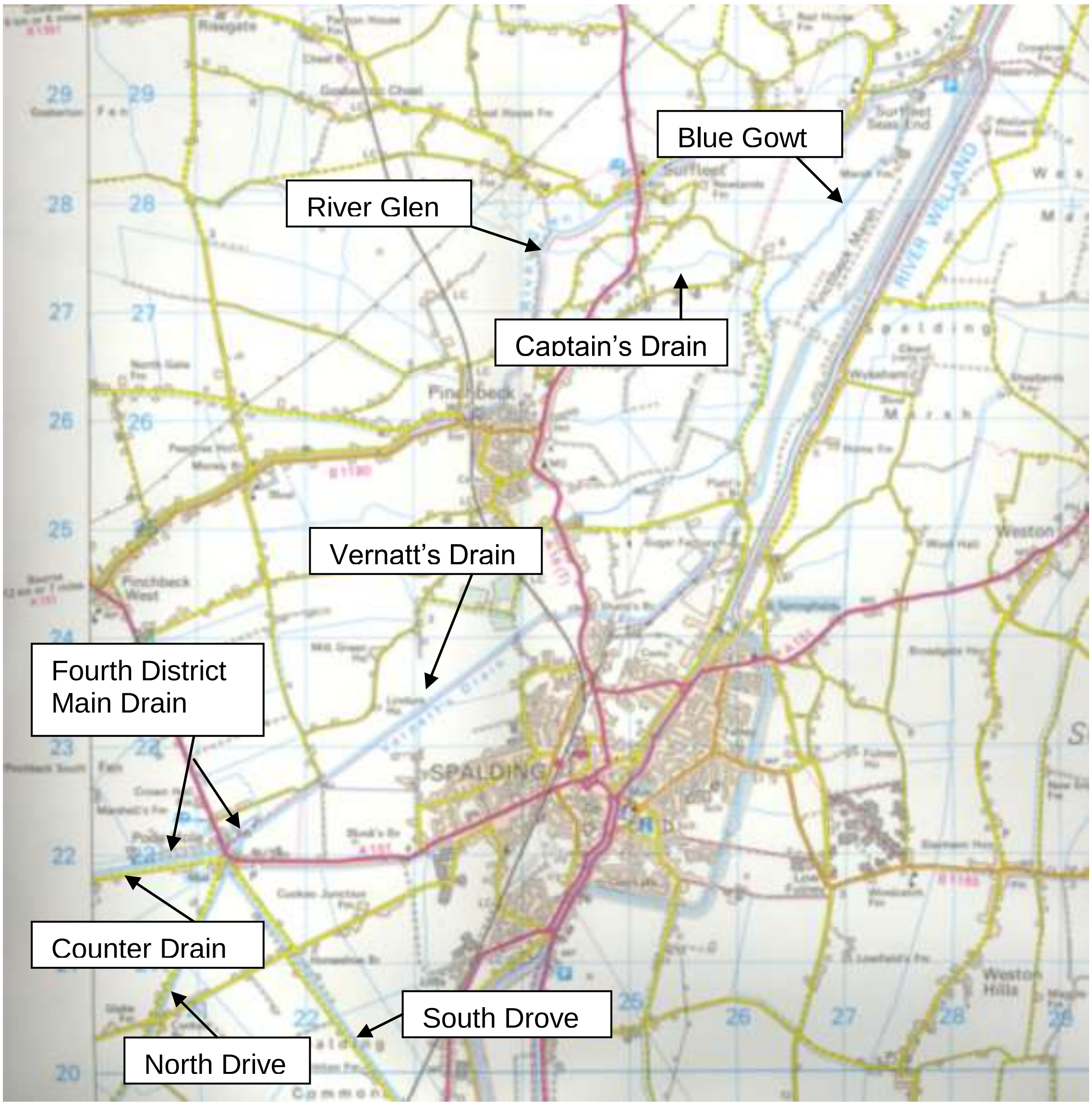

Figure 3 The area north and west of Spalding showing the main surveyed waterbodies

3.1 Ribbon-leaved water-plantain was first found in Lincolnshire in 1955 by Mrs. E.J. Gibbons who then surveyed its distribution in the River Glen and other nearby drains with the help of J.E. Lousley and D. McClintock (Lousley 1957). They found populations in the Blue Gowt, Vernatt's and the Old Sea (Clink's/ Captain's) drains and along "a distance of over two miles" of the River Glen downstream from Surfleet village to "the bridge where the stream becomes tidal" (Lousley 1957). Ribbon-leaved water-plantain was recorded in the downstream end of the Blue Gowt (where it now runs alongside the golf course), between 1955 and 1970 (Palmer 2006). The next record in the area was from 1991, when 40 plants were found by surveying the whole length of the Blue Gowt (Wells *et al*. 1992) during a survey of all the sites listed by Lousley (1957) as having supported plants. In 1992 only six plants were found on the same length of the Blue Gowt (Wells *et al*. 1993). In 2006 approximately 50 plants were found in the Blue Gowt where it runs alongside the golf course, of which 26 were flowering. These records and those for 2009 are summarised in Table 3.

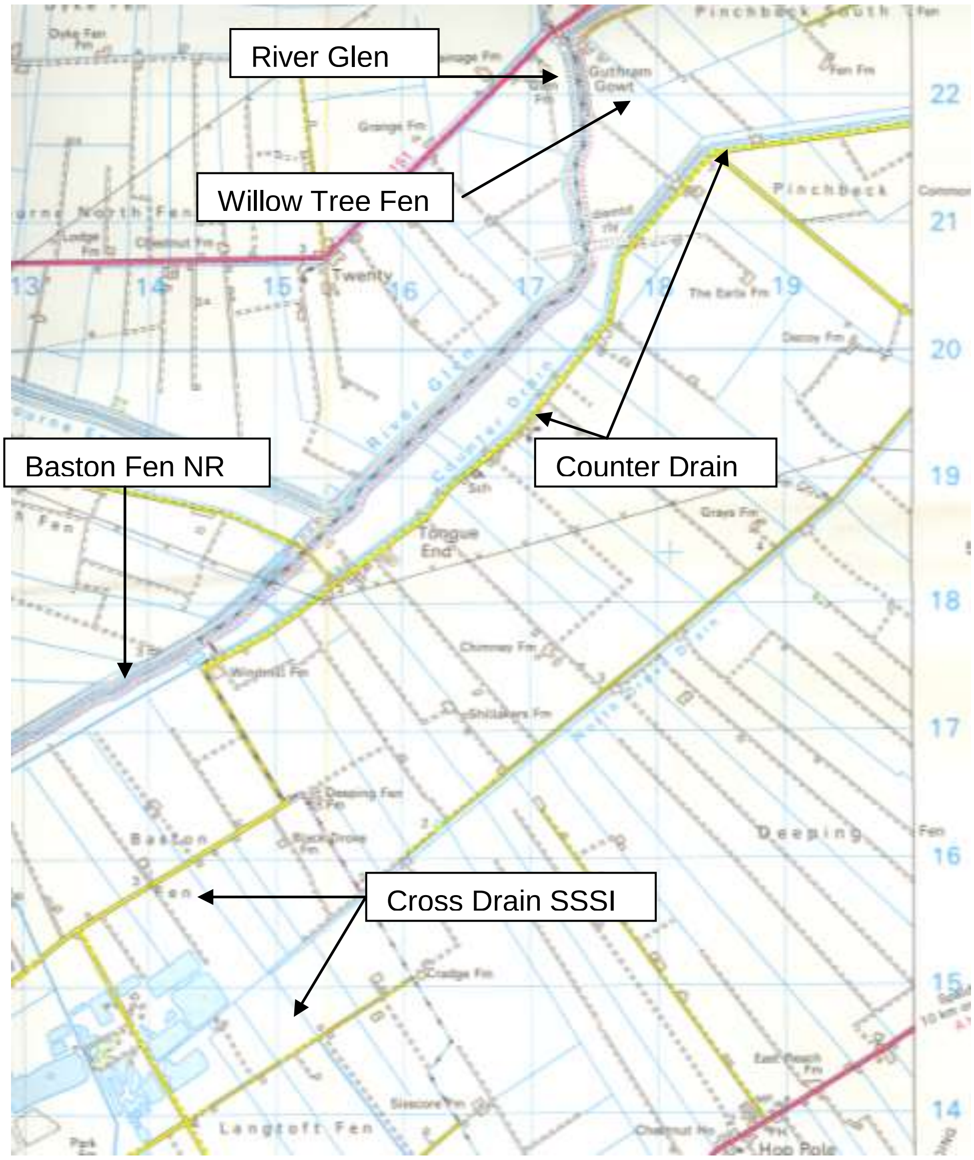

Figure 4 The area between Spalding and Bourne, showing main named sites

Table 3 Records of ribbon-leaved water-plantain in the Spalding area

Year	Blue Gowt	Old Sea	Vernatt's	Counter	R. Glen	Notes
1955	✓	✓	✓		✓[1]	Lousley (1957)
1970			✓			Palmer (2006)
1991	✓[2]					Palmer (2006)
1992	✓[2]					Wells *et al.* (1993)
2006	✓[2]					Palmer (in litt. 2007)
2009			✓			This study
2010				✓[3]		"

1 = specimen in **K** labelled "in R. Glen, Surfleet, 8 September 1955, J.E. Lousley" (conf. I. Björkqvist 1967)

2 = specimens not confirmed, either not flowering or no voucher retained

3 = voucher retained at the British Museum (Natural History) (BM)

3.2 During preliminary work on this project, looking at specimens in the herbarium of the Royal Botanic Gardens Kew, the author noted that a plant collected by J.E. Lousley from the River Glen showed the growth form typical of deep water plants. This was confirmed by Lousley's (1957) publication, where he states that "The plant in the new locality is the deep water submerged form growing in streams and the antithesis of the form previously known from shallow water on the margin of an artificial lake in Worcestershire". Such plants are unlikely to be visible from the banks unless the vegetation in the margins is heavily controlled. The author was aware that previous surveys of the water bodies in the Spalding area had been carried out from the banks and Richard Chadd of the Environment Agency confirmed that no water-based survey of ribbon-leaved water-plantain had been carried out in recent years. The decision was therefore taken to survey as much of the drains and river from which the species has been reported as possible, using a dry suit and snorkel.

3.3 In 2009, reaches of Vernatt's and the Old Sea drains, the Blue Gowt and the River Glen were surveyed which corresponded to sections shown as supporting ribbon-leaved water-plantain by Lousley (1957). One difference is that the River Glen was sampled at intervals rather than along its whole length between Surfleet and the tidal limit, this is mainly because of the time and effort required to survey using a dry suit. Instead four sections of the River Glen were surveyed, each about 500m in length.

Plate 2 Submerged flowering plant of ribbon-leaved water-plantain (Vernatt's Drain, 2009)

3.4 There is a marked similarity in the range of species recorded in 1957 and in 2009 (Table 4), apart from the record of opposite-leaved pondweed (*Groenlandia densa*) in the River Glen in 1957. Opposite-leaved pondweed is a species which appears to be declining throughout its range and it is not very surprising that it was not encountered in 2009. Most of the species recorded are typical of lowland meso-eutrophic water bodies in the UK, however opposite-leaved pondweed, whorled water milfoil (*Myriophyllum verticillatum*), flat-stalked pondweed (*Potamogeton friesii*), fan-leaved water-crowfoot (*Ranunculus circinatus*) and great tassel stonewort (*Tolypella prolifera*) may be considered characteristic of fenland drains and it is possible that ribbon-leaved water-plantain could be included in this list.

Table 4 Species recorded in The River Glen in 1957 and in 2009 (Sections I-IV)

Species	River Glen				
	Lousley (1957)	2009			
		I	II	III	IV
Alisma gramineum	✓				
Callitriche sp.	✓	✓	✓	✓	✓
Ceratophyllum demersum		✓	✓	✓	✓
Chara globularis		✓		✓	✓
Chara vulgaris		✓			✓
Cladophora glomerata		✓			
Elodea canadensis	✓				
Elodea nuttallii		✓	✓	✓	✓
Fontinalis antipyretica			✓	✓	✓
Glyceria maxima		✓			
Groenlandia densa	✓				
Hippuris vulgaris	✓				
Lemna gibba		✓			✓
Lemna minor				✓	
Lemna minuta				✓	✓
Lemna trisulca			✓	✓	
Myriophyllum alterniflorum	✓				
Myriophyllum spicatum		✓	✓	✓	
Myriophyllum verticillatum		✓	✓	✓	✓
Nuphar lutea				✓	
Persicaria amphibia		✓	✓		
Potamogeton berchtoldii / pusillus	✓				
Potamogeton crispus	✓	✓		✓	✓
Potamogeton friesii	✓			✓	

Table continued...

Species	River Glen				
	Lousley (1957)	2009			
		I	II	III	IV
Potamogeton pectinatus	✓	✓	✓		✓
Potamogeton perfoliatus	✓		✓		
Potamogeton trichoides		✓	✓	✓	✓
Ranunculus circinatus	✓	✓	✓	✓	✓
Ranunculus sp.				✓	
Rorippa nasturtium-aquaticum agg.			✓		✓
Sagittaria sagittifolia	✓	✓		✓	
Sparganium erectum					✓
Sparganium sp.	✓				
Tolypella intricata / prolifera				✓	
Ulva flexuosa					✓
Zannichellia palustris	✓				

In 1957, the *Callitriche* was recorded as *C. stagnalis* but this is likely to be an error; the record was made before *Elodea nuttalli* had been recognised in Europe and it could refer either of the two species; the *Potamogeton* recorded by Lousley as *P. pusillus* may well be correct but *P. berchtoldii* can not be excluded.

3.5 It is very likely that ribbon-leaved water-plantain occurs in the seed bank in the bed of the various drains and River Glen but only grows when the triggers necessary for germination occur. The main influence on the drains and River Glen is the management, particularly as, if unmanaged, the drains silt up and return to the historic pre-drainage state. It is very likely that the chances of finding growing plants of ribbon-leaved water-plantain are almost completely dependent upon the timing of surveys in relation to the timing of management actions. For this reason, the main emphasis in the following account is on management of the water bodies

3.6 One of the main aims of the 2010 element of the SRP was to collect samples of non-flowering *Alisma* plants from water bodies in the Spalding area, for molecular analysis ("DNA bar-coding"), as well as surveying water bodies to record the presence of flowering ribbon-leaved water-plantain plants. In September 2010, twelve sections of drain were surveyed and the plants present documented, while three sections of the River Glen were surveyed to try to locate non-flowering *Alisma* plants. Plants in the sections of the River Glen were not documented as this had been done in 2009 and to repeat it in 2010 would have meant that fewer new sections of drain could be surveyed.

3.7 There are two organisations responsible for managing the waterbodies that were surveyed in August 2009-2010; the Environment Agency of England and Wales (EA) and The Welland and Deepings Internal Drainage Board (IDB). The EA are an executive non-departmental public body responsible to the Secretary of State for Environment, Food and Rural Affairs and an Assembly Sponsored Public Body responsible to the National Assembly for Wales. The Welland and Deepings IDB is a public body responsible for land drainage and flood defence in and around the Spalding area. Clink's (Captain's/Old Sea) Drain, Blue Gowt Outfall, Blue Gowt Drain, Vernatt's Drain, South Drove Drain, Counter Drain, Fourth District Main Drain, ditches around Tanglewood and Cross Drain are managed by the Welland and Deepings IDB. The general management practices of the Welland and Deepings IDB are described here (all IDBs operate independently and with some differences in practice). Detailed practices are described in relation to different water bodies below.

3.8 All IDB operations are response-driven, being undertaken depending on necessity. However in the past, management actions were carried out on a set routine from which no deviations were made. Four main types of management action are employed:

- Weed cutting (Roding) by boat is usually restricted to the centre of the channel, leaving at least 1 metre reed fringe at the toe. Blades are set to cut at a specified depth and some plant growth therefore remains in the bottom of the channel. Cutting using an excavator involves working when the water levels are dropped; the furthest bank is cut down in towards the channel and then the nearest bank; using a 4 metre weed basket and leaving a fringe at the toe. It can be cut once or twice a year dependent on growth and tends to be earlier in the more urban areas. The bucket is a basket used to remove the cut material with a fixed blade or a reciprocating knife in its leading edge. It can be very selective dependent on the size of bucket and operator skill (Buisson *et al*. 2008). Material is piled on the bank to rot naturally and is understood to provide good habitat for snakes;
- Flail mowing: of bankside vegetation;
- 'Mudding Out' (de-silting or 'slubbing'-out): removal of accumulated silt from the drain bed; and
- 'Cotting': removal of blanketweed.

Site data

The River Glen

Sections surveyed on 21st August 2009: TF246278 - TF248280, TF256285 - TF258285, TF264287 - TF266286, TF273289 - TF276291

3.9 Flood water in the River Glen has to be managed by releasing water and lowering the water level to make room for the flood surge' (N. Riches pers. comm. to H. Tucker 2009). Weed in the River Glen is cut by boat twice a year (in June and September) along its full length. Cutting starts at the upstream end and takes 3-4 weeks to work downstream to Surfleet. Cut weed is placed on the bank sides at intervals and left to rot down (there are no set places at which the weed is placed, simply where convenient). A stall is placed across the channel to catch the weed; the team in the boat work down, floating the weed to the stall where it is removed. Cutting started in June 2009 but in the past cutting started in July. There is a problem with seepage in the Bourne Eau, due to too much weed in the Bourne Eau and Glen channels which leads to raised water levels and affects the water table.

3.10 Ideally, all aquatic vegetation in the central 80% of the channel is cut and removed (although in practice it may be less than this due to difficulty getting knives through the tough reed fringe). Where the channel is less than 10m wide, a minimum of 10% of the marginal vegetation is retained; where the channel is wider than 10m the retained width increases to 20%. Floating mats of algae (termed "blanket weed") are not removed in a separate operation but are moved down the channel along with the boat which is weed cutting and removed with the rest of the weed. Some of the vegetation on the banks is cut by tractor and flail between July and September, but not down to the toe where the reed fringe is left. Herbicides are generally not used as the boat work keeps the weed under control efficiently. The Glen channel is not de-silted or "mudded out" as it is sufficiently deep that this is not needed. There are no current plans to change any of the management methods or timings along the Glen.

3.11 The River Glen is a broad, relatively shallow fenland river, with slow flow and a more or less trapezoid section with steep banks. In many places there has been fairly significant reinforcement of the banks and in one place (within the main distribution area of ribbon-leaved water-plantain in 1955) the bed has also been reinforced. The River Glen supports diverse and species-rich aquatic vegetation at least between Baston Fen and Surfleet Seas End. This includes a number of relict fenland species, such as ribbon-leaved water-plantain (last recorded in 1955), whorled water-milfoil, flat-stalked pondweed, hair-like pondweed (*P. trichoides*) and fan-leaved water crowfoot (Table 4). The vegetation along the banks is heavily dominated by coarse grasses, including common reed (*Phragmites australis*) and reed canary-grass (*Phalaris arundinacea*), consequently the margins have very low species diversity. Below this, there is often a zone which is mainly bare but may support charophytes, water-plantain, fan-leaved water crowfoot and flat-stalked pondweed. Below this zone and into the deepest part along the centre of the river is usually a dense growth of algae, but in places there are dense stands of species such as rigid hornwort (*Ceratophyllum demersum*), spiked water-milfoil (*Myriophyllum spicatum*) and whorled water-milfoil.

3.12 Some taxa are local or very scattered and this is particularly the case with the *Tolypella* population, which was found only immediately upstream (west) of the A16(T) bridge at Surfleet (TF265286). This material is tall, fine and open, resembling tassel stonewort (*T. intricata*), however the dimensions are closer to great tassel stonewort. In 2010 a very healthy population of great tassel stonewort was found in a ditch in Surfleet (TF260286, see below), only 500m away from the population in the River Glen. More of the fine material was found in the River Glen a short distance upstream of the South Fen Road bridge (TF152184), the latter again with dimensions closer to those of great tassel stonewort. A population of great tassel stonewort had been found in the South Forty-foot Drain in 2007 (Greenall 2007). With the two confirmed populations in the area and the large scale of the two populations in the River Glen, it seems likely that they too are great tassel stonewort.

Plate 3 The River Glen at Surfleet

Plate 4 The River Glen between Surfleet and the tidal limit

The Blue Gowt

Sections surveyed on 20th August 2009: TF263262 - TF263266, TF274284 - TF275288

Table 5 Species recorded in The Blue Gowt in 1992 and in 2009 (sections I and II)

Species	Blue Gowt Wells et al. (1992)	2009 I	 II
Alisma gramineum	✓		
Callitriche sp.		✓	
Ceratophyllum demersum		✓	✓
Chara globularis			✓
Elodea nuttallii	✓		✓
Fontinalis antipyretica			✓
Indet. filamentous algae			✓
Lemna gibba			✓
Lemna trisulca			✓
Lemna turionifera			✓
Myriophyllum spicatum	✓	✓	✓

Table continued...

Species	Blue Gowt		
	Wells et al. (1992)	2009	
		I	II
Persicaria amphibia	✓	✓	✓
Potamogeton berchtoldii / pusillus			✓
Potamogeton pectinatus		✓	✓
Potamogeton perfoliatus	✓		
Potamogeton trichoides			✓
Ranunculus circinatus		✓	✓
Ranunculus sp.			✓

Plate 5 The Blue Gowt looking downstream toward and about 500m from the outfall

3.13 The Blue Gowt is a typical broad, quite shallow fenland drain with a trapezoid section and dense growth of common reed on the banks. It functions as an elongated storage pool with pumped in-flow and gravity outflow. Depth and flow are fairly constant except in high rainfall periods, and due to the nature of the land can be slightly above baseline salinity level (Nick Morris pers. comm. to H. Tucker 2009). For management purposes the Blue Gowt is divided into two reaches. The reach referred to as the Blue Gowt Outfall, which represents the main outlet for the Pinchbeck Marsh Pumping Station, conveys water from the station into the River Glen. On this section, "roding" (weed cutting) is carried out once per year using an excavator and 4m weed basket; material is placed on the bank top to degrade naturally. "Mudding out" was last performed on a rolling programme between 2002 and 2007. On the remainder of the Blue Gowt, roding involves a combination of an excavator with a 4m basket and tractor with 3m basket, once or twice per year dependent on growth, with material placed on the bank top to rot down. Mudding out was carried out on various sections in 2002, 2003 and 2007, with material placed on adjacent agricultural land to be ploughed in. It is of note that the Blue Gowt can be dammed at various points when working and cutting so the levels don't have to be dropped too low throughout.

3.14 With regard to the vegetation, the most notable aspect of the Blue Gowt is that it supports few notable taxa; in particular the only fenland relict species recorded were hair-like pondweed (*Potamogeton trichoides*) and fan-leaved water-crowfoot (*Ranunculus circinatus*). Apart from these, it supports a flora characteristic of nutrient rich waters, including species such as rigid hornwort (*Ceratophyllum demersum*), Nuttall's waterweed (*Elodea nuttallii*) and fennel pondweed (*Potamogeton pectinatus*).

3.15 In 1991 ribbon-leaved water-plantain was recorded in the Blue Gowt when "according to the local drainage officer, the Blue Gowt drain had been cleaned out two years previously with a drag-line, with the marginal vegetation cut and removed every year since" (Wells et al. 1992). The plants were described as "growing in about 60cm of water, rooted in a sticky grey calcareous clay" (Wells et al. 1992) and as "growing about 60cm from the bank in about 30cm of water" (Wells et al. 1993). Following de-silting (slubbing) in 2006, in July 2007, Margaret Palmer and Stan Pywell of the Welland and Deepings Internal Drainage Board, found more than 20 young, emergent plants. A re-visit in August by Margaret Palmer, Stan Pywell and Peter Stroh from NE found that the water level within the Drain had risen by c.20cm, and the plants were now fully submerged. The population was estimated at 50 plants with a total of 36 fruiting inflorescences. In 2009, two sections were surveyed, both approximately 500m in length, one downstream from Pinchbeck Marsh Pumping Station, the other upstream of the outfall; no *Alisma* plants were found.

Plate 6 Surveying the Blue Gowt with dense plant growth in deep water

The Old Sea /Captain's (Clink's) Drain

Section surveyed on 20th August 2009: TF262262 - TF263266; sections surveyed on 15th September 2010: TF245277 - TF246276; TF246276 - T250274

3.16 The Old Sea Drain (referred to as Clink's Drain by Lousley 1957 and also known in part as the Captain's Drain) is a narrow, steep-sided ditch; it is fed by the River Glen and management has recently involved mudding out in 2000 and 2003, with further mudding out unlikely before 2020. A section between the River Glen and Cuckoo Lane was surveyed in 2010; no notable taxa were recorded. A second section between Cuckoo Lane and the B1356 showed slightly greater species-diversity but again no notable taxa. There were a few flowering emergent plants of water-plantain (*Alisma plantago-aquatica*); a linear-leaved, fully submerged *Alisma* plant was collected (Plate 7) and subsequently identified by DNA bar-coding as water-plantain (*Alisma plantago-aquatica*).

3.17 The only record of ribbon-leaved water-plantain in this drain is from the 1955 survey (Lousley 1957) with no details. It is likely that the Old Sea Drain was surveyed in 1991 (Wells et al 1992) and 1992 (Wells *et al*. 1993), but no detail is provided. In 2009, a length of approximately 300m was surveyed but the bed of deep silt and ± 100% cover of floating *Ulva flexuosa* made this difficult.

Table 6 Species recorded in The Old Sea/ Captain's Drain in 2009 and 2010

Section	**1** **2009**	**1** **2010**	**2** **2010**
Algae			
Chara vulgaris	✓		
Ulva flexuosa	✓	✓	✓
Bryophytes			
Fontinalis antipyretica	✓		
Vascular plants			
Agrostis stolonifera			✓
Alisma plantago-aquatica			✓
Apium nodiflorum			✓
Callitriche sp.	✓	✓	✓
Elodea canadensis	✓		
Elodea nuttallii	✓	✓	
Equisetum arvense			✓
Glyceria maxima			✓
Lemna gibba	✓	✓	✓
Lemna turionifera	✓		
Myriophyllum spicatum	✓		
Persicaria amphibia			✓
Phalaris arundinacea			✓
Phragmites australis		✓	✓
Potamogeton pectinatus	✓		
Potamogeton trichoides	✓		
Ranunculus sceleratus		✓	
Rorippa nasturtium-aquaticum agg.		✓	✓
Scrophularia auriculata		✓	
Veronica catenata		✓	

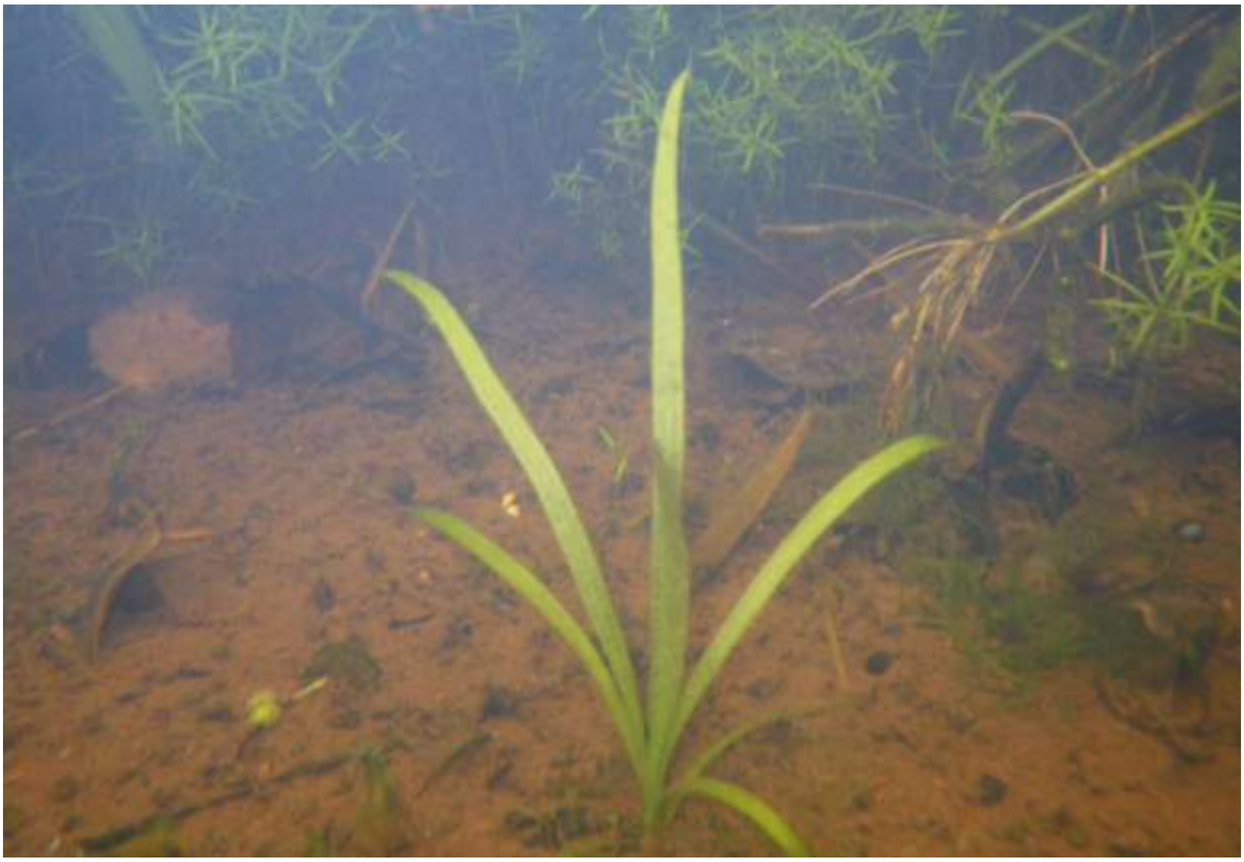

Plate 7 Narrow-leaved plant of *Alisma plantago-aquatica* from the Old Sea Drain

Plate 8 The Old Sea Drain taken from the Pinchbeck Marsh Pumping Station

Plate 9 Surveying the Old Sea Drain through a dense mass of *Ulva flexuosa*

Vernatt's Drain

Sections surveyed on 20th August 2009: TF243242 - TF246243, TF259251 - TF260253

Plate 10 Vernatt's Drain section I near Spalding Hospital

Plate 11 Vernatt's Drain section II near the STW

3.18 Vernatt's Drain is much larger than the Old Sea drain or Blue Gowt and with a greater resemblance to the River Glen. However, It resembles the Blue Gowt in that it functions as an elongated storage pool with pumped in-flow and gravity outflow, but differs in substantial natural inflow from groundwater via the Counter Drain; depth and flow are fairly constant except in high rainfall periods and due to the nature of the land can be slightly above baseline salinity level (Nick Morris pers. comm. to H. Tucker 2009). Between Pode Hole Pumping Station and the outfall to the tidal part of the River Welland (TF213220 to TF281291), vegetation is cut by boat using trailing knives and a side cutter. Weed is then raked out at strategic points by excavator and basket and placed in heaps. Roding takes place in May or June and has been the management routine here for at least 10 years. Mudding out was last carried out in 1991-1994 and will not be repeated until the need becomes evident through ongoing monitoring. Floating filamentous algae are removed independently.

3.19 Vernatt's Drain supports diverse aquatic vegetation, including a number of relict fen species. The most significant record was of a large, flowering, totally submerged plant of ribbon-leaved water-plantain (see below). Other notable species include: water violet (*Hottonia palustris*), flat-stalked pondweed (*Potamogeton friesii*), hair-like pondweed (*Potamogeton trichoides*) and fan-leaved water-crowfoot (*Ranunculus circinatus*). It is highly likely that some of these species derive from the Counter Drain, the North Drove Drain or the South Drove Drain, described later in this report.

Table 7 Species recorded in Vernatt's Drain in 2009

Vascular plants	
Alisma gramineum	*Myriophyllum spicatum*
Alisma sp.	*Phalaris arundinacea*
Bolboschoenus maritimus	*Phragmites australis*
Butomus umbellatus	*Potamogeton berchtoldii / pusillus*
Callitriche sp.	*Potamogeton crispus*
Ceratophyllum demersum	*Potamogeton friesii*
Elodea nuttallii	*Potamogeton pectinatus*
Fontinalis antipyretica	*Potamogeton perfoliatus*
Glyceria maxima	*Potamogeton trichoides*
Hippuris vulgaris	*Ranunculus circinatus*
Hottonia palustris	*Sagittaria sagittifolia*
Lemna gibba	*Sparganium erectum*

Algae	
Chara vulgaris	*Ulva flexuosa*
Cladophora glomerata	

3.20 The only detailed information regarding ribbon-leaved water-plantain in Vernatt's Drain that I have been able to find is the record cited by Lousely (1957) of a population found by E.J. Gibbons and J.E. Lousley in 1955, although Palmer (2006) suggests that it was found in Vernatt's Drain until 1971. In 2009 two sections were surveyed; the upstream section covered approximately 200m upstream from the bridge over the B1356 near Spalding Hospital and the downstream section covered approximately 200m upstream from the B1180 bridge near the sewage treatment works (STW). In the upstream section, a number of deep-submerged, narrow leaved plants were found which could have been ribbon-leaved water-plantain but their identity could not be confirmed. In the downstream section a single completely submerged flowering plant was found, as well as a number of narrow-leaved plants. The flowering plant was approximately 2m high with broadly-linear leaves and at least 200 inflorescences. With an average of 15.5 carpels per flower (Björkvist 1967), this would suggest that the plant may have borne more than 3,000 carpels.

South Drove Drain

Sections surveyed on 14th September 2010: TF213217 - TF213218

Plate 12 The South Drove Drain showing a boom installed to collect cut vegetation

Table 8 Species recorded in the South Drove Drain in 2010

Vascular plants	
Carex sp.	*Phragmites australis*
Glyceria maxima	

Bryophytes
Fontinalis antipyretica

Plate 13 The gates at the upstream end of the pump basin on the Counter Drain at Pode Hole

Plate 14 Weed-cutting in the Counter Drain at Pode Hole (8th July 2010)

3.21 The South Drove Drain is one of three drains which flow into Vernatt's Drain at Pode Hole; the other two are the Counter Drain (see below) and the North Drive Drain. The aim of surveying these three drains was to attempt to find flowering ribbon-leaved water-plantain plants to establish the location of the upstream end of the population found in Vernatt's Drain in 2009. A section of the South Drove Drain 200m upstream from the downstream end at the Pode Hole was surveyed, including the pump basin. The channel vegetation had been cut in the previous week and so little information could be gained from the survey. The extreme downstream end of the North Drove Drain was also inspected, from the pump basin but as it too had been cut in the previous week, there was no merit in surveying it in detail.

Counter Drain

Plate 15 Linear-leaved plant growing submerged in the Counter Drain near Baston Fen

Plate 16 Kate Fagan and Richard Chadd at the access bridge to Baston Fen

3.22 A short section of the Counter Drain was surveyed approximately 300m upstream from Pode Hole (TF208219 - TF212220; 14th September 2010) as well as the pump basin, a longer section downstream from the access bridge at Baston Fen (TF145176 - TF154182; 14th September 2010) and another short section upstream from the road bridge (TF1562181 - TF153182; 14th September 2010). The Counter Drain is one of the three drains that flow into Vernatt's Drain at Pode Hole. Weed was cut in the Counter Drain in July 2010, floated downstream to a boom near the Pode Hole pump basin and then removed onto the bank (Plate 14).

3.23 The main aim of surveying the sections around Pode Hole in 2010 was to try to find flowering plants of ribbon-leaved water-plantain to establish the upstream end of the population found in Vernatt's Drain in 2009. In the event, a single plant with two fruiting inflorescences was found at TF2096321986 within five minutes of entering the water, indicating that the ribbon-leaved water-plantain occurred at least from the STW on Vernatt's Drain upstream to Pode Hole. The survey was therefore moved upstream to the Baston Fen end of the Counter Drain.

Table 9 Species recorded in the Counter Drain downstream from Baston Fen (Tunnel Bank Road)

Vascular plants	
Eleocharis acicularis	*Potamogeton berchtoldii/ pusillus*
Elodea nuttallii	*Potamogeton friesii*
Glyceria maxima	*Potamogeton pectinatus*
Hottonia palustris	*Potamogeton perfoliatus*
Nuphar lutea	*Ranunculus circinatus*
Oenanthe fluviatile	*Sagittaria sagittifolia*
Persicaria amphibia	*Schoenoplectus lacustris*
Phalaris arundinacea	*Utricularia vulgaris*

Bryophytes
Fontinalis antipyretica

Algae	
Chara globularis	*Chara vulgaris*

3.24 Four non-flowering *Alisma* plants were collected from the section downstream from the access bridge at Baston Fen (labelled as "Indet., Counter Drain, Baston Fen d/s (1-4), 14 September 2010"). Two of these were identified through DNA bar-coding as water-plantain (*Alisma plantago-aquatica*) and two as narrow-leaved water-plantain (*Alisma lanceolatum*).

Plate 17 The Counter Drain looking east (downstream) from the access bridge at Baston Fen

3.25 The Counter Drain is remarkable for the range of aquatic plant species that it supports, which include a wide range of relict fen species such as needle spike-rush (*Eleocharis acicularis*), water-violet (*Hottonia palustris*), river water-dropwort (*Oenanthe fluviatilis*), flat-stalked pondweed (*Potamogeton friesii*), fan-leaved water-crowfoot (*Ranunculus circinatus*) and greater bladderwort (*Utricularia vulgaris*) as well as ribbon-leaved water-plantain. Greater water-parsnip (*Sium latifolium*) was also found in the Counter Drain alongside Baston Fen, although this may derive from reintroductions at the Fen, rather than native populations.

Fourth District Main Drain

Section surveyed on 14th September 2010: TF212220 - TF214221, TF210220; TF184216

Table 10 Species recorded in the Fourth District Main Drain in 2010

	West of Pode Hole	East of Pode Hole	Junction with Bank's Cradge Drain
Algae			
Chara vulgaris	✓		
Ulva flexuosa	✓		
Bryophytes			
Fontinalis sp.	✓		
Leptodictyum riparium	✓		
Vascular plants			
Azolla filiculoides		✓	
Callitriche obtusangula		✓	

Table continued...

	West of Pode Hole	East of Pode Hole	Junction with Bank's Cradge Drain
Callitriche platycarpa		✓	
Ceratophyllum demersum		✓	
Eleocharis acicularis	✓		
Hippuris vulgaris		✓	
Lemna gibba		✓	
Lemna trisulca		✓	
Lemna turionifera		✓	
Myriophyllum spicatum		✓	✓
Myriophyllum verticillatum		✓	
Nymphoides peltata	✓	✓	
Phragmites australis	✓	✓	
Potamogeton berchtoldii/ pusillus	✓		
Potamogeton crispus		✓	
Potamogeton friesii			✓
Potamogeton pectinatus		✓	
Potamogeton perfoliatus	✓		
Potamogeton trichoides	✓		
Ranunculus circinatus		✓	✓
Sagittaria sagittifolia	✓		
Sparganium erectum	✓		

3.26 The Fourth District Main Drain runs approximately 20m north of and parallel to the Counter Drain from Willow Tree Fen until a short distance east of the A151 at Pode Hole, where it turns north and becomes part of the drain network between Vernatt's Drain and the River Glen. From TF204219, where it turns away from the Counter Drain to TF225213, where it again heads north, the Fourth District Main Drain is a broad, trapezoidal ditch, approximately 4m wide at the water surface and 1-2m deep.

Plate 18 Surveying the Fourth District Main Drain at Pode Hole

3.27 The section immediately west of the A151 at the Pode Hole is most species-rich, including a large population of fringed water-lily (*Nymphoides peltata*), as well as needle spike-rush (*Eleocharis acicularis*) and hair-like pondweed (*Potamogeton trichoides*), as well as species more typical of nutrient-rich water. In addition, a large pleurocarpous moss was found growing on the clay bed in approximately 2m depth of water. Although it was clearly a *Fontinalis*, it was neither of the two native British species; *F. antipyretica* or *F. squamosa*. The material resembled *F. hypnoides* var. *duriaei* seen by the author in Portugal in May 2010 and was sent to Tom Blockeel, moss recorder for the Bryological Society (BBS) for his opinion. His response was as follows:

"I've had another look at your Lincolnshire *Fontinalis*, but have come to no firm conclusion. When I originally looked at the specimen it seemed to fit *F. hypnoides* because of its habitat in a lowland drain and the complete absence of any trace of keeled leaves.

I've now trawled through the literature that I have, and have compared your plant with a specimen I collected in Portugal in 1989. The Portuguese plant is well marked by its distantly spaced, widely spreading, and almost completely flat leaves (even at the shoot tips the leaves are only slightly concave). It also has reddish stems. The median leaf cells are lax (narrowly rhomboidal rather than linear). In contrast, your Lincolnshire plant has more crowded and less consistently flat leaves, many of them in fact being distinctly concave. The median leaf cells are linear. Most authors stress the flat leaves as a primary diagnostic character of *F. hypnoides/duriaei*, though some allow for occasionally concave leaves. Cell shape seems to be more variable. In comparison with my Portuguese specimen I don't think your plant is altogether convincing as *F. hypnoides*. But I don't know what else to call it. The habitat is wrong for *F. squamosa* and *dalecarlica*. The latter species in turn are difficult morphologically, as it is not always possible to demonstrate the differentiated marginal leaf cells."

3.28 The truth is that *Fontinalis*, like many aquatics, is morphologically very variable, and there is evidence from molecular analysis that the currently defined morphospecies are artificial. It may be that the Lincolnshire plants can't be satisfactorily placed without DNA study." A recent molecular study of the Fontinalaceae, in fact, found that identification of plants on morphological grounds does not agree with distinction at a molecular level (Shaw and Allen 2000). There is a need for a combined molecular and morphological study of the genus to clarify its taxonomy. The specimen will be lodged in the BBS herbarium (BBSUK) housed at the National Museum of Wales, Cardiff.

Cross Drain SSSI (Rose Cottage)

Sections surveyed on 14th September 2010: TF1533014852 - TF1517614852

Plate 19 Cross Drain SSSI from the road bridge near Rose Cottage (September 2010)

3.29 Cross Drain SSSI is a narrow, severely trapezoidal ditch running south-north and eventually meeting the western end of the Counter Drain. It is designated both for the invertebrate fauna that it supports and for "a rich flora typical of relict fenland", including narrow-leaved water plantain (*Alisma lanceolatum*), lesser water-plantain (*Baldellia ranunculoides*), needle spike-rush (*Eleocharis acicularis*), fine-leaved water-dropwort (*Oenanthe aquatica*), fen pondweed (*Potamogeton coloratus*), various-leaved pondweed (*P. gramineus*) and greater bladderwort (*Utricularia vulgaris*).

3.30 Only the section north from Rose Cottage was surveyed. Cross Drain clearly still supports some of the species for which it was noted, such as narrow-leaved water-plantain and needle spike-rush. However, at the time of the survey (September 2010), the substrate and much of the vegetation was obscured by fine silt and there was evidence that the original vegetation was becoming obscured by silt-loving species such as common club-rush (*Schoenoplectus lacustris*).

3.31 Two linear-leaved *Alisma* specimens were collected, labelled "Indet., Cross Drain, Rose Cottage (1 and 2), 14 September 2010. These were both identified through DNA bar-coding as narrow-leaved water-plantain (*Alisma lanceolatum*).

Table 11 Species recorded in Cross Drain SSSI in 2010

Vascular plants	
Alisma lanceolatum	*Myosotis sp.*
Alisma plantago-aquatica	*Phalaris arundinacea*
Apium nodiflorum	*Potamogeton berchtoldii/ pusillus*
Callitriche sp.	*Potamogeton natans*
Eleocharis acicularis	*Potamogeton pectinatus*
Elodea nuttallii	*Ranunculus circinatus*
Galium palustre	*Sagittaria sagittifolia*
Glyceria maxima	*Schoenoplectus lacustris*
Hippuris vulgaris	*Sparganium emersum*
Mentha aquatica	

Charophytes
Chara vulgaris

Ditch between Tanglewood and Pinchbeck South Fen

3.32 This is a series of small ditches, mostly less than 1m wide at the base, in the area between the River Glen and the Counter Drain, east of Willow Tree Fen and west of the A151. No notable taxa were recorded, the banks of most of the ditches had been cut and some had been de-silted shortly before the survey. However, the species recorded suggest that if they were inundated for longer periods or surveyed within a year of de-silting, they may support more interesting aquatic and wetland vegetation. The ditch at Pinchbeck South Fen was more or less dry at the time of survey, but when wet, may connect to the Bank's Cradge Drain which runs alongside Willow Tree Fen.

Table 12 Species recorded in ditches around Pinchbeck South Fen in 2010

Vascular plants	
Lemna gibba	*Ranunculus sceleratus*
Potamogeton berchtoldii/ pusillus	*Ranunculus subgenus Batrachium sp.*

Bryophytes
Pohlia melanodon

Algae
Chara vulgaris

Willow Tree Farm

Bank’s Cradge Drain

Sections surveyed on 15th September 2010: TF173224 - TF184216

3.33 This is a small drain running from Willow Tree Farm to join the Fourth District Main Drain alongside the Counter Drain Drove. It was mechanically de-silted in July 2010, which means that all plants recorded in September were either able to re-grow after de-silting from buried rhizomes (e.g. *Groenlandia densa*) or germinated after de-silting from the seed- or spore-bank.

3.34 The drain supports a remarkable range of plant species, many of which are typical relict fen species. These included abundant lesser water-plantain (*Baldellia ranunculoides*), opposite-leaved pondweed (*Groenlandia densa*), which was locally dominant, blunt-flowered rush (*Juncus subnodulosus*), brookweed (*Samolus valerandii*) and clustered stonewort (*Tolypella glomerata*). The diversity and species-richness of the vegetation declined toward the confluence with the Fourth District Main Drain, but is of exceptional local conservation value. There are plans by Lincolnshire Wildlife Trust to flood the two fields immediately south-west of this drain to enable restoration of fenland on the land of Willow Tree Farm. It is critical that this action does not adversely affect the vegetation of the Banks Cradge Drain which could be extremely important in the restoration, providing a close and species-rich source for colonisation.

Plate 20 Banks Cradge Drain, showing differences due to the nature of bank vegetation

Table 13 Species recorded from the Bank's Cradge Drain in 2010

Vascular plants	
Alisma lanceolatum	*Juncus subnodulosus*
Alisma plantago-aquatica	*Lemna gibba*
Baldellia ranunculoides	*Mentha ×piperita*
Callitriche sp.	*Mentha aquatica*
Eleocharis palustris	*Potamogeton pectinatus*
Elodea nuttallii	*Potamogeton trichoides*
Equisetum palustre	*Ranunculus sceleratus*
Glyceria fluitans	*Ranunculus subgenus Batrachium sp.*
Glyceria maxima	*Samolus valerandii*
Groenlandia densa	*Sparganium erectum*
Juncus ×surrejanus	*Typha latifolia*
Juncus effusus	*Zannichellia palustris*

Bryophytes	
Drepanocladus aduncus	*Pellia endiviifolia*
Leptodictyum riparium	*Pohlia melanodon*

Algae	
Chara contraria	*Chara vulgaris var. papillata*
Chara virgata	*Tolypella glomerata*
Chara vulgaris var. longibracteata	*Ulva flexuosa*

Plate 21 Lesser water-plantain (*Baldellia ranunculoides*) in the Bank's Cradge Drain

3.35 Two linear-leaved plants were collected (as Indet., Willow Tree Fen, , Banks Cradge Drain (1-2), 15 September 2010), one identified as lesser water-plantain (*Baldellia ranunculoides*) in the field because of apparent stoloniferous spread (Plate 21) was confirmed as such by DNA bar-coding; the other was identified by the same technique as water-plantain (*Alisma plantago-aquatica*).

Outflow drain from pond

3.36 The drain flowing out from the pond at Willow Tree Farm and parallel to the Banks Cradge Drain (TF174219 - TF181213) was also surveyed on the 15th September 2010. However this drain had not been de-silted for some time. Consequently, it was heavily overgrown with tall monocots. It would be interesting to survey this ditch shortly after de-silting to see whether it too supports species-rich relict fenland vegetation.

Table 14 Species recorded from the outflow Drain from the pond at Willow Tree Farm in 2010

Vascular plants	
Alisma plantago-aquatica	*Sparganium erectum subsp. erectum*
Lemna gibba	*Typha latifolia*
Phragmites australis	

Ditch at Surfleet

3.37 Whilst moving between survey areas on 17th September 2010, a stand of emergent *Alisma* plants was spotted growing in a ditch at TF260286, beside the road in Surfleet. This ditch supported two taxa of note, in particular, it supported a strong population of greater tassel-stonewort (*Tolypella prolifera*), including approximately 10 plants. However a tall plant subsequently identified through DNA bar-coding as water-plantain (*Alisma plantago-aquatica*), showed typical emergent leaves, as well as almost linear emergent leaves (Plate 22). There were also a number of small plants growing submerged in approximately 30cm of water with broadly linear leaves. It is clear that something is unusual about this plant and it should be investigated further in the future.

Plate 22 *Alisma plantago-aquatica* plant from the ditch at Surfleet showing variation in leaf outline

The results of surveys in the Spalding area

Figure 5 The location of ribbon-leaved water-plantain plants found during this study

3.38 Surveys carried out in 2009-2010 have shown that:

- A population of ribbon-leaved water-plantain persists in the Counter Drain - Vernatt's Drain sequence, which flowers and seeds good quantities of seed.

- Ditches and drains in the area support a remarkable flora, including many fenland relic species, these include lesser water-plantain (*Baldellia ranunculoides*) (Near Threatened), bristly stonewort (*Chara hispida*), needle spike-rush (*Eleocharis acicularis*), opposite-leaved pondweed (*Groenlandia densa*) (Vulnerable), whorled water-milfoil (*Myriophyllum verticillatum*) (Vulnerable), fringed water-lily (*Nymphoides peltata*), river water-dropwort (*Oenanthe fluviatilis*), flat-stalked pondweed (*Potamogeton friesii*) (Near Threatened), hair-like pondweed (*Potamogeton trichoides*), fan-leaved water-crowfoot (*Ranunculus circinatus*), greater water-parsnip (*Sium latifolium*) (Endangered), clustered stonewort (*Tolypella glomerata*) (Nationally Scarce), greater tassel-stonewort (*Tolypella prolifera*) (Endangered) and greater bladderwort (*Utricularia vulgaris*), as well as a population of a *Fontinalis* which does not accord with any currently known taxon.
- DNA bar-coding has been used to demonstrate that both water-plantain (*Alisma plantago-aquatica*) and narrow-leaved water-plantain (*Alisma lanceolatum*) can survive for extended periods (probably years) as permanently submerged, linear-leaved plants with leaves more than 50cm long.

3.39 Clearly these drains are of exceptional conservation value, not least due to the considered management practised by the Welland and Deepings IDB, such as the timing and approach to weed cutting.

Ribbon-leaved water-plantain: The current situation in Lincolnshire

3.40 The current condition of ribbon-leaved water-plantain in ditches and drains in the Spalding area can be summarised as follows:

- A population of ribbon-leaved water-plantain survives in the Counter Drain - Vernatt's Drain sequence, over a length of at least 6 km. In any year, plants appear to be sparse and flower only in the margins but may set large number of seed;
- Ribbon-leaved water-plantain grows in the Blue Gowt for a short period following mudding out, with the last populations recorded in 2006;
- There is no evidence to suggest that populations in the Glen or the Captain's/Clink's/Old Sea Drain survive; and
- The aquatic and wetland vegetation of the area is of high conservation value and merits further survey.

3.41 It seems clear that while there may be restoration of the seed-bank in the Blue Gowt at intervals and relatively continuously in the Counter - Vernatt's Drain sequence, ribbon-leaved water-plantain is not increasing and can barely be described as stable, it can certainly not be described as being in favourable condition.

The future of ribbon-leaved water-plantain in the Spalding area

The influence of current management practices

3.42 It seems likely that ribbon-leaved water-plantain is largely dependent upon anthropogenic activities to maintain the exposure of bare substrate necessary for germination. It is certain that the practices of the Welland and Deeping IDB have been instrumental in maintaining the current population.

Weed cutting

3.43 At present, most weed-cutting is carried out by boat and restricted to the centre of the channel. As a consequence, a tall and dense fringe of vegetation, typically dominated by *Phragmites australis* develops along the margins, precluding colonisation of the shallow margins by ribbon-leaved water-plantain and leading to the development of fairly dense shade over much of the channel. The same effect will result from leaving a fringe at the toe when cutting bank vegetation.

3.44 When weed is cut, rather than dredged, this allows plants in the centre of the channel to survive and would intuitively lead to development of dense beds of vegetation in the centre of the channel. Interestingly, in practice the centre of the channel in most of the water bodies surveyed was largely bare of vegetation or vegetation was represented by a dense mass of filamentous algae. This is presumably because the water is sufficiently turbid to preclude the growth of vascular plants and charophytes on the bed. Another potential consequence of weed cutting is that if ribbon-leaved water-plantain plants develop inflorescences within the centre of the channel, it is unlikely that they will persist long enough for fruit to ripen, particularly if cutting is carried out twice in a summer season. If the vegetation at the toe of the bank is not cut or removed, then a fringe of tall grasses, usually dominated by common reed will develop which will shade the channel and preclude colonisation by ribbon-leaved water-plantain as is the case along most of the River Glen.

3.45 Where stalls are placed across the channel to trap weed which is then dragged onto the bank, this appears to lead to seed deposition from cut weed in extremely localised areas. It seems extremely likely that the large ribbon-leaved water-plantain plant found in Vernatt's Drain in 2009 originated from seed deposited during weed cutting because it was at the precise spot where a stall had been used in previous years (R. Chadd pers. comm. to R.V. Lansdown). The small plant found in 2010 in the Counter drain was toward the downstream end of a cutting section near the point where cut weed is extracted, but not precisely at that spot.

3.46 When cut weed is placed on the bank to rot down, this removes material from the water body and is presumed to provide suitable habitat for grass snakes (*Natrix natrix*). However, this practice means that any seed or viable plants caught up in cut weed will be isolated from the water body and will die. This could have a significant adverse effect on the survival of growing plants and also recruitment into the seed bank.

"Mudding out"

3.47 The removal of built up sediment from the bed of channels can establish areas of bare silt suitable for colonisation by ribbon-leaved water-plantain and can expose parts of the seed bank, thus promoting germination. However where sediment is dug out and placed on agricultural land to be ploughed in (as is the case on the Blue Gowt), this may simply mean that recent horizons of the seed bank are removed from the water body and placed in a situation where they can no longer contribute to the survival of the species. Where "mudding out" is carried out on a rolling programme, a cycle of habitat suitability is created such that the number of growing plants may peak shortly after works and then decline until the next round of works.

3.48 It is of note that although the River Glen is not de-silted, the bed is still predominantly bare. It is not clear whether this is due mainly to high turbidity or whether the lack of disturbance to substrate reduces germination from the seed bank. It is also possible that boat traffic may reduce plant growth in the centre of the channel.

Intervention and modification of the current management practices

3.49 Whilst current management practices appear to enable ribbon-leaved water-plantain to survive in the complex of ditches and drains around Spalding, it appears possible that the population remains stable (in the Counter - Vernatt's sequence) or sporadic (in the Blue Gowt) under this management. Consideration of management practices and conditions elsewhere in the range of the species may indicate ways in which the management may be modified to improved conditions for ribbon-leaved water-plantain:

Plate 23 Ribbon-leaved water-plantain growing in a ditch (Oude Leede, Netherlands, 2000)

Plate 24 Ribbon-leaved water-plantain in a field (Staartjeswaard, Netherlands, 2000)

- In ditch systems around Oude Leede in the Netherlands, where ribbon-leaved water-plantain is abundant (Plate 23), there is a widespread and long-established tradition of annual management. The management involves a digger with perforated bucket working along the ditch, removing vegetation but (theoretically) allowing the water and silt to drain back into the ditch. This practise generates extensive amounts of bare, submerged substrate every year. In all of the ditches sampled (Lansdown 2011) in the lower Rhineland, this management generated a thick 'soup' of suspended peaty material that filled the bottom 80-90% of the water column; emergent ribbon-leaved water-plantain plants grew suspended in this and could flow some distance with the water, up and down ditches, as sluices and pumps were brought into action;

Plate 25 Ribbon-leaved water-plantain in a drained carp pond (Czech Republic, 1999)

- At Staartjeswaard, north-east of Beuningen, also in the Netherlands, R.V. Lansdown and T.J. Pankhurst were shown a population of approximately 2,000,000 ribbon-leaved water-plantain plants (Plate 24). These were growing in a field that may have been fertilised and had been planted with maize, but had been flooded by the River Rhine, suppressing the crop but encouraging growth of a wide diversity of wetland plants;
- In the Czech Republic (as well as in a variety of other sites in continental Europe) fish-rearing ponds are drained at intervals to reduce the build-up of nutrients associated with fish farming. In 1999 R.V. Lansdown visited one such pond that when drained, supported more than 1,000,000 plants of ribbon-leaved water-plantain (Plate 25); and
- In 2010, Király Gergely found millions of plants at each of ten sites in Slovakia whilst surveying seasonally inundated ploughed fields.

3.50 It is clear from these examples, that ribbon-leaved water-plantain can tolerate, and may even benefit from, extremely intensive substrate disturbance. It is possible that if the Blue Gowt was "mudded out" each year, or even every two years, ribbon-leaved water-plantain would increase.

3.51 The current management of the Counter - Vernatt's Drain sequence involves weed cutting in July. This is likely to cut flowering heads of ribbon-leaved water-plantain and does not disturb the substrate. It is possible that delaying the weed cut until September would result in greater seed-set in ribbon-leaved water-plantain, whilst more frequent mudding out might lead to better germination. If either of these modifications to current practice is implemented, then the Counter Drain - Vernatt's sequence needs to be surveyed each year to assess the response.

3.52 The River Glen is not currently mudded out and vegetation is retained along the toe. As a consequence, the substrate has little or no disturbance and the margins are shaded. Localised modification of this regime by mudding out and scraping the margins could allow existing seed to germinate and lead to re-establishment of a viable population. If either of these modifications to current practice is implemented, then the River Glen needs to be surveyed each year to assess the response.

3.53 Mudding out is currently undertaken on the Blue Gowt every four or five years. Increasing the frequency of mudding out might allow ribbon-leaved water-plantain a competitive advantage and stimulate more frequent growth. If this modification to the existing practice is implemented, then the Blue Gowt must be surveyed in each year that mudding out is performed and the subsequent year, to measure the response.

Monitoring in the Spalding area

3.54 Information available on the Spalding metapopulation and overall ecology of ribbon-leaved water-plantain is inadequate to implement an effective monitoring programme based on quantifiable targets. Before monitoring can begin, it will be necessary to establish a baseline from which to develop a meaningful set of targets. Two aspects of the known behaviour of ribbon-leaved water-plantain in the area are pertinent.

1) Ribbon-leaved water-plantain has flowered within the metapopulation in 1955, 1970, 1991, 1992, 2006, 2009 and 2010. The metapopulation still contains plants, but the resilience of the populations within the metapopulation is unknown.
2) Plants grew in the Blue Gowt following draw-down and de-silting but in Vernatt's Drain they appear to grow independent of de-silting.

3.55 There are again two questions which will dictate the nature of monitoring:

1) Is there still genetic exchange between the populations that make up the metapopulation?
2) What is the dormancy capacity of ribbon-leaved water-plantain seed?

3.56 If there is genetic exchange between different populations of the metapopulation, then good seed set need only occur once within the seed dormancy capacity for the whole metapopulation to be in good condition. However, if populations that formerly made up the metapopulation become genetically isolated, then the potential for a population to be restored from another part of the metapopulation is lost, so that each population becomes extremely vulnerable. Until these two questions are answered, monitoring is likely to be onerous and expensive.

3.57 In the absence of modification of existing practices or other intervention, the following actions will provide a measure of the condition of the population:

- Every five years, survey by snorkelling Vernatt's drain upstream from the bridge at the STW and similarly survey the Counter Drain upstream from the Pode Hole until a flowering plant of ribbon-leaved water-plantain is found. If no such plants are found, then the population may be considered to be in unfavourable condition and action should be taken to address this.
- Each time that mudding out is performed on the Blue Gowt, at least the length from Pinchbeck Engine to the confluence with the River Glen should be surveyed by snorkelling (unless water levels are artificially lowered at the time of survey) both in the year during which mudding out is performed and the subsequent year, to count flowering plants. Once some indication can be gained of the normal response to such management, then a measure of seed-set could be employed similar to that proposed for Westwood Great Pool, to inform condition assessment.

4 Vermuyden's Drain, The Ouse Washes

4.1 Vermuyden's Drain is a large, steep-sided ditch, trapezoid in section and running west to east to enter the water body sequence: Counter Drain -> Old Bedford River -> Delph, which itself runs parallel to the western bank of the Ouse Washes. At its eastern end and its confluence with the Counter Drain, boat traffic along Vermuyden's Drain was controlled by a lock. In 1998 the lock was struck and damaged by a boat. Consequently, water levels in the lock are now very low. Responsibility for management of Vermuyden's Drain lies with the EA, but there is no evidence of active management and the drain is very overgrown by common reed. It currently appears unsuitable for ribbon-leaved water-plantain and will remain so until action is taken to suppress the common reed. It is possible that the drain will not be suitable for ribbon-leaved water-plantain until the lock gates are repaired and water levels can be raised.

4.2 Reporting on a survey of all ditches and pools in the Ouse Washes in 1992, Cadbury *et al*. (1993) state that ribbon-leaved water-plantain was found "in the Forty Foot (Vermuyden's) Drain close to Welches Dam (TL468859), just outside the Ouse Washes. Here it appeared well established and was flowering, though submerged in 1.2m of water in a steep sided drain (Libby and Swann 1973). It was reputedly seen there in 1975 (R.E. Randall) and 1976 (J.R. Palmer), but searches in recent years, including 1992, have failed to refind it at this station". All other reports also suggest that ribbon-leaved water-plantain although abundant, only occurred in the end of the drain near Welches Dam (see below). However, I have been unable to find records of surveys for this or any other plant species in the water body sequence: Counter Drain -> Old Bedford River -> Delph into which Vermuyden's Drain flows.

Plate 26 Plantlife staff considering survey and monitoring options at the Ouse Washes

4.3 From herbarium specimens (see Appendix A) it is clear that ribbon-leaved water-plantain was found by R.P. Libbey during a BSBI excursion on the 18th September 1972, Libbey returned with E.L. Swann on the 21st September 1972 and collected more specimens, one of which was sent to J.E. Lousley. Subsequently, a few spikes were apparently seen on 25th August 1975 (R.E. Randall pers. comm. to G. Crompton) and J.R. Palmer reported finding ribbon-leaved water-plantain, apparently in the same part of Vermuyden's Drain in June 1976. Crompton (2006) presents a note that the drain was "somewhat eutrophied - [no ribbon-leaved water-plantain was] found in c.100 paces at S end of drain”, [TF]468.858, AC Leslie & DR Donald, 18.8.1978 and there have been no subsequent records.

4.4 In 2006 plants were found in the drain that were identified as ribbon-leaved water-plantain but they were not flowering and so the identification could not be confirmed (Palmer 2006). In 2009, only the extreme eastern end of the drain was surveyed because of 100% growth of common reed. The remainder of the drain was viewed from the bank but no *Alisma* plants were found. The only species seen in the drain in 2009 were common reed and *Ricciocarpos natans*.

Table 15 Records of ribbon-leaved water-plantain in Vermuyden’s Drain

Year	Vermuyden’s Drain	Notes
1972	✓	R.P. Libby and E.L. Swann (specimen in NWH)
1975	✓	Crompton (2001)
1977	✓	Crompton (2001)
2006	✓[1]	M.A. Palmer pers. comm. to T.J. Pankhurst 2006

4.5 There is no obvious reason for ribbon-leaved water-plantain to occur in Vermuyden’s Drain and not in the Counter Drain, or other water bodies in the area. In 2009 a section of the Counter Drain approximately 500m long upstream from the confluence of the Counter and Vermuyden’s Drains was surveyed, as was a short length of the Old Bedford River upstream and downstream of the bridge at Welches Dam. No ribbon-leaved water-plantain was found, but a more thorough and extensive survey would be needed before the results could reliably suggest that the plant is not growing in one of the water bodies. The following species were recorded in the Counter Drain in 2009.

Elodea nuttallii
Fontinalis antipyretica
Hippuris vulgaris
Hottonia palustris
Lemna gibba
Nuphar lutea
Nymphoides peltata
Oenanthe aquatica
Phragmites australis
Potamogeton lucens
Potamogeton perfoliatus
Potamogeton × salicifolius
Ranunculus circinatus
Ranunculus sp.
Ricciocarpos natans
Sagittaria sagittifolia
Sium latifolium
Sparganium erectum

[1] = specimens not confirmed, either not flowering or no voucher retained

The current situation

4.6 Available information suggests that:

- Ribbon-leaved water-plantain occurred in Vermuyden's Drain between 1972 and 1977 and may have occurred in 2006, but this last record is unconfirmed. The management of the drain currently appears unsuitable and ribbon-leaved water-plantain must be considered to be in unfavourable condition in the drain.
- Available evidence suggests that none of the deep (i.e. over 1m) drains along the northern margin of the Ouse Washes around Welches Dam have been surveyed employing snorkel or scuba equipment, apart from some short sections surveyed opportunistically by R.V. Lansdown in 2009. It is possible that ribbon-leaved water-plantain persists as a totally submerged population in these drains but this requires confirmation.

The future of ribbon-leaved water-plantain in the Ouse Washes

4.7 It may be concluded that ribbon-leaved water-plantain is extinct in the Ouse Washes, however it seems unwise to write it off without some attempt to investigate more thoroughly. Two actions could be undertaken on Vermuyden's Drain to increase the likelihood of ribbon-leaved water-plantain growing there again:

- Cleaning out the vegetation from the drain. Each time that the silt is cleaned out the drain should be surveyed by snorkelling, both in the year during which mudding out is performed and the subsequent year, to count flowering plants.
- Restoration of the function of the lock gates. If the 2006 record is correct, then this action may not be necessary, equally, cleaning out the drain may show that restoration of the lock gates is not necessary. However, in the absence of such information it would appear likely that the hydrology of the drain prior to damage to the lock gates may have been important for the survival of ribbon-leaved water-plantain at the site.

Monitoring at Vermuyden's Drain

4.8 Information on ribbon-leaved water-plantain at the site is currently inadequate as a basis for a monitoring protocol. The following can be said:

- If Vermuyden's Drain is cleaned out, then it must be surveyed as outlined above. If no plants are found following cleaning out, then other action(s) will be required. If plants are found then it should be possible to establish what level of population growth is normal at the site and then develop a monitoring protocol along the lines of that proposed for Westwood Great Pool.
- If no snorkel or scuba-based surveys of the deep drains around the Ouse Washes are carried out or if such surveys fail to locate submerged populations of ribbon-leaved water-plantain, then there will be no basis for monitoring. If submerged plants are found then recording must be used to inform design of a monitoring protocol.
- If the lock gates on Vermuyden's Drain are restored to their former function, then the drain should be surveyed by snorkelling annually for a number of years to establish whether there is germination in response. If so, then recording must be used to inform design of a monitoring protocol.

5 Langmere, Norfolk

5.1 Langmere is one of the Breckland “meres” and is a broad, shallow lake with a maximum depth of only a few metres. The meres are unusual in that they are mainly aquifer fed so that the level of water in the meres does not respond directly to rainfall, but is delayed, such that levels may be high in summer but low in the autumn and early winter. The periods that each mere spends dry or wet depend on its depth; the altitude of its base in relation to the water table; and the conductivity of the surrounding substrates. Extended periods of drought and inundation are both important in their ecology. Drought periods allow accumulated organic sediments to rot down, be absorbed by terrestrial plants and possibly blow away as well as allowing livestock and other animals to penetrate to the centre and disturb the substrate. Conversely, prolonged deep inundation not only kills off terrestrial plants but also marginal plants where they might normally become established.

5.2 The distinctive flora of the meres is characterised by early successional stages after re-inundation when they exploit reduced competition. If the inundation lasts for a long time, the community succeeds to one of more permanent waters and indicative of the relatively high nutrient status of the water (which may also increase with the deposition of organic matter). The characteristic species occur not only because they exploit the lack of competition but also because they may often have the capacity to exploit or tolerate aquatic, marginal and damp terrestrial conditions. The substrate may be important in this; at Langmere for instance, the marginal slopes are sandy and water will rise through capillary action through the sand allowing plants that require a lot of water to grow some distance above the open water. This also apparently occurs along the Rhine where species such as ribbon-leaved water-plantain may grow in terrestrial forms several metres above the water along the sandy flood banks, provided competition is suppressed by other factors, such as grazing.

Plate 27 Langmere in September 2009

5.3 Ribbon-leaved water-plantain was first reported from Langmere in 1972, but Swan (1975) states that it had been found 12 years previously by Mrs. P.A. Willé. Swann (1975) also claims that two taxa were found, now usually treated as two species: ribbon-leaved water-plantain and *A. wahlenbergii*. The only specimen of the plant identified as *A. wahlenbergii* cannot now be found (Lansdown 2011) and so its identity cannot be confirmed. This record seems unlikely to have actually been *A. wahlenbergii* so should be treated with scepticism unless the specimen can be found. In NWH there is a single large ribbon-leaved water-plantain plant collected from Langmere (see Appendix 1) with some ribbon leaves as well as a couple which are slightly expanded. The plant has obviously undergone vegetative propagation with two growth centres and two inflorescences, all joined at the base, the inflorescences are between 400 and 500mm long.

Table 16 Records of ribbon-leaved water-plantain in Langmere

Year	Langmere	Notes
1960	✓	Swann (1975) recorded by P.A. Willé
1972	✓	R.P. Libby and E.L. Swann (specimen in NWH)

Table 17 Plant species recorded in Langmere in 2000 and 2009

	2000	2009		2000	2009
Chara vulgaris	✓		*Myosotis arvensis*	✓	
Riccia cavernosa	✓		*Myosotis laxa*	✓	✓
Bryum argenteum			*Myosoton aquaticum*		✓
Calliergon stramineum	✓		*Persicaria amphibia*	✓	
Drepanocladus revolvens	✓		*Persicaria maculosa*		✓
Drepanocladus sp.	✓		*Phalaris arundinacea*	✓	✓
Agrostis canina		✓	*Potamogeton gramineus*	✓	
Agrostis stolonifera	✓	✓	*Potamogeton lucens*	✓	
Carex hirta	✓		*Potamogeton pectinatus*	✓	
Ceratophyllum submersum		✓	*Potamogeton × angustifolius*	✓	
Chenopodium rubrum		✓	*Plantago major*		✓
Cirsium arvense	✓		*Potentilla anserina*	✓	
Cirsium vulgare		✓	*Potentilla reptans*	✓	✓
Climacium dendroides	✓		*Ranunculus flammula*	✓	
Eleocharis palustris	✓		*Ranunculus sceleratus*	✓	✓
Epilobium montanum		✓	*Ranunculus trichophyllus*	✓	✓
Epilobium palustris	✓		*Rorippa amphibia*	✓	
Epilobium parviflorum		✓	*Rorippa palustris*		✓
Galium palustre	✓		*Rumex crispus*	✓	
Galium uliginosum	✓		*Rumex maritimus*	✓	✓
Holcus lanatus	✓	✓	*Sagina procumbens*		✓
Juncus articulatus	✓	✓	*Schoenoplectus lacustris*	✓	
Juncus bulbosus		✓	*Senecio vulgaris*		✓
Juncus conglomeratus	✓		*Stellaria graminea*	✓	
Lemna gibba	✓		*Stellaria media*		✓
Lemna trisulca	✓	✓	*Urtica dioica*		✓
Medicago lupulina		✓	*Veronica scutellata*	✓	
Mentha aquatica	✓		*Veronica serpyllifolia*		✓
Mentha arvensis	✓	✓	*Vicia sativa*	✓	

5.4 In 2000, nine meres in the Breckland area were surveyed in an attempt to locate additional populations of ribbon-leaved water-plantain, these were Corkmere Bottom (three meres), Devil's Punchbowl, Fen Mere, Fowlmere and a small subsidiary mere, Home Mere and West Mere by the author and T.J. Pankhurst. No new populations were found, however given the sporadic nature of ribbon-leaved water-plantain populations, it is important that such a survey is repeated. In September 2009, Langmere was resurveyed. It was largely dry at the time of survey (Plate 24); in perfect condition for growth of terrestrial forms of ribbon-leaved water-plantain. The entire lake was carefully searched and no plants were found. It is possible that the plant simply did not grow in 2000 or 2009, equally it is possible that Langmere represents one of a number of sites supporting a dynamic metapopulation of ribbon-leaved water-plantain in the area, but without compelling evidence that this is the case it should probably be assumed that ribbon-leaved water-plantain is extinct in Norfolk.

The future of ribbon-leaved water-plantain at Langmere

5.5 Adequate survey of Langmere has been carried out on at least three occasions since 2000 and no ribbon-leaved water-plantain found (see above). It seems likely that repeated surveys for this species cannot be justified. However, anyone visiting the site to record Norfolk Bladder-moss (*Physcomitrium eurystomum),* (a UK Biodiversity Action Plan priority species), could be provided with information on the identification of ribbon-leaved water-plantain in case material is encountered.

5.6 The 2000 Breckland Meres survey for the ribbon-leaved water-plantain SRP was carried out before information in the ecology of the species in continental Europe was readily available; consequently it could easily have missed small or terrestrial ribbon-leaved water-plantain plants. Equally, the 2005-6 survey covered only the inundated zones of the meres surveyed. It would therefore be appropriate to consider a survey of these other meres, both to document their flora for general conservation purposes and to survey specifically for ribbon-leaved water-plantain.

6 Introduction Attempts

Introduction

6.1 There have been a number of planned and one unplanned introduction of ribbon-leaved water-plantain in the UK. The following account brings together all the information that it has been possible to locate on these actions, to inform conservation of the species in the UK.

6.2 There are reports that a John Turner, a horticulturist specialising in water-lilies, based in north Lincolnshire grew populations of ribbon-leaved water-plantain, at least during the 1990s. The seed for this was apparently supplied by T.C.E. Wells for the purposes of preparing plants for introduction. I have been unable to find documentation of this or establish what has happened to the plants grown.

Kingfishers Bridge, Cambridgeshire

6.3 Kingfishers Bridge NR is a small artificial wetland complex created through reclamation of arable land. It lies approximately 2km north of Wicken Fen NNR. A total of twelve ribbon-leaved water-plantain plants were introduced into two ponds at this site in 1998, two of which flowered in the year of planting. Only three plants were present in 1999 but, again, two flowered. No plants have been seen subsequently and the successional state of the ponds in 2002 suggests that may have succumbed to competition (T.J. Pankhurst pers. comm.).

Hauxley NR, Northumberland

Plate 28 The main lagoon, Hauxley NR (2 August 2010)

6.4 Palmer (2006: page 13) stated that, "There is an additional record around 1987 for Northumberland, believed to be a planting into the wild", without further explanation. It has been extremely difficult to clarify this record, however the following information appears to be relevant:

- Hauxley NR is one of four wetland complexes developed from past open cast mine sites and now managed by the Northumberland Wildlife Trust between Alnwick and Ashington, Ambleside, Northumberland;
- The reserve was purchased by the Northumberland Wildlife Trust (NWT) in 1983; at the time of the record, the water bodies at Hauxley were all freshwater and were being established with plants, mainly those native to Northumberland but some brought in from elsewhere;
- In the early 1990s the sluice which held back sea water and maintained the freshwater on site was removed and the lagoons became saline, with the loss of the freshwater flora which had established; and
- In August 2010, a survey of the main lagoon (Plate 25) found only plants with a degree of salt tolerance greater than that of ribbon-leaved water-plantain: Bladder Wrack (*Fucus vesiculosus*), fennel pondweed (*Potamogeton pectinatus*), *Ulva flexuosa* and horned pondweed (*Zannichellia palustris*). However a few small, shallow wetlands remain which could still support ribbon-leaved water-plantain.

6.5 It has not been possible to establish exactly who introduced the material and therefore the source of the material that led to the report. Someone who worked on site at the time said "My suspicion is that it would have come from a volunteer who helped us a lot […] who is a professional wildlife gardener, or was, and was in the habit of introducing exotic native plants. He used some of our artificial pools as a way of building up his stocks. It was me that insisted that everything was recorded".

6.6 It is likely that the ribbon-leaved water-plantain has died out, either because in the long-term the introduction was unsuccessful or because of the saline water incursion. It is difficult to imagine where a wildlife gardener might have obtained material of ribbon-leaved water-plantain in 1987.

Baston Fen LWT Reserve and SSSI

6.7 Baston fen is a Lincolnshire Wildlife Trust (LWT) reserve and Site of Special Scientific Interest (SSSI) which lies in an area in South Lincolnshire characterised by arable fields bounded by ditches, many of which could provide suitable habitat for ribbon-leaved water-plantain. The LWT currently own 45 ha. of Baston Fen, but there are plans to increase this, initially by 39 ha. of reedbed and wet grassland at Willow Tree Farm and eventually to 875 ha. of varied fenland habitats. Successful establishment of a self-sustaining metapopulation of ribbon-leaved water-plantain within the current fen could lead eventually to its spread into these new areas as they become available and possibly into the wider ditch network. The current boundary of the nature reserve and SSSI includes four fields (Figure 6), two represent the High Wash which is at the driest end of the Fen, a single field represents the Middle Wash and another single field the Low Wash. Both the Middle and Low Washes have a fairly high water-table and are inundated throughout much of the winter.

6.8 In 1998 11 plants were introduced into deep peat in a pool at Baston Fen (Wells 1998). The following year there was a total of 16 plants, of which 12 flowered (Lansdown 2011), showing both that the original plants had survived and that some had germinated. Subsequently, no plants have been seen at the site. It is possible that the introduction failed in the long-term partly because the pool was heavily vegetated and plants could not compete with grasses; an alternative is simply that too few plants were introduced over too short a period, simply because it takes more plants and longer to establish a population. Most successful reintroduction programmes, such as that of the white-tailed eagles (*Haliaetus albicillus*) in Scotland and red kite (*Milvus milvus*) elsewhere in the UK, have shown that repeated introduction of large numbers of individuals has the best chance of success. For ribbon-leaved water-plantain it is also possible that the introduction needs to be into sites where the substrate is disturbed by cattle to reduce competition. In a study of a total of 24 ribbon-leaved water-plantain populations in the UK and continental Europe (Lansdown 2011), no populations were found in peat apart from those in ditches at Oude Leede in the Netherlands, all were in clays, even where the surface horizons were peaty.

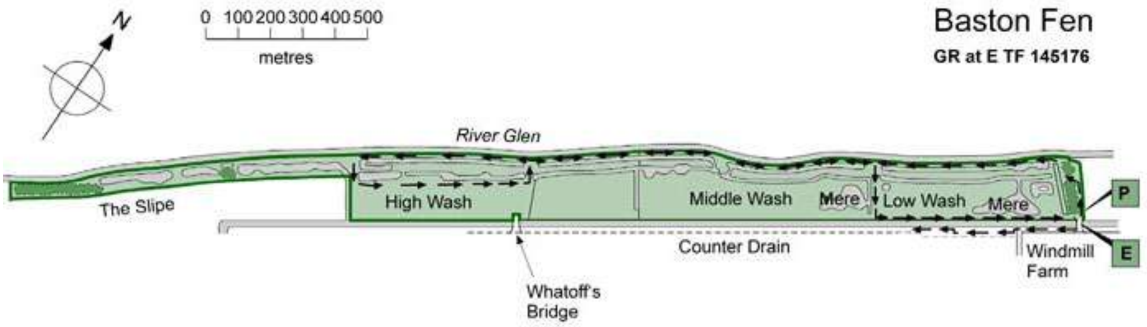

Figure 6 Baston Fen NR showing management units

Plate 29 Baston Fen, taken from the Glen embankment near the western end of the Middle Wash

Plate 30 Ribbon-leaved water-plantain plants in cultivation in trays in Cambridge Botanic Gardens

6.9 In early 2009, an introduction protocol was drafted (Appendix 2). For the purposes of this reintroduction, Baston Fen was seen as consisting of five units, grading from the High Wash which is ± dry and semi-improved at the western end, to the Low Wash which is wet fen with an area of woodland in the east. The surface soil horizon is mainly peat, grading to loam in places, locally poached or excavated to expose underlying clay. The reintroduction protocol was designed taking into consideration all previous attempts to establish new populations of ribbon-leaved water-plantain in the UK; available information on the habitat and ecology of populations in continental Europe; and demonstrated success with reintroduction of other taxa.

6.10 2009 presented the first opportunity to test the proposed protocol. As is always the case, practice showed that some elements of the protocol were impractical while others were simply unnecessary. The project had three main elements:

1) Plants and trays in which plants had been grown under the ribbon-leaved water-plantain SRP were transferred from cultivation by T.J. Pankhurst to Cambridge Botanic Gardens.
2) On the 4th August 2009, 200 plants were "pricked out" into plant pots (generally one per pot) and grown on.
3) On the 3rd September 2009, the potted plants were transferred to Baston Fen and planted out, approximately half of the plants were small and poorly developed, the other half were more robust and many were flowering.

6.11 The plants were divided ± equally between the four fields which comprise the fen (two parts of the High Wash, the Middle Wash and the Low Wash). An assessment was made on site of the most appropriate locations for planting based partly on available habitat and partly on the aim of achieving a reasonable spread of plants.

Plate 31 Pete Michna and Julie Clos of Cambridge Botanic Gardens preparing pots and compost

Plate 32 Pricking out seedlings into pots

Plate 33 Ribbon-leaved water-plantain plants in pots ready for transport to Baston Fen

Plate 34 Making a scrape in dense vegetation in the Low Wash

6.12 To introduce the plants, first an excavator was used to scrape back or dig out vegetation, exposing the underlying soil. Wherever scrapes were created on the edge of ditches or ponds, the substrate was removed so that the plants could be established at or only slightly above the water level in the adjacent water body at the time of planting. To minimise the amount of disturbance to the site, a total of 10 scrapes were created and 20 plants were planted into each scrape by digging a hole by hand that enabled the soil containing the plant to be inserted, without disturbing the roots of the plant itself. A total of 200 plants was planted out on 3rd September 2009.

Plate 35 Making a scrape in a short, grazed sward in the Middle Wash

Plate 36 Environment Agency team planting and documenting the reintroduction

Plate 37 Rosie Glossop (née Blackman) and Holly Tucker planting ribbon-leaved water-plantain

High Wash - three scrapes planted on the edge of ditches, because apart from the ditches there were no evident sites with surface water.

Middle Wash - two scrapes on the margins of ditches and one on the edge of a shallow pool.

Low Wash - one scrape on the edge of a ditch, two in dense tall grasses on the edge of ponds and one in a low-lying grassy depression.

6.13 A visit was made on the 15th October 2009 by the EA and a detailed record made of the condition of plants in the scrapes (Table 18).

6.14 Table 18 shows that forty days after the planting exercise, only 22 plants could be located, of which only two were fruiting. This suggests a very low rate of success, however whether or not plants survived, there should have been some contribution to the establishment of a seed bank. By August 2010, overall species diversity in scrapes created in 2009 was much higher than in the surrounding vegetation (although this was not quantified) and a wide variety of taxa germinated in the scrapes, including flowering rush (*Butomus umbellatus*), water forget-me-not (*Myosotis scorpioides*) and water plantain (*Alisma plantago-aquatica*).

Table 18 Status of ribbon-leaved water-plantain in scrapes at Baston Fen in October 2009

No.	Grid reference	15 October 2009 observations
1	TF1449117633	1 plant visible; plugs uprooted and site used by swans; *Glyceria maxima* encroaching (8 plants); water level 2-3 cm below scrape
2	TF1443017597	No plants visible; 20% growth of *Carex* sp., substantial poaching by cattle; many small dicotyledon seedlings; some *Equisetum* sp. in scrape; wetter than when planted.
3	TF1401917379	No plants visible; extensive poaching by cattle; over-run by hay cut; very dry
4	TF1395317365	No plants visible; poached by cattle throughout; 10% encroachment by *Glyceria maxima*
5	TF1394017395	5 plants visible; no poaching; some encroachment by *Carex* sp. (2 plants)
6	TF1393917419	1 plant visible; extensively poached by cattle; negligible encroachment by *Carex* sp.
7	TF1393917433	2 plants visible and some apparently uprooted; use of scrape by swans; many small dicotyledon seedlings; negligible encroachment by *Carex* sp.
8	TF1387217295	1 plant visible; extensively and deeply poached by cattle; many dicotyledon and monocotyledon seedlings; fairly dry
9	TF1387417351	No plants visible; extensively poached by cattle and grazed
10	TF1352317224	5 plants visible, 2 with fruit; little poaching by cattle; some encroachment by *Carex* sp.
11	TF1341717160	1 uprooted plug; very extensively and deeply poached by cattle
12	TF1337617136	No plants visible; very extensively and deeply poached by cattle
13	TF1332717103	No plants visible; extensively and deeply poached by cattle; spoil collapsed onto scrape
14	TF1328817081	No plants visible; extensively and deeply poached by cattle; spoil collapsed onto scrape
15	TF1324817050	No plants visible; extensively and deeply poached by cattle; spoil collapsed onto scrape
16	TF1319817016	No plants visible; extensively and deeply poached by cattle; spoil collapsed onto scrape
17	TF1315516991	No plants visible; extensively and deeply poached by cattle; spoil collapsed onto scrape
18	TF1312116983	3 plants visible; lightly poached by cattle; very wet
19	TF1306916962	3 plants visible; lightly poached by cattle; very wet
20	TF1301116923	(only 8 seedlings planted) no plants visible, lightly poached by cattle

6.15 There are a number of factors in 2009-10 which could have reduced the survival of plants as well as possibly reducing contribution to the seed bank:

1) Seedlings were not "pricked out" until August, consequently they were not ready for planting out until September and even then, many were poorly established and most not flowering. This could be resolved in future years by pricking out plants in May, as soon as they are large enough.

2) The late planting means that cattle needed to be introduced shortly after planting, and as a consequence most of the scrapes were heavily poached within one month. In the future, enough time should be left between planting out and introduction of cattle to the fen for seedlings to establish and flower.
3) At a number of scrapes the spoil and vegetation removed fell or was knocked back by cattle onto the scrape, probably killing seedlings. This could be avoided in the future by moving the excavated spoil further from the scrapes.

6.16 Baston Fen was visited twice during the late summer of 2010 to record whether plants of ribbon-leaved water-plantain had grown in the scrapes created in 2009. The first visit was on the 2nd August by R.V. Lansdown, R. Chadd and R. Glossop and the second visit on the 13th September by R.V. Lansdown, R. Chadd and K. Fagan.

Plate 38 Scrape number 1 in September 2009 (left) and September 2010 (right)

Plate 39 Scrape 2 showing ribbon-leaved water-plantain with inflorescence (2^{nd} of August 2010)

Plate 40 Flowering ribbon-leaved water-plantain in scrape number 2 (2^{nd} August 2010)

Plate 41 Scrape number 1 showing dense growth of *Glyceria maxima* (13th September 2010)

Plate 42 Spoil and vegetation collapsed back into a scrape created in 2009 (October 2009)

Plate 43 Spoil knocked by cattle back into a scrape created in 2009 (October 2009)

6.17 In 2003, two large scrapes were made on the margins of ditches on Baston Fen (R. Chadd and J. Redwood pers. comm.); one on the ditch between the High and Middle Washes and one on the ditch running parallel to the River Glen on the northern side of the site. These scrapes were approximately four times the size of those made specifically for ribbon-leaved water-plantain in 2009 and were dug down to slightly below the summer water level in the ditch.

Plate 44 One of the scrapes created in 2003 (2nd August 2010)

6.18 In August 2010, plants of ribbon-leaved water-plantain were found in three of the 10 scrapes created in 2009. Four flowering plants were found in the scrape in the Low Wash (eastern field, scrape number 2) (Plates 39 and 40). A further four flowering plants were in one and a single plant in another of the scrapes on the ditch running parallel to the River Glen in the Middle Wash. All of the plants had flowered; the leaves were brown and largely decayed and fruit beginning to fall. On the second visit, in September 2010, no more plants had germinated and all the plants found on the 2nd of August had decayed further, depositing seed. In comparison, water-plantain (*Alisma plantago-aquatica*) and narrow-leaved water-plantain (*Alisma lanceolatum*) on the Fen were still producing buds and flowers at the time of the survey.

6.19 In all cases, by September 2010, vegetation (mainly tall monocots) had grown over the scrapes (Plates 38 and 40), except where the scrapes had fallen in (Plate 42) or been damaged by cattle in 2009 (Plate 43).

Two aspects of the larger scrapes created in 2003 are significant:

- They are both still largely open, even after seven years (Figure 44); and
- Both support a diverse range of aquatic and marginal plants including four species of charophyte (including clustered stonewort, *Tolypella glomerata*), while one supports the only population of least bur-reed (*Sparganium natans*) (Figure 45) currently known in south Lincolnshire, all of which have probably germinated from the seed bank as a result of creation of the scrape.

6.20 Conclusions that can reasonably be reached from the reintroduction attempt made in 2009, as well as the *ad hoc* creation of scrapes in 2003 are:

- Ribbon-leaved water-plantain germinated and set seed in at least three scrapes in 2010, the season subsequent to that in which seedlings were planted;
- The small scrapes created in 2009 were rapidly colonised by tall monocots, whereas those created in 2003 have only recently begun to be so colonised after seven years; and
- Both sizes of scrape resulted in dramatic germination from the seedbank, including some notable taxa.

6.21 The overall conclusions that can be reached from this are:

- It is possible that a self-sustaining population of ribbon-leaved water-plantain could be established at Baston Fen, however this would require repeated planting, use of larger scrapes, removal of spoil further from each scrape and that the whole exercise is completed early enough for cattle not to be on site for at least a month after planting; and
- Creation of more scrapes similar to those created in 2003 could lead to an overall increase in the plant diversity of the site and probably also the invertebrate diversity.

6.22 The discovery in 2009 of totally submerged flowering plants of ribbon-leaved water-plantain in the Counter Drain suggests that it may not be appropriate to artificially establish a population of ribbon-leaved water-plantain at Baston Fen. It is certainly appropriate to refrain from further introduction until the need is absolutely unambiguous. However, independent of the issues of reintroduction of ribbon-leaved water-plantain, it is clear that the condition and species diversity of Baston Fen, and eventually Willow Tree Fen and the wider landscape, would benefit from the creation of more scrapes on Baston Fen. The decision that need to be taken, with great care, is how to achieve the best result not just for those taxa which will benefit, but for all the other aspects of the fen.

Plate 45 *Sparganium natans* growing in a scrape created in 2003 (2nd August 2010)

Monitoring at Baston Fen

6.23 Monitoring at Baston Fen must involve a combination of inspection of the scrapes created in 2009 and an overall survey of suitable habitat to record any growing plants, employing the following data collection and analysis protocol:

1) Record the number of plants growing which can be confirmed as this species (i.e. flowering plants) (F).
2) Record the average number of flowers produced per plant (A).
3) Calculate the estimated seed-set for the year (A x F).
4) Between 2010 and 2014, any seed-set can be seen as indicating that the population is in good condition. Subsequently, review data for the preceding four years, if there has been an estimated seed-set greater than 10,000 then the population can be considered to be in good condition.

7 Ribbon-leaved water-plantain in Britain

The current situation

Table 19 Records of apparently native populations of ribbon-leaved water-plantain

Year	Westwood	Counter	Blue Gowt	Old Sea	Vernatt's	R. Glen	Vermuyden's	Langmere
1920	✓							
1930	✓							
1932	✓							
1939	✓							
1948	✓							
1950	✓							
1953	✓							
1955	✓		✓	✓	✓	✓		
1957	✓							
1960								✓
1970	✓				✓			
1972							✓	✓
1975							✓	
1977							✓	
1980	✓							
1985	✓							
1988	✓							
1989	✓							
1991	✓		✓*					
1992	✓		✓*					
1993	✓							
1997	✓							
1998	✓							
2000	✓*							
2006	✓		✓				✓*	
2009					✓			
2010		✓						

* = unconfirmed records

7.1 The current status of all known populations of ribbon-leaved water-plantain in the UK can be summarised as follows:

- Ribbon-leaved water-plantain has been present at Westwood Great Pool for at least 80 years, growing or in the seed bank; the last confirmed massive growth of an emergent or terrestrial population was in 1991.
- In 2000 and 2004 perennial, submerged populations of ribbon-leaved water-plantain were found at Westwood Great Pool, many of which were flowering in 2004.
- Ribbon-leaved water-plantain has been present in the Spalding area for at least 50 years growing or in the seed bank and a population survives in the Counter Drain - Vernatt's Drain sequence, over a length of at least 6 km. It also grows in the Blue Gowt for a short period following mudding out, with the last populations recorded in 2006. However, there is no evidence to suggest that populations in the Glen or the Captain's/ Clink's/ Old Sea Drain survives.
- Ribbon-leaved water-plantain occurred in Vermuyden's Drain between 1972 and 1977 and may have occurred in 2006, but this last record is unconfirmed.
- Ribbon-leaved water-plantain was confirmed in Langmere only on two occasions or over a period of 12 years. Adequate survey of the site has been carried out on at least three occasions since 2000 and no ribbon-leaved water-plantain found, this population should therefore be considered extinct.
- Plants have been introduced into Baston Fen twice, on the first occasion they persisted for only one year, on the second occasion they were only introduced in 2009 and so there has been insufficient time for monitoring.

7.2 Ribbon-leaved water-plantain is clearly not in favourable condition in the UK. There are a number of actions that could be taken to improve the condition of populations, none of which are worthwhile unless the response is measured by adequate follow up monitoring.

Management and other intervention

7.3 Without intervention, it appears unlikely that the marginal population at Westwood Great Pool will recover, leaving the submerged population vulnerable to localised catastrophic events. Options for intervention include:

- Establish the capacity to artificially draw-down the lake to relatively precise levels by installing a controlled outflow (for which a costed design was prepared in 2003) on the existing dam. This could be combined with scraping or ploughing all or part of the bed and could include raising a crop on part of the site to reduce nutrient levels in the sediment.
- Creation of a subsidiary basin on the northern or western side of the lake, which would be at a level to be flooded in winter, but dry in mid-summer. This should probably include introduction of seed, but does run the risk of becoming a gardening exercise.
- Use of barley straw to control algal blooms to buy time whilst more long-term decisions on control of nutrient levels are reviewed.

7.4 It is likely that populations of ribbon-leaved water-plantain in the Spalding area are largely dependent upon anthropogenic activities to maintain the exposure of bare substrate necessary for germination. Without intervention, it is likely that populations in the Counter Drain - Vernatt's sequence will persist at their current very small scale and that the population in the Blue Gowt will grow and set seed in response to management. It is unlikely that the populations in the area will increase without intervention. Options for intervention include:

- Increasing the frequency of mudding out of the Blue Gowt to resemble that of ditches in the Oude Leede area of the Netherlands;

- Mudding out the Counter Drain - Vernatt's sequence and delaying weed cutting until September to encourage greater germination and allow plants in the centre of the channel to set seed; and
- Mudding out the River Glen and scraping the toe of the banks to encourage germination for areas currently too stable or shaded for ribbon-leaved water-plantain.

7.5 Without intervention, it is likely that the ribbon-leaved water-plantain population in Vermuyden's Drain will die out, or at best germinate only rarely in response to water-level fluctuations resulting from hot summers. Options for intervention include:

- Cleaning out the vegetation from the drain; and
- Restoration of the function of the lock gates.

7.6 It is likely that the ribbon-leaved water-plantain population at Langmere is extinct and it is difficult to justify more intervention, however it would be possible to plough part of the mere in an attempt to stimulate germination from the seed-bank.

Survey and monitoring requirements

7.7 At least in the short-term, it will be necessary to monitor each year if the future of ribbon-leaved water-plantain in the UK is to be assured. Currently, none of the available staff in government agencies is sufficiently familiar with the species to be able to carry out such monitoring. Initially, it will therefore be necessary to bring in specialists. The solution to this is to include an element of species-specific identification training for EA and NE staff in all survey or monitoring contracts. The monitoring requirements for the foreseeable future are:

- Record emergent / terrestrial plants at Westwood Great Pool and record seed-set against the targets recommended in chapter 2.
- Record submerged plants at Westwood Great Pool and record the number surviving.
- Every five years, survey by snorkelling Vernatt's drain upstream from the bridge at the STW and similarly survey the Counter Drain upstream from the Pode Hole.
- Each time that mudding out is performed on the Blue Gowt, at least the length from Pinchbeck Engine to the confluence with the River Glen should be surveyed by snorkelling (unless water levels are artificially lowered at the time of survey) both in the year during which mudding out is performed and the subsequent year, to count flowering plants.
- If no intervention is made at Vermuyden's Drain, then there is little to be gained from recording, although further detailed (snorkel) surveys of the deep Ouse washes drains are needed to establish the limits of the population in the Ouse Washes.
- There is little to be gained from surveying Langmere in the absence of intervention. Subsequent meres surveys should look out for *A. gramineum* (e.g. when monitoring for *Physcomitrium eurystomum*).

Outstanding research requirements

7.8 In spite of reference to the need for research into the dormancy capacity of ribbon-leaved water-plantain at least since 2000, we cannot confirm that it has a long-term dormancy capacity. Without this information, we cannot allow time to pass when populations of ribbon-leaved water-plantain are not setting seed and consider the species to be in favourable condition. This means that any realistic monitoring requirement will be onerous and expensive. It is critical that an effort is initiated to clarify the seed dormancy capacity of ribbon-leaved water-plantain as soon as is possible. It is possible that seed from the Millennium Seed Bank could be used for this purpose, however given that storage conditions at the MSB are designed to maximise dormancy and not to mimic nature, it would be wise to test other seed as well.

7.9 It appears likely that there is an intimate and possibly dependent relationship between perennial submerged and emergent / terrestrial populations. It is also possible that in the long-term conservation of submerged perennial population will ensure the survival of emergent / terrestrial populations, without action directed to the latter. However, we still have no information on the relationship between these. Clarification of the relationship between such populations is probably critical for the conservation of this species in the UK.

7.10 In the past, there was evidently a metapopulation of ribbon-leaved water-plantain in the Spalding area, with populations in at least four water bodies. Such metapopulations are generally much less vulnerable to short-term catastrophic events, as loss of a single sub-population can be countered by colonisation from one of the other sub-populations. If the exchange of genetic material between sub-populations ceases, then each of the sub-populations loses this robustness and becomes more vulnerable. It would therefore be useful to establish whether there is still exchange of genetic material between the populations in the Blue Gowt and the Counter - Vernatt's sequence. This could be achieved through allozyme analysis.

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Appendix 1 Herbarium specimens located

The following specimens have been located (square brackets indicate information not included with the specimen):

Vice County 28 West Norfolk

(As *A. gramineum* subsp. *gramineum*) submerged in 18" of water, northern shore of Langmere, 15th Sept. 1972, Libbey, R.P. and Swann and E.L. 563/3, confirmed by E.J. Clements, 19th April 1977 (**NWH**).

Vice County 29 Cambridgeshire

South bank of the Forty Foot or Vermuyden's Drain, Welches Dam, nr Manea, [TL]468.859, RP Libbey, 9.1972, (**CGE**).

Drain between Byall fen & Langwood fen, South bank, extending some 50 yds from the Eastern end, RP Libbey,18.9.1972 (BSBI Exc.) (**CGE**).

In deep water, Welches Dam, 21.9.1972, leg. Libbey, R.P. (Hb JE Lousley) (**RNG**).

Submerged in 4 ft. (120cm.) of water, Welches Dam, Manea, Cambs, 21st September 1972, Libbey, R.P. and Swann, E.L. No. 563/3 (**NWH**). [This specimen includes three apparently submerged plants, one of which is in fruit, T.J. Pankhurst pers. comm.. 2009].

Vice county 37 Worcestershire

(As *Alisma plantago-aquatica* L. f. *graminifolium*) muddy edge of Westwood Park pool Droitwich in several inches of water, Aug 15 1948, R.C.L. Burgess, "all the plants found in the pool appear to belong to this form although some of the leaf shapes approach f. *lanceolatum*" (**K**, **BM**).

Westwood Pool, Droitwich, 15 August 1953, C.C. Townsend, (conf. I. Björkqvist 1967) (**K**).

(As f. *arcuatum*) margin of Westwood Park Pool, Droitwich, 17 August 1957, N.Y. Sandwith No. 4833, "petals pale whitish lilac, style falcate, no leaves with this material but see corresponding sheet with quite large elliptic leaves in hb. Sandwith", (conf. I. Björkqvist 1967) (**K**).

Westwood Pool, Droitwich, 24 July 1960, J.E. Lousley - linear-leaved plant, leaves c 30 cm, inflorescence c 40cm (**BM**).

Westwood Great pool, nr. Droitwich, 2 September 1991, T. Wells, (conf. R.K. Brummitt 26th February 2004), voucher for Millennium Seed Bank Project (**K**).

Tufted, 4-9 in perennial, muddy shore of pool, Westwood Pool, Droitwich, D.P. Young no. 4889 - terrestrial, broad-leaved plant (**BM**).

Vice county 53 South Lincolnshire

In R. Glen, Surfleet, 8 September 1955, J.E. Lousley, (conf. I. Björkqvist 1967) [leaves all lingulate, up to 80cm long] (**K**).

Surfleet, in River Glen, vc. 53, S. Lincolnshire, 8 September 1955, Ex. herb. J.E. Lousley - deep-submerged plant leaves c 50cm, inflorescence c 1m (**BM**).

Pinchbeck, beside the Roman Bank, 2 1/2 miles north of Spalding, Sept., 1956, J. Gibbins, deep-submerged plant, leaves c 50cm inflorescence c30cm much shorter than leaves (**BM**).

Appendix 2 Strategy for reintroduction of *Alisma gramineum* lej. to Baston Fen NR, Lincolnshire

Introduction

Baston fen lies in an area in South Lincolnshire, characterised by arable fields bounded by ditches, many of which could provide suitable habitat for *Alisma gramineum*. The Lincolnshire Wildlife Trust currently own 45 ha. of Baston Fen, but there are plans to increase this, initially by 39 ha. of reedbed and wet grassland and eventually to 875 ha. of varied fenland habitats. Successful establishment of a self-sustaining metapopulation of *A. gramineum* within the current fen should lead eventually to its spread into these new areas as they become available and possibly into the wider ditch network. For the purposes of this strategy, Baston Fen can be seen as consisting of five units, grading from the High Wash which is ± dry and semi-improved at the western end, to the Low Wash which is wet fen and an area of woodland in the east. The surface soil horizon is mainly peat, grading to loam in places, locally poached or excavated to expose underlying clay.

In 1996 11 *Alisma gramineum* plants were introduced to the margin of a pool toward the eastern end of the site and in 1997 there was a total of 16 plants, of which 12 flowered (Pankhurst and Lansdown *in litt.* 2004). Subsequently, no *A. gramineum* plants have been seen in the area. There are a number of possible explanations for the failure of this introduction, including:

- Single-event introductions very rarely succeed in establishing self-sustaining populations.
- Planting was into peat which may be a poor rooting medium.

In a study of a total of 24 *A. gramineum* populations in the UK and continental Europe (Pankhurst and Lansdown *in litt.* 2004), no populations were found in peat, all were in clays, even where the surface horizons were peaty.

This document represents a recommended strategy for a second attempt at introduction of *Alisma gramineum* to Baston Fen, including proposals for studies to establish the reasons for success or failure of the reintroduction. The overall aim of this reintroduction attempt is to establish a self-sustaining population of *A. gramineum* within Baston Fen but with the possibility that populations will be established outside the fen and so develop a self-sustaining metapopulation over a much wider area.

To maximise the chances of success, recommendations are made to exploit all possible habitats, not only those from which *A. gramineum* has been recorded elsewhere. It is also proposed to introduce plants which already bear ripe, or nearly ripe fruit, with the intention that even if the adult plants do not survive, a good seed-set will occur.

If the reintroduction fails, then the only benefit to be gained from the exercise will be through comprehensive documentation of the work undertaken. Similarly, if successful, one of the greatest benefits from the work will be through thorough documentation of all action taken and the results. For this reason, the reintroduction attempt should not be undertaken unless there is a guarantee of comprehensive documentation of the work.

The reintroduction strategy will be deemed a success if a population is established within which seed is set without intervention within the dormancy capacity of the species.

Planting protocol

Location

The number of possible introduction locations will clearly be dictated by the number of plants available. However, an ideal strategy would be to aim for the plants to be divided equally between the four fields (two parts of the High Wash, Middle Wash and Low Wash), at least up to the first hundred plants. Beyond this number, preference should be shown to planting in parts of the site which meet at least one of the following criteria:

- areas that are heavily poached.
- areas mainly or exclusively on clay, or where poaching breaks through overlying peat to the clay.
- areas where ditches run mainly or exclusively through clays.

The highest priority areas for planting additional plants (i.e. those remaining after 100 plants have been introduced) are the entrances and exits to fields and the shallow parts of ponds that are heavily poached as the site dries in summer. The aim of concentrating on these sites is partly because *A. gramineum* may be a poor competitor and may therefore colonise more effectively where poaching suppresses coarse grasses and partly because it is hoped that mud carried in the hair of cattle will transport seed throughout the site, ensuring the best possibility of plants germinating into suitable habitats.

Number

Each locus for planting should receive at least five plants. In practice, this means that the first hundred plants will be established as five plants at each of five loci in each of the four fields. Subsequent planting may add to these or establish new loci as relevant.

Methods

Experience with cultivation of a wide variety of aquatic and wetland plant species (R.V. Lansdown unpublished data) suggests that the following information may be pertinent to the reintroduction strategy:

- If transplanting mature plants fails, then the seed borne by the mature plants can still enable establishment.
- Repeated wetting and drying appears to have a significant influence over germination, thus drying and subsequent re-wetting of substrate into which a cultivated plant has set seed can result in strong establishment of seedlings.
- Transplanting the substrate in which a plant is growing is a more reliable means of establishment than transplanting bare-rooted plants (even seedlings).
- Transplanting into wet but not submerged soil is more often successful than planting into deeper water, as the combination of buoyancy and wave action can prevent submerged plants developing strong roots when submerged.

It is therefore recommended that transplanting involve:

- Individual plants or groups of plants in a reasonably coherent substrate that can be transplanting into the receptor substrate intact, including the plants.
- Transplanting should take place into substrate at or above water-level at the time of planting.
- Plants should be transplanted when bearing at least some mature seeds.

Experience with other species, such as otter (*Lutra lutra*) and white-tailed eagle (*Haliaeetus albicilla*), has shown that repeated introductions are needed before a self-sustaining population can establish. In the case of the white-tailed eagle, a total of 140 birds was released between 1975 and 1998 (source www.rspb.org.uk). It is very likely that even if there is germination of *A. gramineum* seedlings in the first few years, populations will be very vulnerable until a substantial seed bank can establish. It is therefore proposed that at least 100 plants are introduced to the site each year for five years, with potential to increase this or desist on review at the end of this period. This will depend upon an annual supply of plants as has been established for the 2009 season by Plantlife at the Cambridge Botanical Gardens in 2009.

There are three possible outcomes of the proposed planting:

1) That the transplanted individuals will die and no seeds germinate.
2) That the transplanted individuals will die and some seed germinates.
3) That the transplanted individuals survive and some seed germinates.

Recommended survey and monitoring

As noted in the introduction to this strategy, the long-term value of this introduction from comprehensive documentation is likely to be equal to that of establishing of a self-sustaining population of *A. gramineum*, particularly if the reintroduction is not successful. To be effective, documentation must include the following data collected on each set of transplants:

- number of plants.
- proportion of plants bearing seed.
- approximate number of flowers on each plant (estimate).
- precise location (GPS).
- depth of water at transplant site at time of planting (as well as subsequent levels over the site).
- nature of receptor site substrate (there is no information available on the sort of data that it would be useful to record and so, initially, a thorough characterisation of the substrate, including factors such as particle size/category, moisture, pH and organic matter content would be valuable).
- shading.

Equally, the following information needs to be gathered on any plants that establish (given that plants cannot reliably be identified when not flowering this will only apply to flowering plants.

- number of plants.
- approximate number of flowers on each plant.
- precise location (GPS).
- depth of water at flowering.
- nature of receptor site substrate (as above).
- shading.

To locate plants that establish, it is vital that all suitable habitat is surveyed in June and again in September during each of the five years. The June survey is intended to locate plants that germinate early in the year and which might not survive the summer, while the September survey is intended to locate plants which germinate late in the season.

Surveys in the wider area

It is clear that the previous introduction attempt was unsuccessful in establishing a self-sustaining population of *A. gramineum* on Baston Fen. However, the species is sufficiently poorly known and difficult to identify that it is not easy to be absolutely certain that there are no populations in the wider area.

The idea of establishing a self-sustaining population of *A. gramineum* at Baston Fen is potentially a very positive exercise. However, if a self-sustaining meta-population exists in the Surfleet area, less than

15km to the north-east, then the relationship between the two populations needs to be clear before a reintroduction attempt is made. In 2006 plants were found within the area containing the metapopulation near Surfleet which were thought to be this species; however doubts have subsequently been expressed as to the identity of the plants (M.A. Palmer pers. comm. to T.J. Pankhurst 2006). It is clearly extremely important to clarify the condition of the Surfleet metapopulation before introduction of plants to Baston obscures the natural situation.

Lousley (1957) illustrated the known distribution of *A. gramineum* in 1955 and his map apparently shows an established metapopulation. A specimen in the herbarium of the Royal Botanic Gardens at Kew (labelled “In R. Glen, Surfleet, 8 September 1955, J.E. Lousley” and confirmed by the monographer of the genus Ingmar Björkqvist in 1967) has only lingulate leaves, which are up to 80cm long and overtop the inflorescence, clearly indicating that this was a perennial submerged plant (*sensu* Pankhurst and Lansdown *in litt*. 2004). Whilst some of the waterbodies indicated as supporting this species on the map have been surveyed since 1990, there has apparently been no comprehensive survey of these sites by someone familiar with the species in recent years and in particular, there appears to have been no water-based (i.e. dry suit, scuba or suitable boat) survey of the River Glen to locate perennial submerged populations.

The reintroduction strategy proposed here involves Baston Fen, however not only is the fen linked to the ditch network outside the site, but there is a possibility that animals may transport seed outside the site into suitable habitat. It is important that surveys to locate establishment of the species as part of this reintroduction include:

- All potentially suitable habitat within the fen, including water-based survey of deep or broad watercourses.
- All waterbodies with a direct hydrological link downstream of Baston Fen, including water-based survey of deep or broad watercourses.
- Waterbodies outside the site that are sufficiently close to risk receiving seed transport by animals (e.g. pools in the arable fields south of Baston Fen, gravel pits toward Baston and Cross Drain SSSI, including water-based survey of deep or broad watercourses).

The future - Baston and beyond

The sheer enormity of the area of potentially suitable; the limited familiarity of most surveyors with the species; the fact that it can occur in many different forms, some of which are very difficult to see, means both that it would be prohibitively expensive to survey comprehensively for *A. gramineum* and that it is not very likely to be found by non-specialised surveys. However, it is very possible that populations exist outside the known areas, both in South Lincolnshire and elsewhere in the fens. Successful establishment of a metapopulation at Baston Fen could obscure information gathered in the future on new and even existing populations. It is therefore important to minimise the potential effect of the reintroduction programme.

The ecological profile, drafted by Pankhurst and Lansdown (*in litt.* 2004) has not been completed and badly needs to be brought up to date. The information that it contains should provide all the information necessary to produce this strategy, as well as informing associated work both at Baston Fen and elsewhere. Elements of this strategy depend at least in part upon factors which are either unknown or, at best, insufficiently known, at least in part because the profile has not been completed. The following actions should be as high priority to support and inform work on this species:

1) Survey known sites to establish current situation prior to introduction at Baston.
2) Preparation of a field crib sheet or *aide memoire* for all ditch surveyors.
3) Investigate potential dispersal agents.
4) Investigate dormancy capacity.
5) Complete Ecological profile.

References

Lousley, J.E. 1957. Alisma gramineum in Britain. Proc. BSBI 2, 346-353.

Pankhurst, T.J. and Lansdown, R.V. (*in litt*. 2004). The ecology and conservation status of ribbon-leaved water-plantain (*Alisma gramineum* Lej.) in the United Kingdom. Copy on Natural England files.

Appendix 3 A report on the application of DNA sequence analysis to determine the species status of field collected specimens of *Alisma*

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Summary

DNA barcoding techniques are shown to give a quick and reliable method for discriminating between morphologically indeterminable vegetative material of a range of aquatic macrophytes. The rDNA ITS region was identified as a candidate marker for the analysis of DNA sequence variation in *Alisma*, based on earlier published molecular phylogenetic analysis of the genus. The DNA region was interrogated by standard PCR and DNA sequencing methodologies and six major sequence groups were recovered among the 21 samples provided. Four samples had sequence types determined to be consistent with ITS sequences published for aquatic macrophytes: *Sparganium erectum* (n=2), *Baldellia ranunculoides* (n=1), and a *Sagittaria* sp. (n=1). All remaining samples had ITS sequence types highly consistent with those published for *Alisma* (n=17). The ITS sequences generated from the morphologically determined reference samples of *A. lanceolatum* and *A. plantago-aquatica* had virtually identical sequences to those published for each species on the Genbank database. None of the 21 samples provided generated ITS DNA sequences consistent with published sequences for *A. gramineum* (GenBank accessions DQ339088, DQ468391), based on the two private nucleotide substitutions that distinguish the ITS sequences of *A. gramineum* from *A. lanceolatum* and *A. plantago-aquatica*. Multiple nucleotide substitutions were identified from the DNA alignments to allow for reliable separate ITS sequence groups for *A. lanceolatum* and *A. plantago-aquatica*, and to assign a DNA-based species diagnosis for each specimen.

Introduction

Ecologists, field surveyors and conservationists are often faced with the necessity of identifying material which lacks key diagnostic characters. Experience may enable qualified guesses to be made but these are largely subjective and where expensive resource implications, or legal issues associated with protected species are involved, a more objective and evidence based method is necessary. A case in point is that of *Alisma gramineum*, believed to be Critically Endangered, and protected under the Wildlife and Countryside Act, Schedule 8. This, like many submerged macrophytes, apparently shows considerable morphological plasticity, but its rarity and our inability to accurately identify non-flowering examples compromises our ability to assess the true extent, distribution ,abundance and even the ecology of this plant.

Expert taxonomists and field researchers suggest it is not currently possible to identify *Alisma* plants by morphological means when they are growing submerged and not flowering. Many indeterminable plants now occur which may be *A. gramineum* in areas in which it has hitherto been recorded, or from where we might conceivably expect it from our limited understanding of its ecology. Some method is thus needed whereby an unequivocal determination can be made for any sampled individual. Various molecular techniques may be applicable to address this, including studies of protein genetic variation (allozymes), as well as a range of DNA based approaches. For practical reasons the latter now generally afford a more robust and cost-effective approach. Furthermore, recently published phylogenetic studies in the Alismatales (Jacobsen and Hedren, 2007) have provided the necessary framework for comparative and analytical purposes, whereas the adoption of an allozymic approach would necessitate potentially lengthy optimisation of protocols and the need for broader sampling to create baselines by which comparable taxon specific markers might be identified.

Through parallel evolution many aquatic macrophytes show considerable morphological similarity as a consequence of the adoption of similar strategies to deal with particular suites of environmental variables. The difficulty for the field investigator thus extends beyond the discrimination of closely related congeneric taxa to even discriminating plants of different families. DNA based approaches, such as DNA barcoding, are an excellent way to resolve this and can then help inform and revise morphologically based methods.

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. Until now, biological specimens have primarily been identified using morphological features, however, if a specimen is damaged, or is in an immature stage of development, eg. non-flowering *Alisma* , even specialists may be unable to make identifications. DNA based approaches such as DNA Barcoding may solve these problems (where clear taxon specific differences have been identified) because even non-specialists can obtain DNA from tiny amounts of tissue and then compare the sequence data generated with that already published.

DNA Barcoding has fixed upon standard regions of the genome for wide-scale cross comparability. The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene (“CO1”). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have, for their broad applicability, been approved as the barcode regions for plants. However, other regions may be more suited to particular taxonomic issues, their utility is however restricted by the availability of accurate comparative data upon which to make analyses. This study uses the internal transcriber spacer (ITS) of the ribosome DNA repeat (rDNA), a region already shown (Jacobsen and Hedrén, 2007) to evolve at a rate ideal to discriminate taxa at the specific level in this particular group of organisms. They showed that the biggest distinction in *Alisma* lies between the *A. gramineum*/ *A. wahlenbergii* group and the *A. plantago-aquatica* group (including the other UK native *A. lanceolatum*). Therefore, using the same markers, the distinction between *A. gramineum* plants and other *Alisma* species occurring in the same area should be unambiguous.

Specific Objectives

To apply molecular markers as a diagnostic tool to assess the species status of morphologically undetermined field collections with special reference to candidate populations of the UK threatened taxon, *A. gramineum*.

Methods

Selection of candidate marker

The molecular systematic and taxonomic literature was surveyed for previously published molecular analysis of *Alisma* to identify candidate DNA markers for DNA-sequenced bases species discrimination. Searches of electronic scientific publication databases identified one publication on the molecular phylogenetic analysis of the genus *Alisma* (Jacobson and Hedrén 2007). This study had sampled eleven species of *Alisma*, including all three diploid species reported in the British Isles (following Stace, 2010): *A. gramineum*, *A. lanceolatum* and *A. plantago-aquatica*. This study utilised RAPD fragment length variation markers and in addition two DNA sequenced-based markers: transfer RNA leucene intron (*trn*L) region of the chloroplast genome and the internal transcriber spacer (ITS) of the ribosome DNA repeat (rDNA). Published DNA sequences were retrieved from online DNA sequence database (GenBank) and were aligned using clustal W automated alignment algorithm (Thompson 1994) and MEGALIGN software v. 6.00 (Lasergene, DNAstar). This identified the *trn*L intron had insufficient nucleotide variation to reliably discriminate between *Alisma* taxa, while the ITS fragment had up to 26 fixed nucleotide differences among the pairwise comparisons of GenBank submissions for *A. gramineum*, *A. lanceolatum*, *A. plantago-aquatica*. On the assumption that the original species identification used by Jacobson and Hedrén (2007) was correct, this finding strongly indicated that sequence differences in the ITS fragment could function to discriminate between *Alisma* taxa present in the UK, and diagnostic as a diagnostic tool to verify the presence of *A. gramineum* among the field collected specimens.

Laboratory procedures

Unique accession codes were applied to each of the 21 specimens provided by Richard Lansdown (remaining samples and original packaging available on request). Total genome DNA was isolated by Biosprint Plant DNA extraction kit and the Biosprint automated DNA extraction robot, following to the manufactures specifications (Qiagen). DNA quantity and purity was determined by micro-spectrophotometry (Nana-Drop) (Table 1). DNA dilutions of 50ng/µl were prepared for each sample prior to amplification of the ITS rDNA fragment by the polymerase chain reaction (PCR). The ITS rDNA fragment was amplified using the ITS4 and ITS17SE primers, sequences, as specified in Jacobson and Hedrén (2007), under the following thermocycle conditions: 1 cycle of 94ºC 1 minute, followed 30 cycles of by 94ºC 1 minute, 54ºC minute, 72ºC 3 minutes, and 1 cycle of 72ºC 8 minutes. Amplifications were performed in a reaction volume of 25µl and included the following: 1µl 50ng DNA, 9µl water, 5µl Betaine, 2.5µl 100mM $(NH_4)SO_4$ buffer, 1.5µl 50mM $MgCl_2$, 2.5µl dNTPs (5mM of each nucleotide), 1 µl 10µM of each primer and 5units of Taq polymerase (Bioline). In addition to the 21 test samples, additional reactions were constructed from the same master-mix of reagents to function as negative controls, substituting the DNA extract for an additional volume of molecular biology grade water. PCR products were fractionated by agarose gel electrophoresis, using gels of 0.75% concentration and 1xTAE buffer, and a constant 100 volts. The PCR products were visualised under UV light and Gel Red reagent and the fragment sizes were determined using side-by-side comparison to a DNA ladder (Bioline Hyperladder I) that was loaded on the gel prior to electrophoresis. The remaining volume (21µl) of amplification reactions was dispatch to the NHM sequencing facility, for purification of PCR products (Millipore), sequence reaction assembly, thermocycling and sequence reaction assay. Both DNA strands were sequenced using BigDye Terminator cycle sequencing kit v1.3 (Applied Biosystems, ABI) and analysed on an ABI 3730 capillary sequencing analyzer.

Sequence assembly

Contigs were constructed from electrophoretograms of the forward and reverse strands sequences of each of the 21 PCR products, using SEQMAN software v. 6.00 (Lasergene, DNAstar), employing conservative manually edited to correct for minor base-call error. The resulting consensus sequences for the 21 PCR products were aligned by Clustal W automated alignment algorithm (Thompson 1994) and the MEGALIGN software (Lasergene, DNAstar). Alignments were then manually adjusted after visual inspection, whereby the bases calls of individual positions for individual consensus sequences were edited, following cross-checking of the original electrophoretograms.

Data analysis

The previously published ITS sequences for *Alisma* (Jacobson and Hedrén 2007) were retrieved from GenBank (DQ339079-DQ339090) and were aligned against the newly generated sequences by Clustal W automated alignment algorithm (Thompson 1994) and the MEGALIGN software v. 6.00 (Lasergene, DNAstar). Alignments were further manually adjusted to minimise remaining local alignment inconsistencies. ITS sequences which deviated substantially from those previously published as *Alisma* (Jacobson and Hedrén 2007) were used to interrogate the GenBank database of published DNA sequences. BLAST searches were performed using the nBLAST tool. Further BLAST searches of the GenBank database were performed to validity that ITS sequences specific to *Alisma* had been generated among the remaining test samples. Finally, nucleotide positions with fixed character state differences that distinguished between morphologically verified specimens of *Alisma lanceolatum* and *A. plantago-aquatica* (samples AL2-AL7) were then used to infer the species status of morphologically undetermined specimens (AL8-AL21).

Results

Total genomic DNA was isolated from each of the 21 specimens at a concentration of between 47 and 239 ng/µl, and at a 260/280nm measure of DNA/RNA purity of 1.78 to 2.16, consistent with a good quality of DNA extraction (Table 1). Amplification of the ITS rDNA fragment by PCR yielded a single-banded PCR product for each of the 21 DNA extractions, at a fragment size of c.800-c.900 base pairs in length (Fig. 1). Visual inspection of the agarose gels also determined there were no PCR products where present in the samples that lacked DNA (negative controls, Fig. 1), confirming the PCR products of the test samples were due to the presence of added DNA [*Alisma*] only.

Contigs were constructed from the forward and reverse DNA strand sequences of all 21 test samples. For all samples the forward and reverse DNA sequences had a minimum of 97% sequence identity in the region where the two strands overlapped. After minor editing of sequences, the alignment of 21 DNA sequences recovered nine unique ITS sequence variants. A full alignment of sequences is presented in Appendix 1. Three sequences formed a distinct haplogroup that was designated as type A, differing only by nucleotide changes at alignment positions 408 and 848. Samples of haplogroup type A included those previously morphological determined by R. Lansdown as *A. lanceolatum* (AL2, AL3, AL4). Three additional sequences formed a distinct haplogroup that was designated as type B, and which differ by a nucleotide change at a single alignment position 164. Samples of haplogroup B included those previously morphological determined by R. Lansdown as *A. plantago-aquatica* (AL5, AL6, AL7). Of the remaining three sequence types (C, D, E), type C was present in the sample morphologically determined by R. Lansdown as *Sparganium erectum* (AL1), and also morphologically undetermined sample AL9. The remaining sequences types D and E were recovered from samples AL12 and AL16 respectively, and each differs significantly from all other sequences, indicating a more distant taxonomic relationship to *Alisma*.

BLAST searches of the GenBank online DNA sequence database, using sequence type D (AL12) as the query sequence resulted in a highest total score of 1256, corresponding to accession entry AY395996, having a maximum sequence identity of 97% and query sequence coverage of 88%. This GenBank entry is published as the ITS rDNA region of *Sagittaria natans*. In a second search, the Genebank database was queried using sequence E (AL16)., and resulted in the highest total score of 1279, corresponding to accession entry DQ339092, having a maximum sequence identity of 99% and query sequence coverage of 77%. This GenBank entry is published as the ITS rDNA region of *Baldellia ranunculoides*. In both searches of the GenBank, accessions entries for *Alisma* samples showed high maximum sequence identity (96-100%), but only for a query coverage of 21-33% of the total nucleotide sequence length submitted, corresponding to the conserved 5.8s gene coding domain that separates the two non-coding sub-regions (ITS1, ITS2) of the ITS rDNA region. In this respect, the higher sequence scores over the much higher fraction of the submitted query sequence are more strongly indicative of identifying the correct species/sequence.

To further confirm that the correct DNA fragmented had been amplified and sequenced from each *Alisma* field collected sample, putative *Alisma* ITS sequences were used to interrogate the Genbank database. Sequence type A (putative *A. lanceolatum*) of sample AL2 was used to query GenBank by BLAST search. Accession number DQ339079 gave the highest total score of 1284 and a maximum sequence identity of 100% and query sequence coverage of 79%, and is recorded as the ITS rDNA region of *Alisma lanceolatum*, submitted by Jacobson and Hedrén (2007). This process was repeated using sequence type B (putative *A. plantago-aquatica*) of sample AL5.

Accession number AY588940 gave the highest total score of 1424 and a maximum sequence identity of 99% and query sequence coverage of 88%, and is recorded as the ITS rDNA region of *Alisma plantago-aquatica*, submitted by Chen *et al*. (unpublished, 2004). A slightly lower total score and sequence identity was achieved for the *Alisma plantago-aquatica* ITS sequence submitted by Jacobson and Hedrén (2007), due to this entries shorter DNA sequence length compared to that of Chen *et al*. These findings corroborate the previous morphological determinations for AL2 and AL5, and that haplogroup A sequences can be assigned to *A. lanceolatum*, and haplogroup B can be assigned to *A. plantago-aquatica.* Furthermore, in conjunction with the earlier BLAST results, this would indicate that none of the six sequence types recovered correspond to submit ITS sequences for *A. gramineum* (GenBank submissions; DQ339088, DQ468391). ITS sequences of *A. gramineum* can be distinguished from both *A. lanceolatum* and *Alisma plantago-aquatica* by two private nucleotide substitutions at alignment positions 214 and 678 (Appendix 1).

Having established the validity of the ITS sequences for the morphological reference samples of *A. lanceolatum* and *Alisma plantago-aquatica* (AL2-AL7), it was then possible to identify diagnostic nucleotide differences to separate the species and reliably assign species status to the morphological undetermined samples (AL8-AL21). Alignment positions with fixed nucleotide differences to separate the two species were present at 26 positions. As an illustration, a summary of the distribution of several diagnostic nucleotide changes among the 21 specimens is presented in Table 1, along with the inferred species status of each sample. This covers only a subset of the total nucleotide variation, predominantly over positions 1-300, and further informative positions can be observed from the complete alignment (Appendix 1).

Conclusions

The ITS rDNA region amplified by ITS4 and ITS17SE primers identified nucleotide sequence variation that could discriminate between samples of *Alisma* and other genera of aquatic macrophytes among 21 specimens supplied by R. Lansdown. This include two anomalous samples (AL12, AL16), most likely of *Baldellia ranunculoides* and a species of *Sagittaria*. Exact determination of the *Sagittaria* was not possible, due to an absence of ITS sequences in the GenBank database for all four species recorded as present in the British Isles (Stace, 2010).

Of the remaining ITS sequences variation compatible with *Alisma* taxa, these sequences were highly consistent with those reported for *A. lanceolatum* and *Alisma plantago-aquatica* only (Jacobson and Hedrén 2007). Assuming the latter study to have correctly morphologically determined specimens of *A. gramineum*, we did not recover the nucleotide variations at critical alignment positions 214 and 678 to identify *A. gramineum* among the 21 field samples supplied. This however does not indicate that *A. gramineum* is absent among the sampling site populations, merely among the samples provided.

References

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Specimen Nos.	Taxa/unknown	Locality	Coll. Date	ng/ul	260/280 ratio	PCR product size	Forward Strand start/stop trimming	Reverse Strand start/stop trimming	% match in overlapped regions
AL1	Sparganium erectum	TF21501	16.09.10	239.50	2.06	c.850bp	20/780	20/779	98
AL2	A. lanceolatum	Sheepwalk lake	02.08.10	204.80	2.12	c.900bp	21/890	22/820	98
AL3	A. lanceolatum	Basten Fen	02.08.10	129.40	2.00	c.900bp	23/850	20/720	98
AL4	A. lanceolatum	Over/Alney	03.08.10	194.00	2.04	c.900bp	21/900	20/720	98
AL5	A.plantago-aquatica	Over/Alney	03.08.10	236.50	2.09	c.900bp	21/895	23/840	98
AL6	A.plantago-aquatica	Basten Fen	02.08.10	150.70	2.05	c.900bp	20/880	21/825	98
AL7	A.plantago-aquatica	Loch Tsmia	31.07.10	231.70	1.64	c.900bp	20/885	20/815	98
AL8	Old Sea Drain	Cuckoo Lane	15.09.10	130.90	1.78	c.900bp	20/820	20/780	100
AL9	West of Surfleet	TL24785	16.09.10	150.20	2.16	c.850bp	22/785	21/780	98
AL10	Surfleet Ditch	TL26017 28686	16.09.10	166.20	2.14	c.900bp	18/905	12/785	98
AL11	Surfleet Ditch	TL26017 28686	16.09.10	147.50	1.94	c.900bp	24/895	20/820	98
AL12	Surfleet Sea End	TL26537 28720	16.09.10	114.30	1.95	c.850bp	20/820	13/810	98
AL13	Cross Drain	Rose Cottage 1	14.09.10	70.35	1.81	c.900bp	20/625	14/780	99
AL14	Cross Drain	Rose Cottage 2	14.09.10	94.67	2.03	c.850bp	19/905	14/790	98
AL15	Willow Tree Fen	Drain From N5 1	15.09.10	200.20	1.84	c.900bp	20/905	19/780	98
AL16	Willow Tree Fen	Drain From N5 2	15.09.10	153.00	2.06	c.850bp	20/892	18/870	99
AL17	Basten Fen	Counter Ditch	09.09.10	120.50	2.00	c.900bp	19/890	20/840	98
AL18	Counter Drain	Basten Fen 1	14.09.10	94.23	2.01	c.900bp	20/893	20/350	97
AL19	Counter Drain	Basten Fen 2	14.09.10	47.37	1.88	c.900bp	18/896	16/887	98
AL20	Counter Drain	Basten Fen 3	14.09.10	165.10	2.01	c.900bp	20/890	18/750	98
AL21	Counter Drain	Basten Fen 4	14.09.10	107.20	2.03	c.900bp	20/750	15/636	99

Table 1A Sample information, DNA concentration, PCR fragment sizes and initial DNA sequence editing information.

BM code	Taxon Field ID	Locality	DNA ID
AL1	Sparganium emersum	River Glen, TF21501,25555	Sparganium ?erectum
AL2	Alisma lanceolatum	Sheepwalk Lake	Alisma lanceolatum
AL3	Alisma lanceolatum	Baston Fen	Alisma lanceolatum
AL4	Alisma lanceolatum	Alney Island, Over	Alisma lanceolatum
AL5	Alisma plantago-aquatica	Alney Island, Over	Alisma plantago-aquatica
AL6	Alisma plantago-aquatica	Baston Fen	Alisma plantago-aquatica
AL7	Alisma plantago-aquatica	Loch Tima, Langholm	Alisma plantago-aquatica
AL8	Indet.	Old Sea Drain, Cuckoo Lane	Alisma plantago-aquatica
AL9	Indet	W. of Surfleet, TF 24785,27981	Sparganium ?erectum
AL10	Indet	Surfleet Ditch, TF26017,28686	Alisma plantago-aquatica
AL11	Indet	Surfleet Ditch, TF26017,28686	Alisma plantago-aquatica
AL12	Indet	Surfleet Seas End, TF26537,28720	Sagittaria sp.
AL13	Indet	Cross Drain, Rose Cottage #1	Alisma lanceolatum
AL14	Indet	Cross Drain , Rose Cottage #2	Alisma lanceolatum
AL15	Indet	Willow Tree Fen	Alisma plantago-aquatica
AL16	Indet	Willow Tree fen	Baldellia ranunculoides
AL17	Indet	Baston Fen, Counter Ditch	Alisma plantago-aquatica
AL18	Indet	Counter Drain, Baston Fen	Alisma lanceolatum
AL19	Indet	Counter Drain, Baston Fen	Alisma plantago-aquatica
AL20	Indet	Counter Drain, Baston Fen	Alisma lanceolatum
AL21	Indet	Counter Drain, Baston Fen	Alisma plantago-aquatica

Table 1B Summary of sample identities as confirmed by DNA

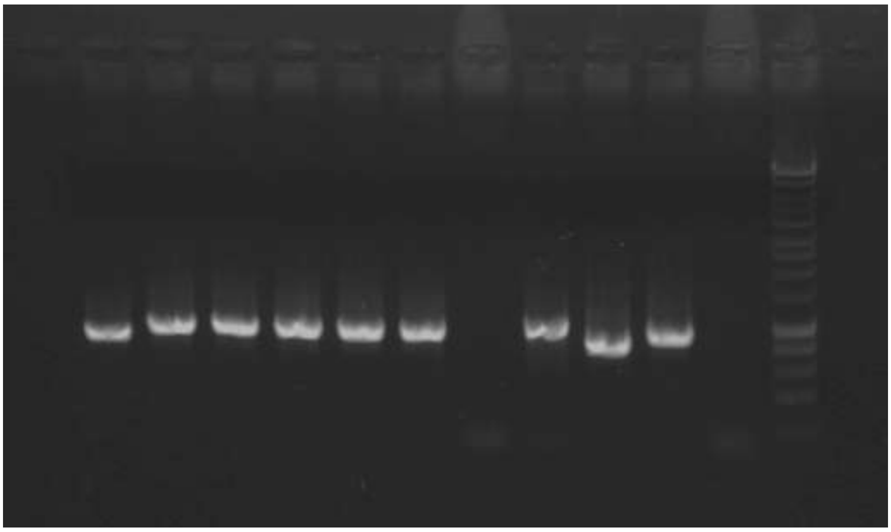
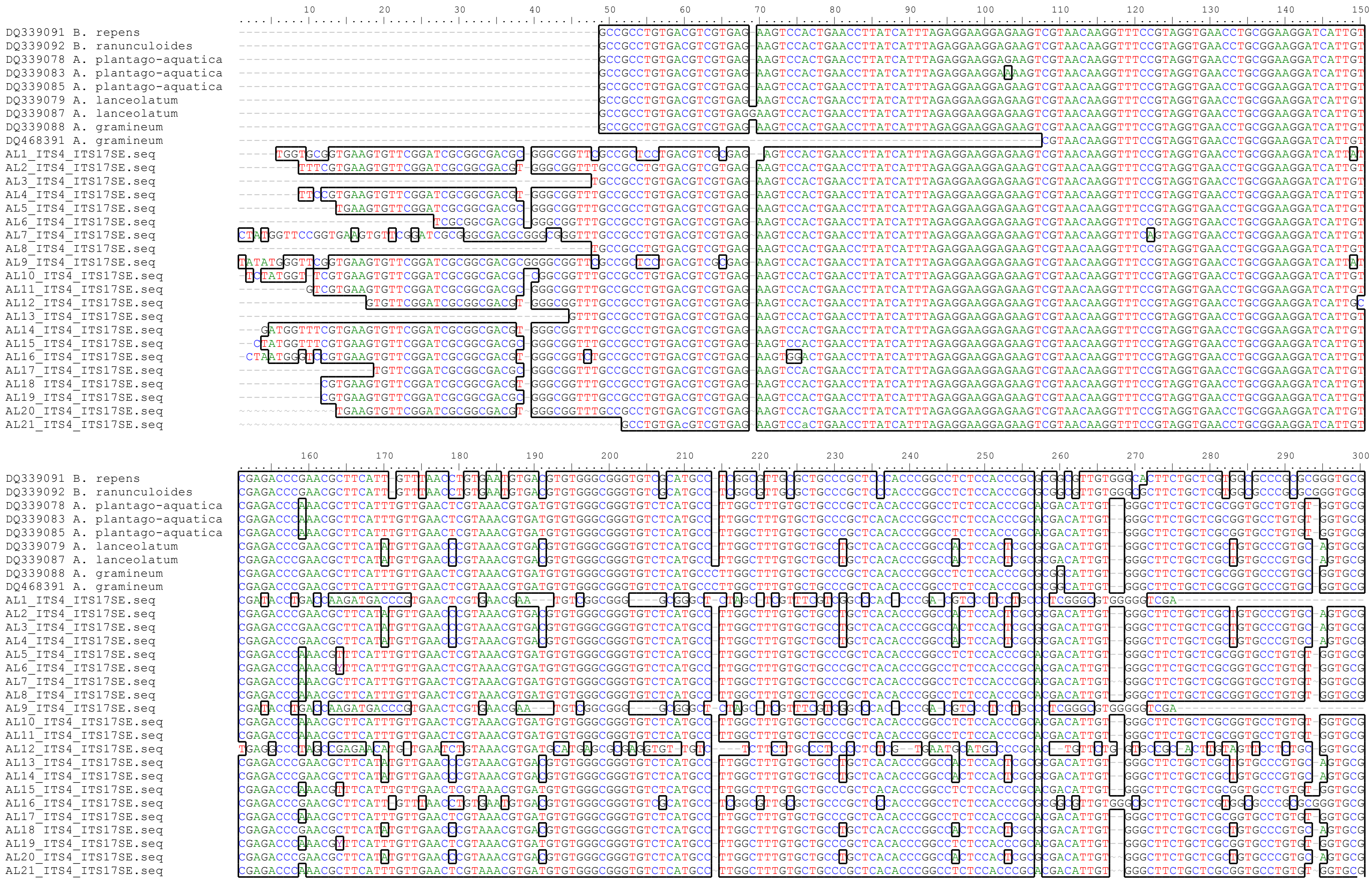
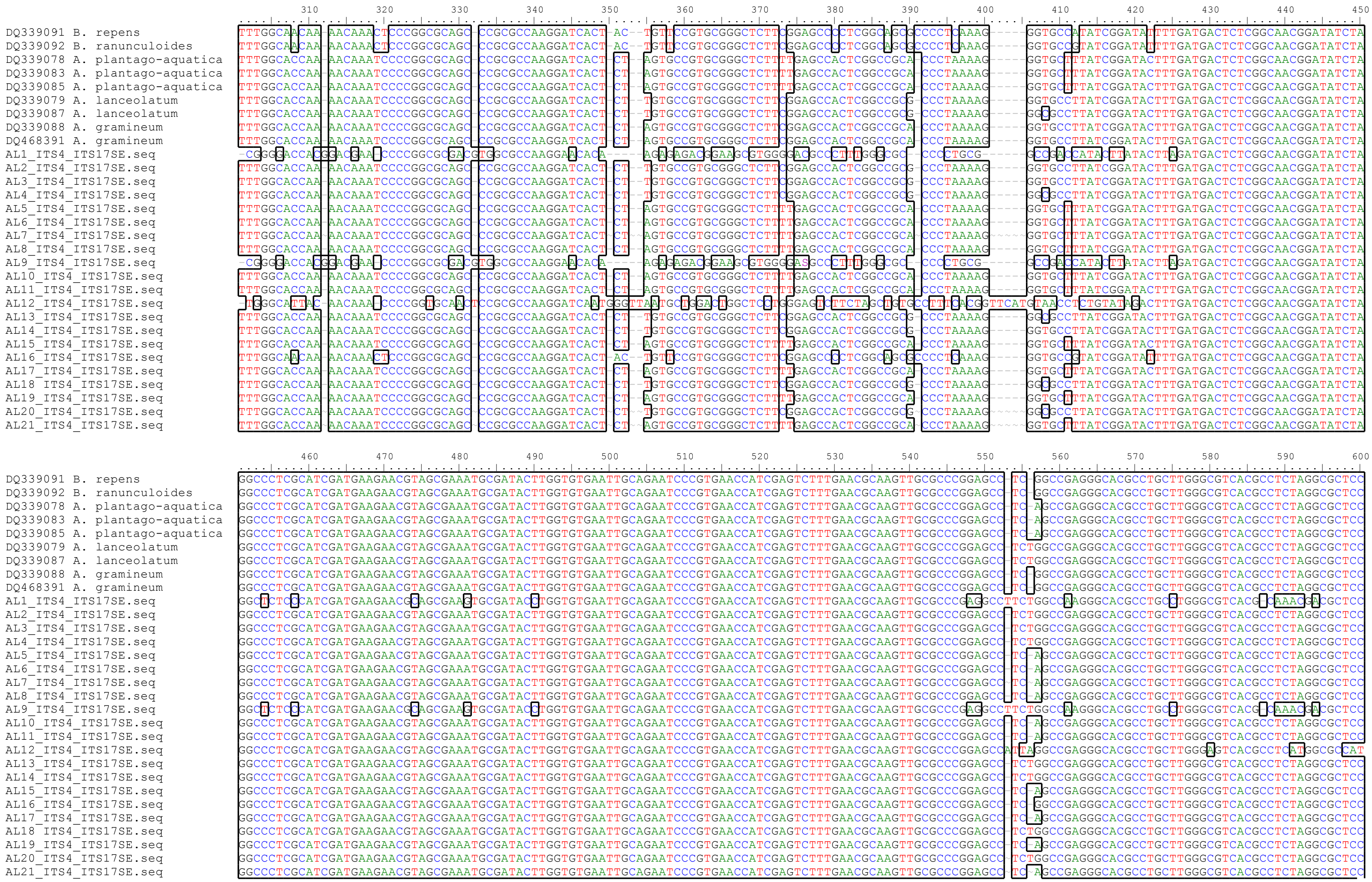


Figure 1 An example of agarose gel electrophoresis assay of ITS PCR products. Lane order left to right, AL1 to AL10, negative control, DNA ladder. AL7 has failed to amplify, and was further diluted to 25ng/µl to counter excessive salt carry-over from the DNA extraction procedure, and subsequently generated a single-banded PCR product of c.900bp, following the outlined laboratory procedures.

			Diagnositic DNA alignment positions																																					
Source	**Taxon**	**data source**	**54**	**55**	**56**	**65**	**74**	**75**	**149**	**150**	**151**	**155**	**158**	**159**	**161**	**163**	**164**	**165**	**166**	**167**	**168**	**169**	**170**	**171**	**179**	**191**	**214**	**220**	**224**	**236**	**231**	**246**	**253**	**257**	**260**	**283**	**289**	**293**	**295**	**678**	**Inferred DNA diagnosis**
Published		**Genbank**																																							
Jacobson &	Baldellia repens	DQ339091	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	T	-	C	C	-	G	C	C	C	T	C	G	G	G	C	C	G	C	Baldellia repens
	Baldellia ranunculoides	DQ339092	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	T	-	C	C	-	G	C	C	C	T	C	G	G	G	C	C	G	C	Baldellia ranunculoides
Hedren 2007	Alisma plantago-aquatica	DQ339078	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Alisma plantago-aquatica	DQ339083	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Alisma plantago-aquatica	DQ339085	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Alisma lanceolatum	DQ339079	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Alisma lanceolatum	DQ339087	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Alisma gramineum	DQ339088	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	T	T	T	T	G	T	T	A	C	T	C	G	G	G	C	C	G	T	Alisma gramineum
unpublished	Alisma gramineum	DQ468391	?	?	?	?	?	?	G	T	C	A	C	G	A	G	C	T	T	C	A	T	T	T	T	T	G	T	T	A	C	T	C	G	G	G	C	C	G	T	Alisma gramineum
Contract		**Sample**																																							
reference	Sparganium erectum	AL1	T	C	C	C	C	C	A	T	C	A	T	G	C	A	A	G	A	T	G	A	C	C	C	-	-	-	T	A	G	G	C	C	C	G	-	-	-	C	Sparganium erectum
	Alisma lanceolatum	AL2	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Alisma lanceolatum	AL3	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Alisma lanceolatum	AL4	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Alisma plantago-aquatica	AL5	C	T	G	T	C	C	G	T	C	A	C	A	A	G	T	T	T	C	A	T	T	T	T	T	-	T	T	A	C	G	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Alisma plantago-aquatica	AL6	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C/T	T	T	C	A	T	T	T	T	T	-	T	T	A	C	G	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Alisma plantago-aquatica	AL7	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	G	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
Contract Test																																									
	Unknown	AL8	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL9	T	C	C	C	C	C	A	T	C	A	T	G	C	A	A	G	A	T	G	A	C	C	T	-	-	-	T	A	G	G	C	C	C	G	-	-	-	C	Sparganium erectum
	Unknown	AL10	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL11	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL12	C	T	G	T	C	C	G	C	T	G	C	T	G	C	G	A	G	A	A	C	A	T	C	C	-	T	T	A	C	G	C	A	A	A	C	C	G	T	Sagittaria species#
	Unknown	AL13	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Unknown	AL14	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Unknown	AL15	C	T	G	T	C	C	G	T	C	A	C	A	A	G	T	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	C	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL16	C	T	G	T	G	G	G	T	C	A	C	G	A	G	C	T	T	C	A	T	T	-	C	C	-	G	C	C	C	T	C	G	G	C	C	C	G	C	Baldellia ranunculoides
	Unknown	AL17	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	C	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL18	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Unknown	AL19	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C/T	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	G	A	G	T	T	G	C	Alisma plantago-aquatica
	Unknown	AL20	C	T	G	T	C	C	G	T	C	A	C	G	A	G	C	T	T	C	A	T	A	T	C	C	-	T	T	A	T	A	T	G	A	T	C	C	A	C	Alisma lanceolatum
	Unknown	AL21	C	T	G	T	C	C	G	T	C	A	C	A	A	G	C	T	T	C	A	T	T	T	T	T	-	T	T	A	C	T	C	A	A	G	T	T	G	C	Alisma plantago-aquatica

Table 2 Summary of nucleotide variation (incomplete) in the ITS rDNA fragment, and inferences of species status for samples AL1-AL21 with reference to GenBank accessions of ITS for *Alisma* and other moncotelydenous macrophytes. A complete alignment of ITS sequences is provided in Appendix 1.

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DQ339092 B. ranunculoides
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DQ339083 A. plantago-aquatica
DQ339085 A. plantago-aquatica
DQ339079 A. lanceolatum
DQ339087 A. lanceolatum
DQ339088 A. gramineum
DQ468391 A. gramineum
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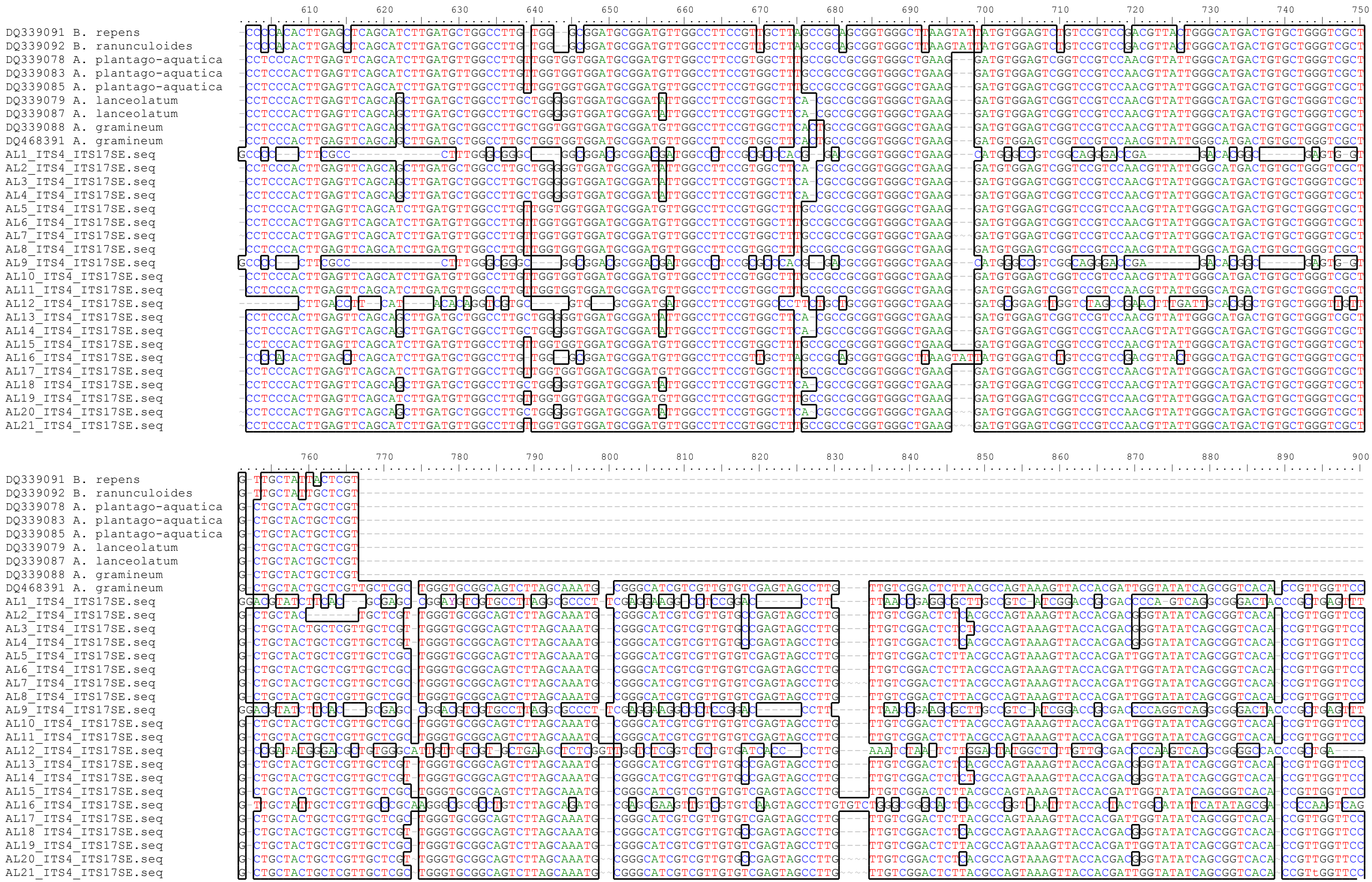
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DQ339078 A. plantago-aquatica
DQ339083 A. plantago-aquatica
DQ339085 A. plantago-aquatica
DQ339079 A. lanceolatum
DQ339087 A. lanceolatum
DQ339088 A. gramineum
DQ468391 A. gramineum
AL1_ITS4_ITS17SE.seq
AL2_ITS4_ITS17SE.seq
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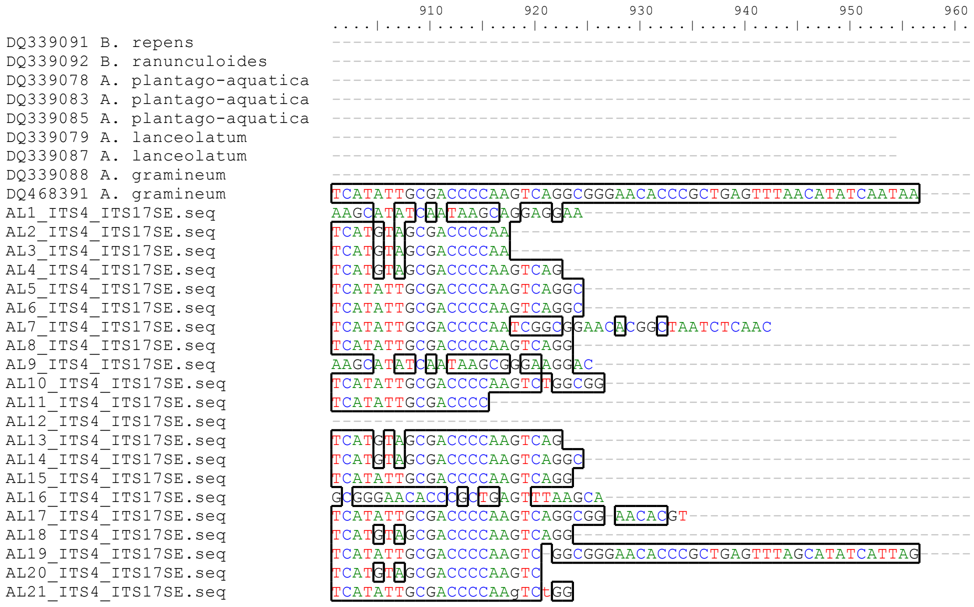

Appendix 1 Alignment of ITS sequences from selected GenBank accessions of *Alisma* and other macrophytes from the molecular phylogenetic study of Jacobson and Hedrén (2007) and the 21 test samples provided by R. Lansdown. Nucleotide mismatches are outlined.