Pond survey and DNA sampling

Towards DNA-based metrics for assessment of pond condition

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Foreword

DNA-based methods offer a significant opportunity to change how we monitor and assess our natural environment. Natural England has been exploring the potential of these methods for environmental monitoring for several years.

One area of particular interest is the development of novel indicators and metrics, which are based on DNA data. We are looking to develop novel metrics for the assessment of pond ecological condition and have brought together a partnership to explore this. We plan to analyse a subset of existing pond data and DNA samples, which are being collected in current pond surveys. One such is the England Ecosystem Survey (EES) ponds element, which is part of the Defra funded Natural Capital and Ecosystem Assessment (NCEA) programme. The NCEA has been set up to collect data on the extent, condition and change over time of England's ecosystems and natural capital, and the benefits to society.

In discussion with the partnership, we realised that supplementing our existing datasets with data from additional 'high quality' ponds (those in near-pristine condition) would aid metric development. This project has selected and surveyed a set of 'high quality' ponds across the country, covering a range of land classes relevant to pond typology. The survey methodology was matched to that of the EES, helping to ensure the datasets are comparable.

Freshwater Habitats Trust have delivered this work in partnership with Natural England, as part of this wider DNA-based pond metric development project. The data collected in this project (DNA and other survey data) will now be analysed alongside data from existing pond surveys, to begin to derive novel metrices for pond condition based on DNA data. This analysis work will be published when available.

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Executive summary

Project background and aims

Natural England is seeking to develop ways to assess the ecological condition of ponds using data derived from DNA sequencing. To do so, they hope to analyse data from the England Ecosystem Survey (EES), which is currently collecting environmental DNA (eDNA) samples from ponds across England. However, initial evaluation suggests that the EES surveys alone are unlikely to include as many 'high-quality' ponds as will be needed to develop an effective eDNA pond condition assessment method.

The aim of this project has been to identify, and then survey, an additional set of 45 high-quality ponds to collect data that will best complement the information being gathered by the England Ecosystem Survey.

Method development

EES survey monads were assessed in order to estimate the minimum number of high-quality ponds likely to be sampled for eDNA in this survey. This was done by overlaying the location of EES survey monads with Natural England's SSSI boundary layer and a pond layer provided by JNCC. The analysis produced a list of 101 monads where most or all ponds were located on SSSIs, and therefore likely to be of high-quality.

In order to investigate whether ponds in these monads are likely to be representative of the broad range of pond types present in Britain, the 101 monads were classified into one of 10 Freshwater Land Class types based on landscape-scale hydrology, geology and morphology (see Appendices 2 &3). The results suggest that the EES dataset is likely to have relatively few high-quality ponds in four of the 10 Freshwater Land Classes: LC3 Sandlands, LC4 Eutrophic till landscapes, LC6 Pre-Quaternary loam landscapes and LC9 Mixed, hard, fissured rock & clay landscapes. The focus of site selection for the current project was, therefore, to fill these gaps and ensure a greater number, and more even distribution, of high-quality sites across the Freshwater Land Class classification.

A list of 152 ponds known to be high quality sites was collated from Freshwater Habitat Trust's databases. Classification of these ponds into Freshwater Land Classes also showed an uneven distribution, and additional ponds were identified for survey from desk-study evaluation of ponds on SSSIs for Freshwater Land Classes where numbers were low.

Field surveys, data collection and analysis

Preliminary analysis suggested that a balance of high quality sites could be achieved by ensuring at least 20 high-quality ponds were included from each of the 10 Defra Freshwater Landscape Classes using both EES data and newly collected data in the

present project. In practice there were insufficient project resources to achieve this. However, surveys of the project's additional 45 high-quality ponds made it possible to ensure that, for 8 Land Classes, there are likely to be between 10 and 23 high-quality sites in the combined EES / Freshwater Habitats Trust dataset. For two Land Classes it was only possible to ensure a total of eight sites in the combined dataset. Of the list of sites initially identified for survey, around 35% proved unsuitable because of issues related to permissions, lack of water and poor quality.

In order to assess the 45 survey ponds' actual (rather than predicted) quality, wetland plant data was analysed using the PSYM algorithm. Of these, 43 ponds classified into PSYM's Good category, indicating that these ponds are high-quality. The two remaining ponds classified as Moderate on the basis of a lower-than-expected number of uncommon plant species. It is recommended that one of these sites (Goremire, Yorkshire), is retained as a high-quality site, but that the second site (Shelve Pool, Shropshire), should be treated with caution as a high-quality site.

Analysis of physico-chemical data from the survey ponds showed that there was a broad distribution of values for each of the main variables that are though to affect pond community type at a local level, including seasonality, shade, the extent of emergent vegetation cover, the presence of grazing, and the presence of an inflow. This suggests that natural variation in these attributes is well represented across the 45 sites.

Contents

1 Introduction

1.1 Aims

The aim of this project has been to identify, and then survey, a set of 'high-quality' ponds in good ecological condition to complement pond data being collected as part of the England Ecosystem Survey (EES).

The project objectives have been to:

(i) Review the quality of ponds being surveyed through the planned EES surveys.

(ii) Identify a set of 45 ponds that will help to fill gaps in the EES dataset, focussing on the provision of a set of high-quality ponds that represent the range of pond types present in England.

(iii) Survey these additional ponds, including:

- Collecting a filtered eDNA sample
- Undertaking a plant PSYM (Predictive SYstem for Multimetrics) analysis
- Collecting water chemistry and other pond environmental data following EES protocols.

1.2 Background

Natural England is seeking to develop novel metrics that can be used to assess pond ecological condition, based on data derived from DNA sequencing.

Metric development will be based on analysis of pre-existing pond DNA samples together with data being collected as part of the England Ecosystem Survey (EES). EES is part of the Natural Capital and Ecosystem Assessment (NCEA) which is a science innovation and transformation programme, that spans across land and water environments. It has been set up to collect data on the extent, condition and change over time of England's ecosystems and natural capital, and the benefits to society.

Initial evaluation suggests that the EES sampling strategy is unlikely to provide as many 'high-quality' ponds (those in near-pristine condition) as will be needed for metric development.

The aim of this project has, therefore, been to identify, and collect data from, a complementary set of 'high-quality' ponds in good ecological condition which can be combined with EES data to create a larger dataset for numerical analysis. The information

gathered from these complementary ponds includes: a filtered eDNA sample, a plant list that can be used for PSYM analysis of pond quality, including Great Crested Newt Habitat Suitability Index (HSI) variables, water chemistry data and standard pond environmental variables.

2 Evaluating the range of ponds currently planned for EES

2.1 Aim

The initial phase of this project aimed to assess the quality of EES survey ponds in order to: (i) identify the likely proportion of high-quality sites, and (ii) assess whether the high-quality ponds in the dataset are likely to represent the range of pond types that occur in England.

2.2 What are pond condition and pond type?

Pond condition

Pond condition is a term that is, here, used synonymously with pond quality and defined in a biological sense as the extent to which a pond differs from its minimally impaired, or pristine state (i.e. its reference condition). In England and Wales, the PSYM system is used as the standard method for assessing pond quality and is based on comparison of a pond's observed plant and macroinvertebrate assemblages with the assemblages that would be expected if the pond was minimally impaired (Freshwater Habitats Trust, 2002).

For ponds where biotic data are not available, the Priority Pond Assessment (PASS) method has recently been developed to identify high-quality ponds based on predictive abiotic factors such as shade, isolation, land cover, plant cover, presence of an inflow etc. (Biggs and Williams, 2023). However, PASS assessments require a summer field visit to ponds in order to adequately assess plant cover, so this method cannot be used to assess pond quality remotely.

Analysis of pond data for PASS and other studies have shown that, amongst abiotic variables, the most effective single predictor of pond quality is **land cover**; with location in a **semi-natural habitat** particularly strongly correlated with the occurrence of high-quality ponds (Davis *et al.*, 2008, Biggs and Williams 2023). Hence, in the current analysis, pond quality has been assessed using surrounding semi-natural land use as a proxy.

Pond type

The aim of a pond typology is to describe the full range of pond types based on the natural variation in their biotic and abiotic characteristics. Britain does not have a single, widely

used pond typology. However, a draft typology has been developed for Natural England as part of the project 'Developing a SSSI Series for Ponds' (Williams and Biggs in prep.).

Table 1. Pond typology: hierarchical abiotic variables which define the pond typology

Hierarchical classification of abiotic variables that determine pond type			
Level 1a (can be included within a GIS land class classification)	**Level 1b** (needs site knowledge)	**Level 2**	**Level 3**
• Altitude • Latitude and longitude • Near surface geology • Alkalinity and hardness • Ponds on and off the floodplain • Also includes water source	• Depth and degree of permanence	• Size • Emergent vegetation • Grazing • Inflow present	• Shade • Surrounding land use type

This project has:

(i) collated information on pre-existing pond typologies based on biotic data

(ii) identified the most significant abiotic attributes that have been shown to shape biotic communities

(iii) refined the list of abiotic attributes that define the typology to a final selection based on: (a) predictive power (b) broad relevance across biotic groups, and (c) measurability.

The results suggest that to fully represent the natural range of pond types present in England, there needs to be adequate representation of ponds at a range of levels, leading to a hierarchical classification: Level 1 variables are the most significant factors shaping pond communities on a national and regional scale. Level 2 and 3 attributes have more site-based relevance and are separated based on the number of biotic groups to which they are relevant, with Level 2 attributes relevant to more biotic groups.

Table 1 lists the variables that are included in the typology. A more detailed overview is given in Appendix 1. Most of the significant factors influencing pond type at a national and regional level (i.e. Level 1 attributes) are amenable to geospatial mapping with the exception of water depth and permanence.

Freshwater Land Class map layer

As part of the 'Developing a SSSI Series for Ponds' project, a GIS base layer was developed to capture Level 1 attributes. This layer was modified from a Freshwater Land Class map created in association with University of York for Defra Project PN0931 (Brown *et al.*, 2006, Biggs *et al.*, 2007). The map has 11 categories (Appendix 2) and describes the co-variance of hydrogeology, soils, topography and land use across the landscape of Great Britain. Appendix 3 describes the broad attributes for each Land Class category. The development of this map is described in Brown *et al.*, 2006 and Biggs *et al.*, 2007.

2.3 Method development for the current project

For the current project, Natural England provided information on the location of monads that would be targeted for survey by the EES team. It was not possible to provide data to identify which monads would be surveyed in 2023 rather than 2024 nor to identify the specific one or two pond(s) that EES would select for survey within each monad. As a result, analyses of EES ponds were undertaken for the whole dataset, at monad, rather than pond level.

The original plan for classifying pond type and assessing quality had been to identify pond type and (likely) condition based on the land cover type surrounding each pond using the Land Cover Map 2020 produced by the UK Centre for Ecology and Hydrology (UKCEH). This map has a classification with 10 categories and an additional 'Other' category for remaining land covers including urban and coastal zones.

However, because the location of survey ponds within the EES monad was not known, this method was not viable. Specifically: analysis of EES monads showed that 99% had multiple land uses. This meant that it was not possible to accurately predict the land cover that would be present around the pond that EES would eventually select for survey.

As an alternative, a two-stage protocol was developed, first to assess the proportion of EES monads that would be likely to support high-quality ponds, and second to assess the balance of high-quality monads likely to support ponds across a range of pond types.

Stage 1: Identifying high-quality ponds

As discussed in Section 2.2, the best single predictor of a high-quality pond is its location within an area of semi-natural land use. At monad level, the best mapping tool to describe this is Natural England's SSSI data layer which shows the spatial distribution of 4,123 SSSIs in England[1]. Initially, the Priority Habitat Inventory was also investigated as a source of information to describe the location of high-quality habitat. However, it proved to be insufficiently discriminatory because of the inclusion of considerable areas of more degraded habitat such as agricultural grassland on floodplains and in coastal lowlands, and a wide range of woodland included from the forest inventory.

To identify ponds likely to have semi-natural land cover in their surrounds, a three-way intersect was undertaken between the geospatial layers: EES monads, Natural England's SSSI boundary data and a national pond layer held by Freshwater Habitats Trust. The latter is based on an OS Master Map Ordnance Survey freshwater layer provided under

[1] Sites of Special Scientific Interest (England) | Sites of Special Scientific Interest (England) | Natural England Open Data Geoportal (arcgis.com). downloaded June 2023.

Table 2. Number of EES monads with ponds in SSSIs present within each Freshwater Land Class

Land Class number	Freshwater Land Class	Number of EES monads
1	River floodplains and low terraces	8
2	Fenlands, warplands & associated low terraces	20
3	Sandlands	2
4	Till landscapes (eutrophic)	3
5	Till landscapes (oligotrophic)	6
6	Pre-Quaternary Clay landscapes	6
7	Chalk and Limestone plateaux and coombe valleys	21
8	Pre-Quaternary 'Loam' landscapes	2
9	Mixed, hard, fissured rock and clay landscapes	4
10	Hard rock landscapes	20
11	Other: salt marsh, sand dunes and estuarine	9
	TOTAL	**101**

licence from JNCC, with an algorithm used to differentiate ponds from other freshwater waterbodies based on the size and shape of the water body polygons.

The results of this analysis showed that, of the 1,742 monads identified by EES for survey, 101 (5.8%) of monads had ponds located on SSSIs.

Stage 2 Ensuring representation of pond types in high-quality ponds

As described in Section 2.2 above, it is possible to assess the extent to which the main national and regional variations in pond type are included in pond selection by ensuring that there is a balanced coverage of ponds across the 11 Defra Freshwater Land Class types.

Analysis of the 101 ESS monads in SSSIs, in terms of their Freshwater Land Class indicates some imbalance across the Land Class types (Table 2). Three Land Classes are represented by at least 20 SSSI monads (LC 2 Fenlands & warplands, LC 9 Chalk & limestone landscapes and LC10 Hard rock landscapes). However, four Land Classes have four or fewer EES monads (LC3 Sandlands, LC4 Eutrophic till landscapes, Pre-Quaternary loam landscapes and LC9 Mixed, hard, fissured rock & clay landscapes).

3 Ponds proposed to supplement the existing dataset

The current project provides for the selection and survey of 45 high-quality ponds that can best supplement the dataset of high-quality ponds likely to be collected through EES surveys. The aim is, ultimately, to create a combined dataset that is representative of high-quality ponds in England.

The analysis described in Section 2 suggests that the current distribution of high-quality EES sites is likely to have an uneven representation across England in terms of the main factors that drive national and regional community type (see Table Level 1a factors and Table 2).

The focus of site selection for this project has, therefore, been to fill gaps in order to ensure a greater number, and more even distribution, of high-quality sites across the Freshwater Land Class classification. To achieve more-or less complete parity with at least 20 ponds in each of the Freshwater Land Class types would require surveys of an additional 120 high-quality ponds. This was not feasible in the current project given the limit of 45 additional survey ponds. The compromise was to ensure that there are data from at least 10 ponds for each Freshwater Land Class type. The number of sites required to do this is shown in the final column of Table 3, and includes a total of 39 ponds, leaving an additional 6 ponds to make up the available tally of 45 ponds. Note that this total excludes the LC11 ‘Other’ category because the majority of pools in this land class type are not freshwater bodies.

A decision was made to spread the remaining 6 ponds amongst better represented Land Classes, to ensure that the best examples of ponds in these land classes were not missed. For example, the outstanding Main Pond at Castor Hanglands National Nature Reserve (LC7 Chalk and limestone plateaux and coombe valleys), one of England’s highest quality ponds, would otherwise not be included in site selection.

Other considerations in site selection

The Freshwater Land Class GIS layer (Section 2.2) can be used to classify ponds in terms of the main factors that determine pond community type. However, as discussed in Section 2.2, a range of other variables also influence pond type at regional, local and site level (Table 1, Levels 1b, 2 and 3). Of these, water depth and seasonality are the most important, but factors such as shade, the presence of grazing or an inflow can also have significant effects. Ensuring adequate representation of these factors in site selection requires prior knowledge of the ponds, which is not always possible. However, where ponds were already known to Freshwater Habitats Trust, efforts were made to ensure selection of sites with different characteristics across the natural range of these variables.

Table 3. Number of additional high-quality ponds required to achieve greater parity with at least 10 ponds per Freshwater Land Class type

Land Class number	Freshwater Land Class	Number of EES monads	Number of extra ponds to give 10+ ponds/Land Class
1	River floodplains and low terraces	8	2
2	Fenlands, warpland & associated low terraces	20	0
3	Sandlands	2	8
4	Till landscapes (eutrophic)	3	7
5	Till landscapes (oligotrophic)	6	4
6	Pre-Quaternary Clay landscapes	6	4
7	Chalk & Limestone plateaux and coombe valleys	21	0
8	Pre-Quaternary 'Loam' landscapes	2	8
9	Mixed, hard, fissured rock and clay landscapes	4	6
10	Hard rock landscapes	20	0
11	Other: salt marsh, sand dunes and estuarine	9	n/a
	TOTAL	**101**	**39**

4 Pond survey preparation

4.1 Site selection

A list of known high-quality ponds was collated from Freshwater Habitat Trust's databases including: minimally impaired ponds surveyed for the National Pond Survey, ponds identified as high-quality Flagship Sites in the People, Ponds and Water project, and from a range of other projects including the Million Ponds Project and national surveys undertaken for the National Trust. All ponds on this list had Good quality PSYM scores indicating that they were close to reference condition. In total this provided a list of 152 sites with one or more ponds.

The 152 sites were classified into the 11 Freshwater Land Classes. As with the EES survey, the results showed an uneven distribution. LC1 River floodplains, LC2 Fenlands and LC10 Hard rock Land classes were all well represented. However, given the need to include at least an extra 10% 'spare' sites in case survey permissions were refused or ponds proved unsuitable, there were an insufficient number of sites for LC3 Sandlands, LC4 Eutrophic tills, LC5 Oligotrophic tills and LC9 Mixed fissured rocks.

To identify sufficient additional ponds on land classes with too few ponds, it was necessary to scan the intersect between the SSSI boundary layer and the pond layer for these land classes. This was done by eye, to ensure that selected ponds were likely to be high-quality, and did not, for example, occur on the border of SSSIs where they were likely to be impacted by adjacent agricultural or urban land. Satellite imagery and information from SSSI citations was also used to inform the selection of these ponds.

4.2 Permissions

Pond landowners were identified through a mixture of personal knowledge, information obtained from contacts, internet searches and the Land Registry.

Survey permissions were predominantly obtained by email, with a covering text and permission proforma. Permission was refused for around 10% of sites. However, the spring and early summer period in 2023 coincided with a period of low rainfall and hot weather, and for an additional 10% of sites, owners or managers reported that the pond was dry at the time of enquiry.

4.3 Selection of survey variables

The aim of the field survey was to collect: (a) a filtered eDNA sample, (b) a plant list to be used for PSYM analysis of pond quality (c) water chemistry and other pond environmental data.

The eDNA kits used were Sylphium Dual Filter Capsule with valves, 0.8 µm PES (SYL013-08). In total, 50 filter kits were obtained: 45 for one sample per pond, plus 5 negative control samples to be collected at random after the pond eDNA filter was taken. Negative controls were filtered from a single 6 lt pack of Scottish Highland bottled water.

The PSYM wetland plant list was used as the basis for plant survey data collection. Additional wetland plants, such as charophyte species, which are included on Freshwater Habitat Trust's extended wetland plant list, were also identified[2].

The list of environmental variables recorded from each pond is shown in the Supplementary information. These variables were derived from the pond attributes measured in EES and include: PSYM predictive variables and Great Crested Newt Habitat Suitability Index (HSI) variables, together with standard pond environmental data such as

[2] https://freshwaterhabitats.org.uk/advice-resources/survey-methods-hub/freshwater-plant-surveys/

permanence, tree cover, surrounding land use, etc. Water chemistry was assessed using field kits to measure phosphate, nitrate, alkalinity and pH[3].

A small number of additional variables were also included to provide information that could potentially be of relevance to eDNA data interpretation, including pond age, and surrounding land cover within 5 m and 100 m of the pond.

5 Field surveys, data collection and analysis

Ponds were surveyed between July and early October 2023. Around 35% of sites originally identified for survey were not suitable either because permissions were refused, sites were dry on arrival, or pond quality was assessed as being too poor during the site visit. The latter was a particular issue for ponds on SSSIs which were selected 'site-unseen' from remote sensing, map and other data.

The final balance of sites across the Freshwater Land Class groups is shown in Table 4. Ultimately it was possible to ensure that, of the 10 Land Classes, there are likely to be 10 or more high-quality sites in the combined EES dataset for eight of the Land Classes. For two Land Classes it was only possible to ensure a total of eight sites. These were LC5 Oligotrophic Till landscapes, and LC8 Pre-Quaternary Loam landscapes. LC5 is one of the smallest landscape types in terms of area, providing fewer opportunities to identify high quality sites and, in addition, over 50% of sites visited for the current project were unsuitable. LC8 also has a relatively small spatial footprint, but the main issue in finding sites was that this fertile loam landscape is dominated by intensive agriculture with few SSSIs. As noted above, Land Class 11 was not included as the sites were usually not freshwaters, being either brackish or estuarine habitats.

The spatial distribution of the 45 survey sites is shown in Figure 1, together with the location of the EES monads on SSSIs that have ponds. As a principle in the current project, an effort was made to minimise spatial autocorrelation by ensuring that ponds on the same Freshwater Land Class were not located in close proximity. However, spatial autocorrelation is itself a phenomenon that is of interest when developing site quality metrics, and to provide anecdotal data, two sites of contrasting type but in close proximity (0.7 km) were surveyed on the Lizard. These were the large, ancient and usually seasonal Ruan Pool located in heathland, and a grassland pond on Cornwall Wildlife Trust's adjacent Windmill Farm reserve which is relatively small, man-made and permanent.

[3] Phosphate: PoolCheck® Phosphate (Bottle of 50 tests), Nitrate: WaterWorks™ Nitrate/Nitrite (Bottle of 50 tests), Alkalinity: Spa Check™ 3 in1, pH: WaterWorks™ Extended Range pH (2-12pH - Bottle of 50 tests)

Table 4. Number of high-quality ponds surveyed per Freshwater Land Class type

Land Class no.	Freshwater Land Class	Number of EES monads	Number of extra ponds surveyed	Total number of ponds
1	River floodplains and low terraces	8	5	13
2	Fenlands, warpland & associated low terraces	20	3	23
3	Sandlands	2	8	10
4	Till landscapes (eutrophic)	3	8	11
5	Till landscapes (oligotrophic)	6	2	8
6	Pre-Quaternary Clay landscapes	6	4	10
7	Chalk & Limestone plateaux & coombe valleys	21	1	22
8	Pre-Quaternary 'Loam' landscapes	2	6	8
9	Mixed, hard, fissured rock & clay landscapes	4	7	11
10	Hard rock landscapes	20	1	21
11	Other: salt marsh, sand dunes and estuarine	9	n/a	n/a
	TOTAL	**101**	**45**	**146**

The Supplementary information that accompanies this report provides the physico-chemical data collected from each survey pond. Amongst these data, analysis of the Level 1b, 2 and 3 attributes known to affect pond type (Table 1), shows that most variables have a reasonable distribution of values.

In terms of water permanence, 53% of sites were assessed as being fully permanent, 20% as drying in exceptionally dry years, and 27% as drying in some, or all, years. As noted above, the inclusion of sites across the permanence gradient is important, and the current dataset appears to represent this well with 53% of the waterbodies permanent and 47% drying at least occasionally.

The surface area of the survey ponds ranged from 30 m^2 to 7,500 m^2 with a median value of 1,000 m^2. Across this range, 31% of sites had an area of less than 500 m^2, 40% were between 500m^2 and 2000m^2 and 29% between 2000m^2 and 10,000 m^2 (1 ha). This suggests broad representation amongst ponds of different sizes within the dataset. However, it is worth noting that, in the wider countryside, smaller ponds (less than 500 m^2) predominate, which is not reflected in the current dataset.

The pH range amongst the ponds was 4.0 to 9.2. Alkalinity ranged from zero to 240 ppm[4]. Phosphate and nitrate was undetectable for the majority (80-84%) of ponds, as might be expected from minimally impaired waterbodies. The maximum values were: phosphate 0.2 ppm (200 µg L^{-1}); nitrate 2 ppm (2 mg L^{-1}).

In total, 18% of survey sites had an inflow. Inflow streams are amongst the most common sources of pollutants in ponds, and the limited number of ponds with inflows reflects the need to include only high-quality ponds in the current survey.

The extent of tree shade overhanging the ponds ranged from 0-80% (average 17%). Marginal shade values ranged from 0-95% (average 27%). There was a bias towards more open ponds, with 78% of survey ponds shaded over less than a third of their area, 16% shaded between one-third to two-thirds of their area and 7% shaded over more than two-thirds of the pond. In practice, the proportions observed are similar to the extent of shade observed in ponds in the wider landscape (Williams *et al*., 2010, Ruth Hall *pers comm*), and this partly reflects the relative paucity of trees in some landscape types such as heathlands and upland grassland.

The majority of sites (68%) were grazed, usually by livestock but in some cases by deer or wild boar. It would perhaps have been preferable to include a more even split of grazed and un-grazed ponds. However, this was difficult to achieve in practice, given an autocorrelation between grazing and the location of the ponds in areas of semi-natural land use.

The representation of ponds with differing amounts of emergent plant cover was reasonably balanced. Average cover by emergent plants was 43%, with 42% of ponds having less than a third of the pond occupied by emergent plants, 33% between a third and two thirds, and 24% more than two-thirds.

Plant surveys

In total the survey recorded just over 200 wetland plant species from the 45 ponds. This represents around 50% of the wetland plant species present in the UK, and is an exceptionally high total for such a small number of sites.

The species list had a relatively high proportion of rare and declining plants including: Pillwort (*Pilularia globulifera*), Hampshire-purslane (*Ludwigia palustris*), Translucent Stonewort (*Nitella translucens*), Lesser Water-plantain (*Baldellia ranunculoides*), Great Fen-sedge (*Cladium mariscus*), Round-fruited Rush (*Juncus compressus*), Tubular Water-dropwort (*Oenanthe fistulosa*) and three species of Bladderwort (*Utricularia* spp.).

[4] Colour changes on the alkalinity test strips often proved hard to interpret.

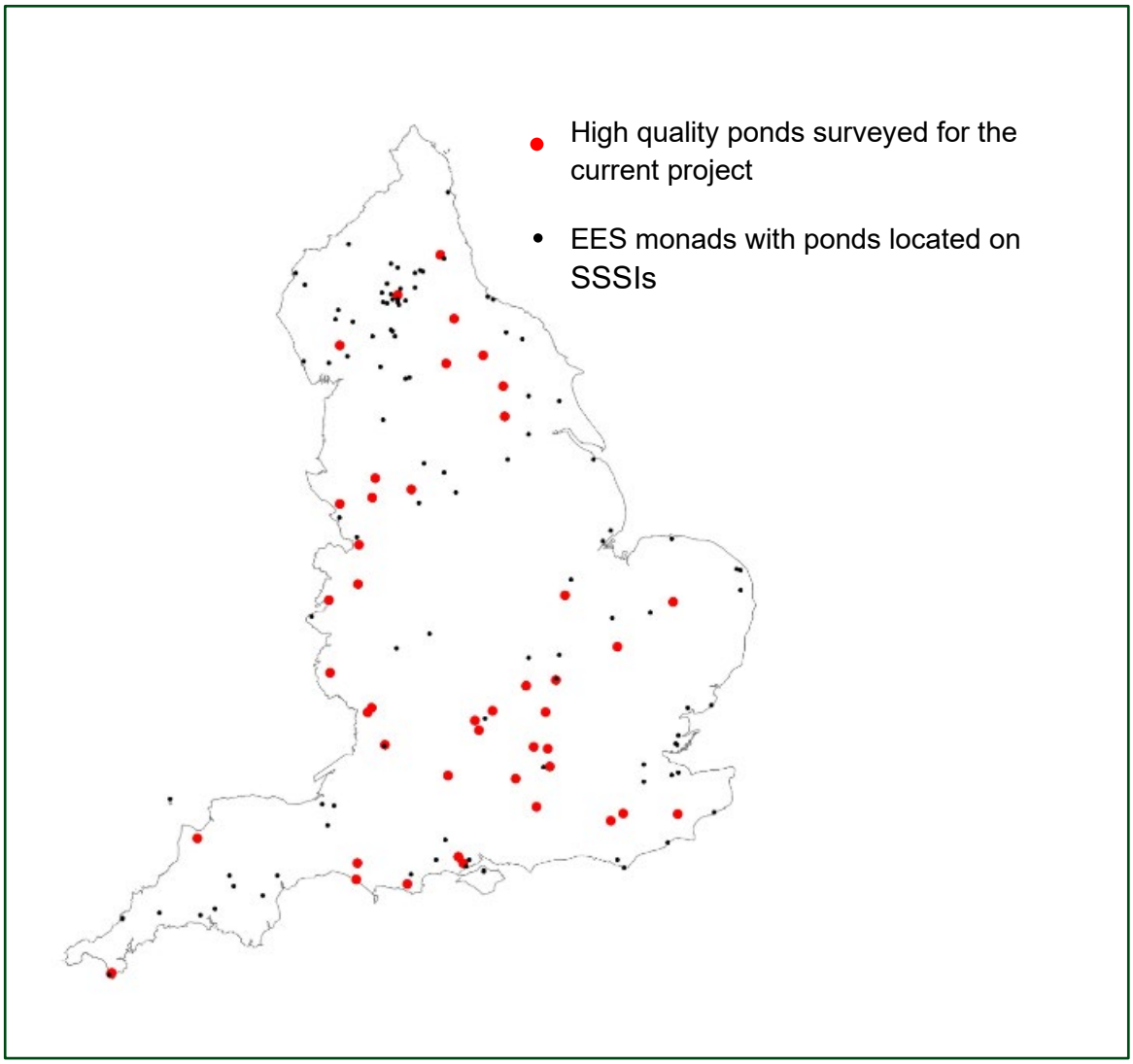

Figure 1. The location of ponds surveyed for the current project and EES monads with ponds on SSSIs.

PSYM analysis

Wetland plant assemblage data from the survey ponds was analysed using PSYM, with ponds categorised in terms of the four PSYM quality categories: Good, Moderate, Poor and Very Poor.

All but two of the 45 ponds classified as Good on the basis of their plant assemblages, indicating that these ponds fell within the boundary for high-quality, minimally impaired ponds that are at, or close to, reference condition.

The two remaining ponds classified as Moderate. In both cases this was due to a lower-than-expected number of uncommon plant species. Of these ponds, Goremire (Yorkshire), was a large, shaded pond with restricted access. In terms of its catchment and other potential impacts, this pond looked to be minimally impaired, and it is recommended that it is retained as a high-quality site.

The second site, Shelve Pool in Shropshire, was surrounded by a floristically rich, high quality, floating mat. However, the pond itself had some issues with blue-green algae and may have had some impairment. It is therefore recommended that this pond is treated with caution as a high-quality site.

Survey issues

A small number of additional issues relating to the collection and interpretation of eDNA samples are worth considering.

As noted above, the spring and early summer of 2023 was hot and dry, and this was followed by a wetter period from mid-summer onwards. It is therefore possible that shallow ponds surveyed in late summer 2023 has dried up in previous weeks and then re-wetted. If so, this is likely to have affected pond assemblages by, for example, reducing macroinvertebrate richness. Ponds where re-wetting could have occurred are identified in the Supplementary information.

The eDNA filters used in the project had a relatively wide water intake aperture. This had two impacts that could potentially affect the eDNA results. Firstly, small animals were easily and unavoidably drawn up, whole, into the filters. This included zooplankton as well as very tiny, early instar damselflies, water boatmen, fly larvae, mayflies and water hog-lice. It is possible that the preservation of these animals in the filter itself will influence the likelihood of their eDNA being detected in the sample. To help assess this, the presence of trapped species was noted in a proportion (c60%) of samples.

A second impact of the wide intake aperture was that organic matter in the water was easily drawn up into the filter. This was a particular issue in more acid ponds which often have a high proportion of suspended organic matter. The extent to which this will influence eDNA results is not known and to aid interpretation, all filters, were given a rank, based on the extent of filter discolouration, on a 1-8 scale, where 1 was little or no discolouration, and 8 extensive amounts of organic detritus in the filter. If the wide filter aperture proves to be an issue, one option would be to add a micro-mesh pre-filter to the intake valve.

The eDNA kit packaging included one of the filter caps as a loose item, rather than being separately packaged. When the pack contents were emptied onto the ground prior to donning gloves, it was easy for this cap to become contaminated with pond bankside mud. This occurred on a number of occasions, including for at least one of the control blanks, and was a particular issue during or after rain when the bank was wet. For this reason, eDNA kits often include the filter cap in separate packaging, and this would be preferable for surveys going forwards.

The filters have been sent to our partners laboratories at Cefas, for analysis via DNA metabarcoding. The molecular and survey data will then be analysed, alongside the EES dataset and other pond datasets, to begin to develop DNA based metrics for pond condition.

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Appendix 1. About the pond typology

Whilst a small number of pond typologies have been developed in the UK based on specific biotic groups, there is no single broad-based typology for ponds. The principles of a draft typology developed for Natural England as part of the project: 'Developing a SSSI series for ponds' is outlined below (Williams and Biggs, in prep).

This project:

(i) collated information on pre-existing pond typologies based on biotic data

(ii) identified the range of abiotic attributes that have been shown to shape biotic communities

(iii) refined the abiotic attributes that define the topology to a final selection based on (a) predictive power (b) broad relevance across biotic groups, and (c) measurability.

The result is a hierarchical classification with three levels:

Level 1 variables are the most significant factors shaping pond communities on a national scale. Level 2 and 3 attributes have more site-based relevance and are separated on the basis of the number of biotic groups to which they are relevant, with Level 2 attributes relevant more biotic groups.

Four of the six Level 1 attributes that influence the pond typology are geographic variables amenable to mapping: latitude/longitude, altitude, near surface geology and floodplain location. Alkalinity and hardness can also be included here because they depend largely on underlying geology and topography. The only Level 1 attribute not easily amenable to spatial analysis is water depth/permanence.

The dominance of mappable variables as the primary factors influencing pond community types enables the most important variables influencing pond type to be encompassed within a simple spatial stratification that can be analysed using GIS.

Doing so depends on finding, or creating, a GIS base layer that captures Level 1 attributes. To do this, the project created a freshwater land class map was derived from DEFRA Project PN0931, (Brown *et al*., 2006, Biggs *et al.*, 2007), where the aim was to capture broad differences in types, properties and abundance of waterbodies, routes of movement of water and potential for exposure to pesticides (i.e. agricultural land-use). To develop the map, the extent to which hydrogeology, soils, topography and cropping patterns co-vary across the landscape was assessed visually using the legend attributes from the 1:250,000 soil maps of England, Wales and Scotland. Descriptions of landscapes were set out using broad types of soil parent material as a link between topography and hydrogeology (expressed as the likelihood of presence of different types of waterbodies) and including elements of a classification of soil types according to their hydrological response (Lilly *et al*., 1998). A digital dataset was generated using the national soil maps of England and Wales and of Scotland at scales of 1:250,000. Non- agricultural areas were identified by combining urban and inland water polygons with all soil association map

units with no significant agricultural usage. All remaining soil associations were assigned to one of 12 agricultural landscape classes using soil parent material as the classifier.

For the project two land classes were removed from the classification as these occur only in Scotland. Non-agricultural areas were then re-classified based on pre-existing categories using national soil association maps and BGS solid and drift maps. Urban areas were identified using the Land Cover Map 2015 produced by the Centre for Ecology and Hydrology (CEH). This created a classification with 10 categories and an additional 'Other' category for remaining land covers including urban, coastal and open water zones.

Appendix 2 shows the final freshwater land class map. Appendix 3 describes their broad attributes of each category.

Practical application of the typology

Practical implementation of the typology to ensure adequate inclusion of pond types requires a three-step process:

Step 1. Evaluate ponds data using the freshwater land class classification and ensure that there is good representation of ponds in each land class. This step requires no data other than pond grid reference.

Step 2. Ensure that the ponds within each land class have a wide range of depths (typically averaging 0.25 m - 1.5 m) and, particularly, permanence categories including permanent, semi-permanent and temporary ponds.

Step 3. Within each land class, and amongst ponds of different permanence types ensure that there is good representation of other factors that are strong drivers of community type. Specifically:

(a) *Size:* ponds that fall into different size classes – though note that this does not presume an *even* distribution, and that in natural landscapes most ponds are small

(b) *Marginal vegetation:* ponds spanning the range from largely unvegetated to 'overgrown'

(c) *Grazing*: ponds that are grazed and ungrazed

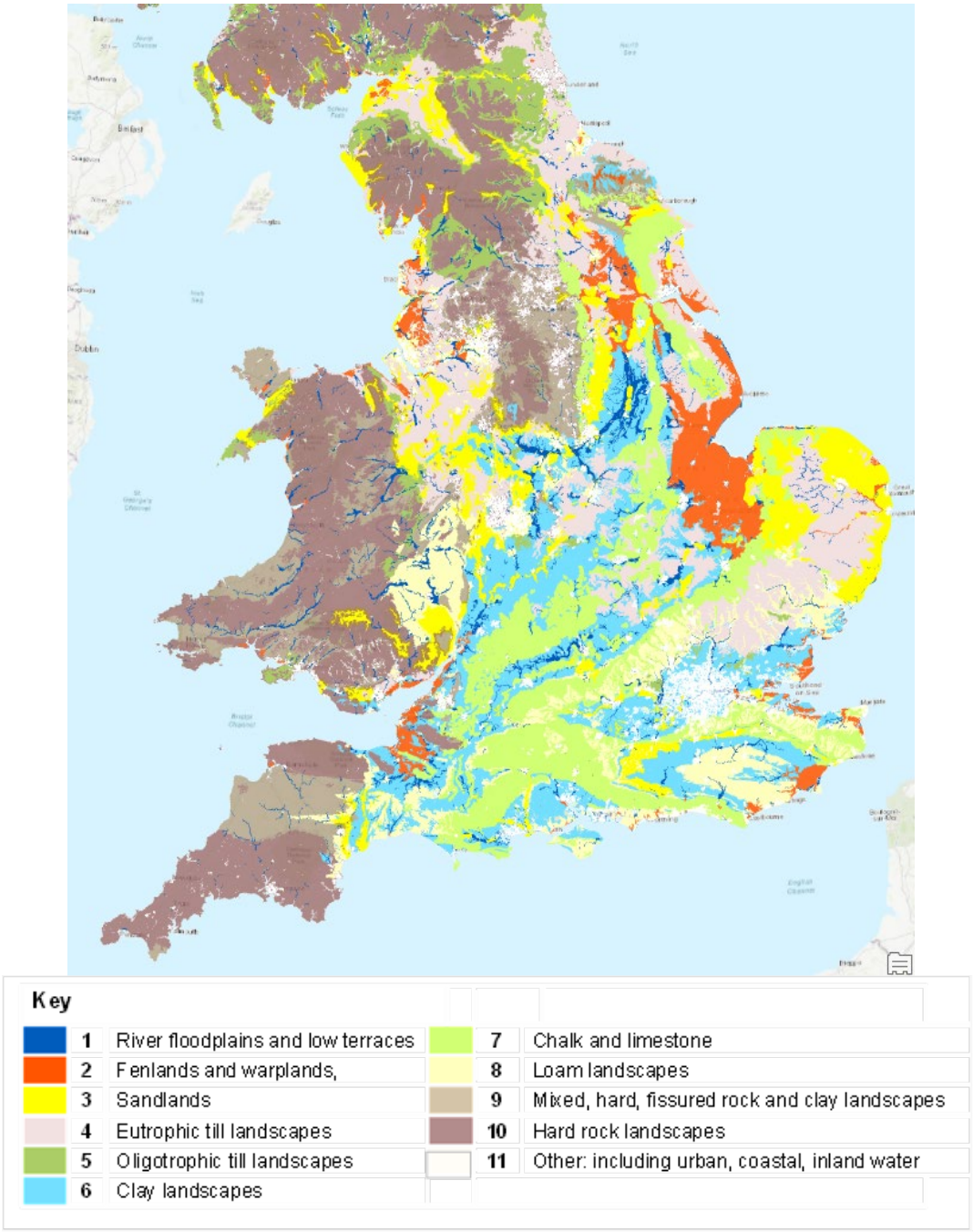
(d) *Inflow:* ponds that do and do not have inflows

(e) *Shade:* ponds spanning the range from unshaded to heavily shaded

(f) *Surrounding land use:* ponds located within a range of semi-natural land-use types e.g. woodland, unimproved grassland, heath, fen, bog.

In practice it is possible to combine many of the attributes in Step 3 by including ponds in different semi-natural landscape types (e.g. woodland, grazed grassland), with different sizes and at a range of successional stages from early succession ponds with little shade or emergent vegetation to late succession ponds which are full of emergent vegetation ('overgrown') or heavily shaded.

Appendix 2 Freshwater land class map



Map of England and Wales, showing the 11 different Freshwater Land Class categorisations used in this project. The categories are described in more detail in Appendix 3. Republished with permission from © Freshwater Habitats Trust.

Appendix 3 Freshwater Land Class categorisation used in the current project

No.	Land class name	Description
1	**River floodplains and low terraces**	River alluvial floodplains and low terraces. Level to very gently sloping ground
2	**Fenlands and warplands**	Lowland peat, and alluvial fine sands, silts and clays Level, broad ‘flats’
3	**Sandlands**	Glacial and post glacial sands and light loams forming level to moderately sloping, rolling hills and broad terraces.
4	**Eutrophic till landscapes**	Glacial till with high base status. Medium loams, clays and chalky clays, Some lighter textured soils on outwash. Level to gently sloping plains.
5	**Oligotrophic till landscapes**	Glacial till with low base status. Medium loams and clays. Some lighter textured soils on outwash
6	**Clay landscapes**	Mesozoic and pre-Quaternary Cenozoic clays with high base status. Slowly permeable (often calcareous). Level to gently sloping vales
7	**Chalk and limestone**	Chalk and limestone overlain by shallow to moderately deep loams Rolling ‘wolds’ and plateaux with ‘dry’ valleys
8	**Loam landscapes**	Mesozoic and pre-Quaternary Cenozoic sands and siltstones. Deep, free-draining and moderately permeable silts and loams. Gently to moderately sloping ridges and vales and plateaux.
9	**Mixed, hard, fissured rock and clay landscapes**	Palaeozoic rocks with moderately deep free-draining loams mixed with heavy loams and clays in vales. Gently to moderately sloping hills, ridges and vales.
10	**Hard rock landscapes**	Hard Palaeozoic and Pre-Cambrian rocks and upland peat. Steeply to gently sloping hills and valleys. Moderately deep free-draining loams and some slowly permeable heavy loams on lower slopes and valleys
11	**Other**	Including, urban, coastal, open water

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