Brecks Fen Edge & Rivers Landscape Partnership

CITIZEN SCIENCE: TESTING THE WATER

eDNA Survey Results 2022

Version 2

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Executive Summary

In this regional survey of an Important Freshwater Landscape, volunteers helped to gather freshwater biodiversity data, through Freshwater Habitats Trust's Citizen Science: Testing the Water project, a part of The Brecks Fen Edge & Rivers Landscape Partnership Scheme. Cutting-edge biological survey and water testing technologies revealed that freshwater fish, amphibians and mammals rely on a range of different waterbodies in The Brecks landscape, and that clean water is most often found in small waterbodies.

The study found that most species were making use of both standing waterbodies, including ponds and lakes, and running waters, such as streams and rivers. The study, which was the first of its kind in The Brecks, found that even fish such as the European Eel, which are usually associated with rivers rather than ponds, are using the whole freshwater environment.

The research also revealed standing waters to be the cleanest freshwater habitats in The Brecks. Surveys conducted across more than 70 sites, found 71% of standing waterbodies to be clean and unpolluted, compared with just 17% of running waterbodies. Most clean waterbodies were in nature reserves or on military land; areas of semi-natural habitat mosaics managed for wildlife conservation.

Volunteers carried out water quality testing to measure levels of two pollutants: nitrate and phosphate in ponds, streams, rivers, lakes and ditches. They also used eDNA testing to detect which species of fish, amphibians and aquatic mammals were living in or around the edge of the waterbodies. This cutting-edge technique makes it possible to gather DNA in small water samples, by detecting traces of an animal, such as its shed skin, hair or eggs.

The most frequently detected amphibian was the common toad, which was found at 20 standing and running waterbodies. Although still widespread, common toads have declined in recent years and, in Britain, the species is now protected, by law, under the Natural Environment and Rural Communities Act 2006. Another protected species, the great crested newt, was recorded at three sites within the wetland rich semi-natural habitats of Stanford Training Area (STANTA) and on Thompson Common, in standing waterbodies only. Evidence was also found of the presence of northern pool frog, the rarest of the UK amphibians, which appears to have naturally dispersed to a new site, adjacent to one of its reintroduction sites.

A total of 29 fish species were recorded, with the most common being the three-spined stickleback and nine-spined stickleback. These small predators were found in a range of waterbodies, including ponds, lakes, rivers and ditches. Understanding the distribution of fish can help to detect trends in other species, such as great crested newts, which are usually intolerant to the presence of fish. Fish species of conservation concern included European eel and lamprey species. Volunteers also found evidence of several aquatic mammals, with water vole, water shrew and otter, found at 11 sites, and many different bird species at a range of both still and standing waterbodies.

On the national register of Priority Ponds collated by Freshwater Habitats Trust and Natural England (2019), prior to this eDNA project there were 153 Priority Ponds identified in the Brecks area. The Project helped to identify seven new Priority Ponds in the Brecks area, based on a combination of clean water and the presence of species of high conservation importance.

This research has provided fascinating insights into how animals are using a range of different waterbodies and highlights the importance of protecting the whole freshwater environment. Polluted water is known to be the main cause of the sharp decline in freshwater biodiversity. Most freshwater plants and animals have evolved over millions of years in naturally low nutrient environments and are now struggling to adapt to the high levels of nutrient pollution, particularly caused by phosphate and nitrate, which are now found in most waterbodies. It is very difficult to clean up rivers and streams, so a more realistic way of giving The Brecks freshwater wildlife a refuge would be to create more clean standing waters – and protect the clean waterbodies we already have. Protecting the best and building out from there will be critical for the conservation of The Brecks freshwater wildlife into the future and this survey has provided invaluable data to help us achieve that.

BFER CITIZEN SCIENCE: TESTING THE WATER

eDNA RESULTS 2022

1.0 Brecks Fen Edge and Rivers Landscape Partnership Scheme

The Brecks Fen Edge and Rivers Landscape Partnership (BFER LP), is a Landscape Partnership scheme hosted by Suffolk Country Council and focused on The Brecks' fen edge and river corridors area (Figure 1). It aims to deliver a series of new and exciting community-based landscape and heritage conservation projects, over a 5-year period (2020-2024). The partnership is made up of regional, national and local organisations with an interest in the area, community groups and members of the community.

Figure 1: Brecks Fen Edge and Rivers Landscape Partnership Scheme area

Freshwater Habitats Trust is leading on one of these projects, named 'Testing the Water'. This Citizen Science project aims to gather valuable data and raise awareness of habitat loss, pollution and the distribution of rare species, involving people in practical activities to get new information about the project area. Using cutting-edge Environmental DNA (eDNA) sampling and rapid water quality test kits (testing for nutrient pollution), volunteers have been undertaking surveys of freshwaters. Unlike many traditional survey methods, these new technologies make visible the pollution that is otherwise invisible and largely unknown, as well as opening up biological recording of freshwater species to a non-technical audience.

1.1 Freshwaters within the BFER Scheme

The Brecks is a relatively dry region when compared to its surrounding landscapes, and is often quoted as having the lowest recorded rainfall of any area in the UK. However, in an interim analysis of Important Freshwater Landscapes, undertaken by Freshwater Habitats Trust, Breckland was one of approximately 24 landscapes in England and Wales recognised for its exceptional freshwater biodiversity (Figure 2).

Figure 2: The Important Freshwater Landscapes in England and Wales Number 11 The Brecks and surroundings

The network of fenland fringe, freshwater habitats and river corridors in The Brecks' Fen Edge and Rivers landscape is a conduit for biodiversity in this part of the country. These habitats are sandwiched between the internationally famous wetland landscapes of The Broads and The Fens. There are also a number of Important Freshwater Areas[1] within the region: a product of its geology and management history. Freshwaters are a critical part of the 1000km^2 Brecks National Character Area, fundamental to its unique biodiversity, habitats and cultural heritage.

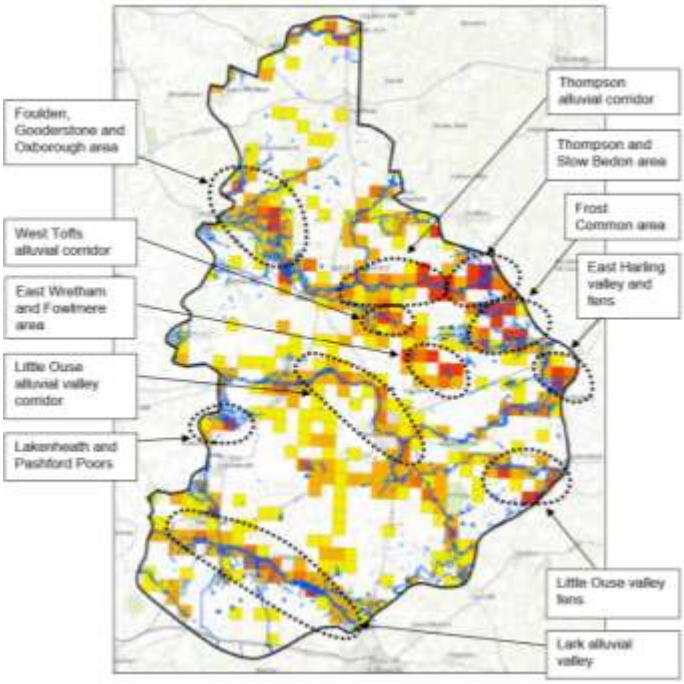


Figure 3: The Brecks Important Freshwater Areas assessment (2018) identified eleven areas that are high priorities for freshwater biodiversity and areas which were data deficient in between

The Brecks is particularly known for its pingos: natural ponds that were formed by freezing and thawing of upwelling groundwater during the last glacial period. The area also contains a number of fluctuating meres: shallow lakes which naturally have no inflows and which would have dried down completely in some years (but are now artificially maintained). The unique hydrology of these waterbodies supports a suite of plant and invertebrate communities, which are nationally rare. The Brecks has a number of other important running water waterbodies, including the chalk rivers and streams of the River Little Ouse, River Lark, River Wissey and associated valley fens, springs and ditch networks and cut drains.

The diversity of freshwater communities, and rarity of a number of species found within them, is due in part to the long history of wetlands in this part of England, and the presence of some large patches of uncultivated land, which have remained unimpacted by pollution and development. However, both the running and standing waterbodies in The Brecks are vulnerable to fragmentation, abstraction, pollution, changes in land-use and development, and therefore remain under threat.

In spite of the high biodiversity value and potential threats, information on aquatic habitats is generally scarce in The Brecks, and even more so on small waters: headwater streams, ditches, ponds, springs and flushes. Currently, only a small a proportion of the waterbodies in The Brecks are regularly monitored for the Water Framework Directive, essentially the main rivers and larger tributary streams and two lakes (Stanford Water and Thompson Water).

As part of the Landscape Conservation Action Plan (LCAP) development work for this area, Freshwater Habitats Trust undertook an Important Freshwater Areas assessment (Figure 3), to review existing information and propose a strategy to address data gaps, potentially by engaging communities in citizen science data gathering, to be delivered as part of The Brecks Fen Edge and Rivers Landscape Partnership Scheme.

[111] Important Freshwater Areas – high priorities for freshwater biodiversity, based on concentrations of freshwater species and habitats, designated sites, ponds, waterbodies or sites that have been identified as having high freshwater biodiversity using a set of nationally agreed criteria.

1.2 Priority Ponds within the BFER Scheme

Priority ponds are waterbodies that have a particularly high conservation value: usually because they support important freshwater species or rare community types. These ponds can be permanent or seasonal waterbodies and can span a wide size range: from $1m^2$ to 2ha in area.

The criteria used to identify 'Priority Ponds' under the UKHabs[2] (formerly the UK Biodiversity Action Plan), are listed below. It is estimated that around 20% of the 500,000 or so ponds in the UK (excluding garden ponds) will meet one or more of the criteria, however only a small proportion of sites have been identified nationally.

1. Habitats of high conservation importance. Ponds with specialist habitats that meet criteria under Annex 1 of the Habitats Directive.

2. Ponds supporting species of high conservation importance. These are Red Data Book species, BAP species, species fully protected under the Wildlife and Countryside Act Schedule 5 and 8, Habitats Directive Annex II species, a Nationally Scarce wetland plant species, or Nationally Scarce aquatic invertebrate species.

3. Ponds with exceptional populations or numbers of key species. This is based on:
    i. Criteria specified in guidelines for the selection of biological Sites of Special Scientific Interest (currently amphibians and dragonflies only).
    ii. Exceptionally rich sites for plants or invertebrates (supporting 30 or more wetland plant species or 50 or more aquatic macroinvertebrate species).

4. Ponds of high ecological quality. These are ponds classified in the top category for ecological quality (a score of 75% or more), as assessed by the standardised method for assessing the biological quality of still waters in England and Wales – the Predictive System for Multimetrics (PSYM).

5. Other important ponds. These are individual ponds or groups of ponds with a limited geographic distribution recognised as important because of their age, rarity of type or landscape context e.g. pingos, dune slack ponds, machair ponds.

6. Clean Water Provisional Priority Ponds. Clean Water is freshwater that is free from nutrient pollution which can pose a major risk to wildlife. It is defined as having a nutrient status of <0.5 mg/l nitrate as nitrogen and <0.05 mg/l phosphate as phosphorous.

On the national register of Priority Ponds collated by Freshwater Habitats Trust and Natural England (2019), prior to this eDNA project there were 153 Priority Ponds identified in the Brecks area (Figure 4)

Of these ninety-five are based on ***Criteria 5 (irreplaceable habitats) – pingo pools.*** These are a very rare type of pond, with The Brecks in Norfolk, holding the largest density in the UK, most of which are around Thompson Common. Created at the end of the last ice age, as the glaciers retreated they left hard lenses of ice pressed into the ground, with soil over the top of them. When things warmed

[2] The UK Habitat Classification (UKHab) is a unified and comprehensive habitat classification system for all terrestrial, freshwater and coastal habitats in the United Kingdom. It has been developed to improve habitat categorisation, recording and geospatial analysis for land use and habitat survey assessment.

up it caused the lenses to melt forming a depression filled with water. In the Brecks area, especially around Thompson and in pockets elsewhere, pingo ponds have been left almost untouched, where in other areas of the UK most pingos have been ploughed up.

Fourteen Priority Ponds in the area are based on ***Criteria 2 – Great Crested Newt presence.*** Of these eleven were identified from District Level Licencing records, (regional surveys to inform DL a new approach to reduce the impact of development pressure on GCN), and 3 from FHT's PondNet GCN Monitoring Programme, the national annual survey for this European protected species.

A further three Priority Ponds are based on ***Criteria 2 – Common Toad presence.*** The records where all taken from the annual Freshwater Habitats Trust Spawn Survey which maps the sightings of Common Frog and Common Toad spawn across the country.

The final forty-one Priority Ponds are based are based on ***Criteria 4 – PSYM (the Predictive SYstem for Multimetrics)*** from FHT's surveys commissioned as part of the People, Ponds and Water HLF project 2015-17.

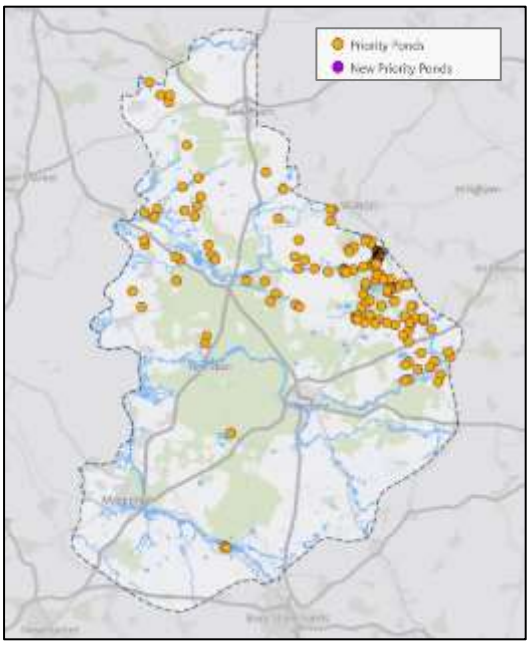


Figure 4: Priority Ponds in The Brecks area prior to eDNA survey

One of the above ponds qualified for Priority Pond Status under both Great Crested Newt presence and a high PSYM score.

2.0 Introduction

This report presents the results of eDNA (environmental DNA) testing that was carried out by volunteers in 2022 as part of the **Citizen Science: Testing the Water** Project. The aim was to assess the distribution of vertebrates - fish, amphibians, birds and aquatic mammals across all waterbody types in The Brecks' Fen Edge and River landscape.

The surveys form part of a monitoring programme that is helping to add to baseline data and to inform the delivery of river restoration activities to guide future landscape conservation activity in the scheme area.

2.1 Environmental DNA

Environmental DNA, or 'eDNA', is genetic material released by an organism into its surrounding environment. Sources of eDNA include faeces, shed skin and hair, mucus and gametes (eggs and sperm). In ponds, lakes, rivers, streams and ditches, eDNA can persists for up to one month, depending on environmental conditions, leaving a unique trace of the plant or animal in the water.

Advances in DNA technology mean it is now possible to detect freshwater animals by collecting and analysing a simple water sample. Freshwater Habitat Trust has collaborated with Nature Metrics, an eDNA specialist company, which has undertaken analyses for multiple projects by Freshwater Habitats Trust, including Saving Oxford's Wetland Wildlife (SOWW).

2.2 A novel technique

The following results provide examples of simultaneous sampling for fish, amphibians, birds and aquatic mammals, all obtained from a single water sample at each sampling location. Owing to the novelty of this sampling method, researchers are still in the process of understanding how best to interpret these data.

We can:

- Report on presence of a particular species, but as with biological recording, a negative result is not proof of absence.
- Report on the presence of a group of species, when genetic separation of similarly related species is not yet possible.
- Identify potential false positives – where the presence of an unlikely species can be explained by contamination in the field or lab.
- Use these results to get a snapshot of the use of ALL freshwaters (ponds, lakes, rivers, streams and ditches) across a landscape – previously impossible with traditional techniques.

As these surveys become more commonplace, we will be better able to compare eDNA results to records obtained using current traditional methods, and thus gain a more detailed understanding of what the eDNA records show. For more information on the limitations of eDNA multispecies analysis, please refer to Appendix 4.

Freshwater Habitats Trust believes that environmental DNA surveys have the potential to revolutionise monitoring of freshwater biodiversity worldwide, given that many aquatic species are typically elusive, difficult to identify or time-consuming and costly to survey. We are excited to be at the forefront of the application of this new survey method. Many thanks to our fantastic volunteers for their hard work and enthusiasm when undertaking the eDNA surveys for Citizen Science: Testing the Water.

3.0 Methodology

A total of 71 survey sites were pre-selected by Freshwater Habitats Trust and landowner access permission confirmed. We recruited volunteers to the survey from those, which had already taken part in clean water testing (using nutrient test kits), and trained them via an online training course. Kits were then distributed to the volunteers via the Royal Mail. Volunteer packs, health and safety guidance, eDNA survey form, online training information and protocols can be found here: freshwaterhabitats.org.uk/bfer-lps-citizen-science-testing-the-water.

The sites were located in and around the BFER scheme area, and encompassed six waterbody types: ponds, streams, rivers, lakes, ditches and the cut-off channel. Sampling was undertaken between April and July 2022 by our team of over 60 volunteers. Volunteers used eDNA 'multi-species' kits to test for vertebrates (amphibians, mammals, birds and fish). Analysis of samples and provision of kits was by Nature Metrics www.naturemetrics.co.uk.

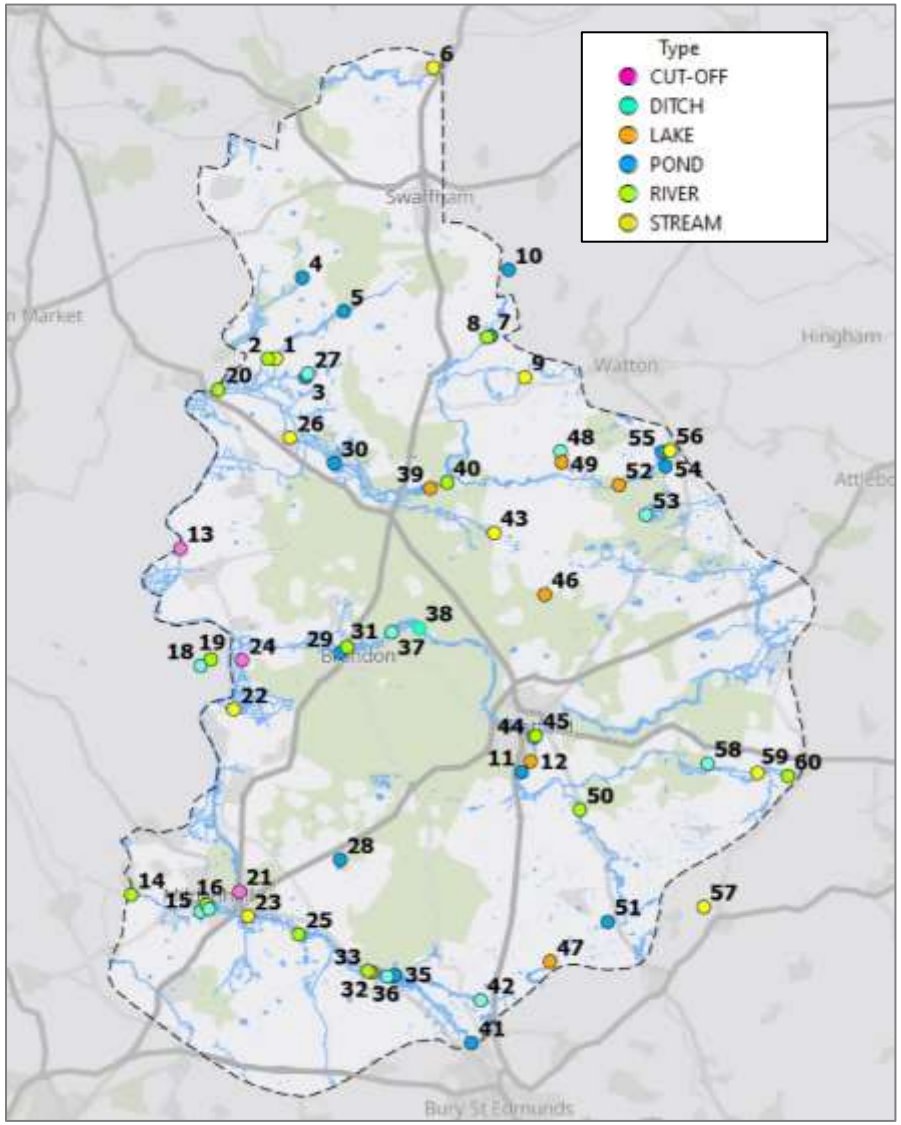
At each site, water samples were collected using a plastic sample pot and transferred into a collection bag (total volume of water collected up to a maximum two litres). To ensure we had a representative sample at each site on linear waterbodies (streams, ditches and rivers), samples were taken at equidistant points along the length of the waterbody. For ponds, samples were taken at 20 evenly spaced points around the pond edge, and for lakes, subsamples were taken from the inflow and outflow (if there was one) and then at 10 metre intervals around the lake perimeter.

The collection bag was shaken to mix the water and any eDNA contained therein. Water was then drawn up using a 50ml syringe, and pushed through a filter to trap any DNA that was present in the sample. This was repeated until the entire sample had been processed, or until the filter became clogged due to silt (at which point filtering was stopped), and the total volume processed then recorded.

Air was then repeatedly pushed through the filter using the 50ml syringe, so that any residual water had been expelled. A preservative solution was pushed through the filter to stabilise the DNA, and lock caps were fitted to each end of the filter to prevent the preservative from leaking. Each filter and accompanying data sheet (Appendix 1) was then sent to Nature Metrics, for analysis using standard metabarcoding techniques. Volunteers were responsible for completion of the test and postage back to the lab, with the regional project officer on hand to help with their enquiries.

Figure 5: eDNA multi species kits from NatureMetrics).

Figure 6: Volunteer filtering water sample thought the filter to capture DNA



No.	Site Name	No.	Site Name
1	TF7401 Oxborough STREAM	31	TL7971 West Stow RIVER LARK
2	TF7401 Oxborough RIVER GADDER	32	TL8070 Culford STREAM
3	TF7600 Foulden Common POND	33	TL8070 Fullers Mill POND
4	TF7605 Shingham POND	34	TL8070 Lackford Lakes DITCH
5	TF7803 Cockley Cley POND	35	TL8087 Santon Downham DITCH
6	TF8215 Castle Acre STREAM	36	TL8187 Santon Downham LITTLE OUSE RIVER
7	TF8600 Watton Brook STREAM	37	TL8194 Lynford Water LAKE
8	TF8605 Houghton Springs POND	38	TL8395 Lynford RIVER WISSEY
9	TF8680 Nunnery Lakes POND	39	TL8467 Suffolk Golf Club POND
10	TF8781 Nunnery Lakes LAKE	40	TL8502 Great Cressingham POND
11	TL 7091 Feltwell CUT OFF CHANNEL	41	TL8502 Great Cressingham RIVER WISSEY
12	TL6774 Jude's Ferry Bridge RIVER LARK	42	TL8469 Timworth DITCH
13	TL7173 Barton Mills DITCH	43	TL8592 Great Carr STREAM
14	TL7174 Barton Mills RIVER LARK	44	TL8782 Nuns Bridge LITTLE OUSE RIVER
15	TL7174 Norah Hanbury NR DITCH	45	TL8782 Nuns Bridge RIVER THET
16	TL7186 Lakenheath Fen DITCH	46	TL8789 Fowl Mere LAKE
17	TL7186 Lakenheath Fen LITTLE OUSE RIVER	47	TL8871 Broad Water LAKE
18	TL7199 Wittington RIVER WISSEY	48	TL8895 Sturton Carr DITCH
19	TL7274 Mildenhall CUT OFF CHANNEL	49	TL8896 West Mere LAKE
20	TL7283 North Fen STREAM	50	TL8978 Euston BLACK BOURNE RIVER
21	TL7373 Tuddenham Mill STREAM	51	TL9073 Pit Plantation POND
22	TL7386 Hockwold cum Wilton CUT OFF CHANNEL	52	TL9194 Thompson Water LAKE
23	TL7572 Temple Bridge RIVER LARK	53	TL9293 Cranberry Rough DITCH
24	TL7597 Northwold STREAM	54	TL9395 Stow Bedon POND
25	TL7600 Foulden common DITCH	55	TL9396 Thompson Common POND
26	TL7776 Canada Farm POND	56	TL9496 Thompson Common STREAM
27	TL7786 Brandon Staunch POND	57	TL9574 Stanton Chare STREAM
28	TL7795 Cranwick POND	58	TL9581 Knettishall DITCH
29	TL7886 Brandon LITTLE OUSE RIVER	59	TL9880 Gasthorpe STREAM
30	TL7970 Alder Carr LAKE	60	TL9980 Scarfe Meadow LITTLE OUSE RIVER

Figure 7: Locations of the 60 sites sampled using eDNA kits for BFER Citizen Science: Testing the Water project

4.0 Results

4.1 Overview

Of the **71** sample sites surveyed (see Appendix 2 for grid references and sample dates), **60** came back with positive records for one or more species (Figure 5). For individual site reports, see Appendix 6. Eleven samples were void (Appendix 3). There could be a number of possible reasons:

- Insufficient or no DNA in the sample: no amplification despite trouble shooting in the lab
- Inhibited samples: for example too much sediment which prevents detection of eDNA
- Non-target DNA present: only human or domestic animals were detected in the sample
- Lab error: the eDNA procedure fails to detect DNA
- Sampler error: field methods fail to collect or fix the sample and degradation occurs before the sample is analysed

The total number of vertebrate species recorded across the 60 sample sites was **69 species - 29** fish (39% of all species recorded), **29** birds (39%), **12** mammals (16%) and **five** amphibians (7%) (Figure 6). Across all waterbody types (standing and running waterbodies) fish species were found in 87% of sites, birds 98%, mammals 53% and amphibians 50% (Figure 7). The number of vertebrate species recorded at a single site ranged from one to 27 (Figure 8).

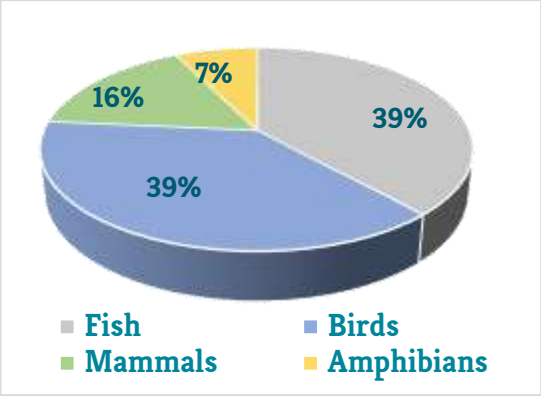


Figure 8: Percentage of vertebrate taxa found across all the eDNA survey sites

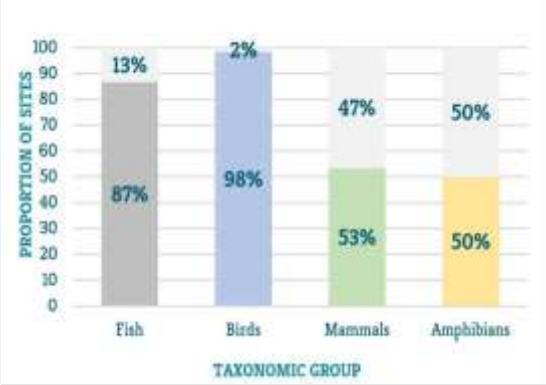


Figure 9: Percentage of taxon groups found in waterbodies in the Breaks area

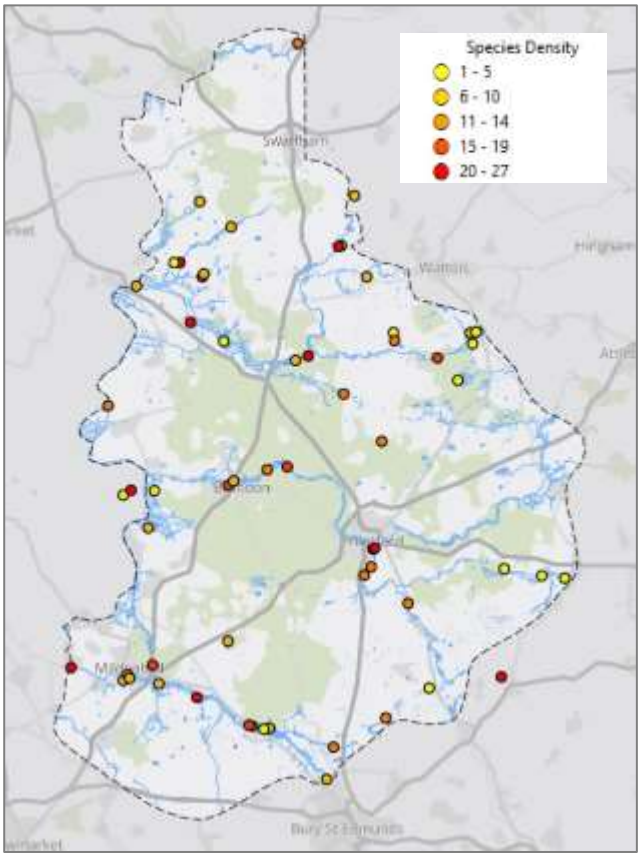


Figure 10: Number of vertebrate species per survey site recorded using eDNA

Species were categorised into aquatic species – species which you would typically expect to find living in or on water (e.g. fish, amphibians, aquatic mammals and wetland birds) and those which were non-aquatic (e.g. red fox, long-tailed tit, etc.). Forty aquatic vertebrates were recorded using

eDNA (60% of the species recorded). However, the eDNA of 28 non-aquatic species (40% of the species recorded) were recorded using waterbodies in The Brecks landscape.

Importantly the results showed that species are rarely confined to only standing water, or running water habitats (Figure 9). Only a handful of fish species (spined loach, sea lamprey, ruffe, common dace and bitterling) were found in running water only, and only two amphibian species (pool/marsh frog and great crested newt) were restricted to standing water (Figure 11).

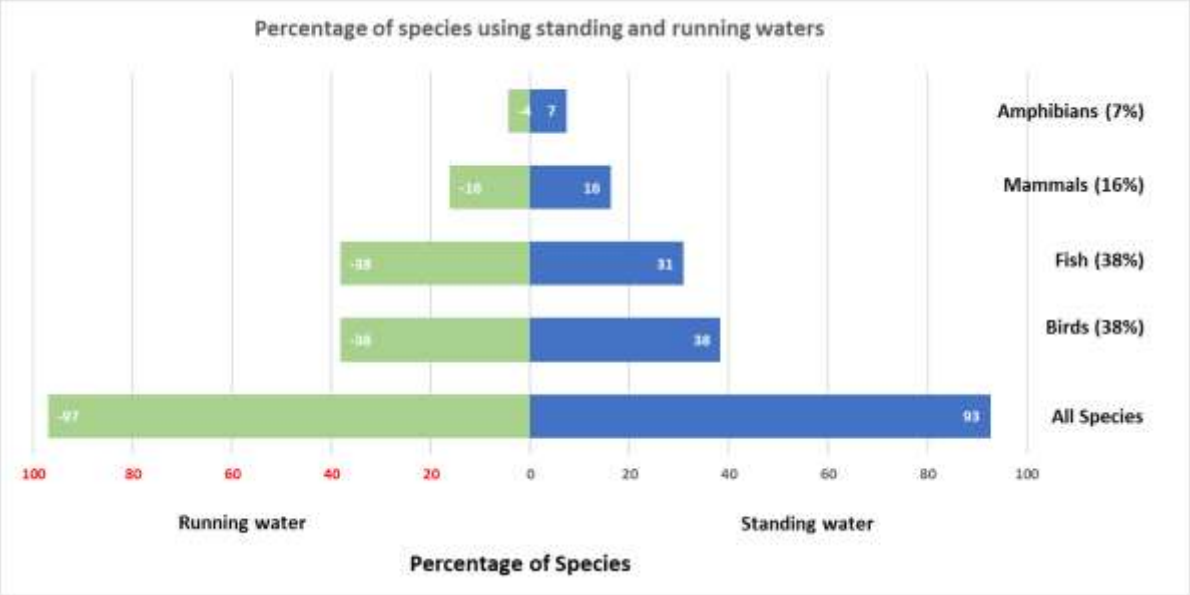


Figure 11: Percentage of species (total species and within taxonomic group) using running waters and standing waters

Some taxa were 'unresolved', i.e. not identified to species level, due to limitations in the DNA technology to separate closely related taxa. All taxa could be identified to family, 90% could be identified to genus, and 88% could be identified to species level.

Identification to family level: some taxa could only be identified to family level, where the markers for their sequence was an equally close match to several different species within the same family.

- *Anatidae sp.* (Waterfowl species)
- *Columbidae sp*. (Pigeon species)
- *Sturnidae sp*. (Starling species)
- *Laridae sp.* (Gull species)
- Corvidae sp. (Covid species)
- Cyprinidae sp. (Cyprinid species)
- *Mustelidae sp*. (Mustelid species)
- *Bovidae sp.* (Bovid species)

Identification to species level: Some animals could not be identified to an exact species, as they are so closely related ('paired species') that they are undistinguishable based on the DNA markers.

- *Lampetra planeri/ Lampetra fluviatilis* (Brook/River lamprey)
- *Pelophylax lessonae/Pelophylax ridibundus* (Pool/Marsh frog)
- *Corvus corax/Corvus corone* (Raven/Common crow)
- *Apodemus sylvaticus/Apodemus flavicollis* (Wood mouse/yellow necked mouse)
- *Callonetta leucophrys/Cygnus olor* (Ringed teal/Mute swan)

With regards to the Ringed teal/Mute swan record, Mute swan is a resident species in the area, whereas ringed teal is a species only found in collections in the UK (with rare escapees), so it can be assumed that the record is for Mute swan (per com Ian Henderson, BTO).

For more information on other paired species, see Appendix 5.

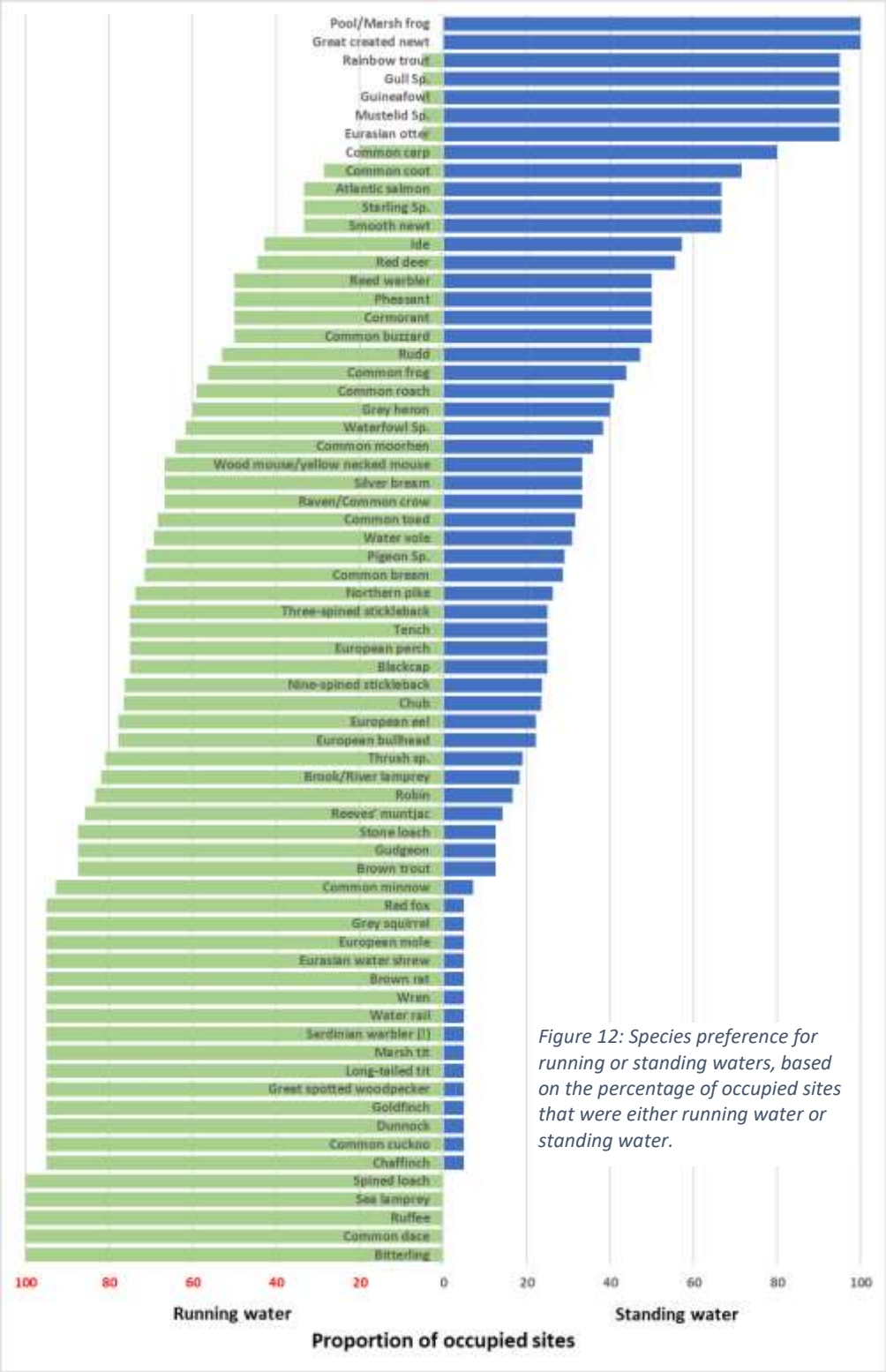


Figure 12: Species preference for running or standing waters, based on the percentage of occupied sites that were either running water or standing water.

4.2 Amphibians

A total of five amphibian species were recorded across the 60 sample sites:

- **Common toad** *Bufo bufo*
- **Common frog** *Rana temporaria*
- **Smooth newt** *Lissotriton vulgaris*
- **Great crested newt** *Triturus cristatus*
- **Pool/Marsh frog** *Pelophylax ridibundus/Pelophylax lessonae*

The most commonly detected amphibian species was common toad, which was recorded at 33% of the survey sites (20), followed by common frog 27% (16 sites), smooth newt 15% (nine sites), great crested newt 5% (three sites), and pool/marsh frog 3% (two sites). At one site, ***TL8789 Fowl Mere Lake*** four amphibian species were recorded (common toad, common frog, smooth newt and great crested newt). No amphibians were recorded at 50% of the sites surveyed (30 sites) (Figure 13). Palmate newts were not recorded at any of the sites, which is in line with what one might expect, given that there are very few records of this native amphibian in The Brecks area.

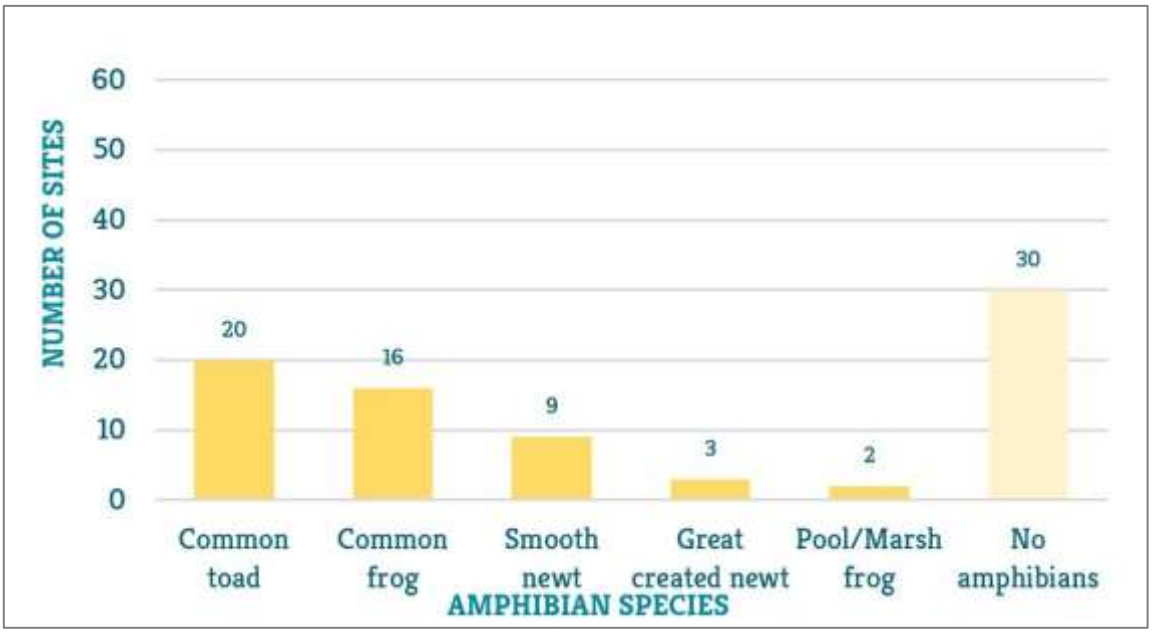


Figure 13: Number of amphibian species found across eDNA survey sites (n=60)

4.2.1. Common toad *Bufo bufo*

Common toads are a widespread amphibian found throughout mainland Britain. However, the number and quality of preferred breeding sites, deeper waterbodies such as farm/village ponds and area of terrestrial habitats, e.g. connected deciduous woodlands, have declined in many parts of the UK. There may also be a link between amphibian declines and the use of agricultural slug pellets. As a result, toad numbers have declined in recent years and in Britain, the species is now protected by law, as a *species of principal importance* under the Natural Environment and Rural Communities (NERC) Act (2006), formally the list of UK BAP species.

In our study, common toad was the most commonly detected amphibian species, occupying 33% of the survey sites. Toads were found in all freshwater habitat types tested including some running waters such as river, streams and ditches. In The Brecks area, many running waters are largely slow

flowing and shallow. Common toads appear to be using these habitats as well as their generally understood preference for standing waters, but they may also be passing through these habitats and not actually using them. Common toads can survive in waterbodies with fish, because they are distasteful or toxic to many predators, so it is possible that they were using both running and standing waters to breed.

4.2.2. Common frog *Rana temporaria*

The common frog is another widespread amphibian species in Britain, and can be found in almost any habitat where suitable breeding ponds are nearby. In the UK, it is protected under Schedule 5 of the Wildlife and Countryside Act (1981).

In our study, common frogs were also found in running waters, as well as in ponds and lakes. Overall their distribution was very similar to common toad (28% of surveyed sites), and the reasons for their distribution pattern are likely to be similar in these two species (Figure 12).

4.2.3. Smooth newt *Lissotriton vulgaris*

Smooth newts are the UK's most widespread newt species. Like the common frog, smooth newts can be found in a wide variety of pond habitats - e.g. large/small or permanent/temporary.

In this study, smooth newts occupied 16% of the survey sites. Of these, 68% were standing water sites and 32% of records were running water (small streams only). Unlike common frogs and common toads, which were both widespread throughout the BFER area, 67% of smooth newts were located within the Stanford Training Area (STANTA) and around Thompson Common (Figure 12), both of which contain a mosaic of semi-natural habitats and include areas of Breckland grassland and heath, as well as standing water, wetlands and many springs and streams. In these semi-natural sites, water quality was good - free from the widespread nutrient pollution recorded across many of the other sites in the study area.

4.2.4. Great crested newt *Triturus cristatus*

Great crested newts were once common and widely distributed throughout lowland Great Britain. However, in the last century the species has declined across Europe, mainly as a result of pond loss, terrestrial habitat loss and habitat deterioration. Great crested newts, their eggs, breeding sites and resting places are now strictly protected by British and European law. They are listed as a European Protected Species under Annex IV of the European Habitats Directive and a species of principal importance under the Natural Environment and Rural Communities (NERC) Act (2006), formally the list of UK BAP species.

Great crested newts were recorded at only three sites (5%) of the sampled sites. In the national monitoring scheme for great crested newts (coordinated by Freshwater Habitats Trust), great crested newts were estimated to occupy on average 13% (6, 19) of ponds in England (2015-2022) (Freshwater Habitats Trust 2022). The results from the Testing the Waters project (great crested newts were recorded in three out of 20 (15%) of the standing waters tested) indicates that occupancy in The Brecks area is in line with the national survey data.

In The Brecks study area, all of the great crested newts were recorded within the wetland rich semi-natural habitats of Stanford Training Area (STANTA) and on Thompson Common, (**TL8789 Fowl Mere LAKE, TL8896 West Mere LAKE** and **TL9396 Thompson Common POND**) with none recorded in the wider Brecks landscape. No great crested newts were recorded in any of the running waters.

It is likely that the historic distribution and pond occupancy of great crested newts in The Brecks was historically higher. In addition to the landscape scale negative changes recorded above, the results from the eDNA testing revealed that all but two of the standing waters **(TL7795 Cranwick POND** and **TL9395 Stow Bedon POND)** contained between two and 12 fish species. Great crested newt larvae and their eggs, are extremely vulnerable to predation by fish. Both three-spined and nine-spined sticklebacks were also recorded in two of the known great crested newt waterbodies. These predatory fish species are known to be particularly harmful to great crested newts and may indicate the beginning of a declining population at these sites.

All amphibians have a preference for high quality pond complexes within and adjacent to semi-natural/unimproved terrestrial habitat. These is huge potential to use the results of this survey to identify Wetland Opportunity Areas adjacent to known locations, where pond creation/restoration will strengthen populations and allow range expansion from existing sites to new areas.

4.2.5. Marsh/Pool frog *Pelophylax ridibundus/ Pelophylax lessonae*

The 'green frogs' form an unusual species complex which it is not possible to separate using eDNA. The group includes two non-native species marsh frog (*Pelophylax ridibundus*) and edible frog (*Pelophylax esculentus*), as well as the native northern pool frog (*Pelophylax lessonae*). Pool frogs became extinct in the UK, but have since been reintroduced to East Anglia. Pool frog is now protected by law, as a *species of principal importance* under the Natural Environment and Rural Communities (NERC) Act (2006), formally the list of UK BAP species.

There was one record for marsh/pool frog at Nunnery Lakes. In The Brecks survey area, there are no records for the non-native marsh frog on the NBN Atlas and no records of the species at Nunnery Lakes, which are well monitored by the BTO, which manage the site (per com Ian Henderson, BTO).

A false positive cannot be entirely ruled out; as there is a chance that DNA contamination may have been brought in by visitors (on boots for example). However, spread of invasive non-native species can happen rapidly and it may be that an unwanted pet marsh frog may have been released into this publicly accessible reserve (per com John Baker, Amphibian & Reptile Conservation).

Having highlighted a potential problem, staff at Nunnery Lakes will be hyper vigilant to the possibility of the presence of marsh frog, an easily identifiable species because of loud vocalisations during the daytime.

The record on Stow Bedon Common is a potential good news story, as native northern pool frogs have recently been re-introduced to ponds on Thompson Common. If this result were not a false positive, then it would indicate that pool frogs have naturally dispersed and expanded their population to an adjacent site. Follow up surveys will be carried out at Stow Bedon Common to confirm whether northern pool frog are now present, which would be an excellent outcome for the future of this species in the region.

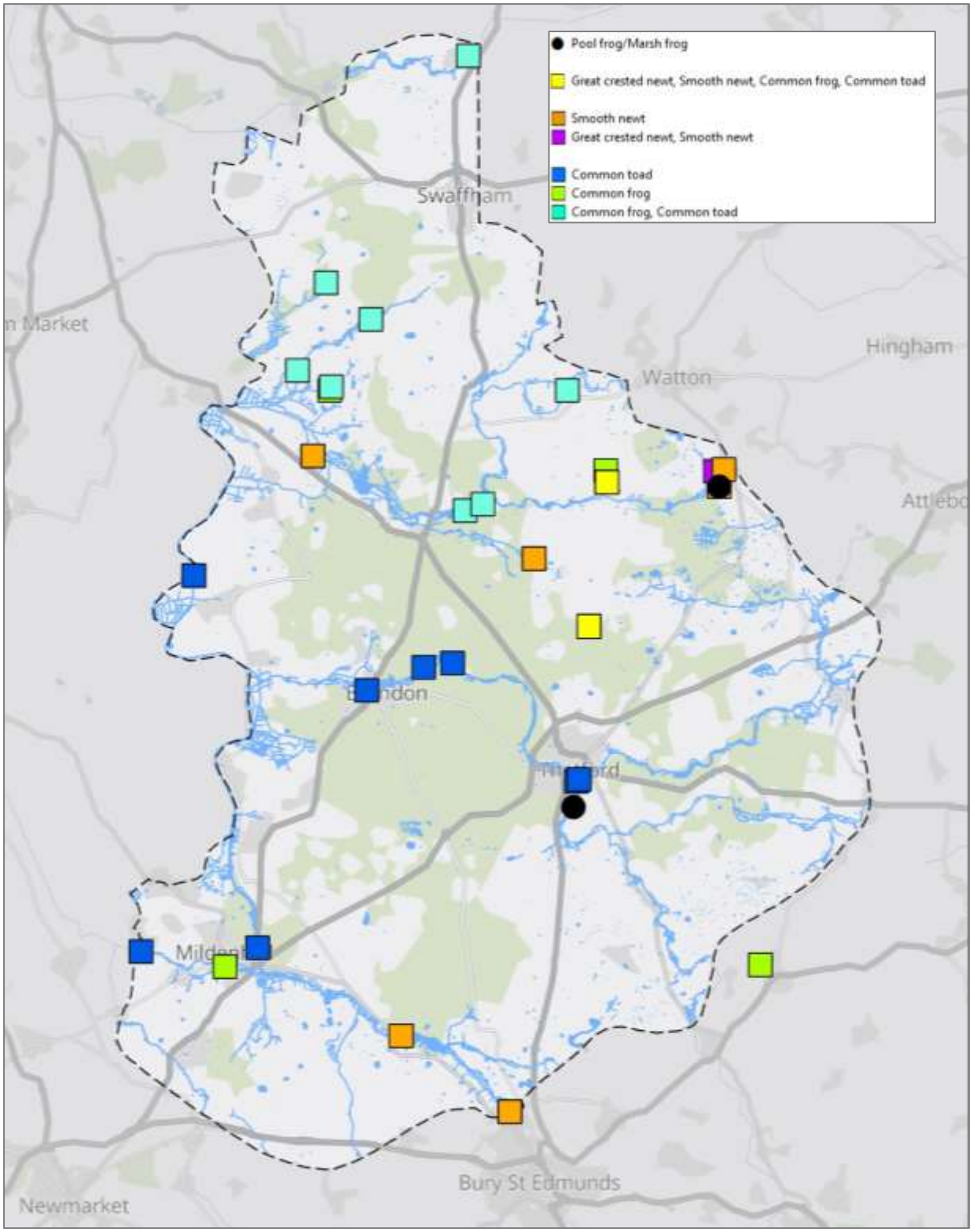


Figure 14: Distribution of amphibians in The Brecks Fen Edge and Rivers Landscape survey area

4.3 Fish

A total of 29 fish species were recorded from the 60 sample sites (Figure 15). eDNA surveys allow landscape-level surveying of fish – something that has been difficult to achieve up until now. Previously, most records were obtained from a subset of the water environment, by anglers and Environment Agency electrofishing surveys. eDNA surveys allow a wider range of freshwater habitats to be sampled across an entire catchment or landscape, from ponds and ditches, to rivers and lakes.

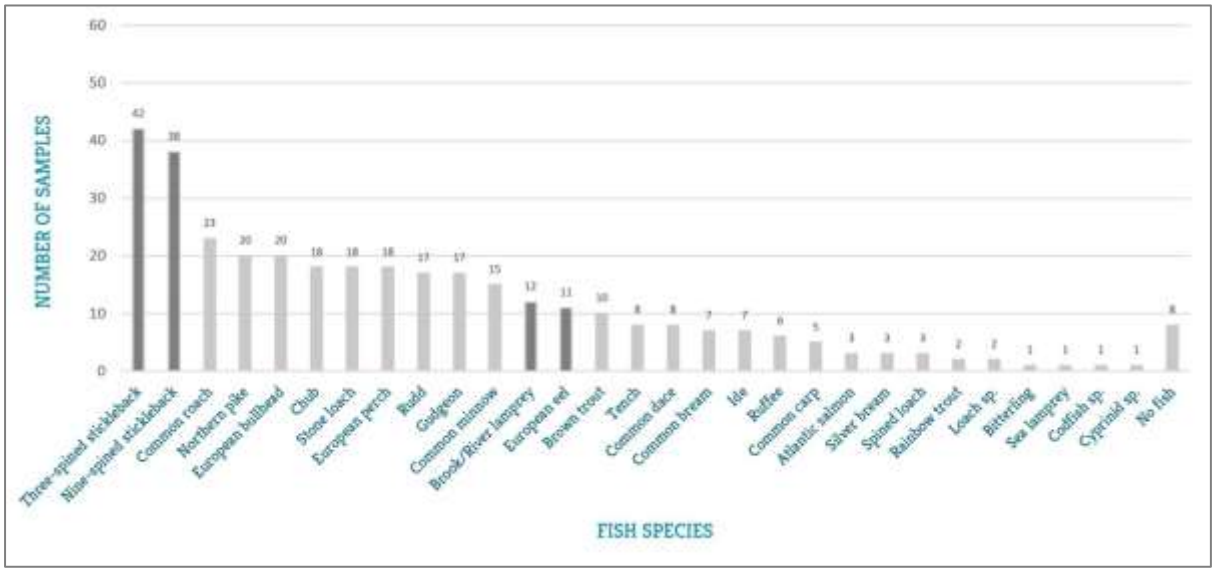


Figure 15: Number of fish species found across eDNA survey sites (n=60)

4.3.1 Three-spined and nine-spined stickleback *Gasterosteus aculeatus/Pungitius pungitius*

The most frequently recorded fish species in the study were three-spined stickleback and nine-spined stickleback which occurred in 42 and 38 sites respectively out of the 60 sites sampled (Figure 13). These two common and widespread species were found in all freshwater habitat types sampled, which is not surprising, as the species are known to reside in ponds, lakes, ditches and rivers. Three-spined sticklebacks, although small, are aggressive predators, feeding on invertebrates and other small animals, including tadpoles and smaller fish. Their presence, as discussed above, may reduce the likelihood of colonisation by amphibians.

4.3.2 European eel *Anguilla anguilla*

The European eel is a long-lived narrow fish that can grow to over a metre in length. They are found in rivers and ditches, but will also spend their lives in ponds and lakes, including waterbodies which are not connected to the running water network. They are capable of traveling overland during wet weather nights, provided there are no significant barriers to their journey. Eel grow to maturity in freshwaters, but leave their freshwater homes after around 15 to 20 years, to breed in the Sargasso Sea in the West Atlantic Ocean. Young eels (known as 'elvers') return to freshwater habitats to live, but the numbers returning have declined dramatically in recent years due to a number of issues. They are a species of principal importance under the Natural Environment and Rural Communities (NERC) Act (2006), formally the UK BAP species list, and are a critically endangered species on the IUCN Red List of Threatened Species.

In this study, eel were found in 11 of the 60 sample sites, nine of which were in running waters, including the River Lark, Little Ouse, River Wissey and River Thet. Two sites, a pond and a lake also supported eel.

4.3.3 Brook/River lamprey Lampetra planeri/Lampetra fluviatilis

Brook/River lamprey, are small eel-like jawless fish with a toothed, sucker-mouth. In terms of eDNA, these species are 'paired species' that are so closely related that they are indistinguishable based on the DNA markers. Both are Annex II species under The Habitats Directive.

Brook/river lamprey were found in 11 of the 57 sample sites (19%) (Figure 16). Nine lamprey sites were in running waters (82% of occupied sites), including the River Lark, Little Ouse and River Wissey, but two sites were in standing waters (18% of occupied sites). This species is usually restricted to running waters, and occasionally online lakes, therefore the standing water records should be viewed as possible false positives.

Interestingly, brook/river lamprey and European eel occurred in nine of the same sites (Figure 14):

- **TF7600 Foulden Common POND**
- **TL6774 Jude's Ferry Bridge RIVER LARK**
- **TL7186 Lakenheath Fen LITTLE OUSE RIVER**
- **TL7572 Temple Bridge RIVER LARK**
- **TL8187 Santon Downham LITTLE OUSE RIVER**
- **TL8395 Lynford RIVER WISSEY**
- **TL8782 Nuns Bridge LITTLE OUSE RIVER**
- **TL8782 Nuns Bridge RIVER THET**
- **TL7597 Northwold STREAM**

River lamprey are found only in Western Europe, where it has a wide distribution from southern Norway to the western Mediterranean. The UK populations are considered important for the conservation of the species at an EU level. Adult river lampreys live in the sea and return to freshwater to spawn. They are found in coastal waters, estuaries and accessible rivers, but have declined where pollution and or artificial obstacles such as weirs or dams, impede migration.

Brook lamprey are the smallest species of lamprey found in the UK and are a non-migratory freshwater species, occurring in streams and occasionally in lakes in north-west Europe. Although the species has declined in parts of the UK, it is the most abundant and widespread of the British lampreys and is often found in the absence of the other two species, for example above a barrier that precludes the presence of the migratory species.

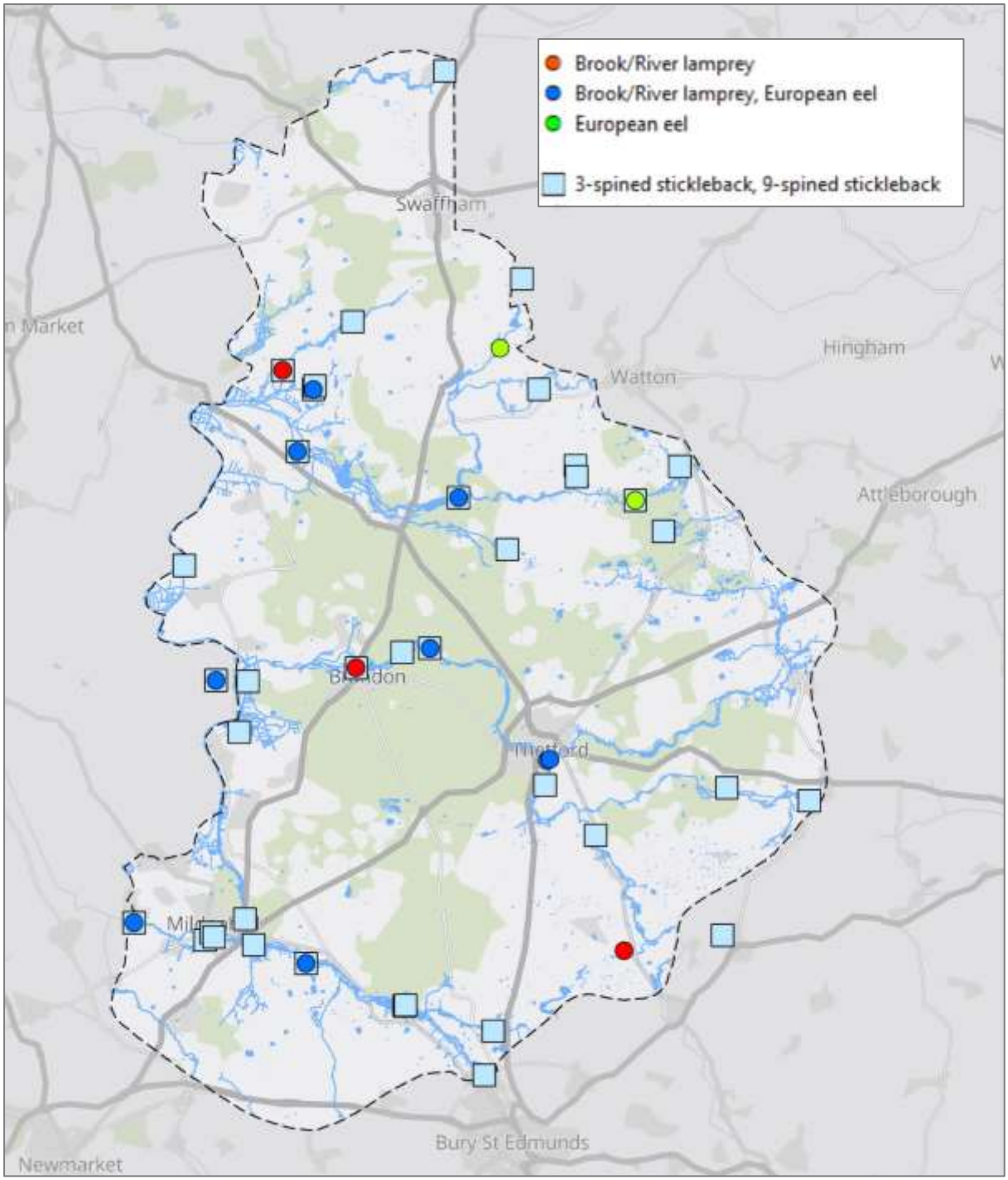


Figure 16: Distribution of three and nine spined stickleback, European eel and lamprey species in The Brecks survey area

4.4 Mammals

A total of 12 mammal species were recorded across the 60 sample sites, including three aquatic mammals. The most commonly detected aquatic mammal species was water vole, which was recorded at 15 of the sites, followed by Eurasian water shrew (four sites) and Eurasian otter (one site) (Figure 17).

- **Water vole** *Arvicola amphibius*
- **Eurasian otter** *Lutra lutra*
- **Eurasian water shrew** *Neomys fodiens*

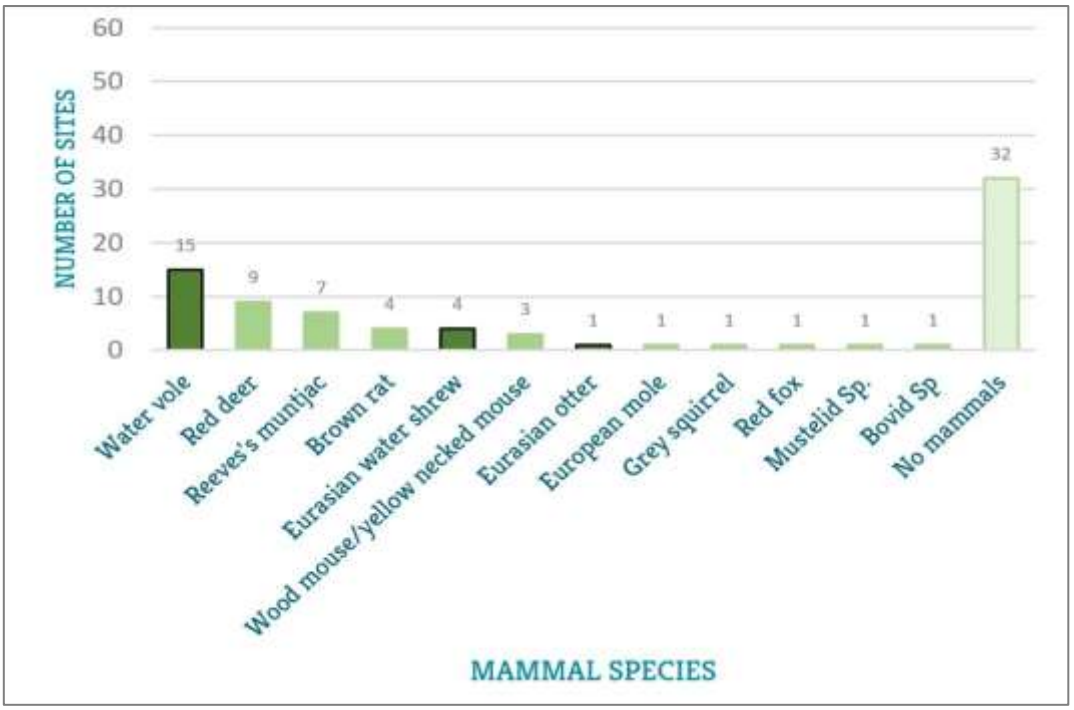


Figure 17: Number of mammal species found across eDNA survey sites (n=60)

4.4.1 Water vole *Arvicola amphibious*

Water vole live along rivers, streams and ditches, around ponds and lakes, in marshes and in reed beds. The species is under serious threat from habitat loss and predation by the American mink. In the UK, it is protected under Schedule 5 of the Wildlife and Countryside Act (1981) and is a species of principal importance under the Natural Environment and Rural Communities (NERC) Act (2006), formally the list of UK BAP species.

Water vole were found in 15 of the 60 sample sites (25%), which is an important result for The Brecks Fen Edge and Rivers Landscape; highlighting the significance of this area for freshwater species of conservation concern.

Eleven of the occupied sites were running waters, including the River Lark and associated ditch network, Little Ouse, River Wissey and River Gadder. Two ponds where the species occurred were adjacent to and directly connected to running water. However, two records of water voles were from standing water habitats, in the wetland rich semi-natural habitats of Thompson Common **TL9194 Thompson Water LAKE** and **TL9396 Thompson Common POND**, and not connected to running water.

4.4.2 Eurasian otter *Lutra lutra*

The elusive otter is one of our top aquatic predators, feeding mainly on fish, water birds, amphibians and crustaceans. They require clean rivers, with an abundant source of food with plenty of bankside vegetation to hide their secluded holts. Rare, due to historic persecution, but still a widespread species, the otter is found throughout the country and is increasing in distribution and abundance. In the UK, otter is protected under Schedule 5 of the Wildlife and Countryside Act (1981) and is a species of principal importance under the Natural Environment and Rural Communities (NERC) Act (2006), formally the UK BAP. It is also a European Protected Species under Annex IV of the European Habitats Directive and listed as Near Threatened on the global IUCN Red List of Threatened Species.

Otter were found in only one of the 57 sample sites, **TL7786 Brandon Staunch POND**, which is connected to Brandon Lock on the Little Ouse. Otters are often recorded from ponds and lakes, as well as running water habitats.

4.4.3 Eurasian water shrew *Neomys fodiens*

The water shrew is our largest shrew species, and is found throughout mainland Britain. However, water shrew are a cryptic species and are difficult to survey. They are often solitary and, because they are semi-aquatic, signs are hard to detect with traditional survey methods. eDNA opens up the possibility of detecting more sites where they are present and updating information on their distribution.

Water shrews live almost entirely in wetland habitats, such as streams, ponds, fens, drainage ditches and reed beds. They spend much of their time swimming underwater to hunt for aquatic insects. In the UK, water shrew are protected under Schedule 5 of the Wildlife and Countryside Act (1981).

In this study, water shrew were found in four of the 60 sites sampled (7%):

- **TF7401 Oxborough RIVER GADDER**
- **TF8215 Castle Acre STREAM**
- **TL8502 Great Cressinham RIVER WISSEY**
- **TL9574 Stanton Chare STREAM**

Interestingly water vole were also found in all four of these waterbodies (Figure 18). Recording two or more priority freshwater mammals at a site is additional evidence of their importance for freshwater wildlife.

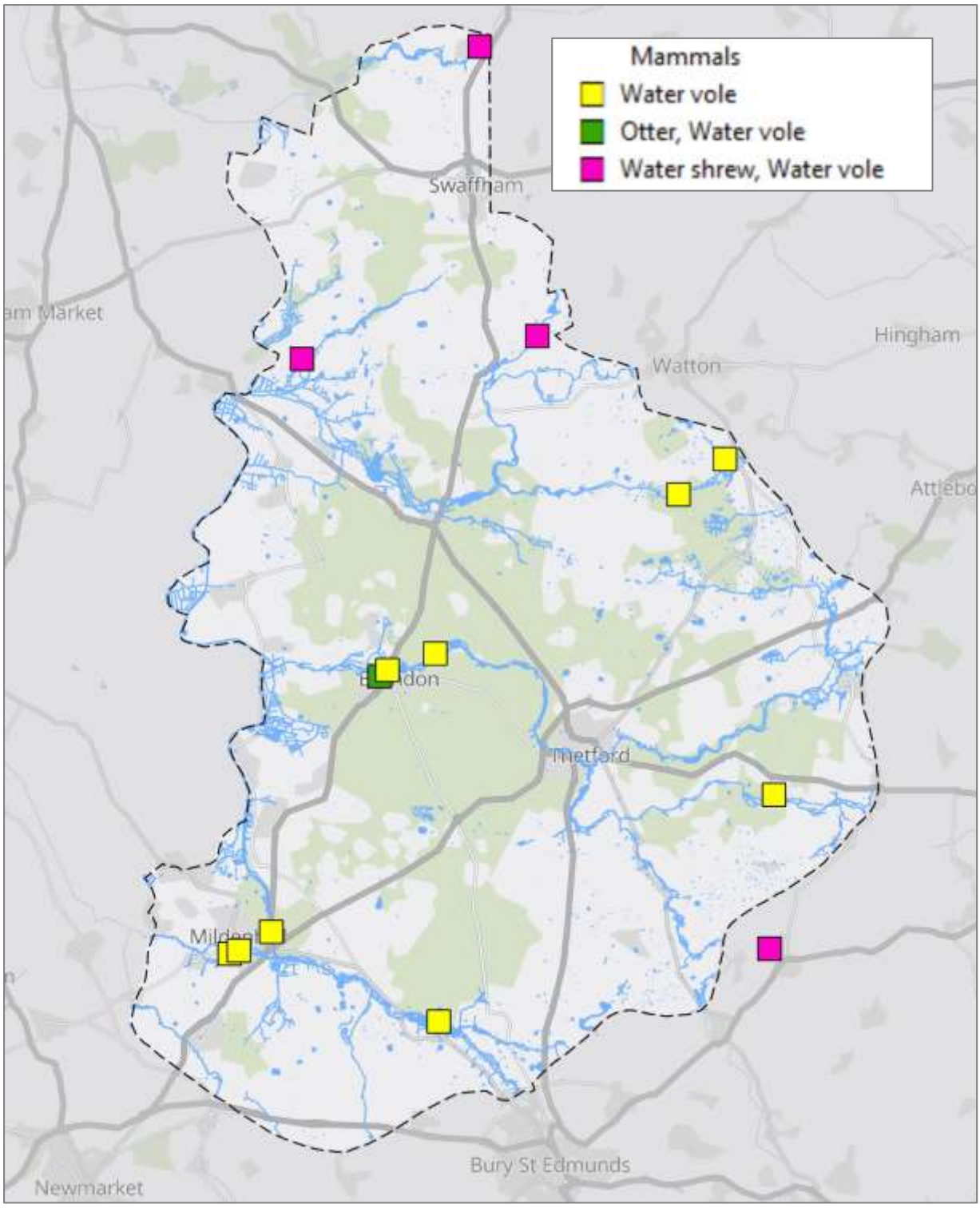


Figure 18: Distribution of aquatic mammals in The Brecks survey area

4.5 Water birds

A total of 26 bird species were recorded across the 60 sample sites (Figure 18). Twenty-one species were identified to species level, with a further five identified to family or genus level. Of the total birds identified, six were water birds:

- **Waterfowl sp.** ducks, geese and swan species
- **Common moorhen** *Gallinula chloropus*
- **Grey heron** *Ardea cinerea*
- **Common coot** *Fulica atra*
- **Cormorant** *Phalacrocorax carbo*
- **Water rail** *Rallus aquaticus*

The most commonly detected water birds were from the **waterfowl family** *Anatidae* recorded at 49 of the 60 sites (82% of sites). This family includes ducks, geese, and swans but the DNA markers for these sequences are too close to identify them to a genus or species.

The second most commonly detected water bird species was **common moorhen,** recorded at 42 sites (72%). **Grey heron** were recorded at 10 sites (17%), **common coot** at 7 sites (12%), **cormorant** at 5 sites (8%) and **water rail** and **green-winged teal** at 2 sites each (3%) and mandarin duck at just 1 site (2%). A further two birds species associated with water were also identified, **reed warbler** *Acrocephalus scirpaceus* and **common cuckoo** *Cuculus canorus.* These were recorded at two (4%) and one (2%) out of the 60 sites respectively (Figure 19).

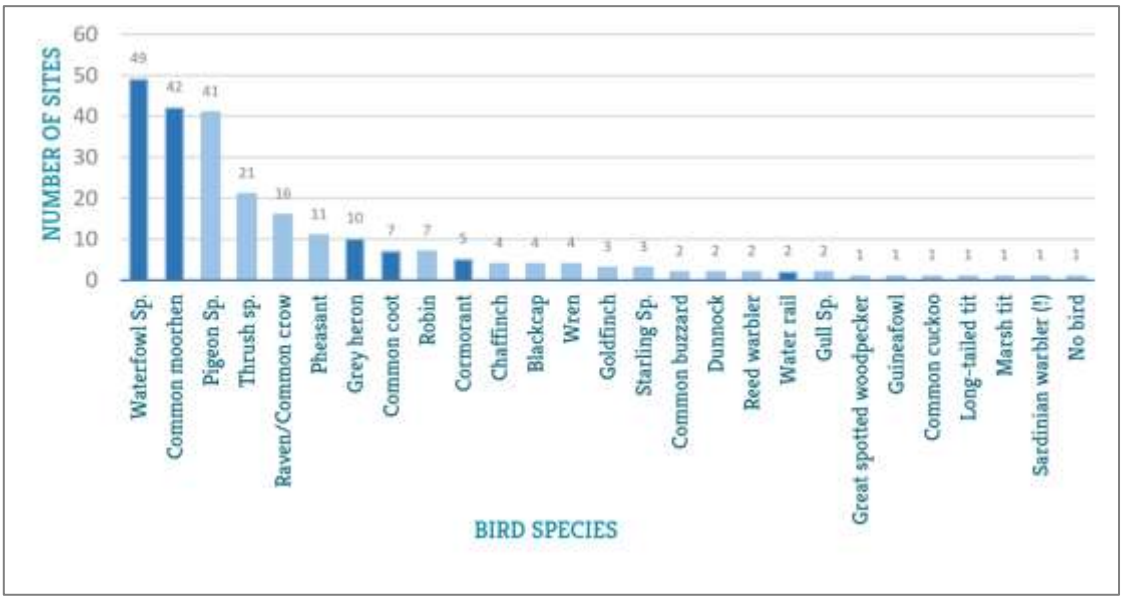


Figure 19: Number of bird species found across eDNA survey sites (n=60)

4.5.1. Waterfowl species *Anatidae sp.*

Ducks, geese and swans, which make up this family, are widespread and can be found in a variety of wetland habitats from small ditches, ponds, lakes and rivers to coastlines. All species are aquatic, with webbed feet and a broad, flattened bill. Nearly all nest on, or beside the water. It is not surprising, therefore, that in the study waterfowl species were recorded at 49 out of the 60 sites sampled, including all of the freshwater habitat types, both running and standing.

4.5.2. Common moorhen *Gallinula chloropus*

Common moorhens can be seen around any pond, lake, stream or river, or even ditches in farmland. In the UK, they breed in lowland areas, especially in central and eastern England. UK breeding birds are residents and tend not to travel far. In the UK, they are protected by the Wildlife and Countryside Act (1981) and classified in the UK as AMBER under the Birds of Conservation Concern: the Red List for Birds (2021). Moorhen, like the waterfowl species, were recorded in all of the waterbody types sampled and occurred at 39 of the 57 study sites.

4.5.3 Grey heron *Ardea cinerea*

Grey herons are widespread common birds, which spend most of their time alone, feeding mainly on fish, although they will take a wide variety of amphibian, other vertebrate and invertebrate species. Heron can be found on canals, ponds, lakes and rivers across the UK. In the UK, they are protected by the Wildlife and Countryside Act (1981), and classified as GREEN under the Birds of Conservation Concern: the Red List for Birds (2021). In this study, the species was found in 10 of the 57 sample sites, six of which were in running waters (rivers and streams) and four standing waters (ponds and lakes), distributed evenly across The Brecks area.

4.5.4 Common coot *Fulica atra*

The common coot is much larger than its cousin the moorhen. An all-black bird with a distinctive white beak, this widespread water bird can be seen on freshwater lakes, gravel pits, reservoirs, ponds and rivers. In the UK, it is protected by the Wildlife and Countryside Act (1981), and classified GREEN under the Birds of Conservation Concern: the Red List for Birds (2021). Coot were found in seven sample sites, five of which were on lakes and larger ponds, and two of which were on rivers.

4.5.5 Cormorant *Phalacrocorax carbo*

Cormorant are large, black water birds with a primitive reptilian appearance. They feed on fish, which they catch with their long, hook-tipped bills while swimming underwater. Being excellent fishers has brought them into conflict with anglers, thus causing them to be persecuted in the past. They can be found around the UK coastline on rocky shores, coastal lagoons and estuaries, and increasingly on inland rivers, reservoirs, lakes and gravel pits. The UK holds internationally significant wintering numbers. In the UK, cormorant are protected by the Wildlife and Countryside Act (1981), and classified as GREEN under the Birds of Conservation Concern: the Red List for Birds (2021). Cormorants were found in four sample sites, including a lake and two rivers.

4.5.6 Water rail *Rallus aquaticus*

Smaller than the moorhen, the water rail is a fairly common but highly secretive inhabitant of freshwater wetlands, more often heard than seen. Difficult to see in the breeding season, it is relatively easier to find in winter, when it is also more numerous and widespread. Water rail can be seen widely but are thinly distributed as breeding birds across the UK. They are most abundant in eastern England and among suitable habitat along the south coast. In the UK, they are protected by the Wildlife and Countryside Act (1981), and classified GREEN under the Birds of Conservation Concern: the Red List for Birds (2021). The species was only found at two sample sites, both on running water.

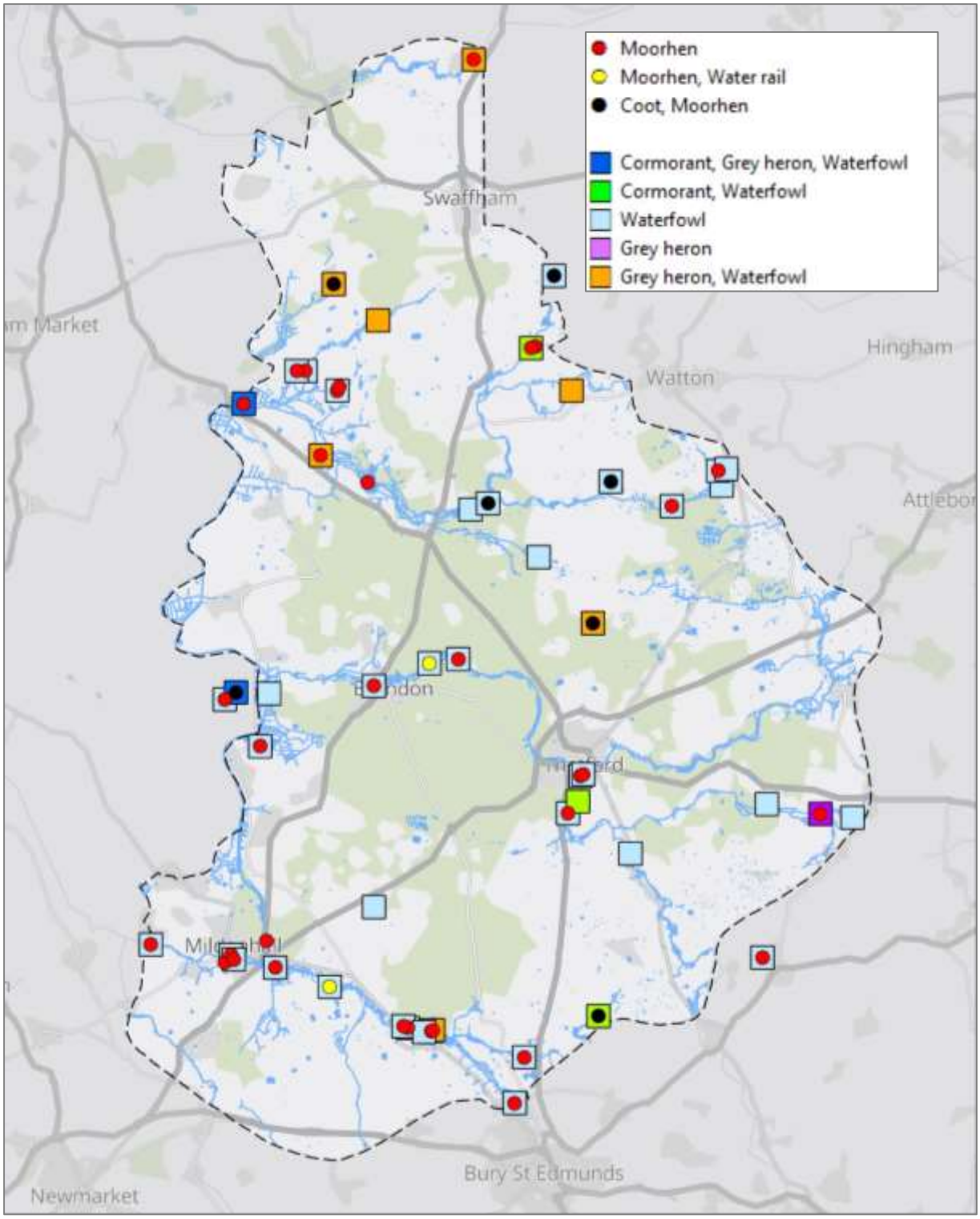


Figure 20: Distribution of aquatic birds in The Brecks survey area

4.6 Other species

In theory, vertebrate DNA can wash into water from anywhere in the landscape, and degradation/transport pathways for terrestrial species DNA entering the freshwater network is currently unknown. However, records of species through their DNA can help to build a picture of landscape scale biodiversity.

As well as the three aquatic mammal species, nine other non-aquatic mammals were recorded, the most abundant being two deer species red deer (*Cervus elaphus*) recorded at nine sites, and Reeves's muntjac (*Muntiacus reevesi*) recorded at seven sites. All non-aquatic mammal species are probably using waterbodies to drink, or shedding DNA as they pass through waterbodies.

A suite of non-aquatic birds was detected (21 species in total), including two birds species associated with water, reed warbler (Acroc*ephalus scirpaceus*) and common cuckoo (*Cuculus canorus*). The reed warbler is a summer breeding visitor to the UK, with the largest concentrations in East Anglia and along the south coast. It is found almost exclusively found in reed beds, building its nest in vegetation over water. Cuckoos are also summer visitors and are well-known brood parasites. Instead of building their own nest, the females lay their eggs in other birds' nests, especially those of reed warblers, meadow pipits and dunnock. Non-aquatic bird DNA could have arrived in the water in many different ways, from visiting the waterbody to drink or wash, or from dropping faeces as they pass over the water.

A few species in the results are being treated as false positives, as it is highly unlikely that they are present at the sample sites:

- Atlantic salmon *Salmo salar*, was detected at three sites (**TL8070 Culford STREAM, TL8789 Fowl Mere Lake** and **TF8781 Nunnery Lakes LAKE**), most probably a result of contamination, possibly from fishing bait.

- Codfish species *Gadidae sp*. was also detected at one site **TL8502 Great Cressingham River Wissey.** Again, most probably a result of contamination, possibly from fishing bait.

- Helmeted guineafowl (*Numida meleagris*) also appeared at one site **TL8070 Fullers Mill Pond**. Originating from Africa, this bird is widespread in the UK as a domesticated species. However, the land manager has confirmed that the bird is not present on this site. It is unclear how DNA has arrived, but it may have come in from the boots of a visitor, as the site is publicly accessible.

Whilst further clarity is required to fully understand what these records mean (for example, how far DNA can travel and the extent of the area it may have washed in from), multi-species eDNA surveys have the potential to provide a useful snapshot of entire communities.

4.7 Water quality testing

As well as collecting eDNA data, volunteers also collected water quality data at the same time. The survey uses 'quick kits' to assess the level of nitrate and phosphate pollution: two nutrients which can pose a major risk to wildlife if they are above natural levels.

Freshwater plants and animals need nutrients to grow; they have evolved over millions of years in a world where water naturally had very low nutrient levels. When we add more - even the smallest amount - it causes profound changes to the freshwater environment. Excess nutrients cause algae, fungi, bacteria and some water plants to grow rapidly. This smothers slower growing and more delicate species, eventually killing them off, which has a knock on effect for the animals that depend on them.

As nutrient levels increase, sensitive species are lost and the habitat slowly becomes poorer in wildlife. Many polluted habitats will still have some wildlife, but they will not have the rich diversity or sensitive species that depend on clean water.

In lowland England and Wales, there are such large quantities of nutrients draining from farmland and urban areas that it is hard to find any rivers or large streams which are free from these pollutants. This is a serious issue for both our own health and the health of the environment.

The cleanest sites are likely to be the smaller freshwaters: the ponds and small lakes, smaller streams and ditches, located in places, which are isolated from pollution sources. Volunteers surveyed 20 standing waterbodies, of which 71% were classified as having clean water. In running water sites (ditch, stream, and river habitats), only 17% were classified as clean (Figure 21).

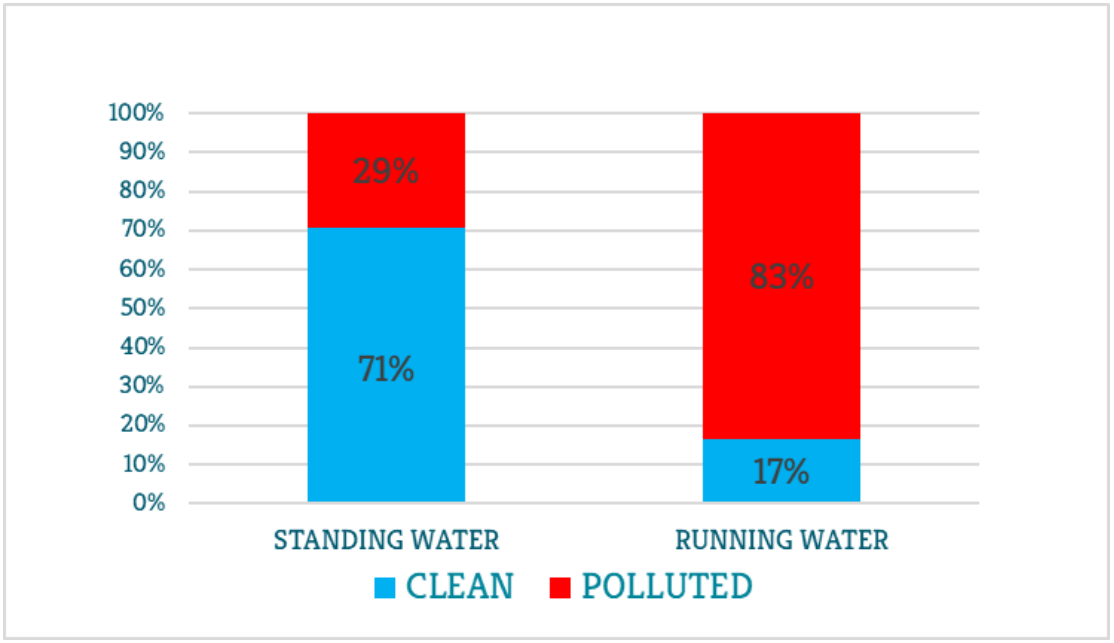


Figure 21: Percentage of clean and polluted water across standing and running waterbodies in The Brecks study area.

Figure 22: Clean water sample from a pond in The Brecks study area.

Figure 23: Polluted water sample from a ditch in The Brecks study area.

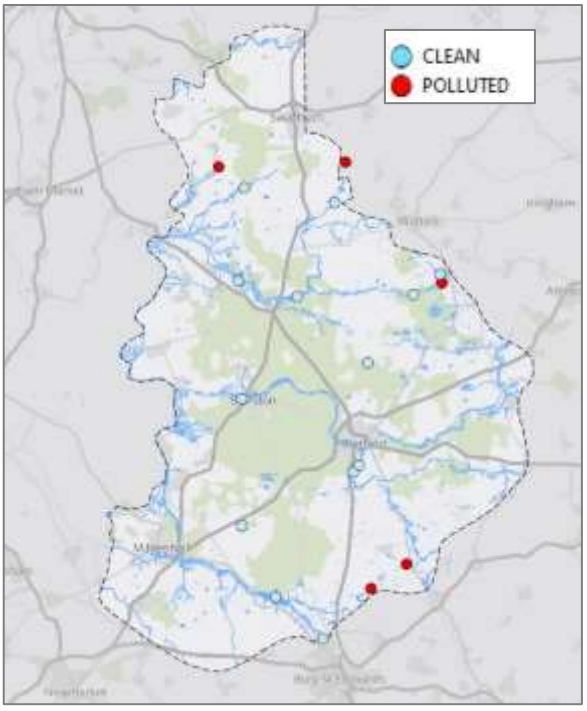


Figure 24: Distribution of clean and unpolluted water in standing waterbodies (ponds & lakes)

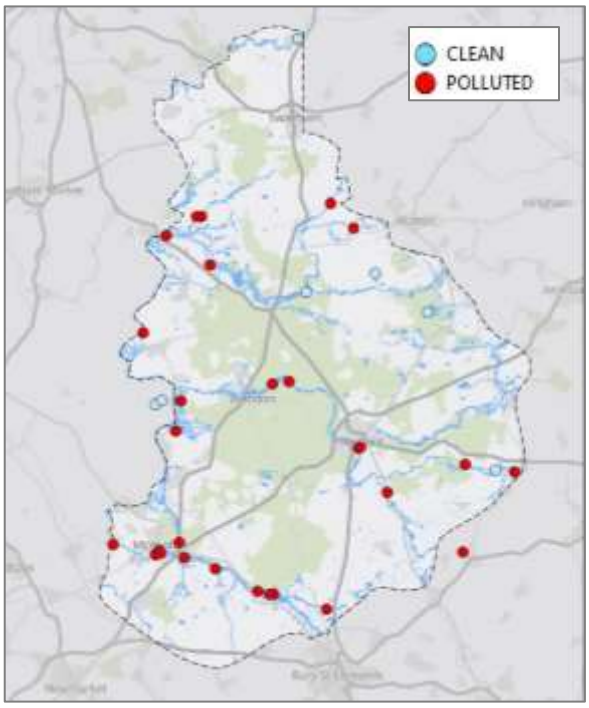


Figure 25: Distribution of clean and polluted water in running waterbodies (rivers, streams, ditches & cut-off channel)

4.8 New Priority Ponds

The BFER Testing the Water project identified a further 7 ponds qualifying as a Priority Pond under **Criteria 2 – Great Crested Newt, Common Toad, Pool Frog, Eurasian Otter, Water Vole, European eel.** However, one of these, *TL9396 Thompson Common Pond*, overlapped with one of the already mapped Priority Ponds.

No.	1km Grid Square and Site Name	Grid Reference	Species of high conservation importance
3	TF7600 Foulden Common POND	TF 76194 00194	European Eel
4	TF7605 Shingham POND	TF 76030 05068	Common Toad
5	TF7803 Cockley Cley POND	TF 78057 03406	Common Toad
27	TL7786 Brandon Staunch POND	TL 77872 86643	Common Toad, Eurasian otter
33	TL8070 Fullers Mill POND	TL 80574 70887	Water vole
54	TL9395 Stow Bedon POND	TL9395	Pool Frog
55	TL9396 Thompson Common POND	TL 93632 96523	Great Crested Newt

A further pond, sampled for vertebrates, in the study, was also identified as a Priority Pond based on **Criteria 2 - an RDB plant species Water-violet (*Hottonia palustris*).** The eDNA result for this pond was very poor with just two species of bird identified during the survey, moorhen and pigeon sp. However, the pond had clean unpolluted water and the owner and surveyor sent us a photo of the pond in question **TL8502 Great Cressingham POND**, which clearly identified Water-violet in abundance in the pond. Water-violet is widespread in the southeast of England but has undergone a significant decline in recent years and is now included on the Red Data List for England. Threats include habitat loss, drainage and poor management. The submerged nature of Water-violet make it very susceptible to water quality. This pond had already qualified as a Provisional Priority Pond due to its status as a clean water pond, which would have flagged a desire to follow up with further surveys, including PSYM assessment and other priority species surveys to confirm Priority Pond status.

Figure 26: Water-violet on TL8502 Great Cressingham POND CREDIT: Rosselle Chapman

The BFER Testing the Water Project has produced a new Priority Ponds map for The Brecks Area and had brought the total number of Priority Ponds in this Important Freshwater Landscape up to 159 Priority Ponds.

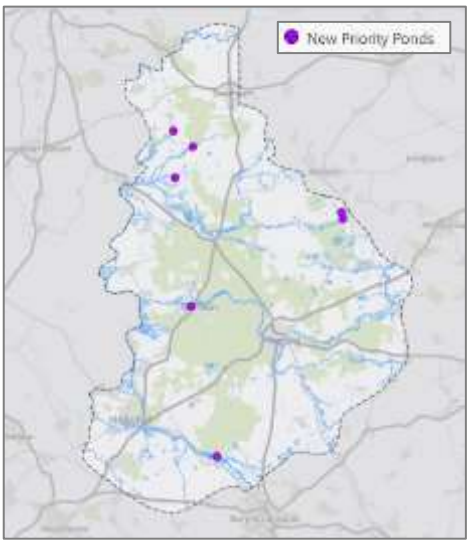


Figure 27: New Priority Ponds in The Brecks area discovered during eDNA surveys

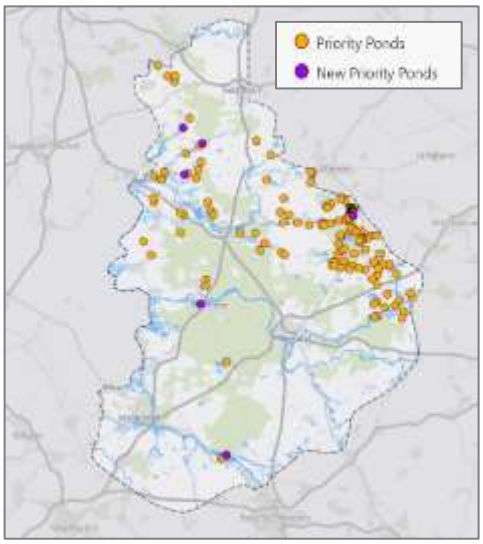


Figure 28: All Priority ponds in The Brecks area post eDNA survey

The majority of identified Priority Ponds, are located to the East of the of the BFER scheme area in the Thompson and Stow Bedon, Frost Common and East Harling valley areas. These where all identified as Important Freshwater Areas - IFA's (Biggs & Dunn, 2028) which are high priority areas for freshwater biodiversity.

Thompson and Stow Bedon Commons, managed by Norfolk Wildlife Trust, contain the highest density of pingo ponds in Breckland. The sites contain a mosaic of both dry and unimproved neutral grasslands, oak and birch woodland and hawthorn scrub. Thirty-four Priority Ponds have currently been identified within the Thompson Water, Carr and Common (SSSI) including eDNA survey ponds TL9396 **Thompson Common POND** and **TL9395 Stow Bedon POND**.

Frost's Common is an area of mixed plantation owned by Forestry England, and forms part of the Breckland Forest (SSSI). The site has over 40 natural pingo ponds, with six currently designated as Priority Ponds. These support many rare freshwater plants and animals including invertebrates associated with pingo systems. A further five Priority Ponds can also be found in the adjacent woodland/heathland compartments of Hockham Heath, Bragmere Pits and Queen's Close, which are all within the Breckland Forest (SSSI).

East Harling Common, situated on chalk on the eastern edge of the Norfolk Breckland, is also of great importance for its pingo ponds and its many scarce species of beetles. Floristically rich fen, a declining habitat, has developed in and around many of the ponds. Middle Harling Fen is a small calcareous valley fen situated at the head of a tributary of the River Thet. The site lies in a shallow valley and a number of springs, bearing water from the underlying chalk, emerge on the sloping ground. A wide range of grassland types is present including both wet and dry communities. The species-rich fen vegetation includes several plants that are now uncommon in East Anglia due to the drainage of many similar areas. Both East Harling Common and Middle Harling Fen are designated at SSSI's.

Thirteen Priority ponds have been identified within the STANDA Military Training around the Thompson and West Tofts alluvial corridor of The River Wissey, also IFA's. STANTA encompasses a large area of semi-natural habitat, including extensive areas of Breckland grassland and heath which is largely unaffected by intensive agriculture, drainage, pollution, eutrophication or water abstraction. Moving west from STANTA along the line of the River Wissey more Priority Ponds can be found around the Didlington area, all in areas of woodland including Forestry England's Spring Covert and Moor Plantation near Didlington Hall.

To the North East of the BFER scheme area, a cluster of Priority Ponds can be found around the Foulden, Gooderstone and Oxborouge area, which has also been identified as an IFA. Several Priority ponds, including new eDNA Priority Ponds **TF7600 Foulden Common POND** and **TF7803 Cockley Cley POND** are located in the area close to the floodplains of the River Gadder and Oxborough Drain, both tributaries of the River Wissey. Foulden Common is a large SSSI site encompassing a wide mosaic of habitats, including open water, rich fen, both acidic and calcareous grassland, birch woodland and pingo ponds. However only two Priority Ponds have so far been identified in this site.

To the south of the BFER scheme area, only four Priority Ponds have been identified, all around the West Stow and Lackford area. Three of these are within the Suffolk Wildlife Trust's Lackford Lakes Nature Reserve and one, an eDNA Priority Pond **TL8070 Fullers Mill POND**, at Fullers Mill Gardens. Conversely, all these ponds sit just outside the boundaries of Lackford Lakes SSSI and Breckland Forest SSSI.

Out of all the Priority Ponds so far mapped 42% sit within protected areas (SSSI's) however the remaining 58% of ponds, all sit within areas of woodland or semi natural habitats. Highlighting the importance of surrounding good quality terrestrial habitat for freshwater wildlife.

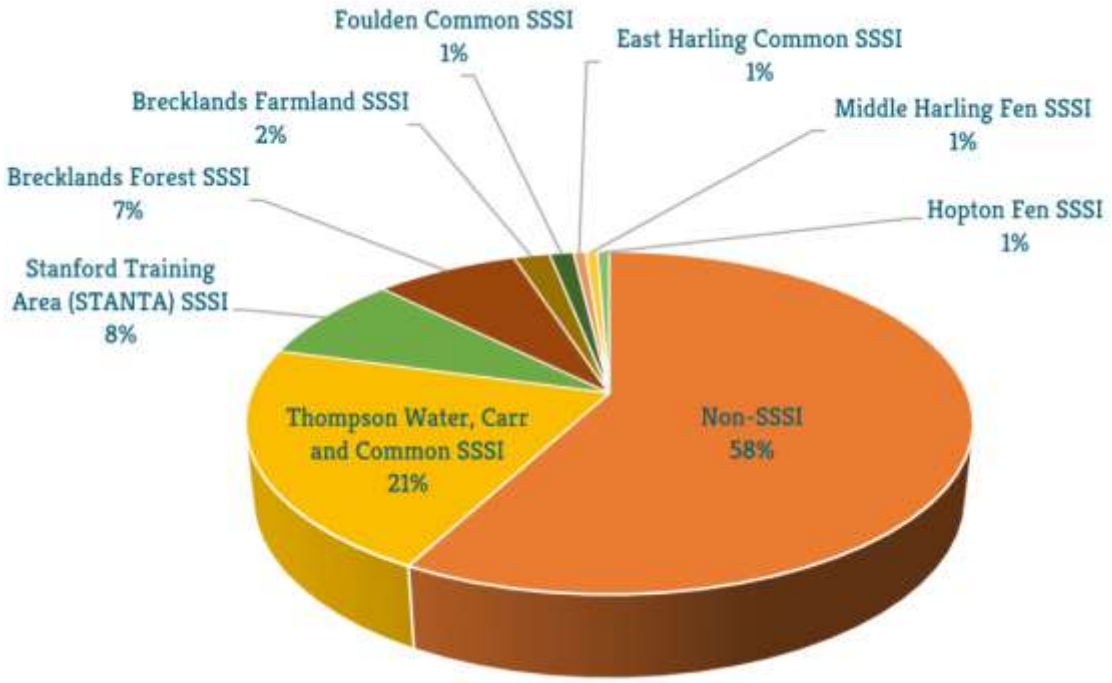


Figure29: Percentage of Priority Ponds in the BFER Scheme Area that fall within Sites of Special Scientific Interest (SSSI's)

Within many if these sites including within the pond rich STANTA, Frost's Common and Foulden Common areas, there are, potentially many more Priority Ponds, which have not been identified. It is a recommendation from this project to continue surveys of ponds in these areas and the wider BFER scheme area to include PSYM assessment and other priority species surveys to confirm the location of other Priority Ponds.

5.0 Discussion

The Testing the Water project has helped to engage volunteers in collecting valuable, accurate data on the distribution of vertebrate species in The Brecks Fen Edge and Rivers landscape area. It also helped to identify seven new Priority Ponds in the Brecks area, based on a combination of clean water and the presence of species of high conservation importance.

The results of eDNA surveys have revealed that a large number of vertebrate species are using the freshwater environment in The Brecks area. As well as fully aquatic animals, such as fish and aquatic and semi-aquatic animals from the mammal, bird and amphibian families, an abundance of non-aquatic species were also found to be using water, probably to drink and wash, but this highlights the importance of freshwater habitats in the environment.

The results also reveal that most of the species detected were found across all the freshwater habitat types, including standing waters (ponds and lakes) and running waters (rivers, streams, ditches and cut-off channels). This suggests that the whole freshwater environment is an important

resource for all vertebrate species. Even species traditionally associated with rivers, such as European eel, were detected in standing waterbodies.

Most freshwater plants and animals have evolved over millions of years in a world where the natural level of nutrients in ponds, lakes, streams and rivers was very low. When more nutrients, like phosphate and nitrate, are added to the freshwater environment this can cause profound changes and freshwater wildlife struggles to adapt.

Although in this study, nutrient pollution was found in all the freshwater habitat types tested, standing waterbodies, including ponds and lakes were overall the cleanest freshwater habitats found in The Brecks. Like most of lowland Britain, rivers and some ditches and streams in the area were found to be proportionally more polluted by nutrients.

It is very difficult to improve the water quality of running waters, as rivers and streams drain large areas of land and are exposed to multiple sources of pollution from urban and agricultural areas. Ponds and lakes are also affected by the surrounding land use, but they naturally drain smaller areas of land so are less likely to be affected by pollution problems if they are located in areas of semi-natural or uncultivated land.

Building up the network of standing waterbodies in the landscape, by creating more ponds in clean catchments would therefore provide a solution to increase freshwater biodiversity in The Brecks area, a refuge for a multitude of species away from pollution issues associated with running waters. Clean water is vital for freshwater biodiversity and these potentially unpolluted small freshwater waterbodies could support rich and valuable wildlife communities.

6.0 References

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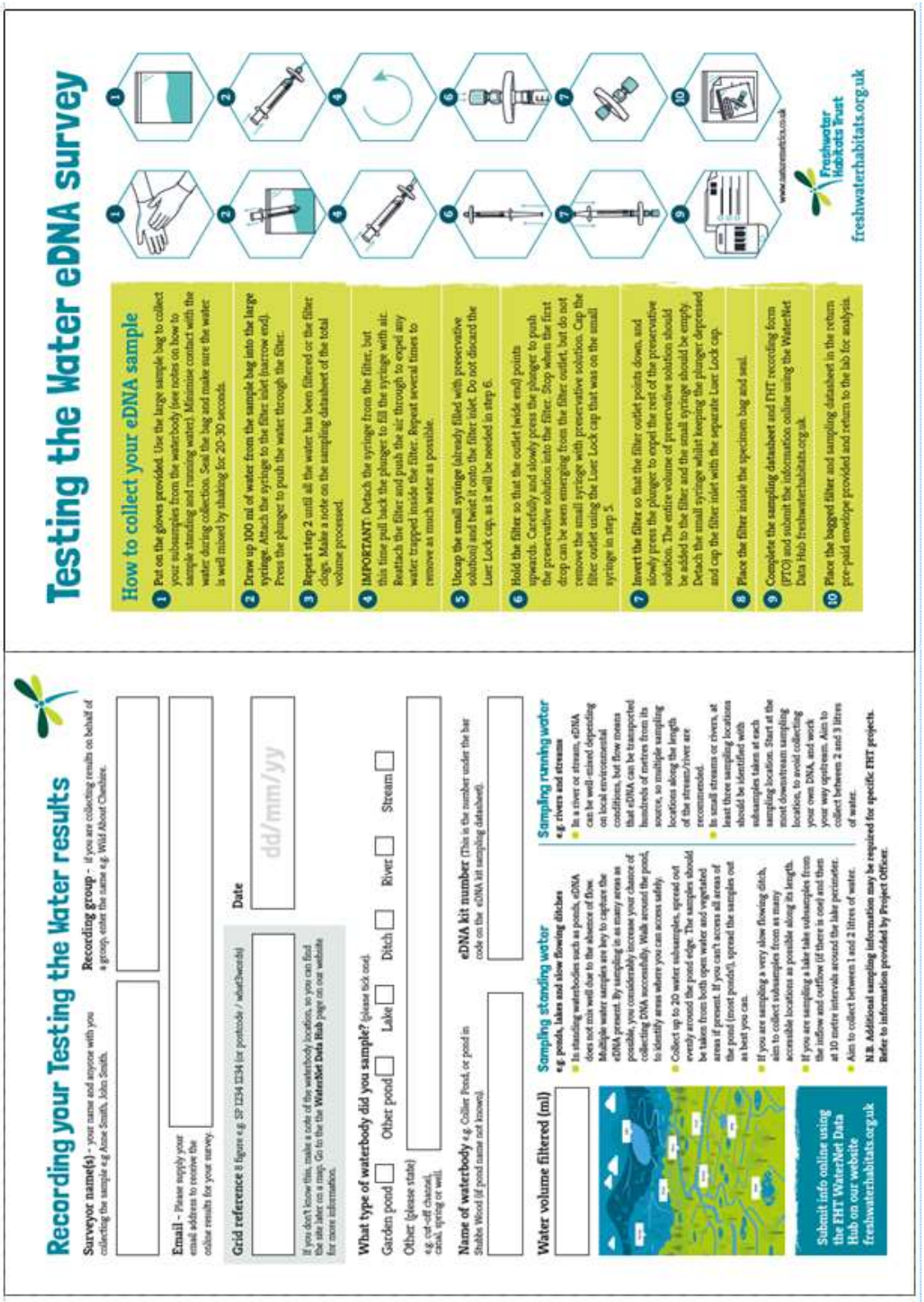
7.0 Appendices

Appendix 1: eDNA survey form returned by volunteers along with their completed eDNA sample kit to Nature Metrics for analysis



Testing the Water eDNA survey

How to collect your eDNA sample

1. Put on the gloves provided. Use the large sample bag to collect your subsamples from the waterbody (see notes on how to sample standing and running water). Minimise contact with the water during collection. Seal the bag and make sure the water is well mixed by shaking for 20-30 seconds.
2. Draw up 100 ml of water from the sample bag into the large syringe. Attach the syringe to the filter inlet (narrow end). Press the plunger to push the water through the filter.
3. Repeat step 2 until all the water has been filtered or the filter clogs. Make a note on the sampling datasheet of the total volume processed.
4. IMPORTANT: Detach the syringe from the filter, but this time pull back the plunger to fill the syringe with air. Reattach the filter and push the air through to expel any water trapped inside the filter. Repeat several times to remove as much water as possible.
5. Uncap the small syringe (already filled with preservative solution) and twist it onto the filter inlet. Do not discard the Luer Lock cap, as it will be needed in step 6.
6. Hold the filter so that the outlet (wide end) points upwards. Carefully and slowly press the plunger to push the preservative solution into the filter. Stop when the first drop can be seen emerging from the filter outlet, but do not remove the small syringe with preservative solution. Cap the filter outlet using the Luer Lock cap that was on the small syringe in step 5.
7. Invert the filter so that the filter outlet points down, and slowly press the plunger to expel the rest of the preservative solution. The entire volume of preservative solution should be added to the filter and the small syringe should be empty. Detach the small syringe whilst keeping the plunger depressed and cap the filter inlet with the separate Luer Lock cap.
8. Place the filter inside the specimen bag and seal.
9. Complete the sampling datasheet and FHT recording form (PTO) and submit the information online using the WaterNet Data Hub freshwaterhabitats.org.uk
10. Place the bagged filter and sampling datasheet in the return pre-paid envelope provided and return to the lab for analysis.

www.naturemetrics.co.uk

Freshwater Habitats Trust

freshwaterhabitats.org.uk

Recording your Testing the Water results

Surveyor name(s) - your name and anyone with you collecting the sample e.g Anne Smith, John Smith.

Recording group - if you are collecting results on behalf of a group, enter the name e.g. Wild About Cheshire.

Email - Please supply your email address to receive the online results for your survey.

Grid reference 8 figure e.g. SP 1234 1234 (or postcode / what3words)

Date dd/mm/yy

If you don't know this, make a note of the waterbody location, so you can find the site later on a map. Go to the the WaterNet Data Hub page on our website for more information.

What type of waterbody did you sample? (please tick one).

Garden pond ☐ Other pond ☐ Lake ☐ Ditch ☐ River ☐ Stream ☐

Other (please state) e.g. cut-off channel, canal, spring or well.

Name of waterbody e.g. Collier Pond, or pond in Stubbs Wood (if pond name not known).

eDNA kit number (This is the number under the bar code on the eDNA kit sampling datasheet).

Water volume filtered (ml)

Sampling standing water
e.g. ponds, lakes and slow flowing ditches

- In standing waterbodies such as ponds, eDNA does not mix well due to the absence of flow. Multiple water samples are key to capture the eDNA present. By sampling in as many areas as possible, you considerably increase your chance of collecting DNA successfully. Walk around the pond, to identify areas where you can access safely.
- Collect up to 20 water subsamples, spread out evenly around the pond edge. The samples should be taken from both open water and vegetated areas if present. If you can't access all areas of the pond (most ponds!), spread the samples out as best you can.
- If you are sampling a very slow flowing ditch, aim to collect subsamples from as many accessible locations as possible along its length.
- If you are sampling a lake take subsamples from the inflow and outflow (if there is one) and then at 10 metre intervals around the lake perimeter.
- Aim to collect between 1 and 2 litres of water.

N.B. Additional sampling information may be required for specific FHT projects. Refer to information provided by Project Officer.

Sampling running water
e.g. rivers and streams

- In a river or stream, eDNA can be well-mixed depending on local environmental conditions, but flow means that eDNA can be transported hundreds of metres from its source, so multiple sampling locations along the length of the stream/river are recommended.
- In small streams or rivers, at least three sampling locations should be identified with subsamples taken at each sampling location. Start at the most downstream sampling location, to avoid collecting your own DNA, and work your way upstream. Aim to collect between 2 and 3 litres of water.

Submit info online using the FHT WaterNet Data Hub on our website freshwaterhabitats.org.uk

Appendix 2. Sample site details – eDNA of one or more species detected

No.	1km Grid Square and Site Name	Type	Grid Reference	Date
1	TF7401 Oxborough STREAM	Stream	TF 74734 01093	06/05/22
2	TF7401 Oxbrough RIVER GADDER	River	TF 74328 01088	07/05/22
3	TF7600 Foulden Common POND	Pond	TF 76194 00194	10/06/22
4	TF7605 Shingham POND	Pond	TF 76030 05068	06/06/22
5	TF7803 Cockley Cley POND	Pond	TF 78057 03406	08/05/22
6	TF8215 Castle Acre STREAM	Stream	TF 82445 15344	06/05/22
7	TF8502 Great Cressingham POND	Pond	TL 85260 02198	23/04/22
8	TF8502 Great Cressingham RIVER WISSE	River	TL 85071 02136	24/04/22
9	TF8600 Watton Brook STREAM	Stream	TF 86924 00194	07/05/22
10	TF8605 Houghton Springs POND	Pond	TF 86121 05448	17/05/22
11	TF8680 Nunnery Lakes POND	Pond	TL 86762 80814	16/05/22
12	TF8781 Nunnery Lakes LAKE	Lake	TL 87217 81355	05/05/22
13	TL7091 Feltwell CUT OFF CHANNEL	Cut off	TL 70041 91807	22/04/22
14	TL6774 Jude's Ferry Bridge RIVER LARK	River	TL 67650 74807	24/04/22
15	TL7173 Barton Mills DITCH	Ditch	TL 71027 73984	06/07/22
16	TL7174 Barton Mills RIVER LARK	River	TL 71319 74341	06/07/22
17	TL7174 Norah Hanbury NR DITCH	Ditch	TL 71452 74126	20/06/22
18	TL7186 Lakenheath Fen DITCH	Ditch	TL 71038 86021	26/04/22
19	TL7186 Lakenheath Fen LITTLE OUSE RIV	River	TL 71552 86346	26/04/22
20	TL7199 Wittington RIVER WISSEY	River	TL 71909 99589	04/05/22
21	TL7274 Mildenhall CUT OFF CHANNEL	Cut off	TL 72930 74984	06/07/22
22	TL7283 North Fen STREAM	Stream	TL 72655 83879	12/05/22
23	TL7373 Tuddenham Mill STREAM	Stream	TL 73345 73753	09/05/22
24	TL7386 Hockwold cum Wilton CUT OFF (	Cut off	TL 73071 86293	03/06/22
25	TL7572 Temple Bridge RIVER LARK	River	TL 75844 72871	06/07/22
26	TL7597 Northwold STREAM	Stream	TL 75434 97225	06/05/22
27	TL7600 Foulden common DITCH	Ditch	TF 76264 00361	10/06/22
28	TL7776 Canada Farm POND	Pond	TL 77871 76527	06/07/22
29	TL7786 Brandon Staunch POND	Pond	TL 77872 86643	03/05/22
30	TL7795 Cranwick POND	Pond	TL77587 95982	03/06/23

No.	1km Grid Square and Site Name	Type	Grid Reference	Date
31	TL7886 Brandon LITTLE OUSE RIVER	River	TL 78232 86928	29/06/22
32	TL7970 Alder Carr LAKE	Lake	TL 79416 70992	28/04/22
33	TL7971 West Stow RIVER LARK	River	TL 79222 71071	28/04/22
34	TL8070 Culford STREAM	Stream	TL 80514 70857	28/04/22
35	TL8070 Fullers Mill POND	Pond	TL 80574 70887	27/04/22
36	TL8070 Lackford Lakes DITCH	Ditch	TL 80172 70801	27/05/22
37	TL8087 Santon Downham DITCH	Ditch	TL 80416 87681	13/05/22
38	TL8187 Santon Downham LITTLE OUSE	River	TL 81726 87871	06/05/22
39	TL8194 Lynford Water LAKE	Lake	TL 82295 94748	07/06/22
40	TL8395 Lynford RIVER WISSEY	River	TL 83096 95036	10/05/22
41	TL8467 Suffolk Golf Club POND	Pond	TL 84325 67554	23/05/22
42	TL8469 Timworth DITCH	Ditch	TL 84752 69652	12/05/22
43	TL8592 Great Carr STREAM	Stream	TL85418 92573	14/05/22
44	TL8782 Nuns Bridge LITTLE OUSE RIVER	River	TL 87334 82513	24/05/22
45	TL8782 Nuns Bridge RIVER THET	River	TL 87465 82606	07/06/22
46	TL8789 Fowl Mere LAKE	Lake	TL 87905 89517	27/04/22
47	TL8871 Broad Water LAKE	Lake	TL 88156 71530	27/04/22
48	TL8895 Sturton Carr DITCH	Ditch	TL88669 965607	13/05/22
49	TL8896 West Mere LAKE	Lake	TL 88717 96029	14/05/22
50	TL8978 Euston BLACK BOURNE RIVER	River	TL 89623 78971	27/04/22
51	TL9073 Pit Plantation POND	Pond	TL 90984 73492	08/05/22
52	TL9194 Thompson Water LAKE	Lake	TL 91514 94907	07/07/22
53	TL9293 Cranberry Rough DITCH	Ditch	TL 92856 93439	13/06/22
54	TL9395 Stow Bedon POND	Pond		13/05/22
55	TL9396 Thompson Common POND	Pond	TL 93632 96523	13/05/22
56	TL9496 Thompson Common STREAM	Stream	TL 94016 96609	09/05/22
57	TL9574 Stanton Chare STREAM	Stream	TL 95668 74210	03/05/22
58	TL9581 Knettishall DITCH	Ditch	TL 95874 81234	25/04/22
59	TL9880 Gasthorpe STREAM	Stream	TL 98328 80788	25/04/22
60	TL9980 Scarfe Meadow LITTLE OUSE RIV	River	TL 99797 80620	05/05/22

Appendix 3. Sample site details – eDNA detection failed

No.	1km Grid Square and Site Name	Type	Grid Reference	Date
61	TF7504 Shingham Wood Pond	Pond	TF 75852 04741	05/06/22
62	TL7472 Tuddenham Heath Pond	Pond	TL 74500 72800	13/05/22
63	TL7672 Secret Fen Ditch	Ditch	TL 76467 72797	26/04/22
64	TL7697 Northwold River Wissey	River	TL 76010 97376	04/05/22
65	TL8084 Go Ape Pond	Pond	TL 80907 84923	29/06/22
66	TL8592 Bodney Pond	Pond	TL 85824 92872	08/05/22
67	TL8692 Bagmore Ditch	Ditch	TL 86061 92338	08/05/22
68	TL9295 Thompson Common Ditch	Ditch	TL 92549 95287	01/05/22
69	TL9390 Queens Close Stream	Stream	TL 93364 90614	02/05/22
70	TL9391 Queens Close Pond	Pond	TL 93573 91011	02/05/22
71	TL9484 Brettenham River Thet	River	TL 94178 84209	28/04/22

Appendix 4: Limitations of the eDNA survey method

Limitation	Explanation
No systematic comparison with conventional methods	As yet, there has been no systematic comparison between multi-species eDNA survey results and results obtained using traditional survey methods. This has meant the results have been difficult to interpret, as we are currently unable to validate records. In 2014, single-species eDNA kits used to survey great crested newts were systematically compared with traditional survey methods for the species, and as a result the eDNA surveys were confirmed as a highly effective method for confirming presence or absence of great crested newt in the breeding season (Biggs *et al.,* 2014). A similar comparison is required for the multi-species eDNA kits to understand the effectiveness of the survey method. However, given that the eDNA kits detect a vast range of vertebrate species, such a systematic comparison would be both expensive and complex.
Precise source of DNA not clear	It is not currently clear i) how far DNA can be transported downstream, and ii) the extent of the area surrounding the waterbody that DNA can be washed in from following rainfall. Therefore, it cannot be assumed that the records originate from the immediate vicinity of the sampling point.
DNA longevity not accurately known	The longevity of DNA, once it has been released into the aquatic environment, is not yet known with certainty. Whilst this will vary depending on environmental conditions and the source of the DNA (e.g. hair, skin, mucus, corpse), further clarity as to how long eDNA persists in different aquatic environments is required (for example, how eDNA persistence in a turbid stream environment varies from that in an offline pond).
Not all vertebrates could be identified to species level	Limitations in the DNA technology used in the metabarcoding analysis resulted in some taxa being 'unresolved', i.e. not identified to species level. Traditional surveys do generally allow the identification of vertebrates to species level (although this does require the surveyor to be skilled in species identification, which is not required for eDNA surveys). However, the continued advancement of DNA technology is likely to ameliorate this over time.
Access to all 20 equidistant sampling points was not possible at every site	One limitation of the survey method employed during eDNA sampling was access. It was not always possible for volunteers to access each equidistant sampling point due to barriers and/or impenetrable vegetation. In this case, the nearest accessible point was sampled instead, but in more extreme cases, it may mean a significant proportion of the waterbody was not sampled.

Appendix 5: Paired species in eDNA analysis

Common name	Binomial name	Explanation
Common carp / Amur carp	*Cyprinus carpio* / *Cyprinus rubrofuscus*	These congeneric species are morphologically very similar, especially immature individuals. It is possible that entries in the reference database for which the molecular identifications are based on may include errors, or that they are indistinguishable based on this particular barcode.
European bitterling / Amur bitterling	*Rhodeus amarus* / *Rhodeus sericeus*	This sequence is either *Rhodeus amarus* or *Rhodeus sericeus*, which are indistinguishable using this marker.
Perch / Zander	*Perca fluviatilis* / *Sander lucioperca*	This sequence is either *Perca fluviatilis* or *Sander lucioperca*, which are indistinguishable using this marker.
Brook lamprey / River lamprey	*Lampetra planeri* / *Lampetra fluviatilis*	This sequence is an exact match to either river lamprey (*Lampetra fluviatilis*) or brook lamprey (*Lampetra planeri*). These species are 'paired species' which some have considered to be the same species. They are so closely related that they are indistinguishable based on this marker.
Marsh frog / Pool frog	*Pelophylax lessonae* / *Pelophylax ridibundus*	This sequence is either *Pelophylax lessonae* or *Pelophylax ridibundus*, which are indistinguishable using this marker.
Pigeon species	*Columba*	The marker for this sequence is an equally close match to multiple *Columba* species, thus NatureMetrics conservatively identified this sequence as a pigeon species (*Columba sp.*).
Pigeon species	*Columbidae*	The marker for this sequence is an equally close match to multiple *Columbidae* species, thus NatureMetrics conservatively identified this sequence as a pigeon species (*Columbidae sp.*).
Duck/goose/swan species	*Anatidae*	The marker for this sequence is an equally close match to several different *Anatidae* species, thus NatureMetrics conservatively identified this sequence as a duck/goose/swan species (*Anatidae sp.*).
Gull species	*Laridae*	This sequence is an exact match to several *Laridae* species, thus NatureMetrics conservatively identified this sequence as a gull species (*Laridae sp.*). Importantly, many species within the family *Laridae* are missing reference sequences for this marker, further complicating taxonomic assignment.
Common raven / Carrion crow	*Corvus corax* / *Corvus corone*	This sequence is either *Corvus corax* or *Corvus corone*, which are indistinguishable using this marker.
Common chaffinch / Brambling	*Fringilla coelebs* / *Fringilla montifringilla*	This sequence is either *Fringilla coelebs* or *Fringilla montifringilla*, which are indistinguishable using this marker.
Sardinian warbler / Eastern subalpine warbler	*Curruca melanocephala* / *Curruca cantillans*	This sequence is either *Curruca melanocephala* or *Curruca cantillans*, which are indistinguishable using this marker.
Starling species	*Sturnidae*	The marker for this sequence is an equally close match to several different *Sturnidae* species,

		thus NatureMetrics conservatively identified this sequence as a starling species (*Sturnidae sp*.).
Thrush species	*Turdus*	The marker for this sequence is an equally close match to several different *Turdus* species, thus NatureMetrics conservatively identified this sequence as a thrush species (*Turdus sp*.).
Mustelid species	*Mustelidae*	This sequence is an exact match to a number of *Mustelidae* species, thus NatureMetrics conservatively identified this sequence as a Mustelid species (*Mustellidae sp*.).
Wood mouse / Yellow-necked mouse	*Apodemus sylvaticus / Apodemus flavicollis*	This sequence is either *Apodemus sylvaticus* or *Apodemus flavicollis*, which are indistinguishable using this marker.

Appendix 6: Species recorded per site

SITE 1: TF7401 Oxborough STREAM (TF 74734 01093)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
Mammals	*Cervus elaphus*	Red deer
	Apodemus sylvaticus/Apodemus flavicollis	Wood/Yellow-necked mouse
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Columba sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Carduellis carduelis	European goldfinch
	Troglodytes troglodytes	Eurasian wren
	Turdus sp.	Thrush species
Fish	*Cyprinus carpio/Cyprinus rubrofuscus*	Common/Amur carp
	Gobio gobio	Gudgeon
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Salmo trutta	Trout
	Cottus gobio	European bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 2: TF7401 Oxbrough RIVER GADDER (TF 74328 01088)		
Group	**Species**	**Common Name**
Mammals	*Arvicola amphibius*	Water vole
	Aneomys fodiens	Water shrew
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen

SITE 3: TF7600 Foulden Common POND (TF 76194 00194)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common frog
Mammals	*Cervus elaphus*	Red deer
	Muntiacus reevesi	Reeves'muntjac
	Apodemus sylvaticus/Apodemus flavicollis	Wood/Yellow-necked mouse
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Erithacus rubecula	European robin
	Sylvia atricapilla	Eurasian blackcap
	Turdus sp.	Thrush species
Fish	*Anguilla anguilla*	European eel
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Cottus gobio	European bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 4: TF7605 Shingham POND (TF 76030 05068)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common Frog
Mammals	*Cervus elaphus*	Red deer
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
	Ardea cinerea	Grey heron
Fish	*Gasterosteus aculeatus*	Three-spined stickleback

SITE 5: TF7803 Cockley Cley POND (TF 78057 03406)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common Frog
Birds	*Anatidae sp.*	Wildfowl species

	Ardea cinerea	Grey heron
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Scardinius erythropthalmus	Rudd

SITE 6: TF8215 Castle Acre STREAM (TF 82445 15344)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common Frog
Mammals	*Arvicola amphibius*	Water vole
	Aneomys fodiens	Water shrew
Birds	Buteo buteo	Buzzard
	Anatidae sp.	Wildfowl species
	Columba sp.	Pigeon species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Fringilla coelebs/ Fringilla montifringilla	Chaffinch/Brambling
	Troglodytes troglodytes	Eurasian wren
	Turdus sp.	Thrush species
	Ardea cinerea	Grey heron
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 7: TL8502 Great Cressingham Pond (TF 85260 02198)		
Group	**Species**	**Common Name**
Birds	*Gallinula chloropus*	Moorhen
	Columbidae sp.	Pigeon species

SITE 8: TL8502 Great Cressingham RIVER WISSEY (TF 85071 02136)		
Group	**Species**	**Common Name**
Mammals	*Bovidae sp.*	Bovid species
	Arvicola amphibius	Water vole
	Rattus norvegicus	Brown rat
	Neomys fodiens	Water shrew
Birds	*Aix galericulata*	Mandarin duck

	Anas carolinensis	Green-winged teal
	Callonetta leucophrys/Cygnus olor	Ringed teal/Mute swan
	Anatidae sp.	Wildfowl species
	Columbidae sp.	Pigeon species
	Erithacus rubecula	European robin
	Phalacrocorax carbo	Great cormorant
Fish	*Anguilla anguilla*	European eel
	Leuciscus leuciscus	Dace
	Barbatula barbatula	Stone loach
	Barbatula sp.	Loach species
	Gadidae sp.	Codfish species
	Gasterosteus aculeatus	Three-spined stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo trutta	Trout
	Cottus gobio	European bullhead

SITE 9: TF8600 Watton Brook STREAM (TF 86924 00194)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common Frog
Mammals	*Rattus norvegicus*	Brown rat
Birds	*Anatidae sp.*	Wildfowl species
	Ardea cinerea	Grey heron
Fish	*Scardinius erythropthalmus*	Rudd
	Tinca tinca	Tench
	Pungitius pungitius	Nine-spine stickleback

SITE 10: TF8605 Houghton Springs POND (TF 86121 05448)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
Fish	*Cyprinus carpio/Cyprinus rubrofuscus*	Common/Amur carp
	Pungitius pungitius	Nine-spine stickleback

SITE 11: TF8680 Nunnery Lakes POND (TL 86762 80814)		
Group	**Species**	**Common Name**
Birds	Buteo buteo	Buzzard
	Anatidae sp.	Wildfowl species
	Gallinula chloropus	Moorhen
	Acrocephalus scirpaceus	Reed-warbler
	Corvus corax/ Corvus corone	Raven/Crow
	Turdus sp.	Thrush species
Fish	*Leuciscus idus*	Ide
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Esox lucius	Northern pike

SITE 12: TF8781 Nunnery Lakes LAKE (TL 87217 81355)		
Group	**Species**	**Common Name**
Amphibians	*Pelophylax lessonae/Pelophylax ridibundus*	Pool/Marsh frog
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phalacrocorax carbo	Great cormorant
Fish	*Abramis brama*	Bream
	Blicca bjoerkna	Silver bream
	Cyprinus carpio/Cyprinus rubrofuscus	Common/Amur carp
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Tinca tinca	Tench
	Esox lucius	Northern pike
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo salar	Atlantic salmon

SITE 13: TL 7091 Feltwell CUT OFF CHANNEL (TL 70041 91807)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Birds	*Columbidae sp.*	Pigeon species
	Corvus corax/ Corvus corone	Raven/Crow
Fish	*Abramis brama*	Bream
	Blicca bjoerkna	Silver bream
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Cottus gobio	European Bullhead

SITE 14: TL6774 Jude's Ferry Bridge RIVER LARK (TL 67650 74807)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Turdus sp.	Thrush species
Fish	*Anguilla anguilla*	European eel
	Abramis brama	Bream
	Gobio gobio	Gudgeon
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

	Gymnocephalus cernua	Ruffe
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo trutta	Trout
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 15: TL7173 Barton Mills DITCH (TL 71027 73984)		
Group	**Species**	**Common Name**
Mammals	*Arvicola amphibius*	Water vole
	Rattus norvegicus	Brown rat
Birds	*Columbidae sp.*	Pigeon species
	Gallinula chloropus	Moorhen
	Carduellis carduelis	European goldfinch
	Turdus sp.	Thrush species
Fish	*Barbatula barbatula*	Stone loach
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 16: TL7174 Barton Mills RIVER LARK (TL 71319 74341)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
Fish	*Abramis brama*	Bream
	Gobio gobio	Gudgeon
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis/Sander lucioperca	Perch/Zander

SITE 17: TL7174 Norah Nanbury NR DITCH (TL71452 74126)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common Frog
Mammals	*Arvicola amphibius*	Water vole
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Troglodytes troglodytes	Eurasian wren
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 18: TL7186 Lakenheath Fen DITCH (TL 71038 86021)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Gallinula chloropus	Moorhen

SITE 19: TL7186 Lakenheath Fen LITTLE OUSE RIVER (TL 71552 86346)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Ardea cinerea	Grey heron
	Phalacrocorax carbo	Great cormorant
Fish	*Anguilla anguilla*	European eel
	Cobitis taenia	Spined loach
	Blicca bjoerkna	Silver bream
	Abramis brama	Bream
	Gobio gobio	Gudgeon
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rhodeus amarus/Rhodeus sericeus	European/Amur bitterling
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd

	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 20: TL7199 Wittington RIVER WISSEY (TL 71909 99589)		
Group	**Species**	**Common Name**
Mammals	*Vulpes vulpes*	Red Fox
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Ardea cinerea	Grey heron
	Phalacrocorax carbo	Great cormorant
Fish	*Gobio gobio*	Gudgeon

SITE 21: TL7274 Mildenhall CUT OFF CHANNEL (TL 72930 74984)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Mammals	*Arvicola amphibius*	Water vole
Birds	*Cuculus canorus*	Cuckoo
	Gallinula chloropus	Moorhen
	Turdus sp.	Thrush species
Fish	*Gobio gobio*	Gudgeon
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

	Gymnocephalus cernua	Ruffe
	Cottus gobio	European Bullhead

SITE 22: TL7283 North Fen STREAM (TL 72655 83879)		
Group	**Species**	**Common Name**
Mammals	*Muntiacus reevesi*	Reeves'muntjac
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Erithacus rubecula	European robin
	Prunella modularis	Dunnock
	Turdus sp.	Thrush species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 23: TL7373 Tuddenham Mill STREAM (TL 73345 73753)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
Fish	*Phoxinus phoxinus*	Minnow
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Cottus gobio	European Bullhead

SITE 24: TL7386 Hockwold cum Wilton CUT OFF CHANNEL (TL 73071 86293)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 25: TL7572 Temple Bridge RIVER LARK (TL 75844 72871)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Rallus aquaticus	Western water rail
	Acrocephalus scirpaceus	Reed-warbler
Fish	*Anguilla angulla*	European eel
	Gobio gobio	Gudgeon
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo trutta	Trout
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 26: TL7597 Northwold STREAM (TL 75434 97225)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Lissotriton vulgaris	Smooth newt
Birds	*Anatidae sp.*	Wildfowl species
	Columba sp.	Pigeon species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Aegithalos caudatus	Long-tailed tit
	Corvus corax/ Corvus corone	Raven/Crow
	Turdus sp.	Thrush species
	Ardea cinerea	Grey heron

Fish	*Anguilla angulla*	European eel
	Cobitis taenia	Spined loach
	Gobio gobio	Gudgeon
	Leuciscus idus	Ide
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo trutta	Trout
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 27: TL7600 Foulden common DITCH (TF 76264 00361)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
Birds	*Columbidae sp.*	Pigeon species
	Gallinula chloropus	Moorhen
	Turdus sp.	Thrush species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 28: TL7776 Canada Farm POND (TL 77871 76527)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Sturnidae sp.	Starling species
	Turdus sp.	Thrush species
Fish	Cyprinus carpio/Cyprinus rubrofuscus	Common /Amurcarp
	Leuciscus idus	Ide

	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub

SITE 29: TL7786 Brandon Staunch POND (TL 77872 86643)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Mammals	*Cervus elaphus*	Red deer
	Lutra lutra	Eurasian otter
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
Fish	*Gobio gobio*	Gudgeon
	Leuciscus idus	Ide
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Cottus gobio	European Bullhead

SITE 30: TL7795 Cranwick POND (TL77587 95982)		
Group	**Species**	**Common Name**
Birds	*Gallinula chloropus*	Moorhen

SITE 31: TL7886 Brandon LITTLE OUSE RIVER (TL 78232 86928)		
Group	**Species**	**Common Name**
Mammals	*Muntiacus reevesi*	Reeves'muntjac
	Arvicola amphibius	Water vole
Birds	*Columba sp.*	Pigeon species
	Columbidae sp.	Pigeon species
	Sylvia atricapilla	Eurasian blackcap
	Turdus sp.	Thrush species

Fish	*Phoxinus phoxinus*	Minnow
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 32: TL7970 Alder Carr LAKE (TL 79416 70992)		
Group	**Species**	**Common Name**
Amphibians	*Lissotriton vulgaris*	Smooth newt
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
Fish	*Leuciscus idus*	Ide
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Esox lucius	Northern pike
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Oncorhynchus mykiss	Rainbow trout

SITE 33: TL7971 West Stow RIVER LARK (TL 79222 71071)		
Group	**Species**	**Common Name**
Mammals	*Muntiacus reevesi*	Reeves'muntjac
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
Fish	*Gobio gobio*	Gudgeon
	Leuciscus idus	Ide
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Gasterosteus aculeatus	Three-spined stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander

	Salmo trutta	Trout
	Cottus gobio	European Bullhead

SITE 34: TL8070 Culford STREAM (TL 80514 70857)		
Group	**Species**	**Common Name**
Mammals	*Muntiacus reevesi*	Reeves'muntjac
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
Fish	*Gobio gobio*	Gudgeon
	Phoxinus phoxinus	Minnow
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo salar	Atlantic salmon

SITE 35: TL8070 Fullers Mill POND (TL 80574 70887)		
Group	**Species**	**Common Name**
Mammals	*Arvicola amphibius*	Water vole
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Numida meleagris	Helmeted guineafowl
	Gallinula chloropus	Moorhen
	Ardea cinerea	Grey heron
Fish	*Rutilus rutilus*	Roach
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Cottus gobio	European Bullhead

SITE 36: TL8070 Lackford Lakes DITCH (TL 80172 70801)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species

SITE 37: TL8087 Santon Downham DITCH (TL 80416 87681)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Mammals	*Muntiacus reevesi*	Reeves'muntjac
	Arvicola amphibius	Water vole
	Apodemus sylvaticus/Apodemus flavicollis	Wood/Yellow-necked mouse
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Rallus aquaticus	Western water rail
	Fringilla coelebs/Fringilla montifringilla	Chaffinch/Brambling
	Turdus sp.	Thrush species
Fish	*Esox lucius*	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Petromyzon marinus	Sea lamprey

SITE 38: TL8187 Santon Downham LITTLE OUSE RIVER (TL 81726 87871)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Curruca melanocephala/Curruca cantillans	Sardinian/Subalpine warbler
Fish	*Anguilla anguilla*	European eel
	Gobio gobio	Gudgeon
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach

	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander

SITE 39: TL8194 Lynford Water LAKE (TL 82295 94748)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
Fish	*Abramis brama*	Bream
	Rutilus rutilus	Roach
	Esox lucius	Northern pike

SITE 40: TL8395 Lynford RIVER WISSEY (TL 83096 95036)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Fringilla coelebs/Fringilla montifringilla	Chaffinch/Brambling
	Erithacus rubecula	European robin
	Turdus sp.	Thrush species
Fish	*Anguilla anguilla*	European eel
	Gobio gobio	Gudgeon
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander

	Salmo trutta	Trout
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 41: TL8467 Suffolk Golf Club POND (TL 84325 67554)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common frog
	Lissotriton vulgaris	Smooth newt
Birds	*Anatidae sp.*	Wildfowl species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 42: TL8469 Timworth DITCH (TL 84752 69652)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Turdus sp.	Thrush species
Fish	*Rutilus rutilus*	Roach
	Tinca tinca	Tench
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander

SITE 43: TL8592 Great Carr STREAM (TL85418 92573)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
	Lissotriton vulgaris	Smooth newt
Mammals	Cervus elaphus	Red Deer
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species

	Corvus corax/ Corvus corone	Raven/Crow
	Erithacus rubecula	European robin
	Poecile palustris	Marsh tit
	Turdus sp.	Thrush species
	Dendrocopos major	Great spotted woodpecker
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 44: TL8782 Nuns Bridge LITTLE OUSE RIVER (TL 87334 82513)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Mammals	*Arvicola amphibius*	Water vole
Birds	*Anas carolinensis*	Green-winged teal
	Callonetta leucophrys/Cygnus olor	Ringed teal/Mute swan
	Anatidae sp.	Wildfowl species
	Laridae sp.	Gull species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Corvidae sp.	Corvid species
Fish	*Anguilla anguilla*	European eel
	Gobio gobio	Gudgeon
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Cyprinidae sp.	Cyprinid species
	Barbatula barbatula	Stone loach
	Barbatula sp.	Loach species
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Salmo trutta	Trout
	Cottus gobio	European bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 45: TL8782 Nuns Bridge RIVER THET (TL 87465 82606)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
Fish	*Anguilla angulla*	European eel
	Cobitis taenia	Spined loach
	Abramis brama	Bream
	Gobio gobio	Gudgeon
	Leuciscus idus	Ide
	Leuciscus leuciscus	Dace
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Cottus gobio	European Bullhead
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 46: TL8789 Fowl Mere LAKE (TL 87905 89517)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
	Lissotriton vulgaris	Smooth newt
	Triturus cristatus	Great crested newt
Mammals	Cervus elaphus	Red Deer
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
	Sturnidae sp.	Starling species
	Ardea cinerea	Grey heron
Fish	*Salmo salar*	Atlantic salmon

SITE 47: TL8871 Broad Water LAKE (TL 88156 71530)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Laridae sp.	Gull species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Phalacrocorax carbo	Great cormorant
Fish	*Cyprinus carpio/Cyprinus rubrofuscus*	Common/Amur carp
	Scardinius erythropthalmus	Rudd
	Gasterosteus aculeatus	Three-spined stickleback

SITE 48: TL8895 Sturton Carr DITCH (TL88669 965607)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common frog
Mammals	Cervus elaphus	Red Deer
Birds	*Turdus sp.*	Thrush species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 49: TL8896 West Mere LAKE (TL 88717 96029)		
Group	**Species**	**Common Name**
Amphibians	*Bufo bufo*	Common toad
	Rana temporaria	Common frog
	Lissotriton vulgaris	Smooth newt
	Triturus cristatus	Great crested newt
Mammals	Cervus elaphus	Red Deer
Birds	*Anatidae sp.*	Wildfowl species
	Phasianus colchicus	Pheasant
	Fulica atra	Coot
	Gallinula chloropus	Moorhen
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 50: TL8978 Euston BLACK BOURNE RIVER (TL 89623 78971)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Corvus corax/ Corvus corone	Raven/Crow
	Turdus sp.	Thrush species
Fish	*Gobio gobio*	Gudgeon
	Phoxinus phoxinus	Minnow
	Rutilus rutilus	Roach
	Barbatula barbatula	Stone loach
	Esox lucius	Northern pike
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Cottus gobio	European Bullhead

SITE 51: TL9073 Pit Plantation POND (TL 90984 73492)		
Group	**Species**	**Common Name**
Fish	*Rutilus rutilus*	Roach
	Lampetra planeri/Lampetra fluviatilis	Brook/River lamprey

SITE 52: TL9194 Thompson Water LAKE (TL 91514 94907)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
	Columbidae sp.	Pigeon species
	Phasianus colchicus	Pheasant
	Gallinula chloropus	Moorhen
	Turdus sp.	Thrush species
Fish	*Anguilla anguilla*	European eel
	Gobio gobio	Gudgeon
	Rutilus rutilus	Roach
	Scardinius erythropthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone loach
	Gasterosteus aculeatus	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

	Perca fluviatilis/Sander lucioperca	Perch/Zander
	Oncorhynchus mykiss	Rainbow trout
	Salmo trutta	Trout
	Cottus gobio	European Bullhead

SITE 53: TL9293 Cranberry Rough DITCH (TL 92856 93439)		
Group	**Species**	**Common Name**
Birds	*Phasianus colchicus*	Pheasant
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 54: TL9395 Stow Bedon POND		
Group	**Species**	**Common Name**
Amphibians	*Pelophylax lessonae/Pelophylax ridibundus*	Pool/Marsh frog
	Lissotriton vulgaris	Smooth newt
Birds	*Anatidae sp.*	Wildfowl species

SITE 55: TL9396 Thompson Common POND (TL 93632 96523)		
Group	**Species**	**Common Name**
Amphibians	*Lissotriton vulgaris*	Smooth newt
	Triturus cristatus	Great crested newt
Mammals	*Arvicola amphibius*	Water vole
Birds	*Anatidae sp.*	Wildfowl species
	Gallinula chloropus	Moorhen
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 56: TL9496 Thompson Common STREAM (TL 94016 96609)		
Group	**Species**	**Common Name**
Amphibians	*Lissotriton vulgaris*	Smooth newt
Birds	*Anatidae sp.*	Wildfowl species
	Erithacus rubecula	European robin

SITE 57: TL9574 Stanton Chare STREAM (TL 95668 74210)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common frog
Mammals	*Muntiacus reevesi*	Reeves'muntjac
	Arvicola amphibius	Water vole
	Rattus norvegicus	Brown rat
	Sciurus carolinensis	Grey squirrel
	Neomys fodiens	Water shew
	Talpa europaea	Mole
Birds	*Anatidae sp.*	Wildfowl species
	Columba sp.	Pigeon species
	Columbidae sp.	Pigeon species
	Gallinula chloropus	Moorhen
	Corvus corax/ Corvus corone	Raven/Crow
	Carduelis carduelis	European goldfinch
	Fringilla coelebs/Fringilla montifringilla	Chaffinch/Brambling
	Erithacus rubecula	European robin
	Prunella modularis	Dunnock
	Sturnidae sp.	Starling species
	Sylvia atricapilla	Eurasian blackcap
	Troglodytes troglodytes	Eurasian wren
	Turdus sp.	Thrush species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback
	Cottus gobio	European Bullhead

SITE 58: TL9581 Knettishall DITCH (TL 95874 81234)		
Group	**Species**	**Common Name**
Mammals	*Arvicola amphibius*	Water vole
Birds	*Anatidae sp.*	Wildfowl species
	Turdus sp.	Thrush species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback

SITE 59: TL9880 Gasthorpe STREAM (TL 98328 80788)		
Group	**Species**	**Common Name**
Birds	*Gallinula chloropus*	Moorhen
	Ardea cinerea	Grey heron

SITE 60: TL9980 Scarfe Meadow LITTLE OUSE RIVER (TL 99797 80620)		
Group	**Species**	**Common Name**
Birds	*Anatidae sp.*	Wildfowl species
Fish	*Gasterosteus aculeatus*	Three-spined stickleback
	Pungitius pungitius	Nine-spine stickleback