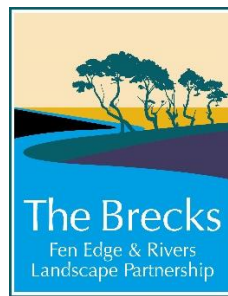




Brecks Fen Edge & Rivers Landscape
Partnership
CITIZEN SCIENCE: TESTING THE WATER
eDNA Survey Results 2022



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Oxford
2022

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This report should be cited as:

Carter A. and Ewald N. 2022. eDNA Survey Results 2022. Citizen Science: Testing the Water. Brecks Fen Edge & Rivers Landscape Partnership. Freshwater Habitats Trust, Oxford.

Acknowledgements

Freshwater Habitats Trust would like to acknowledge the help of our dedicated volunteers who undertook the eDNA surveys around in The Brecks area, and all the landowners who gave us access to the sites. Additionally, we are grateful for the support from Nature Metrics, an eDNA specialist company.

The project is funded by The National Lottery Heritage Fund.



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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 19 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 26 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, three sites and one site respectively; and many different bird species at a range of still and standing waterbodies.

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 County 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.

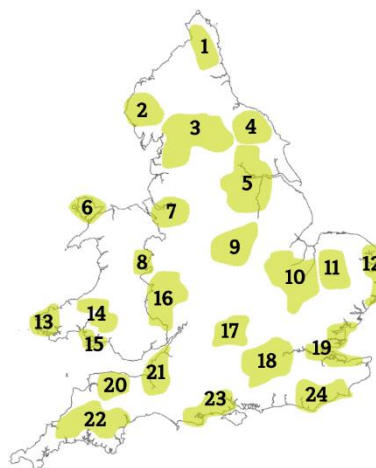


Figure 1: Brecks Fen Edge and Rivers Landscape Partnership Scheme area

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).



- | | |
|--|----------------------------------|
| 1. Cheviots and surroundings | 13. Pembrokeshire heaths |
| 2. North Lake District | 14. South West Wales rivers |
| 3. Yorkshire Dales and Forest of Bowland | 15. Gower |
| 4. North York Moors and surroundings | 16. Shropshire rivers |
| 5. Yorkshire Lowlands | 17. Oxford area |
| 6. Anglesey | 18. Thames Basin |
| 7. North West England | 19. Thames Estuary |
| 8. Montgomery Canal | 20. Exmoor area |
| 9. East Midlands | 21. Avon and Somerset Levels |
| 10. Cambridgeshire Fens | 22. Dartmoor and Mid Cornwall |
| 11. The Brecks and surroundings | 23. New Forest and Dorset Heaths |
| 12. Norfolk and Suffolk Broads | 24. South Coast and the Weald |

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 Areas¹ within the region: a product of its geology and management history. Freshwaters are a critical part of the 1000km² Brecks National Character Area, fundamental to its unique biodiversity, habitats and cultural heritage.

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.

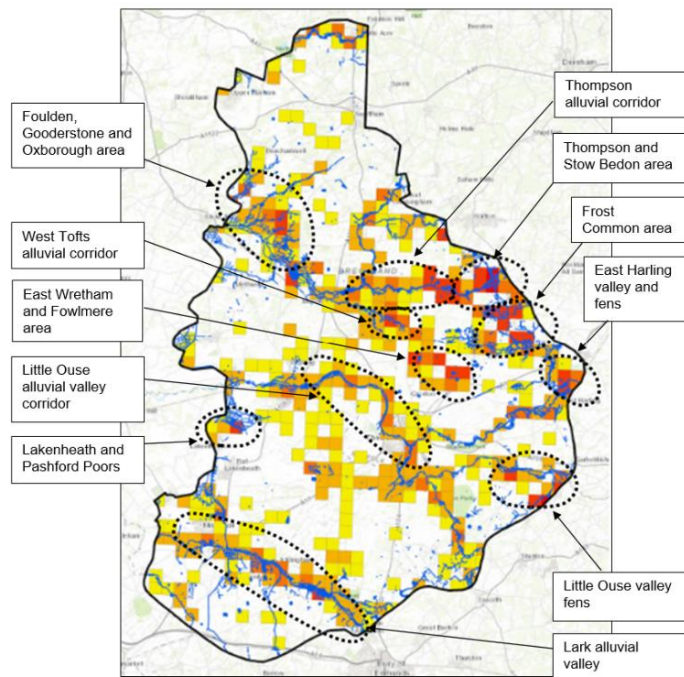


Figure 3: The Brecks Important Freshwater Areas assessment (2018) identified eleven areas which are high priorities for freshwater biodiversity and areas which were data deficient in between

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

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 persist 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 partnered 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 most 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.

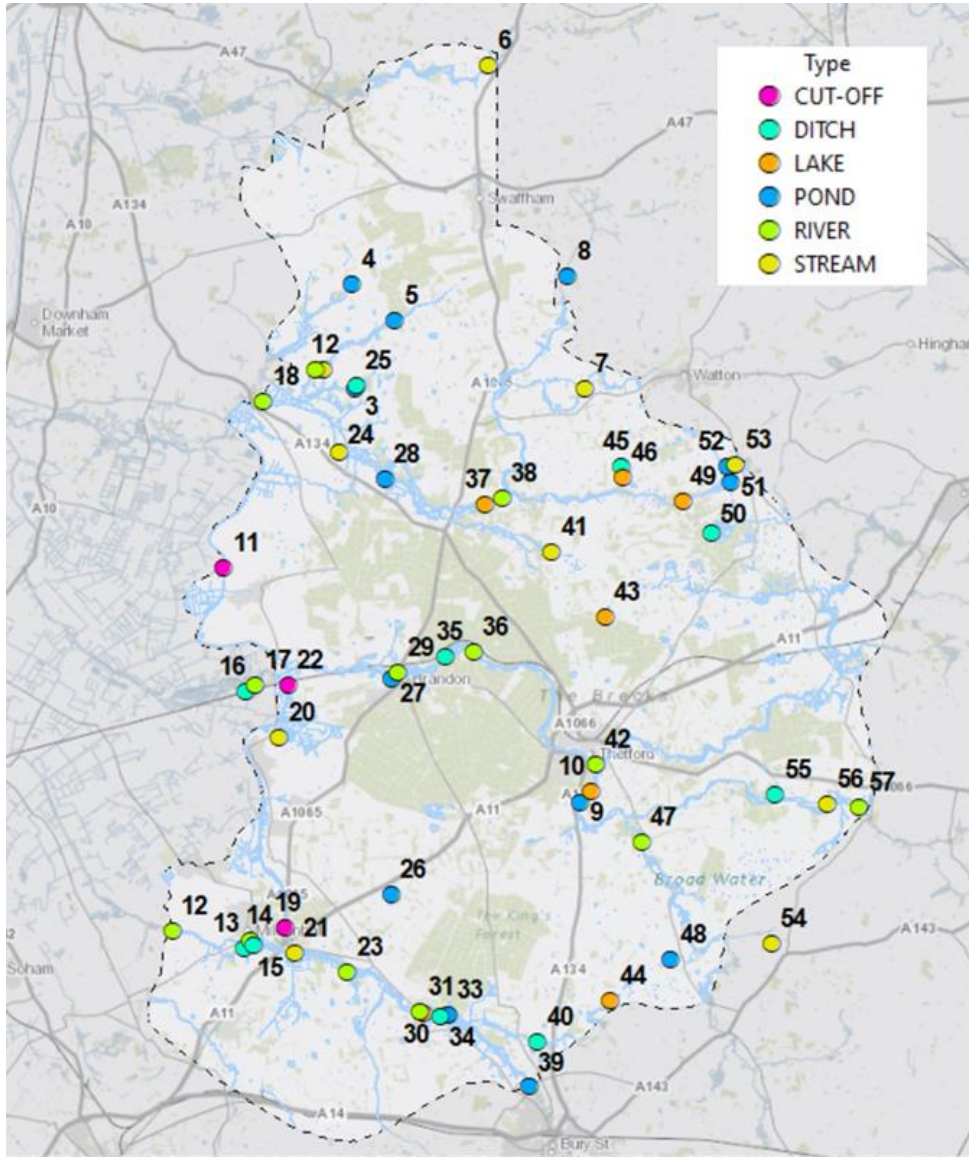
The collection bag was shaken to mix the water and any eDNA contained therein. Water was then drawn up using a 50ml syringe, and pushed through a filter to trap any DNA that was present in the sample. This was repeated until the entire sample had been processed, or until the filter became clogged due 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 4: A filter with preserving fluid in small syringe supplied by NatureMetrics (left) and a volunteer filtering water sample to trap DNA for analysis (right).



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

Figure 5: Locations of the 57 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), **57** came back with positive records for one or more species (Figure 5). For individual site reports, see Appendix 6. Fourteen 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 57 sample sites was **68 species - 26 fish** (38% of all species recorded), **26 birds** (38%), **10 mammals** (16%) and **five amphibians** (7%) (Figure 6). Across all waterbody types (standing and running waterbodies) fish species were found in 88% of sites, birds 98%, mammals 46% and amphibians 51% (Figure 7). The number of vertebrate species recorded at a single site ranged from one to 27 (Figure 8).

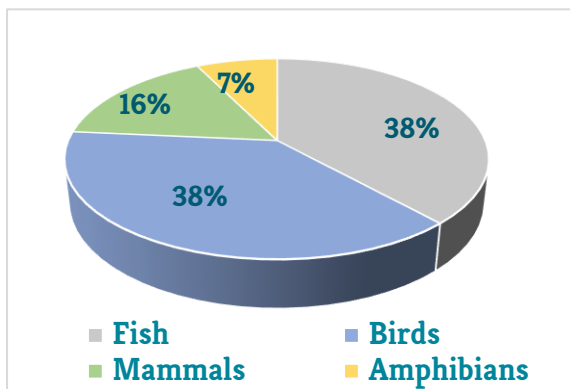


Figure 6: Percentage of vertebrate taxa found across all the eDNA survey sites

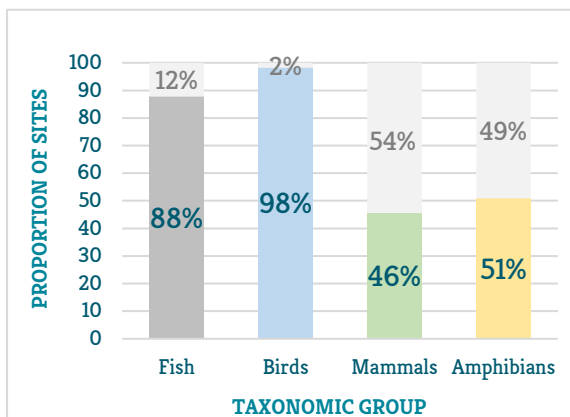


Figure 7: Percentage of taxon groups found in waterbodies in the Brecks area

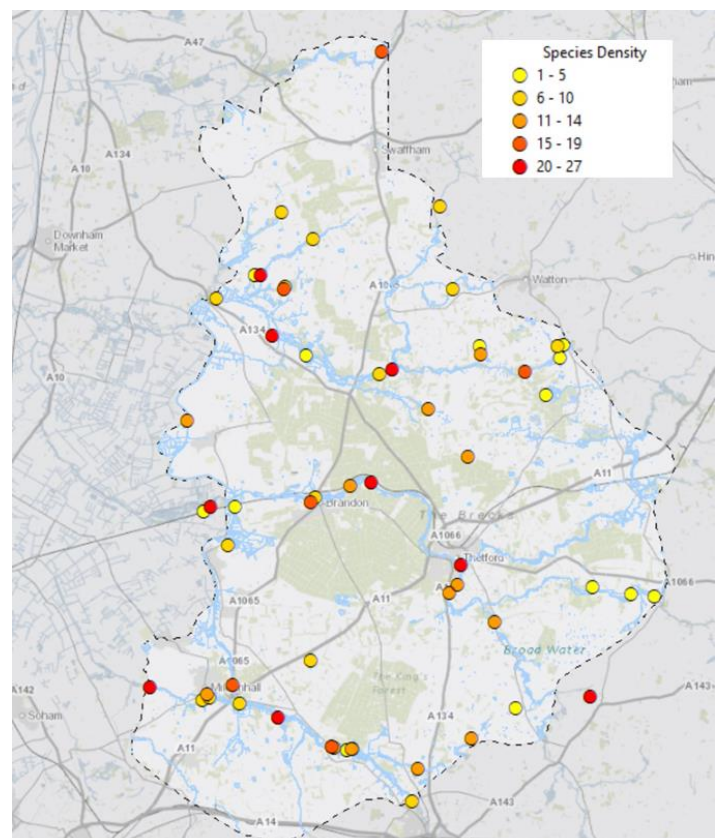


Figure 8: Number of vertebrate species per survey site recorded using eDNA

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 10).

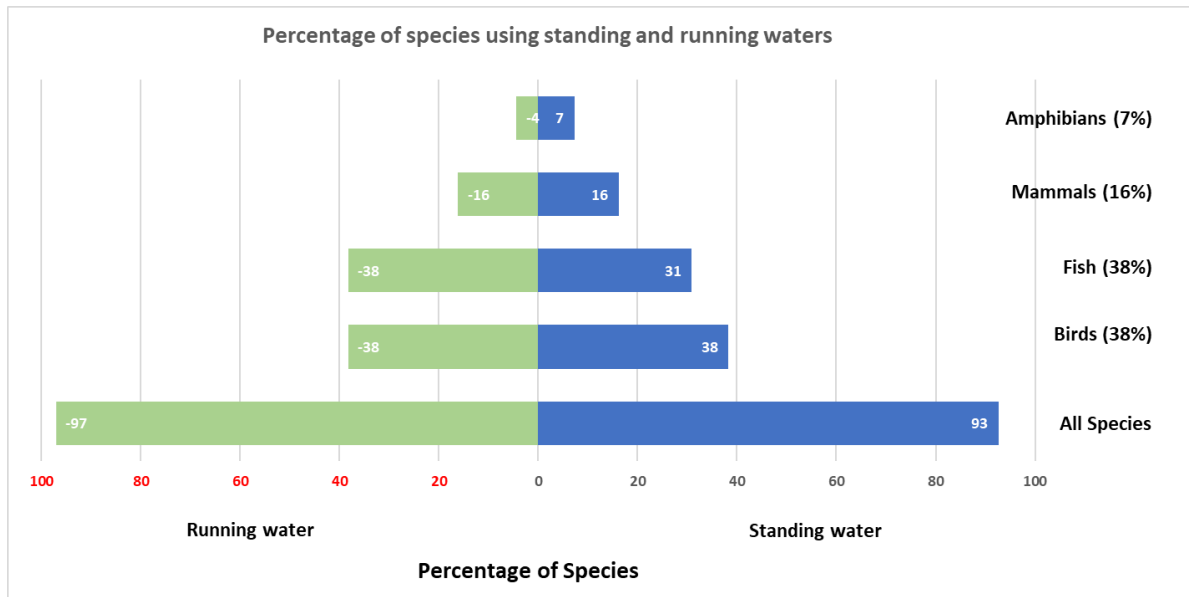


Figure 9: Percentage of species (total species and within taxonomic group) using running waters and standing waters

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)
- *Mustelidae sp.* (Mustelid 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)

For more information on paired species see Appendix 5.

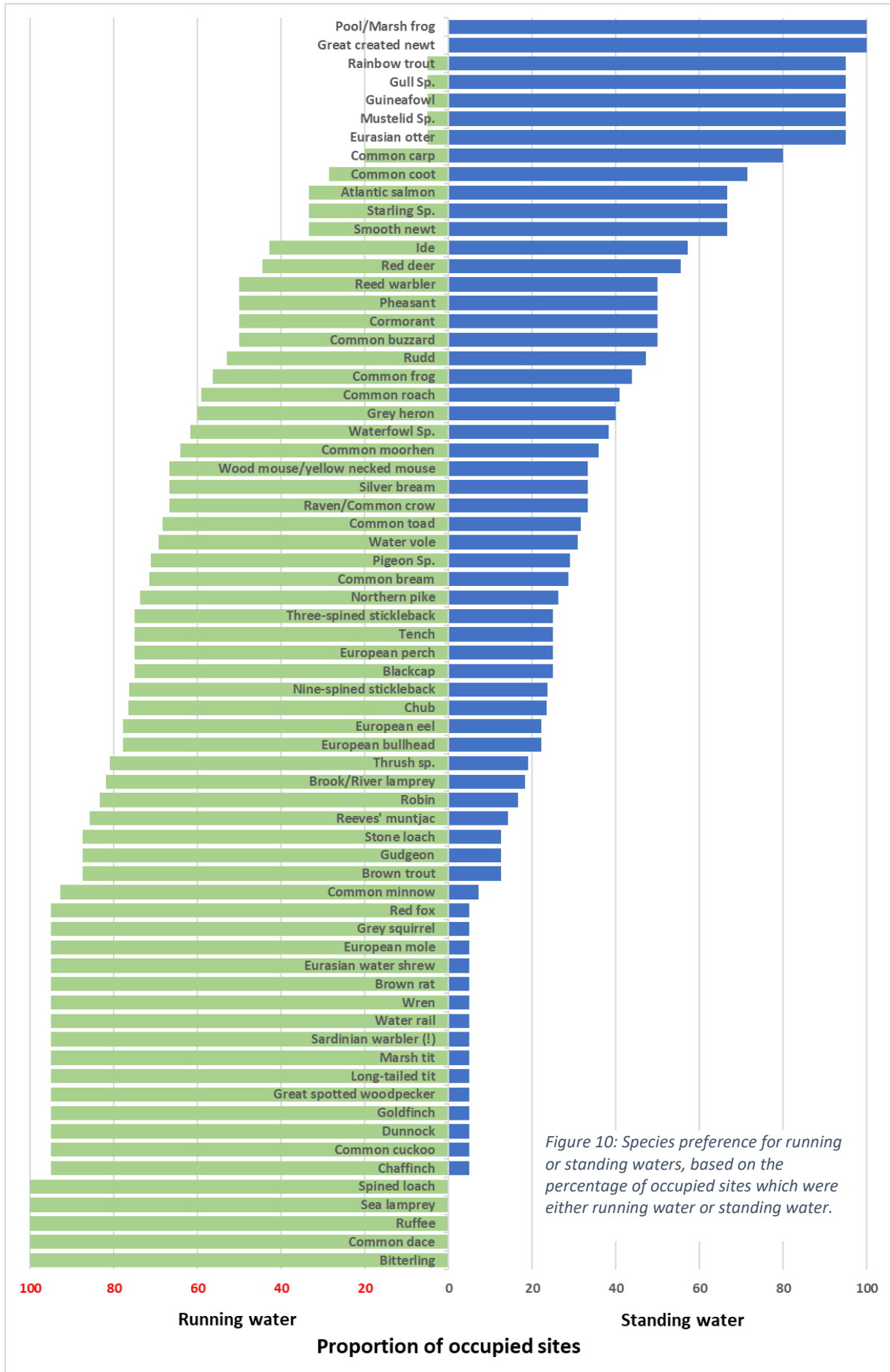


Figure 10: Species preference for running or standing waters, based on the percentage of occupied sites which were either running water or standing water.

4.2 Amphibians

A total of five amphibian species were recorded across the 57 sample sites (Figure 12):

- **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 (19), followed by common frog 28% (16 sites), smooth newt 16% (nine sites), great crested newt 5% (three sites), and pool/marsh frog 4% (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 49% of the sites surveyed (28 sites) (Figure 11). 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.

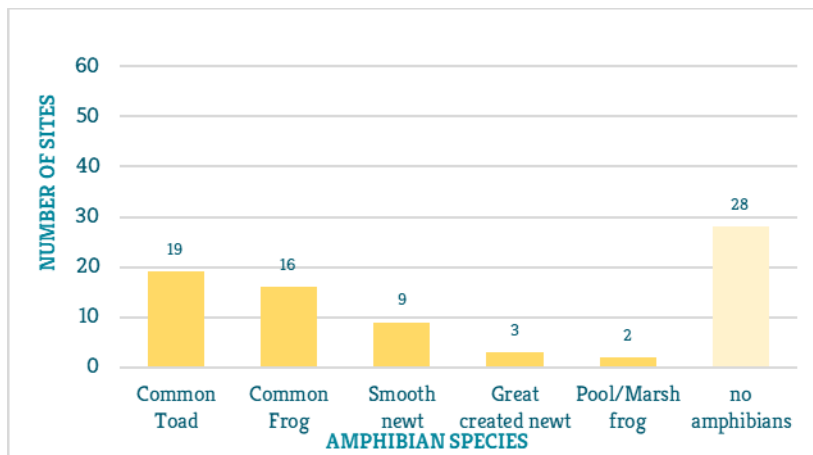


Figure 11: Number of amphibian species found across eDNA survey sites (n=57)

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 part 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 the UK, common frogs are protected under Schedule 5 of the Wildlife and Countryside Act (1981).

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 between 6% and 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. There 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.

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. The site is unsuitable for pool frog and the nearest reintroduction sites are too far away for self-dispersal (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 is 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.

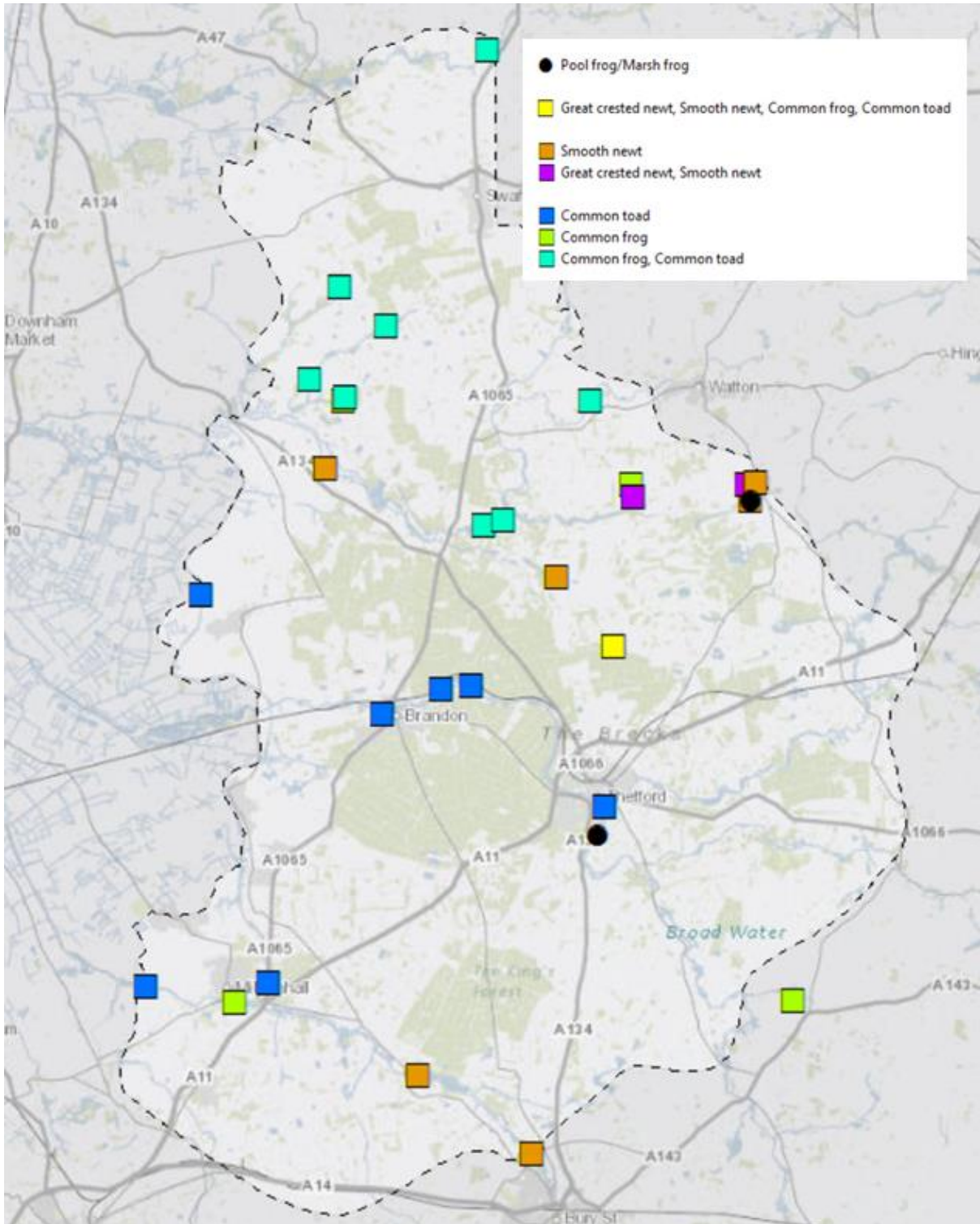


Figure 12: Distribution of amphibians in The Brecks Fen Edge and Rivers Landscape survey area

4.3 Fish

A total of 26 fish species were recorded from the 57 sample sites (Figure 14). 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.

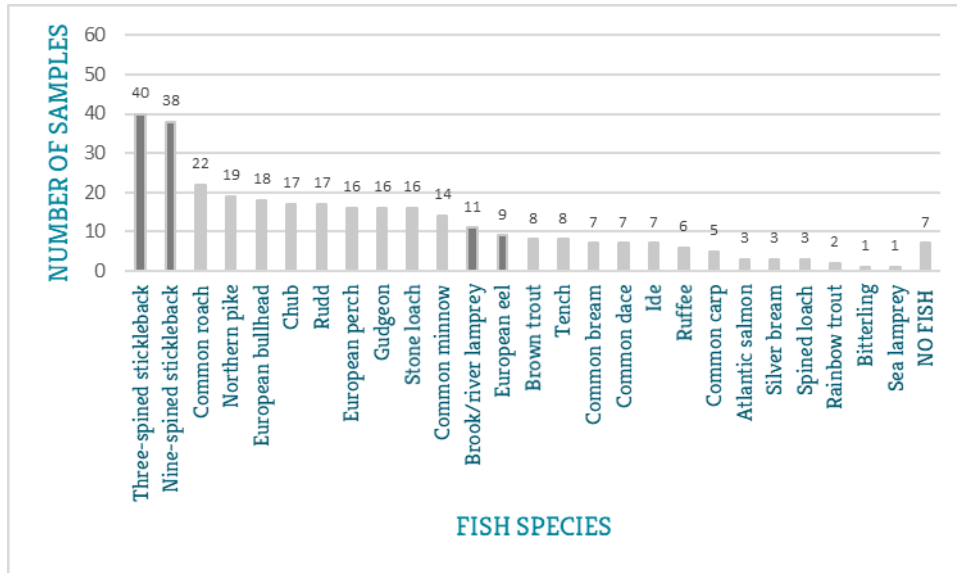


Figure 13: Number of fish species found across eDNA survey sites (n=57)

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 40 and 38 sites respectively out of the 57 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, and 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 nine of the 57 sample sites, 7 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 13). 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 eight 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 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.

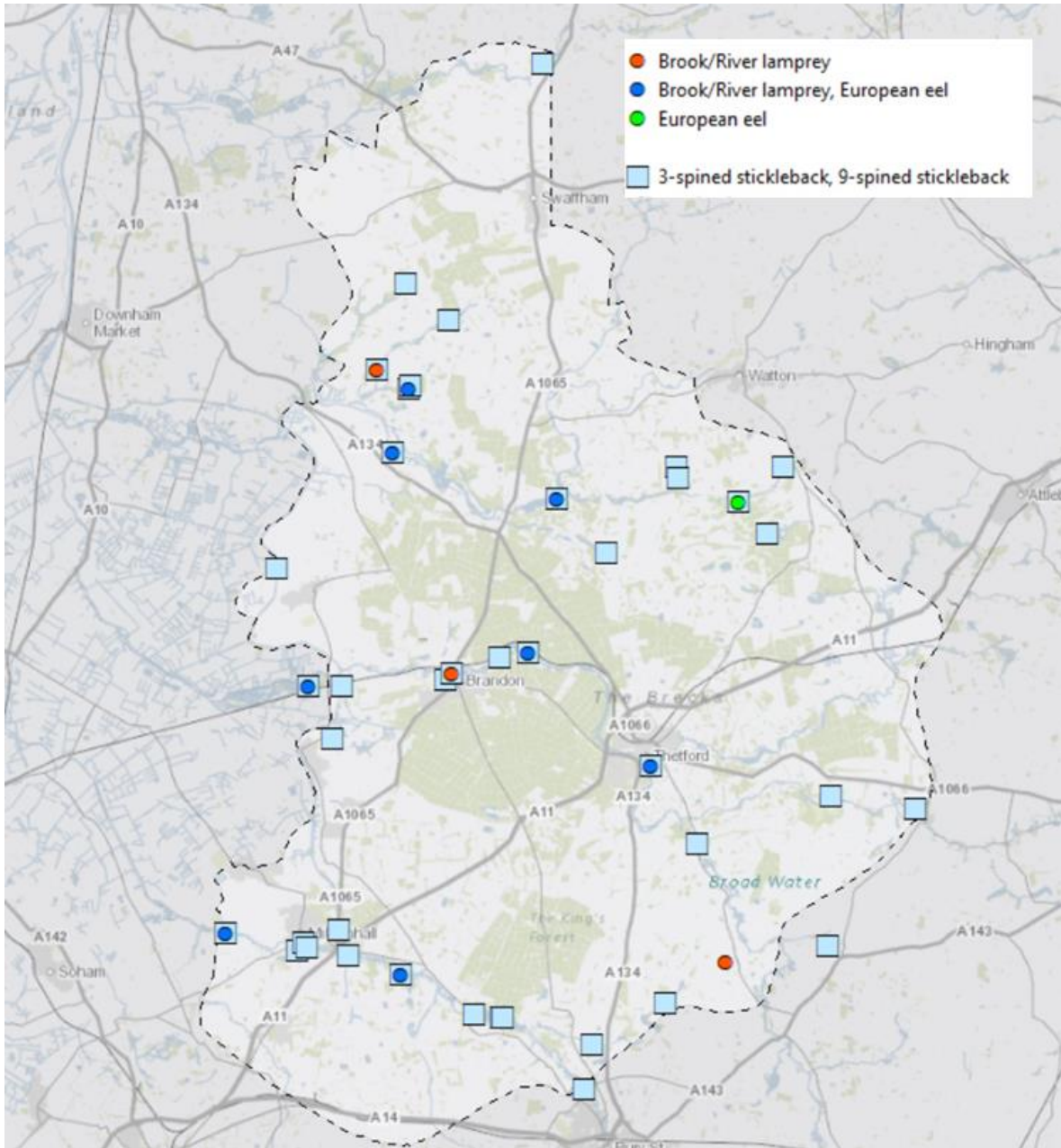


Figure 14: Distribution of three and nine spined stickleback, European eel and lamprey species in The Brecks survey area

4.4 Mammals

A total of 11 mammal species were recorded across the 57 sample sites, including three aquatic mammals (Figure 16). The most commonly detected aquatic mammal species was water vole, which was recorded at 13 of the sites, followed by Eurasian water shrew (three sites) and Eurasian otter (one site) (Figure 15).

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

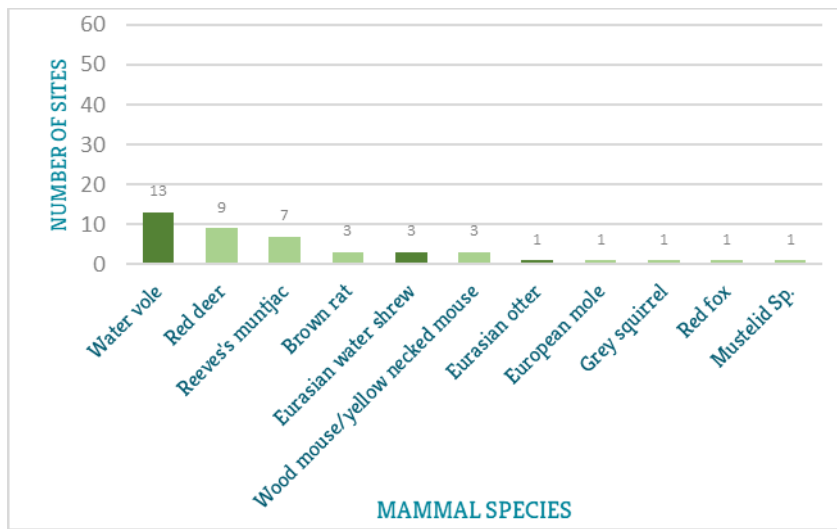


Figure 151: Number of mammal species found across eDNA survey sites (n=57)

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 13 of the 57 sample sites (23%), 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.

Nine of the occupied sites were running waters, including the River Lark and associated ditch network, Little Ouse and River Gadder. Two ponds where the species occurred were adjacent to and directly connected to running water. But, 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 three of the 57 sites sampled (5%):

- **TF7401 Oxborough RIVER GADDER**
- **TF8215 Castle Acre STREAM**
- **TL9574 Stanton Chare STREAM**

Interestingly water vole were also found in all three of these waterbodies (Figure 16).

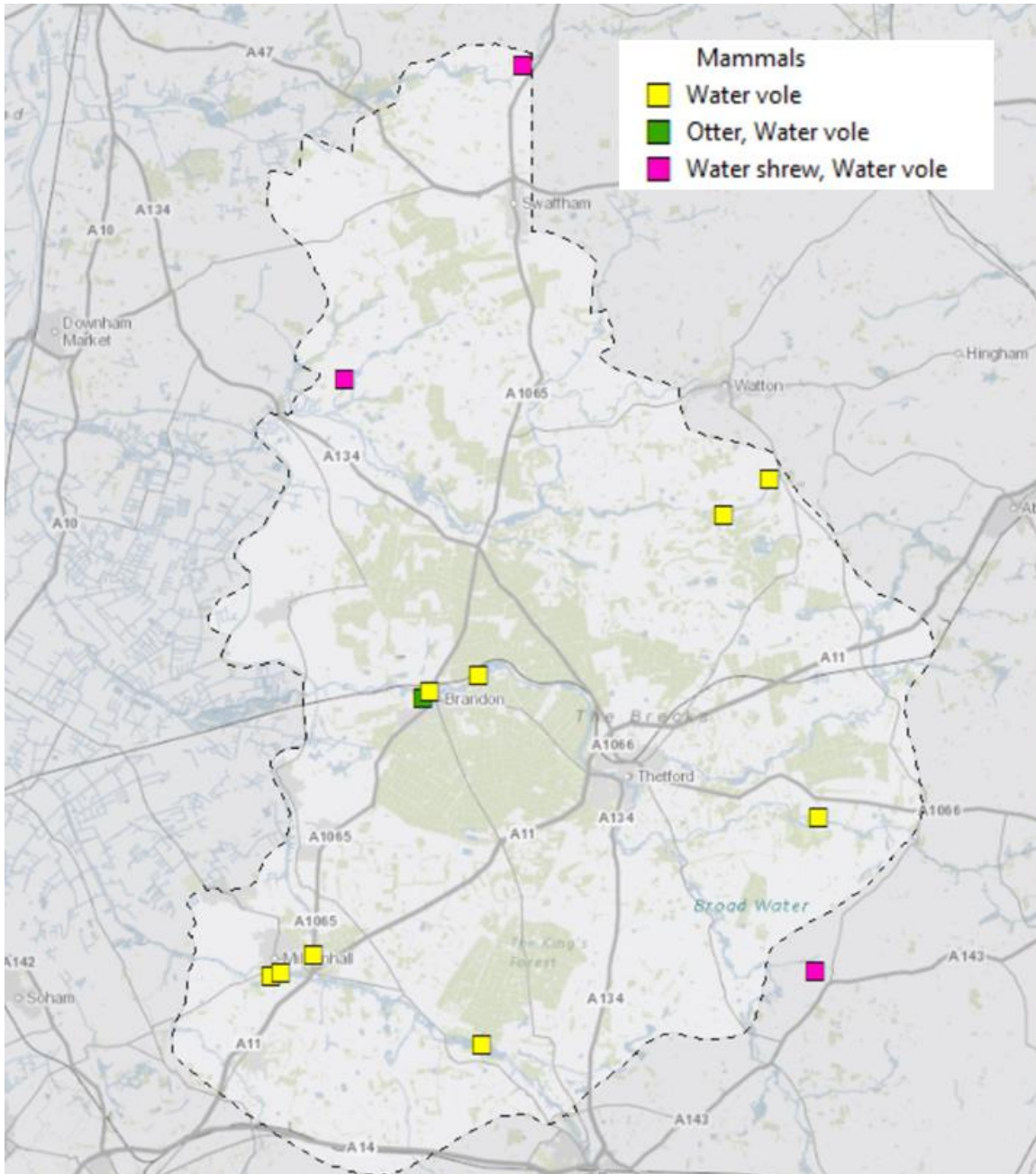


Figure 16: Distribution of aquatic mammals in The Brecks survey area

4.5 Water birds

A total of 26 bird species were recorded across the 57 sample sites (Figure 18). Twenty-one species were identified to species level, with a further 7 identified to family or genus level. Of the total birds identified, 6 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 47 of the 57 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 bird species was **common moorhen**, recorded at 39 sites (68%). **Grey heron** were recorded at 10 sites (40%), **common coot** at 6 sites (12%), **cormorant** at 4 sites (7%) and **water rail** at just 2 sites (4%). 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 57 sites respectively (Figure 17).

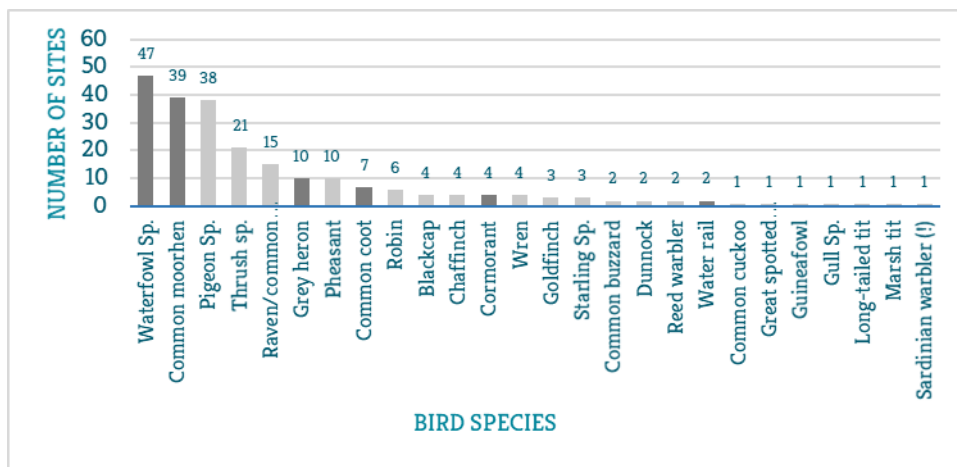


Figure 17: Number of bird species found across eDNA survey sites (n=57)

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 this study waterfowl species were recorded at 47 out of the 57 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 4: the Red List for Birds. 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 4: the Red List for Birds. 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 4: the Red List for Birds. 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 4: the Red List for Birds. 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 4: the Red List for Birds. The species was only found at two sample sites, both on running water.

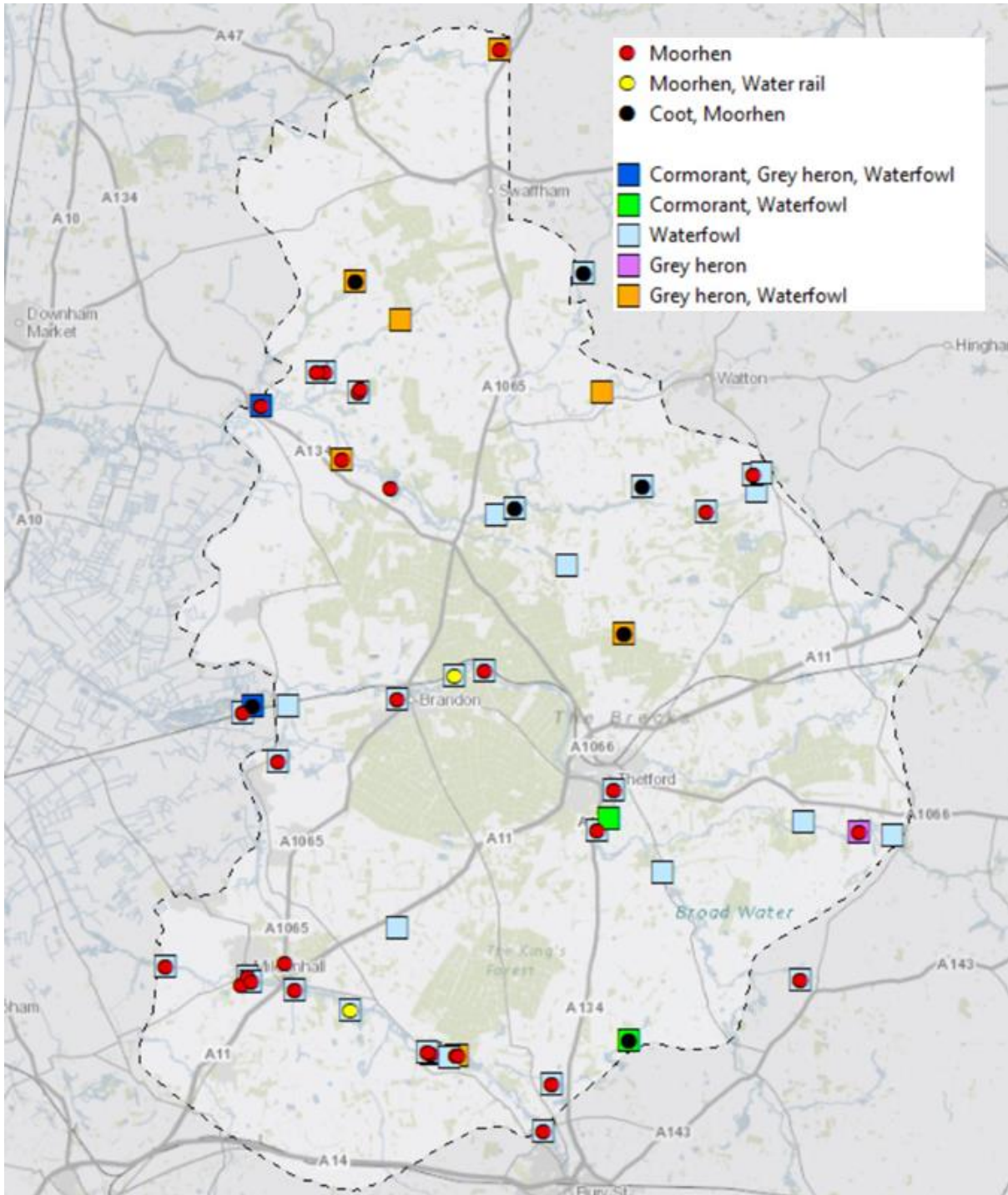


Figure 182: Distribution of aquatic birds in The Brecks survey area

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. But, records of species through their DNA can help to build a picture of landscape scale biodiversity.

As well as the three aquatic mammal species, seven 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 (22 species in total), including two birds species associated with water, reed warbler (*Acrocephalus 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 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 couple of 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.
- 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 won’t 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’s 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, river habitats) only 17% were classified as clean (Figure 19).

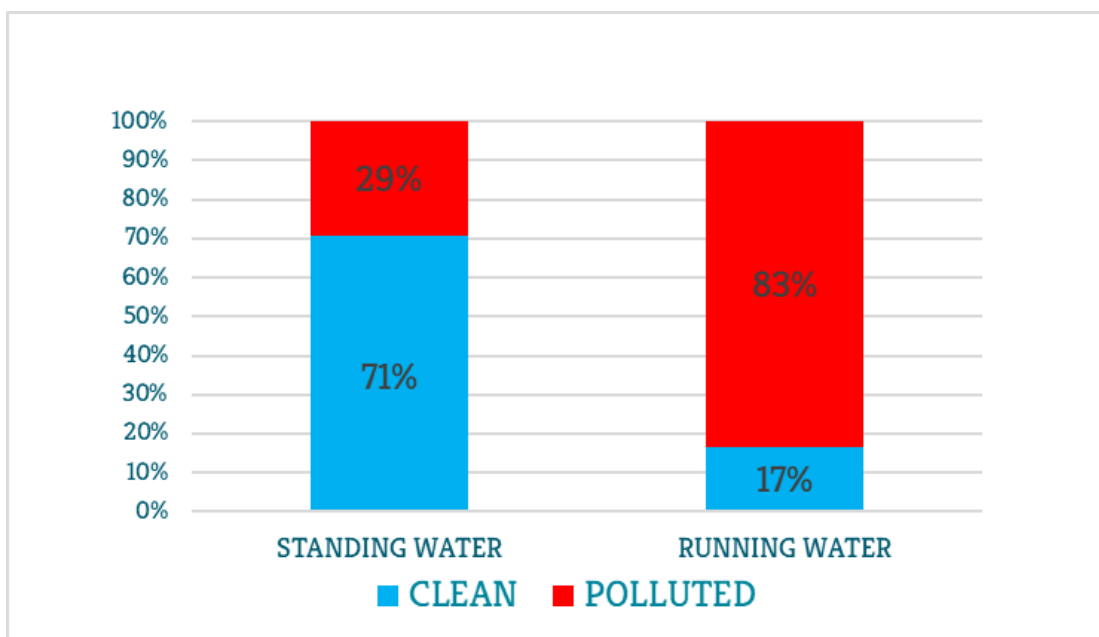


Figure 19: Percentage of clean and polluted water across standing and running waterbodies in The Brecks Fen Edge and Rivers Landscape study area.



Figure 20: Clean water sample from a pond in the Brecks study area.



Figure 21: Polluted water sample from a ditch in the Brecks study area.

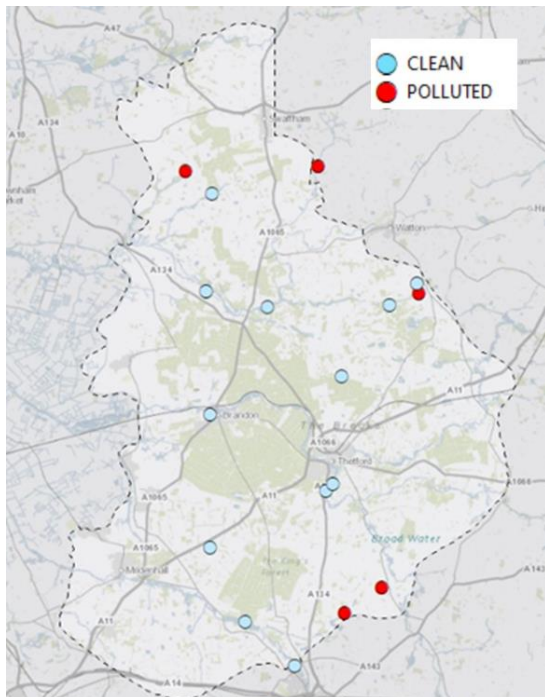


Figure 22: Distribution of clean and polluted water in standing waterbodies (ponds & lakes)

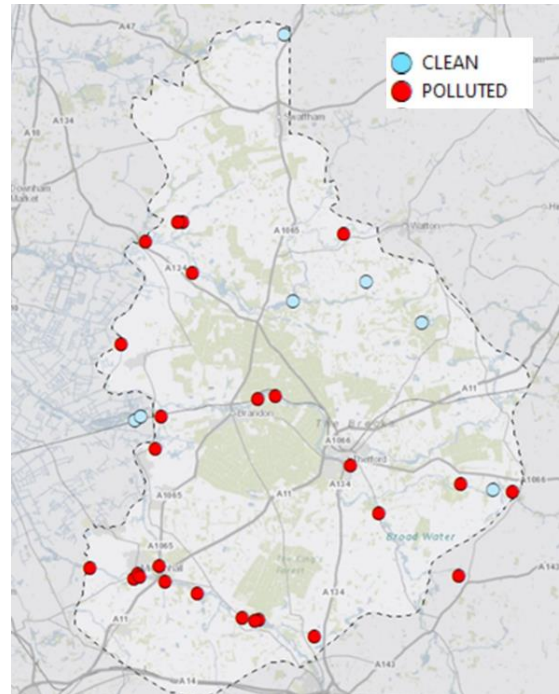


Figure 233: Distribution of clean and polluted water in running waterbodies (rivers, streams, ditches & cut-off channel)

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.

The results of eDNA surveys has 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. So, whilst some species, such as toads, have a preference for standing waters, they are capable of occupying both standing and running waterbodies. Even species traditionally associated with rivers, such as European eel and brook/river lamprey, 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.

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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

Recording your Testing the Water results

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

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

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

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

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 [WaterMet 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 Stable Hood (if pond name not known).

eDNA kit number (this is the number under the bar code on the eDNA kit sampling database).

Testing the Water eDNA survey

- 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 bend 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 EHT recording form (PTO) and submit the information online using the WaterMet 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.

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Recording your Testing the Water results

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.

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.

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

Submit info online using the EHT WaterMet 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	No.	1km Grid Square and Site Name	Type	Grid Reference	Date
1	TF7401 Oxborough Stream	Stream	TF 74734 01093	23/5	30	TL7970 Alder Carr Lake	Lake	TL 79416 70992	12/5
2	TF7401 Oxborough River Gadder	River	TF 74328 01088	23/5	31	TL7971 West Stow River Lark	River	TL 79222 71071	12/5
3	TF7600 Foulden Common Pond	Pond	TF 76194 00194	30/6	32	TL8070 Culford Stream	Stream	TL 80514 70857	11/7
4	TF7605 Shingham Pond	Pond	TF 76030 05068	8/6	33	TL8070 Fullers Mill Pond	Pond	TL 80574 70887	12/5
5	TF7803 Cockley Cley Pond	Pond	TF 78057 03406	12/5	34	TL8070 Lackford Lakes Ditch	Ditch	TL 80172 70801	15/6
6	TF8215 Castle Acre Stream	Stream	TF 82445 15344	9/5	35	TL8087 Santon Downham Ditch	Ditch	TL 80416 87681	17/5
7	TF8600 Watton Brook Stream	Stream	TF 86924 00194	23/5	36	TL8187 Santon Downham Little Ouse	River	TL 81726 87871	10/5
8	TF8605 Houghton Springs Pond	Pond	TF 86121 05448	26/5	37	TL8194 Lynford Water Lake	Lake	TL 82295 94748	8/6
9	TF8680 Nunnery Lakes Pond	Pond	TL 86762 80814	20/5	38	TL8395 Lynford River Wissey	River	TL 83096 95036	12/5
10	TF8781 Nunnery Lakes Lake	Lake	TL 87217 81355	9/5	39	TL8467 Suffolk Golf Club Pond	Pond	TL 84325 67554	24/5
11	TL7091 Feltwell Cut Off Channel	Cut off	TL 70041 91807	25/4	40	TL8469 Timworth Ditch	Ditch	TL 84752 69652	23/5
12	TL6774 Jude's Ferry Bridge River Lark	River	TL 67650 74807	25/4	41	TL8592 Great Carr Stream	Stream	TL 85418 92573	19/5
13	TL7173 Barton Mills Ditch	Ditch	TL 71027 73984	13/7	42	TL8782 Nuns Bridge River Thet	River	TL 87465 82606	9/6
14	TL7174 Barton Mills River Lark	River	TL 71319 74341	13/7	43	TL8789 Fowl Mere Lake	Lake	TL 87905 89517	11/5
15	TL7174 Norah Hanbury Ditch	Ditch	TL 71452 74126	23/6	44	TL8871 Broad Water Lake	Lake	TL 88156 71530	12/5
16	TL7186 Lakenheath Fen Ditch	Ditch	TL 71038 86021	10/5	45	TL8895 Sturton Carr Ditch	Ditch	TL 88669 96560	18/5
17	TL7186 Lakenheath Fen Little Ouse	River	TL 71552 86346	10/5	46	TL8896 West Mere Lake	Lake	TL 88717 96029	18/5
18	TL7199 Wittington River Wissey	River	TL 71909 99589	16/5	47	TL8978 Euston Black Bourne River	River	TL 89623 78971	12/5
19	TL7274 Mildenhall Cut Off Channel	Cut off	TL 72930 74984	13/7	48	TL9073 Pit Plantation Pond	Pond	TL 90984 73492	13/5
20	TL7283 North Fen Stream	Stream	TL 72655 83879	16/5	49	TL9194 Thompson Water Lake	Lake	TL 91514 94907	23/5
21	TL7373 Tuddenham Mill Stream	Stream	TL 73345 73753	12/5	50	TL9293 Cranberry Rough Ditch	Ditch	TL 92856 93439	11/7
22	TL7386 Hockwold-cum-wilton	Cut off	TL 73071 86293	8/6	51	TL9395 Stow Bedon Pond	Pond	Location removed	16/5
23	TL7572 Temple Bridge River Lark	River	TL 75844 72871	13/7	52	TL9396 Thompson Common Pond	Pond	TL 93632 96523	16/5
24	TL7597 Northwold Stream	Stream	TL 75434 97225	9/5	53	TL9496 Thompson Common Stream	Stream	TL 94016 96609	6/5
25	TL7600 Foulden common Ditch	Ditch	TF 76264 00361	30/6	54	TL9574 Stanton Chare Stream	Stream	TL 95668 74210	6/5
26	TL7776 Canada Farm Pond	Pond	TL 77871 76527	13/7	55	TL9581 Knettishall Ditch	Ditch	TL 95874 81234	12/5
27	TL7786 Brandon Staunch Pond	Pond	TL 77872 86643	4/5	56	TL9880 Gasthorpe Stream	Stream	TL 98328 80788	12/5
28	TL7795 Cranwick Pond	Pond	TL77587 95982	11/7	57	TL9980 Scarfe Meadow Little Ouse	River	TL 99797 80620	10/5
29	TL7886 Brandon Little Ouse River	River	TL 78232 86928	6/7					

Appendix 3. Sample site details - eDNA detection failed

No.	1km Grid Square and Site Name	Type	Grid Reference	Date
58	TF7504 Shingham Wood Pond	Pond	TF 75852 04741	11/5
59	TL7472 Tuddenham Heath Pond	Pond	TL 74500 72800	23/5
60	TL7672 Secret Fen Ditch	Ditch	TL 76467 72797	4/5
61	TL7697 Northwold River Wissey	River	TL 76010 97376	16/5
62	TL8084 Go Ape Pond	Pond	TL 80907 84923	6/7
63	TL8502 Great Cressingham Pond	Pond	TF 85260 02198	27/4
64	TL8502 Great Cressingham R. Wissey	River	TF 85071 02136	27/4
65	TL8592 Bodney Pond	Pond	TL 85824 92872	11/5
66	TL8692 Bagmore Ditch	Ditch	TL 86061 92338	11/5
67	TL8782 Nuns Bridge Little Ouse	River	TL 87334 82513	26/4
68	TL9295 Thompson Common Ditch	Ditch	TL 92549 95287	9/5
69	TL9390 Queens Close Stream	Stream	TL 93364 90614	6/5
70	TL9391 Queens Close Pond	Pond	TL 93573 91011	6/5
71	TL9484 Brettenham River Thet	River	TL 94178 84209	3/5

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 <i>et al.</i> , 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	<i>Cyprinus carpio</i> / <i>Cyprinus rubrofuscus</i>	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	<i>Rhodeus amarus</i> / <i>Rhodeus sericeus</i>	This sequence is either <i>Rhodeus amarus</i> or <i>Rhodeus sericeus</i> , which are indistinguishable using this marker.
Perch / Zander	<i>Perca fluviatilis</i> / <i>Sander lucioperca</i>	This sequence is either <i>Perca fluviatilis</i> or <i>Sander lucioperca</i> , which are indistinguishable using this marker.
Brook lamprey / River lamprey	<i>Lampetra planeri</i> / <i>Lampetra fluviatilis</i>	This sequence is an exact match to either river lamprey (<i>Lampetra fluviatilis</i>) or brook lamprey (<i>Lampetra planeri</i>). 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	<i>Pelophylax lessonae</i> / <i>Pelophylax ridibundus</i>	This sequence is either <i>Pelophylax lessonae</i> or <i>Pelophylax ridibundus</i> , which are indistinguishable using this marker.
Pigeon species	<i>Columba</i>	The marker for this sequence is an equally close match to multiple <i>Columba</i> species, thus NatureMetrics conservatively identified this sequence as a pigeon species (<i>Columba sp.</i>).
Pigeon species	<i>Columbidae</i>	The marker for this sequence is an equally close match to multiple <i>Columbidae</i> species, thus NatureMetrics conservatively identified this sequence as a pigeon species (<i>Columbidae sp.</i>).
Duck/goose/swan species	<i>Anatidae</i>	The marker for this sequence is an equally close match to several different <i>Anatidae</i> species, thus NatureMetrics conservatively identified this sequence as a duck/goose/swan species (<i>Anatidae sp.</i>).
Gull species	<i>Laridae</i>	This sequence is an exact match to several <i>Laridae</i> species, thus NatureMetrics conservatively identified this sequence as a gull species (<i>Laridae sp.</i>). Importantly, many species within the family <i>Laridae</i> are missing reference sequences for this marker, further complicating taxonomic assignment.
Common raven / Carrion crow	<i>Corvus corax</i> / <i>Corvus corone</i>	This sequence is either <i>Corvus corax</i> or <i>Corvus corone</i> , which are indistinguishable using this marker.
Common chaffinch / Brambling	<i>Fringilla coelebs</i> / <i>Fringilla montifringilla</i>	This sequence is either <i>Fringilla coelebs</i> or <i>Fringilla montifringilla</i> , which are indistinguishable using this marker.
Sardinian warbler / Eastern subalpine warbler	<i>Curruca melanocephala</i> / <i>Curruca cantillans</i>	This sequence is either <i>Curruca melanocephala</i> or <i>Curruca cantillans</i> , which are indistinguishable using this marker.
Starling species	<i>Sturnidae</i>	The marker for this sequence is an equally close match to several different <i>Sturnidae</i> species,

		thus NatureMetrics conservatively identified this sequence as a starling species (<i>Sturnidae sp.</i>).
Thrush species	<i>Turdus</i>	The marker for this sequence is an equally close match to several different <i>Turdus</i> species, thus NatureMetrics conservatively identified this sequence as a thrush species (<i>Turdus sp.</i>).
Mustelid species	<i>Mustelidae</i>	This sequence is an exact match to a number of <i>Mustelidae</i> species, thus NatureMetrics conservatively identified this sequence as a Mustelid species (<i>Mustellidae sp.</i>).
Wood mouse / Yellow-necked mouse	<i>Apodemus sylvaticus</i> / <i>Apodemus flavicollis</i>	This sequence is either <i>Apodemus sylvaticus</i> or <i>Apodemus flavicollis</i> , 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	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Mammals	<i>Cervus elaphus</i>	Red deer
	<i>Apodemus sylvaticus</i> / <i>Apodemus flavicollis</i>	Wood/Yellow-necked mouse
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Columba sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Gallinula chloropus</i>	Moorhen
	<i>Carduelis carduelis</i>	European goldfinch
	<i>Troglodytes troglodytes</i>	Eurasian wren
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Cyprinus carpio</i> / <i>Cyprinus rubrofuscus</i>	Common/Amur carp
	<i>Gobio gobio</i>	Gudgeon
	<i>Rutilus rutilus</i>	Roach
	<i>Squalius cephalus</i>	Chub
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Salmo trutta</i>	Trout
	<i>Cottus gobio</i>	European bullhead
	<i>Lampetra planeri</i> / <i>Lampetra fluviatilis</i>	Brook/River lamprey

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

SITE 3: TF7600 Foulden Common POND (TF 76194 00194)		
Group	Species	Common Name
Amphibians	<i>Rana temporaria</i>	Common frog
Mammals	<i>Cervus elaphus</i>	Red deer
	<i>Muntiacus reevesi</i>	Reeves' muntjac
	<i>Apodemus sylvaticus</i> / <i>Apodemus flavicollis</i>	Wood/Yellow-necked mouse
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Gallinula chloropus</i>	Moorhen
	<i>Erithacus rubecula</i>	European robin

	<i>Sylvia atricapilla</i>	Eurasian blackcap
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Cottus gobio</i>	European bullhead
	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey

SITE 4: TF7605 Shingham POND (TF 76030 05068)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Mammals	<i>Cervus elaphus</i>	Red deer
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback

SITE 5: TF7803 Cockley Cley POND (TF 78057 03406)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Scardinius erythrophthalmus</i>	Rudd

SITE 6: TF8215 Castle Acre STREAM (TF 82445 15344)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Mammals	<i>Arvicola amphibius</i>	Water vole
	<i>Aneomys fodiens</i>	Water shrew
Birds	<i>Buteo buteo</i>	Buzzard
	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columba sp.</i>	Pigeon species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Fringilla coelebs/ Fringilla montifringilla</i>	Chaffinch/Brambling
	<i>Troglodytes troglodytes</i>	Eurasian wren
	<i>Turdus sp.</i>	Thrush species
<i>Ardea cinerea</i>	Grey heron	
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback

	<i>Pungitius pungitius</i>	Nine-spined stickleback
SITE 7: TF8600 Watton Brook STREAM (TF 86924 00194)		
Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Mammals	<i>Rattus norvegicus</i>	Brown rat
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Tinca tinca</i>	Tench
	<i>Pungitius pungitius</i>	Nine-spined stickleback
SITE 8: TF8605 Houghton Springs POND (TF 86121 05448)		
Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Cyprinus carpio/Cyprinus rubrofuscus</i>	Common/Amur carp
	<i>Pungitius pungitius</i>	Nine-spined stickleback
SITE 9: TF8680 Nunnery Lakes POND (TL 86762 80814)		
Group	Species	Common Name
Birds	<i>Buteo buteo</i>	Buzzard
	<i>Anatidae sp.</i>	Wildfowl species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Acrocephalus scirpaceus</i>	Reed-warbler
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Leuciscus idus</i>	Ide
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Tinca tinca</i>	Tench
	<i>Esox lucius</i>	Northern pike
SITE 10: TF8781 Nunnery Lakes LAKE (TL 87217 81355)		
Group	Species	Common Name
Amphibians	<i>Pelophylax lessonae/Pelophylax ridibundus</i>	Pool/Marsh frog
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phalacrocorax carbo</i>	Great cormorant
Fish	<i>Abramis brama</i>	Bream
	<i>Blicca bjoerkna</i>	Silver bream
	<i>Cyprinus carpio/Cyprinus rubrofuscus</i>	Common/Amur carp
	<i>Rutilus rutilus</i>	Roach

	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Tinca tinca</i>	Tench
	<i>Esox lucius</i>	Northern pike
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo salar</i>	Atlantic salmon

SITE 11: TL 7091 Feltwell CUT OFF CHANNEL (TL 70041 91807)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Birds	<i>Columbidae sp.</i>	Pigeon species
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
Fish	<i>Abramis brama</i>	Bream
	<i>Blicca bjoerkna</i>	Silver bream
	<i>Rutilus rutilus</i>	Roach
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Cottus gobio</i>	European Bullhead

SITE 12: TL6774 Jude's Ferry Bridge RIVER LARK (TL 67650 74807)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Abramis brama</i>	Bream
	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
<i>Salmo trutta</i>	Trout	
<i>Cottus gobio</i>	European Bullhead	

	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey
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SITE 13: TL7173 Barton Mills DITCH (TL 71027 73984)

Group	Species	Common Name
Mammals	<i>Arvicola amphibius</i>	Water vole
	<i>Rattus norvegicus</i>	Brown rat
Birds	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Carduellis carduelis</i>	European goldfinch
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Barbatula barbatula</i>	Stone loach
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 13: TL7174 Barton Mills RIVER LARK (TL 71319 74341)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Abramis brama</i>	Bream
	<i>Gobio gobio</i>	Gudgeon
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander	

SITE 15: TL7174 Norah Hanbury NR DITCH (TL71452 74126)

Group	Species	Common Name
Amphibians	<i>Rana temporaria</i>	Common frog
Mammals	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Gallinula chloropus</i>	Moorhen
	<i>Troglodytes troglodytes</i>	Eurasian wren
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 16: TL7186 Lakenheath Fen DITCH (TL 71038 86021)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Gallinula chloropus</i>	Moorhen

SITE 17: TL7186 Lakenheath Fen LITTLE OUSE RIVER (TL 71552 86346)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Ardea cinerea</i>	Grey heron
	<i>Phalacrocorax carbo</i>	Great cormorant
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Cobitis taenia</i>	Spined loach
	<i>Blicca bjoerkna</i>	Silver bream
	<i>Abramis brama</i>	Bream
	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rhodeus amarus/Rhodeus sericeus</i>	European/Amur bitterling
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Tinca tinca</i>	Tench
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
<i>Cottus gobio</i>	European Bullhead	
<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey	

SITE 18: TL7199 Wittington RIVER WISSEY (TL 71909 99589)

Group	Species	Common Name
Mammals	<i>Vulpes vulpes</i>	Red Fox
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Ardea cinerea</i>	Grey heron
	<i>Phalacrocorax carbo</i>	Great cormorant
Fish	<i>Gobio gobio</i>	Gudgeon

SITE 19: TL7274 Mildenhall CUT OFF CHANNEL (TL 72930 74984)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Mammals	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Cuculus canorus</i>	Cuckoo
	<i>Gallinula chloropus</i>	Moorhen
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gobio gobio</i>	Gudgeon

	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
	<i>Cottus gobio</i>	European Bullhead

SITE 20: TL7283 North Fen STREAM (TL 72655 83879)

Group	Species	Common Name
Mammals	<i>Muntiacus reevesi</i>	Reeves'muntjac
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Erithacus rubecula</i>	European robin
	<i>Prunella modularis</i>	Dunnock
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 21: TL7373 Tuddenham Mill STREAM (TL 73345 73753)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
Fish	<i>Phoxinus phoxinus</i>	Minnow
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Cottus gobio</i>	European Bullhead

SITE 22: TL7386 Hockwold cum Wilton CUT OFF CHANNEL (TL 73071 86293)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 23: TL7572 Temple Bridge RIVER LARK (TL 75844 72871)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Rallus aquaticus</i>	Western water rail
	<i>Acrocephalus scirpaceus</i>	Reed-warbler
Fish	<i>Anguilla anguilla</i>	European eel

	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Tinca tinca</i>	Tench
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo trutta</i>	Trout
	<i>Cottus gobio</i>	European Bullhead
	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey

SITE 24: TL7597 Northwold STREAM (TL 75434 97225)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Lissotriton vulgaris</i>	Smooth newt
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columba sp.</i>	Pigeon species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Aegithalos caudatus</i>	Long-tailed tit
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Turdus sp.</i>	Thrush species
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Cobitis taenia</i>	Spined loach
	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus idus</i>	Ide
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Tinca tinca</i>	Tench
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo trutta</i>	Trout
<i>Cottus gobio</i>	European Bullhead	
<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey	

SITE 25: TL7600 Foulden common DITCH (TF 76264 00361)		
Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Birds	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 26: TL7776 Canada Farm POND (TL 77871 76527)		
Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Sturnidae sp.</i>	Starling species
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Cyprinus carpio/Cyprinus rubrofuscus</i>	Common /Amur carp
	<i>Leuciscus idus</i>	Ide
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub

SITE 27: TL7786 Brandon Staunch POND (TL 77872 86643)		
Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Mammals	<i>Cervus elaphus</i>	Red deer
	<i>Lutra lutra</i>	Eurasian otter
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
Fish	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus idus</i>	Ide
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
<i>Cottus gobio</i>	European Bullhead	

SITE 28: TL7795 Cranwick POND (TL77587 95982)		
Group	Species	Common Name
Birds	<i>Gallinula chloropus</i>	Moorhen

SITE 29: TL7886 Brandon LITTLE OUSE RIVER (TL 78232 86928)		
Group	Species	Common Name
Mammals	<i>Muntiacus reevesi</i>	Reeves' muntjac

	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Columba sp.</i>	Pigeon species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Sylvia atricapilla</i>	Eurasian blackcap
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Phoxinus phoxinus</i>	Minnow
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey

SITE 30: TL7970 Alder Carr LAKE (TL 79416 70992)

Group	Species	Common Name
Amphibians	<i>Lissotriton vulgaris</i>	Smooth newt
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
Fish	<i>Leuciscus idus</i>	Ide
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Esox lucius</i>	Northern pike
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Oncorhynchus mykiss</i>	Rainbow trout

SITE 31: TL7971 West Stow RIVER LARK (TL 79222 71071)

Group	Species	Common Name
Mammals	<i>Muntiacus reevesi</i>	Reeves' muntjac
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus idus</i>	Ide
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo trutta</i>	Trout
	<i>Cottus gobio</i>	European Bullhead

SITE 32: TL8070 Culford STREAM (TL 80514 70857)

Group	Species	Common Name
Mammals	<i>Muntiacus reevesi</i>	Reeves' muntjac

Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Gobio gobio</i>	Gudgeon
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo salar</i>	Atlantic salmon

SITE 33: TL8070 Fullers Mill POND (TL 80574 70887)

Group	Species	Common Name
Mammals	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Numida meleagris</i>	Helmeted guineafowl
	<i>Gallinula chloropus</i>	Moorhen
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Rutilus rutilus</i>	Roach
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Cottus gobio</i>	European Bullhead

SITE 34: TL8070 Lackford Lakes DITCH (TL 80172 70801)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species

SITE 35: TL8087 Santon Downham DITCH (TL 80416 87681)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Mammals	<i>Muntiacus reevesi</i>	Reeves' muntjac
	<i>Arvicola amphibius</i>	Water vole
	<i>Apodemus sylvaticus/Apodemus flavicollis</i>	Wood/Yellow-necked mouse
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Rallus aquaticus</i>	Western water rail
	<i>Fringilla coelebs/Fringilla montifringilla</i>	Chaffinch/Brambling
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Petromyzon marinus</i>	Sea lamprey

SITE 36: TL8187 Santon Downham LITTLE OUSE RIVER (TL 81726 87871)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Curruca melanocephala/Curruca cantillans</i>	Sardinian/Subalpine warbler
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander

SITE 37: TL8194 Lynford Water LAKE (TL 82295 94748)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
Fish	<i>Abramis brama</i>	Bream
	<i>Rutilus rutilus</i>	Roach
	<i>Esox lucius</i>	Northern pike

SITE 38: TL8395 Lynford RIVER WISSEY (TL 83096 95036)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Fringilla coelebs/Fringilla montifringilla</i>	Chaffinch/Brambling
	<i>Erithacus rubecula</i>	European robin
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Gobio gobio</i>	Gudgeon
	<i>Squalius cephalus</i>	Chub
	<i>Tinca tinca</i>	Tench
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike

	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Salmo trutta</i>	Trout
	<i>Cottus gobio</i>	European Bullhead
	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey

SITE 39: TL8467 Suffolk Golf Club POND (TL 84325 67554)

Group	Species	Common Name
Amphibians	<i>Rana temporaria</i>	Common frog
	<i>Lissotriton vulgaris</i>	Smooth newt
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 40: TL8469 Timworth DITCH (TL 84752 69652)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Gallinula chloropus</i>	Moorhen
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Rutilus rutilus</i>	Roach
	<i>Tinca tinca</i>	Tench
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander

SITE 41: TL8592 Great Carr STREAM (TL85418 92573)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
	<i>Lissotriton vulgaris</i>	Smooth newt
Mammals	<i>Cervus elaphus</i>	Red Deer
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Erithacus rubecula</i>	European robin
	<i>Poecile palustris</i>	Marsh tit
	<i>Turdus sp.</i>	Thrush species
	<i>Dendrocopos major</i>	Great spotted woodpecker
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 42: TL8782 Nuns Bridge RIVER THET (TL 87465 82606)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Cobitis taenia</i>	Spined loach
	<i>Abramis brama</i>	Bream
	<i>Gobio gobio</i>	Gudgeon
	<i>Leuciscus idus</i>	Ide
	<i>Leuciscus leuciscus</i>	Dace
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Gymnocephalus cernua</i>	Ruffe
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
<i>Cottus gobio</i>	European Bullhead	
<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey	

SITE 43: TL8789 Fowl Mere LAKE (TL 87905 89517)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
	<i>Lissotriton vulgaris</i>	Smooth newt
	<i>Triturus cristatus</i>	Great crested newt
Mammals	<i>Cervus elaphus</i>	Red Deer
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
	<i>Sturnidae sp.</i>	Starling species
	<i>Ardea cinerea</i>	Grey heron
Fish	<i>Salmo salar</i>	Atlantic salmon

SITE 44: TL8871 Broad Water LAKE (TL 88156 71530)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Laridae sp.</i>	Gull species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Phalacrocorax carbo</i>	Great cormorant
Fish	<i>Cyprinus carpio/Cyprinus rubrofuscus</i>	Common/Amur carp
	<i>Scardinius erythrophthalmus</i>	Rudd

	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
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SITE 45: TL8895 Sturton Carr DITCH (TL88669 965607)

Group	Species	Common Name
Amphibians	<i>Rana temporaria</i>	Common frog
Mammals	<i>Cervus elaphus</i>	Red Deer
Birds	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 46: TL8896 West Mere LAKE (TL 88717 96029)

Group	Species	Common Name
Amphibians	<i>Bufo bufo</i>	Common toad
	<i>Rana temporaria</i>	Common frog
	<i>Lissotriton vulgaris</i>	Smooth newt
	<i>Triturus cristatus</i>	Great crested newt
Mammals	<i>Cervus elaphus</i>	Red Deer
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Fulica atra</i>	Coot
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 47: TL8978 Euston BLACK BOURNE RIVER (TL 89623 78971)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gobio gobio</i>	Gudgeon
	<i>Phoxinus phoxinus</i>	Minnow
	<i>Rutilus rutilus</i>	Roach
	<i>Barbatula barbatula</i>	Stone loach
	<i>Esox lucius</i>	Northern pike
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Cottus gobio</i>	European Bullhead

SITE 48: TL9073 Pit Plantation POND (TL 90984 73492)

Group	Species	Common Name
Fish	<i>Rutilus rutilus</i>	Roach
	<i>Lampetra planeri/Lampetra fluviatilis</i>	Brook/River lamprey

SITE 49: TL9194 Thompson Water LAKE (TL 91514 94907)

Group	Species	Common Name
Mammals	<i>Arvicola amphibius</i>	Water vole
	<i>Mustelidae sp.</i>	Mustelid species
Birds	<i>Anatidae sp.</i>	Wildfowl species

	<i>Columbidae sp.</i>	Pigeon species
	<i>Phasianus colchicus</i>	Pheasant
	<i>Gallinula chloropus</i>	Moorhen
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Anguilla anguilla</i>	European eel
	<i>Gobio gobio</i>	Gudgeon
	<i>Rutilus rutilus</i>	Roach
	<i>Scardinius erythrophthalmus</i>	Rudd
	<i>Squalius cephalus</i>	Chub
	<i>Barbatula barbatula</i>	Stone loach
	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Perca fluviatilis/Sander lucioperca</i>	Perch/Zander
	<i>Oncorhynchus mykiss</i>	Rainbow trout
	<i>Salmo trutta</i>	Trout
	<i>Cottus gobio</i>	European Bullhead

SITE 50: TL9293 Cranberry Rough DITCH (TL 92856 93439)

Group	Species	Common Name
Birds	<i>Phasianus colchicus</i>	Pheasant
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 51: TL9395 Stow Bedon POND

Group	Species	Common Name
Amphibians	<i>Pelophylax lessonae/Pelophylax ridibundus</i>	Pool/Marsh frog
	<i>Lissotriton vulgaris</i>	Smooth newt
Birds	<i>Anatidae sp.</i>	Wildfowl species

SITE 52: TL9396 Thompson Common POND (TL 93632 96523)

Group	Species	Common Name
Amphibians	<i>Lissotriton vulgaris</i>	Smooth newt
	<i>Triturus cristatus</i>	Great crested newt
Mammals	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Gallinula chloropus</i>	Moorhen
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 53: TL9496 Thompson Common STREAM (TL 94016 96609)

Group	Species	Common Name
Amphibians	<i>Lissotriton vulgaris</i>	Smooth newt
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Erithacus rubecula</i>	European robin

SITE 54: TL9574 Stanton Chare STREAM (TL 95668 74210)

Group	Species	Common Name
Amphibians	<i>Rana temporaria</i>	Common frog
Mammals	<i>Muntiacus reevesi</i>	Reeves' muntjac

	<i>Arvicola amphibius</i>	Water vole
	<i>Rattus norvegicus</i>	Brown rat
	<i>Sciurus carolinensis</i>	Grey squirrel
	<i>Neomys fodiens</i>	Water shrew
	<i>Talpa europaea</i>	Mole
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Columba sp.</i>	Pigeon species
	<i>Columbidae sp.</i>	Pigeon species
	<i>Gallinula chloropus</i>	Moorhen
	<i>Corvus corax/ Corvus corone</i>	Raven/Crow
	<i>Carduelis carduelis</i>	European goldfinch
	<i>Fringilla coelebs/Fringilla montifringilla</i>	Chaffinch/Brambling
	<i>Erithacus rubecula</i>	European robin
	<i>Prunella modularis</i>	Dunnock
	<i>Sturnidae sp.</i>	Starling species
	<i>Sylvia atricapilla</i>	Eurasian blackcap
	<i>Troglodytes troglodytes</i>	Eurasian wren
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback
	<i>Cottus gobio</i>	European Bullhead

SITE 55: TL9581 Knettishall DITCH (TL 95874 81234)

Group	Species	Common Name
Mammals	<i>Arvicola amphibius</i>	Water vole
Birds	<i>Anatidae sp.</i>	Wildfowl species
	<i>Turdus sp.</i>	Thrush species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback

SITE 56: TL9880 Gasthorpe STREAM (TL 98328 80788)

Group	Species	Common Name
Birds	<i>Gallinula chloropus</i>	Moorhen
	<i>Ardea cinerea</i>	Grey heron

SITE 57: TL9980 Scarfe Meadow LITTLE OUSE RIVER (TL 99797 80620)

Group	Species	Common Name
Birds	<i>Anatidae sp.</i>	Wildfowl species
Fish	<i>Gasterosteus aculeatus</i>	Three-spined stickleback
	<i>Pungitius pungitius</i>	Nine-spined stickleback