Saving Oxford's Wetland Wildlife Environmental DNA Results 2019

Final Report

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Saving Oxford's Wetland Wildlife
eDNA Results 2019

Summary

Environmental DNA (eDNA) is increasingly being used to assess the presence of target species and the diversity of target taxonomic groups in aquatic environments. eDNA surveys do not require specialist knowledge and the methodology is relatively simple. This makes eDNA an excellent survey tool for volunteers, who are often pivotal to monitoring and surveying efforts. In this project, a team of dedicated volunteers obtained eDNA samples using multi-species eDNA kits from 28 sites in and around the proposed Oxford Flood Alleviation Scheme area. All 28 samples were analysed by eDNA company NatureMetrics for vertebrate eDNA, and two samples were analysed for freshwater mussel eDNA.

A total of 78 species were detected across the 28 sites: 23 fish, 4 amphibians, 4 freshwater mussels, 33 birds (of which 10 were water birds) and 14 mammals (of which two were aquatic). Species detected that are of particular conservation interest included Great Crested Newt, Common Toad, Water Vole, Water Shrew and lampreys (brook, river and sea lampreys cannot currently be separated by eDNA). The richest sites for fish were the Bulstake and Seacourt streams, where between 17 and 19 species were recorded. 16 fish species were detected in the main Thames using eDNA. The most widely recorded fish species were Roach and Perch, recorded at 57% of sites, followed by Pike, which was recorded in 47% of waterbodies. Tench, Barbel and Nine-spined Stickleback were the least frequently recorded fish, being detected at 10-15% of sites. We recorded four common species of freshwater mussel, but not the rarer Depressed River Mussel, which is known to occur in the area. As has commonly been seen in other projects, more species of fish were detected using eDNA than were recorded in traditional electrofishing surveys from the same area. This probably reflects the effect of DNA being sampled from a larger area than is covered by electrofishing surveys, and the ability of eDNA kits to detect small and cryptic species (such as lampreys) which may be harder to catch in traditional surveys.

Amphibians were recorded at half of the sites, but evidence from other surveys shows that they are probably under-represented by multi-species eDNA tests (unlike the single-species test for Great Crested Newts, which is slightly more effective than traditional surveys). Of the four amphibians species present, none were found in more than 30% of sites, and for the two widespread species (Common Frog and Smooth Newt), this is almost certainly lower than their true frequency in the waterbodies in this landscape (respectively Smooth Newts: 30%; Common Frogs: 22%; Common Toads: 17% and Great Crested Newt: 9%). The most widely detected water birds were Moorhen and Mallard, detected in 85% of the waterbodies.

eDNA surveys have the potential to revolutionise monitoring of freshwater biodiversity. Entire aquatic communities can be surveyed by obtaining a simple water

sample. Such samples provide a snapshot of an entire ecosystem, offer an easy way to survey species that are elusive or difficult-to-survey using traditional survey methods, and are both time and cost-effective compared to traditional survey methods (such as electrofishing or standard amphibian surveys with torches and bottle traps). However, there are a number of caveats which concern the validity of the eDNA data presented in this report. Firstly, no one has yet conducted a systematic comparison of multi-species eDNA kit data and data collected using traditional survey methods, so records obtained using eDNA surveys are difficult to interpret. How many species did we miss? Are all the species we recorded truly present, or were some so-called 'false positives? Secondly, whilst DNA technology is advancing rapidly, not all eDNA can be identified to species level, and there is still some uncertainty over the veracity of certain records. For example, is the Atlantic Salmon record in the Thames really true? Lastly, as eDNA can travel downstream, drop into waterbodies or be washed into waterbodies from surrounding land, there is still quite a lot of uncertainty over where eDNA originated from, and whether a species has been present at the sampling point, or if the genetic material originated from elsewhere. We expect that further development of eDNA survey methods, and careful comparisons with traditional methods, will increase the value of landscape-wide eDNA surveys. eDNA has the potential to be a highly cost-effective and accessible survey technique that could lead to a paradigm shift in the way freshwater biodiversity is monitored.

Saving Oxford's Wetland Wildlife
eDNA Results 2019

1.0 Introduction

Freshwater Habitats Trust undertook multi-species **environmental DNA surveys,** as part of the Saving Oxford's Wetland Wildlife project, to assess the distribution of **fish, amphibians and aquatic mammals** around the proposed Oxford Flood Alleviation Scheme area. The surveys form part of a monitoring programme that is helping to add to baseline data from the flood scheme area, which will be used to underpin the long-term monitoring of the flood scheme.

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, mucous and gametes (eggs and sperm). In ponds, lakes, rivers, streams and ditches, eDNA can persists for up to one month, depending on environmental conditions, leaving a **unique trace** of the plant or animal in the water.

Advances in DNA technology mean **it is now possible to detect freshwater animals by collecting and analysing a simple water sample**. Freshwater Habitats Trust have partnered with Nature Metrics, an eDNA specialist company, who have undertaken analyses for multiple projects by Freshwater Habitats Trust, including the Saving Oxford's Wetland Wildlife results presented here.

A Novel Technique

The following results provide **some of the very first examples in Britain of simultaneous sampling of fish, amphibians and aquatic mammals**, all obtained from a single water sample. Owing to the novelty of this sampling method, Freshwater Habitats Trust is still in the process of understanding how best to interpret these data. As these surveys become more commonplace, we expect our understanding of how eDNA data compares to data from current traditional survey methods (such as electrofishing or standard amphibian surveys with torches and bottle traps) to improve, giving a better understanding of the reliability of the eDNA records.

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 Saving Oxford's Wetland Wildlife.

2.0 Methodology

A total of **28 survey sites** were selected by Freshwater Habitats Trust (see Figure 2 for a site location map). The sites were primarily located in and around the Oxford Flood Alleviation Scheme area, and encompassed five waterbody types: **ponds, streams, rivers, lakes and ditches**. Sampling was undertaken between April and July 2019 by our team of dedicated volunteers. Volunteers used eDNA 'multi-species' kits from NatureMetrics.

At each site, a total of **20 x 50 ml water samples** were collected using a plastic sample pot attached to a bamboo cane (total volume collected 1 litre), and placed into a collection bag. For linear waterbodies (streams, ditches and rivers), samples were taken at 20 equidistant points along the length of the waterbody (one sample per 5 metres). For ponds (up to 1 hectare in size), samples were taken at 20 evenly-spaced points around the pond edge.

The collection bag was shaken to mix the water. Water was then drawn up using a 50 ml syringe, and **pushed through the filter to trap any DNA**. This was repeated until the entire 1 litre sample had been processed, except in infrequent situations where the filter became clogged due to silt (at which point filtering was stopped). Following this, the total volume of water filtered was recorded.

Air was then repeatedly pushed through the filter using the 50 ml syringe, until residual water had been expelled. A **preservative solution** was pushed through the filter to **stabilise the DNA**, and the lock caps were fitted to each end of the filter. Each filter and accompanying data sheet was then sent to NatureMetrics for analysis using **metabarcoding**, to enable the detection of a range of vertebrate species from low concentration DNA.

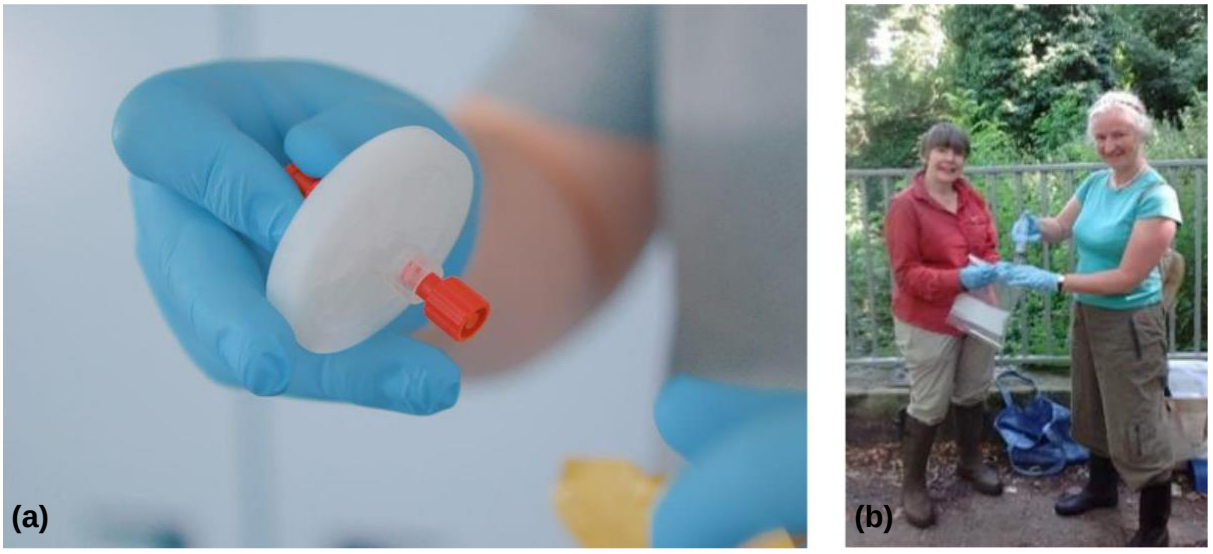

Figure 1. (a) A filter fitted with lock caps from a NatureMetrics multi-species eDNA kit. **(b)** Volunteers filtering a water sample to trap DNA for analysis.

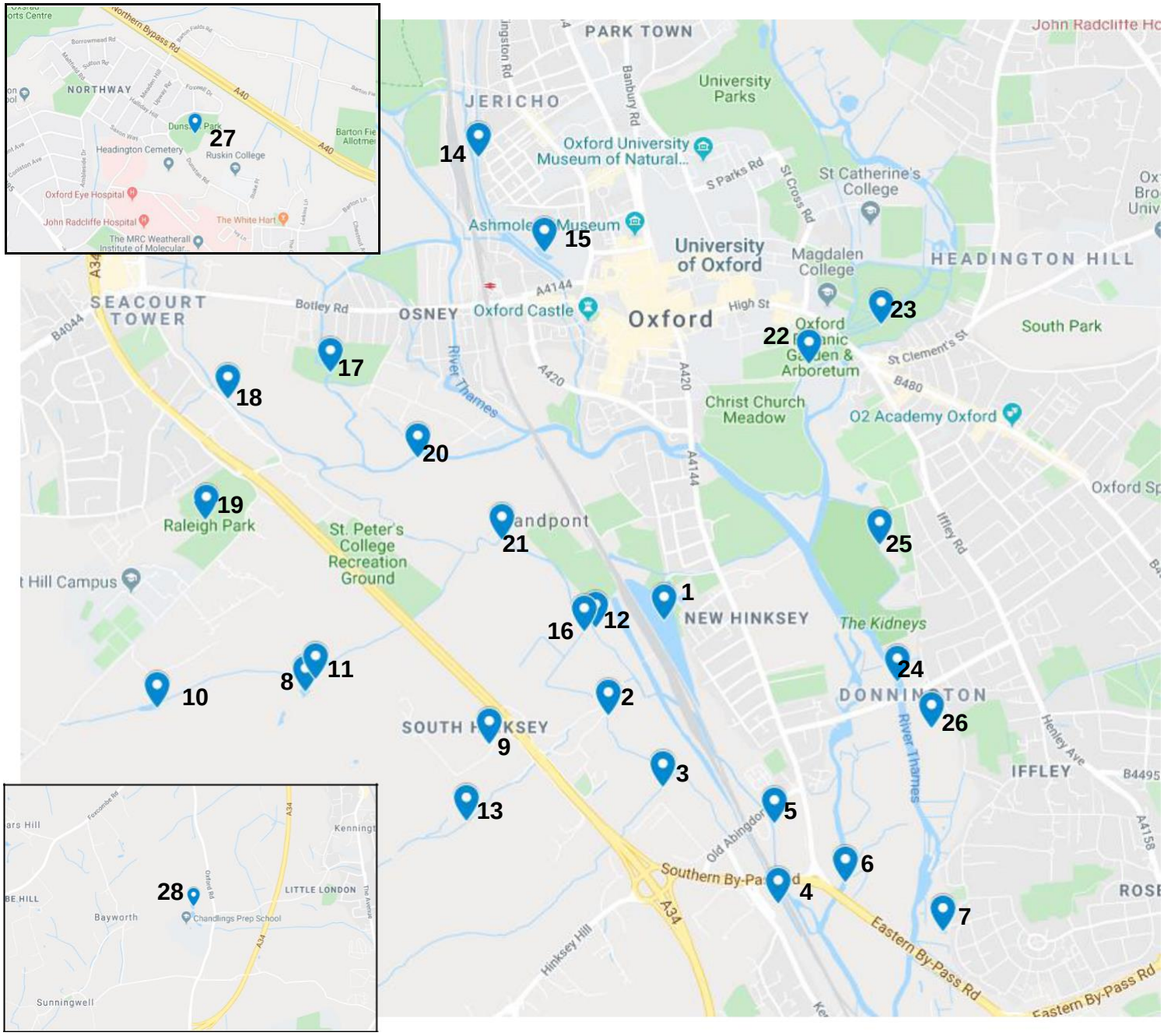

Figure 2. Locations of the 28 sites sampled using eDNA kits for Saving Oxford's Wetland Wildlife in 2019.

Sites 27 and 28 are featured on separate maps due to their distance from the rest of the sample sites. See Table 1 for site names. Maps were created using Google MyMaps.

Table 1. The numbers and names assigned to each eDNA sample site.

No.	Site Name
1	Hinksey Lake
2	South Hinksey Drain 1
3	South Hinksey Drain 2
4	Kennington Pit
5	Hinksey Stream at Redbridge
6	Weirs Mill Stream
7	Rivermead Pond
8	Hinksey Heights Golf Club Pond 1
9	Hinksey Heights Golf Club Pond 2
10	Hinksey Heights Golf Club Pond 3
11	Hinksey Heights Stream
12	South Hinksey Drain 3
13	Chilswell Stream
14	Oxford Canal
15	Worcester College Pond
16	Bulstake Stream 1
17	Bulstake Stream 2
18	Seacourt Stream
19	Raleigh Park Pond
20	Bulstake Stream 3
21	Hinksey Stream
22	Oxford Botanic Garden Pond
23	River Cherwell
24	River Thames (Donnington Bridge)
25	Shire Lake Ditch
26	Boundary Brook
27	Dunstan Park Pond
28	Chandlings School Pond

3.0 Results

3.1 Overview of Sample Sites

The total number of vertebrate species recorded using eDNA kits across all 28 sample sites was 74: **23 fish, 4 amphibians, 14 mammals and 33 birds**. The number of vertebrate species recorded at a single site ranged from 1 to 32, and the median number of species recorded per site was 14.

The four sites with the highest recorded number of vertebrate species were:

- **Bulstake Stream 3** (site 20) with **32** species
- **Hinksey Heights Stream** (site 11) with **30** species
- **Bulstake Stream 2** (site 17) with **26** species
- **Seacourt Stream** (site 18) with **26** species

Although worldwide research suggests that fish eDNA results are quite well aligned with traditional fish data – and we know that single species eDNA tests for Great Crested Newt are very reliable – at present much less is known about how much weight to put on mammal and bird eDNA data.

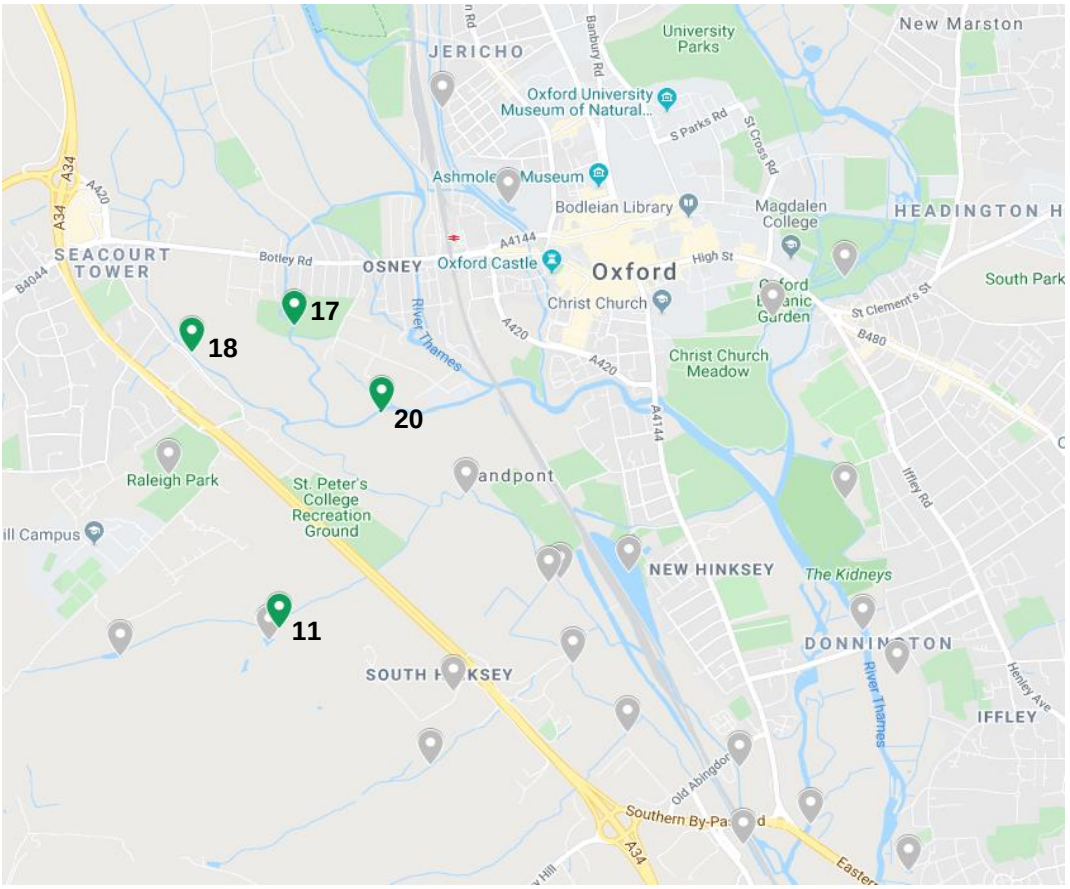

Figure 3. Locations of the four sites with the highest recorded number of vertebrate species (green markers, numbered) and the remainder of the eDNA sampling sites (grey markers).

All four species groups – fish, amphibians, mammals and birds – were detected at just 6 of the sites (refer to Figure 4). The most commonly detected species group was fish (185 records across all 28 sites), followed by birds (154 records), mammals (50 records) and amphibians (18 records).

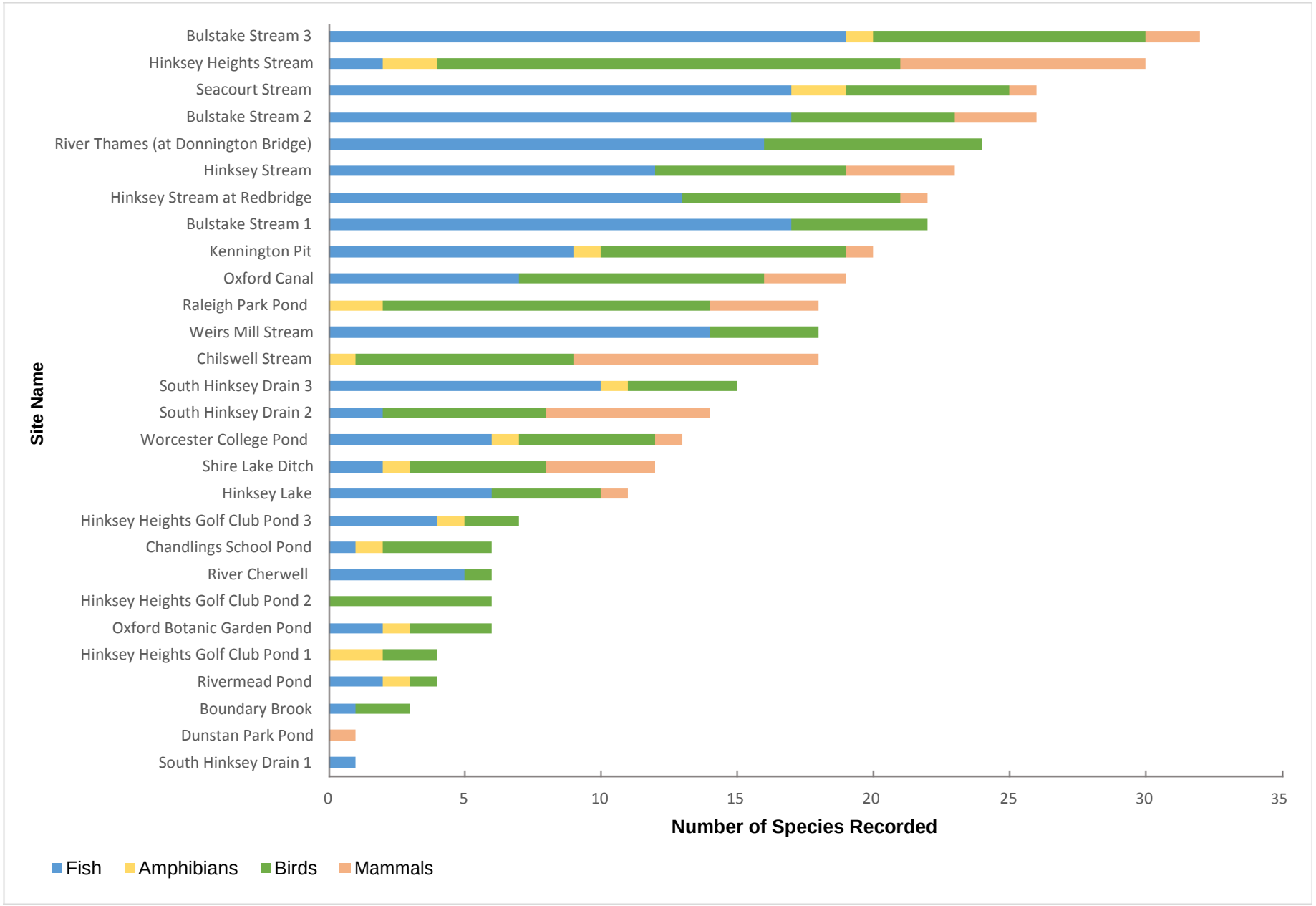

Figure 4. The total number of vertebrate species recorded, and the species group composition of these totals – the number of fish, amphibian, bird and mammal species – for each of the 28 sites sampled using eDNA kits.

3.2 Amphibians

A total of 4 amphibian species were recorded across the 28 sample sites:

- **Common Frog**
- **Common Toad**
- **Smooth Newt**
- **Great Crested Newt.**

The most commonly detected amphibian species was Smooth Newt, which was recorded at 25% of sites, followed by Common Frog (17%) and Common Toad (14%). Smooth Newt is Britain's commonest newt species, so we would expect it to be the most frequently recorded by eDNA. Palmate Newt was not recorded at any of the sites, which is in line with what one might expect, given that this species is the rarest native amphibian in the Oxford area. None of the sites surveyed are known to support Palmate Newt.

Smooth Newt	Common Frog	Common Toad	Great Crested Newt
25%	**17%**	**14%**	**7%**

Figure 5. The percentage (%) of the 28 sample sites where each of the four amphibian species – Smooth Newt, Common Frog, Common Toad and Great Crested Newt - were recorded using eDNA kits. Smooth Newt and Great Crested Newt images © Neil Phillips.

Great Crested Newt

Figure 6. Great Crested Newt (left) and Smooth Newt (right) for size comparison.

Great Crested Newt was recorded at 7% of sites. This is lower than might be expected – Great Crested Newts were recorded at 24% of 131 randomly selected 1 km grid squares spread across England in 2019 using eDNA (Freshwater Habitats Trust, PondNet). This difference can partly be explained by the nature of the landscape in the Oxford area, much of which is urban or floodplain (and thus typically unsuitable for Great Crested Newt).

However, the relatively low number of records may also be linked to the use of multi-species kits, as opposed to single-species kits. The multi-species kits use DNA analyses technology called metabarcoding, whereas the single-species kits use qPCR (quantitative Polymerase Chain Reaction). It is not yet clear how accurate metabarcoding analysis is – in fact, there is evidence to suggest it is not as accurate at detecting Great Crested Newts as qPCR, although this may not be the case for other vertebrate species. For example, in 2017, Newt Conservation Partnership undertook Great Crested Newt eDNA surveys across the South Midlands using single-species kits, and Great Crested Newt was detected at 31% of the 629 ponds sampled. However, when the same samples were re-analysed using metabarcoding, the detection rate of Great Crested Newt was just 20% (Newt Conservation Partnership, unpublished data).

Common Frog

Rana temporaria

Common Frog was recorded at 5 sites:

- Hinksey Heights Stream (site 11)
- Chilswell Stream (site 13)
- Seacourt Stream (site 18)
- Raleigh Park (site 19)
- Shire Lake Ditch (site 25)

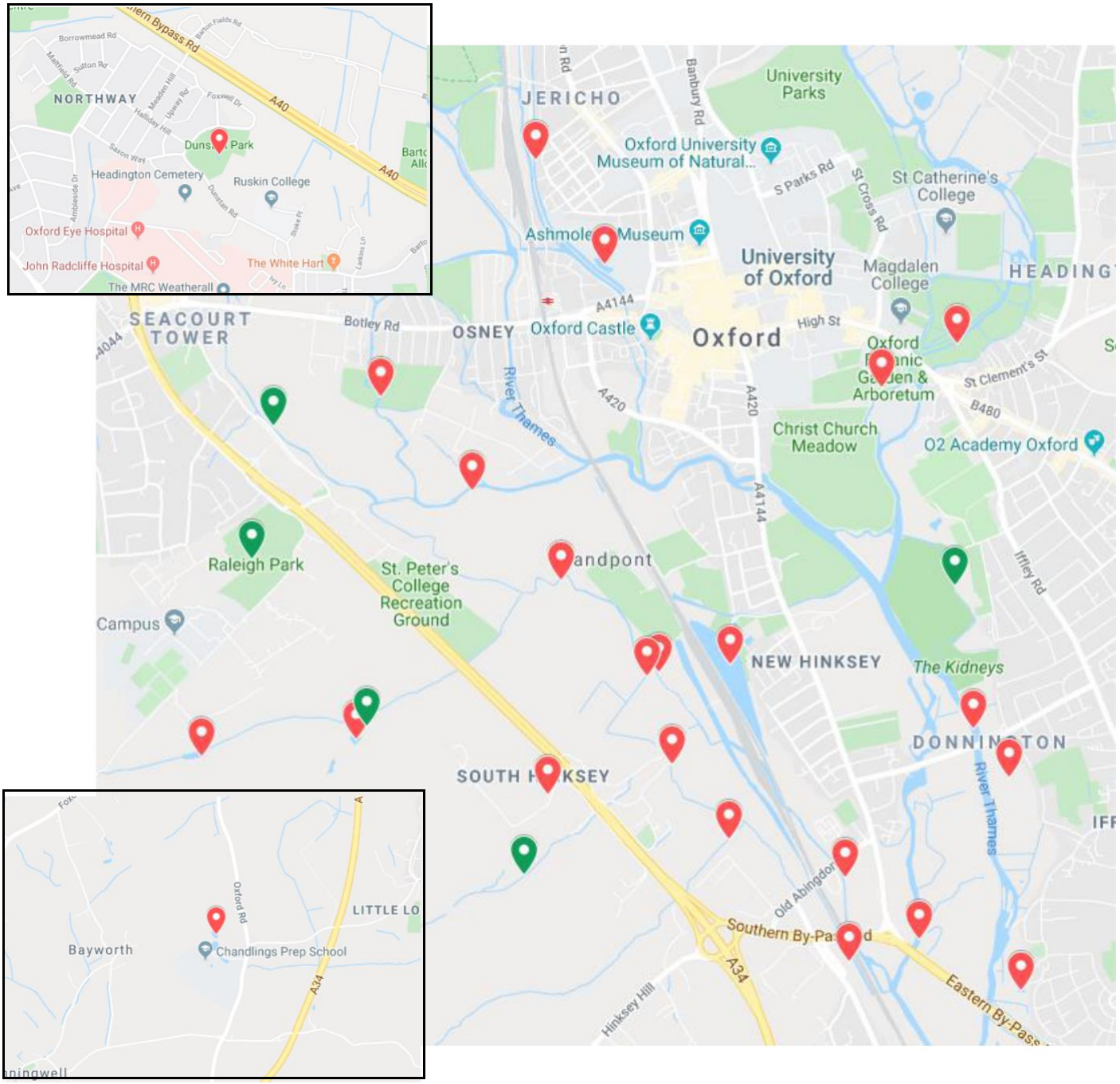

Figure 7. The 5 locations where Common Frog, *Rana temporaria*, was recorded as present using eDNA kits (indicated by green markers), and the 23 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Common Toad

Bufo bufo

Common Toad was recorded at 4 sites:

- Kennington Pit (site 4)
- Rivermead Pond (site 7)
- Bulstake Stream 3 (site 20)
- Hinksey Stream (site 21)

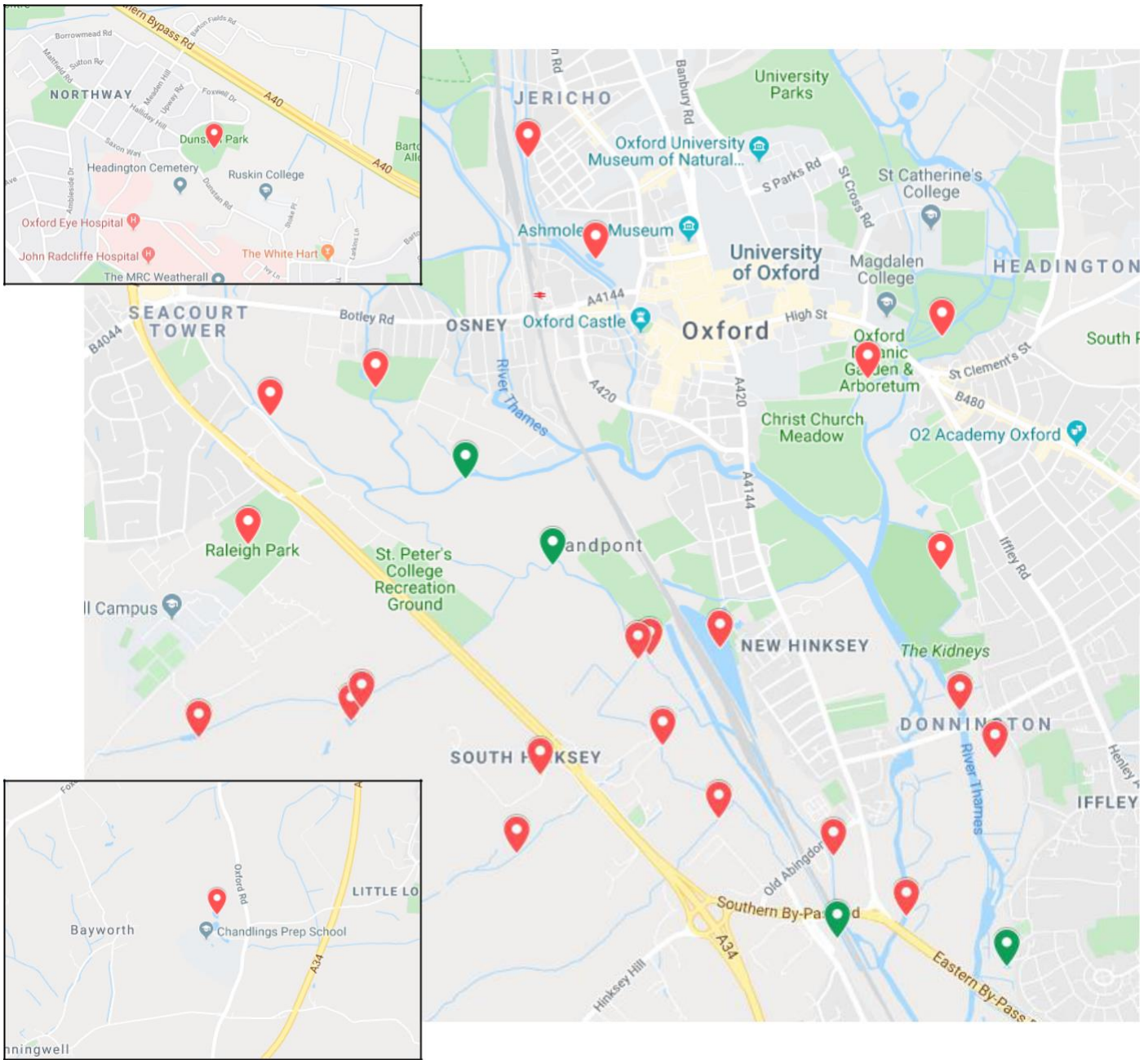

Figure 8. The 4 locations where Common Toad, *Bufo bufo*, was recorded as present using eDNA kits (indicated by green markers), and the 24 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Smooth Newt

Lissotriton vulgaris

Smooth Newt was recorded at 7 sites:

- Hinksey Heights Golf Club Pond 1 (site 8)
- Hinksey Heights Golf Club Pond 3 (site 10)
- Worcester College Pond (site 15)
- Seacourt Stream (site 18)
- Raleigh Park Pond (site 20)
- Oxford Botanic Gardens Pond (site 22)
- Chandlings School Pond (site 28)

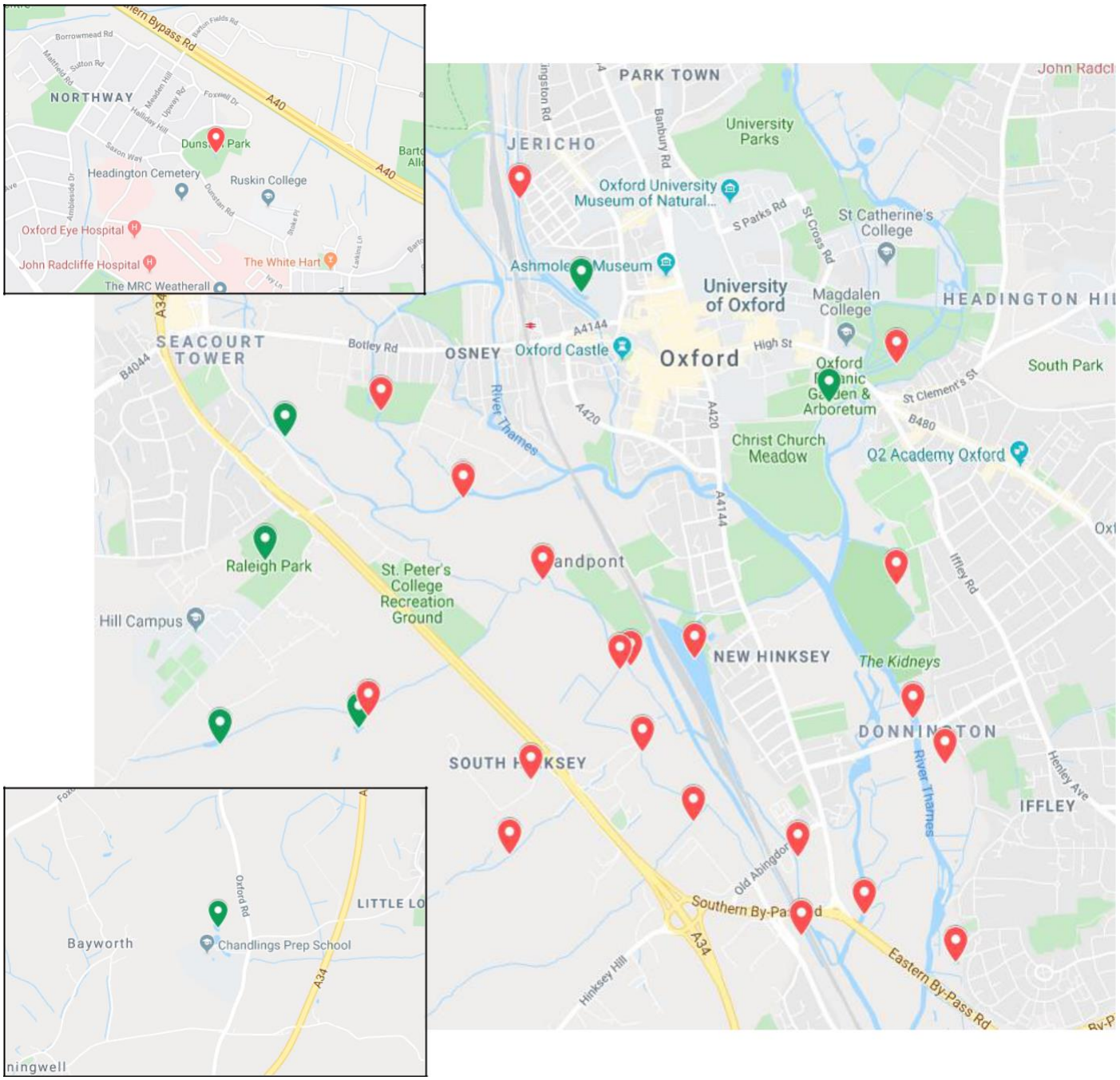

Figure 9. The 7 locations where Smooth Newt, *Lissotriton vulgaris*, was recorded as present using eDNA kits (indicated by green markers), and the 21 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Great Crested Newt

Triturus cristatus

Great Crested Newt was recorded at 2 sites:

- Hinksey Heights Golf Club Pond 1 (site 8)
- Hinksey Heights Stream (site 11)

It would be very unusual for Great Crested Newt to be present in a stream. This may be a case of eDNA being washed in from surrounding land or other ponds.

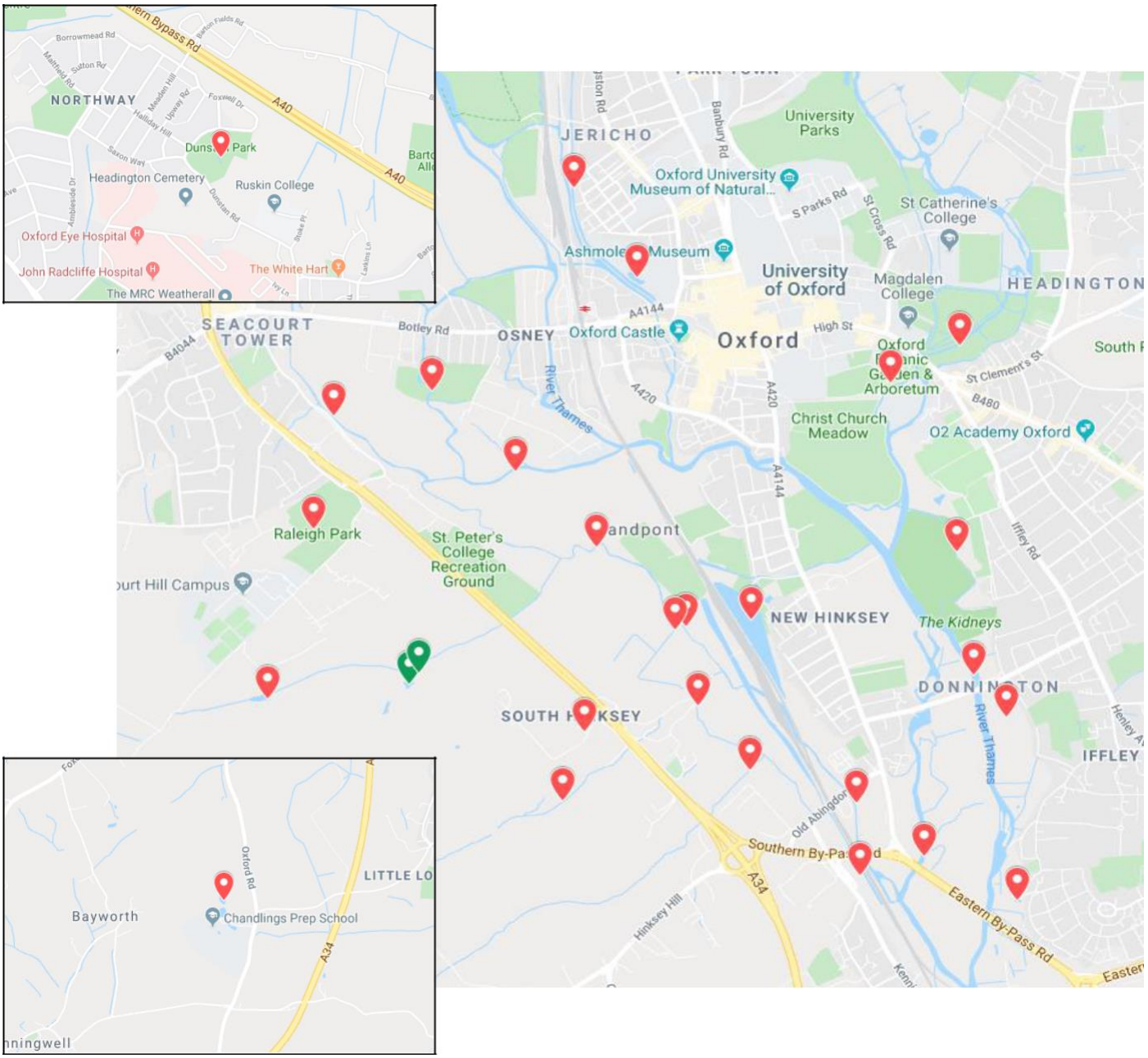

Figure 10. The 2 locations where Great Crested Newt, *Triturus cristatus*, was recorded as present using eDNA kits (indicated by green markers), and the 26 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

3.3 Fish

A total of 23 fish species were recorded across the 28 sample sites. eDNA surveys allow landscape-level surveying of fish – something that has been time-consuming and costly to achieve up until now. Previously, most records were obtained from a subset of the water environment, by anglers and 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.

The eDNA kits recorded a higher number of fish species than the electrofishing surveys undertaken by the Environment Agency in the flood scheme area. This is a commonly observed pattern when comparing data from the two survey methods.

The most commonly recorded fish were **Roach**, **Perch**, **Orfe/Dace** (indistinguishable – see Appendix 2) and **Pike**. The fish species recorded least frequently were **Nine-spined Stickleback**, **Tench** and **lamprey** species.

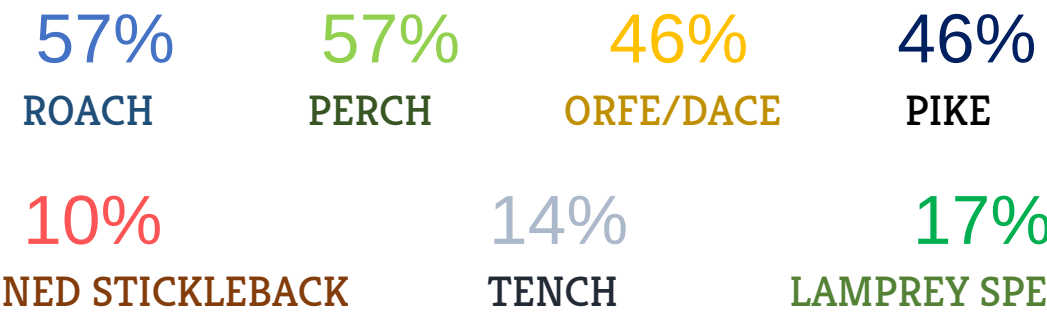

Figure 11. The percentage (%) of the 28 sample sites where the four most commonly recorded and three least frequently recorded fish species were detected using eDNA kits.

An invasive non-native species, Sunbleak (*Leucaspius delineates*), was detected at nine of the sites. Freshwater Habitats Trust has been investigating these records, as it is not clear whether the species is present in the study area. It hasn't been recorded previously, so we are treating this detection with caution. At present we will not treat these records as confirmed until some 'real' fish are captured.

Figure 12. Both 3-spined and 9-spined Sticklebacks were recorded in the Oxford area using eDNA kits. Image © Neil Phillips.

Atlantic Salmon, *Salmo salar*, was detected at one site (Hinksey Stream at Redbridge), and this record is being treated with caution too. A salmon was seen in the Thames in West London, near Chertsey, in 2019, but local fish experts think it is unlikely a salmon was present as far up as Oxford. This detection is currently unverifiable, as there are no traditional fish survey records from the same time to compare the eDNA results against. This raises the possibility that the record may have been a result of contamination or derived from a non-wild source (e.g. eDNA from salmon eaten as a foodstuff reaching the environment through sewage effluents). More matching with data collected using traditional methods, such as electrofishing, would help to establish the way we should treat records such as these.

Stone Loach was recorded at 6 sites, and lamprey was detected at 5 sites. Interestingly, the two species coincided at all 5 sites where lamprey were recorded. This may be because both of these fish species are sensitive to water quality, and are therefore only found in higher quality freshwater habitats. There are three species of lamprey in the UK: River Lamprey (*Lampetra fluviatilis*), Brook Lamprey (*Lampetra planeri*) and Sea Lamprey (*Petromyzon marinus*). DNA technology does not currently enable the different species to be distinguished.

European Perch & Common Roach

Perca fluviatilis & Rutilus rutilus

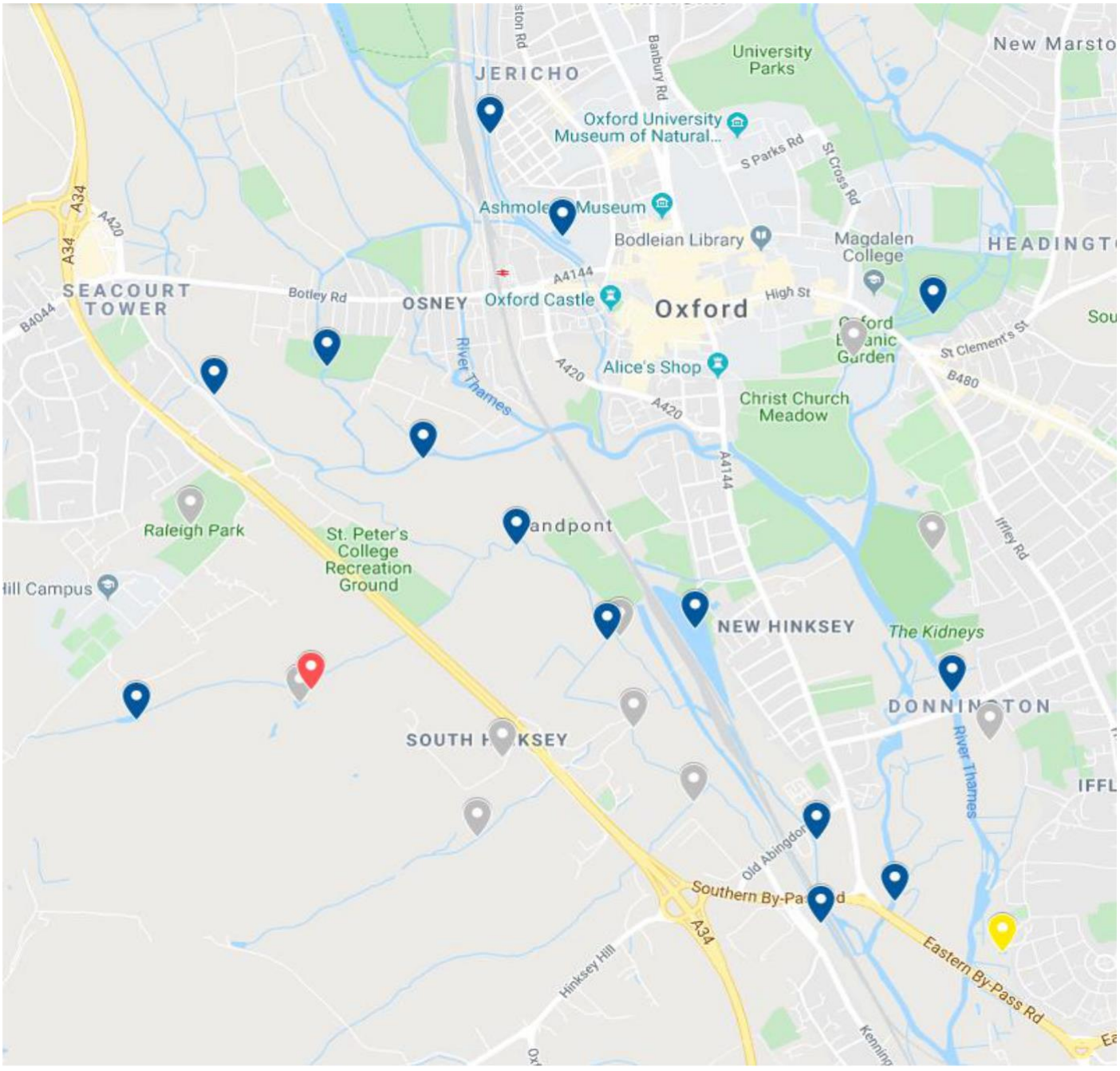

Figure 13. The locations where: **(i)** both European Perch, *Perca fluviatilis*, and Common Roach, *Rutilus rutilus*, were recorded as present using eDNA kits (indicated by blue markers); **(ii)** where Perch was recorded and Roach was not (red marker); **(iii)** where Roach was recorded and Perch was not (yellow marker), and **(iv)** where neither species was recorded (grey markers). Neither species was recorded at sites 27 (Dunstan Park) or 28 (Chandlings School Pond). Map created using Google MyMaps.

Lamprey species

Lampetra sp. and/or *Petromyzon* sp.

Lamprey was recorded at 5 sites:

- Bulstake Stream 1 (site 16)
- Bulstake Stream 2 (site 17)
- Seacourt Stream (site 18)
- Bulstake Stream 3 (site 20)
- River Thames (at Donnington Bridge) (site 24)

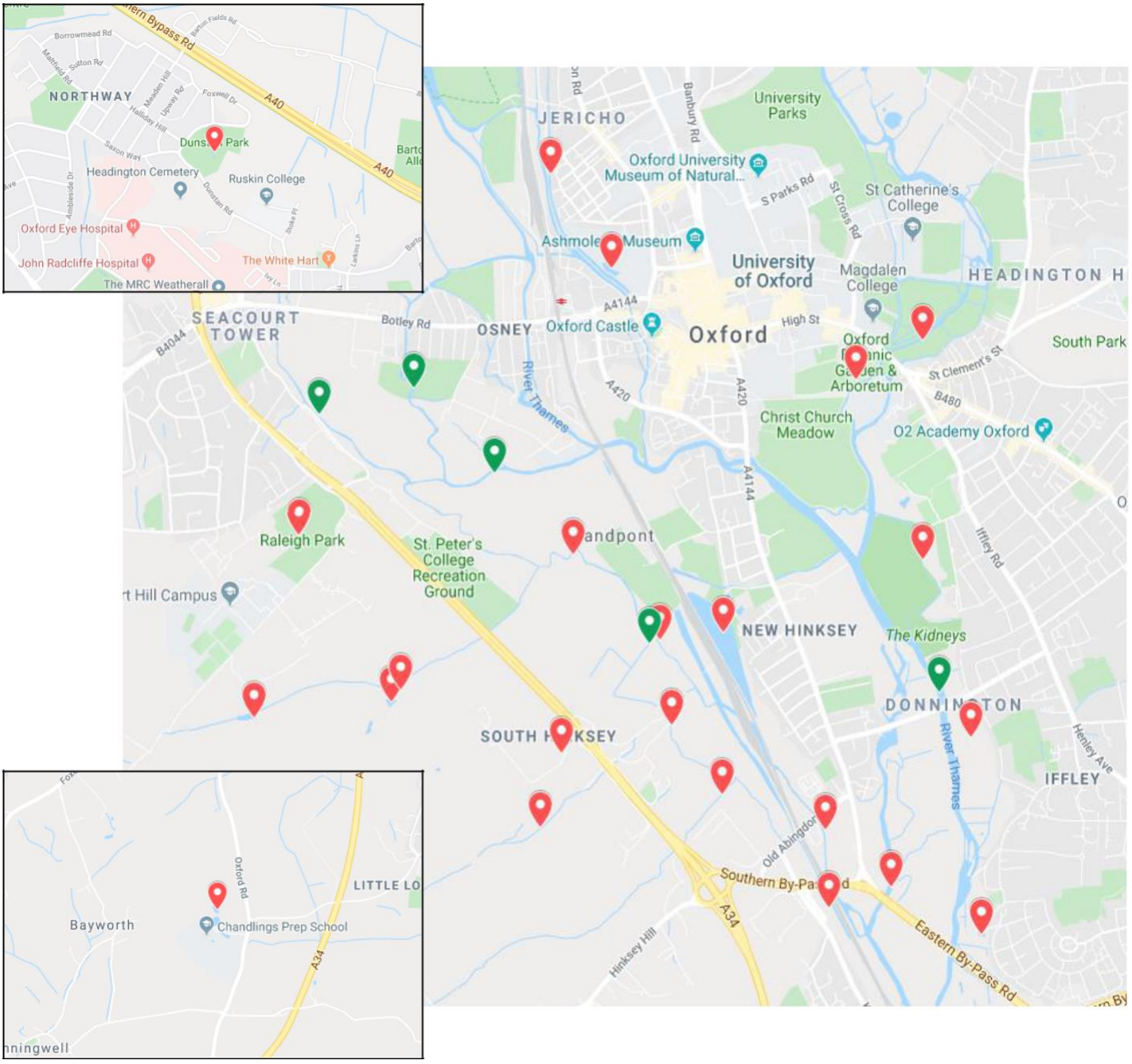

Figure 14. The 5 locations where lamprey, *Lampetra* sp. and/or *Petromyzon* sp., was recorded as present using eDNA kits (indicated by green markers), and the 23 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Tench

Tinca tinca

Tench was recorded at 4 sites:

- Hinksey Lake (site 1)
- Kennington Pit (site 4)
- Bulstake Stream 2 (site 17)
- Bulstake Stream 3 (site 20)

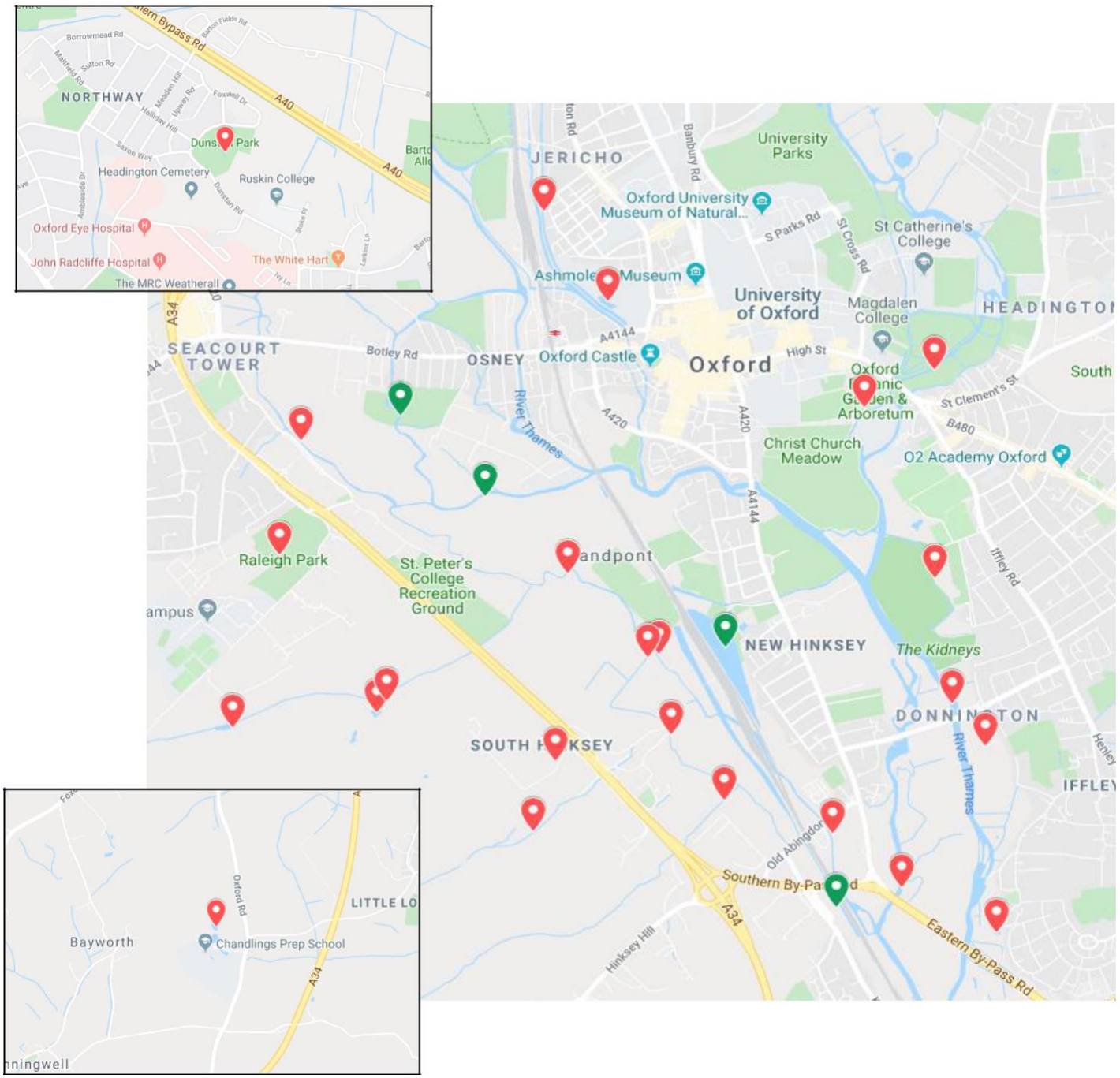

Figure 15. The 4 locations where Tench, *Tinca tinca,* was recorded as present using eDNA kits (indicated by green markers), and the 24 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Nine-spined Stickleback

Pungitius pungitius

Nine-spined Stickleback was recorded at 3 sites:

- Seacourt Stream (site 18)
- Oxford Botanic Garden Pond (site 22)
- Shire Lake Ditch (site 25)

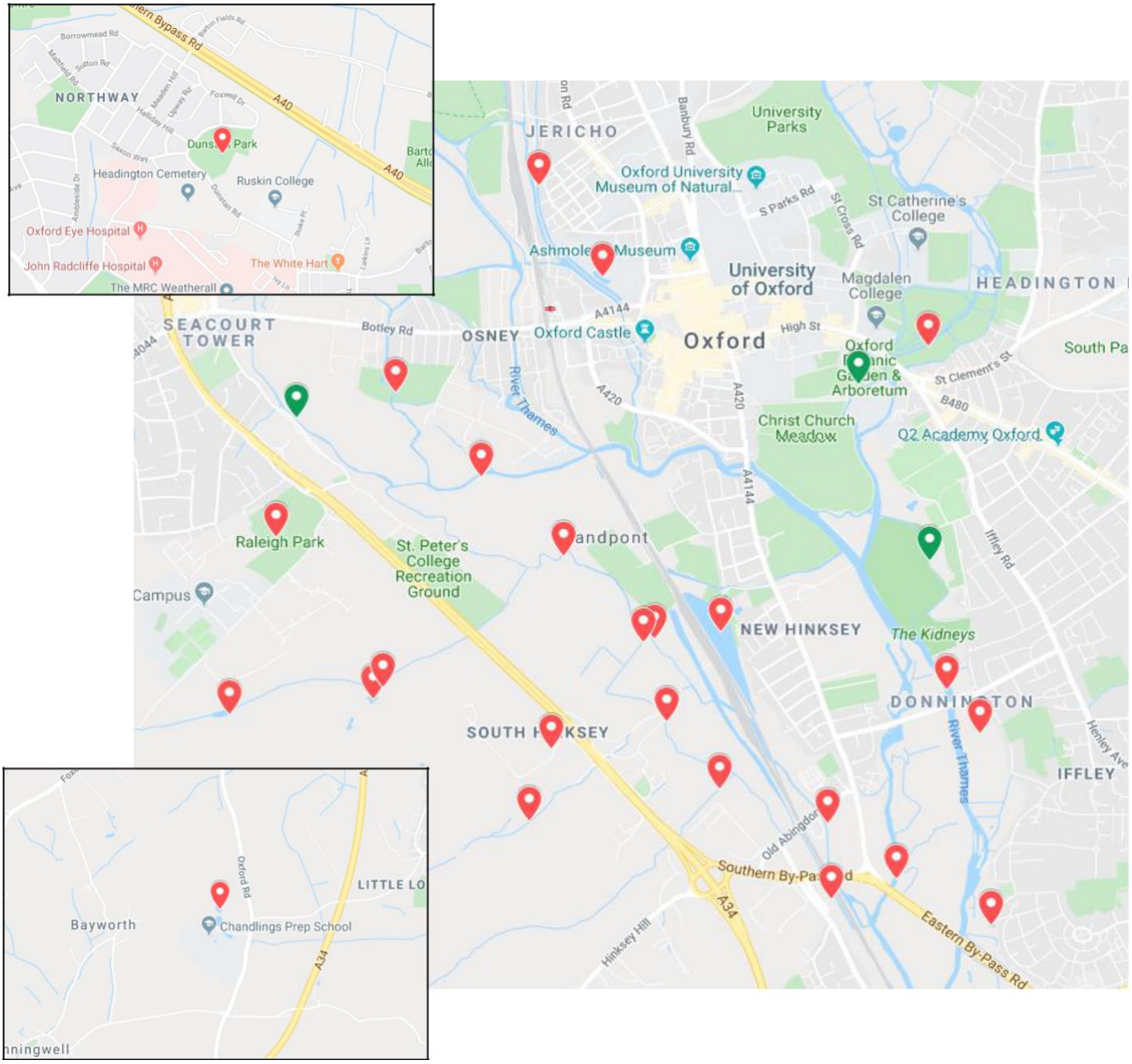

Figure 16. The 3 locations where Nine-spined Stickleback, *Pungitius pungitius,* was recorded as present using eDNA kits (indicated by green markers), and the 25 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

3.4 Aquatic Mammals

Two aquatic mammal species were recorded: Water Vole (Figure 17) and Water Shrew.

Figure 17. Water Vole © William Richardson.

Water Vole

Arvicola amphibius

Water Vole was recorded in a place where they were not previously thought to be present – Hinksey Stream (site 21). Freshwater Habitats Trust is currently investigating this record. It is currently unclear whether Water Vole DNA could be transported from higher up the Thames to this site. The nearest records upstream of Oxford in waterways directly connected to this site are a long way off (perhaps as far as Chimney Meadow). The Oxford Canal (where there is a known population) is not connected directly to the Hinksey Stream.

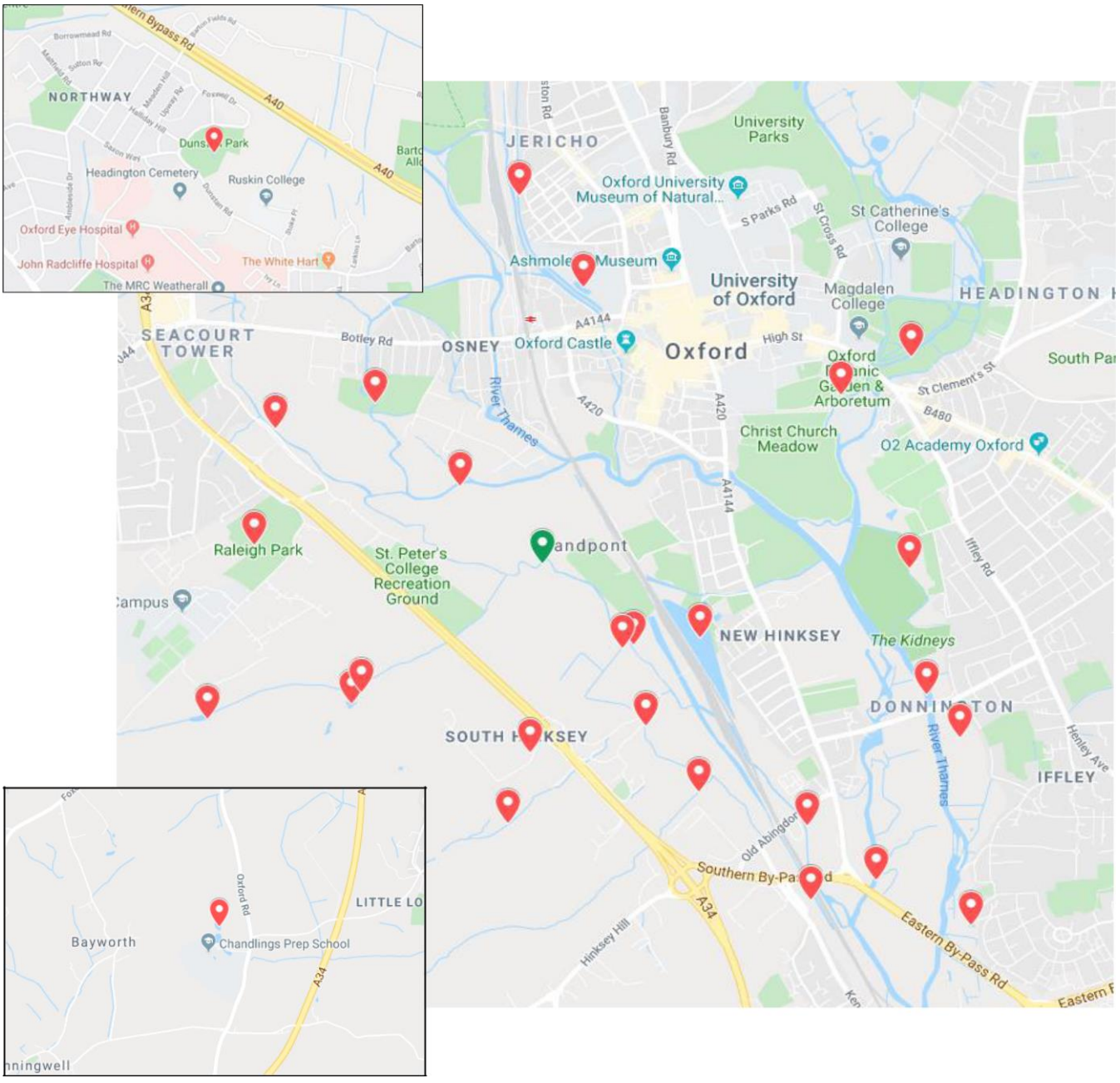

Figure 18. The location where Water Vole, *Arvicola amphibius,* was recorded as present using eDNA kits (indicated by green marker), and the 27 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

Water Shrew

Neomys fodiens

Water Shrew was recorded at 2 sites:

- Hinksey Heights Stream (site 11)
- Chilswell Valley (site 13)

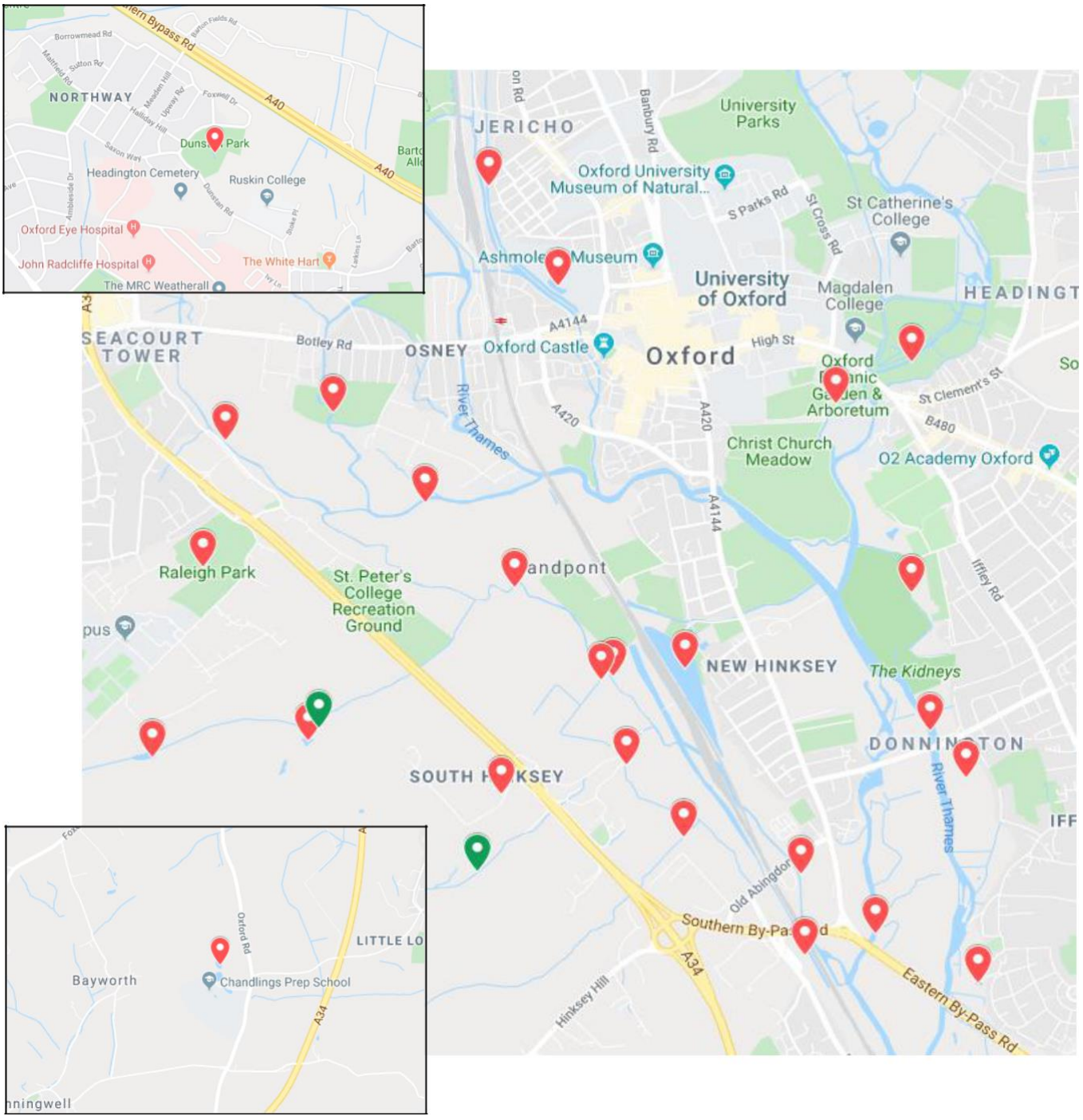

Figure 19. The 2 locations where Water Shrew, *Neomys fodiens,* was recorded as present using eDNA kits (indicated by green markers), and the 26 locations where the species was not recorded (indicated by red markers). Maps created using Google MyMaps.

3.5 Freshwater Mussels

There are six species of freshwater mussel in the United Kingdom. Two of these – the Depressed River Mussel, *Pseudanodonta complanata*, and the Freshwater Pearl Mussel, *Margaritifera margaritifera* – have undergone significant declines in recent years. Whilst the Freshwater Pearl Mussel is confined to the highest quality upland rivers and streams, the Depressed River Mussel occurs in the Oxford area. Two of the eDNA samples were analysed for freshwater mussel DNA:

- River Thames at Donnington Bridge (site 24)
- Hinksey Lake (site 1)

Figure 20. A Depressed River Mussel, *Pseudanodonta complanata*

A total of **four freshwater mussel species** were recorded across the two sites. Three species were detected at **River Thames at Donnington Bridge** (site 24):

- **Duck Mussel** (*Anodonta anatina*)
- **Painter's Mussel** (*Unio pictorum*)
- **Swollen River Mussel** (*Unio tumidus*)

The Depressed River Mussel is known to be present in the River Thames, although it was not detected in the sample from Donnington Bridge. One freshwater mussel species was detected in **Hinksey Lake** (site 1), the non-native **Zebra Mussel** (*Dreissena polymorpha*). This was in line with what we expected, as Hinksey Lake is a known site for Zebra Mussel.

3.6 Water Birds

A total of 7 water birds were recorded to species level, with a further 3 identified to family or genus level. Moorhen was the most commonly detected water bird, and was recorded at a total of 18 sites. Mallard (*Anas platyrhynchos*) and Common Shelduck (*Tadorna tadorna*) cannot be differentiated based on genetic markers, as they are so closely related (see Appendix 2). However, Mallard and/or Shelduck was also widely recorded, present at 18 of the 28 sample sites. These records presumably refer to Mallard as Shelduck are infrequent around Oxford. Grey Heron, Common Kingfisher, Mandarin Duck, Coot and Great Cormorant were the least commonly recorded water birds, detected at just one site.

Figure 21. A Coot, *Fulica atra.* Image © Charlotte Foote.

The water birds recorded were:

- **Grey Heron** *Ardea cinerea*
- **Common Kingfisher** *Alcedo atthis*
- **Mandarin Duck** *Aix galericulata*
- **Swan species** *Cygnus* sp.
- **Mallard Duck/ Common Shelduck** *Anas platyrhynchos/ Tadorna tadorna*
- **Waterfowl species** *Anatidae* sp.
- **Egyptian Goose** *Alopochen aegyptiaca*
- **Great Cormorant** *Phalacrocorax carbo*
- **Common Moorhen** *Gallinula chloropus*
- **Coot** *Fulica atra*

3.7 Other Species

In theory, **vertebrate DNA can wash into water from anywhere in the landscape**. Because of this, a range of non-aquatic mammals were recorded, including Roe Deer (*Capreolus capreolus*), Grey Squirrel (*Sciurus carolinensis*) and Red Fox (*Vulpes vulpes*). Similarly, lots of non-aquatic birds were detected (21 species in total), such as Sparrowhawk (*Accipiter nisus*), Long-tailed Tit (*Aegithalos caudatus*) and undetermined species of pigeon (*Columba* sp.). Their DNA could have arrived in the water in several different ways:

- Washing in from the surrounding area after rain
- When the animal visited the waterbody to drink, or (perhaps more likely) bathe
- Faeces dropping into the water from passing birds flying overhead.

At present, there is no information with which to assess the comparative likelihood of any of these routes of entry of eDNA into the water.

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 ecosystems**.

4.0 About the Project

Saving Oxford's Wetland Wildlife is helping to maximise the environmental and community benefit of the Oxford Flood Alleviation Scheme. The project is helping people and communities get involved in conserving the rare and varied freshwater wildlife found in and around Oxford's wetlands.

The project is generously supported by Thames Water and the Trust for Oxfordshire's Environment (TOE) with funding from Grundon Waste Management Ltd. through the Landfill Communities Fund.

For more information on Saving Oxford's Wetland Wildlife, please visit https://freshwaterhabitats.org.uk/saving-oxfords-wetland-wildlife.

5.0 Acknowledgements

Freshwater Habitats Trust would like to acknowledge the help of our dedicated volunteers who undertook the eDNA surveys around Oxford, including members of the Kennington Toad Patrol, Oxford Amphibian and Reptile Group (OxARG) and the 2nd Wallingford Scout Group. Additionally, we are grateful for the support from our partner, Nature Metrics, an eDNA specialist company.

6.0 References

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths R A, Foster J, Wilkinson J, Arnett A., Williams P and Dunn F. 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust, Oxford.

Appendix 1.

Sample Site Details

Table displaying the name and grid reference of each sampling point.

No.	Site Name	Grid Reference
1	Hinksey Lake	SP 51315 04710
2	South Hinksey Drain 1	SP 51064 04269
3	South Hinksey Drain 2	SP 51314 03932
4	Kennington Pit	SP 51863 03389
5	Hinksey Stream at Redbridge	SP 51838 03764
6	Weirs Mill Stream	SP 52178 03495
7	Rivermead Pond	SP 52645 03268
8	Hinksey Heights Golf Club Pond 1	SP 49648 04369
9	Hinksey Heights Golf Club Pond 2	SP50504 04123
10	Hinksey Heights Golf Club Pond 3	SP48950 04286
11	Hinksey Heights Stream	SP4969 0443
12	South Hinksey Drain 3	SP5100 0468
13	Chilswell Stream	SP 50417 03769
14	Oxford Canal	SP 50426 06960
15	Worcester College Pond	SP 50738 06507
16	Bulstake Stream 1	SP 50944 04661
17	Bulstake Stream 2	SP 49742 05895
18	Seacourt Stream	SP 49258 05762
19	Raleigh Park Pond	SP 49170 05179
20	Bulstake Stream 3	SP 50151 05489
21	Hinksey Stream	SP 50554 05092
22	Oxford Botanic Garden Pond	SP 51981 05968
23	River Cherwell	SP 52319 06176
24	River Thames (Donnington Bridge)	SP 52410444
25	Shire Lake Ditch	SP 52319 05085
26	Boundary Brook	SP 52570 04229
27	Dunstan Park Pond	SP 54097 07985
28	Chandlings School Pond	SP 5073 0138

Appendix 2.

Notes on Results

Some taxa were ‘unresolved’, i.e. not identified to species level, due to limitations in the DNA technology used in the analyses. All taxa could be identified to order, 99% could be identified to family, 95% could be identified to genus, and 81% could be identified to species level. The remainder were identified to the lowest taxonomic level. Explanations below are as supplied by Nature Metrics.

Identification	Explanation
Orfe/Dace	These congeneric (belonging to the same genus) species are morphologically very similar, especially immature individuals. It is possible that entries in the reference database for which the molecular identifications are based may include errors, or that they are indistinguishable based on this particular DNA barcode.
Cyprinid species	This sequence is either Vimba Bream (*Vimba vimba*) or Silver Bream (*Abramis bjoerkna*), which are indistinguishable based on this particular DNA barcode. Nature Metrics therefore conservatively identified this sequence as a bream species.
Lamprey	This sequence is an exact match to either River Lamprey (Lampetra fluviatilis) or Brook Lamprey (Lampetra planeri). These species are 'paired species' that some have considered to be the same species. Nevertheless, they are so closely related that they are indistinguishable based on these markers.
Mallard/Common Shelduck	This sequence is an exact match to either Mallard *(Anas platyrhynchos)* or Common Shelduck (*Tadorna tadorna*). These species are so closely related that they are able to hybridise in the wild.
Swan species	The marker for this sequence is an equally close match to many different *Cygnus* species, so Nature Metrics conservatively identified this sequence as a Swan species (*Cygnus* sp.).
Pigeon species	The marker for this sequence is an equally close match to several different *Columba* species, so Nature Metrics conservatively identified this sequence as a Pigeon species (*Columba* sp.).
Swan/Goose species	This sequence is an exact match to a number of Anser, Cygnus, and Branta sequences. These include: Mute Swan, Whooper, Swan, Tundra Swan, Bewick's Swan, Bean Goose, Greylag Goose, Brent Goose, Greater White-fronted Goose, and Greater Canada Goose. Nature Metrics conservatively identified this sequence as a swan or goose species.
Gull species	This sequence is an exact match to a number of *Larus* sequences. These include: Great Black-backed Gull, Iceland Gull, Black-headed Gull, Sabine's Gull, Ross's Gull, Ivory Gull, and Black-legged Kittiwake. This particular primer set cannot differentiate between these taxa, and so Nature Metrics conservatively identified this sequence as a gull species.
Corvid species	This sequence is an exact match to several corvid species, including Common Raven (Corvus corax). This particular primer set cannot differentiate between these taxa, and so Nature Metrics conservatively identified this sequence as a corvid species.

Sparrow species	This sequence is an exact match to several Passeridae species, including Rock Sparrow (Petronia petronia), Tree Sparrow (Passer montanus), and House Sparrow (Passer domesticus). Nature Metrics conservatively identified this sequence as a Passeridae species.
Passeriformes species	This sequence is an exact match to several Passeriformes species, which belong to different families and include White (a.k.a. Pied) Wagtail (Motacilla alba), Grey Wagtail (Motacilla cinerea), and Bullfinch (Pyrrhula pyrrhula). Nature Metrics conservatively identified this sequence as a passeriformes species.
Warbler species	This sequence is an exact match to several species belonging to the Phylloscopus genus, including Willow Warbler (Phylloscopus trochilus) and Yellow-browed Warbler (Phylloscopus inornatus). Nature Metrics conservatively identified this sequence as a warbler species.
Warbler species	This sequence is an exact match to either Subalpine Warbler (Sylvia cantillans) or Sardinian Warbler (Sylvia melanocephala), which are both non-natives. It is more likely that this sequence belongs to Garden Warbler (Sylvia borin), which is a UK native.
Thrush species	This sequence is an exact match to several thrush species including Common Blackbird (Turdus merula), Redwing (Turdus iliacus), and Song Thrush (Turdus philomelos). This particular primer set cannot differentiate between these taxa and so Nature Metrics conservatively identified this sequence as a thrush species.
Field mouse species	This sequence is an exact match to either Yellow-necked Mouse (Apodemus flavicollis) or Wood Mouse (Apodemus sylvaticus). These species are indistinguishable based on this marker.

Appendix 3.

Species Recorded per Site

Tables containing the vertebrate species (fish, amphibians, birds and mammals) recorded at each of the 28 sample sites.

Site 1: Hinksey Lake

A total of 11 vertebrate species were recorded at Hinksey Lake: six fish, four birds and one mammal. No amphibians were detected.

Site 1: Hinksey Lake (SP 51315 04710)		
Group	**Species**	**Common Name**
Fish	*Rutilus rutilus*	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Esox lucius	Northern Pike
	Perca fluviatilis	European Perch
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba sp.	Pigeon species
	Phalacrocorax carbo	Great Cormorant
Mammals	*Canis lupus*	Dog

Site 2: South Hinksey Drain 1

A total of one vertebrate species was recorded at South Hinksey Drain 1. No amphibians, mammals or birds were detected.

Site 2: South Hinksey Drain 1 (SP 51064 04269)		
Group	**Species**	**Common Name**
Fish	*Gasterosteus aculeatus*	Three-spined Stickleback

Site 3: South Hinksey Drain 2

A total of 14 vertebrate species were recorded at South Hinksey Drain 2: two fish, six birds and six mammals. No amphibians were detected.

Site 3: South Hinksey Drain 2 (SP 51314 03932)		
Group	**Species**	**Common Name**
Fish	*Phoxinus phoxinus*	Common Minnow
	Cottus gobio	European Bullhead
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Cygnus sp.	Swan species
	Columba sp.	Pigeon species
	Pica pica	Eurasian Magpie
	Parus major	Great Tit
	Turdus sp.	Thrush species

Mammals	*Muntiacus reevesi*	Reeves's Muntjac
	Canis lupus	Dog
	Sorex araneus	Common Shrew
	Microtus agrestis	Field Vole
	Myodes glareolus	Bank Vole
	Sciurus carolinensis	Grey Squirrel

Site 4: Kennington Pit

A total of 20 vertebrate species were recorded at Kennington Pit: nine fish, one amphibian, nine birds and one mammal. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution. Common Toad - a declining BAP Priority Species – was recorded at Kennington Pit, which is already a known site for the amphibian.

Site 4: Kennington Pit (SP 51863 03389)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Tinca tinca	Tench
	Esox lucius	Northern Pike
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
Amphibians	*Bufo bufo*	Common Toad
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba sp.	Pigeon species
	Alcedo atthis	Common Kingfisher
	Gallinula chloropus	Common Moorhen
	Corvus sp.	Corvid species
	Pica pica	Eurasian Magpie
	Parus major	Great Tit
	Ardea cinerea	Grey Heron
Mammals	*Microtus agrestis*	Field Vole

Site 5: Hinksey Stream at Redbridge

A total of 22 vertebrate species were recorded at Hinksey Stream at Redbridge: 13 fish, eight birds and one mammal. This was another site where Sunbleak was detected, an invasive fish species not previously recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution. Atlantic Salmon was recorded at this site, though as aforementioned, this record was deemed to be unlikely by local fish experts, and could have been a result of contamination. We are treating this record with caution.

Site 5: Hinksey Stream at Redbridge (SP 51838 03764)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Esox lucius	Northern Pike
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	(Salmo salar	Atlantic Salmon)
	Cottus gobio	European Bullhead
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Larus sp.	Gull species
	Columba sp.	Pigeon species
	Gallinula chloropus	Common Moorhen
	Pica pica	Eurasian Magpie
	Turdus sp.	Thrush species
	Ardea cinerea	Grey Heron
Mammals	*Sciurus carolinensis*	Grey Squirrel

Site 6: Weirs Mill Stream

A total of 18 vertebrate species were recorded at Weirs Mill Stream: 14 fish and four birds. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 6: Weirs Mill Stream (SP 52178 03495)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Barbus barbus	Common Barbel
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Esox lucius	Northern Pike
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba sp.	Pigeon species
	Gallinula chloropus	Common Moorhen

Site 7: Rivermead Pond

Two samples were obtained from Rivermead Pond – the first was deemed inconclusive by the lab as it failed to amplify, so a second sample was obtained in July 2019. A total of four vertebrate species were recorded at Rivermead Pond in the second sample: two fish, one amphibian and one bird.

Site 7: Rivermead Pond (SP 52645 03268)		
Group	**Species**	**Common Name**
Fish	*Rutilus rutilus*	Roach
	Gasterosteus aculeatus	Three-spined Stickleback
Amphibians	*Bufo bufo*	Common Toad
Birds	*Gallinula chloropus*	Common Moorhen

Site 8: Hinksey Heights Golf Club Pond 1

A total of four vertebrate species were recorded at Hinksey Heights Golf Club Pond 1: two amphibians and two birds. Hinksey Heights is already a known site for Great Crested Newt.

Site 8: Hinksey Heights Golf Club Pond 1 (SP 49648 04369)		
Group	**Species**	**Common Name**
Amphibians	*Lissotriton vulgaris*	Smooth Newt
	Triturus cristatus	Great Crested Newt
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Gallinula chloropus	Common Moorhen

Site 9: Hinksey Heights Golf Club Pond 2

A total of six vertebrate species were recorded at Hinksey Heights Golf Club Pond 2, all of which were birds.

Site 9: Hinksey Heights Golf Club Pond 2 (SP 50504 04123)		
Group	**Species**	**Common Name**
Birds	*Accipiter nisus*	Eurasian Sparrowhawk
	Anas platyrhynchos/Tadorna tadorna	Mallard/Common Shelduck
	Cygnus sp.	Swan species
	Columba sp.	Pigeon species
	Aegithalos caudatus	Long-tailed Tit
	Parus major	Great Tit

Site 10: Hinksey Heights Golf Club Pond 3

A total of seven vertebrate species were recorded at Hinksey Heights Golf Club Pond 3: four fish, one amphibian and two birds.

Site 10: Hinksey Heights Golf Club Pond 3 (SP 48950 04286)		
Group	**Species**	**Common Name**
Fish	*Leuciscus idus/Leuciscus leuciscus*	Orfe/Dace
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Perca fluviatilis	European Perch
Amphibians	*Lissotriton vulgaris*	Smooth Newt
Birds	*Columba* sp.	Pigeon species
	Gallinula chloropus	Common Moorhen

Site 11: Hinksey Heights Stream

A total of 30 vertebrate species were recorded at Hinksey Heights Stream: two fish, two amphibians, 17 birds and nine mammals. Great Crested Newt was recorded at Hinksey Heights Stream. Hinksey Heights is already a known site for this protected species.

Site 11: Hinksey Heights Stream (SP 4969 0443)		
Group	**Species**	**Common Name**
Fish	*Carassius* sp.	Crucian Carp species
	Perca fluviatilis	European Perch
Amphibians	*Rana temporaria*	Common Frog
	Triturus cristatus	Great Crested Newt
Birds	*Accipiter nisus*	Eurasian Sparrowhawk
	Columba sp.	Pigeon species
	Phasianus colchicus	Common Pheasant
	Gallinula chloropus	Common Moorhen
	Garrulus glandarius	Eurasian Jay
	Hirundo rustica	Barn Swallow
	Erithacus rubecula	European Robin
	Parus major	Great Tit
	N/A	Passeriformes species
	Phylloscopus sp.	Warbler species
	Prunella modularis	Dunnock
	Regulus regulus	Goldcrest
	Sylvia atricapilla	Blackcap
	Sylvia sp.	Warbler species
	Troglodytes troglodytes	Wren
	Turdus philomelos	Song Thrush
	Turdus sp.	Thrush species
Mammals	*Capreolus capreolus*	Roe Deer
	Muntiacus reevesi	Reeves's Muntjac
	Neomys fodiens	Water Shrew
	Sorex araneus	Common Shrew
	Microtus agrestis	Field Vole
	Myodes glareolus	Bank Vole
	Apodemus flavicollis/ *Apodemus sylvaticus*	Yellow-necked Mouse/ Wood Mouse
	Rattus norvegicus	Brown Rat
	Sciurus carolinensis	Grey Squirrel

Site 12: South Hinksey Drain 3

A total of 15 vertebrate species were recorded at South Hinksey Drain 3: 10 fish, one amphibian, and four birds. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 12: South Hinksey Drain 3 (SP 5100 0468)		
Group	**Species**	**Common Name**
Fish	*Gobio gobio*	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Esox lucius	Northern Pike
	Gasterosteus aculeatus	Three-spined Stickleback
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
Amphibians	*Bufo bufo*	Common Toad
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Columba sp.	Pigeon species
	Gallinula chloropus	Common Moorhen
	Pica pica	Eurasian Magpie

Site 13: Chilswell Stream

A total of 18 vertebrate species were recorded at Chilswell Stream: one amphibian, eight birds and nine mammals.

Site 13: Chilswell Stream (SP 50417 03769)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common Frog
Birds	*Alopochen aegyptiaca*	Egyptian Goose
	Anatidae sp.	Waterfowl species
	Columba sp.	Pigeon species
	Corvus sp.	Corvid species
	Garrulus glandarius	Eurasian Jay
	Erithacus rubecula	European Robin
	Sylvia atricapilla	Blackcap
	Turdus sp.	Thrush species
Mammals	*Capreolus capreolus*	Roe Deer
	Muntiacus reevesi	Reeves's Muntjac
	Vulpes vulpes	Red Fox
	Neomys fodiens	Water Shrew
	Sorex araneus	Common Shrew
	Myodes glareolus	Bank Vole
	Apodemus flavicollis/Apodemus sylvaticus	Yellow-necked Mouse/ Wood Mouse
	Rattus norvegicus	Brown Rat
	Sciurus carolinensis	Grey Squirrel

Site 14: Oxford Canal

A total of 19 vertebrate species were recorded at Oxford Canal: seven fish, nine birds and three mammals.

Site 14: Oxford Canal (SP 50426 06960)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Cyprinidae sp.	Cyprinid species
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
Birds	*Alopochen aegyptiaca*	Egyptian Goose
	Anas platyrhynchos/Tadorna tadorna	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Cygnus sp.	Swan species
	Columba sp.	Pigeon species
	Gallinula chloropus	Common Moorhen
	Corvus sp.	Corvid species
	Pica pica	Eurasian Magpie
	Turdus sp.	Thrush species
Mammals	*Canis lupus*	Dog
	Felis catus	Domesticated Cat
	Sciurus carolinensis	Grey Squirrel

Site 15: Worcester College Pond

A total of 13 vertebrate species were recorded at Worcester College Pond: six fish, 1 amphibian, five birds and one mammal.

Site 15: Worcester College Pond (SP 50738 06507)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Esox lucius	Northern Pike
	Perca fluviatilis	European Perch
Amphibians	*Lissotriton vulgaris*	Smooth Newt
Birds	*Alopochen aegyptiaca*	Egyptian Goose
	Anas platyrhynchos/Tadorna tadorna	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Cygnus sp.	Swan species
	Columba sp.	Pigeon species
Mammals	*Sciurus carolinensis*	Grey Squirrel

Site 16: Bulstake Stream 1

A total of 22 vertebrate species were recorded at Bulstake Stream 1: 17 fish and five birds. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 16: Bulstake Stream 1 (SP 50944 04661)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Barbus barbus	Barbel
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Gasterosteus aculeatus	Three-spined Stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
	Lampetra sp.	Lamprey species
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba livia	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	Pica pica	Eurasian Magpie

Site 17: Bulstake Stream 2

A total of 26 vertebrate species were recorded at Bulstake Stream 2: 17 fish, six birds and three mammals. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 17: Bulstake Stream 2 (SP 49742 05895)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Cyprinus carpio	Common Carp
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
	Lampetra sp.	Lamprey species
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba livia	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	Turdus sp.	Thrush species
	Picus viridis	European Green Woodpecker
Mammals	*Meles meles*	European Badger
	Sorex araneus	Common Shrew
	Microtus agrestis	Field Vole

Site 18: Seacourt Stream

A total of 26 vertebrate species were recorded at Seacourt Stream: 17 fish, two amphibians, six birds and one mammal. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 18: Seacourt Stream (SP 49258 05762)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Gasterosteus aculeatus	Three-spined Stickleback
	Pungitius pungitius	Nine-spined Stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
	Lampetra sp.	Lamprey species
Amphibians	*Rana temporaria*	Common Frog
	Lissotriton vulgaris	Smooth Newt
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba sp.	Pigeon species
	Gallinula chloropus	Common Moorhen
	*Corvus*sp.	Corvid species
	Passer sp.	Sparrow species
Mammals	*Sciurus carolinensis*	Grey Squirrel

Site 19: Raleigh Park Pond

A total of 18 vertebrate species were recorded at Raleigh Park Pond: two amphibians, 12 birds and four mammals.

Site 19: Raleigh Park Pond (SP 49170 05179)		
Group	**Species**	**Common Name**
Amphibians	*Rana temporaria*	Common Frog
	Lissotriton vulgaris	Smooth Newt
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Columba livia	Feral/Rock Pigeon
	*Corvus*sp.	Corvid species
	Pica pica	Eurasian Magpie
	Carduelis carduelis	European Goldfinch
	Erithacus rubecula	European Robin
	Parus major	Great Tit
	Passer sp.	Sparrow species
	N/A	Passeriformes species
	Prunella modularis	Dunnock
	Troglodytes troglodytes	Wren
	Turdus sp.	Thrush species
Mammals	*Vulpes vulpes*	Red Fox
	Sorex araneus	Common Shrew
	Myodes glareolus	Bank Vole
	Sciurus carolinensis	Grey Squirrel

Site 20: Bulstake Stream 3

A total of 32 vertebrate species were recorded at Bulstake Stream 3: 19 fish, one amphibian, 10 birds and two mammals (the highest number of vertebrate species recorded of the 28 sample sites). Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 20: Bulstake Stream 3 (SP 50151 05489)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Barbus barbus	Barbel
	Cyprinus carpio	Common Carp
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Tinca tinca	Tench
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Gasterosteus aculeatus	Three-spined Stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
	Lampetra sp.	Lamprey species
Amphibians	*Bufo bufo*	Common Toad
Birds	*Aix galericulata*	Mandarin Duck
	Anas platyrhynchos/Tadorna tadorna	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba livia	Feral/Rock Pigeon
	Phasianus colchicus	Common Pheasant
	Gallinula chloropus	Common Moorhen
	Sylvia atricapilla	Blackcap
	Sylvia sp.	Warbler species
	Turdus sp.	Thrush species
	Picus viridis	European Green Woodpecker
Mammals	*Microtus agrestis*	Field Vole
	Sciurus carolinensis	Grey Squirrel

Site 21: Hinksey Stream

A total of 23 vertebrate species were recorded at Hinksey Stream: 12 fish, seven birds and four mammals. Sunbleak was detected at this site, which is an invasive species that has not previously been recorded in the Oxford area. The veracity of this record is not currently known, and we are treating it with caution.

Site 21: Hinksey Stream (SP 50554 05092)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Gobio gobio	Gudgeon
	(Leucaspius delineatus	Sunbleak)
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Anatidae sp.	Waterfowl species
	Columba livia	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	Sylvia atricapilla	Blackcap
	Turdus sp.	Thrush species
	Ardea cinerea	Grey Heron
Mammals	*Arvicola amphibius*	Water Vole
	Microtus agrestis	Field Vole
	Myodes glareolus	Bank Vole
	Sciurus carolinensis	Grey Squirrel

Site 22: Oxford Botanic Garden Pond

A total of six vertebrate species were recorded at Oxford Botanic Garden: two fish, one amphibian and three birds.

Site 22: Oxford Botanic Garden Pond (SP 51981 05968)		
Group	**Species**	**Common Name**
Fish	*Gasterosteus aculeatus*	Three-spined Stickleback
	Pungitius pungitius	Nine-spined Stickleback
Amphibians	*Lissotriton vulgaris*	Smooth Newt
Birds	*Columba livia*	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	Pica pica	Eurasian Magpie

Site 23: River Cherwell

A total of six vertebrate species were recorded at River Cherwell: five fish and one bird.

Site 23: River Cherwell (SP 52319 06176)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Rutilus rutilus	Roach
	Squalius cephalus	Chub
	Esox lucius	Northern Pike
	Perca fluviatilis	European Perch
Birds	*Turdus* sp.	Thrush species

Site 24: River Thames (Donnington Bridge)

A total of 24 vertebrate species were recorded at River Thames (Donnington Bridge): 16 fish and eight birds.

Site 24: River Thames (at Donnington Bridge) (SP 5241 0444)		
Group	**Species**	**Common Name**
Fish	*Abramis brama*	Common Bream
	Alburnus alburnus	Bleak
	Cyprinidae sp.	Cyprinid species
	Gobio gobio	Gudgeon
	Leuciscus idus/Leuciscus leuciscus	Orfe/Dace
	Phoxinus phoxinus	Common Minnow
	Rutilus rutilus	Roach
	Scardinius erythrophthalmus	Rudd
	Squalius cephalus	Chub
	Barbatula barbatula	Stone Loach
	Esox lucius	Northern Pike
	Gasterosteus aculeatus	Three-spined Stickleback
	Gymnocephalus cernua	Ruffe
	Perca fluviatilis	European Perch
	Cottus gobio	European Bullhead
	Lampetra sp.	Lamprey species
Birds	*Alopochen aegyptiaca*	Egyptian Goose
	Anatidae sp.	Waterfowl species
	Larus sp.	Gull species
	Columba livia	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	*Corvus*sp.	Corvid species
	Erithacus rubecula	European Robin
	Parus major	Great Tit

Site 25: Shire Lake Ditch

A total of 12 vertebrate species were recorded at Shire Lake Ditch: two fish, one amphibian, five birds and four mammals.

Site 25: Shire Lake Ditch (SP 52319 05085)		
Group	**Species**	**Common Name**
Fish	*Gasterosteus aculeatus*	Three-spined Stickleback
	Pungitius pungitius	Nine-spined Stickleback
Amphibians	*Rana temporaria*	Common Frog
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Cygnus sp.	Black Swan
	Columba livia	Feral/Rock Pigeon
	Gallinula chloropus	Common Moorhen
	Troglodytes troglodytes	Wren
Mammals	*Canis lupus*	Dog
	Vulpes vulpes	Red Fox
	Myodes glareolus	Bank Vole
	Rattus norvegicus	Brown Rat

Site 26: Boundary Brook

A total of three vertebrate species were recorded at Boundary Brook: one fish and two birds.

Site 26: Boundary Brook (SP 52570 04229)		
Group	**Species**	**Common Name**
Fish	*Gasterosteus aculeatus*	Three-spined Stickleback
Birds	*Columba livia*	Feral/Rock Pigeon
	Turdus sp.	Thrush species

Site 27: Dunstan Park Pond

A total of one vertebrate species was recorded at Dunstan Park Pond.

Site 27: Dunstan Park Pond (SP 54097 07985)		
Group	**Species**	**Common Name**
Mammals	*Canis lupus*	Dog

Site 28: Chandlings School Pond

A total of six vertebrate species were recorded at Chandlings School Pond: one fish, one amphibian and four birds.

Site 28: Chandlings School Pond (SP 5073 0138)		
Group	**Species**	**Common Name**
Fish	*Carassius* sp.	Crucian Carp species
Amphibians	*Lissotriton vulgaris*	Smooth Newt
Birds	*Anas platyrhynchos/Tadorna tadorna*	Mallard/Common Shelduck
	Fulica atra	Coot
	Gallinula chloropus	Common Moorhen
	Pica pica	Eurasian Magpie

Appendix 4.

Limitations of Survey Method

The below table summarises some of the limitations of multi-species eDNA surveys.

Limitation	Explanation
No systematic comparison with conventional methods	As of yet, there has been no systematic comparison between multi-species eDNA survey results and results obtained using traditional survey methods. This has meant the results have been difficult to interpret, as we are currently unable to validate records. In 2014, single-species eDNA kits used to survey Great Crested Newts were systematically compared with traditional survey methods for the species, and as a result the eDNA surveys were confirmed as a highly effective method for confirming presence or absence of Great Crested Newt in the breeding season (Biggs *et al.,* 2014). A similar comparison is required for the multi-species eDNA kits to understand the effectiveness of the survey method. However, given that the eDNA kits detect a vast range of vertebrate species, such a systematic comparison would be both expensive and complex.
Precise source of DNA not clear	It is not currently clear i) how far DNA can be transported downstream, and ii) the extent of the area surrounding the waterbody that DNA can be washed in from following rainfall. Therefore, it cannot be assumed that the records originate from the immediate vicinity of the sampling point.
DNA longevity not accurately known	The longevity of DNA once it has been released into the aquatic environment is not yet known with certainty. Whilst this will vary depending on environmental conditions and the source of the DNA (e.g. hair, skin, mucous, 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.