GenePools 2021: Report

Hello GenePools team! Firstly, we would like to say a huge thank you for taking part in this project and your patience in receiving your results. We hope you have enjoyed the process. Now for the exciting bit where you find out the eDNA you retrieved!

What did we do with your sample?

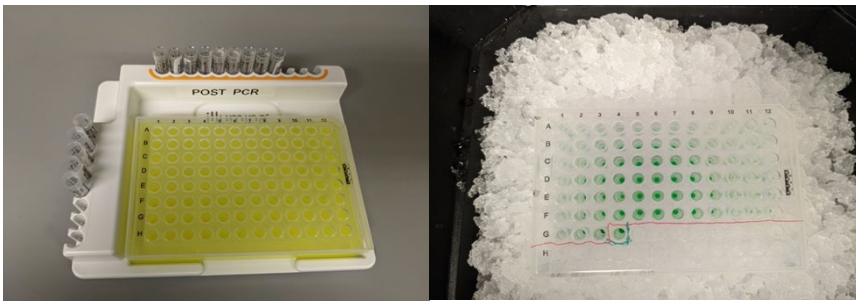
Your samples arrived by post at laboratories and were processed by our team of molecular biologists. Each sample went through several procedures to get the data in your report:

1. Recovering the DNA

First, a solution known as a 'lysis buffer' was added to the filter to extract the DNA[1]. This solution effectively breaks open any cells caught on the filter, releasing their DNA into the solution. The DNA was then purified or cleaned to remove contaminants.

2. Preparing for sequencing

To find out what species are in your pond we took the DNA that we recovered and used PCR (polymerase chain reaction) to copy the 'section' of the DNA that we were interested in multiple times. PCR tests have recently been used to detect very small amounts of the COVID-19 virus from swab samples but, in this project, we were looking for vertebrate, invertebrate and microbial DNA. Each of these groups were processed in separate tests that look for different sections of DNA, which were then multiplied during the PCR amplification.



We set up PCRs in these 96-well plates, allowing us to process hundreds of samples at the same time.

3. Metabarcoding

Instead of asking 'is species X present?', metabarcoding poses the question 'what species are present?'. This information is collected by simultaneously sequencing the different fragments of DNA which have been generated by the PCR process. The sequences generated are then compared against those from a range of species held in databases of

[1] DNA (deoxyribonucleic acid) comprises of a string of four different molecules: 'A', 'T', 'G' and 'C'. Every organism has a unique pattern of molecules known as their genetic code or DNA sequence.

sequences[2] to seek a match. A match allows us to identify the species that the DNA sequences come from, and thus the species present in your pond.

Your results

Before you dive into your pond's results, here are a few things you should know:

Although small particles of DNA may have been found in your pond, this does not necessarily mean they originated there. DNA is shed in the environment by living creatures all the time through normal day-to-day activities, such as shedding skin or hair cells and drops of bodily fluid. This means it's likely there will be species in your results that don't make sense at first. For example, if a bird defecates into your pond as it flies over, you may find the eDNA of creatures it has eaten. If you feed the fish in your pond, you are likely to detect a few other fish species, which are ground up as part of the fish food, as well as species of shrimp, algae and worm. You can play detective with your results! Consider if species could be living in the pond, or just visiting - or if the DNA may have found its way into your pond another way.

Another reason that unexpected species can appear in your eDNA results is that online DNA sequence reference databases don't contain all the species in our ponds. These databases are 'DNA dictionaries', containing millions of sequences from different species, against which we compare our data to see what species they came from. The reference databases are the result of amazing collaborative efforts between researchers around the world, but they still don't cover anywhere near the huge diversity of life that exists on Earth; probably <1% of species on Earth have been described and are represented in sequence databases. If a creature you have in your pond is not yet in the reference library, it may be 'matched' to a close relative who is in the reference library, causing an incorrect species to appear on your list. For this reason, it is important to read through metabarcoding results so that anything unexpected can be checked. Reference libraries are updated with new species every day. This means we could go back to our DNA data in the future and see what other species we can identify.

Equally, your results might be 'missing' species that you think should be there. As above, this can happen if the species isn't yet in the reference library. There are also several reasons why some DNA might be present in very low concentrations which we weren't able to detect. For example, eDNA only stays in the environment for a period of days/weeks before degrading. This means that if a species hasn't visited the pond for more than month, it probably won't show up in the sample.

Finally, in the lab, we ran four different tests on your pond water. We tested for:

1. **Vertebrates** – animals with spines such as mammals, birds and fish.
2. **Invertebrates** – including insects, worms and crustaceans.
3. **Bacteria** – the tiny single-celled creatures which live in your pond.
4. **Microeukaryotes** – more tiny creatures such as fungi, algae and amoebae.

We used small pieces of DNA called 'primers' to select for each of these groups of creatures from the whole mix of DNA in your pond. The primers we chose allowed us to search your pond water for 'all bacterial DNA', for example. Different types of primers are better at detecting different groups of species. In this project we didn't use any plant primers, which is why you won't see many plants in your results.

[2] DNA sequencing is the process by which we 'read' the genetic code of a piece of DNA to identify which species the DNA has come from. We performed DNA sequencing on your pond water samples in a laboratory.

Results overall

Overall, 164 ponds were surveyed, and we found over 2250 species, spanning everything from microbes less than 1 micron in size, to insects, to chickens, foxes and dogs. This included predators and herbivores as well as decomposer species that are vital to recycle nutrients. Being able to find such a range helps explore the full urban pond ecosystem beyond just the more visible species, and this would be almost impossible without eDNA.

Interestingly, we found a lot of creatures that live on land. This might seem surprising but remember their DNA could have entered the pond in lots of ways, like when they visit your pond to drink. So, this might mean we can use eDNA from ponds to help understand urban biodiversity more widely, and the importance of ponds in sustaining this.

City	Total species	No. of bird species	No. of mammal species	No. of insect species	No. of ponds
London	1474	23	8	65	53
Bristol	1473	14	8	64	68
Newcastle	1366	18	10	78	43
Across all 3 cities	2250	28	11	124	164

Each city had different numbers and types of ponds, so we can't make direct comparisons without more sophisticated analyses to account for this. But it's still interesting to see the number of species found in each city.

The table shows that although birds, mammals, and insects are visible and important parts of ponds, a much larger number of species in other groups are also present in ponds. This is especially the case for bacteria, which had far greater diversity than any other group of organisms. For example, we recorded 144 different genera of Gammaproteobacteria alone, more than all of the insect, bird and mammal species combined. We are still only beginning to learn about these microbial groups, so results from urban ponds will help to understand which species are likely to be found where and how they contribute to pond ecosystems.

Vertebrates

The most common vertebrate was human, which is not surprising! This was followed by common frog, pigeon, blackbird and dog. We did also find some species that are listed as a conservation concern (e.g., palmate newt, Eurasian widgeon and song thrush). The birds and mammals probably range over a wider area, so some of these could help to understand what's happening to urban wildlife in your area. However, we need to be aware that sampling took place in winter and it would be interesting to see how these results compare with what we could find in spring and summer.

Invertebrates

We found around 394 species of invertebrate, including earthworms, insects, tardigrades (water bears), molluscs, and crustaceans. We detected hundreds more invertebrate species, which could not be identified to genus or species level because our reference databases don't yet include the huge diversity of life that exists. Each pond contained an average of 12 invertebrate species; most common were insects and worms, which were found in almost every pond. Dragonflies and damselflies were only found in four ponds – it's likely they are present in many more ponds than this, but perhaps at very low abundance during winter and so there was little or no eDNA to detect when the samples were taken.

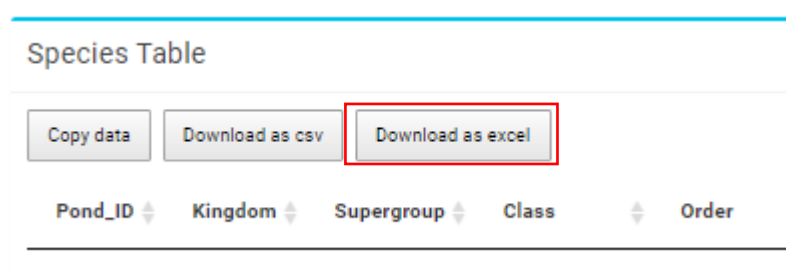
Bacteria and microeukaryotes

There were around 740 bacterial species as well as around 1080 microeukaryotes (microscopic, usually single-celled, eukaryotic organisms). The most diverse group (Gammaproteobacteria) had an amazing 144 different genera (groups). It will be valuable to

explore the microbial results to see what differences there are in the species present between different types of ponds, and what this could tell us about how these ponds function and how they might respond to changing environmental conditions.

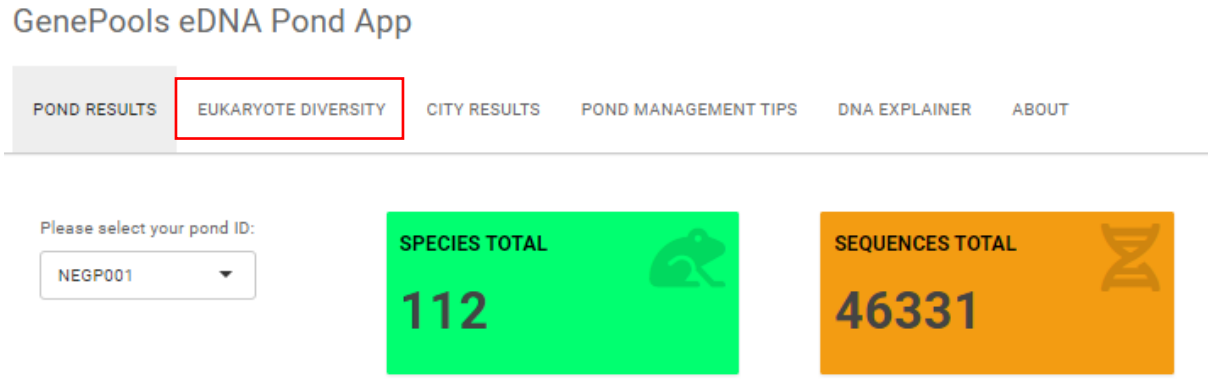
Accessing your results

In the email to which this report is attached, you should have received a link to our **results website** where you will find an interactive sunburst chart that can be opened via any web browser, which allows you to explore your data. Clicking a segment will zoom in on the taxon group of interest – to go up one level click the middle segment. Scroll down below the sunburst chart and you will find your species table. At the top of this, highlighted in red below, **there are also links to download your data in a spreadsheet in case you find that more convenient and intuitive**.

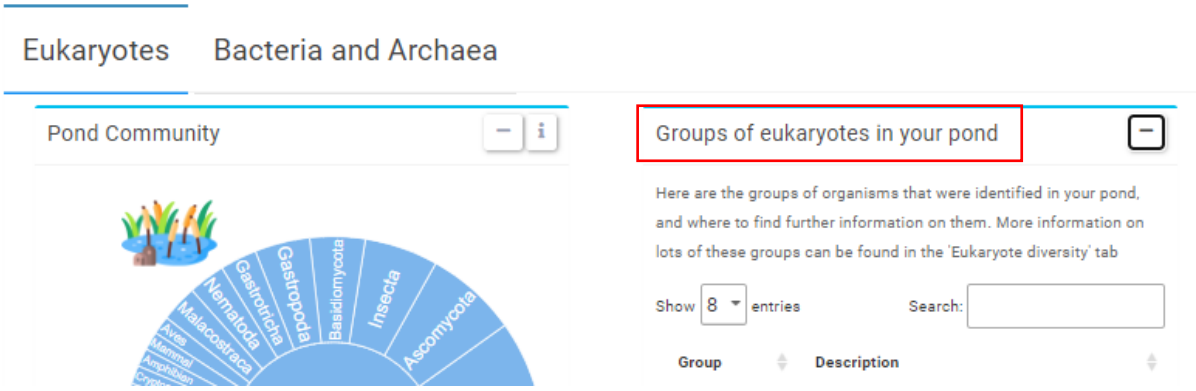


Eukaryotes

When you select a pond, the sunburst plot initially has an outer and inner ring of names. The inner ring comprises the high-level classifications often called 'supergroups'. Information on these is provided in the EUKARYOTE DIVERSITY tab highlighted in red below.

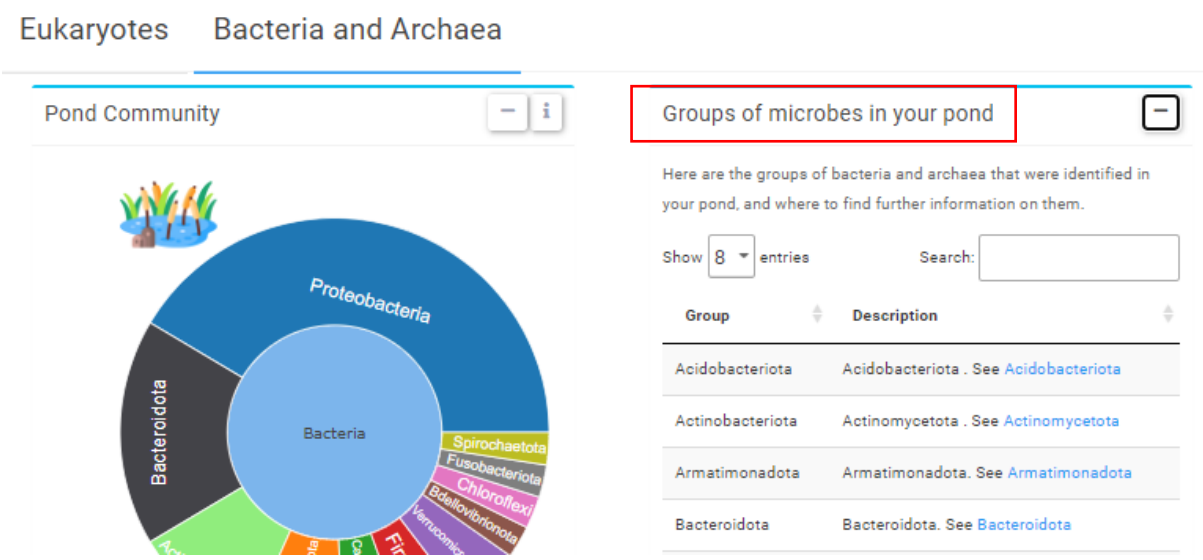


The outer ring has more names, of groups that are contained within the supergroups. These are listed alphabetically to the right of the sunburst plot under the drop-down heading "**Groups of eukaryotes in your pond**".



This list provides access to more information about the groups in the outer ring of the sunburst plot, either on external websites, the EUKARYOTE DIVERSITY tab, or simply providing a simple explanation such as Aves = birds. Under the drop-down heading “Some of the species in your pond” we have picked out a few of the most recognisable species that were detected your pond.

Bacteria
Like the sunburst plot for the eukaryotes, the group names in the outer ring of the bacterial plot are listed alphabetically under the heading “**Groups of microbes in your pond**”, to the right of the plot.



If you have any problems accessing your results please contact us: genepools@nhm.ac.uk.

Pond management tips

A pond is perhaps the most important habitat you can have in a garden. Many species may depend on your pond for survival, so here are some tips to make it even better for wildlife!

Algae
Many ponds are covered in a layer of filamentous algae that can sometimes be a bit of a nuisance if it’s left to grow out of control. Algal growth is often related to high nutrient levels in a pond, which could be due to adding fish food or letting leaf litter build up in the pond. The animals living in your pond will feed on the algae, keeping it at a healthy level, but if your pond is newer then you may want to monitor the algal growth during the summer months when it can build up easily.

Don’t worry if it does. You can remove any build-up of filamentous algae by using a stick (e.g. bamboo poles) or a small pond net to collect the algae out of the pond. Be careful not to catch any invertebrates or amphibians like newts. Leave the algae next to the pond before you get rid of it to allow any animals caught in it to return to the pond.

Invasive plants
It’s important to encourage and plant native plants to grow in your pond, as many species of invertebrates and other animals often feed on them. Non-native species can be difficult for some invertebrates to feed on and they can spread very easily and take over the pond or even worse spread to natural habitats outside your garden.

When clearing your pond area stick to the rule of only clearing one third of the pond area in a year and remember to leave any plants you clear by the side of the pond for any animals to escape back into the pond!

Topping up the water
Many of us panic during the summer months when pond water levels drop, but fear not! It's a natural process of ponds in the UK as they naturally dry and refill with the seasons. Many animals have learned to cope with these seasonal differences in water levels. For example, newts can survive in the muddy areas. If your pond falls significantly low on water, then you can top it up using rainwater collected in water butts. Try not to use tap water as the chemicals used to treat it can harm wildlife.

Animal perches or ramps
One way to help animals access your pond is to put some sort of ramp, plank or rock near the very edge of the pond, so animals can drink without falling in, and if they do fall in, they have a way to climb back out. Similarly, having small perches around the outside of your pond can give small birds a spot to approach the pond safely.

What next?

This project has been funded as part of the Natural Capital and Ecosystem Assessment Programme. Currently in a pilot phase, this is a transformative programme to understand the extent, condition and change over time of environmental assets across England's land and water environments.

This project was presented at the European Citizen Science Association Conference 2022 because it has been a really important step in what can be achieved through citizen science to assess the biodiversity of urban ponds through eDNA. Getting information about so many different groups of species would've been all but impossible without eDNA so we were really pleased with how the methods worked. We're also learning a lot about how to improve in future – making the methods easier to apply and how we process and analyse the samples.

Other activities you can take part in

If you enjoyed this project and would like to carry on learning about the wildlife in your pond, try our suggestions of extra activities and check out the resources listed.

Taxonomy
If you found yourself interested by the species found in your pond, then why not try learning how to identify some of them? Amphibians is a nice group to start with, as there are only a small number of species to learn. Here is a useful resource to start with: https://www.arguk.org/info-advice/id-guides/441-amphibian-id-guide-revised-2019-pdf.

Insects and other invertebrates are also interesting. Although there are many of them to learn, they vary in difficulty to identify – so there really is a group for everyone! The guide to freshwater invertebrates written by OPAL is a great place to start as it has good photographs of the main groups of pond invertebrates: https://www.imperial.ac.uk/media/imperial-college/research-centres-and-groups/opal/WATER-4pp-chart.pdf.

Biological recording
If you find that you enjoy this process of identifying and recording wildlife, you can upload your wildlife photos to iNaturalistUK, which is a website and app designed for anyone to collect and monitor wildlife sightings. Use it to upload photos of the wildlife you see (e.g. plant, insect) alongside a location and date. You don't have to be an expert, as the app will give you some handy suggestions of what it thinks you've seen, before it is shared with the

rest of the iNaturalistUK online community who can confirm your identification or suggest another species. Best of all, the records you submit will help inform local and national conservation work. Find the iNaturalistUK website here: https://uk.inaturalist.org and download the app to your smart phone.

London, Bristol and Northeast England all took part in City Nature Challenge (CNC) 2022 and will likely take part in CNC 2023, 28th April- 1st May. CNC is an annual global celebration of biodiversity in and around urban areas. It's an international collaboration-meets-friendly-competition to find and document wildlife, but also to see which city can gather most observations of nature, find the most species, and engage the most people in the event. What better motivation to start using iNaturalistUK! Find out more here: https://www.bnhc.org.uk/city-nature-challenge/.

Join/volunteer for a local wildlife group

You can also find many local wildlife groups to further your interest, by joining them as a member, participating in conservation efforts and volunteering for example. Here are some examples of groups within your areas:

- Northumbria Natural History Society: https://www.nhsn.org.uk/ including https://www.nhsn.org.uk/citizen-science/
- Northumberland Wildlife Trust
- Avon Wildlife Trust: https://www.avonwildlifetrust.org.uk/
- London Wildlife Trust: https://www.wildlondon.org.uk/
- Friends of City Gardens (London): https://friendsofcitygardens.org.uk/
- Bristol Avon Rivers Trust: https://bristolavonriverstrust.org/
- Bristol Natural History Consortium: https://www.bnhc.org.uk/

Useful resources

- The RSPB guide to pond management for wildlife: https://www.rspb.org.uk/birds-and-wildlife/advice/gardening-for-wildlife/water-for-wildlife/looking-after-your-pond/
- The Royal Horticultural Guide for managing wildlife ponds: https://www.rhs.org.uk/ponds/wildlife-ponds
- The Wildlife Trusts and Royal Horticultural Society guide to ponds, which includes management and identification tips: Wild About Gardens: Big or Small, Ponds for all
- OPAL freshwater invertebrate guide: https://www.imperial.ac.uk/media/imperial-college/research-centres-and-groups/opal/WATER-4pp-chart.pdf
- Amphibian identification guide: https://www.arguk.org/info-advice/id-guides/441-amphibian-id-guide-revised-2019-pdf
- https://freshwaterhabitats.org.uk/
- Citizen Science monitoring of water quality in UK rivers: https://www.riverflies.org/
- https://www.brc.ac.uk/recording-schemes

Appendix: Information on some of the species we found in your ponds

1. **Vertebrates (phylum: Chordata)**
   1.1. **Mammals (class: Mammalia)**
      1.1.1. **Red fox (*Vulpes vulpes*)**
      The red fox is common throughout the UK, and roughly 150,000 of them live in urban areas, where they both hunt and scavenge food. Rodents form a high proportion of their diets. A fox's den (or "earth") is sometimes in a burrow, but in urban areas can also be in crevices and gaps between and under things like garden sheds. Foxes are a fairly common sight in many cities, especially at dawn and dusk in suburban areas.
      1.1.2. **Brown rat *(Rattus norvegicus)***
      Brown rats are common throughout the UK and one of the most abundant mammals in urban areas. They can eat almost anything and thrive on human food waste and refuse. Despite their poor reputation, they are curious, intelligent and highly adaptable animals. They're more common outdoors than in, and frequently seen in urban areas, especially at night. Other signs include well-worn paths ("runs") along fences, water courses and under sheds, and their dark, oily, pointed droppings.
      1.1.3. **Field vole *(Microtus agrestis)***
      Very common throughout the UK (apart from Northern Ireland), field voles are usually found in gardens that have undisturbed long grass. They do not usually damage cultivated plants. They're not easy to see, but they leave field signs: a system of runways at the base of the grass stems, untidy nests made of balls of grass, oval green droppings, and feeding signs in the form of grass blades clipped at a 45° angle. Some of these signs are like those of the bank vole and may be confused.
      1.1.4. **Eastern grey squirrel *(Sciurus carolinensis)***
      Grey squirrels are common throughout English cities, where they are possibly the most frequently seen wild mammal. Though non-native, they thrive in parks and gardens – and on bird tables! - and have generally outcompeted the native red squirrel. Look for their nests ("dreys"), which look like large birds' nests, usually in tree forks, and incorporate leaves as well as twigs. However, they can also damage trees through bark-stripping. They often bury nuts for winter, which can lead to unexpected tree seedlings sprouting in flowerpots.
      1.1.5. **Domestic dog *(Canis familiaris)***
      If you have a dog, this will come as no surprise – if not, remember DNA can get into ponds through many routes! There are 12 million pet dogs in the UK, found in approximately 9% of London households, 25% of South-West households, and a huge 36% of North-East households. Dogs even shape our cities – think about dog walk routes and dog parks. Their number can also put pressure on wildlife when out and about.
   1.2. **Amphibians (class: Amphibia)**
   There are seven species of native amphibians in the UK: two frogs (common and pool); two toads (common and natterjack); and three species of newt (great crested, smooth, and palmate). Numbers of amphibians have been in decline across Europe and the rest of the world since the 1960s and as such the UK's population of great crested newt is both internationally important and highly protected. Our amphibians hibernate in winter; however, some stay in ponds over the winter so you may be one of the lucky few to have detected them.
   1.3. **Ray-finned fishes (class: Actinopterygii)**
   The fish found in urban ponds are many and varied, and some of your ponds will have been designed specifically as fish ponds. You may have stocked your pond with fish, or there may have been fish in it already. DNA from fish may also have entered the pond from fish-eating mammals visiting your pond, even your garden

cat! If you are managing your pond for wildlife, then having too many fish in your pond can cause problems. Fish predate on tadpoles, other amphibian eggs and larvae as well as many insects and plants. Fish foraging for food at the bottom of the pond stir up the sediment which can lead to algae growing, and too much algae can cause problems for wildlife.

1.4. **Birds (class: Aves)**

1.4.1. **Common blackbird (*Turdus merula*)**

One of the most common garden visitors, these familiar birds with a melodic song have adapted well to suburban areas. In folklore, it is a sign of good fortune to have a blackbird nesting near your house! Blackbirds are members of the thrush family (Turdidae), and are UK residents all year round, although increased numbers arrive here in the winter from northern Europe. To help blackbirds in your garden, provide food on a table or on the ground (they are too large to access bird feeders). They are especially keen on earthworms, so you may find them paying a visit after you have dug over the soil.

1.4.2. **Song thrush (*Turdus philomelos*)**

The song thrush was once a common sight in woodland, farmland and gardens, but has sadly declined significantly since 1970. It is now on the amber list of species of conservation concern, probably caused by a loss of habitat due to changing agricultural and woodland management practices. Song thrushes lay bright blue spotted eggs, the shells of which you might find under hedges in springtime. They eat snails by cracking the shell against a stone ‘anvil’, in a behaviour not seen in other birds. Song thrush need plenty of trees, bushes and overgrown hedgerows to nest in, so consider leaving those pruning shears on the shelf if possible!

1.4.3. **Feral pigeon (*Columba livia*)**

Pigeons were originally domesticated to provide food but have also been bred to carry letters and packages due to their strong homing instinct. There are about 1000 different breeds of domestic pigeon. Escaped domestic pigeons then led to large populations of feral pigeons which are a common sight in towns and cities around the world. Feral pigeons are very varied in colour and pattern from blue-black to reddish brown or very pale. Pigeons in towns can be seen as a nuisance, but more pigeons have been awarded the Dickin medal for gallantry during World War Two than any other species! Pigeons can breed at any time of the year, and they usually mate for life.

1.4.4. **Great tit (*Parus major*)**

Great tits are the largest of the UK tits, roughly the size of a robin. Listen out for their distinctive two-tone call which sounds like ‘teacher-teacher’. You may recognise them by their black head, white cheeks and bright green and yellow plumage. All of our UK tit species build their nests in holes within trees - if you have a suitable wall or tree, you could help them to nest by providing a nest box. They will make their nests in April – May and the young will emerge about 5 weeks later.

1.4.5. **Eurasian magpie (*Pica pica*)**

You probably won’t have missed your magpies, with their loud chattering calls and iridescent blue-black feathers. Magpies are common in urban areas as well as semi-open woods and farmland. Look out for their large stick-built nests in tall trees. They are omnivorous, and will scavenge and predate on eggs, baby birds and insects. The Eurasian magpie is thought to be among the most intelligent animals; their brain-to-body mass ratio is similar to that of the great apes. Magpies can recognise themselves in mirrors, use tools, and engage in complex social rituals including grief.

1.4.6. **Common moorhen (*Gallinula chloropus*)**

The common moorhen is a familiar sight on lakes, ponds and rivers. Moorhens have an extremely large range, and are found throughout Europe, Africa and

Asia. Moorhens favour slow flowing water, which is sheltered by trees or scrub. You can recognise a moorhen by its bright red face shield. Male and female moorhens share the task of incubating eggs, which will hatch after three weeks. Outside of breeding season, moorhens can form flocks of 15 – 30 individuals.

1.4.7. **Eurasian robin (*Erithacus rubecula*)**

Robins are a familiar sight in UK gardens and parks and one of the few garden birds whose numbers are increasing. Known as the gardener's best friend, robins are ground feeders and will often appear when a gardener is digging to feast on freshly dug worms. Male and female robins look identical, with their distinctive bright red breasts which they develop in adulthood (juvenile robins are speckled brown). Robins are highly territorial; you may see them singing loudly from a visible perch to make their presence known and warn off intruders.

2. Invertebrates:

2.1. **Snails (Phylum: Mollusca; Class: Gastropoda)**

There are about 40 different species of water snails in the UK, with the largest being the Great Pond Snail, growing up to 4cm. They feed on algae and detritus in the pond and usually enter new ponds as eggs on translocated pond plants or being carried accidentally by visiting birds and amphibians. They need calcium in the water to grow their shells so they may be reduced or absent in naturally acid ponds.

2.2. **Dragonflies/damselflies (Phylum: Arthropoda; Class: Insecta; Order: Odonata)**

Dragonflies and damselflies are most well known for their adults, which are acrobatic flyers and relatively easy to watch, but most of their lives are spent underwater in ponds and rivers. Dragonflies are the bigger and faster of the two groups, while damselflies are typically smaller and slower as adults. Both, however, are voracious predators above and below the water! In the UK there are around 50 species, but the most common dragonflies to visit garden ponds include the southern hawker (*Aeshna cyanea*), common darter (*Sympetrum striolatum*) or the broad-bodied chaser (*Libellula depressa*). The most common damselflies in garden ponds are the large red (*Pyrrhosoma nymphula*), the common blue (*Enallagma cyathigerum*), the azure (*Coenagrion puella*) and the blue-tailed (*Ischnura elegans*).

2.3. **Bugs (Phylum: Arthropoda; Class: Insecta; Order: Hemiptera)**

While most invertebrates are commonly called bugs, in taxonomy the name only applies to the order Hemiptera. There are 70 freshwater species in the UK, and the most common in ponds are the pond skaters (Family: Gerridae) which, as their name suggests, skate on the surface of the pond. They use their feet like this to feel any disturbances on the pond surface that might suggest potential prey, just like a spider feeling its web. Another common group are the backswimmers (Family: Notonectidae) which live upside-down under the pond surface and resemble a rowing boat with two long oars (their hind legs). They usually drop to the bottom of the pond if disturbed but as they breathe air using a bubble trapped to their body, they will bob back up after a few minutes to refresh their air supply.

2.4. **Beetles (Phylum: Arthropoda; Class: Insecta; Order: Coleoptera)**

Beetles are a very diverse group, with over 400 freshwater species in the UK. Some species like the whirligig beetles (Family: Gyrinidae) zoom around on the water surface. Their eyes are divided, with the lower half adapted for looking down through the water, while the upper half is adapted for looking above the surface. In contrast, diving beetles (Family: Dytiscidae) spend most of their time diving under water hunting for prey. Their larvae are also voracious predators, eating various pond inhabitants including tadpoles.

2.5. **Mayflies (Phylum: Arthropoda; Class: Insecta; Order: Ephemeroptera)**

Mayflies are an ancient group of insects, first recorded over 300 million years ago. There are 50 species in the UK but if you find one in your pond it will almost certainly be the pond olive (*Cloeon dipterum*) which is the second most common garden pond invertebrate after the common water slater (see Crustacea below). The

pond olive is the only ovoviviparous mayfly in Europe, meaning their embryos develop within the eggs whilst still in the female and hatch as soon as they hit the water. Male pond olive mayflies have an extra set of well-developed eyes pointing directly up, which help them find females in the confusion of a mating swarm.

2.6. **Flies (Phylum: Arthropoda; Class: Insecta; Order: Diptera)**
Flies are the most diverse group in freshwater habitats, with over 1500 species in the UK alone. Almost all ponds will have non-biting midges (Family: Chironomidae); while they resemble mosquitos they don't bite, rather feeding on nectar and pollen. Other groups include the *craneflies* (Family: Tipulidae) which resemble huge (non-biting) mosquitos as adults, *horseflies* (Family: Tabanidae), and *mosquitos* (Family: Culicidae) which do bite! One of the smaller groups, the phantom midges (Family: Chaoboridae) are unusual in that they live in the water column (i.e., not at the surface or bottom). They are the submarines of the insect world, with small air sacs to help keep them at their chosen depth, and antennae modified like the arms of a praying mantis to grab mosquito larvae and small crustaceans like Daphnia.

2.7. **Caddisflies (Phylum: Arthropoda; Class: Insecta; Order: Trichoptera)**
Caddisflies resemble small moths as adults, though they have hairs rather than scales on their wings and there are just over 200 species in the UK. Their larvae are relatively easy to find in ponds, but the adults can be harder to see as many only fly at dusk or night. Most caddis larvae construct intricate cases to protect them from predators. The cases are made up of small sticks, sand grains or bits of vegetation woven together with their own silk. The fact it is adhesive under water means their silk might have medical uses too!

2.8. **Crustaceans (Phylum: Arthropoda; subphylum: Crustacea)**
Crustaceans are almost always present in a garden pond. The water slater/water louse (*Asellus aquaticus*) is the most common invertebrate in garden ponds, and is found on the sides and bottom of the pond, feeding on detritus where they play a major role in nutrient cycling. The freshwater shrimp (*Crangonyx pseudogracilis*) is also common and has spread throughout the UK following its introduction from the USA in the 1930's. Although they are invasive here, they don't seem to have affected the native freshwater species. If you look closely in your pond, you are very likely to see water fleas (Order: Cladocera), small (1-3mm) crustaceans swimming in the water column, the most common being *Daphnia* species. Water fleas move their legs to create a constant current, allowing them to filter particles from the water column; they can be found closer to the surface at night using a torch.

2.9. **Water mites (Phylum Arthropoda, Class: Arachnida; unranked: Hydrachnidia)**
Water mites are ubiquitous in freshwater habitats, and globally are among the most abundant and diverse freshwater arthropods, though their true diversity is unknown as not many people study them. Water mite larvae are parasitic on other freshwater invertebrates and can often be seen attached to their host or swimming around a pond looking for another host!

3. Archaea

Archaea were previously thought to be a type of bacteria, until DNA sequence analysis in the 1970s showed that they form a whole separate domain of life. Archaea are microscopic single-celled organisms that appear similar to bacteria but are, in fact, more closely related to eukaryotes. Archaea are the least well-studied of the three domains of life, because they are often difficult to grow in the laboratory. They have never been found to cause disease and are often mutualists, helping other species to survive. Archaea have been found in almost every environment on Earth, and can live in extreme heat, salt and acid environments. The archaea in your pond are likely to play important ecological roles such as carbon and nutrient cycling.