GenePools

Investigating the life that dwells in garden ponds

First published May 2023

Natural England Commissioned Report NECR454

www.gov.uk/natural-england

About Natural England

Natural England is here to secure a healthy natural environment for people to enjoy, where wildlife is protected and England's traditional landscapes are safeguarded for future generations.

Further Information

This report can be downloaded from the Natural England Access to Evidence Catalogue. For information on Natural England publications or if you require an alternative format, please contact the Natural England Enquiry Service on 0300 060 3900 or email enquiries@naturalengland.org.uk.

Copyright

ISBN: 978-1-78354-990-2

Catalogue code: NECR454

Report details

Author(s)

Helen C. Rees (helen.rees@adas.co.uk), Claire Baker, Stephen Kane and Jon Owen

Natural England Project Managers

Katie Clark and Harriet Knafler

Contractor

RSK ADAS Ltd.

Keywords

Citizen science, eDNA, freshwater, GenePools, metabarcoding, ponds, sampling

Acknowledgements

We would like to thank Katie Clark of Natural England for commissioning the project; Dr Ben Price from the Natural History Museum and Dr David Bass from Cefas for input into metabarcoding primer selection; and the rest of the GenePools team within Cefas, The Natural History Museum, Natural England and The Joint Nature Conservation Committee for their assistance during the project.

Citation

REES. H.C., BAKER, C.B., KANE, S.D., OWEN, J. 2023. *GenePools – Investigating the life that dwells in garden ponds.* Natural England Commissioned Reports, Number 454.

Executive Summary

DNA-based methods offer a significant opportunity to change how we monitor and assess biodiversity. However, for most techniques, there is still development required before they can be used in routine monitoring. Natural England has been exploring the further use of these methods for environmental monitoring for several years, delivering a series of reports which focus on the development of DNA-based methods with potential in a particular area.

GenePools is a collaborative project between Natural England, the Natural History Museum, Cefas and JNCC. The GenePools project is part of the Natural Capital and Ecosystem Assessment (NCEA). The NCEA is a Defra funded programme launched to transform decision-making by delivering high quality evidence to assess the extent and condition of biodiversity, ecosystems and natural capital assets across our terrestrial, coastal and marine environments.

GenePools was set up to trial the combination of citizen science with eDNA metabarcoding techniques to measure biodiversity within urban ponds. Citizen scientists from London, Bristol and Newcastle were engaged to take water samples from garden and community ponds. These water samples were then analysed using four different DNA metabarcoding assays – for vertebrates; invertebrates; prokaryotes; and microeukaryotes. This report describes the two assays undertaken by RSK ADAS: those for vertebrates and invertebrates. Other aspects of GenePools, including recruitment and engagement of citizen scientists; and reporting of results to citizen scientists, were led by the Natural History Museum and will be published separately.

The work presented here demonstrates that sampling by citizen scientists can be combined with cutting edge metabarcoding technologies, to measure biodiversity within urban ponds. Garden and community ponds form part of a network of freshwater habitats within towns and cities, the value and importance of which is not well understood. Following the success of this pilot project, we hope to continue to engage members of the public around their local freshwater habitats and use this partnership to build a picture of the biodiversity status of our urban ponds.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Contents

1. Introduction

Natural England is the Government's advisor for the natural environment. It provides practical advice on how to safeguard England's natural wealth for the benefit of everyone. ADAS is an environmental consultancy which exists to provide ideas, specialist knowledge and solutions to secure our food and enhance the environment.

1.1 GenePools

The GenePools project brings together citizen scientists from Bristol, London, and Newcastle to investigate the biodiversity within ponds from urban areas. Ponds are home to a variety of wildlife and can act as stepping-stones to allow species to move through towns and cities. Many organisms within ponds are too small to be seen by the naked eye, or are difficult to identify, however they still have an important role to play in the health of ecosystems. The biodiversity within privately owned garden ponds is largely unknown therefore Natural England asked citizen scientists to collect water samples from their garden or community ponds which were then analysed by DNA metabarcoding for the vertebrates, invertebrates and microbes within them. The resulting data will be presented to the citizen scientists and will be used to fill evidence gaps in the Natural Capital and Ecosystem Assessment scheme (NCEA).

1.2 Environmental DNA

Environmental DNA (eDNA) describes the DNA that can be extracted from an environmental sample, for example water, soil or sediment, or air. DNA present within an environmental water sample will originate from the faeces, saliva, urine and skin cells etc. of species occupying the water bodies in question. Similarly, the DNA of animals that visit the environment, such as birds and mammals using the water body to drink can also be present. This means that the eDNA from water bodies can be used for the monitoring of aquatic and semi-aquatic populations. In theory, the presence of a specific species can be detected anywhere within the water body and not just at its point of origin due to the rapid diffusion of DNA from its source (Rees et al. 2014).

1.3 Metabarcoding

DNA metabarcoding combines two techniques: DNA based identification and high-throughput sequencing (Margulies et al. 2005). Using primers that work across a wide range of taxa ('universal' PCR primers), specific target sequences can be amplified, the result being the mass-amplification of the target of interest from multiple species. Ideally, primers should target a hypervariable region (for high resolution taxonomic discrimination) and additionally, should target short DNA fragments (around 400 bp or less). This allows for the recovery of target DNA which may have been degraded over time or through exposure to hostile sample matrices. Some environments will be more detrimental to DNA

quality than others – for example temperature and humidity will affect DNA quality – and are therefore described as ‘hostile’. DNA is also liable to degradation by factors such as enzymes, UV light, and microbial action.

Universal primers have been developed for a wide range of gene fragments including: nuclear 18S and 28S ribosomal RNA markers (Machida et al. 2012a); the mitochondrial 12S rRNA gene (Machida et al. 2012b); and the mitochondrial Cytochrome Oxidase I gene (COI). Next generation DNA sequencing methods are used to return large numbers of high-quality sequence reads from the amplified target sequences. Sequence data is usually reduced down to a single representative of each species’ target DNA sequence - an operational taxonomic unit (OTU). The individual OTUs can then be compared with existing DNA databases to identify the organisms that they represent.

Metabarcoding has proven an effective technique for community biodiversity assessment across a range of taxa and environments (Deiner et al. 2016; Drummond et al. 2015; Hajibabaei et al. 2011; Murray et al. 2012; Valentini et al. 2016). When compared with traditional identification methods, metabarcoding can generate comprehensive data sets many times quicker and is therefore a powerful means to study and understand the diversity and distribution of fauna and flora. Additionally, metabarcoding can be used to study communities which cannot be described by traditional identification methods, such as microbial biodiversity. Given the relative ease of sample collection, citizen scientists have been able to become involved in specific study programmes utilising eDNA, for example the national survey of ponds for great crested newts funded by Defra (Biggs et al., 2015). In combination with biodiversity events such as ‘BioBlitz’, the use of eDNA metabarcoding can therefore be used to help engage the public in biodiversity science (Deiner et al. 2017).

1.4 Aims and objectives

GenePools aims to gather information on pond life through DNA metabarcoding using citizen scientists who have who have access to a pond to collect water samples for this project. The main objectives were:

- to provide suitable eDNA collection kits that are easy and safe for citizen scientists to use including training and engagement materials.
- to extract and analyse DNA present in the water samples and produce a list of species present within the samples, whilst also providing half of the DNA sample to Cefas for microbial analysis.

This report details the methodology employed in this study, the results obtained, and discussion of the results. All data will be made available for further study.

2. Materials and methods

2.1 Sample collection

Sampling kit:

Sampling kits were provided to citizen scientists and contained (Figure 1):

- 2 x filter paper (to be kept stuck together for use)
- Stamped, addressed jiffy envelope
- Test strip colour chart
- A water chemistry test strip
- 1 pair of sterile gloves
- A pre-filled capped 10 mL syringe (containing Longmires solution)
- A 50 mL syringe
- An eDNA filter unit and sealing caps
- 1 sterile 30 mL sampling ladle
- 2 sterile sampling bags

Each sampling kit was labelled with a unique reference number i.e., NEGP001 to NEGP200. Citizen scientists were asked to provide two clean tissues and a pen or pencil.

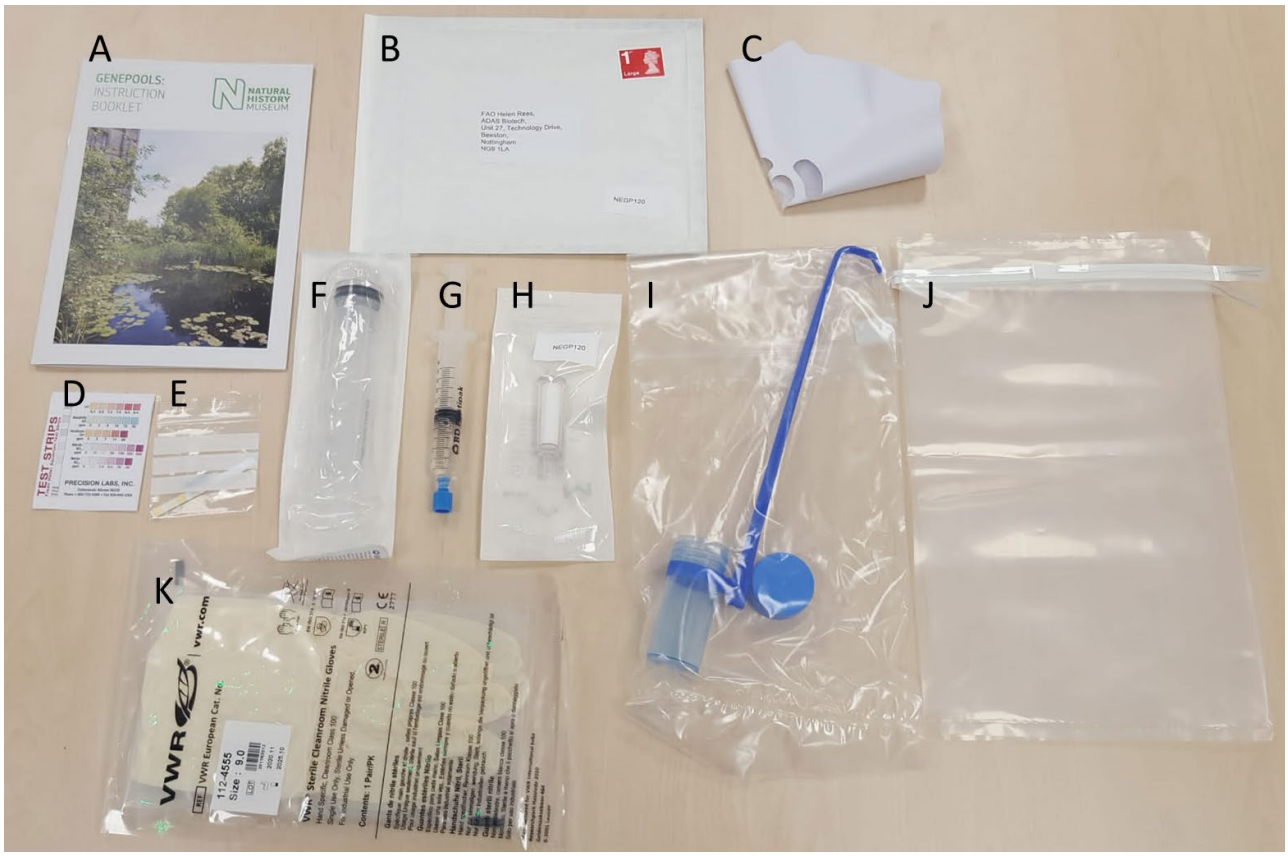


Figure 1. Sampling kit. A. instruction booklet; B. return envelope; C. 2x filter paper; D. test strip colour chart; E. a water chemistry test strip; F. a 50 mL syringe; G. a pre-filled capped 10 mL syringe (containing Longmires solution); H. an eDNA filter and sealing caps; I. 1

sterile 30 mL sampling ladle; J. 2 sterile sampling bags; K. 1 pair sterile gloves. © Helen Rees (ADAS)

Collection of pond water samples:

Samples were taken from the edge of the pond and citizen scientists were asked not to stand in the water. If necessary, the sampling ladle could be attached to a garden cane with tape to allow all areas of the pond to be reached. The sampling procedure was as follows:

1. Open your kit and put on a pair of gloves.
2. Open one of the sterile sampling bags by tearing off the clear plastic strip along the perforated line, then pull the tabs.
3. **Scoop.** Collect 20 samples of 30 mL of water from around the pond (multiple scoops can be taken from the same location) using the sampling ladle. Empty each sample into the sampling bag (Figure 2a).
4. **Remove large particles.** Pour the collected water through the two filter papers into the second sampling bag. This will remove any pond weed and large particles that may clog the eDNA filter (Figure 2b).
5. **Shake.** Close the bag securely using the top tabs (fold over several times and bend tabs over). Shake the sampling bag for 10 seconds. This mixes any DNA across the whole water sample.

Filtering of the sample:

1. Draw up 50 mL water from your bag into the 50 mL syringe and then screw the syringe onto the filter unit. Push the water through the filter back into the pond (you may find it easier to push the end of the syringe against a hard surface like a paving stone) then detach the syringe (Figure 2c). Draw up another 50ml of water from your sampling bad and repeat. Keep repeating this process. Please try to filter 200ml of water, but please do not worry if the filter clogs and you are unable to manage this volume.
2. Record the volume of water filtered.
3. Tap the filter unit on a clean tissue until the water is removed.
4. Take the pre-filled 10ml syringe (containing Longmires solution), twist off the cap (do not throw away the cap as it will be reused to seal one end of the filter unit) and attach the syringe to the filter unit. Hold the filter upside-down (syringe at bottom) and **slowly and gently** push the liquid into the filter until it starts to come out of the top. The filter unit should fill with liquid.
5. Dry your hands on a tissue if needed, and screw/push the caps onto each end of the filter unit.
6. Place the filter unit into the provided jiffy envelope.
7. Place all used gloves, syringes and other rubbish into the sampling bag and dispose of in your normal household waste.

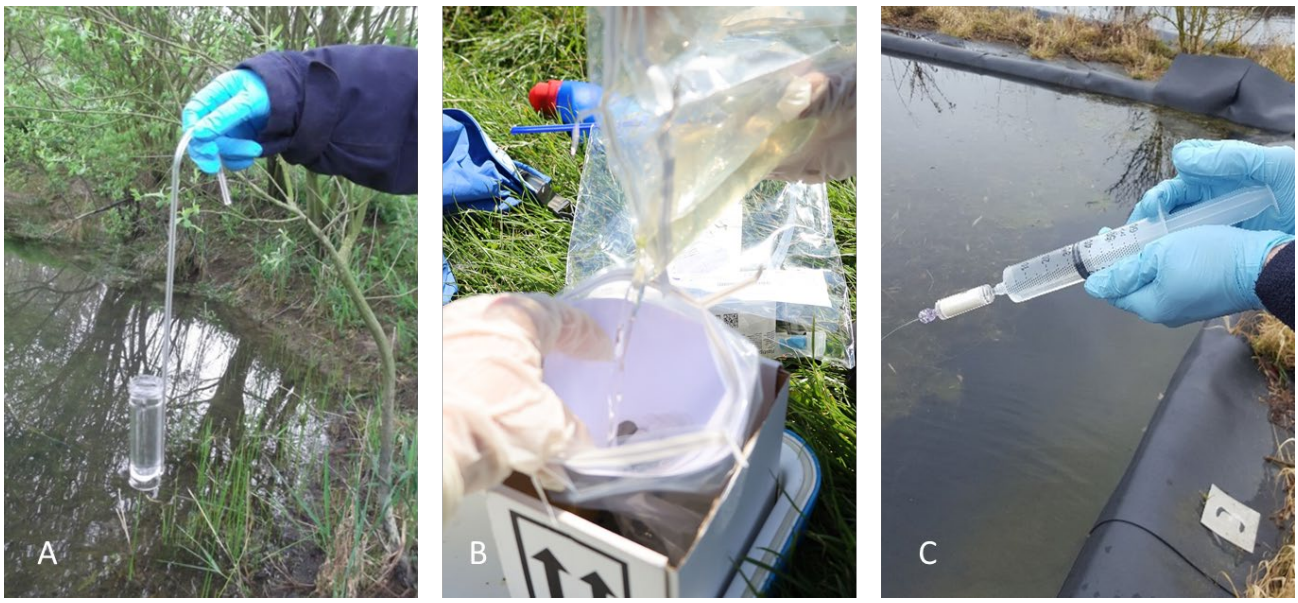


Figure 2. A) Water sample collection; B) water sample filtering to remove large particulates and pond weeds; C) water filtering through a Sterivex filter unit. © Helen Rees (ADAS).

Test the water:

1. Hold the end of the test strip between your index finger and thumb. Do not touch the test pads with your fingers.
2. Dip the test strip into the pond for one second, immersing all five pads.
3. Remove and shake off excess liquid.
4. Hold your test strip next to the colour chart (with your fingers at the bottom).
5. Immediately compare the alkalinity pad to the colour chart supplied, and circle the closest match. Next compare the Nitrate and Nitrite pads and circle the closest colour matches.
6. After one minute, compare the pH and Hardness pads and circle the closest colour match.
7. Return the colour chart supplied along with your filter unit in the stamped addressed jiffy envelope.
8. Empty the remaining pond water from the sampling bag back into the pond.
9. Post the stamped addressed jiffy envelope into any Royal Mail post box.

2.2 Laboratory standard and specification

All laboratory activities associated with DNA analysis are subject to errors if quality control is inadequate. Our DNA analysis follows a unidirectional workflow with separate laboratories and staff to act as a physical separation for the different aspects of the analysis work. This greatly reduces the potential for contamination of samples or the PCR amplicons. ‘Blank’ PCRs (sterile water rather than DNA) are used to monitor for reagent/procedural contamination, and in addition positive control samples are used to increase confidence in the results and identify any cross-contamination issues, should they occur.

2.3 DNA extraction and quantification

Prior to DNA extraction all samples were stored in a fridge at 4°C, length of storage between sample collection and DNA extraction was on average 18 days with the vast majority of samples being stored for between 11 and 27 days. DNA was extracted from filter units using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's instructions (described below), with the exception that 720µL of ATL buffer was added to each sample, along with 40µL of PK. Final resuspension was in 200µL AE buffer. All extractions were quantified using a Qubit 3.0 Fluorometer (Invitrogen) following the manufacturer's instructions then stored at -20 ºC prior to PCR set up (described below).

DNA extraction from Sterivex filters:

1. Remove the preservative solution from the filter by pushing air through the filter with a syringe and collecting in a 1.5mL Eppendorf tube. Record the volume recovered.
2. Add 720µL of pre-warmed ATL buffer and 40µL PK from the DNeasy Blood and Tissue kit to the filter via the top of the unit before sealing the unit with a cap.
3. Place the filter unit into a 50ml falcon tube and place at 56°C in a water bath for 1 hour, vortexing the tube along the length of the filter unit every 10 minutes.
4. An extra 1.5 mL tube was set up to act as an extraction blank for every set of extractions performed. Therefore, add 360 µL of buffer ATL into a 1.5 mL microfuge tube and perform the DNA extraction as per steps below. Label this tube as extraction blank (EB).
5. During the 1 hour incubation, centrifuge the recovered preservative solution for 30 minutes at 13,000xg. Remove the supernatant and retain the pellet in the Eppendorf tube as the ATL/PK mix from the next step will be collected into this tube effectively pooling the two together.
6. After incubation, remove the ATL/PK mix from the filter unit into the 50ml falcon tube by pushing air through the filter with a syringe. Collect into the Eppendorf tube from step 5 and vortex to mix.
7. Wash the filter unit through with 400µL molecular biology grade ethanol and add to the 50ml falcon tube, mix by vortexing.
8. Pipet the mixture into a DNeasy Mini spin column placed in a 2 mL collection tube.
9. Centrifuge at ≥ 6000 xg (8000 rpm) for 1 min. Discard the flow-through and collection tube.
10. Place the spin column in a new 2 mL collection tube. Add 500 µL Buffer AW1.
11. Centrifuge for 1 min at ≥6000 xg. Discard the flow-through and collection tube.
12. Place the spin column in a new 2 mL collection tube, add 500 µL Buffer AW2.
13. Centrifuge for 3 min at 20,000 xg (14,000 rpm). Discard the flow-through and collection tube.
14. Transfer the spin column to a new pre-labelled 1.5 mL microcentrifuge tube.
15. Elute the DNA by adding 200 µL Buffer AE to the centre of the spin column membrane. Incubate for 1 min at room temperature (15–25°C).
16. Centrifuge for 1 min at ≥6000 xg.

DNA quantification:

DNA extracts were quantified using the Qubit® dsDNA BR assay kit and Qubit 3.0 fluorimeter as follows:

1. The Qubit® working solution was prepared by diluting the Qubit® dsDNA BR reagent 1:200 in Qubit® dsDNA BR buffer.
2. Make up two standards by adding 190 µL Qubit® working solution into each of two tubes before adding 10 µL of each Qubit® standard to the appropriate tube. Mix by vortexing.
3. For each extract make up a tube with a final volume of 200 µL containing 2 µL extract and 198 µL Qubit® working solution.
4. Allow all tubes to incubate for two minutes before reading the standards and extracts on the Qubit® 3.0 fluorimeter.

2.4. Metabarcoding PCR

The primer combination used for the first round PCR amplification was fwhF2/fwhR2 (Vamos et al. 2017) for COI PCRs or 12S forward and reverse for 12S PCRs (Riaz et al. 2011). Overhang adapter sequences (Table 1, Figure 3) were included at the 5' end of the primers to ensure compatibility with Illumina index and sequencing adapters (Illumina 2011). PCRs included one negative control (ddH2O in place of DNA); two DNA extraction blanks; a positive control sample (Allolobophora chlorotica DNA (earthworm) for COI PCRs or Oncorhynchus mykiss (rainbow trout) for 12S PCRs) and all pond DNA samples.

Table 1. Primers used for specimen identification and PCR rounds one and two for metabarcoding. For the COI and 12S primers, sequences marked in bold underlined are the first round PCR primer adapter sequences, the remainder are the COI or 12S locus-specific primer sequences. For the Index primers, sequences marked in bold are Illumina overhang adapter sequences, Index 1 and 2 sequences are marked with Xs as this sequence is variable for each different sample, those in normal text are the P5 and P7 sequences. Index 1 (i7) and Index 2 (i5) are examples of the type of primers used with the Index sequence itself being altered for different samples. Letters other than A, C, G, and T in the primer sequences represent degenerate nucleotide codes where at these positions there are several possible bases e.g. R represents an A or G in this position.

Primer Name	Oligonucleotides (5'-3')	Reference
FwhF2 (plus adapter)	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GG DACWGGWTGAACWGTWTAYCCHCC	Vamos (2017)
FwhR2 (plus adapter)	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**G TRATWGCHCCDGCAARWACWGG	Vamos (2017)
12S forward (plus adapter)	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TA GAACAGGCTCCTCTAG	Riaz (2011)
12S reverse (plus adapter)	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**T TAGATACCCCACTATGC	Riaz (2011)
Index 1	CAAGCAGAAGACGGCATACGAGATXXXXXXXX**GTCT CGTGGGCTCGG**	Illumina (2011)
Index 2	AATGATACGGCGACCACCGAGATCTACACXXXXXXX X**TCGTCGGCAGCGTC**	Illumina (2011)

Round 1 PCR

12S PCRs were set up in triplicate in a total volume of 25 µL consisting of:

a. 5 µL of extracted template DNA (0.3ng/µl),
b. 1.25 µL of each primer (0.5 µmol/L),
c. 12.5 µL of TaqMan® Environmental Master Mix 2.0 (containing AmpliTaq GOLD DNA polymerase),
d. 5 µL ddH2O.

For the 12S primers (vertebrates) PCR cycling was as follows: an initial incubation for 5 minutes at 95°C; then 45 cycles with denaturation at 95°C for 30 seconds, 52°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension step at 72°C for 10 minutes before holding at 4°C until collection of PCR products for analysis.

COI PCRs were set up in triplicate in a total volume of 25 µl consisting of:

a. 5 µL of extracted template DNA (0.3 ng/µl),
b. 1 µL of each primer (0.1 µmol/L),
c. 12.5 µL of TaqMan® Environmental Master Mix 2.0 (containing AmpliTaq GOLD DNA polymerase),
d. 5.5 µL ddH2O.

For the COI primers (invertebrates) PCR Touchdown cycling was as follows: an initial incubation for 5 minutes at 95°C; 15 cycles with denaturation at 95°C for 30 seconds, annealing at 68°C for 1:30 mins, reducing by 1°C each cycle to 54°C, and extension at 72°C for 2 minutes. Followed by: 30 cycles with denaturation at 95°C for 30 seconds, annealing at 54°C for 1:30 mins and a final extension step at 72°C for 10 minutes before holding at 4°C until collection of PCR products for analysis.

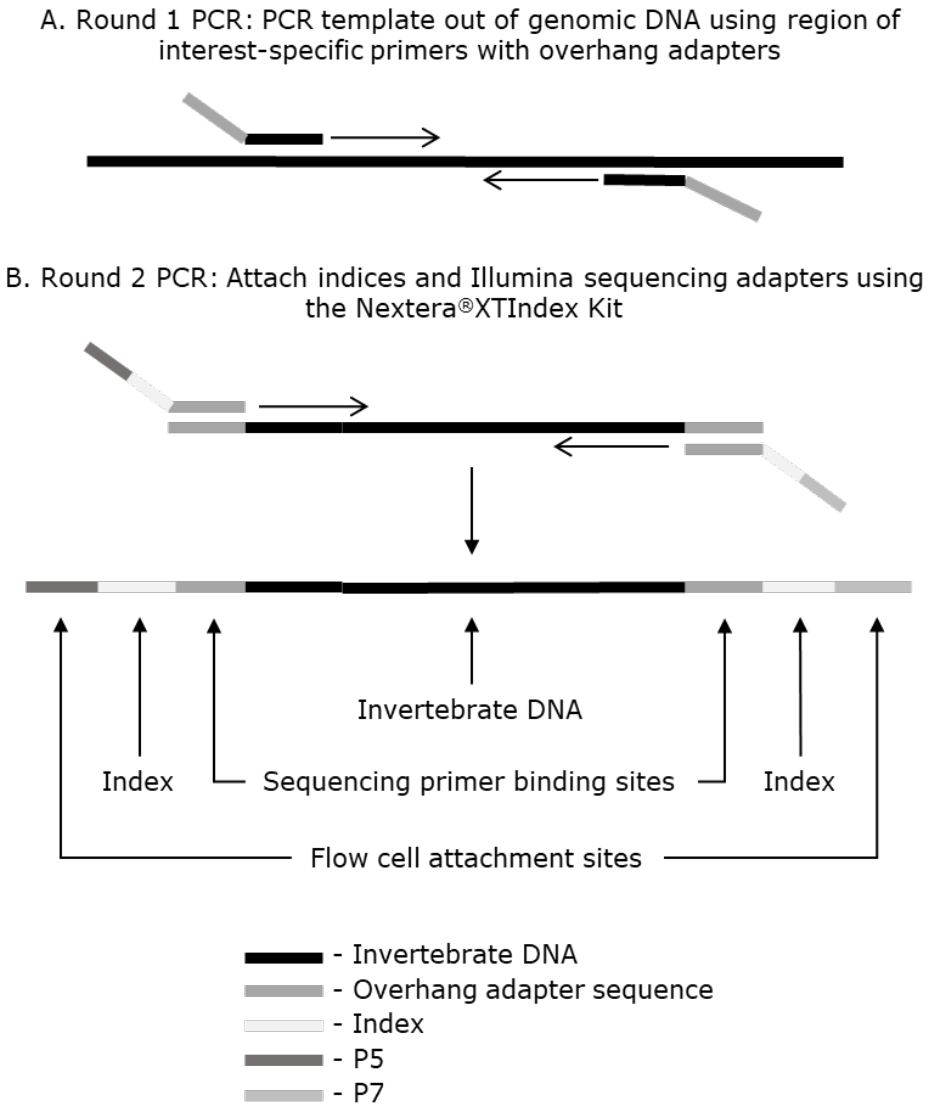


Figure 3. PCR Amplicon workflow A) Forward and reverse primers complementary to the region of interest including overhanging adapters (see Table 1). B) Subsequent amplification step used to add indices and Illumina sequencing adapters.

The first round PCR amplicons for each sample were pooled and run on a 1.5% agarose gel. For COI PCRs any bands of the correct size were excised and purified using NucleoSpin® Gel and PCR Clean-up purification columns (Machery-Nagel) according to the manufacturers' instructions (described below). For 12S PCRs, PCR products were directly cleaned using the NucleoSpin® Gel and PCR Clean-up purification columns.

Nucleospin® gel and PCR cleanup:

For DNA extraction from agarose gels:

1. Excise DNA fragment from gel with a fresh sterile scalpel blade for each sample.
2. Determine the weight of the gel slice and add 200µl buffer NTI for every 100mg of agarose gel
3. Incubate for 5-10 minutes at 50°C vortexing every 2-3 minutes until the gel slice is completely dissolved.
4. Place a NucleoSpin® gel and PCR clean-up column into a collection tube and load 700µl of sample onto the spin column and centrifuge for 30 seconds at 11,000 xg.
5. Wash the silica membrane by adding 700 µL Buffer NT3 to the column and centrifuge for 30 seconds at 11,000 xg.
6. Discard the flow-through and place the column back into the collection tube before repeating this wash step.
7. Dry the silica membrane for one minute at 11,000 xg to remove Buffer NT3 completely.
8. Elute the DNA by placing the column into a fresh 1.5 mL microcentrifuge tube and add 20 µL Buffer NE and incubate at room temperature for one minute before centrifuging for one minute at 11,000 xg.

For PCR product clean up:

1. If using small volumes (< 30 µL) adjust the volume of the reaction mixture to 50-100 µL with ultrapure water.
2. Mix one volume of PCR product with two volumes of Buffer NTI.
3. Place a NucleoSpin® gel and PCR clean-up column into a collection tube and load onto the spin column.
4. Wash the silica membrane by adding 700 µL Buffer NT3 to the column and centrifuge for 30 seconds at 11,000 xg.
5. Discard the flow-through and place the column back into the collection tube before repeating this wash step.
6. Dry the silica membrane for one minute at 11,000 xg to remove Buffer NT3 completely.
7. Elute the DNA by placing the column into a fresh 1.5 mL microcentrifuge tube and add 20 µL Buffer NE and incubate at room temperature for one minute before centrifuging for one minute at 11,000 xg.
8. Quantify the DNA using Qubit (see section 2.3)

2.5 Sequence library preparation and sequencing

The second round of PCR or ‘Index’ PCR was performed using the IDT Illumina DNA/RNA Unique Dual Indexes (Set A) kit (Illumina) to add molecular identification (MID) tags (unique 8-nucleotide sequences) and Illumina MiSeq sequencing adapters to the first round PCR products. In this process a unique Index 1 and Index 2 are added to each first round PCR product on a 96-well plate (Figure 4).

Index PCR

PCRs were set up in a total volume of 50 µL consisting of:

a. 25 µl 2x KAPA HotStart ReadyMix

b. 5 µl Nextera XT Index 1 Primers
c. 5 µL Nextera XT Index 2 Primers
d. 10 µL PCR grade water
e. 5 µL DNA

PCR cycling was as follows: an initial incubation for 3 minutes at 95°C; followed by 8 cycles with denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension step at 72°C for 5 minutes before holding at 4°C until collection of PCR products for analysis.

The indexed amplicons were quantified via a fluorometric method involving QuantiFluor dsDNA assay (Promega); and qualified using electrophoretic separation on the Agilent TapeStation 4200. This concentration and sizing information has been used to calculate the molarity of each sample. Libraries were then pooled in equimolar amounts to create one library for Illumina sequencing. The amplicon library pool was spiked with 20 % PhiX Control v3 library (Illumina) and run on the Illumina MiSeq using a MiSeq Reagent Kit v2 500 cycle kit (Illumina), to generate 250-bp paired-end reads. PhiX DNA is derived from the small, well characterized bacteriophage PhiX genome, it is a concentrated Illumina library (10 nM in 10 µl) that has an average size of 500 bp and consists of balanced base composition at ~45% GC and ~55% AT and serves as an in-run QC for the Illumina sequencing.

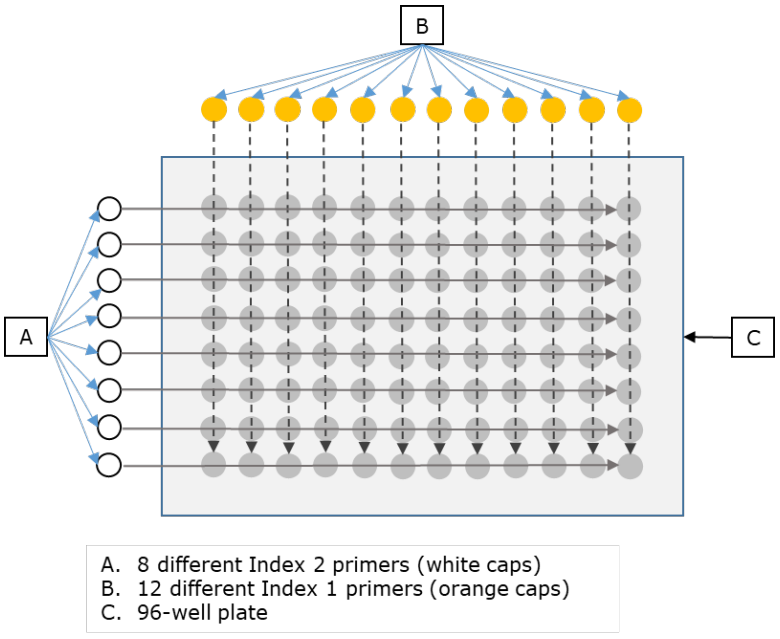


Figure 4. Dual indexing principle for Illumina sequencing. Index 2 primers are added across the plate (arrows) and the Index 1 primers are added down the plate (dashed arrows) resulting in 96 separate combinations of primers

2.6 Bioinformatic processing

Data processing was performed on an Intel i7 PC running Ubuntu Linux 20.04.4 LTS. In an initial step, paired end reads were trimmed using trimmomatic 0.39 (Bolger, Lohse and

Usadel, 2014) to remove Miseq adapters, to clip low quality and unpaired reads, and to truncate the sequence if the average phred score of a 5nt sliding window dropped below 25. An example command line to process a demultiplexed FASTQ sequence file was:

java -jar trimmomatic-0.39.jar PE R1_001.fastq R2_001.fastq read1_paired_R1_001.fastq read1_unpaired_R1_001.fastq read2_paired_R2_001.fastq read2_unpaired_R2_001.fastq ILLUMINACLIP:NexteraPE-PE.fa:2:30:10:2:True LEADING:3 TRAILING:3 MINLEN:36 SLIDINGWINDOW:5:25

Paired reads were merged using FLASH 1.2.11 (Fast Length Adjustment of SHort reads, Magnoc and Salzberg, 2011) to convert paired end reads (R1 and R2 in the MiSeq platform) to a single merged read using a minimum overlap of 80 nucleotides and a maximum of 150 nucleotides.

After converting DNA sequences from FASTQ format to FASTA format using SeqKit (Shen et al, 2016), template specific PCR primers at the 5' and 3' ends were removed using the "linked adapter" option of Cutadapt 3.5 (Martin, 2011) with a 10% error rate within the primer site i.e. 2 bp variants allowed per primer. Only trimmed sequences i.e. those containing both matching primer sequences were retained.

Before taxonomic assignment, standard Linux tools were used to identify 100% identical reads and condense them down to a single read to minimise time-consuming and repetitive BLAST searches, However, a record of the frequency of replicate sequences was maintained. Any reads with less than 5 replicates were excluded from the BLAST search.

A custom COI BLAST database was created from the National Centre for Biotechnology Information (NCBI) database using the search terms 'annelida', 'arthropoda', 'cnidaria', lophophorata', 'mollusca', 'nematoda', 'nematomorpha', 'platyhelminthes', 'porifera', 'rotifera', 'tardigrada', AND 'COI', OR 'cytochrome oxidase 1', NOT 'sea', NOT 'ocean' before downloading the records in FASTA format and combining to create a single database of 3.07 million sequences.

A custom 12S BLAST database was created from the National Centre for Biotechnology Information (NCBI) database using the search terms 'vertebrata' AND '12S' before downloading the records in FASTA format. A total of 190K sequences downloaded from NCBI were included in the final database.

BLAST searching was performed using the "megablast" program which is optimised to identify alignments in highly similar sequences and returned the top hit for each query sequence in a custom tabulated format. An e-value of 1e-15 was set; higher values such as 1 or 10 return a larger list of more low-scoring hits, and actual e-values returned were in the order of 1e-150 for a full-length alignment.

A custom perl script filtered the BLAST output, identifying hits sharing an accession number and passing a set of criteria covering the percentage similarity between the query sequence and the database sequence (typically 97%), and having a query alignment

length difference less than 6 bp. Read counts for each sequence passing the similarity and query alignment length filters were pooled based on accession number to generate a final frequency count for each accession.

3. Results

3.1 DNA extraction

DNA was extracted from individual samples and the DNA quantified (Table 2). Volumes of water sampled by the citizen scientists and the volume of recovered preservative were also recorded.

Table 2. Sample information. Some water volumes were not supplied by the citizen scientists and are marked as 'not supplied'. T1 and T2 were test sampling samples carried out by NHM staff

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
NEGP001	100	1ml	3.52	1.1	37
NEGP002	200	2.5ml	1.28	16.4	2.68
NEGP003	not supplied	2ml	0.91	1.25	7.52
NEGP005	200	150µl	0.338	11.5	13.9
NEGP006	200	1.5ml	2.86	14.3	61.1
NEGP007	85	1.5ml	2.66	4.3	44.5
NEGP008	200	200µl	0.82	12.4	19.3
NEGP009	not supplied	100µl	too low	2.9	20.9
NEGP010	not supplied	400µl	0.081	12.8	23.9
NEGP011	not supplied	0µl	0.68	10.4	14.6
NEGP012	200	1ml	0.37	6.3	67.1
NEGP013	300	1ml	2.59	10.4	19.3
NEGP014	300	500µl	1.98	14.6	6.08
NEGP015	200	500µl	0.91	15.8	30.2
NEGP016	600	2.2ml	5.71	4.18	15
NEGP017	220	300µl	3.24	6.03	34
NEGP018	80	1ml	21.5	5.85	8.55
NEGP020	175	3ml	3.87	14.1	54.1
NEGP021	100	2ml	2.86	2.9	11.5
NEGP022	200	200µl	1.23	7.3	86.1
NEGP023	220	2ml	17	14.3	19.4
NEGP024	90	2.2ml	0.105	13.7	29.5
NEGP025	100	500µl	28.7	3.12	33.9
NEGP026	200	100µl	0.113	3.48	7.61

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
NEGP027	350	600µl	23.5	14.3	46.4
NEGP028	not supplied	2.5ml	0.44	13	95.6
NEGP029	300	2.5ml	2.74	17.4	32.4
NEGP030	200	2.5ml	0.06	13.7	35.7
NEGP031	not supplied	2.5ml	0.48	11.2	2.87
NEGP032	200	2.5ml	0.819	12	54.3
NEGP033	200	1.8ml	1.16	5.31	32.7
NEGP034	not supplied	2ml	0.803	6.12	81.5
NEGP035	200	1.8ml	1.35	11.5	88.8
NEGP036	186	2.5ml	0.802	no PCR product	no PCR product
NEGP037	200	2.3ml	0.31	no PCR product	no PCR product
NEGP039	50	1ml	1.18	7.12	no PCR product
NEGP040	200	2ml	2.43	5.15	78.4
NEGP041	200	2.5ml	1.82	no PCR product	no PCR product
NEGP044	200	2.3ml	2.13	9.03	46.3
NEGP045	200	2ml	1.67	14.4	3
NEGP046	200	400µl	2.24	5.78	47.4
NEGP047	35	2.2ml	0.8	6.63	15.5
NEGP048	200	1.5ml	2.01	no PCR product	no PCR product
NEGP049	150	1ml	0.537	1.9	65.6
NEGP050	200	2.3ml	0.36	no PCR product	no PCR product
NEGP051	240	2ml	0.944	15.8	17
NEGP052	200	600µl	1.76	7.33	6.25
NEGP053	not supplied	200µl	0.534	2.68	70.2
NEGP054	150	3ml	1.69	no PCR product	no PCR product
NEGP055	200	500µl	4.36	10.4	32.5
NEGP056	240	2.3ml	5.43	16.1	36.7
NEGP057	125	500µl	4.73	2.68	53.3
NEGP058	200	2.2ml	0.399	8.57	42.5
NEGP059	135	400µl	8.42	17.9	26.5
NEGP060	200	200µl	0.59	10	86.8

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
NEGP061	200	2ml	0.567	7.03	76.8
NEGP062	140	2ml	2.29	17.8	51.8
NEGP064	not supplied	2.2ml	6.51	13.5	85.5
NEGP065	200	2.3ml	5.09	5.16	55.9
NEGP066	200	2.3ml	too low	2.06	12.3
NEGP068	200	2ml	0.325	15.8	39
NEGP069	200	2.3ml	0.06	no PCR product	30.2
NEGP070	200	2.5ml	4.74	15.6	22.5
NEGP071	not supplied	200µl	2.42	16.9	86.1
NEGP072	not supplied	400µl	0.319	12.7	49.9
NEGP074	34	1ml	9.82	6.19	49.2
NEGP075	not supplied	2ml	1.58	18.1	73.2
NEGP076	100	2.5ml	0.605	5.44	1.396
NEGP077	200	2.2ml	5.72	18	79.1
NEGP078	200	100µl	0.133	14.1	30.1
NEGP079	200	2ml	1.91	12.9	90.1
NEGP080	120	2.2ml	1.38	21.3	33.8
NEGP081	not supplied	600µl	7.29	9.54	17
NEGP084	200	2ml	0.504	no PCR product	no PCR product
NEGP085	250	100µl	2.34	13.5	19.7
NEGP085R	not supplied	500µl	1.85	13.2	39.8
NEGP086	350	500µl	0.72	16.2	9.96
NEGP087	250	500µl	2.42	7.4	61.1
NEGP088	400	100µl	0.249	10.8	49.2
NEGP089	200	2.5ml	0.87	7.01	102
NEGP090	120	2.3ml	1.56	11.4	28.1
NEGP091	200	200µl	0.328	8.13	33.1
NEGP092	200	2.5ml	5.23	4	16.4
NEGP093	200	2.5ml	0.155	5.47	67.3
NEGP094	50	2.1ml	1.95	11.8	19.2
NEGP095	200	2.5ml	3.39	5	90.2
NEGP096	170	800µl	0.98	9.55	no PCR product
NEGP097	200	200µl	2.1	12.6	6.56

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
NEGP098	207	500µl	1.16	14.9	20.4
NEGP099	200	100µl	0.053	2.86	13.7
NEGP104	130	2.5ml	4.17	14	2.97
NEGP105	not supplied	300µl	8.65	13.6	11.1
NEGP106	200	500µl	1.81	9.54	104
NEGP107	200	2ml	1.18	5.49	34.9
NEGP108	130	2.5ml	2.29	10.4	13
NEGP109	600	2.4ml	too low	18.3	86.9
NEGP110	200	1.6ml	0.349	8.13	95.4
NEGP111	200	2.4ml	0.458	7.4	62.8
NEGP112	200	1.5ml	1.21	10.8	13.4
NEGP113	200	2.5ml	9.48	5.56	11.1
NEGP114	200	500µl	0.823	17.3	65.8
NEGP115	160	400µl	9.85	11.8	38
NEGP116	200	2.5ml	2.43	5.86	74.9
NEGP117	220	500µl	0.824	6.4	52.5
NEGP118	60	2.5ml	8.52	15.1	65.1
NEGP119	180	600µl	1.35	10.7	23.8
NEGP121	200	0µl	0.215	1.66	113
NEGP122	200	1.5ml	1.45	10.5	12.7
NEGP123	200	500µl	1.11	1	48.6
NEGP124	200	400µl	3.48	7.56	33.6
NEGP125	200	500µl	0.3	0.47	37
NEGP129	200	300µl	0.564	15	44.6
NEGP130	60	2.3ml	1.6	6.84	no PCR product
NEGP131	200	2.4ml	1.38	14.6	56.8
NEGP132	not supplied	2ml	0.261	3.12	49.9
NEGP133	300	600µl	0.586	10.9	104
NEGP135	35	2.5ml	0.64	1.42	49.5
NEGP136	90	2.5ml	2.41	9.36	91
NEGP137	200	500µl	6.69	9.63	56.8
NEGP138	135	2.5ml	1.17	7.06	7.06
NEGP139	70	1.5 ml	6.44	11.6	17.4
NEGP142	200	2ml	0.199	6.7	58.4
NEGP143	210	2.2ml	0.737	19.2	78.4
NEGP145	200	200µl	2.61	10.5	1.93
NEGP147	200	2ml	0.285	6.53	67.5
NEGP148	200	2.3ml	0.693	9.02	78.6

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
NEGP149	190	2.4ml	0.832	no PCR product	no PCR product
NEGP150	70	2ml	3.87	9.54	44.2
NEGP151	200	2.5ml	0.381	6.6	69.3
NEGP152	200	200µl	1.09	17.2	67.5
NEGP153	240	2.1ml	0.965	7.38	23.5
NEGP154	160	1ml	0.922	7.72	16.8
NEGP155	200	200µl	0.278	12.1	85.5
NEGP156	200	400µl	too low	4.27	11.3
NEGP157	200	1.5ml	0.238	3.04	4.2
NEGP158	not supplied	400µl	0.623	9.8	16.7
NEGP159	110	2.1ml	11.7	16.4	87.5
NEGP162	200	2.3ml	2.19	9.02	103
NEGP163	200	200µl	1.09	3.07	28.4
NEGP164	150	200µl	3.55	8.43	48.5
NEGP165	300	100µl	0.76	10	75.3
NEGP167	not supplied	1ml	1.1	7.73	5.63
NEGP169	85	2ml	1.5	14.6	66.2
NEGP174	200	700µl	1.44	9.86	68.6
NEGP176	not supplied	400µl	1.03	7.87	53.9
NEGP178	175	2ml	6.37	15.4	12.7
NEGP179	200	2.5ml	1.18	10.5	29.6
NEGP180	200	2.5ml	too low	15.5	5.17
NEGP181	1200	700µl	0.096	6.85	52.3
NEGP182	150	400µl	0.813	2.52	70.8
NEGP183	200	2.4ml	0.961	13.3	20.8
NEGP187	not supplied	1ml	0.57	6.07	35.9
NEGP188	200	2ml	1.89	9.67	85.7
NEGP189	250	200µl	1.06	19.2	80
NEGP190	250	2ml	1.63	17.7	59.7
NEGP191	200	150µl	0.085	9.41	19.3
NEGP192	200	150µl	1.6	8.22	77.2
NEGP194	not supplied	500µl	0.46	16.6	14
NEGP195	not supplied	400µl	1.36	18.3	29.9
NEGP197	100	2.5ml	2.64	9.05	23.4
T1	200	1.5ml	0.137	7.46	97

Kit ID	Volume of water filtered (mL)	Volume preservative recovered (approx)	DNA concentration (ng/µl)	DNA concentration after COI (invertebrate) PCR amplicon clean up (ng/µl)	DNA concentration after 12S (vertebrate) PCR amplicon clean up (ng/µl)
T2	200	1ml	2.97	13.6	52

3.2 Metabarcoding PCR and library production

Of 160 returned samples, PCR set up using a non-proof reading Taq was not able to amplify 9 samples with the COI primers and 11 samples with the 12S primers (Table 2). 8 of these samples were in common in that we were not able to amplify the samples with either the COI or the 12S primers. Several of the amplified COI products produced a double band when run on agarose gels, therefore all COI amplicons were gel extracted and purified prior to indexing PCR. For those that failed to amplify with COI and/or 12S primers the PCRs were repeated twice more to attempt to get a useable product.

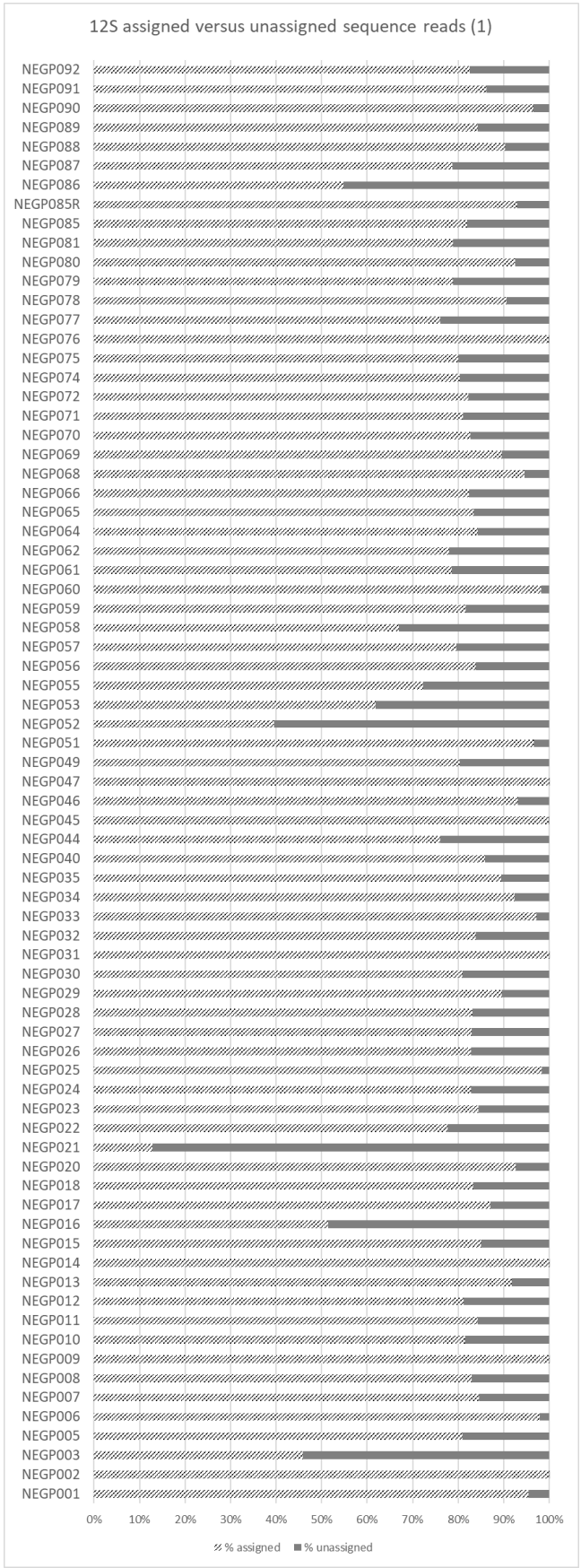
Initial results suggested that the second round PCR or 'indexing' PCR was very inefficient. All primer sequences were cross checked against both the suppliers notes to ensure that the sequence had been synthesised correctly. The primers passed all these checks. In addition, it demonstrated that the amplicons contained the correct PCR adaptors for the second round PCR. It was also discussed whether the Taq polymerase used was not suitable for generating the indexed products or if the purification system that had been used was somehow interfering with the indexing PCR. An extensive troubleshooting of the indexing PCR was carried out with different Taq polymerases and purification methods, a higher number of PCR cycles, and a higher starting concentration of first round PCR amplicons. This troubleshooting of the indexing PCR found that the use of a non-proof reading Taq polymerase rather than the standard proof reading Taq polymerase used by the metabarcoding laboratory (Kapa HiFi mastermix, Roche Molecular Systems) was necessary to allow for efficient index PCR amplification.

3.3 Bioinformatics and data analysis

Quality control reports generated by the sequencing service showed that returned sequences were of good quality with all samples having a Q30 of above 70% (most Illumina runs will generate >70-80% Q30 data) and mean quality scores above 30 (a measure of base calling accuracy equivalent to the probability of an incorrect base call 1 in 1000 times).

3.4 Sequencing results per sample

For the 12S analysis, a total of 7.97M sequences (~88% of sequences) were assigned a taxonomic identification which represented 61 species including fish, birds, mammals, and amphibians. A total of 1.1M sequences (~12% of sequences) were unassigned. The percentage of assigned and unassigned sequence reads per pond are shown in figure 5 (1 and 2). Results are shown in Table 3 and Appendix 1. Ponds contained between one and 17 species of vertebrate with an average of 4 species per pond. The most commonly found species were human (118 samples) and common frog (72 ponds) with 20 other species only being found in one pond sample including the fish species tench, roach and chub. A total of 16 fish species were found the most common of which being goldfish. A total of 28 species of bird were found including sparrows, robins, moorhens and coots indicating that both common garden bird species and waterfowl could be found using metabarcoding. Of the UK's seven amphibian species, the ponds were found to contain common frog, smooth and palmate newts, and common toad but not pool frogs, great crested newts, or natterjack toads. A total of 11 mammal species were found with dog and red fox being most commonly found after human. Metabarcoding was also able to detect badger, wood mouse and squirrel within pond samples. For the full list of 12S metabarcoding results, please see Appendix 1.



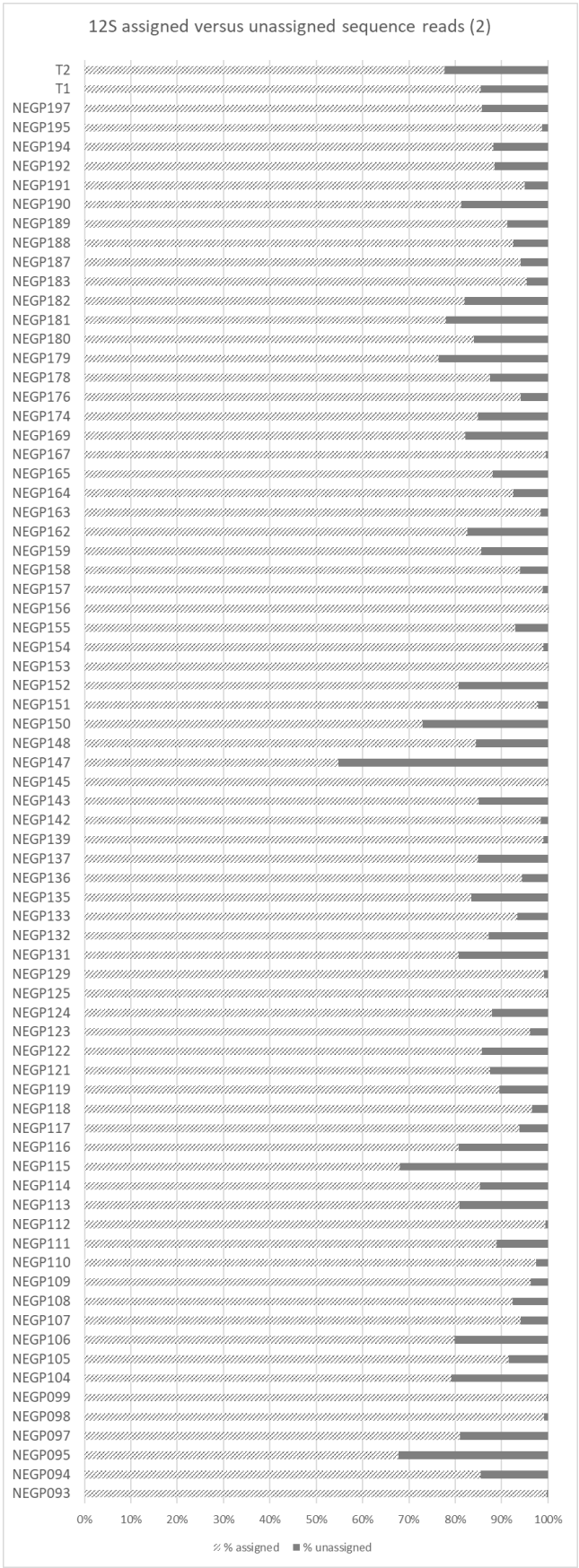


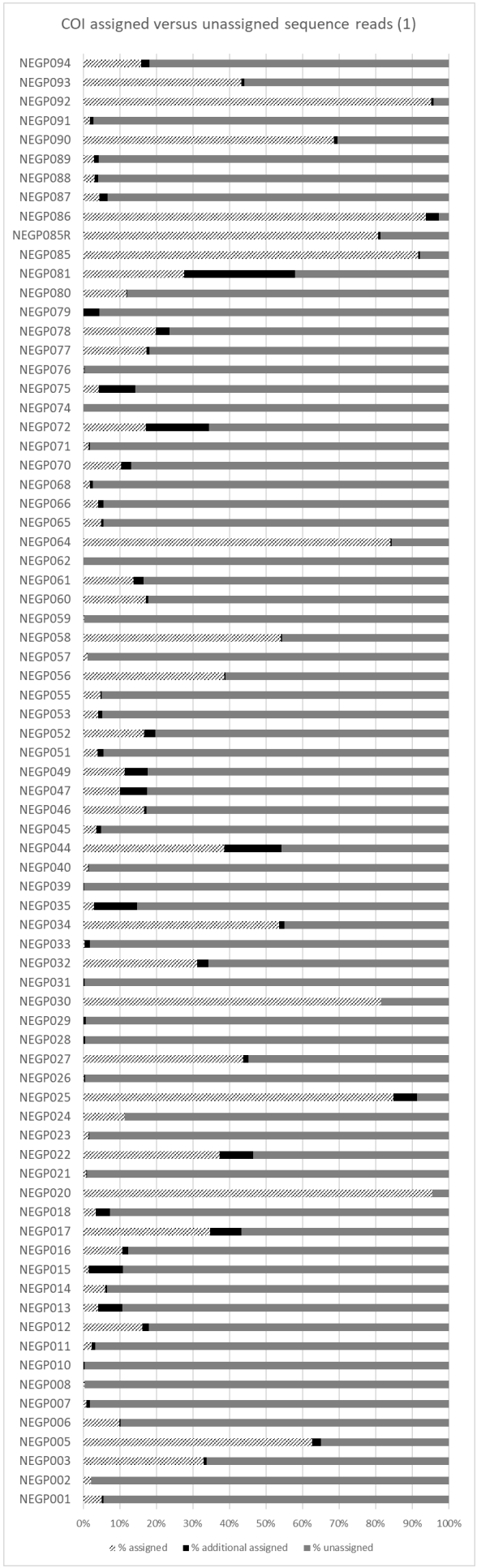
Figure 5 (1 and 2). 12S proportion of assigned and unassigned sequence reads.

Species	Common name	Number of ponds	Species	Common name	Number of ponds
Homo sapiens	Human	118	*Apodemus sylvaticus*	European woodmouse	4
Rana temporaria	Common frog	72	*Pungitius pungitius*	Ninespine stickleback	4
Columba livia	Rock dove	31	*Bufo bufo*	Common toad	3
Turdus merula	Common blackbird	29	*Rattus norvegicus*	Brown rat	3
Canis lupus familiaris	Domestic dog	28	*Scardinius erythrophthalmus*	Rudd	3
Cyprinus sp.	Carp sp.	26	*Troglodytes troglodytes mosukei*	Eurasian wren	3
Turdus philomelos	Song thrush	25	*Anas penelope*	Eurasian widgeon	2
Carassius auratus langsdorfi	Goldfish	23	*Perca fluviatilis*	European perch	2
Vulpes vulpes	Red fox	22	*Phasianus colchicus*	Ring-necked pheasant	2
Parus major	Great tit	16	*Abramis brama*	Common bream	1
Oncorhynchus mykiss	Rainbow trout	15	*Aythya fuligula*	Tufted duck	1
Passer domesticus	House sparrow	15	*Chroicocephalus ridibundus*	Black-headed gull	1
Pica pica	Eurasian magpie	15	*Cygnus olar*	Mute swan	2
Carduelis carduelis	European goldfinch	13	*Emberiza citrinella*	Yellowhammer	1
Sus domesticus	Domestic pig	13	*Fingilla coelebs*	Chaffinch	1
Columba sp.	Dove/pigeon sp.	12	*Fringilla montifringilla*	Brambling	1
Erithacus rubecula	Eurasian robin	10	*Fulica atra*	Eurasian coot	1
Gallinula chloropus	Common moorhen	10	*Gobio gobio*	Gudgeon	1
Gallus domesticus	Domestic chicken	9	*Leuciscus idus*	Ide	1
Anas platyrhynchos	Mallard	8	*Meles meles*	European badger	1
Corvus sp.	Corvus sp.	7	*Microtus agrestis*	Short-tailed field vole	1
Lissotriton helveticus	Palmate newt	7	*Parus ater*	Coal tit	2
Bos taurus	Domestic cattle	6	*Phalacrocorax carbo*	Common cormorant	1
Cyprinus carpio carpio	Eurasian carp	5	*Phoxinus phoxinus*	Minnow	1
Felis catus	Domestic cat	5	*Rutilus rutilus*	Common roach	1

Species	Common name	Number of ponds	Species	Common name	Number of ponds
Gasterosteus aculeatus	Three-spined stickleback	5	*Squalius cephalus*	European chub	1
Lissotriton vulgaris	Smooth newt	5	*Sylvia atricapilla*	Eurasian blackcap	1
Sciurus carolinensis	Eastern grey squirrel	5	*Tinca tinca*	Tench	1
Alburnus alburnus	Common bleak	4			

Table 3. Number of ponds containing each species (vertebrates) using 12S primers.

For the COI analysis, a total of 1.73M sequences (~23% of sequences) were assigned a taxonomic identification which represented 164 to the species level (84% of assigned sequences) and a further 92 to the genus level (16% of assigned sequences). These represented annelids, arthropods, cnidaria, molluscs, nemertea, rotifers and tardigrades. A total of 6M sequences (~77% of sequences) were unassigned. The percentage of assigned and unassigned sequence reads are shown in Figure 6 (1 and 2). Results are shown in Table 4 and Appendix 2. Ponds contained between 1 and 35 species of invertebrate with an average of 12 species per pond i.e. three times the average number of vertebrates found in each pond sample. A comparison of the number of vertebrate and invertebrate species per pond was performed and is shown in Figure 7 (1 and 2).



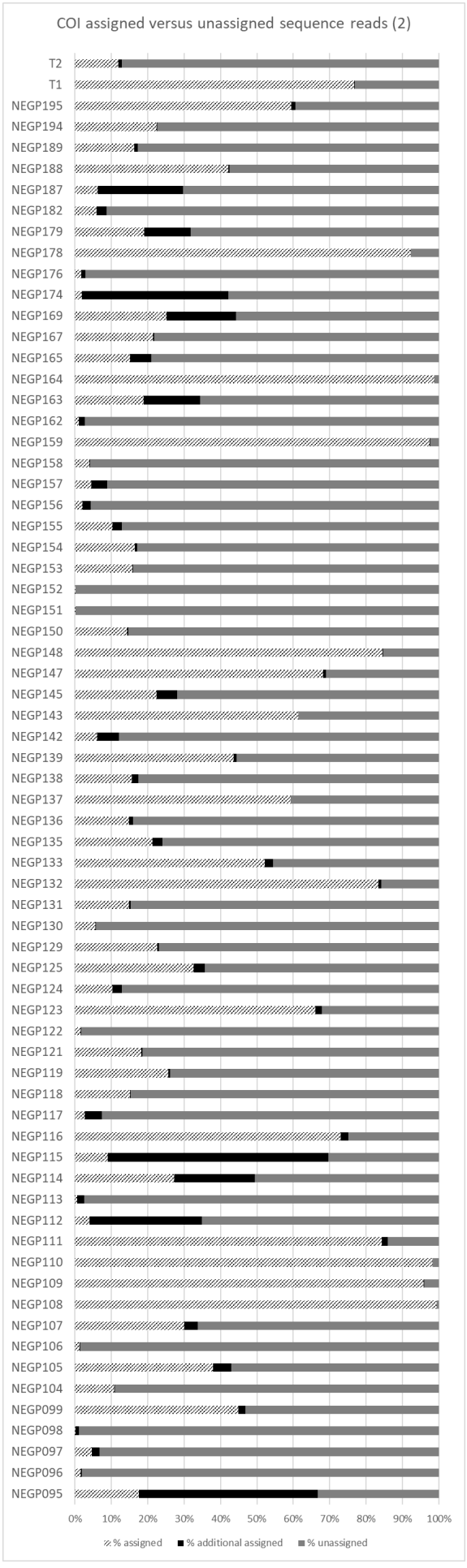


Figure 6 (1 and 2). COI proportion of assigned and unassigned sequence reads.

The most commonly found species were the copepod *Acanthocyclops vernalis* and the barklice species *Ectopsocus briggsi* with 172 other species only being found in one pond (Table 4). For the full list of CO1 metabarcoding results, please see Appendix 2.

Table 4. Number of ponds containing species from each invertebrate family

Family	Number of ponds containing the family	Family	Number of ponds containing the family
Cyclopidae	93	Dytiscidae	3
Lumbricidae	79	Staphylinidae	3
Nadide	78	Drosophilidae	3
Chironomidae	72	Fanniidae	3
Ectopsocidae	46	Muscidae	3
Chydoridae	42	Psychodidae	3
Philodinidae	41	Psyllidae	3
Asellidae	35	Libellulidae	3
Hydroptilidae	28	Acroloxidae	3
Enchytraeidae	26	Stigmaeidae	2
Canthocamptidae	23	Tydeidae	2
Lymnaeidae	23	Geophilidae	2
Physidae	23	Lithobiidae	2
Tomoceridae	22	Curculionidae	2
Culicidae	20	Tenebrionidae	2
Lumbriculidae	19	Trichoceridae	2
Katiannidae	19	Formicidae	2
Baetidae	18	Tortricidae	2
Isotomidae	16	Trogiidae	2
Aphididae	16	Unionidae	2
Hydridae	16	Lineidae	2
Crangonyctidae	15	Testudinellidae	2
Daphniidae	14	Hydrozetidae	1
Tipulidae	14	Liacaridae	1
Hypogastruridae	13	Micreremidae	1
Entomobryidae	13	Himantariidae	1
Cicadellidae	12	Neelidae	1
Noctuidae	12	Brachystomellidae	1
Syrphidae	10	Neanuridae	1
Cyprididae	10	Bourletiellidae	1
Sphaeriidae	10	Sminthurididae	1
Agriolimacidae	10	Orchesellidae	1
Euchlanidae	10	Diaptomidae	1
Simuliidae	9	Phyllognathopodidae	1
Caeciliusidae	8	Agromyzidae	1
Arionidae	8	Anisopodidae	1
Synchaetidae	8	Calliphoridae	1
Hypsibiidae	8	Ephydridae	1
Eupodidae	7	Keroplatidae	1

Family	Number of ponds containing the family	Family	Number of ponds containing the family
Dicyrtomidae	7	Mycetophilidae	1
Sciaridae	7	Phoridae	1
Carabidae	6	Sciomyzidae	1
Lecanidae	6	Sphaeroceridae	1
Erpobdellidae	5	Issidae	1
Humerobatidae	5	Notonectidae	1
Lauxaniidae	5	Triozidae	1
Candonidae	5	Braconidae	1
Helicidae	5	Blastobasidae	1
Hygromiidae	5	Tineidae	1
Milacidae	5	Coniopterygidae	1
Planorbidae	5	Coenagrionidae	1
Tubificidae	4	Gryllidae	1
Phalangiidae	4	Elipsocidae	1
Macrothricidae	4	Stenopsocidae	1
Chaoboridae	4	Trichoniscidae	1
Limoniidae	4	Dytiscidae	1
Tachinidae	4	Bithyniidae	1
Limnephilidae	4	Limacidae	1
Arcitalitridae	4	Testacellidae	1
Brachionidae	4	Pachychilidae	1
Ameronothridae	3	Flosculariidae	1
Cyzicidae	3	Asplanchnidae	1
Coccinellidae	3		

When the bioinformatics was repeated with a COI database containing both vertebrate and invertebrate data a further 138 species/genus were identified. For the full list of additional COI metabarcoding results please see Appendix 3.

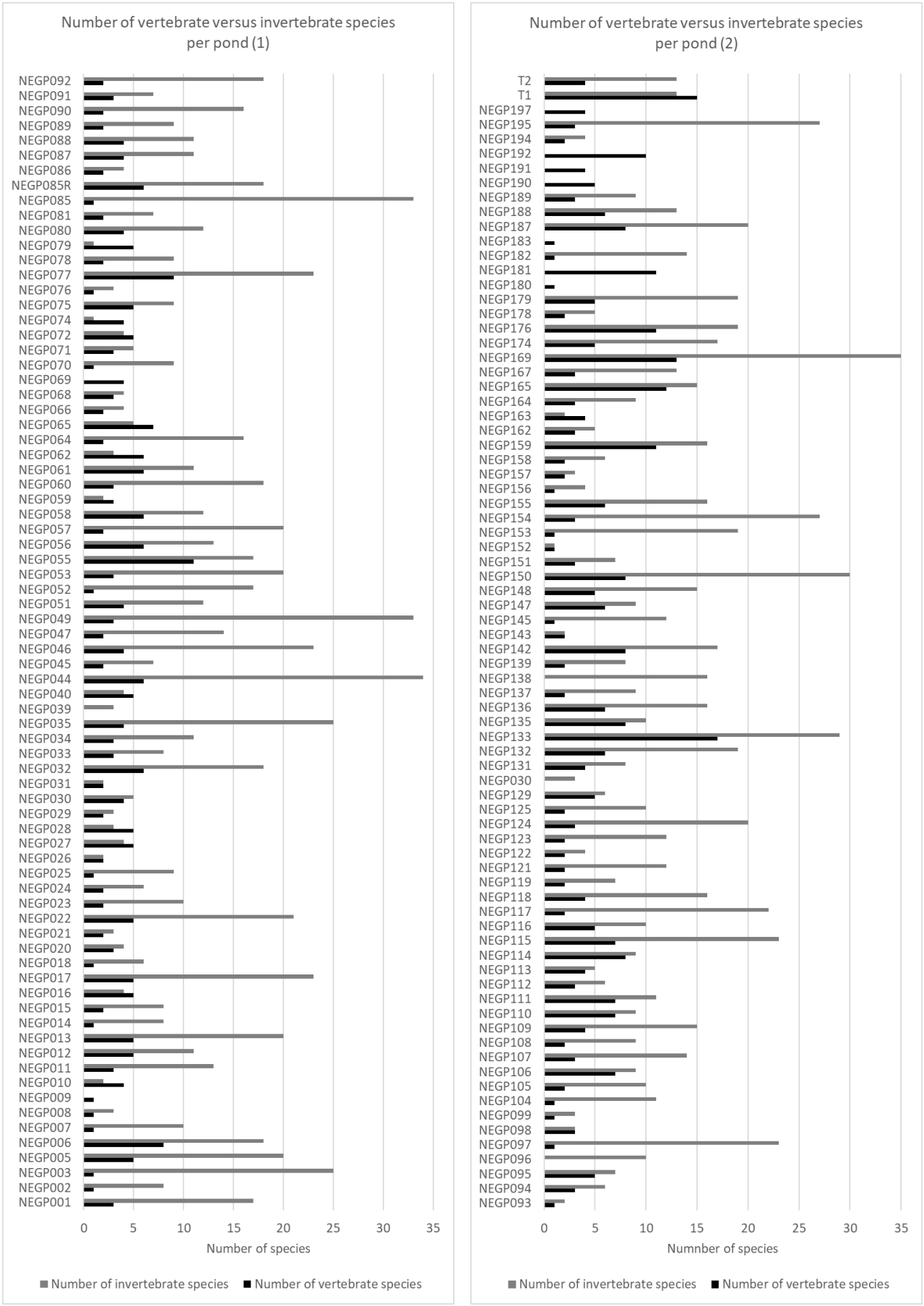


Figure 7. Comparison of the number of vertebrate and invertebrate species per pond.

4. Discussion

This work was undertaken to determine the species residing in garden ponds in Bristol, London, and Newcastle using a metabarcoding approach. At the outset of the project citizen scientists were recruited by The Natural History Museum to take part in the study. Sampling kits were then sent out to the citizen scientists via Royal Mail and their arrival was tracked using the Royal Mail tracking service. Feedback from citizen scientists stated that sampling kits should not have been distributed around the Christmas period which in hindsight was a good point although this was constrained by the timing of the project. The Royal Mail tracking system did not work in all cases, so it is not clear whether some citizen scientists actually received their kits or not, although there were instances where samples were returned to ADAS despite no tracking information on citizen scientists receiving the kits. On the other hand, some samples were not returned by citizen scientists despite tracking information showing that the kits were received. Despite these points around 80% of kits were used and samples returned to ADAS for analysis. Although the sampling instructions appeared to be clearly understood by the citizen scientists further feedback raised concerns over the difficulty of pushing the pond water through the Sterivex filters and also pushing the preservation solution into the filter unit. It is known that pushing water through these filters can be difficult however a larger pore-sized filter could not be sourced at the time of the study. Self-funded work since this study was carried out has indicated that an alternative type of filter with a 0.8 µM pore size allows far more water to be filtered due to a reduced rate of clogging and the filtering is much easier to perform. Macrofauna studies have shown that the efficiency of filtering a larger volume of water through a larger pore sized filter is better than when filtering less water through a smaller pore sized filter (Hosler 2017, Sepulveda et al. 2019). The use of a larger pore sized filter would also not result in a large loss of small sized particles containing eDNA as eDNA loss for pore sizes up to 1 µm has been shown to be minimal (Turner et al. 2014, Wilcox et al. 2015). The addition of the preservative solution to the filter may also be easier when using a larger pore sized filter as the filter is unlikely to be clogged up with any sediment/particles present in the pond which can in turn make it difficult to push the preservative solution into the filter. Some citizen scientists reported that the preservative solution leaked back out of the filter unit indicating that a fuller instruction might be needed in the protocol at this step in the water sampling procedure. Should the study be repeated a larger pore sized filter product is recommended to attempt to alleviate both concerns.

Where possible this project followed previous examples of similar metabarcoding work, instead of designing and trialling new PCR primers, which was beyond the scope of this project. Primers that had previously been described (and are in widespread use) were used to generate PCR amplicons from each sample (Vamos et al 2017; Riaz et al 2011). These primers were selected after discussions with the project members (B. Price personal communications). In carrying out the first round PCR the aim was to capture as much of the sequence diversity as possible that is contained within the samples. The primers used are therefore degenerate that is they contain variations at some of the nucleotide positions within primer sequence (Table 1).

Metabarcoding identified a total of 317 species across the pond samples analysed with approximately 75% of these being invertebrate species. For vertebrates and invertebrates, the number of unassigned sequences corresponded to 12% and 77% of the total number of reads respectively although this ranged from 0.01% to 87.16% for 12S individual pond sequence data and 0.60% to 99.67% for COI individual pond sequence data (Figure 6, 1 and 2). It is known that for the invertebrate primers used around 40% of sequences will usually be assigned to a species (B. Price, personal communication), therefore in this study there were more unassigned sequences than expected and it is not clear why this should be the case. Unassigned reads are due to two factors: firstly, only having created databases containing vertebrate 12S or invertebrate COI sequences; and secondly due to a lack of sequence data availability. The lack of sequence data availability is a known problem which was recently reviewed in Macadam et al. (2020) where a gap analysis was carried out. The bioinformatics was repeated with vertebrate and invertebrate COI or 12S databases to attempt to decrease the number of unassigned reads. No other species were assigned for the 12S primer generated sequences but for the COI primer generated sequences this allowed a further 138 species/genus to be identified (Appendix 3). Over half of these (77) were oomycetes: 23 were Phytopthera sp.; and 45 were Pythium sp. There were a few invertebrates not found previously (14), it is unclear as to why these were not found previously as all sequences were submitted to Genbank prior to the previous search. The remaining species/genus were algae, amoeba, diatoms, fungi, rotifers, and water moulds.

Remaining unassigned sequences were due to a lack of available sequencing data. Although Genbank contains sequence information on more than 270,000 species (Hinchliffe and Smith, 2014) it has been estimated that this figure may only account for 3% of Earth's species (Mora et al. 2011) so it is inevitable that there will be many unassigned sequences in any metabarcoding study. It is also the case that invertebrate species are particularly poorly represented in reference databases – explaining the fact that far more of the invertebrate data was unassigned compared to vertebrates. Over time gaps in sequence databases will be alleviated by the efforts of projects such as the Darwin Tree of Life project which aims to sequence the DNA barcodes and full genomes of all 66000+ described UK species. Over the coming years as more sequence data becomes available it would be useful to repeat the data analysis to investigate whether more of the sequence data generated in this study can be assigned to species or genus.

The results obtained in the study illustrate that although many species were identified, the breadth of species was not entirely as expected. We would expect to see common species such as the common pond skater (*Gerris lacustris*) and lesser water boatman (*Corixa punctata*) which are widespread throughout the UK, but which were not found here at all. Whereas the lesser water boatman can be seen in ponds all year round, the common pond skater, flies away from ponds in the autumn to hibernate before emerging again in April indicating that for this common species at least, the timing of the sampling in winter was not ideal. In a good wildlife pond there should also be lots of different kinds of water beetles (around 350 species in the UK) and according to the Freshwater Habitats Trust 'between a third and a half of all the species of animals you can see will be water beetles'. This was not found to be the case in this study as for example the common black diving

beetle (*Agabus bipustulatus*) was only found in three of the ponds and this species can be found in ponds all year round. Of other species that are expected to be in ponds, the pond olive mayfly (*Cloeon dipterum*) was found in around 20 of the ponds (around one tenth of the ponds surveyed), although it has been suggest that across Britain they are found in two fifths of all ponds (Freshwater Habitats Trust). Dragonflies, damselflies and caddisflies were also found in fewer ponds than expected.

In terms of vertebrates, several amphibian species were seen: newts (smooth and palmate); frogs (common); and toads (common). Newt species found in the UK can overwinter in ponds but are known to hibernate underground, among tree roots and in old walls over the winter. Therefore, a possible explanation for finding smooth and palmate newts but not the great crested newt could be due to their relative numbers in the UK with the great crested newt population having seen significant declines in recent decades. This species would be more likely to be found if sampling took place during the spring/summer. This is also likely the explanation for finding common toads but not Natterjack toads in the ponds surveyed as this species is only found in a handful of sites in the UK.

Several species of bird were found as often the small birds that visit our gardens use small ponds to drink and bathe. It was noted by Genepools partner, Phil Davidson, that there are relatively few 12S sequences for birds within Genbank and this could in part be an explanation for some expected species, such as wood pigeon and blue tit, not being found. Several ponds were found to contain DNA from Red Jungle fowl but as this species was unlikely to be found and the 12S sequence was very similar to that of domestic chicken these records were amended in our results. This was also the case for domestic pig, which initially was found to be wild boar which again is very unlikely to appear in garden ponds.

There was a lot of contamination of samples by human DNA accounting for 45% of all assigned reads and in the worst case (NEGP153) accounting for 99.99% of the sequence reads in the sample. This may be inevitable when looking at water samples in close proximity to where people live, although it could also be due to cross-contamination during sample collection. In order to reduce this human DNA contamination, it is possible to use blocking primers (Seyama et al., 1992; Vestheim and Jarman, 2008; Craig et al., 2014) which effectively prevent human DNA from being amplified during the first round PCR as they contain a 3’ end modification which prevents enzymatic elongation of the primer. As a next step, should this study be repeated, then human blocking primers should be found/developed for both gene targets. This should allow for more efficient amplification of the other species within the sample thus giving us more information about the invertebrate and vertebrate communities within the samples.

It is possible that PCR amplification biases may have led to some species within the pond community not being identified during this study. For the COI primers (Vamos et al. 2017), it was noted by the authors that the primer set Fwh2 used here showed a higher primer bias than other primer sets developed in their study (Fwh1). However, the Fwh2 primer set was still able to identify 98% of the freshwater insects present within the mock communities used, with Perlidae species (stoneflies – the majority of which are found in North America) being underrepresented. For the 12S primer set, although biases are not

specifically discussed by the authors, they were able to amplify 98% of a sequence training set used and when tested on faeces for diet analysis were able to identify both predator and prey species. PCR amplification biases can cause a species DNA that is present in the sample not being detected in the sequencing results. Different species' DNA are in competition to bind to the universal primers, and this competition can prevent the effective amplification of all species present as more common template DNAs are more likely to be amplified (Kelly et al. 2014). This can mean that high abundance species can prevent the detection of low abundance species resulting in 'species masking' (Brandon-Mong et al. 2015; Evans et al. 2016; Kelly et al. 2014). Metabarcoding may therefore be less capable of identifying the DNA of less abundant species within a community than a species-specific qPCR.

The positive control species, *Allolobophora chlorotica* DNA (earthworm) for COI PCRs or *Oncorhynchus mykiss* (rainbow trout) for 12S PCRs were found in 15 and 13 of the samples respectively. For rainbow trout it is unlikely that this species would be found in garden ponds therefore this is possibly a result of contamination and/or barcode leakage, the number of sequences corresponding to rainbow trout ranged from 5 to 7383 accounting for 0.05 to 10.73% of the total number of sequences. For earthworms these results are again likely to be due to contamination and/or barcode leakage although it is possible that earthworms could get into ponds, with the number of sequences corresponding to earthworm ranging from 5 to 508 accounting for 0.02 to 1% of the total number of sequences. In hindsight, the earthworm was not the best positive control to use for this work as it could potentially be found in ponds. We therefore do not know if the earthworm DNA found was as a result of contamination or it really occurred in the samples. A more suitable positive control may be a species only found in salt water for example that would definitely not be found in garden ponds.

Several of the samples could not be amplified with COI and/or 12S primers. Nine samples were not amplified with the COI primers and 11 samples were not amplified with the 12S primers. Of these 8 were not amplified by either COI or 12S primers and in fact were also not amplified using 16S or 18S primers in the Cefas laboratories (one additional sample was not amplified with 16S or 18S primers). Although these samples all had low DNA concentrations after DNA extraction (0.36 to 2.01 ng/µL) there were a total of 79 samples with DNA concentrations in this range, of which 7 were not amplified using the COI primers and 10 were not amplified using 12S primers so DNA concentration does not explain the lack of amplification. This was also the case when the volume of water filtered, or the volume of preservative solution recovered was considered. The chemical analysis carried out on the water samples also did not reveal any explanations as to why these samples failed to amplify with one or more primer set. Tests were carried out to investigate the potential for inhibition of the PCR amplifications due to inhibitors in the extracted DNA, however again, there was no effect of inhibitors therefore it remains unknown as to why amplification of these samples was not possible.

This project has successfully used DNA metabarcoding on freshwater samples collected by citizen scientists, to produce species data for vertebrate and invertebrate communities in urban ponds in Bristol, London and Newcastle. Winter was not an ideal season for

sampling for many species including amphibians which often leave the ponds for terrestrial habitats in the autumn, and for mammals which would usually hibernate. Many invertebrate species will overwinter as eggs or may burrow into the mud which would not have been sampled and therefore not allow their detection. In order to get a more complete representation of the communities within ponds sampling needs to be carried out in the spring/summer when the pond life is more active (or even multiple times during the year depending on the question/s being asked).

Although this report does not contain data on prokaryotes and eukaryotes (as this work was carried out by Cefas), all results from this study will be shared with the citizen scientists who collected the samples during the course of 2022 as and when the data analysis has been completed. The results will be shared as a report to citizen scientists and as a web based R-shiny app which will present the individual pond data as interactive sun-burst plots. It is also intended that comparisons will be made between ponds in each area (Bristol, London, and Newcastle).

5. Recommendations

- We would recommend that human blocking primers are applied, or designed, to prevent the amplification of human DNA which can swamp the other DNA present, and preferentially amplify thus potentially blocking the amplification of other species present.
- We would recommend the sampling being carried out over the spring/summer months as more species should be found at this time.
- Posting of kits/samples around the Christmas period should be avoided; this led to prolonged periods in transit and kits not arriving in time for citizen scientists to take samples, and/or samples not arriving in time to take part in the analysis.
- We also recommend that a filter with a larger pore size e.g. 0.45µm or 0.8 µm and/or a larger pore-sized pre-filter should be used should this study be repeated. This will help to reduce the effects of clogging and should allow a larger volume of water to be filtered which could help to improve the results by increasing the chances of detecting low abundance or low-copy number species.
- Care should be taken when choosing the positive controls to use in COI and 12S PCR amplifications. Species used as positive controls should be those which are unlikely to appear in the actual samples so that if they appear in the actual data they can be removed and further steps taken to reduce cross-contamination and/or barcode leakage.

6. References

BIGGS, J., EWALD, N., VALENTINI, A., GABORIAUD, C., DEJEAN, T., GRIFFITHS, R.A.,...BROTHERTON, P. (2015). Using eDNA to develop a national citi-zen science-

based monitoring programme for the great crested newt (Triturus cristatus).Biological Conservation,183,19–28

BOLGER AM, LOHSE M, USADEL B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. BIOINFORMATICS. 30(15):2114-20. DOI:10.1093/BIOINFORMATICS/BTU170.

CRAIG, C., KIMMERER, W. J., AND COHEN, C. S. (2014). A DNA-based method for investigating feeding by copepod nauplii. J. Plankton. Res. 36, 271–275. doi: 10.1093/plankt/fbt104

DEINER, K., FRONHOFER, E.A., MACHLER, E., WALSER, J.-C., & ALTERMATT, F. (2016). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications, 7, 12544.

DEINER, K., BIK, H.M., MÄCHLER, E., SEYMOUR, M., LACOURSIÈRE-ROUSSEL, A., ALTERMATT, F., CREER, S., BISTA, I., LODGE, D.M., DE VERE, N., PFRENDER, M.E., BERNATCHEZ, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol Ecol, 26: 5872-5895. https://doi.org/10.1111/mec.14350

DRUMMOND, A. J., NEWCOMB, R. D., BUCKLEY, T. R., XIE, D., DOPHEIDE, A., POTTER, B. C.,… GROSSER, S. (2015). Evaluating a multigene environmental DNA approach for biodiversity assessment. GigaScience, 4, 1.

HAJIBABAEI, M., SHOKRALLA, S., ZHOU, X., SINGER, G. A., & BAIRD, D. J. (2011). Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. PLoS ONE, 6, e17497.

HINCHLIFFE, C. E., & SMITH, S. A. (2014). Some limitations of public sequence data for phylogenetic inference (in plants). PloS one, 9(7), e98986. https://doi.org/10.1371/journal.pone.0098986

HOSLER, D. (2017). Where is the body? Dreissenid mussels, raw water testing, and the real value of environmental DNA. Management of Biological Invasions: International Journal of Applied Research on Biological Invasions 8: 335–341. https://doi.org/10.3391/mbi.2017.8.3.07

Kelly, R.P., Port, J.A., Yamahara, K.M. & Crowder, L.B. (2014) Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE, 9, e86175.

ILLUMINA (2012). 16S Metagenomic Sequencing Library Preparation *Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System.*

MACADAM, C., ROBINS, J. & THOMSON, T. (2020). 2020 Gap Analysis of the BOLD Database for Key English Invertebrates, Natural England Commissioned Report Number 32.

MACHIDA, R.J., KNOWLTON, N. (2012a) PCR Primers for Metazoan Nuclear 18S and 28S Ribosomal DNA Sequences. PLoS ONE 7(9): e46180.

MACHIDA, R.J., KWESKIN, M., KNOWLTON, N. (2012b) PCR Primers for Metazoan Mitochondrial 12S Ribosomal DNA Sequences. PLoS ONE 7(4): e35887.

MAGOC, T. & SALZBERG, S. (2011) Fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:21, 2957-63.

MARGULIES, M., EGHOLM, M., ALTMAN, W. E., ATTIYA, S., BADER, J. S., BEMBEN, L. A.,... CHEN, Z. (2005). Genome sequencing in microfabricated high-density picolitre reactors. Nature, 437, 376–380.

MARTIN, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal, 17(1), 10-12.

MORA, C., TITTENSOR, D.P., ADL, S., SIMPSON, A.G.B., WORM, B. (2011). How Many Species Are There on Earth and in the Ocean? PLoS Biology 9: e1001127.

MURRAY, D. C., PEARSON, S. G., FULLAGAR, R., CHASE, B. M., HOUSTON, J., ATCHISON, J.,... MACPHAIL, M. (2012). High-throughput sequencing of ancient plant and mammal DNA preserved in herbivore middens. Quaternary Science Reviews, 58, 135–145.

REES, H.C., MADDISON, B.C., MIDDLEDITCH, D.J., PATMORE, J.R.M., GOUGH, K.C. (2014). The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. Journal of Applied Ecology, 51, 1450-1459. DOI: 10.1111/1365-2664.12306

RIAZ, T., SHEHZAD, W., VIARI, A., POMPANON, F., TABERLET, P., COISSAC, E. (2011). ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acid Research, 39, e145. DOI: 10.1093/nar/gkr732

SEPULVEDA, A.J., SCHABACKER, J., SMITH, S., AL-CHOKHACHY, R., LUIKART, G., AMISH. S.J. (2019). Improved detection of rare, endangered and invasive trout in using a new large-volume sampling method for eDNA capture. Environmental DNA 1: 227–237. https://doi.org/10.1002/edn3.23

SEYAMA, T., ITO, T., HAYASHI, T., MIZUNO, T., NAKAMURA, N., AND AKIYAMA, M. (1992). A novel blocker-PCR method for detection of rare mutant alleles in the presence of an excess amount of normal DNA. Nucleic Acids Res. 20, 2493–2496. doi: 10.1093/nar/20.10.2493

SHEN, W., LE, S., LI, Y., HU, F. (2016) SEQKIT: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. PLoS ONE 11(10): e0163962. https://doi.org/10.1371/journal.pone.0163962

TURNER, C.R., BARNES, M.A., XU, C.C.Y., JONES, S.E., JERDE, C.L., LODGE, D.M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods in Ecology and Evolution 2014: 676–684. https://doi.org/10.1111/2041-210X.12206

VALENTINI, A., TABERLET, P., MIAUD, C., CIVADE, R., HERDER, J., THOMSEN, P.F.,... BOYER, F. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology, 25, 929–942.

VAMOS, E., ELBRECHT, V., & LEESE, F. (2017). Short COI markers for freshwater macroinvertebrate metabarcoding. *Metabarcoding and Metagenomics*, *1*, e14625. https://doi.org/10.3897/mbmg.1.14625

VESTHEIM, H., and JARMAN, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples - a case study on prey DNA in Antarctic krill stomachs. *Front. Zool.* 5:12. doi: 10.1186/1742-9994-5-12

WILCOX, T.M., MCKELVEY, K.S., YOUNG, M.K., LOWE, W.H., SCHWARTZ, M.K. (2015)… Environmental DNA particle size distribution from Brook Trout (Salvelinus fontinalis). Conservation Genetics Resources 7: 639–641. https://doi.org/10.1007/s12686-015-0465-z

List of tables

List of figures

Appendix 1: 12S Metabarcoding Results

Table 5: 12S metabarcoding results. N/A in species column is due to this being a carp hybrid with no species name. T1 and T2 are results from test sampling by NMH staff. The 'unassigned reads' is given once per pond, therefore all further rows for that pond are marked '/' to indicate no data

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
Batch 3 Positive control	Rainbow trout	*Oncorhynchus mykiss*	57653	9131
Batch 4 Positive control	Rainbow trout	*Oncorhynchus mykiss*	74247	11781
NEGP001	Common frog	*Rana temporaria*	35819	8208
NEGP001	Human	*Homo sapiens*	5657	/
NEGP001	Chaffinch	*Fingilla coelebs*	118	/
NEGP002	Human	*Homo sapiens*	41413	16
NEGP003	Common frog	*Rana temporaria*	41583	7466
NEGP005	Common frog	*Rana temporaria*	31299	7949
NEGP005	Common Blackbird	*Turdus merula*	1463	/
NEGP005	Eurasian magpie	*Pica pica*	620	/
NEGP005	Dog	*Canis lupus familiaris*	325	/
NEGP005	Human	*Homo sapiens*	19	/
NEGP005	Song thrush	*Turdus philomelos*	5	/
NEGP006	Human	*Homo sapiens*	6867	156
NEGP006	Rock dove	*Columba livia*	140	/
NEGP006	N/a	*Corvus sp.*	110	/
NEGP006	Eurasian magpie	*Pica pica*	96	/
NEGP006	Eurasian robin	*Erithacus rubecula*	48	/
NEGP006	Song thrush	*Turdus philomelos*	20	/
NEGP006	Common Blackbird	*Turdus merula*	8	/
NEGP006	Eurasian wren	*Troglodytes troglodytes*	6	/
NEGP007	Common frog	*Rana temporaria*	45555	8302
NEGP008	Dog	*Canis lupus familiaris*	38309	7856
NEGP009	Human	*Homo sapiens*	54328	26
NEGP010	Human	*Homo sapiens*	23241	6481
NEGP010	Common Blackbird	*Turdus merula*	3187	/
NEGP010	Domestic pig	*Sus domesticus*	2242	/
NEGP010	Song thrush	*Turdus philomelos*	8	/
NEGP011	Common frog	*Rana temporaria*	34727	6739
NEGP011	Human	*Homo sapiens*	1490	/
NEGP011	Cat	*Felis catus*	84	/
NEGP012	Goldfish	*Carassius auratus langsdorfi*	18517	4391

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP012	Common frog	*Rana temporaria*	455	/
NEGP012	Carp hybrid	*n/a*	46	/
NEGP012	Human	*Homo sapiens*	14	/
NEGP012	Crucian carp	*Carassius carassius*	7	/
NEGP013	Human	*Homo sapiens*	18507	2249
NEGP013	Rock dove	*Columba livia*	6154	/
NEGP013	Eurasian magpie	*Pica pica*	506	/
NEGP013	N/a	*Columba sp.*	13	/
NEGP013	N/a	*Corvus sp.*	12	/
NEGP014	Human	*Homo sapiens*	99677	53
NEGP015	Human	*Homo sapiens*	36637	6908
NEGP015	Domestic pig	*Sus domesticus*	2788	/
NEGP016	Common frog	*Rana temporaria*	11330	18743
NEGP016	Common Blackbird	*Turdus merula*	3732	/
NEGP016	Eurasian wren	*Troglodytes troglodytes*	3586	/
NEGP016	Song thrush	*Turdus philomelos*	1138	/
NEGP016	Great tit	*Parus major*	17	/
NEGP017	Human	*Homo sapiens*	16977	2825
NEGP017	House sparrow	*Passer domesticus*	1609	/
NEGP017	Ring-necked pheasant	*Phasianus colchicus*	389	/
NEGP017	Dog	*Canis lupus familiaris*	59	/
NEGP017	Short-tailed field vole	*Microtus agrestis*	47	/
NEGP018	Common frog	*Rana temporaria*	38637	7727
NEGP020	Human	*Homo sapiens*	14158	1658
NEGP020	Common frog	*Rana temporaria*	6783	/
NEGP020	Rainbow trout	*Oncorhynchus mykiss*	13	/
NEGP021	Rainbow trout	*Oncorhynchus mykiss*	5289	36145
NEGP021	Common frog	*Rana temporaria*	37	/
NEGP022	Common frog	*Rana temporaria*	3919	2206
NEGP022	Human	*Homo sapiens*	3712	/
NEGP022	Rock dove	*Columba livia*	29	/
NEGP022	Song thrush	*Turdus philomelos*	6	/
NEGP022	Rainbow trout	*Oncorhynchus mykiss*	5	/
NEGP023	Common frog	*Rana temporaria*	65936	12469
NEGP023	Human	*Homo sapiens*	1499	/
NEGP024	Common frog	*Rana temporaria*	61539	12806
NEGP024	Human	*Homo sapiens*	248	/
NEGP025	Human	*Homo sapiens*	83275	1348
NEGP026	Human	*Homo sapiens*	22042	8360
NEGP026	Dog	*Canis lupus familiaris*	18226	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP027	Human	*Homo sapiens*	1847	689
NEGP027	Rock dove	*Columba livia*	671	/
NEGP027	House sparrow	*Passer domesticus*	523	/
NEGP027	Red Fox	*Vulpes vulpes*	303	/
NEGP027	Cattle	*Bos taurus*	12	/
NEGP028	Goldfish	*Carassius auratus langsdorfi*	5995	1242
NEGP028	Dog	*Canis lupus familiaris*	74	/
NEGP028	Carp hybrid	*n/a*	19	/
NEGP028	Common toad	*Bufo bufo*	10	/
NEGP028	Human	*Homo sapiens*	9	/
NEGP029	Common frog	*Rana temporaria*	14249	2897
NEGP029	Human	*Homo sapiens*	10285	/
NEGP029	Domestic chicken	*Gallus domesticus*	228	/
NEGP030	Human	*Homo sapiens*	20509	6088
NEGP030	Dog	*Canis lupus familiaris*	3382	/
NEGP030	Cat	*Felis catus*	1863	/
NEGP030	Cattle	*Bos taurus*	22	/
NEGP031	Common Bleak	*Alburnus alburnus*	43093	5
NEGP031	Minnow	*Phoxinus phoxinus*	235	/
NEGP032	Human	*Homo sapiens*	5120	1272
NEGP032	Common frog	*Rana temporaria*	1148	/
NEGP032	Red fox	*Vulpes vulpes*	252	/
NEGP032	Goldfish	*Carassius auratus langsdorfi*	89	/
NEGP032	Common moorhen	*Gallinula chloropus*	6	/
NEGP032	Rock dove	*Columba livia*	6	/
NEGP033	Human	*Homo sapiens*	27178	826
NEGP033	Rock dove	*Columba livia*	1659	/
NEGP033	Cat	*Felis catus*	7	/
NEGP034	Human	*Homo sapiens*	4224	409
NEGP034	Dog	*Canis lupus familiaris*	425	/
NEGP034	Common frog	*Rana temporaria*	354	/
NEGP035	Common frog	*Rana temporaria*	1811	358
NEGP035	Three-spined stickleback	*Gasterosteus aculeatus*	709	/
NEGP035	Human	*Homo sapiens*	470	/
NEGP035	Ninespine stickleback	*Pungitius pungitius*	21	/
NEGP040	Rock dove	*Columba livia*	337	156
NEGP040	Mallard	*Anas platyrhynchos*	222	/
NEGP040	Common moorhen	*Gallinula chloropus*	189	/
NEGP040	Human	*Homo sapiens*	170	/
NEGP040	Tufted duck	*Aythya fuligula*	31	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP044	Three-spined stickleback	*Gasterosteus aculeatus*	5819	3124
NEGP044	Human	*Homo sapiens*	3314	/
NEGP044	Common moorhen	*Gallinula chloropus*	491	/
NEGP044	Mallard	*Anas platyrhynchos*	302	/
NEGP044	Dog	*Canis lupus familiaris*	36	/
NEGP044	Rock dove	*Columba livia*	13	/
NEGP045	Common Blackbird	*Turdus merula*	92438	74
NEGP045	Song thrush	*Turdus philomelos*	532	/
NEGP045	Great tit	*Parus major*	5	/
NEGP046	Common Blackbird	*Turdus merula*	16879	1331
NEGP046	Eurasian robin	*Erithacus rubecula*	317	/
NEGP046	Song thrush	*Turdus philomelos*	312	/
NEGP046	Palmate newt	*Lissotriton helveticus*	306	/
NEGP047	Rock dove	*Columba livia*	29363	9
NEGP047	N/a	*Columba sp.*	152	/
NEGP049	Human	*Homo sapiens*	4513	1357
NEGP049	Palmate newt	*Lissotriton helveticus*	940	/
NEGP049	Domestic pig	*Sus domesticus*	75	/
NEGP051	Human	*Homo sapiens*	23837	1185
NEGP051	Eurasian robin	*Erithacus rubecula*	7511	/
NEGP051	N/a	*Corvus sp.*	1557	/
NEGP051	Eurasian magpie	*Pica pica*	801	/
NEGP052	Common frog	*Rana temporaria*	20279	30921
NEGP053	Human	*Homo sapiens*	2838	2033
NEGP053	Common frog	*Rana temporaria*	409	/
NEGP053	Common Blackbird	*Turdus merula*	50	/
NEGP055	Common frog	*Rana temporaria*	8265	4417
NEGP055	European perch	*Perca fluviatilis*	1356	/
NEGP055	Human	*Homo sapiens*	1271	/
NEGP055	Common Bream	*Abramis brama*	546	/
NEGP055	Common Blackbird	*Turdus merula*	20	/
NEGP055	Rudd or Dace	*Scardinius erythrophthalmus or Leuciscus leuciscus*	10	/
NEGP055	House sparrow	*Passer domesticus*	9	/
NEGP055	Song thrush	*Turdus philomelos*	7	/
NEGP055	Common starling	*Sturnus vulgaris*	7	/
NEGP055	Mallard	*Anas platyrhynchos*	7	/
NEGP055	Rainbow trout	*Oncorhynchus mykiss*	6	/
NEGP056	Goldfish	*Carassius auratus langsdorfi*	7735	2982
NEGP056	Human	*Homo sapiens*	6941	/
NEGP056	Eurasian magpie	*Pica pica*	476	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP056	Dog	*Canis lupus familiaris*	241	/
NEGP056	Carp hybrid	*n/a*	24	/
NEGP056	Crucian carp	*Carassius carassius*	6	/
NEGP057	Common frog	*Rana temporaria*	16433	4345
NEGP057	Great tit	*Parus major*	591	/
NEGP058	Human	*Homo sapiens*	7614	5329
NEGP058	Red fox	*Vulpes vulpes*	2055	/
NEGP058	Domestic pig	*Sus domesticus*	700	/
NEGP058	Cat	*Felis catus*	230	/
NEGP058	Dog	*Canis lupus familiaris*	118	/
NEGP058	Rock dove	*Columba livia*	88	/
NEGP059	Red fox	*Vulpes vulpes*	40940	9259
NEGP059	Carp hybrid	*n/a*	175	/
NEGP059	Crucian carp	*Carassius carassius*	5	/
NEGP060	Human	*Homo sapiens*	7571	153
NEGP060	Common frog	*Rana temporaria*	1067	/
NEGP060	Red fox	*Vulpes vulpes*	6	/
NEGP061	Dog	*Canis lupus familiaris*	1167	633
NEGP061	Human	*Homo sapiens*	706	/
NEGP061	Common frog	*Rana temporaria*	256	/
NEGP061	Common starling	*Sturnus vulgaris*	183	/
NEGP061	Common Blackbird	*Turdus merula*	9	/
NEGP061	Brown rat	*Rattus norvegicus*	8	/
NEGP062	Goldfish	*Carassius auratus*	14630	6443
NEGP062	Human	*Homo sapiens*	3502	/
NEGP062	Eurasian carp	*Cyprinus carpio*	2651	/
NEGP062	Carp hybrid	*n/a*	1944	/
NEGP062	Carp hybrid	*n/a*	7	/
NEGP062	Crucian carp	*Carassius carassius*	7	/
NEGP064	Human	*Homo sapiens*	2144	490
NEGP064	Dog	*Canis lupus familiaris*	501	/
NEGP065	Common frog	*Rana temporaria*	7359	2730
NEGP065	Common Blackbird	*Turdus merula*	1638	/
NEGP065	Rock dove	*Columba livia*	1453	/
NEGP065	Song thrush	*Turdus philomelos*	1120	/
NEGP065	Human	*Homo sapiens*	1099	/
NEGP065	Domestic chicken	*Gallus domesticus*	976	/
NEGP065	Great tit	*Parus major*	78	/
NEGP066	Common frog	*Rana temporaria*	40559	8838
NEGP066	Dog	*Canis lupus familiaris*	676	/
NEGP068	Human	*Homo sapiens*	27074	2279
NEGP068	Common frog	*Rana temporaria*	12798	/
NEGP068	Domestic chicken	*Gallus domesticus*	17	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP069	Human	*Homo sapiens*	24196	3701
NEGP069	Goldfish	*Carassius auratus*	5265	/
NEGP069	Domestic chicken	*Gallus domesticus*	2181	/
NEGP069	Carp hybrid	*n/a*	15	/
NEGP070	Common frog	*Rana temporaria*	47188	9887
NEGP071	Goldfish	*Carassius auratus*	6943	1727
NEGP071	Human	*Homo sapiens*	460	/
NEGP071	Carp hybrid	*n/a*	12	/
NEGP072	Human	*Homo sapiens*	10326	4926
NEGP072	Ninespine stickleback	*Pungitius pungitius*	6287	/
NEGP072	Three-spined stickleback	*Gasterosteus aculeatus*	6078	/
NEGP072	House sparrow	*Passer domesticus*	190	/
NEGP072	Domestic pig	*Sus domesticus*	18	/
NEGP074	Common frog	*Rana temporaria*	10546	3937
NEGP074	Human	*Homo sapiens*	4233	/
NEGP074	Common Blackbird	*Turdus merula*	850	/
NEGP074	Eurasian magpie	*Pica pica*	432	/
NEGP075	Goldfish	*Carassius auratus*	4582	2278
NEGP075	Eurasian carp	*Cyprinus carpio*	2497	/
NEGP075	Carp hybrid	*n/a*	1333	/
NEGP075	Human	*Homo sapiens*	719	/
NEGP075	Common frog	*Rana temporaria*	8	/
NEGP076	Human	*Homo sapiens*	98847	196
NEGP077	Common frog	*Rana temporaria*	9588	3277
NEGP077	Palmate newt	*Lissotriton helveticus*	146	/
NEGP077	Song thrush	*Turdus philomelos*	144	/
NEGP077	Human	*Homo sapiens*	140	/
NEGP077	Common Blackbird	*Turdus merula*	104	/
NEGP077	Great tit	*Parus major*	102	/
NEGP077	Eurasian robin	*Erithacus rubecula*	84	/
NEGP077	Eurasian blackcap	*Sylvia atricapilla*	74	/
NEGP077	House sparrow	*Passer domesticus*	45	/
NEGP078	Human	*Homo sapiens*	43402	5201
NEGP078	Domestic pig	*Sus domesticus*	6992	/
NEGP079	Goldfish	*Carassius auratus*	13178	96938
NEGP079	Eurasian carp	*Cyprinus carpio*	8780	/
NEGP079	Carp hybrid	*n/a*	3877	/
NEGP079	Human	*Homo sapiens*	43	/
NEGP079	Carp hybrid	*n/a*	5	/
NEGP080	Human	*Homo sapiens*	27776	2450
NEGP080	Rock dove	*Columba livia*	1465	/
NEGP080	Red fox	*Vulpes vulpes*	650	/
NEGP080	Dog	*Canis lupus familiaris*	260	/
NEGP081	Common frog	*Rana temporaria*	89119	47160

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP081	Smooth newt	*Lissotriton vulgaris*	87115	/
NEGP085	Red fox	*Vulpes vulpes*	157051	34582
NEGP085R	Human	*Homo sapiens*	37664	3350
NEGP085R	Goldfish	*Carassius auratus*	3904	/
NEGP085R	Great tit	*Parus major*	2254	/
NEGP085R	Eurasian robin	*Erithacus rubecula*	795	/
NEGP085R	Common Blackbird	*Turdus merula*	58	/
NEGP085R	Song thrush	*Turdus philomelos*	24	/
NEGP085R	Carp hybrid	*n/a*	13	/
NEGP086	Red fox	*Vulpes vulpes*	15827	19975
NEGP086	Eastern grey squirrel	*Sciurus carolinensis*	8412	/
NEGP087	Common toad	*Bufo bufo*	64905	21436
NEGP087	Red fox	*Vulpes vulpes*	13914	/
NEGP087	Cat	*Felis catus*	196	/
NEGP087	Rock dove	*Columba livia*	18	/
NEGP088	Human	*Homo sapiens*	92445	10093
NEGP088	Red fox	*Vulpes vulpes*	660	/
NEGP088	Dog	*Canis lupus familiaris*	269	/
NEGP088	Eastern grey squirrel	*Sciurus carolinensis*	25	/
NEGP089	Red fox	*Vulpes vulpes*	11428	2126
NEGP089	Human	*Homo sapiens*	6	/
NEGP090	Human	*Homo sapiens*	170383	6213
NEGP090	Red fox	*Vulpes vulpes*	839	/
NEGP091	Human	*Homo sapiens*	35742	11155
NEGP091	Domestic chicken	*Gallus domesticus*	25894	/
NEGP091	House sparrow	*Passer domesticus*	7556	/
NEGP091	Dog	*Canis lupus familiaris*	599	/
NEGP092	Palmate newt	*Lissotriton helveticus*	60193	13254
NEGP092	Dog	*Canis lupus familiaris*	2749	/
NEGP093	Human	*Homo sapiens*	90345	206
NEGP094	Common frog	*Rana temporaria*	88241	26871
NEGP094	Human	*Homo sapiens*	64100	/
NEGP094	Brown rat	*Rattus norvegicus*	5486	/
NEGP095	Common frog	*Rana temporaria*	6822	6910
NEGP095	Human	*Homo sapiens*	4618	/
NEGP095	Goldfish	*Carassius auratus*	3080	/
NEGP095	Rainbow trout	*Oncorhynchus mykiss*	27	/
NEGP095	Carp hybrid	*n/a*	15	/
NEGP095	Rock dove	*Columba livia*	7	/
NEGP097	Red fox	*Vulpes vulpes*	87065	20442
NEGP098	Eurasian magpie	*Pica pica*	50585	706

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP098	Human	*Homo sapiens*	32871	/
NEGP098	N/a	*Corvus sp.*	27	/
NEGP099	Human	*Homo sapiens*	219449	402
NEGP104	European woodmouse	*Apodemus sylvaticus*	78727	20783
NEGP105	Human	*Homo sapiens*	59106	7673
NEGP105	Domestic pig	*Sus domesticus*	24600	/
NEGP106	Goldfish	*Carassius auratus*	14039	13693
NEGP106	Human	*Homo sapiens*	7901	/
NEGP106	Common frog	*Rana temporaria*	6493	/
NEGP106	Rainbow trout	*Oncorhynchus mykiss*	565	/
NEGP106	Common moorhen	*Gallinula chloropus*	430	/
NEGP106	Rock dove	*Columba livia*	311	/
NEGP106	Carp hybrid	*n/a*	95	/
NEGP107	Human	*Homo sapiens*	67615	5659
NEGP107	Common frog	*Rana temporaria*	20497	/
NEGP107	n/a	*n/a*	3586	/
NEGP108	Human	*Homo sapiens*	63638	5221
NEGP108	Common frog	*Rana temporaria*	17	/
NEGP109	Eurasian carp	*Cyprinus carpio*	52942	2374
NEGP109	Eurasian magpie	*Pica pica*	7257	/
NEGP109	Human	*Homo sapiens*	1541	/
NEGP109	Carp hybrid	*n/a*	15	/
NEGP110	House sparrow	*Passer domesticus*	30706	1255
NEGP110	Human	*Homo sapiens*	14246	/
NEGP110	Dog	*Canis lupus familiaris*	1460	/
NEGP110	Common starling	*Sturnus vulgaris*	1397	/
NEGP110	Common Blackbird	*Turdus merula*	70	/
NEGP110	Song thrush	*Turdus philomelos*	67	/
NEGP110	Rock dove	*Columba livia*	29	/
NEGP111	Common starling	*Sturnus vulgaris*	28069	7023
NEGP111	Human	*Homo sapiens*	20819	/
NEGP111	Common frog	*Rana temporaria*	5481	/
NEGP111	Common blackbird	*Turdus merula*	2311	/
NEGP111	House sparrow	*Passer domesticus*	95	/
NEGP111	Song thrush	*Turdus philomelos*	68	/
NEGP111	Cattle	*Bos taurus*	54	/
NEGP112	Rock dove	*Columba livia*	47535	559
NEGP112	Human	*Homo sapiens*	43292	/
NEGP112	N/a	*Columba sp.*	86	/
NEGP113	Goldfish	*Carassius auratus*	69426	16546
NEGP113	Carp hybrid	*n/a*	440	/
NEGP113	Crucian carp	*Carassius carassius*	26	/
NEGP113	Common frog	*Rana temporaria*	6	/
NEGP114	Rudd	*Scardinius erythrophthalmus*	16737	7373

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP114	Common frog	*Rana temporaria*	8927	/
NEGP114	Human	*Homo sapiens*	7000	/
NEGP114	Tench	*Tinca tinca*	5390	/
NEGP114	Goldfish	*Carassius auratus*	4652	/
NEGP114	Ide	*Leuciscus idus*	30	/
NEGP114	Carp hybrid	*n/a*	28	/
NEGP114	Common Bleak	*Alburnus alburnus*	22	/
NEGP115	Human	*Homo sapiens*	42091	12335
NEGP115	N/a	*Columba sp.*	9043	/
NEGP115	Common frog	*Rana temporaria*	8242	/
NEGP115	Goldfish	*Carassius auratus*	6926	/
NEGP115	Rock dove	*Columba livia*	1939	/
NEGP115	Carp hybrid	*n/a*	32	/
NEGP115	Crucian carp	*Carassius carassius*	6	/
NEGP116	Red fox	*Vulpes vulpes*	29744	12998
NEGP116	Common frog	*Rana temporaria*	12733	/
NEGP116	Human	*Homo sapiens*	8272	/
NEGP116	Common toad	*Bufo bufo*	3012	/
NEGP116	Rainbow trout	*Oncorhynchus mykiss*	640	/
NEGP117	Human	*Homo sapiens*	34179	3380
NEGP117	Common frog	*Rana temporaria*	17839	/
NEGP118	Rock dove	*Columba livia*	48067	2157
NEGP118	Common frog	*Rana temporaria*	9030	/
NEGP118	Eurasian magpie	*Pica pica*	3425	/
NEGP118	N/a	*Columba sp.*	124	/
NEGP119	Human	*Homo sapiens*	46022	10186
NEGP119	Common frog	*Rana temporaria*	40665	/
NEGP121	Common frog	*Rana temporaria*	32074	7386
NEGP121	Human	*Homo sapiens*	19417	/
NEGP122	Common frog	*Rana temporaria*	53218	13093
NEGP122	Human	*Homo sapiens*	25929	/
NEGP123	Human	*Homo sapiens*	73448	3001
NEGP123	Common frog	*Rana temporaria*	14120	/
NEGP124	Human	*Homo sapiens*	54111	10811
NEGP124	Common frog	*Rana temporaria*	18105	/
NEGP124	Domestic pig	*Sus domesticus*	6245	/
NEGP125	Human	*Homo sapiens*	96324	250
NEGP125	Red fox	*Vulpes vulpes*	728	/
NEGP129	Rock dove	*Columba livia*	73159	733
NEGP129	Common blackbird	*Turdus merula*	6176	/
NEGP129	Song thrush	*Turdus philomelos*	3907	/
NEGP129	Great tit	*Parus major*	223	/
NEGP129	N/a	*Columba sp.*	167	/
NEGP131	Goldfish	*Carassius auratus*	41985	11446
NEGP131	Common frog	*Rana temporaria*	5193	/
NEGP131	Red fox	*Vulpes vulpes*	718	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP131	Carp hybrid	*n/a*	252	/
NEGP131	Crucian carp	*Carassius carassius*	16	/
NEGP132	Common frog	*Rana temporaria*	29123	9300
NEGP132	Human	*Homo sapiens*	18526	/
NEGP132	Smooth newt	*Lissotriton vulgaris*	10737	/
NEGP132	Great tit	*Parus major*	2619	/
NEGP132	Common blackbird	*Turdus merula*	1648	/
NEGP132	Brown rat	*Rattus norvegicus*	446	/
NEGP133	Common blackbird	*Turdus merula*	18680	3945
NEGP133	Common frog	*Rana temporaria*	10463	/
NEGP133	Great tit	*Parus major*	8400	/
NEGP133	House sparrow	*Passer domesticus*	5896	/
NEGP133	Human	*Homo sapiens*	4415	/
NEGP133	Song thrush	*Turdus philomelos*	4123	/
NEGP133	Eurasian magpie	*Pica pica*	1027	/
NEGP133	Eurasian robin	*Erithacus rubecula*	999	/
NEGP133	Rock dove	*Columba livia*	321	/
NEGP133	Coal tit	*Parus ater*	314	/
NEGP133	European goldfinch	*Carduelis carduelis*	305	/
NEGP133	Domestic pig	*Sus domesticus*	251	/
NEGP133	Ring-necked pheasant	*Phasianus colchicus*	185	/
NEGP133	Coal tit	*Periparus ater*	47	/
NEGP133	Eastern grey squirrel	*Sciurus carolinensis*	30	/
NEGP133	Common starling	*Sturnus vulgaris*	26	/
NEGP133	Yellowhammer	*Emberiza citrinella*	7	/
NEGP135	Goldfish	*Carassius auratus*	22350	11775
NEGP135	Eurasian magpie	*Pica pica*	16830	/
NEGP135	Mallard	*Anas platyrhynchos*	16451	/
NEGP135	Dog	*Canis lupus familiaris*	2985	/
NEGP135	Human	*Homo sapiens*	627	/
NEGP135	Carp hybrid	*n/a*	157	/
NEGP135	Eurasian widgeon	*Anas penelope*	28	/
NEGP135	N/a	*Corvus sp.*	17	/
NEGP136	Human	*Homo sapiens*	24852	2852
NEGP136	Mallard	*Anas platyrhynchos*	11160	/
NEGP136	Common frog	*Rana temporaria*	5983	/
NEGP136	Dog	*Canis lupus familiaris*	3040	/
NEGP136	Common moorhen	*Gallinula chloropus*	1277	/
NEGP136	Ninespine stickleback	*Pungitius pungitius*	1274	/
NEGP137	Common frog	*Rana temporaria*	33493	7990
NEGP137	Human	*Homo sapiens*	11588	/
NEGP139	Human	*Homo sapiens*	89264	881
NEGP139	Cattle	*Bos taurus*	5447	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP142	Rock dove	*Columba livia*	49144	1005
NEGP142	Human	*Homo sapiens*	8261	/
NEGP142	Great tit	*Parus major*	3325	/
NEGP142	Common blackbird	*Turdus merula*	2344	/
NEGP142	Song thrush	*Turdus philomelos*	1983	/
NEGP142	Cattle	*Bos taurus*	320	/
NEGP142	Dog	*Canis lupus familiaris*	291	/
NEGP142	N/a	*Columba sp.*	97	/
NEGP143	Common frog	*Rana temporaria*	50295	10333
NEGP143	Human	*Homo sapiens*	8749	/
NEGP145	Human	*Homo sapiens*	102114	122
NEGP147	Human	*Homo sapiens*	15656	21991
NEGP147	Domestic chicken	*Gallus domesticus*	5257	/
NEGP147	House sparrow	*Passer domesticus*	4897	/
NEGP147	Smooth newt	*Lissotriton vulgaris*	698	/
NEGP147	Common moorhen	*Gallinula chloropus*	152	/
NEGP147	Domestic pig	*Sus domesticus*	72	
NEGP148	Palmate newt	*Lissotriton helveticus*	22782	8368
NEGP148	Common frog	*Rana temporaria*	21643	/
NEGP148	Common blackbird	*Turdus merula*	853	/
NEGP148	European woodmouse	*Apodemus sylvaticus*	131	/
NEGP148	Song thrush	*Turdus philomelos*	10	/
NEGP150	Human	*Homo sapiens*	27931	19754
NEGP150	Common frog	*Rana temporaria*	11158	/
NEGP150	Eurasian robin	*Erithacus rubecula*	6791	/
NEGP150	European badger	*Meles meles*	5623	/
NEGP150	Rock dove	*Columba livia*	3693	/
NEGP150	Rainbow trout	*Oncorhynchus mykiss*	1340	/
NEGP150	Red fox	*Vulpes vulpes*	305	/
NEGP150	N/a	*Columba sp.*	7	/
NEGP151	Human	*Homo sapiens*	69957	1573
NEGP151	Domestic pig	*Sus domesticus*	1870	/
NEGP151	Dog	*Canis lupus familiaris*	83	/
NEGP152	Common frog	*Rana temporaria*	57762	13739
NEGP153	Human	*Homo sapiens*	86123	9
NEGP154	Human	*Homo sapiens*	46597	652
NEGP154	Common blackbird	*Turdus merula*	22752	/
NEGP154	Song thrush	*Turdus philomelos*	135	/
NEGP155	Human	*Homo sapiens*	20853	3714
NEGP155	Gudgeon	*Gobio gobio*	18590	/
NEGP155	Three-spined stickleback	*Gasterosteus aculeatus*	9747	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP155	Rudd	*Scardinius erythrophthalamus*	189	/
NEGP155	Common Bleak	*Alburnus alburnus*	19	/
NEGP155	European chub	*Squalius cephalus*	11	/
NEGP156	Human	*Homo sapiens*	94653	11
NEGP157	Human	*Homo sapiens*	85322	1060
NEGP157	Cattle	*Bos taurus*	9431	/
NEGP158	Human	*Homo sapiens*	69568	5779
NEGP158	Dog	*Canis lupus familiaris*	21200	/
NEGP159	Domestic chicken	*Gallus domesticus*	19849	7858
NEGP159	Common frog	*Rana temporaria*	12180	/
NEGP159	Human	*Homo sapiens*	8775	/
NEGP159	Common blackbird	*Turdus merula*	1685	/
NEGP159	Eurasian magpie	*Pica pica*	1662	/
NEGP159	N/a	*Corvus sp.*	1072	/
NEGP159	Song thrush	*Turdus philomelos*	765	/
NEGP159	House sparrow	*Passer domesticus*	434	/
NEGP159	Rock dove	*Columba livia*	420	/
NEGP159	Red fox	*Vulpes vulpes*	69	/
NEGP159	Great tit	*Parus major*	14	/
NEGP162	Common frog	*Rana temporaria*	42834	9613
NEGP162	Eurasian robin	*Erithacus rubecula*	1506	/
NEGP162	Human	*Homo sapiens*	1289	/
NEGP163	Human	*Homo sapiens*	45847	1336
NEGP163	Rock dove	*Columba livia*	32415	/
NEGP163	Mute swan	*Cygnus olar*	9654	/
NEGP163	N/a	*Columba sp.*	82	/
NEGP164	Common moorhen	*Gallinula chloropus*	41244	5179
NEGP164	Common frog	*Rana temporaria*	15016	/
NEGP164	Rainbow trout	*Oncorhynchus mykiss*	7383	/
NEGP165	European perch	*Perca fluviatilis*	42275	6906
NEGP165	Mute swan	*Cygnus olar*	2059	/
NEGP165	Mallard	*Anas platyrhynchos*	1523	/
NEGP165	Human	*Homo sapiens*	1090	/
NEGP165	Great cormorant	*Phalacrocorax carbo*	994	/
NEGP165	Black-headed gull	*Chroicocephalus ridibundus*	975	/
NEGP165	Eurasian coot	*Fulica atra*	959	/
NEGP165	Eurasian carp	*Cyprinus carpio carpio*	518	/
NEGP165	Eurasian widgeon	*Anas penelope*	252	/
NEGP165	Common Bleak	*Alburnus alburnus*	83	/
NEGP165	Common roach	*Rutilus rutilus*	70	/
NEGP165	Dog	*Canis lupus familiaris*	30	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP167	Common blackbird	*Turdus merula*	79083	283
NEGP167	Song thrush	*Turdus philomelos*	465	/
NEGP167	Great tit	*Parus major*	6	/
NEGP169	Human	*Homo sapiens*	24244	10729
NEGP169	Rock dove	*Columba livia*	12592	/
NEGP169	Goldfish	*Carassius auratus*	5084	/
NEGP169	Common frog	*Rana temporaria*	4971	/
NEGP169	Eurasian magpie	*Pica pica*	673	/
NEGP169	Palmate newt	*Lissotriton helveticus*	629	/
NEGP169	Common blackbird	*Turdus merula*	396	/
NEGP169	Rainbow trout	*Oncorhynchus mykiss*	233	/
NEGP169	Carp hybrid	*n/a*	221	/
NEGP169	Song thrush	*Turdus philomelos*	196	/
NEGP169	Mallard	*Anas platyrhynchos*	64	/
NEGP169	N/a	*Columba sp.*	35	/
NEGP169	Great tit	*Parus major*	6	/
NEGP174	Goldfish	*Carassius auratus*	30575	9227
NEGP174	Human	*Homo sapiens*	18330	/
NEGP174	Common frog	*Rana temporaria*	1772	/
NEGP174	Carp hybrid	*n/a*	1238	/
NEGP174	Crucian carp	*Carassius carassius*	10	/
NEGP176	Great tit	*Parus major*	28497	3504
NEGP176	Red fox	*Vulpes vulpes*	11264	/
NEGP176	Eurasian robin	*Erithacus rubecula*	3945	/
NEGP176	House sparrow	*Passer domesticus*	2968	/
NEGP176	Human	*Homo sapiens*	2551	/
NEGP176	Rock dove	*Columba livia*	2455	/
NEGP176	Common starling	*Sturnus vulgaris*	2064	/
NEGP176	Common blackbird	*Turdus merula*	1868	/
NEGP176	Song thrush	*Turdus philomelos*	828	/
NEGP176	Eastern grey squirrel	*Sciurus carolinensis*	222	/
NEGP176	N/a	*Columba sp.*	5	/
NEGP178	Common frog	*Rana temporaria*	49927	11293
NEGP178	Human	*Homo sapiens*	28837	/
NEGP179	Human	*Homo sapiens*	45922	21448
NEGP179	Common frog	*Rana temporaria*	15094	/
NEGP179	Song thrush	*Turdus philomelos*	8211	/
NEGP179	Common blackbird	*Turdus merula*	36	/
NEGP179	Great tit	*Parus major*	36	/
NEGP180	Dog	*Canis lupus familiaris*	71991	13717
NEGP181	Human	*Homo sapiens*	12916	11481
NEGP181	Common frog	*Rana temporaria*	13749	/
NEGP181	Smooth newt	*Lissotriton vulgaris*	6786	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP181	Dog	*Canis lupus familiaris*	2956	/
NEGP181	House sparrow	*Passer domesticus*	2677	/
NEGP181	Common moorhen	*Gallinula chloropus*	544	/
NEGP181	Rainbow trout	*Oncorhynchus mykiss*	329	/
NEGP181	Red fox	*Vulpes vulpes*	291	/
NEGP181	Rock dove	*Columba livia*	247	/
NEGP181	Eastern grey squirrel	*Sciurus carolinensis*	42	/
NEGP181	European woodmouse	*Apodemus sylvaticus*	8	/
NEGP182	Common frog	*Rana temporaria*	51284	11187
NEGP183	Human	*Homo sapiens*	86287	4074
NEGP187	Rock dove	*Columba livia*	44059	4818
NEGP187	Human	*Homo sapiens*	18793	/
NEGP187	Red fox	*Vulpes vulpes*	6028	/
NEGP187	Brambling	*Fringilla montifringilla*	5187	/
NEGP187	Eurasian magpie	*Pica pica*	2212	/
NEGP187	N/a	*Corvus sp.*	1164	/
NEGP187	Dog	*Canis lupus familiaris*	186	/
NEGP187	N/a	*Columba sp.*	113	/
NEGP188	Human	*Homo sapiens*	38759	4380
NEGP188	Goldfish	*Carassius auratus*	9193	/
NEGP188	Palmate newt	*Lissotriton helveticus*	4484	/
NEGP188	Common frog	*Rana temporaria*	1889	/
NEGP188	Eurasian wren	*Troglodytes troglodytes*	215	/
NEGP188	Carp hybrid	*n/a*	44	/
NEGP189	Human	*Homo sapiens*	38802	4159
NEGP189	Ninespine stickleback	*Pungitius pungitius*	3958	/
NEGP189	Three-spined stickleback	*Gasterosteus aculeatus*	525	/
NEGP190	Goldfish	*Carassius auratus*	37251	11824
NEGP190	Common frog	*Rana temporaria*	9076	/
NEGP190	Human	*Homo sapiens*	4835	/
NEGP190	Carp hybrid	*n/a*	247	/
NEGP190	Crucian carp	*Carassius carassius*	14	/
NEGP191	Human	*Homo sapiens*	63353	4459
NEGP191	Domestic pig	*Sus domesticus*	12512	/
NEGP191	Common blackbird	*Turdus merula*	8386	/
NEGP191	Song thrush	*Turdus philomelos*	35	/
NEGP192	Common frog	*Rana temporaria*	24983	6776
NEGP192	Human	*Homo sapiens*	17926	/

Pond ID	Common Name	Species Name	Read Count	Unassigned Reads
NEGP192	House sparrow	*Passer domesticus*	3612	/
NEGP192	Rock dove	*Columba livia*	2187	/
NEGP192	Great tit	*Parus major*	1166	/
NEGP192	Domestic chicken	*Gallus domesticus*	937	/
NEGP192	Common moorhen	*Gallinula chloropus*	579	/
NEGP192	Rainbow trout	*Oncorhynchus mykiss*	501	/
NEGP192	Common Blackbird	*Turdus merula*	71	/
NEGP192	European woodmouse	*Apodemus sylvaticus*	32	/
NEGP194	Common frog	*Rana temporaria*	36456	9557
NEGP194	Human	*Homo sapiens*	35294	/
NEGP195	Common starling	*Sturnus vulgaris*	41824	985
NEGP195	Human	*Homo sapiens*	22135	/
NEGP195	European goldfinch	*Carduelis carduelis*	9521	/
NEGP197	Goldfish	*Carassius auratus*	37854	11176
NEGP197	Human	*Homo sapiens*	29636	/
NEGP197	Carp hybrid	*n/a*	244	/
NEGP197	Crucian carp	*Carassius carassius*	16	/
T1	Human	*Homo sapiens*	15354	5641
T1	Common frog	*Rana temporaria*	7801	/
T1	Domestic chicken	*Gallus domesticus*	5168	/
T1	House sparrow	*Passer domesticus*	2881	/
T1	Dog	*Canis lupus familiaris*	409	/
T1	Red fox	*Vulpes vulpes*	329	/
T1	Eurasian magpie	*Pica pica*	251	/
T1	Rock dove	*Columba livia*	217	/
T1	Goldfish	*Carassius auratus*	201	/
T1	Mallard	*Anas platyrhynchos*	135	/
T1	Rainbow trout	*Oncorhynchus mykiss*	131	/
T1	Common moorhen	*Gallinula chloropus*	89	/
T1	Eurasian robin	*Erithacus rubecula*	31	/
T1	Common Blackbird	*Turdus merula*	26	/
T1	Song thrush	*Turdus philomelos*	7	/
T2	Human	*Homo sapiens*	34619	18396
T2	Common frog	*Rana temporaria*	18974	/
T2	Domestic pig	*Sus domesticus*	6684	/
T2	Smooth newt	*Lissotriton vulgaris*	3732	/

Appendix 2: COI Metabarcoding Results

Table 6. COI metabarcoding results. N/A in common name column is due to there being no common name for this species,'?' means that this is unknown for this entry. T1 and T2 are results from test sampling by NMH staff. Where a sequence has not been assigned to species level, the lowest possible taxonomic rank is given. The 'unassigned reads' is given once per pond, therefore all further rows for that pond are marked '/' to indicate no data

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP001	Red worm	*Limnodrilus hoffmeisteri*	1876	88749
NEGP001	American bladder snail	*Physella acuta*	1054	/
NEGP001	N/A (worm)	*Fridericia galba*	702	/
NEGP001	Great pond snail	*Lymnaea stagnalis*	524	/
NEGP001	N/A (copepod)	*Cyclops abyssorum divergens*	153	/
NEGP001	Microflex worms	*Dero digitata*	110	/
NEGP001	N/A (barklice)	*Ectopsocus sp.*	75	/
NEGP001	Springtail	*Tomocerus minor*	72	/
NEGP001	N/A (worm)	*Globulidrilus sp.*	71	/
NEGP001	N/A (worm)	*Pristina aequiseta*	22	/
NEGP001	N/A (worm)	*Potamothrix bavaricus*	22	/
NEGP001	N/A (worm)	*Limnodrilus claparedianus*	13	/
NEGP001	N/A (shrimp)	*Crangonyx pseudogracilis*	11	/
NEGP001	Angle shades moth	*Phlogophora meticulosa*	9	/
NEGP001	N/A (non-biting midge)	*Limnophyes sp.*	9	/
NEGP001	N/A (moth)	*Blastobasis adustella*	7	/
NEGP001	2 spotted water hog-louse	*Asellus aquaticus*	7	/
NEGP002	N/A (crustacean)	*Maxillopoda sp.*	1613	104985
NEGP002	N/A (copepod)	*Canthocamptidae sp.*	230	/
NEGP002	Sludge/sewage worm	*Tubifex tubifex*	184	/
NEGP002	N/A (copepod)	*Acanthocyclops vernalis*	178	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP002	N/A (water flea)	*Chydorus sphaericus*	51	/
NEGP002	red worm	*Limnodrilus hoffmeisteri*	28	/
NEGP002	American bladder snail	*Physa acuta*	7	/
NEGP002	N/A (rotifer)	*Synchaeta pectinata*	5	/
NEGP003	N/A (copepod)	*Acanthocyclops vernalis*	25831	80002
NEGP003	N/A (copepod)	*Canthocamptidae sp.*	11891	/
NEGP003	Green leaf hopper	*Empoasca decipiens*	280	/
NEGP003	red worm	*Limnodrilus hoffmeisteri*	261	/
NEGP003	N/A (non-biting midge)	*Natarsia ep.*	203	/
NEGP003	N/A (tardigrade)	*Ramazzottius sp.*	130	/
NEGP003	N/A (worm)	*Pristina aequiseta*	119	/
NEGP003	N/A (bdelloid rotifer)	*Rotaria rotatoria*	72	/
NEGP003	Salt and pepper microcaddisfly	*Agraylea multipunctata*	62	/
NEGP003	N/A (fly)	*Diptera sp.*	55	/
NEGP003	N/A (ground beetle)	*Dromius meridionalis*	50	/
NEGP003	N/A (earthworm)	*Lumbricidae sp.*	48	/
NEGP003	N/A (barklice)	*Ectopsocus briggsi*	46	/
NEGP003	water flea	*Daphnia pulex*	33	/
NEGP003	N/A (mite)	*Eupodidae sp.*	33	/
NEGP003	Lake limpet	*Acroloxus lacustris*	30	/
NEGP003	Fresh-water polyp	*Hydra magnipapillata*	26	/
NEGP003	2 spotted water hog-louse	*Asellus aquaticus*	26	/
NEGP003	N/A (white lacewing)	*Conwentzia psociformis*	24	/
NEGP003	Lily aphid	*Neomyzus circumflexus*	21	/
NEGP003	N/A (crustacean)	*Maxillopoda sp.*	19	/
NEGP003	Light brown apple moth	*Epiphyas postvittana*	18	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP003	N/A (worm)	*Enchytraeidae sp.*	13	/
NEGP003	N/A (barklice)	*Ectopsocus sp.*	13	/
NEGP003	N/A (barklice)	*Psocoptera sp.*	6	/
NEGP005	N/A (springtail)	*Sminthurinus reticulatus*	10614	10577
NEGP005	N/A (springtail)	*Isotomurus fucicola*	6089	/
NEGP005	Grindal worms	*Enchytraeus buchholzi*	404	/
NEGP005	N/A (springtail)	*Parisotoma notabilis*	164	/
NEGP005	N/A (copepod)	*Canthocamptidae sp.*	106	/
NEGP005	Green worm	*Allolobophora chlorotica*	60	/
NEGP005	N/A (arachnid)	*Arachnida sp.*	59	/
NEGP005	N/A (worm)	*Henlea perpusilla*	55	/
NEGP005	Salt and pepper microcaddisfly	*Agraylea multipunctata*	28	/
NEGP005	Common earthworm	*Lumbricus terrestris*	23	/
NEGP005	Black-headed worm	*Aporrectodea longa*	18	/
NEGP005	?	*UNVERIFIED: Arthropoda environmental sample*	17	/
NEGP005	N/A (worm)	*Enchytraeidae sp.*	13	/
NEGP005	N/A (springtail)	*Willowsia nigromaculata*	12	/
NEGP005	Grey worm	*Aporrectodea caliginosa*	12	/
NEGP005	N/A (springtail)	*Isotomurus cf. unifasciatus*	11	/
NEGP005	?	*Arthropoda environmental sample*	8	/
NEGP005	Rosy-tipped worm	*Aporrectodea rosea*	7	/
NEGP005	N/A (crustacean)	*Haplophthalmus danicus*	5	/
NEGP005	N/A (crane fly)	*Helius sp.*	5	/
NEGP006	N/A (copepod)	*Eucyclops cf. serrulatus*	4948	64673

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP006	Green hydra fresh-water polyp	*Hydra vulgaris*	1535	/
NEGP006	N/A (Psocoptera	*Graphopsocus cruciatus*	133	/
NEGP006	American bladder snail	*Physa acuta*	116	/
NEGP006	N/A (earthworm)	*Aporrectodea nocturna*	65	/
NEGP006	N/A (copepod)	*Cyclops abyssorum divergens*	58	/
NEGP006	N/A (bdelloid rotifer)	*Rotaria rotatoria*	49	/
NEGP006	N/A (worm)	*Dendrobaena cf. attemsi*	43	/
NEGP006	N/A (copepod)	*Paracyclops fimbriatus*	31	/
NEGP006	N/A (barklice)	*Ectopsocus briggsi*	27	/
NEGP006	water flea	*Daphnia pulex*	20	/
NEGP006	N/A (mite)	*Eupodidae sp.*	20	/
NEGP006	N/A (mite)	*Tydeidae sp.*	15	/
NEGP006	N/A (fly)	*Pseudolyciella pallidiventris*	11	/
NEGP006	N/A (fly)	*Limnophyes minimus*	11	/
NEGP006	Hoverfly "the footballer"	*Helophilus pendulus*	9	/
NEGP006	N/A (scuttle fly)	*Megaselia nigra*	7	/
NEGP006	Birch aphid	*Euceraphis betulae*	6	/
NEGP007	N/A (red worm)	*Eiseniella tetraedra*	239	89983
NEGP007	red worm	*Limnodrilus hoffmeisteri*	180	/
NEGP007	N/A (non-biting midge)	*Chironomus luridus*	110	/
NEGP007	N/A (worm)	*Enchytraeidae sp.*	79	/
NEGP007	N/A (springtail)	*Xenylla sp.*	68	/
NEGP007	N/A (earthworm)	*Lumbricus castaneus*	65	/
NEGP007	N/A (barklice)	*Ectopsocus briggsi*	27	/
NEGP007	N/A (crustacean)	*Podocopida sp.*	22	/
NEGP007	N/A (arachnid)	*Arachnida sp.*	19	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP007	N/A (bdelloid rotifer)	*Rotaria rotatoria*	9	/
NEGP008	N/A (rotifer)	*Euchlanis dilatata*	112	45431
NEGP008	?	*UNVERIFIED: Arthropoda environmental sample*	25	/
NEGP008	N/A (arachnid)	*Arachnida sp.*	15	/
NEGP009	----	*Empty*		118886
NEGP010	Green hydra fresh-water polyp	*Hydra vulgaris*	78	65442
NEGP010	N/A (copepod)	*Acanthocyclops vernalis*	12	/
NEGP011	N/A (water flea)	*Chydorus sphaericus*	1272	106100
NEGP011	N/A (worm)	*Allolobophoridella eiseni*	508	/
NEGP011	N/A (non-biting midge)	*Chironomidae sp.*	250	/
NEGP011	N/A (worm)	*Fridericia sp.*	170	/
NEGP011	N/A (worm)	*Enchytraeidae sp.*	131	/
NEGP011	Salt and pepper microcaddisfly	*Agraylea multipunctata*	109	/
NEGP011	N/A (rotifer)	*Lecane closterocerca*	67	/
NEGP011	N/A (copepod)	*Acanthocyclops vernalis*	59	/
NEGP011	N/A (black fly)	*Simulium velutinum*	36	/
NEGP011	N/A (non-biting midge)	*Orthocladius sp.*	9	/
NEGP011	N/A (springtail)	*Parisotoma notabilis*	6	/
NEGP011	N/A (non-biting midge)	*Micropsectra atrofasciata*	6	/
NEGP011	N/A (water flea)	*Chydorus sphaericus*	5	/
NEGP012	Black-headed worm	*Aporrectodea longa*	8453	49268
NEGP012	N/A (bdelloid rotifer)	*Rotaria rotatoria*	712	/
NEGP012	N/A (worm)	*Bothrioneurum vejdovskyanum*	92	/
NEGP012	N/A (worm)	*Chaetogaster diastrophus*	74	/
NEGP012	N/A (worm)	*Nais communis*	63	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP012	Blackworm	*Lumbriculus variegatus*	21	/
NEGP012	N/A (midge)	*Cricotopus sylvestris*	20	/
NEGP012	N/A (non-biting midge)	*Eukiefferiella claripennis*	10	/
NEGP012	Microflex worms	*Dero digitata*	10	/
NEGP012	N/A (worm)	*Dero obtusa*	9	/
NEGP012	2 spotted water hog-louse	*Asellus aquaticus*	7	/
NEGP013	N/A (non-biting midge)	*Chironomidae sp.*	353	38052
NEGP013	N/A (worm)	*Dendrobaena cf. attemsi*	154	/
NEGP013	N/A (rotifer)	*Synchaeta pectinata*	150	/
NEGP013	N/A (hydra)	*Hydra circumcincta*	138	/
NEGP013	N/A (non-biting midge)	*Psectrocladius platypus*	134	/
NEGP013	N/A (copepod)	*Acanthocyclops vernalis*	124	/
NEGP013	American bladder snail	*Physella acuta*	120	/
NEGP013	American bladder snail	*Physa acuta*	94	/
NEGP013	Common maple aphid	*Periphyllus testudinaceus*	62	/
NEGP013	Harelquin ladybird	*Harmonia axyridis*	58	/
NEGP013	Phantom midge	*Chaoborus crystallinus*	52	/
NEGP013	Salt and pepper microcaddisfly	*Agraylea multipunctata*	49	/
NEGP013	N/A (barklice)	*Ectopsocus briggsi*	47	/
NEGP013	Springtail	*Tomocerus minor*	36	/
NEGP013	N/A (shrimp)	*Crangonyx pseudogracilis*	31	/
NEGP013	N/A (copepod)	*Eucyclops cf. serrulatus*	16	/
NEGP013	Girdled snail	*Hygromia cinctella*	10	/
NEGP013	Green cellar slug/Irish yellow slug	*Limacus maculatus*	7	/
NEGP013	Southern house mosquito	*Culex pipiens*	7	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP013	Backswimming water boatman	*Notonecta maculata*	6	/
NEGP014	N/A (copepod)	*Acanthocyclops vernalis*	3593	61514
NEGP014	N/A (non-biting midge)	*Chironomidae sp.*	302	/
NEGP014	N/A (shrimp)	*Crangonyx pseudogracilis*	24	/
NEGP014	N/A (non-biting midge)	*Chironomus pseudothummi*	18	/
NEGP014	N/A (springtail)	*Sminthurinus reticulatus*	8	/
NEGP014	N/A (barklice)	*Ectopsocus briggsi*	7	/
NEGP014	N/A (barklice)	*Valenzuela flavidus*	6	/
NEGP014	water flea	*Daphnia pulex*	6	/
NEGP015	Girdled snail	*Hygromia cinctella*	252	36368
NEGP015	N/A (planthopper)	*Issus coleoptratus*	81	/
NEGP015	Southern house mosquito	*Culex pipiens*	76	/
NEGP015	N/A (mayfly)	*Cloeon dipterum*	76	/
NEGP015	American bladder snail	*Physella acuta*	69	/
NEGP015	water flea	*Daphnia pulex*	24	/
NEGP015	River limpet	*Ancylus fluviatilis*	12	/
NEGP015	Chestnut slug	*Deroceras invadens*	5	/
NEGP016	N/A (bdelloid rotifer)	*Rotaria rotatoria*	3360	53906
NEGP016	N/A (copepod)	*Acanthocyclops vernalis*	2744	/
NEGP016	Banded mosquito	*Culiseta sp.*	343	/
NEGP016	N/A (crustacean)	*Chydorus brevilabris*	5	/
NEGP017	N/A (crane fly)	*Tipula oleracea*	3635	19552
NEGP017	N/A (copepod)	*Acanthocyclops vernalis*	3630	/
NEGP017	2 spotted water hog-louse	*Asellus aquaticus*	832	/
NEGP017	Salt and pepper microcaddisfly	*Agraylea multipunctata*	691	/
NEGP017	N/A (springtail)	*Willowsia nigromaculata*	523	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP017	Blackworm	*Lumbriculus variegatus*	347	/
NEGP017	Bank worm	*Bimastos rubidus*	188	/
NEGP017	Chestnut slug	*Deroceras invadens*	99	/
NEGP017	N/A (arachnid)	*Arachnida sp.*	92	/
NEGP017	N/A (fly)	*Satchelliella sp.*	88	/
NEGP017	N/A (non-biting midge)	*Limnophyes pentaplastus*	54	/
NEGP017	N/A (springtail)	*Entomobryidae sp.*	43	/
NEGP017	N/A (shrimp)	*Crangonyx pseudogracilis*	40	/
NEGP017	N/A (leech)	*Erpobdella testacea*	39	/
NEGP017	N/A (tardigrade)	*Diphascon higginsi*	25	/
NEGP017	N/A (harvestman)	*Paroligolophus agrestis*	20	/
NEGP017	N/A (barklice)	*Ectopsocus briggsi*	17	/
NEGP017	N/A (bdelloid rotifer)	*Rotaria rotatoria*	12	/
NEGP017	N/A (barklice)	*Ectopsocus sp.*	12	/
NEGP017	?	*UNVERIFIED: Arthropoda environmental sample*	11	/
NEGP017	?	*Arthropoda environmental sample*	11	/
NEGP017	N/A (mite)	*Ameronothrus sp.*	9	/
NEGP017	N/A (diving beetle)	*Agabus bipustulatus*	6	/
NEGP018	N/A (earthworm)	*Dendrodrilus rubidus*	589	24397
NEGP018	N/A (barklice)	*Valenzuela flavidus*	149	/
NEGP018	Wooly apple aphid	*Eriosoma lanigerum*	71	/
NEGP018	red worm	*Limnodrilus hoffmeisteri*	54	/
NEGP018	N/A (aphid)	*Tuberculatus annulatus*	17	/
NEGP018	Large yellow underwing moth	*Noctua pronuba*	9	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP020	N/A (crustacean)	*Diacyclops bicuspidatus*	103470	4882
NEGP020	N/A (copepod)	*Canthocamptidae sp.*	111	/
NEGP020	N/A (copepod)	*Acanthocyclops vernalis*	77	/
NEGP020	Common branding worm	*Eisenia sp.*	6	/
NEGP021	N/A (water flea)	*Chydorus sphaericus*	886	110584
NEGP021	N/A (crustacean)	*Cypridopsis sp.*	97	/
NEGP021	N/A (mayfly)	*Cloeon dipterum*	11	/
NEGP022	N/A (copepod)	*Acanthocyclops verN/Alis*	19756	/
NEGP022	N/A (non-biting midge)	*Paratanytarsus sp.*	1130	/
NEGP022	N/A (crustacean)	*Maxillopoda sp.*	820	/
NEGP022	N/A (non-biting midge)	*Chironomidae sp.*	316	/
NEGP022	N/A (non-biting midge)	*Corynoneura scutellata*	291	/
NEGP022	N/A (non-biting midge)	*Corynoneura lobata*	116	/
NEGP022	N/A (midge)	*Cricotopus sylvestris*	92	/
NEGP022	N/A (rotifer)	*Philodinidae sp.*	79	/
NEGP022	N/A (black fly)	*Simulium velutinum*	47	/
NEGP022	N/A (non-biting midge)	*Zavrelimyia sp.*	25	/
NEGP022	N/A (springtail)	*Sminthurinus reticulatus*	15	/
NEGP022	N/A (caddis fly)	*Glyphotaelius pellucidus*	15	/
NEGP022	N/A (fly)	*Fannia pallitibia*	15	/
NEGP022	N/A (fungus gnat)	*Exechia fusca*	15	/
NEGP022	N/A (worm)	*Chaetogaster diastrophus*	14	/
NEGP022	N/A (barklice)	*Ectopsocus sp.*	13	/
NEGP022	Harelquin ladybird	*Harmonia axyridis*	11	/
NEGP022	N/A (non-biting midge)	*Metriocnemus eurynotus*	10	/
NEGP022	N/A (crustacean)	*Diacyclops bicuspidatus*	7	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP022	White-lipped snail	*Cepaea hortensis*	7	/
NEGP022	N/A (non-biting midge)	*Chironomus luridus*	6	/
NEGP023	N/A (black fly)	*Simulium velutinum*	261	54383
NEGP023	N/A (non-biting midge)	*Micropsectra atrofasciata*	203	/
NEGP023	N/A (non-biting midge)	*Chironomidae sp.*	111	/
NEGP023	2 spotted water hog-louse	*Asellus aquaticus*	96	/
NEGP023	N/A (worm)	*Nais communis*	72	/
NEGP023	red worm	*Limnodrilus hoffmeisteri*	69	/
NEGP023	N/A (black fly)	*Simulium noelleri*	29	/
NEGP023	N/A (non-biting midge)	*Rheocricotopus fuscipes*	17	/
NEGP023	Sludge/sewage worm	*Tubifex tubifex*	12	/
NEGP023	N/A (copepod)	*Acanthocyclops vernalis*	5	/
NEGP024	N/A (copepod)	*Acanthocyclops vernalis*	7940	65406
NEGP024	N/A (water flea)	*Chydorus sphaericus*	304	/
NEGP024	Sludge/sewage worm	*Tubifex tubifex*	70	/
NEGP024	N/A (worm)	*Pristina aequiseta*	17	/
NEGP024	N/A (worm)	*Nais communis*	6	/
NEGP024	N/A (crustacean)	*Daphnia sp.*	6	/
NEGP025	Common earthworm	*Lumbricus terrestris*	42882	7943
NEGP025	N/A (barklice)	*Psocoptera sp.*	791	/
NEGP025	Spotted-winged Drosophila	*Drosophila suzukii*	359	/
NEGP025	N/A (fly)	*Hebecnema vespertina*	357	/
NEGP025	Salt and pepper microcaddisfly	*Agraylea multipunctata*	175	/
NEGP025	N/A (harvestman)	*Paroligolophus agrestis*	102	/
NEGP025	N/A (barklice)	*Ectopsocus briggsi*	99	/
NEGP025	N/A (shrimp)	*Crangonyx pseudogracilis*	83	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP025	N/A (fly)	*Leptocera caenosa*	18	/
NEGP026	?	*UNVERIFIED: Arthropoda environmental sample*	106	56539
NEGP026	N/A (copepod)	*Acanthocyclops vernalis*	25	/
NEGP027	N/A (copepod)	*Eucyclops cf. serrulatus*	14868	19580
NEGP027	N/A (springtail)	*Sminthurinus sp.*	365	/
NEGP027	Common earthworm	*Lumbricus terrestris*	29	/
NEGP027	N/A (crustacean)	*Diacyclops bicuspidatus*	23	/
NEGP028	N/A (worm)	*Nais sp.*	19	40758
NEGP028	N/A (worm)	*Bothrioneurum vejdovskyanum*	18	/
NEGP028	N/A (midge)	*Cricotopus sylvestris*	10	/
NEGP029	American bladder snail	*Physella acuta*	16	30719
NEGP029	Great pond snail	*Lymnaea stagnalis*	14	/
NEGP029	American bladder snail	*Physa acuta*	8	/
NEGP030	N/A (crustacean)	*Chydorus brevilabris*	39281	12612
NEGP030	N/A (crustacean)	*Maxillopoda sp.*	14634	/
NEGP030	N/A (water flea)	*Chydorus sphaericus*	1587	/
NEGP030	N/A (arachnid)	*Arachnida sp.*	34	/
NEGP030	N/A (rotifer)	*Synchaeta pectinata*	15	/
NEGP031	N/A (crustacean)	*Macrothrix sp.*	10	105826
NEGP031	N/A (rotifer)	*Asplanchna sieboldi*	6	/
NEGP032	N/A (rotifer)	*Synchaeta pectinata*	22986	64840
NEGP032	Netted slug	*Deroceras reticulatum*	5636	/
NEGP032	American bladder snail	*Physa acuta*	162	/
NEGP032	N/A (mayfly)	*Cloeon dipterum*	128	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP032	Common earthworm	*Lumbricus terrestris*	121	/
NEGP032	N/A (mayfly)	*Cloeon dipterum*	107	/
NEGP032	Chestnut slug	*Deroceras invadens*	47	/
NEGP032	N/A (worm)	*Chaetogaster diaphanus*	43	/
NEGP032	Keeled slug	*Tandonia sowerbyi*	42	/
NEGP032	N/A (crustacean)	*Pleuroxus denticulatus*	41	/
NEGP032	N/A (crustacean)	*Cypridopsis sp.*	32	/
NEGP032	N/A (non-biting midge)	*Metriocnemus eurynotus*	23	/
NEGP032	N/A (crustacean)	*Maxillopoda sp.*	13	/
NEGP032	Garden snail	*Helix aspersa*	11	/
NEGP032	N/A (crustacean)	*Macrothrix sp.*	11	/
NEGP032	N/A (copepod)	*Eucyclops cf. serrulatus*	8	/
NEGP032	N/A (copepod)	*Acanthocyclops vernalis*	7	/
NEGP032	N/A (springtail)	*Isotomidae sp.*	5	/
NEGP033	N/A (water flea)	*Chydorus sphaericus*	166	71926
NEGP033	Black-headed worm	*Aporrectodea longa*	47	/
NEGP033	N/A (rotifer)	*Testudinella patina*	22	/
NEGP033	N/A (non-biting midge)	*Agraylea multipunctata*	10	/
NEGP033	N/A (slug)	*Arion sp.*	9	/
NEGP033	Hoverfly	*Eupeodes luniger*	7	/
NEGP033	Mushroom springtail	*Ceratophysella denticulata*	6	/
NEGP033	Square-spot rustic moth	*Xestia xanthographa*	5	/
NEGP034	N/A (copepod)	*Acanthocyclops vernalis*	41582	41507
NEGP034	N/A (water flea)	*Chydorus sphaericus*	6026	/
NEGP034	N/A (non-biting midge)	*Limnophyes pentaplastus*	118	/
NEGP034	N/A (water flea)	*Chydoridae sp.*	78	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP034	N/A (copepod)	*Paracyclops fimbriatus*	49	/
NEGP034	Great pond snail	*Lymnaea stagnalis*	45	/
NEGP034	N/A (arachnid)	*Arachnida sp.*	27	/
NEGP034	N/A (non-biting midge)	*Bryophaenocladius sp.*	26	/
NEGP034	Black-headed worm	*Aporrectodea longa*	25	/
NEGP034	N/A (non-biting midge)	*Chironomidae sp.*	13	/
NEGP034	N/A (worm)	*Chaetogaster diastrophus*	8	/
NEGP035	N/A (rotifer)	*Lecane closterocerca*	906	65724
NEGP035	N/A (bdelloid rotifer)	*Rotaria rotatoria*	332	/
NEGP035	Grey worm	*Aporrectodea caliginosa*	147	/
NEGP035	N/A (non-biting midge)	*Limnophyes pentaplastus*	72	/
NEGP035	Great pond snail	*Lymnaea stagnalis*	51	/
NEGP035	N/A (earthworm)	*Dendrobaena octaedra*	45	/
NEGP035	Salt and pepper microcaddisfly	*Agraylea multipunctata*	42	/
NEGP035	N/A (worm)	*Pristina aequiseta*	41	/
NEGP035	N/A (crane fly)	*Tipula lateralis*	32	/
NEGP035	N/A (mayfly)	*Cloeon dipterum*	29	/
NEGP035	N/A (crustacean)	*Chydorus brevilabris*	29	/
NEGP035	N/A (worm)	*Chaetogaster diastrophus*	29	/
NEGP035	Black-headed worm	*Aporrectodea longa*	29	/
NEGP035	N/A (red worm)	*Eiseniella tetraedra*	27	/
NEGP035	Common darter dragonfly	*Sympetrum striolatum*	25	/
NEGP035	N/A (springtail)	*Brachystomella parvula*	24	/
NEGP035	?	*Arthropoda*	21	/
NEGP035	N/A (non-biting midge)	*Metriocnemus eurynotus*	20	/
NEGP035	N/A (copepod)	*Eucyclops cf. serrulatus*	12	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP035	N/A (springtail)	*Parisotoma notabilis*	11	/
NEGP035	N/A (non-biting midge)	*Paraphaenocladius impensus*	11	/
NEGP035	Duck mussel	*Anodonta anatina*	11	/
NEGP035	N/A (worm)	*Fridericia galba*	8	/
NEGP035	Broad-bodied chaser dragonfly	*Ladona depressa*	8	/
NEGP035	N/A (caddis fly)	*Limnephilus lunatus*	7	/
NEGP039	Marsh sN/Ail	*Stagnicola palustris*	75	119262
NEGP039	N/A (non-biting midge)	*Zavrelimyia sp.*	33	/
NEGP039	N/A (springtail)	*Isotoma viridis*	9	/
NEGP040	Green hydra fresh-water polyp	*Hydra viridissima*	107	13243
NEGP040	N/A (non-biting midge)	*Psectrotanypus varius*	54	/
NEGP040	N/A (non-biting midge)	*Chironomidae sp.*	13	/
NEGP040	2 spotted water hog-louse	*Asellus aquaticus*	6	/
NEGP044	N/A (springtail)	*Tomocerus vulgaris*	11710	31148
NEGP044	N/A (water flea)	*Chydorus sphaericus*	4771	/
NEGP044	N/A (copepod)	*Eucyclops cf. serrulatus*	958	/
NEGP044	N/A (bdelloid rotifer)	*Rotaria rotatoria*	382	/
NEGP044	N/A (red worm)	*Eiseniella tetraedra*	326	/
NEGP044	Salt and pepper microcaddisfly	*Agraylea multipunctata*	237	/
NEGP044	N/A (worm)	*Globulidrilus sp.*	220	/
NEGP044	N/A (mosquito)	*Culiseta morsitans*	208	/
NEGP044	N/A (mite)	*Humerobatidae sp.*	93	/
NEGP044	Grey worm	*Aporrectodea caliginosa*	81	/
NEGP044	N/A (mayfly)	*Cloeon dipterum*	62	/
NEGP044	N/A (copepod)	*Canthocamptidae sp.*	61	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP044	N/A (moth)	*Epinotia immundana*	46	/
NEGP044	N/A (Psocoptera)	*Valenzuela atricornis*	39	/
NEGP044	N/A (non-biting midge)	*Paraphaenocladius impensus*	39	/
NEGP044	N/A (arachnid)	*Arachnida sp.*	38	/
NEGP044	Silver slug or Dotted slug	*Arion circumscriptus*	36	/
NEGP044	Green worm	*Allolobophora chlorotica*	34	/
NEGP044	Hoverfly "the footballer"	*Helophilus pendulus*	27	/
NEGP044	2 spotted water hog-louse	*Asellus aquaticus*	21	/
NEGP044	Springtail	*Tomocerus minor*	19	/
NEGP044	N/A (slug)	*Arion sp.*	18	/
NEGP044	N/A (springtail)	*Xenylla sp.*	16	/
NEGP044	N/A (crustacean)	*Chydorus sp.*	16	/
NEGP044	N/A (crustacean)	*Diacyclops bicuspidatus*	15	/
NEGP044	N/A (springtail)	*Tomocerus sp.*	15	/
NEGP044	N/A (rotifer)	*Testudinella patina*	12	/
NEGP044	N/A (caddis fly)	*Limnephilus marmoratus*	10	/
NEGP044	Cosmpolitan springtail	*Entomobrya nivalis*	10	/
NEGP044	N/A (non-biting midge)	*Chironomidae sp.*	9	/
NEGP044	N/A (non-biting midge)	*Metriocnemus eurynotus*	8	/
NEGP044	N/A (caddis fly)	*Glyphotaelius pellucidus*	5	/
NEGP044	N/A (springtail)	*Dicyrtomina ornata*	5	/
NEGP044	N/A (worm)	*Oligochaeta sp.*	5	/
NEGP045	red worm	*Limnodrilus hoffmeisteri*	1061	46288
NEGP045	N/A (arachnid)	*Arachnida sp.*	466	/
NEGP045	?	*UNVERIFIED: Arthropoda environmental sample*	87	/
NEGP045	N/A (bdelloid rotifer)	*Rotaria rotatoria*	71	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP045	N/A (worm)	*Lumbriculidae sp.*	14	/
NEGP045	N/A (red worm)	*Eiseniella tetraedra*	9	/
NEGP045	N/A (copepod)	*Acanthocyclops vernalis*	8	/
NEGP046	N/A (non-biting midge)	*Chironomidae sp.*	2749	32021
NEGP046	N/A (true bug)	*Cacopsylla melanoneura*	1109	/
NEGP046	Winter crane fly	*Trichoceridae sp.*	423	/
NEGP046	N/A (worm)	*Nais sp.*	419	/
NEGP046	N/A (copepod)	*Paracyclops fimbriatus*	380	/
NEGP046	N/A (barklice)	*Ectopsocus briggsi*	309	/
NEGP046	N/A (arachnid)	*Arachnida sp.*	241	/
NEGP046	N/A (earthworm)	*Dendrodrilus rubidus*	201	/
NEGP046	N/A (copepod)	*Cyclopidae sp.*	129	/
NEGP046	N/A (earthworm)	*Lumbricidae sp.*	111	/
NEGP046	N/A (springtail)	*Ceratophysella bengtssoni*	65	/
NEGP046	2 spotted water hog-louse	*Asellus aquaticus*	59	/
NEGP046	N/A (fly)	*Limnophyes minimus*	43	/
NEGP046	Chestnut slug	*Deroceras invadens*	29	/
NEGP046	?	*Arthropoda environmental sample*	29	/
NEGP046	N/A (true bug)	*Drepanosiphum sp.*	26	/
NEGP046	N/A (hover fly)	*Syrphus ribesii*	21	/
NEGP046	Bank worm	*Bimastos rubidus*	15	/
NEGP046	N/A (worm)	*Octolasion cyaneum*	14	/
NEGP046	N/A (fly)	*Lauxaniidae sp.*	11	/
NEGP046	N/A (bdelloid rotifer)	*Rotaria rotatoria*	6	/
NEGP046	N/A (worm)	*Nais communis*	6	/
NEGP046	?	*UNVERIFIED: Arthropoda environmental sample*	5	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP047	Sludge/sewage worm	*Tubifex tubifex*	262	8179
NEGP047	N/A (worm)	*Nais communis*	182	/
NEGP047	N/A (worm)	*Nais sp.*	132	/
NEGP047	N/A (worm)	*Henlea nasuta*	124	/
NEGP047	Rosy-tipped worm	*Aporrectodea rosea*	112	/
NEGP047	2 spotted water hog-louse	*Asellus aquaticus*	34	/
NEGP047	red worm	*Limnodrilus hoffmeisteri*	16	/
NEGP047	N/A (copepod)	*Eucyclops cf. serrulatus*	12	/
NEGP047	N/A (crane fly)	*Tipula oleracea*	8	/
NEGP047	Wandering pond snail	*Radix balthica*	8	/
NEGP047	N/A (copepod)	*Acanthocyclops vernalis*	7	/
NEGP047	N/A (ground beetle)	*Nebria brevicollis*	6	/
NEGP047	Large yellow underwing moth	*Noctua pronuba*	5	/
NEGP047	Common bluebottle fly	*Calliphora vicina*	5	/
NEGP049	N/A (red worm)	*Eiseniella tetraedra*	1578	61764
NEGP049	N/A (crustacean)	*Diacyclops bicuspidatus*	1216	/
NEGP049	Sludge/sewage worm	*Tubifex tubifex*	1112	/
NEGP049	Rosy-tipped worm	*Aporrectodea rosea*	1036	/
NEGP049	Salt and pepper microcaddisfly	*Agraylea multipunctata*	702	/
NEGP049	Green worm	*Allolobophora chlorotica*	697	/
NEGP049	Tapered drone fly	*Eristalis pertinax*	237	/
NEGP049	red worm	*Limnodrilus hoffmeisteri*	236	/
NEGP049	N/A (worm)	*Pristina aequiseta*	190	/
NEGP049	Grey worm	*Aporrectodea caliginosa*	149	/
NEGP049	N/A (fly)	*Xenopelopia nigricans*	108	/
NEGP049	N/A (arachnid)	*Arachnida sp.*	102	/
NEGP049	2 spotted water hog-louse	*Asellus aquaticus*	74	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP049	N/A (shrimp)	*Crangonyx pseudogracilis*	36	/
NEGP049	N/A (centipede)	*Geophilus flavus*	35	/
NEGP049	Black clock beetle	*Pterostichus madidus*	34	/
NEGP049	N/A (earthworm)	*Aporrectodea tuberculata*	33	/
NEGP049	N/A (true bug)	*Ribautiana ulmi*	31	/
NEGP049	N/A (barklice)	*Ectopsocus briggsi*	29	/
NEGP049	N/A (springtail)	*Entomobryidae sp.*	28	/
NEGP049	N/A (worm)	*Fridericia sp.*	27	/
NEGP049	Banded mosquito	*Culiseta sp.*	27	/
NEGP049	N/A (earthworm)	*Dendrodrilus rubidus*	23	/
NEGP049	N/A (crane fly)	*Helius longirostris*	20	/
NEGP049	N/A (fly)	*Scatopsciara vitripennis*	18	/
NEGP049	N/A (hover fly)	*Melanogaster aerosa*	17	/
NEGP049	N/A (caddis fly)	*Glyphotaelius pellucidus*	15	/
NEGP049	N/A (rotifer)	*Euchlanis dilatata*	14	/
NEGP049	N/A (worm)	*Limnodrilus claparedianus*	13	/
NEGP049	Bank worm	*Bimastos rubidus rubidus*	13	/
NEGP049	N/A (springtail)	*Tomocerus sp.*	10	/
NEGP049	N/A (barklice)	*Valenzuela flavidus*	8	/
NEGP049	N/A (diving beetle)	*Colymbetes fuscus*	7	/
NEGP051	N/A (copepod)	*Eucyclops cf. serrulatus*	2338	66649
NEGP051	N/A (copepod)	*Canthocamptidae sp.*	198	/
NEGP051	N/A (non-biting midge)	*Metriocnemus eurynotus*	44	/
NEGP051	N/A (arachnid)	*Arachnida sp.*	36	/
NEGP051	N/A (barklice)	*Cerobasis guestfalica*	23	/
NEGP051	N/A (red worm)	*Eiseniella tetraedra*	22	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP051	N/A (barklice)	*Ectopsocus briggsi*	20	/
NEGP051	2 spotted water hog-louse	*Asellus aquaticus*	16	/
NEGP051	N/A (mite)	*Eupodidae sp.*	12	/
NEGP051	N/A (springtail)	*Willowsia nigromaculata*	11	/
NEGP051	N/A (barklice)	*Ectopsocus sp.*	10	/
NEGP051	N/A (springtail)	*Dicyrtomina saundersi*	5	/
NEGP052	N/A (copepod)	*Canthocamptidae sp.*	4780	38914
NEGP052	Green hydra fresh-water polyp	*Hydra viridissima*	800	/
NEGP052	N/A (non-biting midge)	*Chironomidae sp.*	665	/
NEGP052	N/A (worm)	*Aulodrilus pluriseta*	471	/
NEGP052	N/A (shrimp)	*Crangonyx pseudogracilis*	317	/
NEGP052	Mushroom springtail	*Ceratophysella denticulata*	231	/
NEGP052	2 spotted water hog-louse	*Asellus aquaticus*	178	/
NEGP052	N/A (worm)	*Enchytraeidae sp.*	156	/
NEGP052	N/A (arachnid)	*Arachnida sp.*	74	/
NEGP052	N/A (copepod)	*Eucyclops cf. serrulatus*	49	/
NEGP052	N/A (non-biting midge)	*Agraylea multipunctata*	32	/
NEGP052	red worm	*Limnodrilus hoffmeisteri*	24	/
NEGP052	N/A (barklice)	*Ectopsocus briggsi*	16	/
NEGP052	N/A (worm)	*Nais sp.*	10	/
NEGP052	N/A (leech)	*Erpobdella testacea*	7	/
NEGP052	N/A (worm)	*Potamothrix bavaricus*	5	/
NEGP052	2 spotted water hog-louse	*Asellus aquaticus*	5	/
NEGP053	N/A (crustacean)	*Podocopida sp.*	888	81844
NEGP053	N/A (water flea)	*Chydorus sphaericus*	874	/
NEGP053	N/A (rotifer)	*Synchaeta pectinata*	347	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP053	N/A (non-biting midge)	*Chironomus sp.*	341	/
NEGP053	N/A (mayfly)	*Cloeon dipterum*	309	/
NEGP053	N/A (crane fly)	*Tipula lateralis*	294	/
NEGP053	N/A (crustacean)	*Cypridopsis vidua*	117	/
NEGP053	N/A (worm)	*Dero obtusa*	81	/
NEGP053	N/A (crustacean)	*Maxillopoda sp.*	72	/
NEGP053	N/A (worm)	*Bothrioneurum vejdovskyanum*	71	/
NEGP053	Great pond snail	*Lymnaea stagnalis*	43	/
NEGP053	N/A (copepod)	*Eucyclops cf. serrulatus*	34	/
NEGP053	N/A (crustacean)	*Diacyclops bicuspidatus*	25	/
NEGP053	N/A (cricket)	*Gryllus sp.*	17	/
NEGP053	Large yellow underwing moth	*Noctua pronuba*	13	/
NEGP053	Microflex worms	*Dero digitata*	8	/
NEGP053	Argentine ant	*Linepithema sp.*	8	/
NEGP053	N/A (crustacean)	*Cypridopsis sp.*	8	/
NEGP053	N/A (braconid wasp)	*Praon sp.*	7	/
NEGP053	?	*UNVERIFIED: Arthropoda environmental sample*	5	/
NEGP055	N/A (rotifer)	*Keratella cochlearis*	1205	30062
NEGP055	N/A (worm)	*Buchholzia appendiculata*	55	/
NEGP055	N/A (worm)	*Fridericia paroniana*	45	/
NEGP055	N/A (worm)	*Enchytraeidae gen. sp.*	27	/
NEGP055	Duck mussel	*Anodonta anatina*	24	/
NEGP055	N/A (worm)	*Enchytraeidae sp.*	22	/
NEGP055	N/A (copepod)	*Canthocamptidae sp.*	19	/
NEGP055	N/A (copepod)	*Cyclops strenuus*	13	/
NEGP055	Black-headed worm	*Aporrectodea longa*	13	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP055	N/A (red worm)	*Eiseniella tetraedra*	12	/
NEGP055	N/A (springtail)	*Lepidocyrtus sp.*	12	/
NEGP055	N/A (worm)	*Ophidonais serpentina*	10	/
NEGP055	N/A (worm)	*Enchytraeus bulbosus*	9	/
NEGP055	Redhead worm	*Lumbricus rubellus*	7	/
NEGP055	N/A (springtail)	*Entomobrya albocincta*	6	/
NEGP055	Bank worm	*Bimastos rubidus*	5	/
NEGP055	Salt and pepper microcaddisfly	*Agraylea multipunctata*	5	/
NEGP056	N/A (copepod)	*Eucyclops cf. serrulatus*	11278	26619
NEGP056	N/A (water flea)	*Chydorus sphaericus*	5043	/
NEGP056	N/A (copepod)	*Acanthocyclops vernalis*	268	/
NEGP056	N/A (crustacean)	*Diacyclops bicuspidatus*	43	/
NEGP056	N/A (leech)	*Erpobdella testacea*	26	/
NEGP056	N/A (barklice)	*Ectopsocus sp.*	25	/
NEGP056	2 spotted water hog-louse	*Asellus aquaticus*	23	/
NEGP056	N/A (midge)	*Cricotopus sylvestris*	12	/
NEGP056	Silver slug or Dotted slug	*Arion circumscriptus*	12	/
NEGP056	Rosy-tipped worm	*Aporrectodea rosea*	12	/
NEGP056	Great ramshorn snail	*Planorbarius corneus*	11	/
NEGP056	Fresh-water polyp	*Hydra magnipapillata*	11	/
NEGP056	N/A (arachnid)	*Arachnida sp.*	7	/
NEGP057	N/A (worm)	*Enchytraeidae sp.*	211	103425
NEGP057	N/A (worm)	*Fridericia galba*	165	/
NEGP057	N/A (crane fly)	*Tipula paludosa*	164	/
NEGP057	Green hydra fresh-water polyp	*Hydra vulgaris*	155	/
NEGP057	N/A (rove beetle)	*Xantholinus linearis*	119	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP057	N/A (copepod)	*Paracyclops fimbriatus*	59	/
NEGP057	N/A (earthworm)	*Lumbricidae sp.*	59	/
NEGP057	Black-headed worm	*Aporrectodea longa*	58	/
NEGP057	N/A (barklice)	*Ectopsocus briggsi*	36	/
NEGP057	N/A (springtail)	*Lepidocyrtus sp.*	34	/
NEGP057	N/A (water flea)	*Chydorus sphaericus*	25	/
NEGP057	Brown centipede	*Lithobius forficatus*	20	/
NEGP057	2 spotted water hog-louse	*Asellus aquaticus*	19	/
NEGP057	Mushroom springtail	*Ceratophysella denticulata*	18	/
NEGP057	N/A (crustacean)	*Isopoda sp.*	9	/
NEGP057	N/A (red worm)	*Eiseniella tetraedra*	8	/
NEGP057	N/A (copepod)	*Acanthocyclops vernalis*	8	/
NEGP057	N/A (barklice)	*Ectopsocus sp.*	8	/
NEGP057	Rosy-tipped worm	*Aporrectodea rosea*	5	/
NEGP057	Salt and pepper microcaddisfly	*Agraylea multipunctata*	5	/
NEGP058	Sludge/sewage worm	*Tubifex tubifex*	9421	28381
NEGP058	N/A (copepod)	*Canthocamptidae sp.*	8607	/
NEGP058	N/A (worm)	*Aulodrilus pluriseta*	8077	/
NEGP058	red worm	*Limnodrilus hoffmeisteri*	4444	/
NEGP058	N/A (worm)	*Tubificinae sp.*	1172	/
NEGP058	Black-headed worm	*Aporrectodea longa*	1010	/
NEGP058	Blackworm	*Lumbriculus variegatus*	442	/
NEGP058	N/A (worm)	*Limnodrilus udekemianus*	89	/
NEGP058	Mottled worm	*Aporrectodea icterica*	28	/
NEGP058	Salt and pepper microcaddisfly	*Agraylea multipunctata*	22	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP058	?	*Arthropoda environmental sample*	20	/
NEGP058	N/A (springtail)	*Sminthurinus sp.*	19	/
NEGP059	N/A (mayfly)	*Cloeon dipterum*	93	47939
NEGP059	Marsh snail	*Stagnicola palustris*	9	/
NEGP060	N/A (copepod)	*Acanthocyclops vernalis*	2212	30967
NEGP060	N/A (red worm)	*Eiseniella tetraedra*	1698	/
NEGP060	American bladder snail	*Physa acuta*	1643	/
NEGP060	N/A (worm)	*Enchytraeidae sp.*	351	/
NEGP060	Black-headed worm	*Aporrectodea longa*	125	/
NEGP060	Green worm	*Allolobophora chlorotica*	94	/
NEGP060	N/A (copepod)	*Cyclopoida sp.*	78	/
NEGP060	N/A (crane fly)	*Tipula oleracea*	47	/
NEGP060	N/A (springtail)	*Tomocerus vulgaris*	32	/
NEGP060	N/A (fly)	*Notiphila cinerea*	26	/
NEGP060	N/A (centipede)	*Geophilus flavus*	16	/
NEGP060	Ruddy worm	*Lumbricus festivus*	16	/
NEGP060	N/A (slug)	*Deroceras sp.*	14	/
NEGP060	N/A (rove beetle)	*Quedius levicollis*	12	/
NEGP060	N/A (shrimp)	*Crangonyx pseudogracilis*	12	/
NEGP060	N/A (fly)	*Psychoda phalaenoides*	8	/
NEGP060	American bladder snail	*Physella acuta*	5	/
NEGP060	N/A (brown centipede)	*Lithobius sp.*	5	/
NEGP061	N/A (copepod)	*Acanthocyclops vernalis*	5199	33460
NEGP061	N/A (copepod)	*Canthocamptidae sp.*	46	/
NEGP061	Blackworm	*Lumbriculus variegatus*	27	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP061	Green leaf hopper	*Empoasca decipiens*	19	/
NEGP061	N/A (arachnid)	*Arachnida sp.*	15	/
NEGP061	N/A (shrimp)	*Crangonyx pseudogracilis*	10	/
NEGP061	Phantom midge	*Chaoborus crystallinus*	10	/
NEGP061	Marsh snail	*Stagnicola palustris*	7	/
NEGP061	N/A (fly)	*Drosophila sp.*	7	/
NEGP061	N/A (barklice)	*Ectopsocus briggsi*	5	/
NEGP061	?	*Arthropoda environmental sample*	5	/
NEGP062	N/A (non-biting midge)	*Chironomidae sp.*	122	113190
NEGP062	N/A (worm)	*Nais communis*	14	/
NEGP062	Lake limpet	*Acroloxus lacustris*	8	/
NEGP064	Common earthworm	*Lumbricus terrestris*	17948	9027
NEGP064	N/A (mite)	*Humerobatidae sp.*	15864	/
NEGP064	N/A (springtail)	*Sminthurinus reticulatus*	12301	/
NEGP064	N/A (earthworm)	*Lumbricidae sp.*	792	/
NEGP064	N/A (copepod)	*Acanthocyclops vernalis*	294	/
NEGP064	Rosy-tipped worm	*Aporrectodea rosea*	238	/
NEGP064	N/A (crane fly)	*Tipula oleracea*	176	/
NEGP064	Common branding worm	*Eisenia sp.*	41	/
NEGP064	N/A (springtail)	*Sminthurinus elegans*	27	/
NEGP064	Great pond snail	*Lymnaea stagnalis*	25	/
NEGP064	N/A (barklice)	*Ectopsocus briggsi*	24	/
NEGP064	Microflex worms	*Dero digitata*	24	/
NEGP064	Grey worm	*Aporrectodea caliginosa*	18	/
NEGP064	N/A (non-biting midge)	*Chironomidae sp.*	17	/
NEGP064	Green worm	*Allolobophora chlorotica*	11	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP064	Sludge worm	*Psammoryctides barbatus*	6	/
NEGP065	American bladder snail	*Physella acuta*	148	5822
NEGP065	American bladder snail	*Physa acuta*	64	/
NEGP065	N/A (non-biting midge)	*Chironomus luridus*	35	/
NEGP065	N/A (bdelloid rotifer)	*Rotaria rotatoria*	26	/
NEGP065	N/A (red worm)	*Eiseniella tetraedra*	26	/
NEGP066	N/A (mite)	*Eupodidae sp.*	787	43425
NEGP066	N/A (ground beetle)	*Nebria brevicollis*	527	/
NEGP066	N/A (non-biting midge)	*Chironomidae sp.*	315	/
NEGP066	N/A (copepod)	*Acanthocyclops vernalis*	250	/
NEGP068	N/A (copepod)	*Acanthocyclops vernalis*	1739	92728
NEGP068	Bank worm	*Bimastos rubidus*	33	/
NEGP068	N/A (worm)	*Lumbriculus sp.*	12	/
NEGP068	N/A (rotifer)	*Euchlanis dilatata*	9	/
NEGP070	Common earthworm	*Lumbricus terrestris*	2663	26713
NEGP070	American bladder snail	*Physella acuta*	145	/
NEGP070	red worm	*Limnodrilus hoffmeisteri*	95	/
NEGP070	?	*UNVERIFIED: Arthropoda environmental sample*	94	/
NEGP070	Microflex worms	*Dero digitata*	39	/
NEGP070	N/A (fly)	*Dicranomyia modesta*	31	/
NEGP070	N/A (springtail)	*Tomocerus sp.*	29	/
NEGP070	N/A (shrimp)	*Crangonyx pseudogracilis*	20	/
NEGP070	N/A (worm)	*Eisenia sp.*	9	/
NEGP071	Mint aphid	*Ovatus crataegarius*	393	30100
NEGP071	N/A (non-biting midge)	*Micropsectra atrofasciata*	39	/
NEGP071	N/A (black fly)	*Simulium velutinum*	24	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP071	Common earthworm	*Lumbricus terrestris*	21	/
NEGP071	N/A (midge)	*Cricotopus sylvestris*	5	/
NEGP072	N/A (worm)	*Dero obtusa*	4923	24073
NEGP072	N/A (bdelloid rotifer)	*Rotaria rotatoria*	21	/
NEGP072	?	*UNVERIFIED: Arthropoda environmental sample*	18	/
NEGP072	Landhopper/woodhopper/lawn shrimp	*Arcitalitrus dorrieni*	15	/
NEGP074	----	*Empty*		7079
NEGP075	N/A (copepod)	*Acanthocyclops vernalis*	1179	35011
NEGP075	N/A (rotifer)	*Lecane closterocerca*	106	/
NEGP075	N/A (worm)	*Chaetogaster diaphanus*	91	/
NEGP075	N/A (worm)	*Stylaria lacustris*	66	/
NEGP075	N/A (worm)	*Chaetogaster diastrophus*	48	/
NEGP075	N/A (non-biting midge)	*Chironomidae sp.*	31	/
NEGP075	N/A (worm)	*Nais sp.*	12	/
NEGP075	N/A (hydra)	*Hydra circumcincta*	5	/
NEGP075	N/A (hexapod)	*Cyclopoida environmental sample*	5	/
NEGP076	?	*UNVERIFIED: Arthropoda environmental sample*	60	45510
NEGP076	N/A (earthworm)	*Octolasion lacteum*	48	/
NEGP076	Giant willow aphid	*Tuberolachnus salignus*	6	/
NEGP077	Mottled worm	*Aporrectodea icterica*	3882	42144
NEGP077	Southern house mosquito	*Culex pipiens*	1921	/
NEGP077	N/A (worm)	*Nais communis*	993	/
NEGP077	Black-headed worm	*Aporrectodea longa*	991	/
NEGP077	N/A (copepod)	*Paracyclops fimbriatus*	212	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP077	N/A (copepod)	*Acanthocyclops vernalis*	184	/
NEGP077	Hoverfly "the footballer"	*Helophilus pendulus*	136	/
NEGP077	N/A (barklice)	*Ectopsocus briggsi*	116	/
NEGP077	N/A (crustacean)	*Daphnia sp.*	81	/
NEGP077	Banded mosquito	*Culiseta sp.*	74	/
NEGP077	Common earthworm	*Lumbricus terrestris*	40	/
NEGP077	Compost worm	*Dendrobaena veneta*	38	/
NEGP077	Angle shades moth	*Phlogophora meticulosa*	28	/
NEGP077	N/A (worm)	*Eisenia sp.*	23	/
NEGP077	N/A (worm)	*Chaetogaster diastrophus*	22	/
NEGP077	Great pond snail	*Lymnaea stagnalis*	18	/
NEGP077	N/A (barklice)	*Ectopsocus sp.*	17	/
NEGP077	N/A (non-biting midge)	*Chironomus luridus*	10	/
NEGP077	Atlantic shelled slug	*Testacella maugei*	6	/
NEGP077	N/A (worm)	*Pristina longiseta*	6	/
NEGP077	N/A (non-biting midge)	*Metriocnemus picipes*	6	/
NEGP077	N/A (tardigrade)	*Ramazzottius sp.*	6	/
NEGP077	N/A (mite)	*Eupodidae sp.*	6	/
NEGP078	red worm	*Limnodrilus hoffmeisteri*	1261	6592
NEGP078	N/A (copepod)	*Acanthocyclops vernalis*	133	/
NEGP078	NA (copepod)	*Cyclops abyssorum*	123	/
NEGP078	NA (worm)	*Bothrioneurum vejdovskyanum*	52	/
NEGP078	N/A (mayfly)	*Cloeon dipterum*	24	/
NEGP078	N/A (barklice)	*Ectopsocus briggsi*	23	/
NEGP078	N/A (crustacean)	*Daphnia sp.*	13	/
NEGP078	water flea	*Daphnia pulex*	6	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP078	Great pond snail	*Lymnaea stagnalis*	5	/
NEGP079	N/A (tardigrade)	*Hypsibius cf. dujardini*	27	42342
NEGP080	N/A (water flea)	*Chydorus sphaericus*	3867	44969
NEGP080	N/A (crustacean)	*Podocopida sp.*	1231	/
NEGP080	N/A (copepod)	*Acanthocyclops vernalis*	728	/
NEGP080	N/A (crustacean)	*Cypridopsis vidua*	67	/
NEGP080	N/A (crustacean)	*Maxillopoda sp.*	50	/
NEGP080	N/A (worm)	*Chaetogaster diastrophus*	31	/
NEGP080	Blackworm	*Lumbriculus variegatus*	28	/
NEGP080	Green hydra fresh-water polyp	*Hydra viridissima*	21	/
NEGP080	N/A (bdelloid rotifer)	*Rotaria rotatoria*	10	/
NEGP080	red worm	*Limnodrilus hoffmeisteri*	5	/
NEGP080	N/A (crustacean)	*Cypridopsis sp.*	5	/
NEGP080	N/A (ribbon worm)	*Cerebratulus environmental sample*	5	/
NEGP081	N/A (crustacean)	*Diacyclops bicuspidatus*	5445	15249
NEGP081	N/A (red worm)	*Eiseniella tetraedra*	132	/
NEGP081	2 spotted water hog-louse	*Asellus aquaticus*	89	/
NEGP081	N/A (mollusc)	*Musculium sp.*	70	/
NEGP081	N/A (barklice)	*Ectopsocus briggsi*	35	/
NEGP081	Salt and pepper microcaddisfly	*Agraylea multipunctata*	34	/
NEGP081	N/A (bdelloid rotifer)	*Rotaria rotatoria*	9	/
NEGP085	red worm	*Limnodrilus hoffmeisteri*	31621	7377
NEGP085	N/A (worm)	*Pristina longiseta*	21359	/
NEGP085	N/A (water flea)	*Chydorus sphaericus*	12346	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP085	N/A (crustacean)	*Pseudocandona sp.*	9182	/
NEGP085	N/A (crustacean)	*Pleuroxus denticulatus*	3490	/
NEGP085	Green hydra fresh-water polyp	*Hydra vulgaris*	1516	/
NEGP085	N/A (mollusc)	*Musculium sp.*	437	/
NEGP085	N/A (copepod)	*Acanthocyclops vernalis*	393	/
NEGP085	N/A (springtail)	*Sminthurinus reticulatus*	196	/
NEGP085	N/A (worm)	*Fridericia sp.*	173	/
NEGP085	American bladder snail	*Physa acuta*	157	/
NEGP085	N/A (springtail)	*Sminthurinus sp.*	92	/
NEGP085	N/A (non-biting midge)	*Zavrelimyia sp.*	84	/
NEGP085	Common earthworm	*Lumbricus terrestris*	60	/
NEGP085	Compost worm	*Dendrobaena veneta*	52	/
NEGP085	N/A (worm)	*Quistadrilus sp.*	50	/
NEGP085	N/A (barklice)	*Ectopsocus briggsi*	46	/
NEGP085	N/A (fly)	*Scatopsciara sp.*	45	/
NEGP085	N/A (springtail)	*Willowsia nigromaculata*	39	/
NEGP085	N/A (red worm)	*Eiseniella tetraedra*	39	/
NEGP085	Girdled snail	*Hygromia cinctella*	38	/
NEGP085	American bladder snail	*Physella acuta*	30	/
NEGP085	N/A (non-biting midge)	*Metriocnemus eurynotus*	25	/
NEGP085	Black-headed worm	*Aporrectodea longa*	18	/
NEGP085	N/A (worm)	*Globulidrilus sp.*	17	/
NEGP085	N/A (crustacean)	*Isopoda sp.*	13	/
NEGP085	N/A (non-biting midge)	*Limnophyes sp.*	12	/
NEGP085	N/A (springtail)	*Hypogastrura distincta*	10	/
NEGP085	N/A (true bug)	*Drepanosiphum sp.*	10	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP085	N/A (springtail)	*Desoria sp.*	10	/
NEGP085	N/A (crustacean)	*Chydorus sp.*	7	/
NEGP085	N/A (springtail)	*Entomobryidae sp.*	6	/
NEGP085	N/A (crustacean)	*Pleuroxus sp.*	5	/
NEGP085R	N/A (worm)	*Pristina longiseta*	30093	11687
NEGP085R	Green hydra fresh-water polyp	*Hydra vulgaris*	7075	/
NEGP085R	N/A (water flea)	*Chydorus sphaericus*	3975	/
NEGP085R	N/A (crustacean)	*Pseudocandona sp.*	3948	/
NEGP085R	red worm	*Limnodrilus hoffmeisteri*	1531	/
NEGP085R	American bladder snail	*Physella acuta*	376	/
NEGP085R	N/A (crustacean)	*Pleuroxus denticulatus*	362	/
NEGP085R	American bladder snail	*Physa acuta*	308	/
NEGP085R	N/A (worm)	*Eisenia sp.*	308	/
NEGP085R	N/A (mollusc)	*Musculium sp.*	281	/
NEGP085R	N/A (copepod)	*Acanthocyclops vernalis*	255	/
NEGP085R	N/A (barklice)	*Ectopsocus briggsi*	239	/
NEGP085R	N/A (non-biting midge)	*Metriocnemus eurynotus*	153	/
NEGP085R	Girdled snail	*Hygromia cinctella*	108	/
NEGP085R	N/A (springtail)	*Sminthurinus reticulatus*	78	/
NEGP085R	?	*UNVERIFIED: Arthropoda environmental sample*	7	/
NEGP085R	N/A (red worm)	*Eiseniella tetraedra*	6	/
NEGP085R	Compost worm	*Dendrobaena veneta*	5	/
NEGP086	N/A (crustacean)	*Diacyclops bicuspidatus*	58878	4646
NEGP086	NA (crustacean)	*Pleuroxus denticulatus*	10494	/
NEGP086	NA (crustacean)	*Pleuroxus sp.*	42	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP086	?	*UNVERIFIED: Arthropoda environmental sample*	8	/
NEGP087	N/A (copepod)	*Eucyclops cf. serrulatus*	312	14890
NEGP087	N/A (non-biting midge)	*Zavrelimyia sp.*	177	/
NEGP087	N/A (copepod)	*Paracyclops fimbriatus*	81	/
NEGP087	N/A (worm)	*Pristina aequiseta*	22	/
NEGP087	N/A (copepod)	*Cyclopidae sp.*	16	/
NEGP087	red worm	*Limnodrilus hoffmeisteri*	15	/
NEGP087	N/A (mite)	*Humerobatidae sp.*	15	/
NEGP087	N/A (non-biting midge)	*Chironomidae sp.*	14	/
NEGP087	N/A (ribbon worm)	*Nemertean sp.*	12	/
NEGP087	N/A (arachnid)	*Arachnida sp.*	11	/
NEGP087	N/A (crustacean)	*Macrothrix sp.*	6	/
NEGP088	N/A (springtail)	*Hypogastrura sp.*	1656	78949
NEGP088	N/A (crustacean)	*Cypridopsis sp.*	209	/
NEGP088	American bladder snail	*Physa acuta*	204	/
NEGP088	N/A (barklice)	*Ectopsocus briggsi*	166	/
NEGP088	N/A (springtail)	*Pogonognathellus flavescens*	120	/
NEGP088	N/A (arachnid)	*Arachnida sp.*	87	/
NEGP088	?	*UNVERIFIED: Arthropoda environmental sample*	65	/
NEGP088	Garden snail	*Helix aspersa*	45	/
NEGP088	N/A (worm)	*Eisenia sp.*	34	/
NEGP088	N/A (non-biting midge)	*Zavrelimyia sp.*	13	/
NEGP088	?	*Arthropoda environmental sample*	6	/
NEGP089	Western yellow centipede	*Stigmatogaster subterranea*	1045	42887

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP089	N/A (springtail)	*Sminthurinus sp.*	93	/
NEGP089	Green worm	*Allolobophora chlorotica*	87	/
NEGP089	N/A (springtail)	*Sminthurinus reticulatus*	14	/
NEGP089	Salt and pepper microcaddisfly	*Agraylea multipunctata*	13	/
NEGP089	N/A (garden/pseudo centipedes)	*Symphyla sp.*	13	/
NEGP089	N/A (worm)	*Eisenia sp.*	9	/
NEGP089	?	*UNVERIFIED: Arthropoda environmental sample*	6	/
NEGP089	N/A (springtail)	*Parisotoma notabilis*	5	/
NEGP090	Common earthworm	*Lumbricus terrestris*	23831	11678
NEGP090	Compost worm	*Dendrobaena veneta*	356	/
NEGP090	N/A (springtail)	*Tomocerus sp.*	258	/
NEGP090	N/A (water flea)	*Chydorus sphaericus*	166	/
NEGP090	American bladder snail	*Physa acuta*	152	/
NEGP090	N/A (earthworm)	*Lumbricus castaneus*	133	/
NEGP090	N/A (non-biting midge)	*Chironomidae sp.*	131	/
NEGP090	Southern house mosquito	*Culex pipiens*	89	/
NEGP090	N/A (tardigrade)	*Ramazzottius sp.*	87	/
NEGP090	Hoverfly "the footballer"	*Helophilus pendulus*	47	/
NEGP090	N/A (worm)	*Eisenia sp.*	39	/
NEGP090	N/A (springtail)	*Parisotoma notabilis*	38	/
NEGP090	N/A (leafhopper)	*Aphrodes makarovi*	36	/
NEGP090	N/A (copepod)	*Acanthocyclops vernalis*	23	/
NEGP090	American bladder snail	*Physella acuta*	22	/
NEGP090	N/A (non-biting midge)	*Chironomus luridus*	21	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP091	N/A (worm)	*Chaetogaster diastrophus*	335	30699
NEGP091	Green hydra fresh-water polyp	*Hydra vulgaris*	68	/
NEGP091	N/A (worm)	*Pristina longiseta*	59	/
NEGP091	N/A (crustacean)	*Maxillopoda sp.*	55	/
NEGP091	N/A (worm)	*Ophidonais serpentina*	47	/
NEGP091	N/A (crustacean)	*Pleuroxus denticulatus*	11	/
NEGP091	N/A (crustacean)	*Podocopida sp.*	8	/
NEGP092	N/A (copepod)	*Acanthocyclops vernalis*	79177	4022
NEGP092	N/A (worm)	*Enchytraeidae sp.*	422	/
NEGP092	Blackworm	*Lumbriculus variegatus*	105	/
NEGP092	N/A (bdelloid rotifer)	*Rotaria rotatoria*	87	/
NEGP092	N/A (mayfly)	*Cloeon dipterum*	70	/
NEGP092	Bank worm	*Bimastos rubidus*	55	/
NEGP092	N/A (barklice)	*Ectopsocus briggsi*	20	/
NEGP092	N/A (worm)	*Nais communis*	19	/
NEGP092	2 spotted water hog-louse	*Asellus aquaticus*	15	/
NEGP092	Banded mosquito	*Culiseta sp.*	10	/
NEGP092	Foxglove/glasshouse-potato aphid	*Aulacorthum solani*	7	/
NEGP092	Vine weevil	*Otiorhynchus sulcatus*	6	/
NEGP092	N/A (worm)	*Nais christinae*	6	/
NEGP092	Microflex worms	*Dero digitata*	6	/
NEGP092	N/A (worm)	*Tubificinae sp.*	6	/
NEGP092	N/A (springtail)	*Tomocerus sp.*	6	/
NEGP092	N/A (springtail)	*Dicyrtomina ornata*	5	/
NEGP092	N/A (slug)	*Arion sp.*	5	/
NEGP093	N/A (crustacean)	*Diacyclops bicuspidatus*	23112	30631

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP093	N/A (crustacean)	*Daphnia obtusa*	28	/
NEGP094	Green worm	*Allolobophora chlorotica*	184	2316
NEGP094	N/A (non-biting midge)	*Zavrelimyia sp.*	102	/
NEGP094	Bank worm	*Bimastos rubidus*	91	/
NEGP094	N/A (black fungus gnats)	*Sciaridae sp.*	42	/
NEGP094	Hoverfly "the footballer"	*Helophilus pendulus*	11	/
NEGP094	N/A (non-biting midge)	*Chironomus luridus*	9	/
NEGP095	N/A (worm)	*Dero obtusa*	12165	60935
NEGP095	N/A (mite)	*Tydeidae sp.*	632	/
NEGP095	N/A (worm)	*Chaetogaster diastrophus*	148	/
NEGP095	Green worm	*Allolobophora chlorotica*	28	/
NEGP095	N/A (barklice)	*Ectopsocus briggsi*	19	/
NEGP095	N/A (arachnid)	*Arachnida sp.*	19	/
NEGP095	N/A (tardigrade)	*Hypsibius dujardini*	10	/
NEGP096	N/A (worm)	*Nais christinae*	421	65385
NEGP096	N/A (mayfly)	*Cloeon dipterum*	219	/
NEGP096	Green hydra fresh-water polyp	*Hydra viridissima*	127	/
NEGP096	N/A (non-biting midge)	*Smittia sp.*	103	/
NEGP096	N/A (worm)	*Fridericia sp.*	78	/
NEGP096	N/A (slug)	*Arion sp.*	42	/
NEGP096	Phantom midge	*Chaoborus crystallinus*	19	/
NEGP096	N/A (arachnid)	*Arachnida sp.*	15	/
NEGP096	N/A (copepod)	*Acanthocyclops vernalis*	7	/
NEGP096	N/A (non-biting midge)	*Psectrocladius sp.*	7	/
NEGP097	N/A (worm)	*Chaetogaster diastrophus*	474	38062
NEGP097	Green leaf hopper	*Empoasca decipiens*	412	/
NEGP097	Salt and pepper microcaddisfly	*Agraylea multipunctata*	184	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP097	N/A (springtail)	*Parisotoma notabilis*	114	/
NEGP097	N/A (fly)	*Psychoda phalaenoides*	82	/
NEGP097	N/A (non-biting midge)	*Limnophyes pentaplastus*	81	/
NEGP097	N/A (non-biting midge)	*Zavrelimyia sp.*	69	/
NEGP097	N/A (springtail)	*Ceratophysella bengtssoni*	66	/
NEGP097	N/A (springtail)	*Tomocerus sp.*	65	/
NEGP097	N/A (non-biting midge)	*Metriocnemus albolineatus*	48	/
NEGP097	N/A (mayfly)	*Cloeon dipterum*	39	/
NEGP097	N/A (non-biting midge)	*Metriocnemus eurynotus*	29	/
NEGP097	N/A (springtail)	*Hypogastrura burkilli*	29	/
NEGP097	N/A (non-biting midge)	*Micropsectra atrofasciata*	28	/
NEGP097	Common darter dragonfly	*Sympetrum striolatum*	24	/
NEGP097	N/A (non-biting midge)	*Eukiefferiella claripennis*	21	/
NEGP097	N/A (non-biting midge)	*Conchapelopia melanops*	15	/
NEGP097	N/A (arachnid)	*Arachnida sp.*	13	/
NEGP097	N/A (springtail)	*Isotoma viridis*	9	/
NEGP097	N/A (bdelloid rotifer)	*Rotaria rotatoria*	7	/
NEGP097	?	*UNVERIFIED: Arthropoda environmental sample*	6	/
NEGP097	N/A (black fly)	*Simulium velutinum*	5	/
NEGP097	N/A (non-biting midge)	*Paratanytarsus sp.*	5	/
NEGP098	N/A (bdelloid rotifer)	*Rotaria rotatoria*	53	84805
NEGP098	?	*UNVERIFIED: Arthropoda environmental sample*	39	/
NEGP098	?	*Arthropoda environmental sample*	7	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP099	N/A (water flea)	*Chydorus sphaericus*	7005	16603
NEGP099	N/A (worm)	*Chaetogaster diastrophus*	6426	/
NEGP099	?	*UNVERIFIED: Arthropoda environmental sample*	52	/
NEGP104	N/A (copepod)	*Eucyclops cf. serrulatus*	10995	96596
NEGP104	N/A (bdelloid rotifer)	*Rotaria rotatoria*	168	/
NEGP104	N/A (worm)	*Nais communis*	150	/
NEGP104	N/A (crustacean)	*Maxillopoda sp.*	124	/
NEGP104	N/A (crane fly)	*Tipula confusa*	51	/
NEGP104	2 spotted water hog-louse	*Asellus aquaticus*	33	/
NEGP104	N/A (slug)	*Arion sp.*	29	/
NEGP104	N/A (worm)	*Chaetogaster diastrophus*	28	/
NEGP104	N/A (worm)	*Enchytraeidae sp.*	21	/
NEGP104	N/A (crustacean)	*Diacyclops bicuspidatus*	20	/
NEGP104	N/A (barklice)	*Ectopsocus briggsi*	7	/
NEGP105	American bladder snail	*Physa acuta*	4747	10458
NEGP105	Southern house mosquito	*Culex pipiens*	1022	/
NEGP105	N/A (harvestman)	*Paroligolophus agrestis*	275	/
NEGP105	N/A (barklice)	*Ectopsocus sp.*	149	/
NEGP105	Hoverfly "the footballer"	*Helophilus pendulus*	66	/
NEGP105	Banded mosquito	*Culiseta sp.*	49	/
NEGP105	Banded mosquito	*Culiseta sp.*	47	/
NEGP105	N/A (springtail)	*Tomocerus sp.*	22	/
NEGP105	American bladder snail	*Physella acuta*	11	/
NEGP105	?	*Arthropoda environmental sample*	8	/
NEGP106	N/A (rotifer)	*Synchaeta pectinata*	477	54513

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP106	N/A (rotifer)	*Lecane closterocerca*	42	/
NEGP106	N/A (non-biting midge)	*Chironomidae sp.*	40	/
NEGP106	N/A (non-biting midge)	*Eukiefferiella claripennis*	33	/
NEGP106	N/A (worm)	*Chaetogaster diastrophus*	20	/
NEGP106	N/A (copepod)	*Paracyclops fimbriatus*	17	/
NEGP106	N/A (non-biting midge)	*Metriocnemus eurynotus*	16	/
NEGP106	N/A (fly)	*Parachironomus monochromus*	12	/
NEGP106	N/A (worm)	*Nais communis*	11	/
NEGP107	N/A (copepod)	*Acanthocyclops vernalis*	9269	43562
NEGP107	N/A (crustacean)	*Chydorus sp.*	5379	/
NEGP107	N/A (water flea)	*Chydorus sphaericus*	2437	/
NEGP107	N/A (crustacean)	*Cypridopsis sp.*	655	/
NEGP107	N/A (springtail)	*Hypogastrura burkilli*	289	/
NEGP107	Mint aphid	*Ovatus crataegarius*	151	/
NEGP107	N/A (copepod)	*Paracyclops fimbriatus*	109	/
NEGP107	N/A (crustacean)	*Candona sp.*	91	/
NEGP107	N/A (crane fly)	*Tipula confusa*	76	/
NEGP107	N/A (bdelloid rotifer)	*Philodina citrina*	66	/
NEGP107	Marsh snail	*Stagnicola palustris*	53	/
NEGP107	Sage leaf hopper	*Eupteryx melissae*	47	/
NEGP107	N/A (earthworm)	*Dendrodrilus rubidus*	28	/
NEGP107	N/A (ribbon worm)	*Cerebratulus environmental sample*	6	/
NEGP108	N/A (crustacean)	*Diacyclops bicuspidatus*	96161	624
NEGP108	red worm	*Limnodrilus hoffmeisteri*	1613	/
NEGP108	N/A (worm)	*Tubificinae sp.*	1027	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP108	N/A (copepod)	*Eucyclops cf. serrulatus*	174	/
NEGP108	N/A (springtail)	*Sminthurinus sp.*	12	/
NEGP108	N/A (worm)	*Potamothrix bavaricus*	11	/
NEGP108	Cloaked minor moth	*Mesoligia furuncula*	11	/
NEGP108	N/A (barklice)	*Ectopsocus briggsi*	7	/
NEGP108	water flea	*Daphnia pulex*	7	/
NEGP109	N/A (crustacean)	*Pleuroxus denticulatus*	107404	4809
NEGP109	N/A (crustacean)	*Pleuroxus sp.*	658	/
NEGP109	N/A (black fly)	*Simulium noelleri*	215	/
NEGP109	N/A (worm)	*Nais communis*	206	/
NEGP109	N/A (worm)	*Nais elinguis*	160	/
NEGP109	N/A (worm)	*Chaetogaster diastrophus*	76	/
NEGP109	N/A (non-biting midge)	*Eukiefferiella claripennis*	61	/
NEGP109	N/A (black fly)	*Simulium velutinum*	58	/
NEGP109	red worm	*Limnodrilus hoffmeisteri*	54	/
NEGP109	N/A (water flea)	*Anomopoda sp.*	49	/
NEGP109	N/A (non-biting midge)	*Chironomidae sp.*	29	/
NEGP109	N/A (non-biting midge)	*Cricotopus sp.*	19	/
NEGP109	N/A (crane fly)	*Tipula oleracea*	13	/
NEGP109	N/A (copepod)	*Acanthocyclops vernalis*	11	/
NEGP109	N/A (copepod)	*Eucyclops cf. serrulatus*	8	/
NEGP110	N/A (copepod)	*Acanthocyclops vernalis*	67102	1657
NEGP110	N/A (water flea)	*Chydorus sphaericus*	20218	/
NEGP110	N/A (crustacean)	*Caenestheriella gifuensis*	2524	/
NEGP110	N/A (crustacean)	*Podocopida sp.*	1361	/
NEGP110	N/A (bdelloid rotifer)	*Rotaria rotatoria*	112	/
NEGP110	N/A (fly)	*Siphona cristata*	47	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP110	N/A (rotifer)	*Euchlanis dilatata*	10	/
NEGP110	N/A (water flea)	*Chydorus sp.*	9	/
NEGP110	N/A (crustacean)	*Eucypris pigra*	6	/
NEGP111	N/A (copepod)	*Acanthocyclops vernalis*	38556	10023
NEGP111	N/A (copepod)	*Eucyclops cf. serrulatus*	14858	/
NEGP111	N/A (mite)	*Humerobatidae sp.*	427	/
NEGP111	N/A (non-biting midge)	*Chironomidae sp.*	141	/
NEGP111	N/A (bdelloid rotifer)	*Philodina citrina*	65	/
NEGP111	N/A (midge)	*Cricotopus sylvestris*	32	/
NEGP111	N/A (water flea)	*Chydorus sphaericus*	17	/
NEGP111	N/A (arachnid)	*Arachnida sp.*	14	/
NEGP111	N/A (leech)	*Erpobdella testacea*	12	/
NEGP111	2 spotted water hog-louse	*Asellus aquaticus*	12	/
NEGP111	N/A (mite)	*Hydrozetidae sp.*	7	/
NEGP112	N/A (worm)	*Dero obtusa*	1625	46364
NEGP112	Common branding worm	*Eisenia sp.*	116	/
NEGP112	N/A (springtail)	*Tomocerus sp.*	73	/
NEGP112	N/A (copepod)	*Acanthocyclops vernalis*	57	/
NEGP112	N/A (non-biting midge)	*Chironomidae sp.*	33	/
NEGP112	N/A (non-biting midge)	*Chironomus luridus*	8	/
NEGP113	N/A (worm)	*Dero obtusa*	169	55005
NEGP113	N/A (bdelloid rotifer)	*Rotaria rotatoria*	120	/
NEGP113	Large yellow underwing moth	*Noctua pronuba*	34	/
NEGP113	Microflex worms	*Dero digitata*	23	/
NEGP113	N/A (worm)	*Chaetogaster diastrophus*	8	/
NEGP114	N/A (rotifer)	*Brachionus calyciflorus*	17469	49705
NEGP114	N/A (springtail)	*Hypogastrura burkilli*	835	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP114	Common branding worm	*Eisenia sp.*	198	/
NEGP114	N/A (non-biting midge)	*Limnophyes sp.*	99	/
NEGP114	N/A (non-biting midge)	*Chironomidae sp.*	24	/
NEGP114	N/A (springtail)	*Tomocerus sp.*	7	/
NEGP114	N/A (midge)	*Cricotopus sylvestris*	6	/
NEGP114	N/A (non-biting midge)	*Corynoneura coronata*	5	/
NEGP114	N/A (worm)	*Chaetogaster diastrophus*	5	/
NEGP115	N/A (barklice)	*Caeciliusidae sp.*	845	41191
NEGP115	N/A (bdelloid rotifer)	*Rotaria rotatoria*	835	/
NEGP115	Marsh snail	*Stagnicola palustris*	311	/
NEGP115	Banded mosquito	*Culiseta sp.*	281	/
NEGP115	N/A (worm)	*Chaetogaster diaphanus*	259	/
NEGP115	N/A (springtail)	*Sminthurinus reticulatus*	256	/
NEGP115	Microflex worms	*Dero digitata*	234	/
NEGP115	N/A (worm)	*Chaetogaster diastrophus*	202	/
NEGP115	?	*UNVERIFIED: Arthropoda environmental sample*	174	/
NEGP115	Southern house mosquito	*Culex pipiens*	124	/
NEGP115	Mint aphid	*Ovatus crataegarius*	94	/
NEGP115	N/A (copepod)	*Cyclopidae sp.*	71	/
NEGP115	Budapest keeled slug	*Tandonia budapestensis*	67	/
NEGP115	N/A (non-biting midge)	*Zavrelimyia sp.*	65	/
NEGP115	Landhopper/woodhopper/lawn shrimp	*Arcitalitrus dorrieni*	51	/
NEGP115	N/A (non-biting midge)	*Chironomus luridus*	47	/
NEGP115	Common branding worm	*Eisenia sp.*	47	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP115	2 spotted water hog-louse	*Asellus aquaticus*	23	/
NEGP115	N/A (barklice)	*Ectopsocus briggsi*	19	/
NEGP115	Ornate/violet aphid	*Myzus ornatus*	16	/
NEGP115	Wandering pond snail	*Ampullaceana balthica*	12	/
NEGP115	?	*Arthropoda environmental sample*	9	/
NEGP115	N/A (mite)	*Eupodidae sp.*	8	/
NEGP116	N/A (crustacean)	*Diacyclops bicuspidatus*	43552	20891
NEGP116	N/A (copepod)	*Eucyclops cf. serrulatus*	12624	/
NEGP116	N/A (water flea)	*Chydorus sphaericus*	181	/
NEGP116	N/A (red worm)	*Eiseniella tetraedra*	55	/
NEGP116	N/A (copepod)	*Acanthocyclops vernalis*	55	/
NEGP116	N/A (rotifer)	*Lecane closterocerca*	42	/
NEGP116	N/A (bdelloid rotifer)	*Rotaria rotatoria*	20	/
NEGP116	N/A (crustacean)	*Pleuroxus denticulatus*	7	/
NEGP116	N/A (worm)	*Chaetogaster diastrophus*	7	/
NEGP116	N/A (mayfly)	*Cloeon dipterum*	6	/
NEGP117	N/A (bdelloid rotifer)	*Rotaria rotatoria*	987	90605
NEGP117	N/A (springtail)	*Sminthurinus reticulatus*	606	/
NEGP117	N/A (crustacean)	*Maxillopoda sp.*	238	/
NEGP117	N/A (crustacean)	*Caenestheriella gifuensis*	210	/
NEGP117	N/A (non-biting midge)	*Zavrelimyia sp.*	77	/
NEGP117	N/A (springtail)	*Tomocerus sp.*	63	/
NEGP117	N/A (worm)	*Dendrobaena cf. attemsi*	52	/
NEGP117	N/A (mite)	*Stigmaeidae sp.*	43	/
NEGP117	N/A (non-biting midge)	*Corynoneura scutellata*	39	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP117	Common earthworm	*Lumbricus terrestris*	32	/
NEGP117	N/A (non-biting midge)	*Chironomus luridus*	31	/
NEGP117	N/A (copepod)	*Cyclopidae sp.*	24	/
NEGP117	N/A (springtail)	*Dicyrtomina ornata*	21	/
NEGP117	N/A (worm)	*Chaetogaster diastrophus*	17	/
NEGP117	N/A (springtail)	*Pogonognathellus flavescens*	14	/
NEGP117	N/A (mollusc)	*Musculium sp.*	13	/
NEGP117	N/A (springtail)	*Dicyrtomina saundersi*	12	/
NEGP117	N/A (copepod)	*Acanthocyclops vernalis*	11	/
NEGP117	N/A (springtail)	*Parisotoma notabilis*	9	/
NEGP117	N/A (springtail)	*Dicyrtomidae sp.*	8	/
NEGP117	N/A (non-biting midge)	*Metriocnemus eurynotus*	7	/
NEGP117	N/A (springtail)	*Sminthurinus sp.*	6	/
NEGP118	N/A (earthworm)	*Aporrectodea nocturna*	1912	23380
NEGP118	2 spotted water hog-louse	*Asellus aquaticus*	1353	/
NEGP118	N/A (springtail)	*Sminthurinus reticulatus*	338	/
NEGP118	Common earthworm	*Lumbricus terrestris*	153	/
NEGP118	Green worm	*Allolobophora chlorotica*	92	/
NEGP118	N/A (worm)	*Pristina aequiseta*	82	/
NEGP118	N/A (shrimp)	*Crangonyx pseudogracilis*	70	/
NEGP118	Azure damselfly	*Coenagrion puella*	38	/
NEGP118	N/A (leech)	*Erpobdella testacea*	32	/
NEGP118	Keeled slug	*Tandonia sowerbyi*	27	/
NEGP118	N/A (barklice)	*Ectopsocus briggsi*	23	/
NEGP118	N/A (non-biting midge)	*Chironomidae sp.*	20	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP118	N/A (non-biting midge)	*Chironomus luridus*	15	/
NEGP118	N/A (non-biting midge)	*Zavrelimyia sp.*	15	/
NEGP118	N/A (diving beetle)	*Agabus bipustulatus*	12	/
NEGP118	N/A (worm)	*Octolasion cyaneum*	8	/
NEGP119	N/A (bdelloid rotifer)	*Rotaria rotatoria*	15597	49748
NEGP119	N/A (worm)	*Chaetogaster diastrophus*	1080	/
NEGP119	N/A (copepod)	*Acanthocyclops vernalis*	251	/
NEGP119	Banded mosquito	*Culiseta sp.*	63	/
NEGP119	N/A (non-biting midge)	*Chironomus luridus*	61	/
NEGP119	?	*UNVERIFIED: Arthropoda environmental sample*	35	/
NEGP119	Garden snail	*Helix aspersa*	27	/
NEGP121	N/A (water flea)	*Chydorus sphaericus*	11253	70628
NEGP121	N/A (copepod)	*Eucyclops cf. serrulatus*	3523	/
NEGP121	N/A (crustacean)	*Simocephalus exspinosus*	766	/
NEGP121	N/A (mite)	*Eupodidae sp.*	82	/
NEGP121	N/A (springtail)	*Hypogastrura burkilli*	37	/
NEGP121	N/A (crustacean)	*Diacyclops bicuspidatus*	33	/
NEGP121	N/A (springtail)	*Willowsia nigromaculata*	20	/
NEGP121	N/A (springtail)	*Sminthurinus bimaculatus*	16	/
NEGP121	N/A (copepod)	*Paracyclops fimbriatus*	14	/
NEGP121	Common earthworm	*Lumbricus terrestris*	5	/
NEGP121	N/A (worm)	*Eisenia sp.*	5	/
NEGP121	N/A (springtail)	*Bourletiellidae sp.*	5	/
NEGP122	N/A (copepod)	*Paracyclops fimbriatus*	836	69990
NEGP122	N/A (copepod)	*Acanthocyclops vernalis*	508	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP122	Microflex worms	*Dero digitata*	220	/
NEGP122	N/A (non-biting midge)	*Chironomus luridus*	31	/
NEGP123	N/A (copepod)	*Canthocamptidae sp.*	24214	17248
NEGP123	N/A (copepod)	*Eucyclops cf. serrulatus*	8501	/
NEGP123	Green worm	*Allolobophora chlorotica*	330	/
NEGP123	N/A (worm)	*Nais communis*	266	/
NEGP123	red worm	*Limnodrilus hoffmeisteri*	125	/
NEGP123	N/A (worm)	*Dero obtusa*	81	/
NEGP123	N/A (crane fly)	*Tipula paludosa*	25	/
NEGP123	N/A (worm)	*Nais communis*	25	/
NEGP123	Great ramshorn snail	*Planorbarius corneus*	24	/
NEGP123	Small black ant	*Lasius niger*	17	/
NEGP123	Sludge/sewage worm	*Tubifex tubifex*	8	/
NEGP123	N/A (worm)	*Nais sp.*	5	/
NEGP124	N/A (copepod)	*Canthocamptidae sp.*	3875	65495
NEGP124	Compost worm	*Dendrobaena veneta*	1148	/
NEGP124	Chestnut slug	*Deroceras invadens*	893	/
NEGP124	red worm	*Limnodrilus hoffmeisteri*	614	/
NEGP124	N/A (worm)	*Allolobophoridella eiseni*	211	/
NEGP124	N/A (red worm)	*Eiseniella tetraedra*	202	/
NEGP124	N/A (copepod)	*Acanthocyclops vernalis*	122	/
NEGP124	Great pond snail	*Lymnaea stagnalis*	105	/
NEGP124	N/A (bdelloid rotifer)	*Rotaria rotatoria*	72	/
NEGP124	2 spotted water hog-louse	*Asellus aquaticus*	69	/
NEGP124	N/A (worm)	*Nais sp.*	54	/
NEGP124	N/A (crustacean)	*Isopoda sp.*	54	/
NEGP124	N/A (barklice)	*Ectopsocus briggsi*	53	/
NEGP124	N/A (worm)	*Oligochaeta sp.*	27	/
NEGP124	N/A (jumping plant lice)	*Cacopsylla brunneipennis*	17	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP124	Great pond snail	*Lymnaea stagnalis*	16	/
NEGP124	N/A (crustacean)	*Pleuroxus denticulatus*	14	/
NEGP124	?	*Arthropoda environmental sample*	9	/
NEGP124	N/A (worm)	*Potamothrix bavaricus*	6	/
NEGP124	N/A (true bug)	*Drepanosiphum sp.*	5	/
NEGP125	N/A (crustacean)	*Chydorus brevilabris*	21570	45558
NEGP125	N/A (red worm)	*Eiseniella tetraedra*	195	/
NEGP125	Salt and pepper microcaddisfly	*Agraylea multipunctata*	73	/
NEGP125	Microflex worms	*Dero digitata*	67	/
NEGP125	N/A (crustacean)	*Diacyclops bicuspidatus*	47	/
NEGP125	?	*Arthropoda environmental sample*	15	/
NEGP125	N/A (crustacean)	*Pleuroxus denticulatus*	9	/
NEGP125	N/A (worm)	*Pristina longiseta*	8	/
NEGP125	Green hydra fresh-water polyp	*Hydra vulgaris*	7	/
NEGP125	N/A (water flea)	*Chydorus sphaericus*	5	/
NEGP129	N/A (copepod)	*Paracyclops fimbriatus*	500	2364
NEGP129	N/A (crustacean)	*Podocopida sp.*	114	/
NEGP129	N/A (copepod)	*Acanthocyclops vernalis*	43	/
NEGP129	N/A (rotifer)	*Synchaeta pectinata*	24	/
NEGP129	?	*UNVERIFIED: Arthropoda environmental sample*	6	/
NEGP129	N/A (crustacean)	*Cypridopsis vidua*	5	/
NEGP130	N/A (black fly)	*Simulium velutinum*	3869	69363

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP130	N/A (bdelloid rotifer)	*Rotaria rotatoria*	188	/
NEGP130	N/A (bdelloid rotifer)	*Philodina citrina*	18	/
NEGP131	N/A (non-biting midge)	*Paratanytarsus sp.*	4427	35862
NEGP131	Rosy-tipped worm	*Aporrectodea rosea*	832	/
NEGP131	N/A (copepod)	*Canthocamptidae sp.*	684	/
NEGP131	N/A (non-biting midge)	*Chironomidae sp.*	224	/
NEGP131	N/A (worm)	*Chaetogaster diastrophus*	45	/
NEGP131	N/A (non-biting midge)	*Paratanytarsus sp.*	43	/
NEGP131	N/A (bdelloid rotifer)	*Rotaria rotatoria*	7	/
NEGP131	N/A (non-biting midge)	*Chironomus luridus*	5	/
NEGP132	N/A (copepod)	*Cyclopidae sp.*	51784	13690
NEGP132	N/A (crustacean)	*Maxillopoda sp.*	12069	/
NEGP132	N/A (copepod)	*Canthocamptidae sp.*	2846	/
NEGP132	N/A (copepod)	*Acanthocyclops vernalis*	494	/
NEGP132	2 spotted water hog-louse	*Asellus aquaticus*	463	/
NEGP132	Blackworm	*Lumbriculus variegatus*	401	/
NEGP132	N/A (crustacean)	*Isopoda sp.*	82	/
NEGP132	N/A (bdelloid rotifer)	*Rotaria rotatoria*	44	/
NEGP132	N/A (red worm)	*Eiseniella tetraedra*	40	/
NEGP132	N/A (fly)	*Tetanocera ferruginea*	31	/
NEGP132	Marsh snail	*Stagnicola palustris*	31	/
NEGP132	Salt and pepper microcaddisfly	*Agraylea multipunctata*	30	/
NEGP132	N/A (non-biting midge)	*Micropsectra atrofasciata*	10	/
NEGP132	N/A (crustacean)	*Pleuroxus denticulatus*	9	/
NEGP132	N/A (shrimp)	*Crangonyx pseudogracilis*	8	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP132	N/A (mollusc)	*Musculium sp.*	8	/
NEGP132	N/A (arachnid)	*Arachnida sp.*	7	/
NEGP132	N/A (non-biting midge)	*Zavrelimyia sp.*	6	/
NEGP132	N/A (barklice)	*Ectopsocus briggsi*	5	/
NEGP133	N/A (copepod)	*Cyclopidae sp.*	30145	38243
NEGP133	N/A (red worm)	*Eiseniella tetraedra*	4764	/
NEGP133	N/A (arachnid)	*Arachnida sp.*	2552	/
NEGP133	N/A (copepod)	*Acanthocyclops vernalis*	701	/
NEGP133	N/A (crustacean)	*Podocopida sp.*	483	/
NEGP133	Black-headed worm	*Aporrectodea longa*	446	/
NEGP133	Blackworm	*Lumbriculus variegatus*	432	/
NEGP133	N/A (crustacean)	*Candona candida*	422	/
NEGP133	N/A (copepod)	*Eucyclops cf. serrulatus*	411	/
NEGP133	Great pond snail	*Lymnaea stagnalis*	267	/
NEGP133	N/A (copepod)	*Canthocamptidae sp.*	243	/
NEGP133	2 spotted water hog-louse	*Asellus aquaticus*	139	/
NEGP133	N/A (mosquito)	*Culiseta morsitans*	138	/
NEGP133	Common earthworm	*Lumbricus terrestris*	129	/
NEGP133	N/A (non-biting midge)	*Chironomidae sp.*	82	/
NEGP133	Salt and pepper microcaddisfly	*Agraylea multipunctata*	68	/
NEGP133	red worm	*Limnodrilus hoffmeisteri*	34	/
NEGP133	Mottled worm	*Aporrectodea icterica*	33	/
NEGP133	N/A (barklice)	*Ectopsocus briggsi*	24	/
NEGP133	N/A (fly)	*Lauxaniidae sp.*	15	/
NEGP133	N/A (fly)	*Fannia pallitibia*	14	/
NEGP133	N/A (copepod)	*Cyclops abyssorum divergens*	12	/
NEGP133	N/A (barklice)	*Elipsocus sp.*	10	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP133	?	*Arthropoda environmental sample*	10	/
NEGP133	Shallot aphid	*Myzus ascalonicus*	9	/
NEGP133	N/A (ribbon worm)	*Nemertean sp.*	8	/
NEGP133	N/A (slug)	*Deroceras sp.*	7	/
NEGP133	N/A (mollusc)	*Musculium sp.*	6	/
NEGP133	Banded mosquito	*Culiseta sp.*	5	/
NEGP135	N/A (rotifer)	*Keratella sp.*	6961	26748
NEGP135	American bladder snail	*Physa acuta*	70	/
NEGP135	red worm	*Limnodrilus hoffmeisteri*	51	/
NEGP135	N/A (worm)	*Nais sp.*	45	/
NEGP135	Blackworm	*Lumbriculus variegatus*	32	/
NEGP135	N/A (copepod)	*Acanthocyclops vernalis*	17	/
NEGP135	N/A (rotifer)	*Keratella cochlearis*	16	/
NEGP135	N/A (worm)	*Dero obtusa*	16	/
NEGP135	Sludge/sewage worm	*Tubifex tubifex*	9	/
NEGP135	N/A (rotifer)	*Euchlanis dilatata*	6	/
NEGP136	N/A (worm)	*Allolobophoridella eiseni*	3709	41382
NEGP136	N/A (rotifer)	*Brachionus calyciflorus*	1309	/
NEGP136	American bladder snail	*Physella acuta*	597	/
NEGP136	Sludge/sewage worm	*Tubifex tubifex*	516	/
NEGP136	N/A (copepod)	*Cyclops abyssorum divergens*	300	/
NEGP136	American bladder snail	*Physa acuta*	183	/
NEGP136	Blackworm	*Lumbriculus variegatus*	176	/
NEGP136	N/A (copepod)	*Eucyclops cf. serrulatus*	140	/
NEGP136	N/A (worm)	*Chaetogaster diastrophus*	106	/
NEGP136	red worm	*Limnodrilus hoffmeisteri*	73	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP136	N/A (fly)	*Siphona geniculata*	49	/
NEGP136	Salt and pepper microcaddisfly	*Agraylea multipunctata*	38	/
NEGP136	N/A (worm)	*Limnodrilus claparedianus*	19	/
NEGP136	N/A (non-biting midge)	*Monopelopia tenuicalcar*	7	/
NEGP136	2 spotted water hog-louse	*Asellus aquaticus*	7	/
NEGP136	N/A (barklice)	*Ectopsocus sp.*	6	/
NEGP137	N/A (crustacean)	*Diacyclops bicuspidatus*	40491	28664
NEGP137	N/A (copepod)	*Canthocamptidae sp.*	1023	/
NEGP137	Sludge/sewage worm	*Tubifex tubifex*	122	/
NEGP137	2 spotted water hog-louse	*Asellus aquaticus*	82	/
NEGP137	N/A (copepod)	*Paracyclops fimbriatus*	38	/
NEGP137	red worm	*Limnodrilus hoffmeisteri*	33	/
NEGP137	N/A (leafhopper)	*Eupteryx atropunctata*	22	/
NEGP137	Banded mosquito	*Culiseta sp.*	12	/
NEGP137	N/A (springtail)	*Desoria sp.*	8	/
NEGP138	N/A (worm)	*Enchytraeidae sp.*	2056	23506
NEGP138	N/A (springtail)	*Parisotoma notabilis*	481	/
NEGP138	N/A (springtail)	*Sminthurinus reticulatus*	469	/
NEGP138	N/A (copepod)	*Acanthocyclops vernalis*	412	/
NEGP138	N/A (springtail)	*Pseudosinella alba*	318	/
NEGP138	Common earthworm	*Lumbricus terrestris*	237	/
NEGP138	Redhead worm	*Lumbricus rubellus*	93	/
NEGP138	Angle shades moth	*Phlogophora meticulosa*	73	/
NEGP138	Spotted-winged Drosophila	*Drosophila suzukii*	67	/
NEGP138	N/A (springtail)	*Lepidocyrtus lignorum*	40	/
NEGP138	N/A (arachnid)	*Arachnida sp.*	38	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP138	Square-spot rustic moth	*Xestia xanthographa*	22	/
NEGP138	N/A (springtail)	*Megalothorax sp.*	17	/
NEGP138	N/A (barklice)	*Ectopsocus briggsi*	5	/
NEGP138	Bank worm	*Bimastos rubidus*	5	/
NEGP138	?	*UNVERIFIED: Arthropoda environmental sample*	5	/
NEGP139	N/A (water flea)	*Chydorus sphaericus*	15826	22349
NEGP139	Rosy-tipped worm	*Aporrectodea rosea*	1069	/
NEGP139	Black-headed worm	*Aporrectodea longa*	152	/
NEGP139	Bank worm	*Bimastos rubidus rubidus*	101	/
NEGP139	Banded mosquito	*Culiseta sp.*	72	/
NEGP139	N/A (non-biting midge)	*Chironomidae sp.*	22	/
NEGP139	N/A (crustacean)	*Podocopida sp.*	17	/
NEGP139	N/A (worm)	*Satchellius mammalis*	9	/
NEGP142	Common earthworm	*Lumbricus terrestris*	618	17073
NEGP142	Black-headed worm	*Aporrectodea longa*	129	/
NEGP142	N/A (springtail)	*Sminthurinus reticulatus*	102	/
NEGP142	N/A (worm)	*Chaetogaster diastrophus*	58	/
NEGP142	N/A (springtail)	*Tomocerus sp.*	50	/
NEGP142	N/A (water flea)	*Chydorus sphaericus*	31	/
NEGP142	N/A (rotifer)	*Euchlanis dilatata*	26	/
NEGP142	N/A (non-biting midge)	*Chironomidae sp.*	15	/
NEGP142	N/A (fly)	*Scatopsciara dentifera*	13	/
NEGP142	N/A (worm)	*Pristina aequiseta*	12	/
NEGP142	N/A (barklice)	*Caeciliusidae sp.*	10	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP142	N/A (rove beetle)	*Xantholinus linearis*	9	/
NEGP142	N/A (arachnid)	*Arachnida sp.*	9	/
NEGP142	N/A (non-biting midge)	*Zavrelimyia sp.*	8	/
NEGP142	N/A (mayfly)	*Cloeon dipterum*	7	/
NEGP142	Netted slug	*Deroceras reticulatum*	6	/
NEGP142	N/A (non-biting midge)	*Bryophaenocladius sp.*	6	/
NEGP143	N/A (crustacean)	*Diacyclops bicuspidatus*	37987	23850
NEGP143	2 spotted water hog-louse	*Asellus aquaticus*	17	/
NEGP145	N/A (crustacean)	*Diacyclops bicuspidatus*	3790	22211
NEGP145	Blackworm	*Lumbriculus variegatus*	1402	/
NEGP145	Common earthworm	*Lumbricus terrestris*	616	/
NEGP145	N/A (fly)	*Pseudolyciella pallidiventris*	194	/
NEGP145	Green worm	*Allolobophora chlorotica*	193	/
NEGP145	N/A (springtail)	*Sminthurinus sp.*	92	/
NEGP145	N/A (barklice)	*Ectopsocus briggsi*	73	/
NEGP145	N/A (crustacean)	*Daphnia sp.*	35	/
NEGP145	Banded mosquito	*Culiseta sp.*	16	/
NEGP145	red worm	*Limnodrilus hoffmeisteri*	15	/
NEGP145	N/A (true bug)	*Drepanosiphum sp.*	14	/
NEGP145	N/A (bdelloid rotifer)	*Rotaria rotatoria*	5	/
NEGP147	N/A (copepod)	*Acanthocyclops vernalis*	43232	20325
NEGP147	?	*UNVERIFIED: Arthropoda environmental sample*	111	/
NEGP147	Lake limpet	*Acroloxus lacustris*	107	/
NEGP147	Great ramshorn snail	*Planorbarius corneus*	20	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP147	?	*Arthropoda environmental sample*	18	/
NEGP147	N/A (worm)	*Octolasion cyaneum*	16	/
NEGP147	N/A (springtail)	*Sphaeridia pumilis*	13	/
NEGP147	Black-headed worm	*Aporrectodea longa*	9	/
NEGP147	Marsh snail	*Stagnicola palustris*	6	/
NEGP148	N/A (crustacean)	*Maxillopoda sp.*	71367	14718
NEGP148	N/A (copepod)	*Cyclopidae sp.*	8004	/
NEGP148	N/A (copepod)	*Acanthocyclops vernalis*	545	/
NEGP148	N/A (non-biting midge)	*Corynoneura scutellata*	22	/
NEGP148	Black-headed worm	*Aporrectodea longa*	19	/
NEGP148	N/A (crustacean)	*Diacyclops bicuspidatus*	17	/
NEGP148	N/A (springtail)	*Sminthurinus reticulatus*	13	/
NEGP148	N/A (non-biting midge)	*Metriocnemus eurynotus*	12	/
NEGP148	N/A (crane fly)	*Tipula oleracea*	11	/
NEGP148	red worm	*Limnodrilus hoffmeisteri*	9	/
NEGP148	N/A (crustacean)	*Podocopida sp.*	9	/
NEGP148	Blackworm	*Lumbriculus variegatus*	7	/
NEGP148	N/A (springtail)	*Sminthurinus sp.*	7	/
NEGP148	N/A (mollusc)	*Musculium sp.*	6	/
NEGP148	N/A (worm)	*Pristina longiseta*	5	/
NEGP150	Landhopper/woodhopper/lawn shrimp	*Arcitalitrus dorrieni*	2026	43880
NEGP150	Southern house mosquito	*Culex pipiens*	1874	/
NEGP150	N/A (copepod)	*Canthocamptidae sp.*	1268	/
NEGP150	N/A (barklice)	*Ectopsocus briggsi*	734	/
NEGP150	Winter crane fly	*Trichoceridae sp.*	179	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP150	Banded mosquito	*Culiseta sp.*	170	/
NEGP150	red worm	*Limnodrilus hoffmeisteri*	161	/
NEGP150	N/A (worm)	*Enchytraeidae sp.*	161	/
NEGP150	Budapest keeled slug	*Tandonia budapestensis*	159	/
NEGP150	N/A (non-biting midge)	*Limnophyes pentaplastus*	132	/
NEGP150	Salt and pepper microcaddisfly	*Agraylea multipunctata*	114	/
NEGP150	N/A (arachnid)	*Arachnida sp.*	42	/
NEGP150	Netted slug	*Deroceras reticulatum*	37	/
NEGP150	?	*UNVERIFIED: Arthropoda environmental sample*	33	/
NEGP150	American bladder snail	*Physa acuta*	32	/
NEGP150	N/A (barklice)	*Valenzuela flavidus*	22	/
NEGP150	N/A (fly)	*Fannia pallitibia*	22	/
NEGP150	Keeled slug	*Tandonia sowerbyi*	19	/
NEGP150	?	*Arthropoda environmental sample*	18	/
NEGP150	N/A (springtail)	*Dicyrtoma fusca*	15	/
NEGP150	N/A (bdelloid rotifer)	*Rotaria rotatoria*	12	/
NEGP150	N/A (wasps, bees, ants, sawflies)	*Hymenoptera sp.*	12	/
NEGP150	N/A (earthworm)	*Lumbricus castaneus*	11	/
NEGP150	Strawberry snail	*Trochulus striolatus*	10	/
NEGP150	N/A (crustacean)	*Pleuroxus denticulatus*	10	/
NEGP150	2 spotted water hog-louse	*Asellus aquaticus*	10	/
NEGP150	N/A (slug)	*Arion sp.*	10	/
NEGP150	N/A (ground beetle)	*Nebria brevicollis*	6	/
NEGP150	Green worm	*Allolobophora chlorotica*	6	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP150	Common earthworm	*Lumbricus terrestris*	5	/
NEGP151	N/A (leafhopper)	*Aphrodes makarovi*	38	100820
NEGP151	N/A (mayfly)	*Cloeon dipterum*	12	/
NEGP151	N/A (ground beetle)	*Nebria brevicollis*	11	/
NEGP151	N/A (leafhopper)	*Erythroneurini sp.*	11	/
NEGP151	N/A (non-biting midge)	*Limnophyes pentaplastus*	10	/
NEGP151	N/A (leaf-mining fly)	*Phytomyza ranunculi*	8	/
NEGP151	Bank worm	*Bimastos rubidus*	5	/
NEGP152	N/A (leafhopper)	*Aphrodes makarovi*	25	57331
NEGP153	N/A (worm)	*Fridericia sp.*	1819	16966
NEGP153	N/A (bdelloid rotifer)	*Rotaria rotatoria*	446	/
NEGP153	N/A (worm)	*Enchytraeidae sp.*	247	/
NEGP153	Common earthworm	*Lumbricus terrestris*	141	/
NEGP153	Fern smut moth	*Psychoides filicivora*	137	/
NEGP153	N/A (rotifer)	*Lecane closterocerca*	67	/
NEGP153	Compost worm	*Dendrobaena veneta*	66	/
NEGP153	Garden snail	*Helix aspersa*	49	/
NEGP153	N/A (non-biting midge)	*Chironomus luridus*	46	/
NEGP153	N/A (non-biting midge)	*Zavrelimyia sp.*	32	/
NEGP153	N/A (springtail)	*Entomobrya intermedia*	31	/
NEGP153	Angle shades moth	*Phlogophora meticulosa*	30	/
NEGP153	Budapest keeled slug	*Tandonia budapestensis*	24	/
NEGP153	N/A (barklice)	*Ectopsocus sp.*	11	/
NEGP153	N/A (leafhopper)	*Cicadellidae sp.*	11	/
NEGP153	N/A (crustacean)	*Pleuroxus denticulatus*	10	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP153	Landhopper/woodhopper/lawn shrimp	*Arcitalitrus dorrieni*	6	/
NEGP153	Lily aphid	*Neomyzus circumflexus*	5	/
NEGP153	Banded mosquito	*Culiseta sp.*	5	/
NEGP154	Sludge/sewage worm	*Tubifex tubifex*	6863	54144
NEGP154	N/A (crustacean)	*Candonopsis kingsleii*	2611	/
NEGP154	red worm	*Limnodrilus hoffmeisteri*	240	/
NEGP154	N/A (worm)	*Tubificinae sp.*	145	/
NEGP154	N/A (water flea)	*Chydorus sphaericus*	134	/
NEGP154	Blackworm	*Lumbriculus variegatus*	126	/
NEGP154	N/A (crustacean)	*Diacyclops bicuspidatus*	70	/
NEGP154	N/A (worm)	*Quistadrilus sp.*	70	/
NEGP154	N/A (worm)	*Branchiura sowerbyi*	53	/
NEGP154	Sludge worm	*Psammoryctides barbatus*	36	/
NEGP154	N/A (barklice)	*Ectopsocus briggsi*	29	/
NEGP154	N/A (sludge worm)	*Naididae sp.*	27	/
NEGP154	Mealworm beetle	*Tenebrio molitor*	26	/
NEGP154	Salt and pepper microcaddisfly	*Agraylea multipunctata*	22	/
NEGP154	N/A (non-biting midge)	*Zavrelimyia sp.*	22	/
NEGP154	N/A (mollusc)	*Musculium sp.*	18	/
NEGP154	N/A (copepod)	*Paracyclops fimbriatus*	14	/
NEGP154	N/A (springtail)	*Orchesella villosa*	13	/
NEGP154	N/A (fungus gnat)	*Bradysia impatiens*	11	/
NEGP154	N/A (worm)	*Bothrioneurum vejdovskyanum*	11	/
NEGP154	N/A (worm)	*Oligochaeta sp.*	11	/
NEGP154	N/A (copepod)	*Canthocamptidae sp.*	10	/
NEGP154	N/A (springtail)	*Tomocerus sp.*	8	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP154	Marsh snail	*Stagnicola palustris*	7	/
NEGP154	Lake orb mussel	*Musculium lacustre*	7	/
NEGP154	N/A (non-biting midge)	*Chironomidae sp.*	6	/
NEGP154	?	*Arthropoda environmental sample clone*	6	/
NEGP155	N/A (worm)	*Stylaria lacustris*	993	19881
NEGP155	N/A (non-biting midge)	*Chironomidae sp.*	429	/
NEGP155	N/A (black fly)	*Simulium velutinum*	215	/
NEGP155	N/A (non-biting midge)	*Micropsectra atrofasciata*	165	/
NEGP155	N/A (red worm)	*Eiseniella tetraedra*	139	/
NEGP155	N/A (water flea)	*Chydoridae sp.*	87	/
NEGP155	American bladder snail	*Physella acuta*	80	/
NEGP155	N/A (non-biting midge)	*Micropsectra atrofasciata*	74	/
NEGP155	N/A (worm)	*Eisenia sp.*	21	/
NEGP155	N/A (worm)	*Henlea ventriculosa*	16	/
NEGP155	N/A (rotifer)	*Euchlanis dilatata*	14	/
NEGP155	N/A (black fly)	*Simulium noelleri*	12	/
NEGP155	N/A (non-biting midge)	*Eukiefferiella claripennis*	10	/
NEGP155	N/A (worm)	*Chaetogaster diaphanus*	9	/
NEGP155	N/A (non-biting midge)	*Conchapelopia melanops*	6	/
NEGP155	N/A (fly)	*Lauxaniidae sp.*	5	/
NEGP156	N/A (arachnid)	*Arachnida sp.*	876	46572
NEGP156	N/A (rotifer)	*Euchlanis dilatata*	35	/
NEGP156	?	*Arthropoda environmental sample*	31	/
NEGP156	N/A (non-biting midge)	*Chironomidae sp.*	16	/
NEGP157	N/A (copepod)	*Acanthocyclops vernalis*	2309	60901
NEGP157	N/A (bdelloid rotifer)	*Rotaria rotatoria*	559	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP157	N/A (crustacean)	*Pleuroxus denticulatus*	6	/
NEGP158	N/A (copepod)	*Eucyclops cf. serrulatus*	1220	51158
NEGP158	N/A (copepod)	*Cyclops abyssorum divergens*	809	/
NEGP158	2 spotted water hog-louse	*Asellus aquaticus*	112	/
NEGP158	N/A (worm)	*Nais communis*	16	/
NEGP158	Great pond snail	*Lymnaea stagnalis*	5	/
NEGP158	?	*UNVERIFIED: Arthropoda environmental sample*	5	/
NEGP159	N/A (crustacean)	*Diacyclops bicuspidatus*	61551	1731
NEGP159	N/A (crustacean)	*Chydorus sp.*	4283	/
NEGP159	N/A (worm)	*Nais sp.*	177	/
NEGP159	Sludge/sewage worm	*Tubifex tubifex*	166	/
NEGP159	N/A (copepod)	*Acanthocyclops vernalis*	131	/
NEGP159	Southern house mosquito	*Culex pipiens*	103	/
NEGP159	Blackworm	*Lumbriculus variegatus*	27	/
NEGP159	Great pond snail	*Lymnaea stagnalis*	21	/
NEGP159	American bladder snail	*Physella acuta*	16	/
NEGP159	Banded mosquito	*Culiseta sp.*	14	/
NEGP159	N/A (barklice)	*Ectopsocus briggsi*	13	/
NEGP159	N/A (non-biting midge)	*Paratanytarsus grimmii*	8	/
NEGP159	N/A (midge)	*Cricotopus sylvestris*	7	/
NEGP159	2 spotted water hog-louse	*Asellus aquaticus*	7	/
NEGP159	N/A (crustacean)	*Isopoda sp.*	7	/
NEGP159	N/A (water flea)	*Chydorus sphaericus*	6	/
NEGP162	N/A (worm)	*Fridericia galba*	15	3583

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP162	Mottled worm	*Aporrectodea icterica*	13	/
NEGP162	N/A (barklice)	*Ectopsocus briggsi*	5	/
NEGP162	N/A (arachnid)	*Arachnida sp.*	5	/
NEGP163	Common earthworm	*Lumbricus terrestris*	3924	16950
NEGP163	N/A (crustacean)	*Daphnia magna*	18	/
NEGP164	N/A (crustacean)	*Diacyclops bicuspidatus*	102285	1298
NEGP164	N/A (copepod)	*Eucyclops cf. serrulatus*	282	/
NEGP164	N/A (copepod)	*Canthocamptidae sp.*	126	/
NEGP164	N/A (copepod)	*Macrocyclops albidus*	48	/
NEGP164	N/A (copepod)	*Acanthocyclops vernalis*	39	/
NEGP164	Common bithynia/faucet snail	*Bithynia tentaculata*	35	/
NEGP164	Common bithynia/faucet snail	*UNVERIFIED: Bithynia tentaculata*	26	/
NEGP164	N/A (bdelloid rotifer)	*Rotaria rotatoria*	5	/
NEGP164	N/A (barklice)	*Ectopsocus briggsi*	5	/
NEGP165	?	*Arthropoda environmental sample*	1351	20538
NEGP165	N/A (worm)	*Chaetogaster diastrophus*	526	/
NEGP165	N/A (non-biting midge)	*Psectrocladius sp.*	499	/
NEGP165	N/A (mayfly)	*Cloeon dipterum*	468	/
NEGP165	?	*UNVERIFIED: Arthropoda environmental sample*	310	/
NEGP165	N/A (copepod)	*Eucyclops cf. serrulatus*	178	/
NEGP165	N/A (copepod)	*Eudiaptomus gracilis*	161	/
NEGP165	Fresh-water polyp	*Hydra magnipapillata*	44	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP165	N/A (non-biting midge)	*Chironomus tentans*	26	/
NEGP165	N/A (copepod)	*Acanthocyclops vernalis*	23	/
NEGP165	N/A (copepod)	*Cyclopoida sp.*	23	/
NEGP165	N/A (non-biting midge)	*Chironomus nuditarsis*	19	/
NEGP165	2 spotted water hog-louse	*Asellus aquaticus*	19	/
NEGP165	N/A (copepod)	*Cyclops abyssorum*	14	/
NEGP165	N/A (non-biting midge)	*Acricotopus lucens*	7	/
NEGP167	N/A (worm)	*Chaetogaster diastrophus*	10248	42650
NEGP167	Rosy-tipped worm	*Aporrectodea rosea*	662	/
NEGP167	Great pond snail	*Lymnaea stagnalis*	507	/
NEGP167	N/A (worm)	*Nais communis*	64	/
NEGP167	N/A (red worm)	*Eiseniella tetraedra*	57	/
NEGP167	Salt and pepper microcaddisfly	*Agraylea multipunctata*	25	/
NEGP167	Tawny marbled minor moth	*Oligia latruncula*	20	/
NEGP167	N/A (copepod)	*Acanthocyclops vernalis*	20	/
NEGP167	Marsh snail	*Stagnicola palustris*	18	/
NEGP167	N/A (shrimp)	*Crangonyx pseudogracilis*	14	/
NEGP167	N/A (springtail)	*Vertagopus arboreus*	12	/
NEGP167	N/A (true bug)	*Hemiptera sp.*	5	/
NEGP167	Banded mosquito	*Culiseta sp.*	5	/
NEGP169	N/A (crustacean)	*Diacyclops bicuspidatus*	6045	29499
NEGP169	Black-headed worm	*Aporrectodea longa*	1146	/
NEGP169	N/A (bdelloid rotifer)	*Rotaria rotatoria*	643	/
NEGP169	Common earthworm	*Lumbricus terrestris*	414	/
NEGP169	Harelquin ladybird	*Harmonia axyridis*	294	/
NEGP169	N/A (non-biting midge)	*Bryophaenocladius sp.*	242	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP169	Green worm	*Allolobophora chlorotica*	148	/
NEGP169	red worm	*Limnodrilus hoffmeisteri*	139	/
NEGP169	Grey worm	*Aporrectodea caliginosa*	117	/
NEGP169	Rosy-tipped worm	*Aporrectodea rosea*	100	/
NEGP169	N/A (mite)	*Humerobatidae sp.*	63	/
NEGP169	N/A (hover fly)	*Syrphus ribesii*	61	/
NEGP169	N/A (mite)	*Ameronothrus sp.*	52	/
NEGP169	N/A (springtail)	*Tomocerus vulgaris*	50	/
NEGP169	N/A (crane fly)	*Tipula maxima*	46	/
NEGP169	N/A (worm)	*Octolasion cyaneum*	44	/
NEGP169	N/A (barklice)	*Ectopsocus briggsi*	37	/
NEGP169	N/A (arachnid)	*Arachnida sp.*	36	/
NEGP169	N/A (worm)	*Enchytraeidae sp.*	35	/
NEGP169	N/A (barklice)	*Cerobasis guestfalica*	30	/
NEGP169	N/A (worm)	*Allolobophoridella eiseni*	30	/
NEGP169	N/A (copepod)	*Canthocamptidae sp.*	27	/
NEGP169	Bank worm	*Bimastos rubidus rubidus*	26	/
NEGP169	N/A (fly)	*Phaonia subventa*	18	/
NEGP169	?	*Arthropoda environmental sample*	17	/
NEGP169	Blackworm	*Lumbriculus variegatus*	16	/
NEGP169	N/A (fly)	*Limnophyes habilis*	14	/
NEGP169	N/A (true bug)	*Drepanosiphum sp.*	11	/
NEGP169	N/A (mosquito)	*Culiseta morsitans*	10	/
NEGP169	Salt and pepper microcaddisfly	*Agraylea multipunctata*	10	/
NEGP169	Microflex worms	*Dero digitata*	7	/
NEGP169	Window gnat	*Sylvicola fenestralis*	6	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP169	N/A (weevil)	*Sitona lepidus*	6	/
NEGP169	2 spotted water hog-louse	*Asellus aquaticus*	5	/
NEGP169	N/A (tardigrade)	*Ramazzottius sp.*	5	/
NEGP174	N/A (black fly)	*Simulium velutinum*	461	58462
NEGP174	N/A (non-biting midge)	*Micropsectra atrofasciata*	119	/
NEGP174	N/A (non-biting midge)	*Eukiefferiella claripennis*	81	/
NEGP174	N/A (non-biting midge)	*Chironomidae sp.*	79	/
NEGP174	N/A (rotifer)	*Euchlanis dilatata*	64	/
NEGP174	red worm	*Limnodrilus hoffmeisteri*	62	/
NEGP174	N/A (worm)	*Chaetogaster diastrophus*	45	/
NEGP174	Redhead worm	*Lumbricus rubellus*	40	/
NEGP174	N/A (red worm)	*Eiseniella tetraedra*	27	/
NEGP174	N/A (shrimp)	*Crangonyx pseudogracilis*	25	/
NEGP174	N/A (crustacean)	*Diacyclops bicuspidatus*	24	/
NEGP174	N/A (arachnid)	*Arachnida sp.*	22	/
NEGP174	N/A (crane fly)	*Tipula paludosa*	20	/
NEGP174	N/A (black fly)	*Simulium aureum*	17	/
NEGP174	N/A (springtail)	*Tomocerus vulgaris*	15	/
NEGP174	N/A (worm)	*Nais sp.*	10	/
NEGP174	N/A (crustacean)	*Maxillopoda sp.*	8	/
NEGP176	N/A (crustacean)	*Maxillopoda sp.*	748	104385
NEGP176	N/A (bdelloid rotifer)	*Rotaria rotatoria*	291	/
NEGP176	N/A (springtail)	*Sminthurinus reticulatus*	162	/
NEGP176	N/A (copepod)	*Cyclopidae sp.*	101	/
NEGP176	N/A (mite)	*Stigmaeidae sp.*	88	/
NEGP176	N/A (springtail)	*Pogonognathellus flavescens*	73	/
NEGP176	N/A (copepod)	*Acanthocyclops vernalis*	64	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP176	N/A (worm)	*Chaetogaster diastrophus*	61	/
NEGP176	N/A (crustacean)	*Caenestheriella gifuensis*	60	/
NEGP176	N/A (springtail)	*Parisotoma notabilis*	54	/
NEGP176	N/A (mollusc)	*Musculium sp.*	29	/
NEGP176	N/A (non-biting midge)	*Zavrelimyia sp.*	21	/
NEGP176	N/A (red worm)	*Eiseniella tetraedra*	15	/
NEGP176	N/A (springtail)	*Tomocerus sp.*	15	/
NEGP176	N/A (worm)	*Dendrobaena cf. attemsi*	9	/
NEGP176	N/A (non-biting midge)	*Corynoneura scutellata*	7	/
NEGP176	N/A (springtail)	*Dicyrtomina ornata*	6	/
NEGP176	2 spotted water hog-louse	*Asellus aquaticus*	6	/
NEGP176	Salt and pepper microcaddisfly	*Agraylea multipunctata*	5	/
NEGP178	2 spotted water hog-louse	*Asellus aquaticus*	37418	3457
NEGP178	N/A (copepod)	*Acanthocyclops vernalis*	3542	/
NEGP178	Common earthworm	*Lumbricus terrestris*	152	/
NEGP178	Green worm	*Allolobophora chlorotica*	118	/
NEGP178	N/A (bdelloid rotifer)	*Rotaria rotatoria*	22	/
NEGP179	red worm	*Limnodrilus hoffmeisteri*	2380	33643
NEGP179	American bladder snail	*Physella acuta*	928	/
NEGP179	Microflex worms	*Dero digitata*	877	/
NEGP179	N/A (mayfly)	*Cloeon dipterum*	744	/
NEGP179	Great pond snail	*Lymnaea stagnalis*	645	/
NEGP179	N/A (worm)	*Stylaria lacustris*	573	/
NEGP179	N/A (worm)	*Chaetogaster diaphanus*	445	/
NEGP179	2 spotted water hog-louse	*Asellus aquaticus*	173	/
NEGP179	American bladder snail	*Physa acuta*	150	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP179	N/A (crustacean)	*Macrothrix sp.*	148	/
NEGP179	N/A (non-biting midge)	*Zavrelimyia sp.*	141	/
NEGP179	N/A (worm)	*Pristina longiseta*	140	/
NEGP179	Broad-bodied chaser dragonfly	*Ladona depressa*	132	/
NEGP179	N/A (non-biting midge)	*Corynoneura scutellata*	130	/
NEGP179	Mealworm beetle	*Tenebrio molitor*	116	/
NEGP179	Phantom midge	*Chaoborus crystallinus*	77	/
NEGP179	N/A (non-biting midge)	*Chironomus luridus*	54	/
NEGP179	N/A (diving beetle)	*Agabus bipustulatus*	27	/
NEGP179	N/A (slug)	*Arion sp.*	13	/
NEGP182	N/A (worm)	*Pristina aequiseta*	634	47568
NEGP182	N/A (non-biting midge)	*Chironomidae sp.*	605	/
NEGP182	N/A (crustacean)	*Podocopida sp.*	526	/
NEGP182	N/A (crustacean)	*Daphnia sp.*	468	/
NEGP182	N/A (mayfly)	*Cloeon dipterum*	209	/
NEGP182	N/A (non-biting midge)	*Chironomus luridus*	148	/
NEGP182	N/A (crustacean)	*Pleuroxus denticulatus*	144	/
NEGP182	N/A (copepod)	*Paracyclops fimbriatus*	104	/
NEGP182	N/A (non-biting midge)	*Zavrelimyia sp.*	64	/
NEGP182	N/A (crustacean)	*Cypridopsis sp.*	56	/
NEGP182	N/A (midge)	*Cricotopus sylvestris*	25	/
NEGP182	Angle shades moth	*Phlogophora meticulosa*	17	/
NEGP182	N/A (crustacean)	*Pleuroxus sp.*	10	/
NEGP182	N/A (non-biting midge)	*Eukiefferiella claripennis*	6	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP187	Blackworm	*Lumbriculus variegatus*	1998	51319
NEGP187	Salt and pepper microcaddisfly	*Agraylea multipunctata*	339	/
NEGP187	N/A (arachnid)	*Arachnida sp.*	260	/
NEGP187	N/A (worm)	*Mesenchytraeus sp.*	230	/
NEGP187	N/A (red worm)	*Eiseniella tetraedra*	106	/
NEGP187	N/A (copepod)	*Acanthocyclops vernalis*	104	/
NEGP187	Swamp orb mussel	*Sphaerium nucleus*	79	/
NEGP187	N/A (copepod)	*Cyclopidae sp.*	73	/
NEGP187	N/A (springtail)	*Tomocerus sp.*	46	/
NEGP187	N/A (shrimp)	*Crangonyx pseudogracilis*	28	/
NEGP187	N/A (barklice)	*Ectopsocus briggsi*	27	/
NEGP187	Chestnut slug	*Deroceras invadens*	26	/
NEGP187	N/A (worm)	*Dendrobaena cf. attemsi*	24	/
NEGP187	N/A (crane fly)	*Phylidorea ferruginea*	21	/
NEGP187	N/A (leaf-mining fly)	*Ctenosciara hyalipennis*	15	/
NEGP187	N/A (true bug)	*Typhlocyba sp.*	11	/
NEGP187	?	*UNVERIFIED: Arthropoda environmental sample*	9	/
NEGP187	N/A (root-maggot flies)	*Pegomya flavifrons*	8	/
NEGP187	N/A (barklice)	*Valenzuela flavidus*	7	/
NEGP187	N/A (mite)	*Adoristes poppei*	7	/
NEGP188	N/A (copepod)	*Canthocamptidae sp.*	9108	35386
NEGP188	N/A (crustacean)	*Maxillopoda sp.*	8554	/
NEGP188	N/A (copepod)	*Acanthocyclops vernalis*	4602	/
NEGP188	N/A (water flea)	*Chydorus sphaericus*	2896	/
NEGP188	N/A (non-biting midge)	*Psectrocladius sp.*	202	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP188	red worm	*Limnodrilus hoffmeisteri*	198	/
NEGP188	N/A (rotifer)	*Euchlanis dilatata*	68	/
NEGP188	N/A (non-biting midge)	*Chironomidae sp.*	49	/
NEGP188	N/A (springtail)	*Hypogastrura burkilli*	45	/
NEGP188	2 spotted water hog-louse	*Asellus aquaticus*	42	/
NEGP188	N/A (non-biting midge)	*Corynoneura scutellata*	18	/
NEGP188	N/A (rotifer)	*Synchaeta pectinata*	14	/
NEGP188	N/A (arachnid)	*Arachnida sp.*	7	/
NEGP189	N/A (copepod)	*Canthocamptidae sp.*	5349	31650
NEGP189	N/A (worm)	*Stylodrilus heringianus*	282	/
NEGP189	N/A (springtail)	*Isotomurus fucicola*	188	/
NEGP189	N/A (springtail)	*Neanura muscorum*	98	/
NEGP189	N/A (freshwater snail)	*Sulcospira sp.*	56	/
NEGP189	N/A (worm)	*Pristina aequiseta*	55	/
NEGP189	Salt and pepper microcaddisfly	*Agraylea multipunctata*	53	/
NEGP189	N/A (springtail)	*Tomocerus vulgaris*	23	/
NEGP189	Springtail	*Tomocerus minor*	15	/
NEGP194	N/A (water flea)	*Chydorus sphaericus*	12704	48786
NEGP194	N/A (copepod)	*Acanthocyclops vernalis*	1380	/
NEGP194	Green worm	*Allolobophora chlorotica*	61	/
NEGP194	Great ramshorn snail	*Planorbarius corneus*	8	/
NEGP195	N/A (copepod)	*Acanthocyclops vernalis*	6125	10426
NEGP195	N/A (worm)	*Enchytraeidae sp.*	2313	/
NEGP195	N/A (crustacean)	*Podocopida sp.*	2004	/
NEGP195	N/A (true bug)	*Cacopsylla melanoneura*	1079	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
NEGP195	American bladder snail	*Physella acuta*	743	/
NEGP195	N/A (barklice)	*Ectopsocus briggsi*	591	/
NEGP195	Common earthworm	*Lumbricus terrestris*	408	/
NEGP195	Microflex worms	*Dero digitata*	389	/
NEGP195	American bladder snail	*Physa acuta*	341	/
NEGP195	N/A (worm)	*Satchellius mammalis*	302	/
NEGP195	N/A (mite)	*Ameronothrus sp.*	282	/
NEGP195	N/A (harvestman)	*Paroligolophus agrestis*	156	/
NEGP195	N/A (worm)	*Rhyacodrilus falciformis*	154	/
NEGP195	Redhead worm	*Lumbricus rubellus*	84	/
NEGP195	Green leaf hopper	*Empoasca decipiens*	53	/
NEGP195	N/A (jumping plant lice)	*Trioza urticae*	45	/
NEGP195	N/A (springtail)	*Dicyrtomina saundersi*	28	/
NEGP195	N/A (springtail)	*Entomobryidae*	22	/
NEGP195	N/A (fungus gnat)	*Orfelia nemoralis*	18	/
NEGP195	N/A (bdelloid rotifer)	*Rotaria rotatoria*	17	/
NEGP195	N/A (springtail)	*Entomobrya intermedia*	17	/
NEGP195	N/A (leafhopper)	*Aphrodes makarovi*	13	/
NEGP195	N/A (worm)	*Nais sp.*	12	/
NEGP195	N/A (barklice)	*Ectopsocus sp.*	11	/
NEGP195	N/A (springtail)	*Desoria grisea*	9	/
NEGP195	N/A (springtail)	*Isotoma viridis*	7	/
NEGP195	N/A (red worm)	*Eiseniella tetraedra*	7	/
T1	N/A (water flea)	*Chydorus sphaericus*	46541	14439
T1	N/A (worm)	*Fridericia sp.*	788	/
T1	American bladder snail	*Physa acuta*	178	/
T1	N/A (fly)	*Scatopsciara atomaria*	36	/
T1	N/A (non-biting midge)	*Chironomidae sp.*	21	/

Pond ID	Common Name	Species Name	Num Reads	Unassigned Reads
T1	N/A (earthworm)	*Lumbricidae sp.*	17	/
T1	N/A (tardigrade)	*Ramazzottius sp.*	15	/
T1	N/A (mayfly)	*Cloeon dipterum*	14	/
T1	N/A (crustacean)	*Daphnia magna*	12	/
T1	N/A (non-biting midge)	*Zavrelimyia sp.*	12	/
T1	Springtail	*Tomocerus minor*	8	/
T1	N/A (mosquito)	*Phyllognathopus viguieri*	6	/
T1	Bank worm	*Bimastos rubidus*	5	/
T2	N/A (copepod)	*Acanthocyclops vernalis*	1218	22733
T2	N/A (copepod)	*Canthocamptidae sp.*	478	/
T2	N/A (rotifer)	*Limnias sp.*	470	/
T2	N/A (worm)	*Pristina longiseta*	238	/
T2	N/A (crustacean)	*Cypridopsis sp.*	221	/
T2	N/A (crustacean)	*Podocopida sp.*	217	/
T2	N/A (mite)	*Micreremidae sp.*	92	/
T2	N/A (crustacean)	*Cypridopsis vidua*	59	/
T2	N/A (non-biting midge)	*Paratanytarsus austriacus*	31	/
T2	N/A (bdelloid rotifer)	*Rotaria neptunoida*	22	/
T2	N/A (non-biting midge)	*Chironomidae sp.*	19	/
T2	N/A (barklice)	*Ectopsocus sp.*	9	/
T2	N/A (springtail)	*Pseudosinella alba*	6	/

Appendix 3. Additional Metabarcoding Results

Table 7. Additional metabarcoding results.

Family	Number of ponds containing the species	Family	Number of ponds containing the species
Achlya bisexualis	1	*Phytophthora polonica*	1
Achlya racemosa	1	*Phytophthora rosacearum*	1
Aculops sp.	1	*Phytophthora sp.*	1
Adineta vaga	2	*Phytophthora syringae*	35
Albugo candida	1	*Phytophthora thermophila*	1
Aphanomyces laevis	1	*Phytophthora x stagnum*	4
Arthropoda environmental sample	1	*Phytopythium sp.*	2
Asplanchna sieboldi	1	*Pinnularia cf. gibba*	1
Bradysia strenua	1	*Pinnularia sp.*	1
Cepaea hortensis	1	*Plumatella fungosa*	1
Chaetonotus aff. persimilis	1	*Poteriospumella sp.*	1
Chaoborus crystallinus	1	*Psammoryctides albicola*	1
Cladosporium bruhnei	13	*Psectrocladius limbatellus*	1
Cladosporium sp.	10	*Pseudomonas fluorescens*	1
Cladosporium tenuissimum	5	*Pythium adhaerens*	1
Cochliopodium larifeili	9	*Pythium aff. pachycaule*	18
Corynoneura scutellata	1	*Pythium anandrum*	2
Crangonyx sp.	1	*Pythium angustatum*	19
Cryptomonas curvata	3	*Pythium aphanidermatum*	1
Cyclops abyssorum divergens	1	*Pythium aquatile*	3
Cyclops strenuus	23	*Pythium attrantheridium*	3
Cyclotella cryptica	6	*Pythium biforme*	5
Davidiella tassiana	1	*Pythium brachiatum*	4
Dictyuchus sp.	1	*Pythium contiguanum*	3
Encyonema sp.	2	*Pythium flevoense*	32
Entomobrya multifasciata	2	*Pythium helicandrum*	4
Eunotia bilunaris	3	*Pythium junctum*	14
Eunotia sp.	2	*Pythium monospermum*	8
Fistulifera pelliculosa	2	*Pythium multisporum*	8
Fusarium sp.	1	*Pythium nagaii*	1
Fusarium tricinctum	1	*Pythium oopapillum*	29
Globisporangium heterothallicum	1	*Pythium pachycaule*	44
Globisporangium spinosum	1	*Pythium paroecandrum*	1

Family	Number of ponds containing the species	Family	Number of ponds containing the species
Globisporangium urmianum	2	*Pythium porphyrae*	1
Habrotrocha elusa elusa	1	*Pythium rostratifingens*	1
Habrotrochidae sp.	1	*Pythium segnitium*	2
Korotnevella heteracantha	8	*Pythium senticosum*	2
Korotnevella leshevi	2	*Pythium sp.*	91
Korotnevella sp.	11	*Pythium sukuiense*	18
Korotnevella stella	48	*Pythium torulosum*	1
Lagenidium caudatum	11	*Pythium ultimum*	8
Melampsora magnusiana	4	*Pythium undulatum*	1
Melosira varians	3	*Pythium undulatum*	6
Mischococcus sphaerocephalus	1	*Pythium utonaiense*	3
Monodus sp.	14	*Ripella platypodia*	1
Mucor hiemalis	1	*Rotaria magnacalcarata*	1
Naegleria fultoni	1	*Salisapilia tartarea*	4
Neonectria hubeiensis	7	*Saprolegnia bulbosa*	5
Nitzschia acidoclinata	16	*Saprolegnia delica*	6
Nitzschia cf. recta	1	*Saprolegnia ferax*	4
Nitzschia palea	3	*Saprolegnia parasitica*	22
Paralemanea annulata	7	*Satchelliella trivialis*	1
Paraphysomonas sp.	12	*Sellaphora cf. minima*	7
Pedospumella sp.	3	*Sheathia arcuata*	1
Philodina sp.	2	*Spumella sp.*	2
Philoscia muscorum	5	*Stylodrilus lemani*	1
Phytophthora amnicola x moyootj	3	*Synchaeta tremula*	1
Phytophthora balyanboodjas	1	*Synura sp. strain*	1
Phytophthora bilorbang	32	*Torularia atra*	1
Phytophthora cf. attenuata	1	*Trichoptera sp.*	1
Phytophthora cf. sp. sylvatica	1	*Uncultured fungus*	9
Phytophthora chlamydospora	20	*Uncultured Jaminaea*	13
Phytophthora gallica	14	*UNVERIFIED: Amblyseius herbicolus*	3
Phytophthora gonapodyides	41	*UNVERIFIED: Articulospora atra*	2
Phytophthora gregata	1	*UNVERIFIED: Branchiura sowerbyi*	1
Phytophthora hibernalis	4	*UNVERIFIED: Gomphonema parvulum*	24
Phytophthora inundata	2	*UNVERIFIED: Limnophyes sp.*	1

Family	Number of ponds containing the species	Family	Number of ponds containing the species
Phytophthora lacustris	85	*UNVERIFIED: Phyllognathopus viguieri*	1

Natural England is here to secure a healthy natural environment for people to enjoy, where wildlife is protected and England's traditional landscapes are safeguarded for future generations.

Natural England publications are available as accessible pdfs from www.gov.uk/natural-england.

Should an alternative format of this publication be required, please contact our enquiries line for more information: 0300 060 3900 or email enquiries@naturalengland.org.uk.

ISBN 978-1-78354-990-2

Catalogue code: NECR454

NATURAL ENGLAND