



# Preliminary Environmental Information Report Volume 2

## Appendix 8.5 Baseline Report - eDNA Survey

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**lionlink**!



# Lionlink eDNA study: single species testing and metabarcoding

Lionlink eDNA study

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# 1 INTRODUCTION

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This report provides the environmental DNA (eDNA) analysis results of samples taken from watercourses within the boundary of the LionLink proposed Onshore Scheme. Surveys were completed, where accessed allowed, within the proposed Onshore Scheme Scoping Boundary, which included two options for proposed Landfall Sites (at Southwold and Walberswick) and encompassed an onshore cable corridor that runs from Uggheshall in the north to Friston in the south.

eDNA describes the DNA that can be extracted from an environmental sample, such as soil, sediments, water, or snow. The eDNA present within a waterbody will include that material that has originated from the faeces, saliva, urine, and skin cells of animals occupying that waterbody and may also be from animals that may have visited that environment, such as birds and mammals visiting the waterbody to drink. The rapid diffusion of eDNA from its source means that in theory the presence of a specific animal can be detected anywhere within the waterbody and not just at its point of origin. eDNA released into the environment is likely to be broken down by the action of Ultraviolet (UV) light and microbial activity. Thus, presence of eDNA from a target species can allow us to determine their presence or very recent presence without the need for direct observation or trapping. A further factor for consideration in rivers and streams is that eDNA can be well mixed and flow can transport eDNA some distance from its source.

Analysis of eDNA to determine species presence/absence from water involves: the collection of a sample of water; extraction of the eDNA; and the use of the Polymerase Chain Reaction (PCR) to amplify specific genetic sequences within the eDNA of the target species. Using next-generation sequencing techniques (metabarcoding) allows us to determine complex communities of faunas within water samples collected from a single site.

This report provides the analysis of the eDNA extracted from rivers and streams for invasive species, such as signal crayfish (*Pacifastacus leniusculus*) and zebra mussel (*Dreissena polymorpha*), and cray fish plague, using species-specific real-time PCR assays. The same water extracts were additionally analysed by DNA metabarcoding approaches to characterise the vertebrate; invertebrate; and Unionidae/Veneridae populations that may be present within water collected at the sampling sites.

## 2 METHODS

### 2.1 Location

eDNA sampling was attempted at 30 locations overall, where access allowed. However, of these 30 locations, sampling was only possible at 17 locations. No samples were possible from 13 locations due to the watercourses being dry or due to access restrictions. It should be noted that visits were made to these 13 locations at least twice through the summer period in an attempt to obtain a sample. Please see Table 1, below, for a list of these locations.

**Table 1 Sampling locations.**

| Sample ID          | Name                                    | Grid Reference | eDNA Analysis?           |
|--------------------|-----------------------------------------|----------------|--------------------------|
| LL-ARP_eDNA Pt 180 | Buss Creek                              | TM5046476913   | Yes                      |
| LL-ARP-EDNAPt-181  | River Wang Point 1                      | TM4636679143   | Yes                      |
| LL-ARP_eDNA Pt 182 | River Wang Point 2                      | TM4459579749   | Yes                      |
| LL-ARP_eDNA Pt 183 | River Wang Tributary                    | TM4470079855   | Yes                      |
| LL-ARP_eDNA Pt 184 | River Wang ditch network                | TM4571279456   | Yes                      |
| LL-ARP_eDNA Pt 185 | River Wang feeder ditch                 | TM4541879836   | Yes                      |
| LL-ARP_eDNA Pt 186 | River Wang Tributary ditch network      | TM4468380404   | Yes                      |
| LL-ARP_eDNA Pt 187 | River Wang Tributary at New Valley Farm | TM4444477961   | Yes                      |
| LL-ARP_eDNA Pt 188 | River Blythe Point 1                    | TM4410575396   | Yes                      |
| LL-ARP_eDNA Pt 189 | River Blythe Point 2                    | TM4493475387   | Yes                      |
| LL-ARP-EDNAPt-190  | River Blythe Tributary 1                | TM4493475387   | No – Access restrictions |
| LL-ARP-EDNAPt-191  | River Blythe Tributary 2                | TM4411875306   | No – Access restrictions |
| LL-ARP_eDNA Pt 192 | River Blythe ditch network              | TM4448575367   | Yes                      |
| LL-ARP_eDNA Pt 193 | River Blythe Tributary ditch network    | TM4496271161   | Yes                      |
| LL-ARP_eDNA Pt 194 | Dunwich River Tidal                     | TM4982174386   | Yes                      |

| Sample ID          | Name                                                    | Grid Reference | eDNA Analysis?            |
|--------------------|---------------------------------------------------------|----------------|---------------------------|
| LL-ARP-EDNAPt-195  | Dunwich River                                           | TM4496271161   | No - Watercourse was dry. |
| LL-ARP_eDNA Pt 196 | Minsmere New Cut                                        | TM4298068113   | Yes                       |
| LL-ARP_eDNA Pt 197 | Minsmere New Cut ditch network Point 1                  | TM4229268698   | Yes                       |
| LL-ARP_eDNA Pt 198 | Minsmere New Cut ditch network Point 2                  | TM4244968497   | Yes                       |
| LL-ARP_eDNA Pt 199 | Minsmere New Cut Tributary                              | TM4288567953   | Yes                       |
| LL-ARP-EDNAPt-201  | Minsmere New Cut Tributary north of Pretty road         | TM4320466503   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-202  | Minsmere New Cut Tributary south of Pretty road Point 1 | TM4315465531   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-203  | Minsmere New Cut Tributary south of Pretty road Point 2 | TM4314765490   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-204  | Minsmere New Cut Tributary south of Pretty road Point 3 | TM4342065701   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-205  | Hundred River Point 1                                   | TM4231563791   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-206  | Hundred River Point 2                                   | TM4236562365   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-207  | Hundred River Northern Tributary                        | TM4217163765   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-208  | Hundred River Southern Tributary                        | TM4207563154   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-209  | Fromus catchment ditch network Point 1 (approximate)    | TM4011862902   | No - Watercourse was dry. |
| LL-ARP-EDNAPt-210  | Fromus catchment ditch network Point 2 (approximate)    | TM3953763148   | No - Watercourse was dry. |

## 2.2 Laboratory standard and specifications

All laboratory activities associated with DNA analysis are subject to errors if quality control is inadequate. The 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 Sampling

The sampling kit consisted of two sterile Whirl-Pak bags; one pair of sterile gloves; one sterile sampling ladle; a 50ml syringe, two 0.22 µM polyethersulfone filter (Sterivex, Millipore) and sealing caps; and a 10ml syringe containing an ethanol-based preservative.

Samples of water were collected every two kilometers of main river which fell within the broad survey boundary, as well as the confluence of tributaries. Samples were taken from across a transect of the river/stream (walking diagonally upstream whilst taking the samples), using the sampling ladle. Each ladle full was emptied into the Whirl-Pak bag. Where the minimum of three sub-sample locations from near bank, middle of river, and far bank was used around 10 sub samples were collected in each location. This process was repeated walking back across the river (again diagonally upstream) collecting the water into the second Whirl-Pak bag. This created two independent water samples. Full sampling methodology can be found in Appendix 1.

## 2.4 DNA extraction from filters

DNA was extracted from all filters using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions with minor modifications (below) and finally resuspended in 200 µL of elution buffer. Extraction blanks were included (one per day of DNA extractions) to monitor for any cross-contamination during the DNA extraction.

Protocol modifications:

- 540 µL ATL buffer, 300µL AL buffer, and 50 µL proteinase K (all components of the kit) increased over standard amounts.
- 500 µL 100% ethanol used instead of standard amount.

Upon completion of DNA extraction, the two samples from each location were pooled together and the DNA extracts were quantified using a Qubit 3.0 Fluorometer (Invitrogen) following the manufacturer's instructions then stored at -20 °C prior to PCR set up.

## 2.5 Single species eDNA assays

### 2.5.1 Zebra mussel

This assay followed the methods of Gingera *et al* (2017), utilizing a species-specific qPCR-based eDNA assay for the cytochrome b (cyt b) region of the mitochondrial gene. The final volume was adjusted to 25 µl (all components adjusted accordingly) and used 3 µl of extracted DNA. Twelve replicates were performed using 55 cycles rather than the 35 used in the publication. RSK ADAS uses 55 cycles as standard when a high cycle PCR is required to detect DNA that is likely to be at very low concentrations such as would be the case for the water samples herein. DNA for use as a positive control was extracted from zebra mussels sourced from the Attenborough Nature Reserve, South Nottingham and were confirmed by Sanger sequencing.

### 2.5.2 White-clawed crayfish

This assay followed the methods of Atkinson *et al.* (2019), utilizing a species-specific qPCR-based eDNA assay for the cytochrome oxidase 1 (COI) region of the mitochondrial gene. The final PCR volume was adjusted to 25 µl (all components adjusted accordingly) and used 3 µl of extracted DNA. Twelve replicates were performed using 50 cycles rather than the 40 used in the publication. RSK ADAS uses 55 cycles as standard when a high cycle PCR is required to detect DNA that is likely to be at very low concentrations such as would be the case for the water samples herein. DNA for use as a positive control was extracted from a deceased, white-clawed crayfish sourced from the River Witham, Lincolnshire by a local angler and were confirmed by Sanger sequencing.

### 2.5.3 Signal crayfish

This assay followed the methods of Harper *et al.* (2018), utilizing a species-specific qPCR-based eDNA assay for the cytochrome oxidase 1 (COI) region of the mitochondrial gene. The final volume was adjusted to 25 µl (all components adjusted accordingly) and used 3 µl of extracted DNA. Twelve replicates were performed using 50 cycles rather than the 40 used in the publication. RSK ADAS uses 55 cycles as standard when a high cycle PCR is required to detect DNA that is likely to be at very low concentrations such as would be the case for the water samples herein. DNA for use as a positive control was extracted from a signal crayfish sourced from a licensed trapper and were confirmed by Sanger sequencing.

### 2.5.4 Crayfish plague

This assay followed the methods of Vralstad *et al.* (2009), utilizing a species-specific qPCR-based eDNA assay for the nuclear ribosomal DNA (nrDNA) of the internal transcribed spacer (ITS). The final volume was adjusted to 25µl (all components adjusted accordingly) and used 3 µl of extracted DNA. Twelve replicates were performed using 55 cycles rather than the 40 used in the publication. RSK ADAS uses 55 cycles as standard when a high cycle PCR is required to detect DNA that is likely to be at very low concentrations such as would be the case for the water samples herein. DNA for use as a positive control was synthesised by a commercial company (Integrated DNA Technologies).

## 2.6 Metabarcoding

### 2.6.1 12S metabarcoding for vertebrates

The primer combination used for the first round PCR amplification were the 12S forward and reverse primers of Riaz *et al.* (2011). Overhang adapter sequences were included at the 5' end of the primers to ensure compatibility with Illumina index and sequencing adapters (Illumina, 2012). PCRs included one negative control (ultrapure H<sub>2</sub>O in place of DNA); two DNA extraction blanks; a positive control sample Atlantic mackerel (*Scomber scombrus*); all extracted filter DNA samples were amplified in triplicate using 3 ng extracted DNA per reaction and 40 cycles.

### 2.6.2 COI metabarcoding for invertebrates

The primer combination used for the first round COI PCR amplification was fwhF2/fwhR2 (Vamos *et al.* 2017). Overhang adapter sequences were included at the 5' end of the primers to ensure compatibility with Illumina index and sequencing adapters (Illumina, 2012). PCRs included one negative control (ultrapure H<sub>2</sub>O in place of DNA); two DNA extraction blanks;

a positive control sample from earthworm (*Allolobophora chlorotica*); and all extracted filter DNA samples were amplified in triplicate using 3 ng extracted DNA per reaction and a touchdown PCR (Don *et al.*, 1991).

### 2.6.3 Metabarcoding for Unionidae and Veneridae

The primer combinations used for the first round PCR amplification for Unionidae and Veneridae were those of Prie *et al.* (2020). Overhang adapter sequences were included at the 5' end of the primers to ensure compatibility with Illumina index and sequencing adapters (Illumina, 2012). PCRs included one negative control (ultrapure H<sub>2</sub>O in place of DNA); two DNA extraction blanks; a positive control sample Painters mussel (*Unio pictorum*) or Swamp orb mussel (*Sphaerium nucleus*) respectively; and all extracted filter DNA samples were amplified in triplicate using 3 ng extracted DNA per reaction and 40 cycles.

### 2.6.4 Illumina library production

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. 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 by the supplier Source RSK Bioscience.

### 2.6.5 Bioinformatic processing

Data processing was performed using custom bioinformatic scripts and custom 12S, COI, and Unionidae/Veneridae databases derived from Genbank (online public sequence database).

## 2.7 Survey limitations

Whilst the optimal available sampling location was chosen for each targeted section of watercourse, limitations were encountered at a number of sampling locations. A summary of these are:

- LL-ARP-EDNAPt-182 (River Wang Point 2) – the river could only be accessed from the southern bank.
- LL-ARP-EDNAPt-183 (River Wang Tributary) – the watercourse was too deep to access safely; therefore, all samples were taken from the eastern bank.
- LL-ARP-EDNAPt-184 (River Wang ditch network) – the ditch was heavily vegetated with steep banks making safe access not possible in most locations. Water sampling was restricted to a small area where access was considered safe.
- LL-ARP-EDNAPt-185 (River Wang feeder ditch) – the ditch was heavily silted, as such a poorer quality water sample was collected. Only 100 ml passed through the filter.
- LL-ARP-EDNAPt-186 (River Wang Tributary ditch network) – the silt at this site was too deep to safely access the watercourse; therefore, samples were taken from the bank.
- LL-ARP-EDNAPt-194 (Dunwich River Tidal) – the watercourse was too deep to cross safely; therefore, samples were taken from the north bank only.

- LL-ARP-EDNAPt-196 (Minsmere New Cut) – the watercourse was heavily vegetated and silted which impeded safe access. Samples were only taken from banks.
- LL-ARP-EDNAPt-197 (Minsmere New Cut ditch network Point 1) – the watercourse was too deep to cross safely; therefore, samples were taken from a single bank.
- LL-ARP-EDNAPt-198 (Minsmere New Cut ditch network Point 2) – the watercourse was too deep to cross safely; therefore, samples were taken from a single bank.
- LL-ARP-EDNAPt-199 (Minsmere New Cut Tributary) – the watercourse was too silty to access safely; therefore, samples were taken from the southern bank only.

Despite the above listed limitations at ten of the sampling locations, samples from all locations were successfully analysed and clear results obtained following a rigorous analysis protocol. As such, the limitations experienced during sample collection are not considered to be significant.

## 3 RESULTS

### 3.1 Single-species qPCR

A summary of all single species qPCR eDNA assays is shown in Table 1. Only two samples were positive for crayfish plague DNA, all remaining samples were negative for crayfish plague. All samples were negative for zebra mussel DNA, white-clawed crayfish DNA, and signal crayfish DNA.

**Table 2 Single-species qPCR results**

| Sample ID                                                    | Laboratory ID | Zebra mussel | White clawed crayfish | Signal crayfish | Crayfish plague |
|--------------------------------------------------------------|---------------|--------------|-----------------------|-----------------|-----------------|
| LL-ARP_eDNA Pt 180 (Buss Creek)                              | LL3 B         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 181 (River Wang Point 1)                      | LL4 C         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 182 (River Wang Point 2)                      | LL3 F         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 183 (River Wang Tributary)                    | LL3 D         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 184 (River Wang ditch network)                | LL4 B         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 185 (River Wang feeder ditch)                 | LL4 D         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network)      | LL3 C         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL4 A         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 188 (River Blythe Point 1)                    | LL2 B         | 0/12         | 0/12                  | 0/12            | 7/12            |
| LL-ARP_eDNA Pt 189 (River Blythe Point 2)                    | LL2 A         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 192 (River Blythe ditch network)              | LL2 C         | 0/12         | 0/12                  | 0/12            | 10/12           |
| LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network)    | LL5 A         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 194 (Dunwich River Tidal)                     | LL3 E         | 0/12         | 0/12                  | 0/12            | 0/12            |

| Sample ID                                                      | Laboratory ID | Zebra mussel | White clawed crayfish | Signal crayfish | Crayfish plague |
|----------------------------------------------------------------|---------------|--------------|-----------------------|-----------------|-----------------|
| LL-ARP_eDNA Pt 196<br>(Minsmere New Cut)                       | LL1 A         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 197<br>(Minsmere New Cut ditch network Point 1) | LL1 B         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 198<br>(Minsmere New Cut ditch network Point 2) | LL1 C         | 0/12         | 0/12                  | 0/12            | 0/12            |
| LL-ARP_eDNA Pt 199<br>(Minsmere New Cut Tributary)             | LL3 A         | 0/12         | 0/12                  | 0/12            | 0/12            |

Samples positive for crayfish plague DNA are shown in red text.

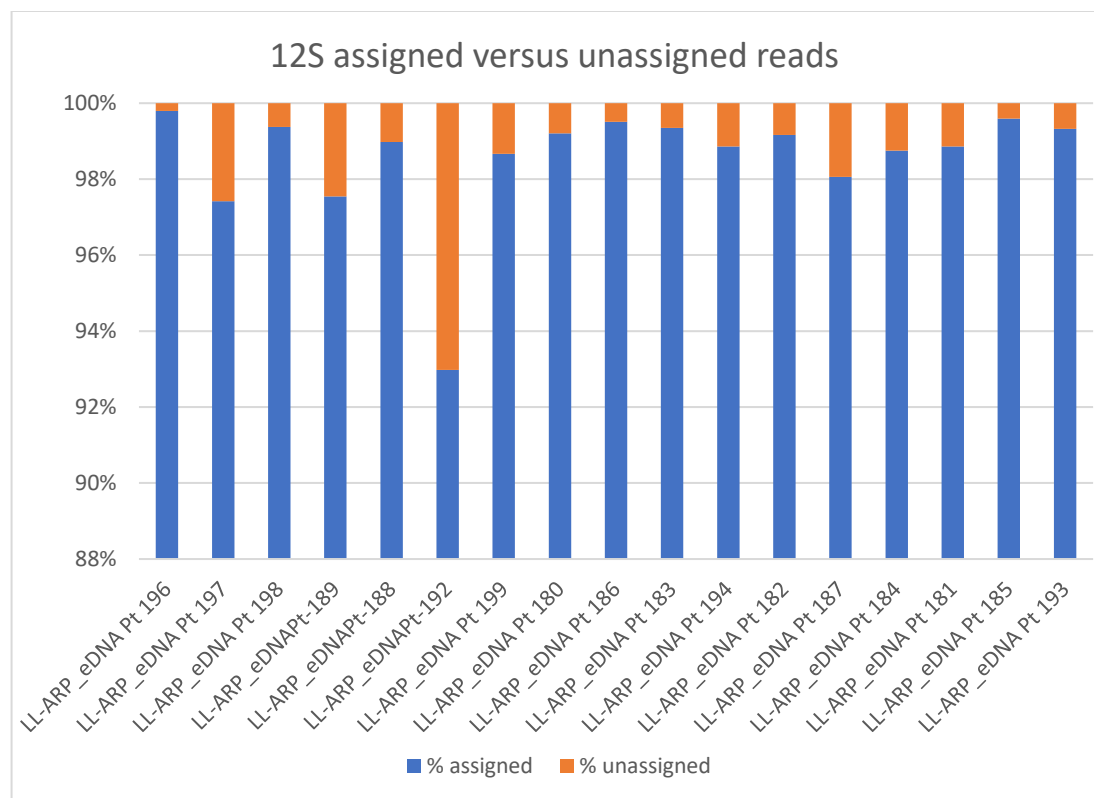
## 3.2 Metabarcoding results

All 17 samples were successfully amplified, DNA quantified, and the amplicon DNA was sent to Source Biosciences for indexing PCR and Illumina sequencing. Quality control reports generated by the sequencing service showed that both the indexed amplicons and the returned sequences were of good quality.

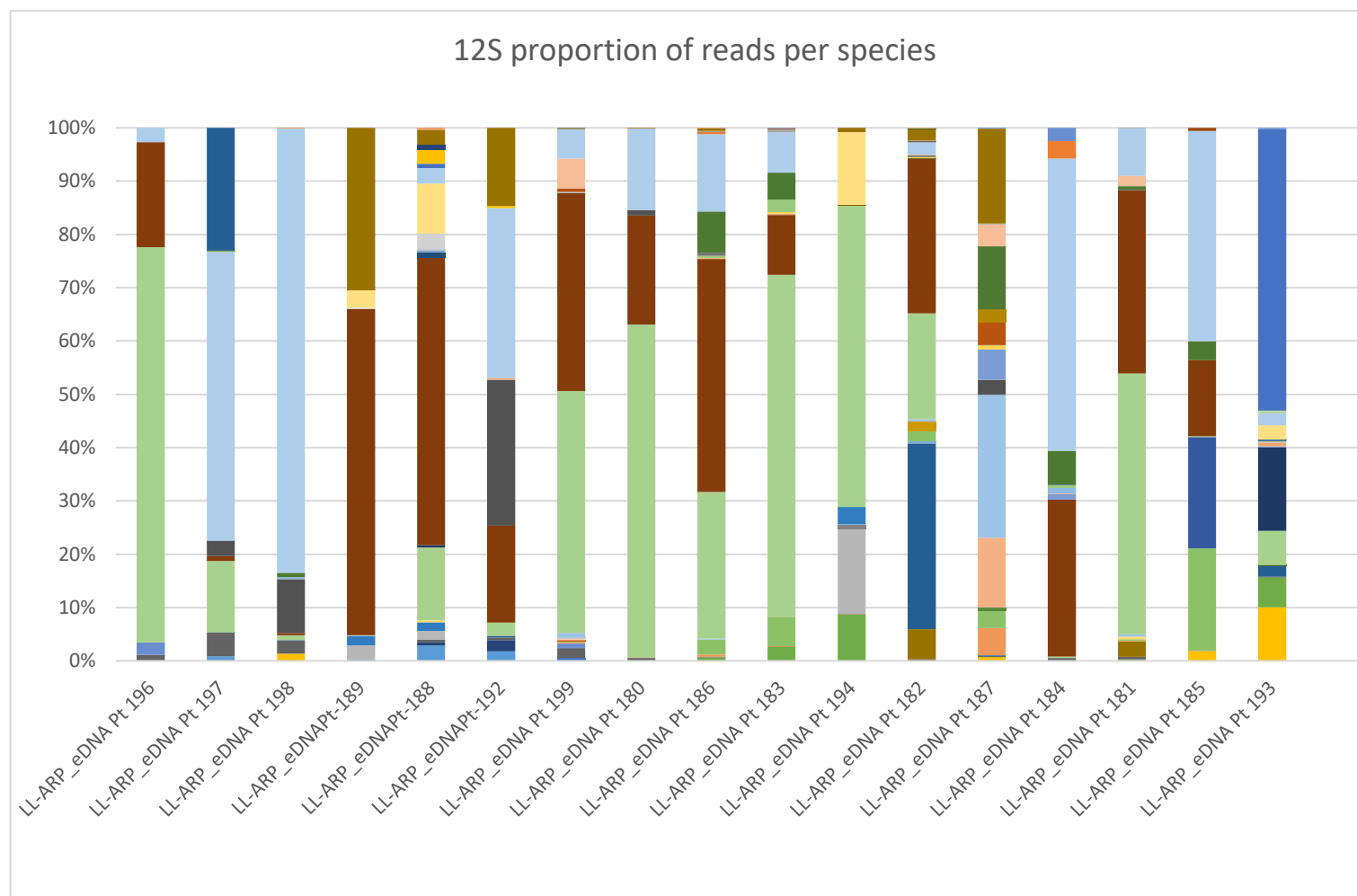
### 3.2.1 12S Vertebrate metabarcoding

For the 12S analysis, of the sequences that passed all filters and went onto taxonomic assignment a total of 1.76M sequences (~98.5% of sequences) were assigned a taxonomic identification which represented 68 species including fish, birds, mammals, and amphibians. A total of 13k sequences (~1.5% of sequences) were unassigned. The percentage of assigned and unassigned sequence reads per sample are shown in Figure 1. Results are shown in Table 3, Figure 2 and Appendix 2. Samples contained between four and 26 species of vertebrate with an average of 15 species per sample. Twenty-three species including common bleak (*Alburnus alburnus*), Eurasian sparrowhawk (*Accipiter nisus*), long-tailed tit (*Aegithalos caudatus*) and European otter (*Lutra lutra*) were only found in one sample. The most recorded species across all samples were European water vole (*Arvicola amphibius*), three-spined stickleback (*Gasterosteus aculeatus*), and ninespine stickleback (*Pungitius pungitius*). For the full list of 12S metabarcoding results, please see Appendix 2.

**Figure 1 12S proportion of assigned and unassigned sequence reads.**



**Figure 2 12S proportion of reads per species**



## Legend - 12S proportion of reads per species

|                                                     |                                                       |                                                          |
|-----------------------------------------------------|-------------------------------------------------------|----------------------------------------------------------|
| ■ <i>Accipiter nisus</i> Eurasian sparrowhawk       | ■ <i>Aegithalos caudatus</i> Long tailed tit          | ■ <i>Alburnus alburnus</i> Bleak                         |
| ■ <i>Anas platyrhynchos</i> Mallard duck            | ■ <i>Anguilla anguilla</i> Freshwater eel             | ■ <i>Anguilla anguilla</i> European eel                  |
| ■ <i>Anser anser</i> Common goose                   | ■ <i>Apodemus sylvaticus</i> European woodmouse       | ■ <i>Arvicola amphibius</i> European water vole          |
| ■ <i>Barbatula barbatula</i> Stone loach            | ■ <i>Bos taurus</i> Cow                               | ■ <i>Botaurus stellaris</i> Eurasian bittern             |
| ■ <i>Bufo bufo</i> Common toad                      | ■ <i>Canis lupus familiaris</i> Domestic dog          | ■ <i>Chelon labrosus</i> Thicklip grey mullet            |
| ■ Chordate environmental sample                     | ■ <i>Chroicocephalus ridibundus</i> Black-headed gull | ■ <i>Columba livia</i> Rock dove                         |
| ■ <i>Columba oenas</i> Stock dove                   | ■ <i>Corvus splendens</i> House crow                  | ■ <i>Cygnus columbianus bewickii</i> Bewick's swan       |
| ■ <i>Dama dama</i> European fallow deer             | ■ <i>Dicentrarchus labrax</i> European seabass        | ■ <i>Emberiza citrinella</i> Yellowhammer                |
| ■ <i>Equus caballus</i> Domesticated horse          | ■ <i>Erithacus rubecula</i> European robin            | ■ <i>Fringilla montifringilla</i> Brambling              |
| ■ <i>Gallinula chloropus</i> Common moorhen         | ■ <i>Gallus domesticus</i> Chicken                    | ■ <i>Gasterosteus aculeatus</i> Three-spined stickleback |
| ■ <i>Gobio gobio</i> Gudgeon                        | ■ <i>Homo sapiens</i> Human                           | ■ <i>Lissotriton vulgaris</i> Smooth newt                |
| ■ <i>Liza aurata</i> Golden grey mullet             | ■ <i>Lutra lutra</i> Otter                            | ■ <i>Meles meles</i> European badger                     |
| ■ <i>Micromys minutus</i> Eurasian harvest mouse    | ■ <i>Microtus agrestis</i> Field vole                 | ■ <i>Muntiacus reevesi</i> Reeves's muntjac              |
| ■ <i>Myodes glareolus</i> Bank vole                 | ■ <i>Neomys fodiens</i> Eurasian water shrew          | ■ <i>Oryctolagus cuniculus</i> European rabbit           |
| ■ <i>Ovis aries</i> Sheep                           | ■ <i>Parus major</i> Great tit                        | ■ <i>Passer montanus</i> Eurasian tree sparrow           |
| ■ <i>Pelecus cultratus</i> Ziege                    | ■ <i>Phalacrocorax carbo</i> Great cormorant          | ■ <i>Phasianus colchicus</i> Common pheasant             |
| ■ <i>Phylloscopus collybita</i> Eurasian chiffchaff | ■ <i>Pica pica</i> Eurasian magpie                    | ■ <i>Pleuronectes platessa</i> European plaice           |
| ■ <i>Pomatoschistus microps</i> Common goby         | ■ <i>Pungitius pungitius</i> Nine-spined stickleback  | ■ <i>Pyrrhula pyrrhula</i> Eurasian bullfinch            |
| ■ <i>Rallus aquaticus</i> Water rail                | ■ <i>Rana temporaria</i> Common frog                  | ■ <i>Rattus norvegicus</i> Brown rat                     |
| ■ <i>Rutilus rutilus</i> Roach                      | ■ <i>Salmo salar</i> salmon                           | ■ <i>Salmo trutta</i> Brown trout                        |
| ■ <i>Scardinius erythrophthalmus</i> Rudd           | ■ <i>Sciurus carolinensis</i> Eastern grey squirrel   | ■ <i>Sorex araneus</i> Common shrew                      |
| ■ <i>Sus scrofa domesticus</i> Pig                  | ■ <i>Tinca tinca</i> Tench                            | ■ <i>Turdus merula</i> Blackbird                         |
| ■ Unknown ungulate                                  | ■ <i>Vulpes vulpes</i> Red fox                        |                                                          |

**Table 3 Number of samples containing species (vertebrates) using 12S primers.**

| Species                                    | Common name              | Number of samples |
|--------------------------------------------|--------------------------|-------------------|
| <i>Accipiter nisus</i>                     | Eurasian sparrowhawk     | 1                 |
| <i>Aegithalos caudatus</i>                 | Long tailed tit          | 1                 |
| <i>Alburnus alburnus</i>                   | Bleak                    | 1                 |
| <i>Anas platyrhynchos</i>                  | Mallard duck             | 5                 |
| <i>Anguilla anguilla</i>                   | European eel             | 9                 |
| <i>Anser anser</i>                         | Common goose             | 3                 |
| <i>Apodemus sylvaticus</i>                 | European woodmouse       | 4                 |
| <i>Arvicola amphibius</i>                  | European water vole      | 15                |
| <i>Barbatula barbatula</i>                 | Stone loach              | 2                 |
| <i>Bos taurus</i>                          | Cow                      | 8                 |
| <i>Botaurus stellaris</i>                  | Eurasian bittern         | 1                 |
| <i>Bufo bufo</i>                           | Common toad              | 3                 |
| <i>Canis lupus familiaris</i>              | Domestic dog             | 9                 |
| <i>Chelon labrosus</i>                     | Thicklip grey mullet     | 3                 |
| Chordate environmental sample <sup>1</sup> | N/A                      | 2                 |
| <i>Chroicocephalus ridibundus</i>          | Black-headed gull        | 1                 |
| <i>Columba livia</i>                       | Rock dove                | 8                 |
| <i>Columba oenas</i>                       | Stock dove               | 2                 |
| <i>Corvus splendens</i>                    | House crow               | 1                 |
| <i>Cygnus columbianus bewickii</i>         | Bewick's swan            | 1                 |
| <i>Dama dama</i>                           | European fallow deer     | 1                 |
| <i>Dicentrarchus labrax</i>                | European seabass         | 3                 |
| <i>Emberiza citrinella</i>                 | Yellowhammer             | 1                 |
| <i>Equus caballus</i>                      | Domesticated horse       | 1                 |
| <i>Erithacus rubecula</i>                  | European robin           | 3                 |
| <i>Fringilla montifringilla</i>            | Brambling                | 1                 |
| <i>Gallinula chloropus</i>                 | Common moorhen           | 2                 |
| <i>Gallus domesticus</i>                   | Chicken                  | 6                 |
| <i>Gasterosteus aculeatus</i>              | Three-spined stickleback | 16                |
| <i>Gobio gobio</i>                         | Gudgeon                  | 2                 |
| <i>Lissotriton vulgaris</i>                | Smooth newt              | 5                 |
| <i>Liza aurata</i>                         | Golden grey mullet       | 1                 |
| <i>Lutra lutra</i>                         | Otter                    | 1                 |
| <i>Meles meles</i>                         | European badger          | 1                 |
| <i>Micromys minutus</i>                    | Eurasian harvest mouse   | 4                 |
| <i>Microtus agrestis</i>                   | Field vole               | 5                 |
| <i>Muntiacus reevesi</i>                   | Reeves's muntjac         | 1                 |
| <i>Myodes glareolus</i>                    | Bank vole                | 6                 |
| <i>Neomys fodiens</i>                      | Eurasian water shrew     | 5                 |

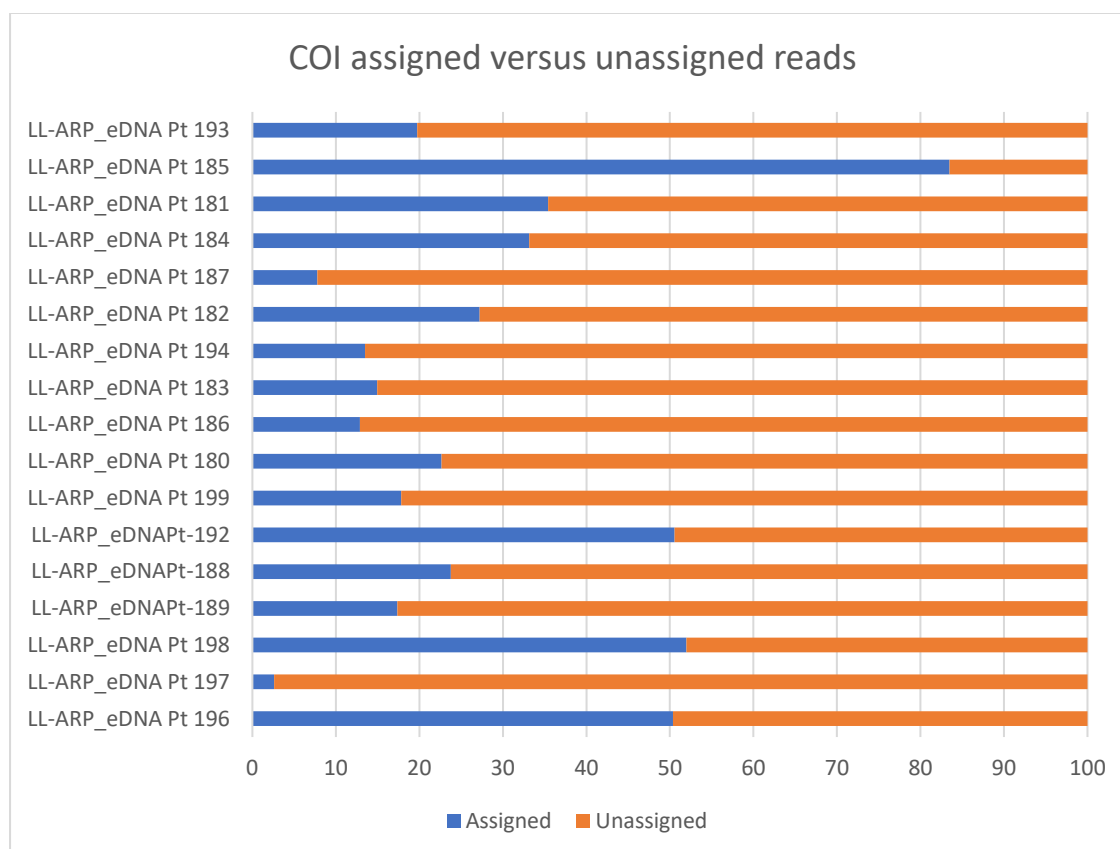
<sup>1</sup> Unknown species from the phylum Chordata.

| Species                            | Common name             | Number of samples |
|------------------------------------|-------------------------|-------------------|
| <i>Oryctolagus cuniculus</i>       | European rabbit         | 5                 |
| <i>Ovis aries</i>                  | Sheep                   | 5                 |
| <i>Parus major</i>                 | Great tit               | 2                 |
| <i>Passer montanus</i>             | Eurasian tree sparrow   | 2                 |
| <i>Pelecus cultratus</i>           | Ziege                   | 1                 |
| <i>Phalacrocorax carbo</i>         | Great cormorant         | 1                 |
| <i>Phasianus colchicus</i>         | Common pheasant         | 7                 |
| <i>Phylloscopus collybita</i>      | Eurasian chiffchaff     | 1                 |
| <i>Pica pica</i>                   | Eurasian magpie         | 5                 |
| <i>Pleuronectes platessa</i>       | European plaice         | 2                 |
| <i>Pomatoschistus microps</i>      | Common goby             | 4                 |
| <i>Pungitius pungitius</i>         | Nine-spined stickleback | 15                |
| <i>Pyrrhula pyrrhula</i>           | Eurasian bullfinch      | 2                 |
| <i>Rallus aquaticus</i>            | Water rail              | 2                 |
| <i>Rana temporaria</i>             | Common frog             | 3                 |
| <i>Rattus norvegicus</i>           | Brown rat               | 6                 |
| <i>Rutilus rutilus</i>             | Roach                   | 2                 |
| <i>Salmo salar</i>                 | salmon                  | 1                 |
| <i>Salmo trutta</i>                | Brown trout             | 1                 |
| <i>Scardinius erythrophthalmus</i> | Rudd                    | 1                 |
| <i>Sciurus carolinensis</i>        | Eastern grey squirrel   | 4                 |
| <i>Sorex araneus</i>               | Common shrew            | 1                 |
| <i>Sus scrofa domesticus</i>       | Pig                     | 9                 |
| <i>Tinca tinca</i>                 | Tench                   | 2                 |
| <i>Turdus merula</i>               | Blackbird               | 1                 |
| <i>Unknown ungulate</i>            | N/A                     | 4                 |
| <i>Vulpes vulpes</i>               | Red fox                 | 4                 |

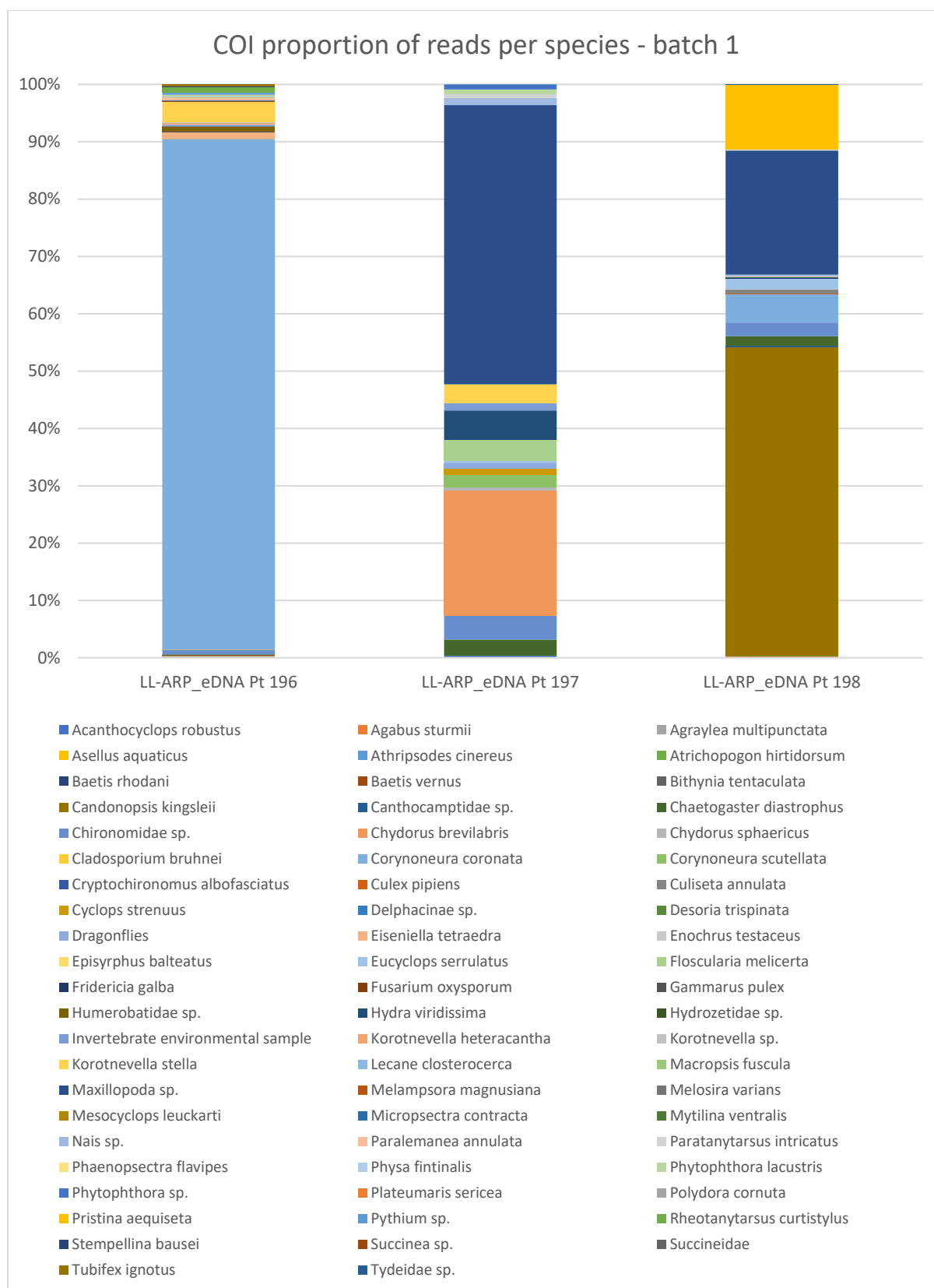
### 3.2.2 COI Invertebrate metabarcoding

For the COI analysis, of the sequences that passed all filters and went onto taxonomic assignment a total of 219k sequences (~37% of sequences) were assigned a taxonomic identification which represented 358 species including arthropods, annelids, and molluscs. A total of 371k sequences (~63% of sequences) were unassigned. The percentage of assigned and unassigned sequence reads per sample are shown in Figure 3. Results are shown in Table 4, Figures 4-8 and Appendix 3. Samples contained between 17 and 101 species of invertebrate with an average of 43 species per sample. Two hundred and ten species were only found in one sample and the most found species were found in at least nine of the samples: Detritus worm (*Chaetogaster diastrophus*), non-biting midge (*Chironomidae* sp.), mould (*Cladosporium* sp.), garden slug (*Deroceras reticulatum*), Rust fungi (*Melampsora magnusiana*), diatom (*Melosira varians*), oomycete (*Pythium* sp. and *Phytophthora lacustris*). For the full list of COI metabarcoding results, please see Appendix 3.

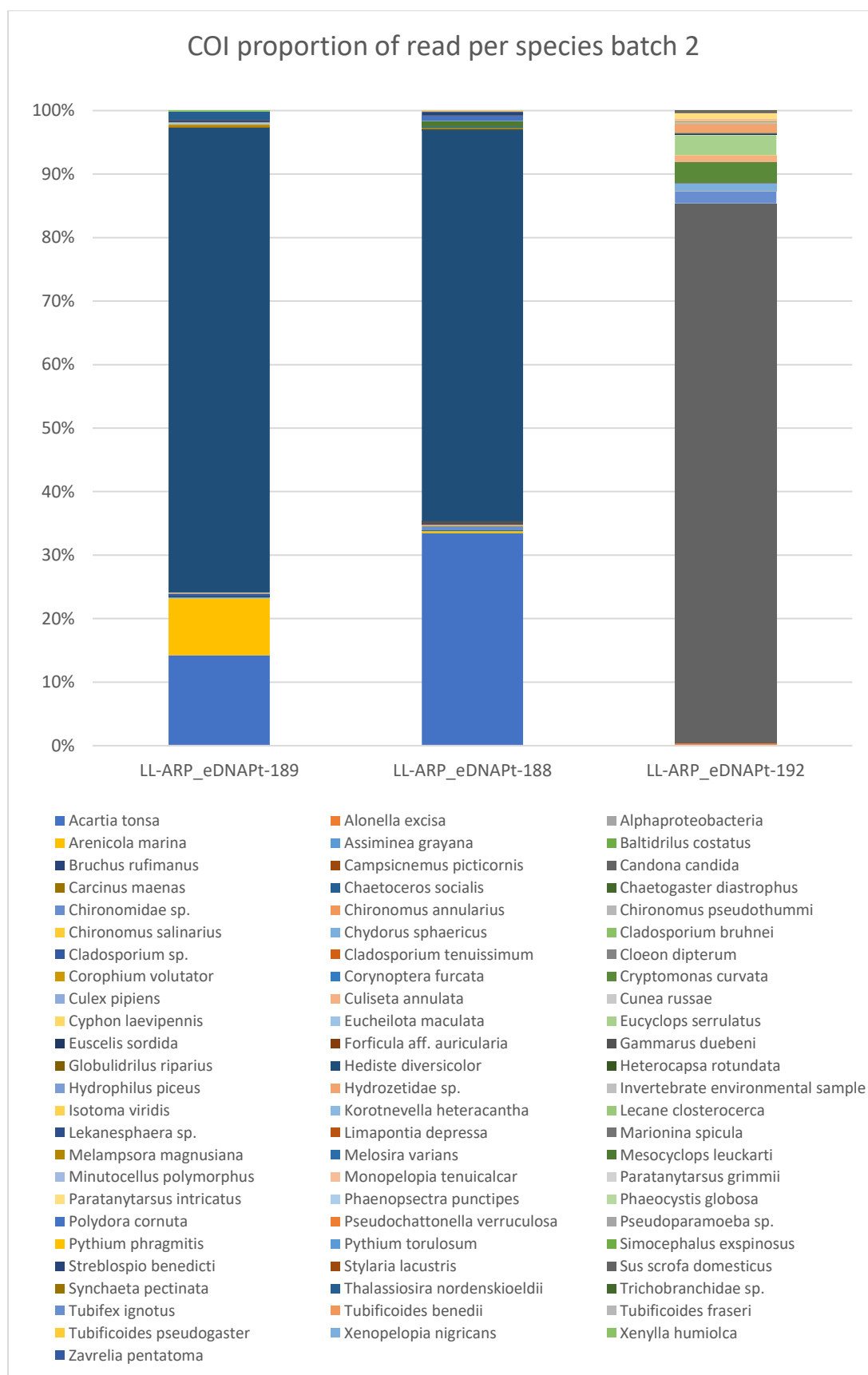
**Figure 3 COI proportion of assigned and unassigned sequence reads.**



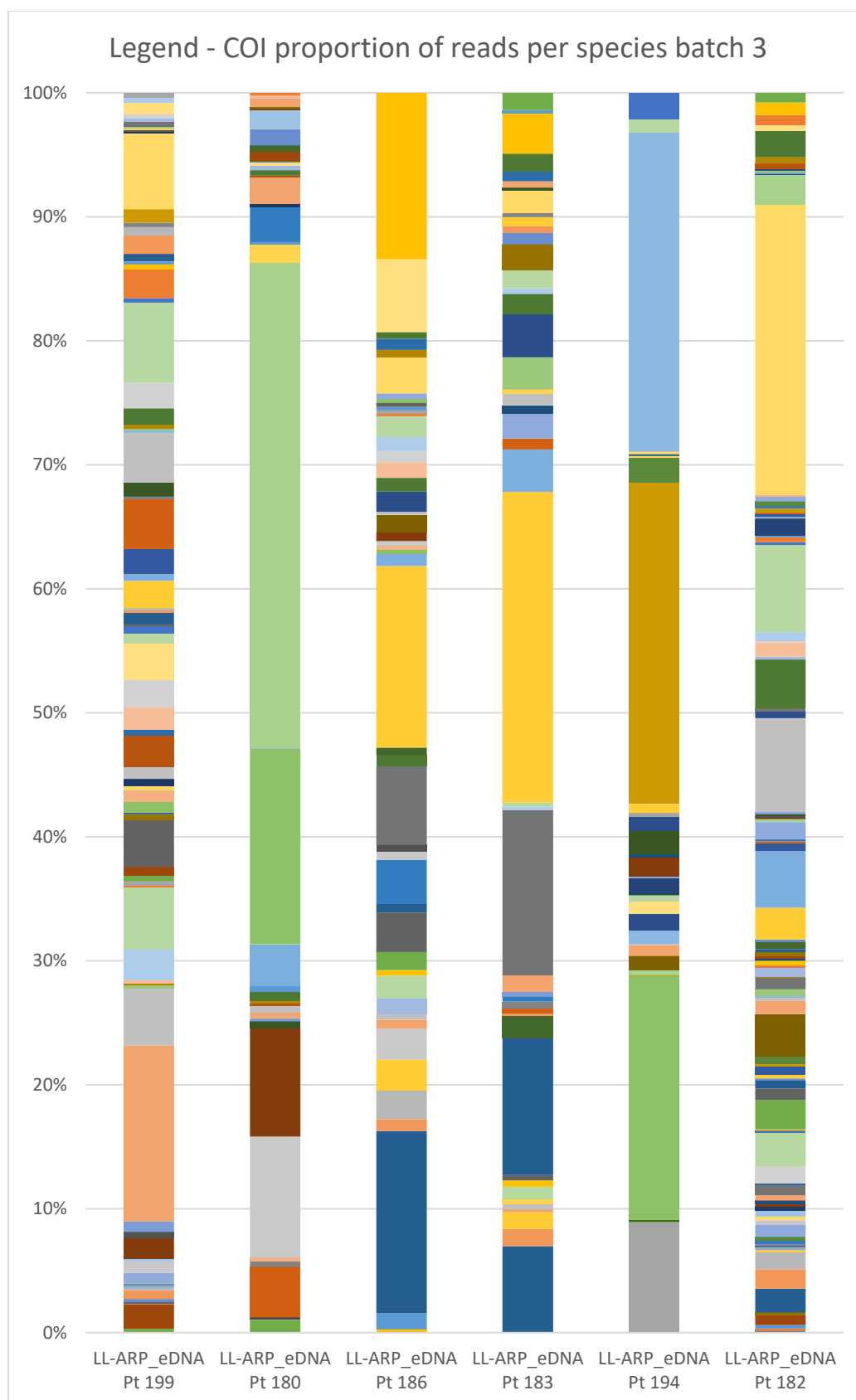
**Figure 4 COI proportion of reads per species batch 1**



**Figure 5 COI proportion of reads per species batch 2**



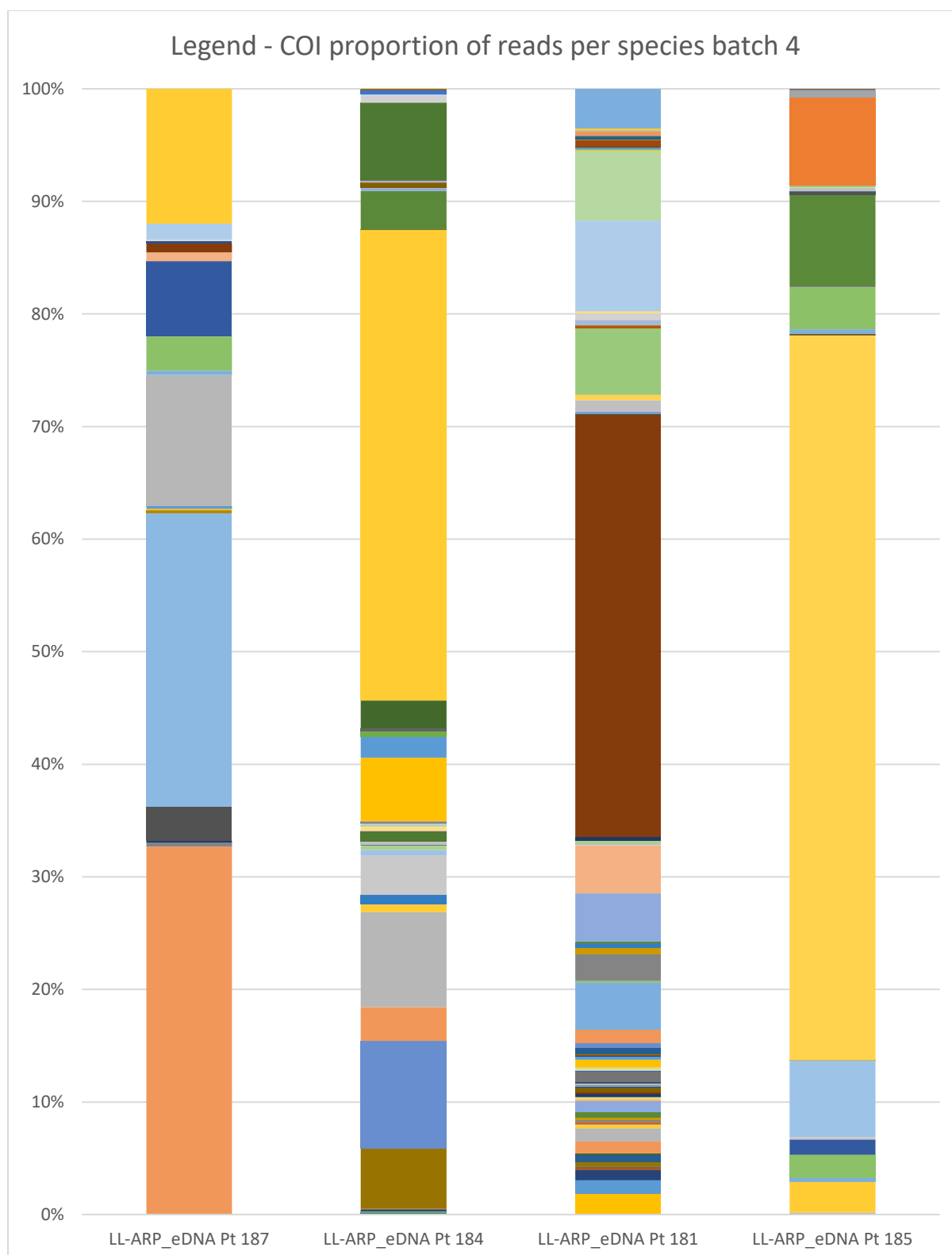
**Figure 6 COI proportion of reads per species batch 3**



## Legend - COI proportion of reads per species batch 3

|                                 |                                     |                                |
|---------------------------------|-------------------------------------|--------------------------------|
| ■ Acalles misellus              | ■ Acanthamoeba hatchetti            | ■ Acrochaetium sp.             |
| ■ Acrotona fungi                | ■ Acrotrichis sp.                   | ■ Adalia decempunctata         |
| ■ Agonum thoreyi                | ■ Agrylea multipunctata             | ■ Albugo candida               |
| ■ Allacma fusca                 | ■ Allolobophora chlorotica          | ■ Amoebozoa sp.                |
| ■ Anania hortulata              | ■ Aporetodea caliginosa             | ■ Aporetodea longa             |
| ■ Aporetodea rosea              | ■ Apsectrotanypus trifascipennis    | ■ Arenicola marina             |
| ■ Arion sp.                     | ■ Arrenurus sp.                     | ■ Asellus aquaticus            |
| ■ Aurelia sp.                   | ■ Baetis rhodani                    | ■ Bimastos eiseni              |
| ■ Brilia bifida                 | ■ Cantharis cryptica                | ■ Canthocamptidae sp.          |
| ■ Cernosvitoviella minor        | ■ Cerobasis guestfalica             | ■ Chaetoceros socialis         |
| ■ Chaetogaster diastrophus      | ■ Chironomidae sp.                  | ■ Chironomus pseudothummi      |
| ■ Chironomus salinarius         | ■ Choleva angustata                 | ■ Chydorus sphaericus          |
| ■ Cladosporium bruhnei          | ■ Cladosporium sp.                  | ■ Cladosporium tenuissimum     |
| ■ Cloeon dipterum               | ■ Clydonella sawyeri                | ■ Coniopteryx tineiformis      |
| ■ Corophium volutator           | ■ Corynoneura coronata              | ■ Corynoneura fittkaui         |
| ■ Cricotopus sp.                | ■ Culex pipiens                     | ■ Cyclops strenuus             |
| ■ Cyclops strenuus              | ■ Cyphoderia ampulla                | ■ Cyphon coarctatus            |
| ■ Delphacinae sp.               | ■ Dendrobaena octaedra              | ■ Deroceras reticulatum        |
| ■ Deroceras sp.                 | ■ Dicranota bimaculata              | ■ Dicyphus epilobii            |
| ■ Dicyrtoma fusca               | ■ Diptera sp.                       | ■ Drosophila suzukii           |
| ■ Ectocarpus subulatus          | ■ Ectopsocus briggsi                | ■ Eiseniella tetraedra         |
| ■ Elasmucha grisea              | ■ Enchytraeidae sp.                 | ■ Enchytraeus bulbosus         |
| ■ Entomobrya intermedia         | ■ Entomobrya nicoleti               | ■ Epistrophe eligans           |
| ■ Eristalis tenax               | ■ Euchlanis dilatata                | ■ Eucyclops serrulatus         |
| ■ Eupteryx calcarata            | ■ Euzetes globulus                  | ■ Fagocyba douglasi            |
| ■ Folsomia quadrioculata        | ■ Fridericia galba                  | ■ Fridericia perrieri          |
| ■ Fusarium diversisporum        | ■ Gammarus pulex                    | ■ Globulidrilus riparius       |
| ■ Glyptobothrus sp.             | ■ Glyptotendipes cauliginellus      | ■ Glyptotendipes sp.           |
| ■ Gonocerus acuteangulatus      | ■ Gonothyræa loveni                 | ■ Graphopsocus cruciatus       |
| ■ Haplophthalmus danicus        | ■ Harpacticoida sp.                 | ■ Hediste diversicolor         |
| ■ Helophilus pendulus           | ■ Henlea perpusilla                 | ■ Humeroibatidae sp.           |
| ■ Hydrozetidae sp.              | ■ Hypogastrura sp.                  | ■ Hypogastrura viatica         |
| ■ Idotea chelipes               | ■ Invertebrate environmental sample | ■ Isotomurus fucicola          |
| ■ Isotomurus unifasciatus       | ■ Jaminæa sp.                       | ■ Javesella dubia              |
| ■ Kleidocerys ericae            | ■ Korotnevelia sp.                  | ■ Korotnevelia stella          |
| ■ Lecane closterocerca          | ■ Lehmannia marginata               | ■ Limnodrilus hoffmeisteri     |
| ■ Limnophyes pentaplastus       | ■ Limonia macrostigma               | ■ Lineus viridis               |
| ■ Lithobius microps             | ■ Lonchoptera uniseta               | ■ Lucilia ampullacea           |
| ■ Lucilia illustris             | ■ Lumbricidae sp.                   | ■ Lumbricus castaneus          |
| ■ Lumbricus ryalis              | ■ Lumbricus rubellus                | ■ Lumbricus terrestris         |
| ■ Lype phaeopa                  | ■ Macropelopia sp.                  | ■ Maxillopoda sp.              |
| ■ Melampsora magnusiana         | ■ Melosira varians                  | ■ Metriocnemus albolineatus    |
| ■ Micropsectra contracta        | ■ Micropsectra notescens            | ■ Micropsectra pallidula       |
| ■ Minutocellus polymorphus      | ■ Mollusca environmental sample     | ■ Monocorophium insidiosum     |
| ■ Neanura muscorum              | ■ Neonectria hubeiensis             | ■ Nesovitreia sp.              |
| ■ Nitzschia longissima          | ■ Nothophytaphthora sp.             | ■ Octolasion tyrtaeum          |
| ■ Odacantha melanura            | ■ Orchesella villosa                | ■ Ormosia hederæ               |
| ■ Oscinella sp.                 | ■ Oxydopa elongatula                | ■ Pantilius tunicatus          |
| ■ Paracyclops fimbriatus        | ■ Paradromius linearis              | ■ Paralemanea annulata         |
| ■ Paranthomyza nitida           | ■ Paraphaenocladus impensus         | ■ Paratanytarsus lauterborni   |
| ■ Parisotoma notabilis          | ■ Pedospumella encystans            | ■ Pedospumella sp.             |
| ■ Phaenopsectra flavipes        | ■ Phaeocystis globosa               | ■ Philoscia muscorum           |
| ■ Physa acuta                   | ■ Phytophthora cryptogea            | ■ Phytophthora gallica         |
| ■ Phytophthora infestans        | ■ Phytophthora inundata             | ■ Phytophthora lacustris       |
| ■ Phytophthora plurivora        | ■ Phytophthora sp.                  | ■ Phytophthora syringae        |
| ■ Phytophthora taxon            | ■ Phytophthora thermophila          | ■ Planorbis planorbis          |
| ■ Pleuropteryx ruralis          | ■ Plumatella fungosa                | ■ Pogonognathellus longicornis |
| ■ Polyarthra sp.                | ■ Populicerus confusus              | ■ Potamothenix bavaricus       |
| ■ Pristina aequisetæ            | ■ Proasellus meridianus             | ■ Prodiamesa olivacea          |
| ■ Psallus falleni               | ■ Psammotettix bavaricus            | ■ Psammotettix sp.             |
| ■ Pseudolyciella pallidiventris | ■ Pseudosinella alba                | ■ Psocoptera sp.               |
| ■ Ptychoptera albimana          | ■ Ptychoptera lacustris             | ■ Pythium cystogenes           |
| ■ Pythium oligandrum            | ■ Pythium periplocum                | ■ Pythium phragmitis           |
| ■ Pythium sp.                   | ■ Pythium torulosum                 | ■ Rheotanytarsus curtistylus   |
| ■ Rhyacodrilus coccineus        | ■ Rotaria rotatoria                 | ■ Satchelliella trivialis      |
| ■ Simocephalus sp.              | ■ Sminthurus viridis                | ■ Stigmatogaster subterranea   |
| ■ Strigamia acuminata           | ■ Stylodrilus heringianus           | ■ Sus scrofa domesticus        |
| ■ Sympetrum sanguineum          | ■ Synchaeta cecilia                 | ■ Tachypodius niger            |
| ■ Tipula lateralis              | ■ Tipula maxima                     | ■ Tipula paludosa              |
| ■ Tomocerus minor               | ■ Tomocerus vulgaris                | ■ Trichoniscus pusillus        |
| ■ Trichoptera sp.               | ■ Trioza urticae                    | ■ Trochulus hispidus           |
| ■ Tubifex tubifex               | ■ Tubificinae sp.                   | ■ Tubificoides benedii         |
| ■ Tubificoides pseudogaster     | ■ Tydeidae sp.                      | ■ Unclutred fungus             |
| ■ Willowsia nigromaculata       | ■ Xantholinus longiventris          | ■ Xenylla sp.                  |

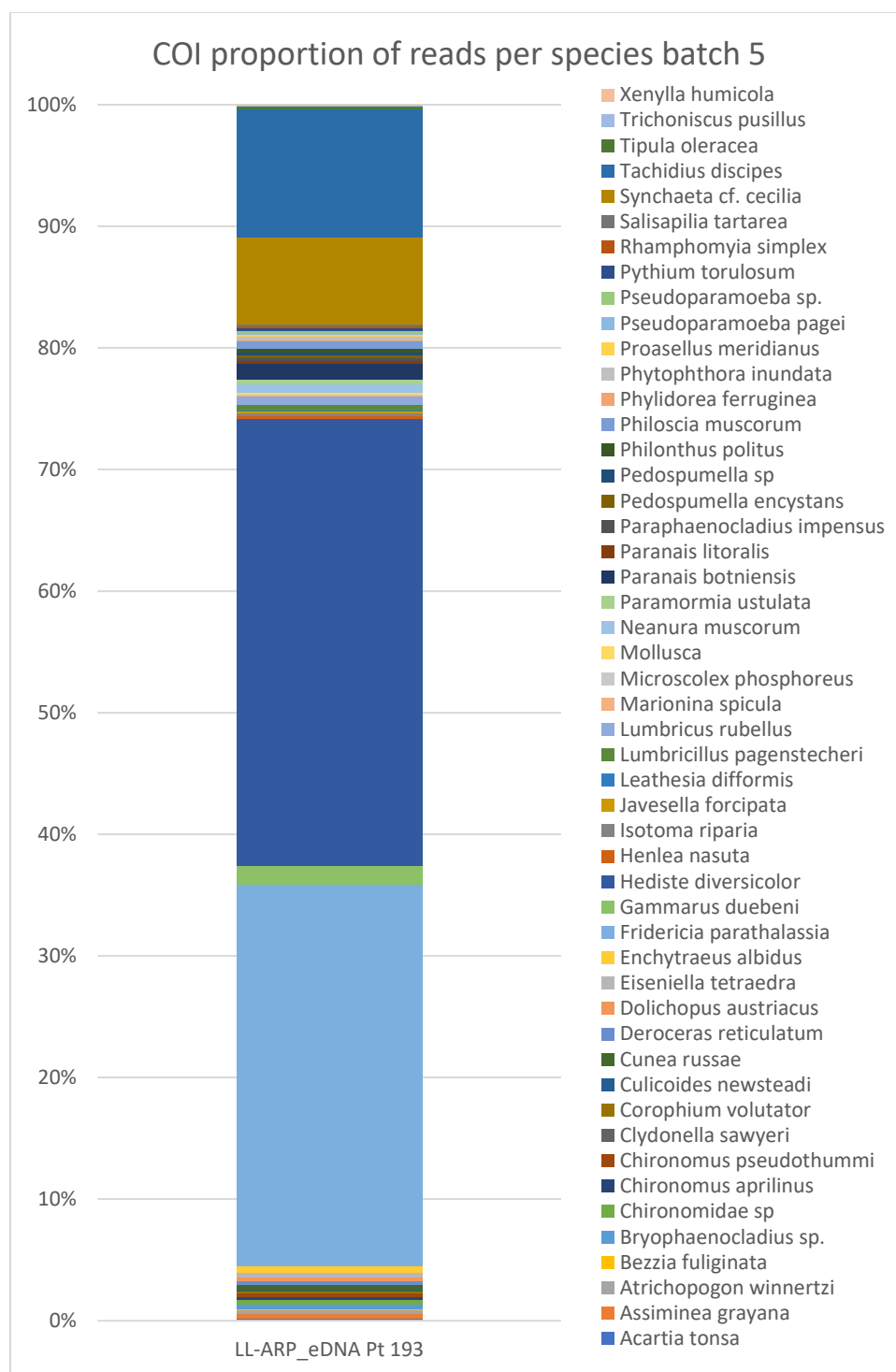
**Figure 7 COI proportion of reads per species batch 4**



## Legend - COI proportion of reads per species batch 4

|                                     |                                 |                                   |
|-------------------------------------|---------------------------------|-----------------------------------|
| ■ Acanthocyclops robustus           | ■ Achlya ambisexualis           | ■ Agabus bipustulatus             |
| ■ Agrylea multipunctata             | ■ Allolobophora chlorotica      | ■ Anopheles claviger              |
| ■ Aporectodea longa                 | ■ Aporectodea rosea             | ■ Arthropoda environmental sample |
| ■ Asellus aquaticus                 | ■ Baetis vernus                 | ■ Bradysia trivittata             |
| ■ Candonopsis kingsleii             | ■ Canthocamptidae sp.           | ■ Chaetogaster diastrophus        |
| ■ Chironomidae sp                   | ■ Chironomus luridus            | ■ Chironomus nr. piger            |
| ■ Chironomus pseudothummi           | ■ Cladosporium bruhnei          | ■ Cladosporium sp                 |
| ■ Cladosporium tenuissimum          | ■ Conosanus obsoletus           | ■ Corynoneura coronata            |
| ■ Corynoneura fittkaui              | ■ Corynoneura lobata v          | ■ Corynoneura scutellata          |
| ■ Culiseta annulata                 | ■ Cyclopidae sp                 | ■ Delphacinae sp.                 |
| ■ Deroceras reticulatum             | ■ Deroceras sp.                 | ■ Diplocladius cultriger          |
| ■ Drosophila suzukii                | ■ Ectopsocus briggsi            | ■ Eiseniella tetraedra            |
| ■ Elasmucha grisea                  | ■ Enchytraeidae sp.             | ■ Erythroneurini sp.              |
| ■ Eucyclops serrulatus              | ■ Eucypris viren                | ■ Eunotia sp.                     |
| ■ Gammarus pulex                    | ■ Gomphonema parvulum           | ■ Graphopsocus cruciatus          |
| ■ Hebecnema vespertina              | ■ Humeroatidae sp.              | ■ Hydra circumcincta              |
| ■ Hydra viridissima                 | ■ Hydrozetidae sp.              | ■ Ilybius ater                    |
| ■ Invertebrate environmental sample | ■ Isotomurus fucicola           | ■ Jaminacea sp.                   |
| ■ Javesella dubia                   | ■ Korotnevella heteracantha     | ■ Korotnevella leshevi            |
| ■ Korotnevella sp                   | ■ Korotnevella stella           | ■ Krenopelopia sp.                |
| ■ Leistus spinibarbis               | ■ Leptolegnia sp.               | ■ Limnodrilus hoffmeisteri        |
| ■ Lucilia caesar                    | ■ Lucilia illustris             | ■ Lumbriculus variegatus          |
| ■ Lumbricus rubellus                | ■ Lumbricus terrestris          | ■ Maso sundevalli                 |
| ■ Maxillopoda sp.                   | ■ Melampsora magnusiana         | ■ Melosira varians                |
| ■ Mesocyclops leuckarti             | ■ Metriocnemus eurynotus        | ■ Micropsectra contracta          |
| ■ Micropsectra notescens            | ■ Mollusca environmental sample | ■ Nais sp.                        |
| ■ Natarsia punctata                 | ■ Neanura muscorum              | ■ Neonectria hubeiensis           |
| ■ Nitzschia cf. fonticola           | ■ Nothophytophthora intricata   | ■ Octolasion tyrtaeum             |
| ■ Pantilius tunicatus               | ■ Paralemanea annulata          | ■ Paratanytarsus austriacus       |
| ■ Paratanytarsus lauterborni        | ■ Pedospumella encystans        | ■ Pentatoma rufipes               |
| ■ Phaenopsectra flavipes            | ■ Physa fontinalis              | ■ Phytophthora bilobang           |
| ■ Phytophthora gallica              | ■ Phytophthora inundata         | ■ Phytophthora lacustris          |
| ■ Phytophthora thermophila          | ■ Pipunculus cf. omissinervis   | ■ Platynothrus peltifer           |
| ■ Podura aquatica                   | ■ Potamothenix bavaricus        | ■ Pristina aequiseti              |
| ■ Proasellus meridianus             | ■ Pythium adhaerens             | ■ Pythium aff. pachycaule         |
| ■ Pythium cystogenes                | ■ Pythium sp.                   | ■ Rheotanytarsus curtistylus      |
| ■ Rhyacodrilus coccineus            | ■ Rotaria rotatoria             | ■ Rotaria sp                      |
| ■ Salpingus planirostris            | ■ Saprolegnia ferax             | ■ Sheathia arcuata                |
| ■ Sminthurus viridis                | ■ Smittia sp                    | ■ Stylodrilus heringianus         |
| ■ Succineidae sp.                   | ■ Tachypodoiulus niger          | ■ Tanytarsus heusdensis           |
| ■ Tipula paludosa                   | ■ Tubifex tubifex               | ■ Typhlocybae sp.                 |
| ■ Valenzuela flavidus               | ■ Willowsia nigromaculata       |                                   |

**Figure 8 COI proportion of reads per species batch 5**



**Table 4 Number of samples containing species (invertebrates) using COI primers.**

| Species                                | Common name/type               | Phylum         | Number of samples |
|----------------------------------------|--------------------------------|----------------|-------------------|
| <i>Acalles misellus</i>                | Weevil                         | Arthropoda     | 1                 |
| <i>Acanthamoeba hatchetti</i>          | Amoebae                        | Amoebozoa      | 1                 |
| <i>Acanthocyclops robustus</i>         | Copepod                        | Arthropoda     | 3                 |
| <i>Acartia tonsa</i>                   | Copepod                        | Arthropoda     | 3                 |
| <i>Achlya ambisexualis</i>             | Water mould                    | Gyrista        | 1                 |
| <i>Acrochaetium sp.</i>                | Seaweed                        | Algae          | 1                 |
| <i>Acrotona fungi</i>                  | Beetle                         | Arthropoda     | 1                 |
| <i>Acrotrichis sp.</i>                 | Beetle                         | Arthropoda     | 2                 |
| <i>Adalia decempunctata</i>            | Ten-spotted ladybird           | Arthropoda     | 3                 |
| <i>Agabus bipustulatus</i>             | Diving beetle                  | Arthropoda     | 1                 |
| <i>Agabus sturmii</i>                  | Diving beetle                  | Arthropoda     | 2                 |
| <i>Agonum thoreyi</i>                  | Black ground beetle            | Arthropoda     | 1                 |
| <i>Agraylea multipunctata</i>          | Salt and pepper microcaddisfly | Arthropoda     | 5                 |
| <i>Albugo candida</i>                  | White rust                     | Gyrista        | 1                 |
| <i>Allacma fusca</i>                   | Springtail                     | Arthropoda     | 1                 |
| <i>Allolobophora chlorotica</i>        | Earthworm                      | Annelida       | 4                 |
| <i>Alonella excisa</i>                 | Water flea                     | Arthropoda     | 1                 |
| <i>Alphaproteobacteria</i>             | Bacteria                       | Pseudomonadota | 1                 |
| <i>Amoebozoa sp.</i>                   | Amoebae                        | Amoebozoa      | 1                 |
| <i>Anania hortulata</i>                | Small magpie                   | Arthropoda     | 1                 |
| <i>Anopheles claviger</i>              | Mosquito                       | Arthropoda     | 1                 |
| <i>Aporrectodea caliginosa</i>         | Earthworm                      | Annelida       | 4                 |
| <i>Aporrectodea longa</i>              | Black-headed worm              | Annelida       | 5                 |
| <i>Aporrectodea rosea</i>              | rosy tipped worm               | Annelida       | 5                 |
| <i>Apsectrotanypus trifascipennis</i>  | Midge                          | Arthropoda     | 2                 |
| <i>Arenicola marina</i>                | Sandworm                       | Annelida       | 3                 |
| <i>Arion sp.</i>                       | Roundback slug                 | Mollusca       | 1                 |
| <i>Arrenurus sp.</i>                   | Arachnid                       | Arthropoda     | 1                 |
| <i>Arthropoda environmental sample</i> | N/A                            | Arthropoda     | 2                 |
| <i>Asellus aquaticus</i>               | 2 spotted water hog-louse      | Arthropoda     | 7                 |
| <i>Assiminea grayana</i>               | Dun sentinel                   | Mollusca       | 3                 |
| <i>Athripsodes cinereus</i>            | Caddisfly                      | Arthropoda     | 1                 |
| <i>Atrichopogon hirtidorsum</i>        | Biting midge                   | Arthropoda     | 1                 |
| <i>Atrichopogon winnertzi</i>          | Biting midge                   | Arthropoda     | 1                 |
| <i>Aurelia sp.</i>                     | Moon jelly                     | Cnidaria       | 1                 |
| <i>Baetis rhodani</i>                  | Large dark olive mayfly        | Arthropoda     | 2                 |
| <i>Baetis vernus</i>                   | Blue-winged olive              | Arthropoda     | 3                 |
| <i>Baltidrilus costatus</i>            | Worm                           | Annelida       | 2                 |
| <i>Bezzia fuliginata</i>               | Biting midge                   | Arthropoda     | 1                 |

| Species                               | Common name/type    | Phylum      | Number of samples |
|---------------------------------------|---------------------|-------------|-------------------|
| <i>Bimastos eiseni</i>                | Lumbricid worm      | Annelida    | 1                 |
| <i>Bithynia tentaculata</i>           | Common faucet snail | Mollusca    | 1                 |
| <i>Bradysia trivittata</i>            | Fungus gnat         | Arthropoda  | 1                 |
| <i>Brillia bifida</i>                 | Non-biting midge    | Arthropoda  | 2                 |
| <i>Bruchus rufimanus</i>              | Broadbean weevil    | Arthropoda  | 1                 |
| <i>Bryophaenocladus sp.</i>           | Non-biting midge    | Arthropoda  | 1                 |
| <i>Campsicnemus picticornis</i>       | Fly                 | Arthropoda  | 1                 |
| <i>Candona candida</i>                | Ostracod            | Arthropoda  | 1                 |
| <i>Candonopsis kingsleii</i>          | Ostracod            | Arthropoda  | 2                 |
| <i>Cantharis cryptica</i>             | Soldier beetle      | Arthropoda  | 1                 |
| <i>Canthocamptidae sp.</i>            | Copepod             | Arthropoda  | 8                 |
| <i>Carcinus maenas</i>                | European green crab | Arthropoda  | 2                 |
| <i>Cernosvitoviella sp.</i>           | Worm                | Annelida    | 1                 |
| <i>Cerobasis guestfalica</i>          | Psocoptera          | Arthropoda  | 2                 |
| <i>Chaetoceros socialis</i>           | Diatom              | Gyrsta      | 2                 |
| <i>Chaetogaster diastrophus</i>       | Detritus worm       | Annelida    | 9                 |
| <i>Chironomidae sp.</i>               | Non-biting midge    | Arthropoda  | 12                |
| <i>Chironomus annularius</i>          | Non-biting midge    | Arthropoda  | 1                 |
| <i>Chironomus aprilinus</i>           | Non biting midge    | Arthropoda  | 1                 |
| <i>Chironomus luridus</i>             | Non biting midge    | Arthropoda  | 1                 |
| <i>Chironomus nr. piger</i>           | Non biting midge    | Arthropoda  | 1                 |
| <i>Chironomus pseudothummi</i>        | Non biting midge    | Arthropoda  | 5                 |
| <i>Chironomus salinarius</i>          | Non-biting midge    | Arthropoda  | 2                 |
| <i>Choleva angustata</i>              | Beetle              | Arthropoda  | 1                 |
| <i>Chydorus brevilabris</i>           | Water flea          | Arthropoda  | 1                 |
| <i>Chydorus sphaericus</i>            | Water flea          | Arthropoda  | 3                 |
| <i>Cladosporium bruhnei</i>           | Mould               | Gyrsta      | 5                 |
| <i>Cladosporium sp.</i>               | Mould               | Gyrsta      | 10                |
| <i>Cladosporium tenuissimum</i>       | Pigmented mould     | Gyrsta      | 8                 |
| <i>Cloeon dipterum</i>                | Mayfly              | Arthropoda  | 2                 |
| <i>Clydonella sawyeri</i>             | Amoeba              | Amoebazoa   | 2                 |
| <i>Coniopteryx tineiformis</i>        | Waxfly              | Arthropoda  | 1                 |
| <i>Conosanus obsoletus</i>            | Leafhopper          | Arthropoda  | 1                 |
| <i>Corophium volutator</i>            | Mud scud            | Arthropoda  | 4                 |
| <i>Corynoneura coronata</i>           | Non-biting midge    | Arthropoda  | 4                 |
| <i>Corynoneura fittkaui</i>           | Non biting midge    | Arthropoda  | 2                 |
| <i>Corynoneura lobata v</i>           | Non biting midge    | Arthropoda  | 1                 |
| <i>Corynoneura scutellata</i>         | Non-biting midge    | Arthropoda  | 3                 |
| <i>Corynoptera furcata</i>            | Fly                 | Arthropoda  | 1                 |
| <i>Cricotopus sp.</i>                 | Chironomidae        | Arthropoda  | 2                 |
| <i>Cryptochironomus albofasciatus</i> | Chironomid          | Arthropoda  | 1                 |
| <i>Cryptomonas curvata</i>            | Photolithotroph     | Cryptophyta | 1                 |

| Species                        | Common name/type            | Phylum     | Number of samples |
|--------------------------------|-----------------------------|------------|-------------------|
| <i>Culex pipiens</i>           | Southern house mosquito     | Arthropoda | 3                 |
| <i>Culicoides newsteadi</i>    | Biting midge                | Arthropoda | 1                 |
| <i>Culiseta annulata</i>       | Mosquito                    | Arthropoda | 3                 |
| <i>Cunea russae</i>            | Amoeba                      | Amoebozoa  | 2                 |
| <i>Cyclopidae sp.</i>          | Copepods                    | Arthropoda | 2                 |
| <i>Cyclops strenuus</i>        | Copepod                     | Arthropoda | 3                 |
| <i>Cyphoderia ampulla</i>      | Cyphoderia                  | Cercozoa   | 1                 |
| <i>Cyphon coarctatus</i>       | Beetle                      | Arthropoda | 1                 |
| <i>Cyphon laevipennis</i>      | Leaf beetle                 | Arthropoda | 1                 |
| <i>Delphacinae sp.</i>         | Plant hopper                | Arthropoda | 4                 |
| <i>Dendrobaena octaedra</i>    | Earthworm                   | Annelida   | 1                 |
| <i>Deroceras reticulatum</i>   | Garden slug                 | Mollusca   | 9                 |
| <i>Deroceras sp.</i>           | Slug                        | Mollusca   | 2                 |
| <i>Desoria trispinata</i>      | Collembola                  | Arthropoda | 1                 |
| <i>Dicranota bimaculata</i>    | Crane fly                   | Arthropoda | 1                 |
| <i>Dicyphus epilobii</i>       | Miridae                     | Arthropoda | 1                 |
| <i>Dicyrtoma fusca</i>         | Springtail                  | Arthropoda | 3                 |
| <i>Diplocladius cultriger</i>  | Chironomid-non biting midge | Arthropoda | 1                 |
| <i>Diptera sp.</i>             | Fly                         | Arthropoda | 1                 |
| <i>Dolichopus austriacus</i>   | Long legged fly             | Arthropoda | 1                 |
| <i>Drosophila suzukii</i>      | Spotted wing drosophila     | Arthropoda | 5                 |
| <i>Ectocarpus subulatus</i>    | Brown algae                 | Gyrsta     | 1                 |
| <i>Ectopsocus briggsi</i>      | Psocoptera                  | Arthropoda | 2                 |
| <i>Ectopsocus californicus</i> | Barklouse                   | Arthropoda | 1                 |
| <i>Eiseniella tetraedra</i>    | Red worm                    | Annelida   | 7                 |
| <i>Elasmucha grisea</i>        | Parent bug                  | Arthropoda | 2                 |
| <i>Enchytraeidae sp.</i>       | Pot worm                    | Annelida   | 5                 |
| <i>Enchytraeus albidus</i>     | White worm                  | Annelida   | 1                 |
| <i>Enchytraeus bulbosus</i>    | White worm                  | Annelida   | 1                 |
| <i>Enochrus testaceus</i>      | Beetle                      | Arthropoda | 1                 |
| <i>Entomobrya intermedia</i>   | Springtail                  | Arthropoda | 1                 |
| <i>Entomobrya nicoleti</i>     | Springtail                  | Arthropoda | 1                 |
| <i>Epistrophe eligans</i>      | Hoverfly                    | Arthropoda | 1                 |
| <i>Episyrphus balteatus</i>    | Marmalade hoverfly          | Arthropoda | 1                 |
| <i>Eristalis tenax</i>         | Drone fly                   | Arthropoda | 1                 |
| <i>Erythroneurini sp.</i>      | Leaf hopper                 | Arthropoda | 1                 |
| <i>Eucheilota maculata</i>     | Hydrozoa                    | Cnidaria   | 1                 |
| <i>Euchlanis dilatata</i>      | Rotifer                     | Rotifera   | 1                 |
| <i>Eucyclops serrulatus</i>    | Copepod                     | Arthropoda | 8                 |
| <i>Eucypris virens</i>         | Ostracod                    | Arthropoda | 1                 |
| <i>Eunotia sp.</i>             | Diatom                      | Gyrsta     | 1                 |

| Species                                  | Common name/type              | Phylum       | Number of samples |
|------------------------------------------|-------------------------------|--------------|-------------------|
| <i>Eupteryx calcarata</i>                | Leafhopper                    | Arthropoda   | 1                 |
| <i>Euscelis sordida</i>                  | Leafhopper                    | Arthropoda   | 1                 |
| <i>Euzetes globulus</i>                  | Mite                          | Arthropoda   | 1                 |
| <i>Fagocyba douglasi</i>                 | Short-horned bug              | Arthropoda   | 1                 |
| <i>Floscularia melicerta</i>             | Rotifer                       | Rotifera     | 1                 |
| <i>Folsomia quadrioculata</i>            | Springtail                    | Arthropoda   | 1                 |
| <i>Forficula aff. auricularia</i>        | Common earwig                 | Arthropoda   | 1                 |
| <i>Fridericia galba</i>                  | Pot worm                      | Annelida     | 2                 |
| <i>Fridericia parathalassia</i>          | Pot worm                      | Annelida     | 1                 |
| <i>Fridericia perrieri</i>               | Pot worm                      | Annelida     | 1                 |
| <i>Fusarium diversisporum</i>            | Fungi                         | Gyrsta       | 1                 |
| <i>Fusarium oxysporum</i>                | Fungi                         | Ascomycota   | 1                 |
| <i>Gammarus duebeni</i>                  | Crustacean                    | Arthropoda   | 2                 |
| <i>Gammarus pulex</i>                    | Crustacean                    | Arthropoda   | 3                 |
| <i>Globulidrilus riparius</i>            | Segmented worm                | Annelida     | 2                 |
| <i>Glyptobothrus jacobsi</i>             | Grasshopper                   | Arthropoda   | 1                 |
| <i>Glyptotendipes cauliginellus</i>      | Non-biting midge              | Arthropoda   | 1                 |
| <i>Glyptotendipes sp.</i>                | Non-biting midge              | Arthropoda   | 1                 |
| <i>Gomphonema parvulum</i>               | Diatom                        | Gyrsta       | 1                 |
| <i>Gonocerus acuteangulatus</i>          | Box bug                       | Arthropoda   | 1                 |
| <i>Gonothyraea loveni</i>                | Cnidaria                      | Cnidaria     | 1                 |
| <i>Graphopsocus cruciatus</i>            | Barkfly                       | Annelida     | 2                 |
| <i>Haplophthalmus danicus</i>            | Spurred ridgeback isopod      | Arthropoda   | 1                 |
| <i>Harpacticoida sp.</i>                 | Copepod                       | Arthropoda   | 1                 |
| <i>Hebecnema vespertina</i>              | Fly                           | Arthropoda   | 1                 |
| <i>Hediste diversicolor</i>              | Ragworm                       | Annelida     | 4                 |
| <i>Helophilus pendulus</i>               | The footballer                | Arthropoda   | 1                 |
| <i>Henlea nasuta</i>                     | Worm                          | Annelida     | 1                 |
| <i>Henlea perpusilla</i>                 | Worm                          | Annelida     | 2                 |
| <i>Heterocapsa rotundata</i>             | Dinoflagellate                | Myzozoa      | 1                 |
| <i>Humerobatidae sp.</i>                 | Mite                          | Arthropoda   | 4                 |
| <i>Hydra circumcincta</i>                | Freshwater hydra              | Cnidaria     | 1                 |
| <i>Hydra viridissima</i>                 | Green hydra fresh-water polyp | Cnidaria     | 3                 |
| <i>Hydrophilus piceus</i>                | Great silver water beetle     | Arthropoda   | 1                 |
| <i>Hydrozetidae sp.</i>                  | Oribatids                     | Arthropoda   | 4                 |
| <i>Hypogastrura sp.</i>                  | Springtail                    | Arthropoda   | 1                 |
| <i>Hypogastrura viatica</i>              | Springtail                    | Arthropoda   | 1                 |
| <i>Idotea chelipes</i>                   | Myriapod                      | Arthropoda   | 1                 |
| <i>Ilybius ater</i>                      | Water beetle                  | Arthropoda   | 1                 |
| <i>Invertebrate environmental sample</i> | N/A                           | Invertebrate | 7                 |

| Species                           | Common name/type       | Phylum        | Number of samples |
|-----------------------------------|------------------------|---------------|-------------------|
| <i>Isotoma riparia</i>            | Springtail             | Arthropoda    | 1                 |
| <i>Isotoma viridis</i>            | Springtail             | Arthropoda    | 1                 |
| <i>Isotomurus fucicola</i>        | Springtail             | Arthropoda    | 4                 |
| <i>Isotomurus unifasciatus</i>    | Springtail             | Arthropoda    | 1                 |
| <i>Jaminalia sp.</i>              | Yeast                  | Basidiomycota | 3                 |
| <i>Javesella dubia</i>            | Plant Hopper           | Arthropoda    | 2                 |
| <i>Javesella forcipata</i>        | plant hopper           | Arthropoda    | 1                 |
| <i>Kleidocerys ericae</i>         | Birch catkin bug       | Arthropoda    | 1                 |
| <i>Korotnevella heteracantha</i>  | Amoeba                 | Amoebozoa     | 3                 |
| <i>Korotnevella leshevi</i>       | Amoeba                 | Amoebozoa     | 1                 |
| <i>Korotnevella sp.</i>           | Amoeba                 | Amoebozoa     | 6                 |
| <i>Korotnevella stella</i>        | Amoeba                 | Amoebozoa     | 6                 |
| <i>Krenopelopia sp.</i>           | Non-biting midge       | Arthropoda    | 1                 |
| <i>Leathesia difformis</i>        | Sea cauliflower        | Gyrista       | 1                 |
| <i>Lecane closterocerca</i>       | Rotifer                | Rotifera      | 3                 |
| <i>Lehmanna marginata</i>         | Tree slug              | Mollusca      | 1                 |
| <i>Leistus spinibarbis</i>        | Ground beetle          | Arthropoda    | 1                 |
| <i>Lekanesphaera sp.</i>          | Sea slater             | Arthropoda    | 1                 |
| <i>Leptolegnia sp.</i>            | Oomycete               | Gyrista       | 1                 |
| <i>Limapontia depressa</i>        | Sea slug               | Mollusca      | 1                 |
| <i>Limnodrilus hoffmeisteri</i>   | Red worm               | Annelida      | 3                 |
| <i>Limnophyes pentaplastus</i>    | Non-biting midge       | Arthropoda    | 1                 |
| <i>Limonia macrostigma</i>        | Crane fly              | Arthropoda    | 1                 |
| <i>Lineus viridis</i>             | Ribbon worm            | Annelida      | 1                 |
| <i>Lithobius microps</i>          | Stone centipede        | Arthropoda    | 1                 |
| <i>Lonchoptera uniseta</i>        | Spear-winged fly       | Arthropoda    | 1                 |
| <i>Lucilia ampullacea</i>         | Streakless greenbottle | Arthropoda    | 1                 |
| <i>Lucilia caesar</i>             | blow fly               | Arthropoda    | 1                 |
| <i>Lucilia illustris</i>          | Green bottle fly       | Arthropoda    | 2                 |
| <i>Lumbricidae sp.</i>            | Earthworm              | Annelida      | 1                 |
| <i>Lumbricillus pagenstecheri</i> | Worm                   | Annelida      | 1                 |
| <i>Lumbriculus variegatus</i>     | Black worm             | Annelida      | 2                 |
| <i>Lumbricus castaneus</i>        | Chestnut worm          | Annelida      | 1                 |
| <i>Lumbricus ryalis</i>           | Earthworm              | Annelida      | 1                 |
| <i>Lumbricus rubellus</i>         | Red earthworm          | Annelida      | 4                 |
| <i>Lumbricus terrestris</i>       | Common earthworm       | Annelida      | 3                 |
| <i>Lype phaeopa</i>               | Caddisfly              | Arthropoda    | 1                 |
| <i>Macropelopia sp.</i>           | Non-biting midge       | Arthropoda    | 1                 |
| <i>Macropsis fuscata</i>          | Rubus leafhopper       | Arthropoda    | 1                 |
| <i>Marionina spicula</i>          | Pot worm               | Annelida      | 2                 |
| <i>Maso sundevalli</i>            | Dwarf spider           | Arthropod     | 1                 |
| <i>Maxillopoda sp.</i>            | Crustacean             | Arthropoda    | 4                 |
| <i>Melampsora magnusiana</i>      | Rust fungi             | Basidiomycota | 12                |

| Species                              | Common name/type | Phylum           | Number of samples |
|--------------------------------------|------------------|------------------|-------------------|
| <i>Melosira varians</i>              | Diatom           | Gyrsta           | 9                 |
| <i>Mesocyclops leuckarti</i>         | Copepod          | Arthropoda       | 2                 |
| <i>Metriocnemus albolineatus</i>     | Chironomid       | Arthropoda       | 1                 |
| <i>Metriocnemus eurynotus</i>        | Non-biting midge | Arthropoda       | 1                 |
| <i>Micropsectra contracta</i>        | Non-biting midge | Arthropoda       | 5                 |
| <i>Micropsectra notescens</i>        | Chironomidae     | Arthropoda       | 4                 |
| <i>Micropsectra pallidula</i>        | Chironomidae     | Arthropoda       | 1                 |
| <i>Microscolex phosphoreus</i>       | Earthworm        | Annelida         | 1                 |
| <i>Minutocellus polymorphus</i>      | Diatom           | Heterokontophyta | 3                 |
| <i>Mollusca environmental sample</i> | N/A              | Mollusca         | 4                 |
| <i>Monocorophium insidiosum</i>      | Crustacean       | Arthropoda       | 1                 |
| <i>Monopelopia tenuicalcar</i>       | Non-biting midge | Arthropoda       | 1                 |
| <i>Mytilina ventralis</i>            | Rotifer          | Rotifera         | 1                 |
| <i>Nais sp.</i>                      | Detritus worm    | Annelida         | 5                 |
| <i>Natarsia punctata</i>             | Non-biting midge | Arthropoda       | 3                 |
| <i>Neanura muscorum</i>              | Springtail       | Arthropoda       | 5                 |
| <i>Neonectria hubeiensis</i>         | Fungi            | Gyrsta           | 2                 |
| <i>Nesovitrea sp.</i>                | Snail            | Mollusca         | 1                 |
| <i>Nitzschia cf. fonticola</i>       | Diatom           | Gyrsta           | 1                 |
| <i>Nitzschia longissima</i>          | Braown algae     | Gyrsta           | 1                 |
| <i>Nothophytophthora intricata</i>   | Oomycete         | Gyrsta           | 1                 |
| <i>Nothophytophthora sp.</i>         | Oomycete         | Gyrsta           | 1                 |
| <i>Octolasion tyrtaeum</i>           | Earthworm        | Anneilda         | 2                 |
| <i>Odacantha melanura</i>            | Ground beetle    | Arthropoda       | 1                 |
| <i>Orchesella villosa</i>            | Springtail       | Arthropoda       | 2                 |
| <i>Ormosia hederæ</i>                | Hexapod          | Arthropoda       | 1                 |
| <i>Oscinella sp.</i>                 | Fly              | Arthropoda       | 1                 |
| <i>Oxypoda elongatula</i>            | Beetle           | Arthropoda       | 1                 |
| <i>Pantilius tunicatus</i>           | Beetle           | Arthropoda       | 3                 |
| <i>Paracyclops fimbriatus</i>        | Copepod          | Arthropoda       | 1                 |
| <i>Paradromius linearis</i>          | Ground beetle    | Arthropoda       | 1                 |
| <i>Paralemanea annulata</i>          | Red algae        | Rhodophyta       | 7                 |
| <i>Paramormia ustulata</i>           | Moth fly         | Arthropoda       | 1                 |
| <i>Paranais botniensis</i>           | Worm             | Annelida         | 1                 |
| <i>Paranais litoralis</i>            | Worm             | Annelida         | 1                 |
| <i>Paranthomyza nitida</i>           | Diptera          | Arthropoda       | 1                 |
| <i>Paraphaenocladus impensus</i>     | Non-biting midge | Arthropoda       | 2                 |
| <i>Paratanytarsus austriacus</i>     | Non-biting midge | Arthropoda       | 1                 |
| <i>Paratanytarsus grimmii</i>        | Non-biting midge | Arthropoda       | 1                 |
| <i>Paratanytarsus intricatus</i>     | Non-biting midge | Arthropoda       | 3                 |
| <i>Paratanytarsus lauterborni</i>    | Non-biting midge | Arthropoda       | 3                 |
| <i>Parisotoma notabilis</i>          | Springtail       | Arthropoda       | 3                 |

| Species                             | Common name/type          | Phylum     | Number of samples |
|-------------------------------------|---------------------------|------------|-------------------|
| <i>Pedospumella encystans</i>       | Golden algae              | Gyrsta     | 3                 |
| <i>Pedospumella sp.</i>             | Golden algae              | Gyrsta     | 2                 |
| <i>Pentatoma rufipes</i>            | Forest bug                | Arthropoda | 1                 |
| <i>Phaenopsectra flavipes</i>       | Non-biting midge          | Arthropoda | 4                 |
| <i>Phaenopsectra punctipes</i>      | Midge                     | Arthropoda | 1                 |
| <i>Phaeocystis globosa</i>          | Algae                     | Gyrsta     | 2                 |
| <i>Philonthus politus</i>           | Rove beetle               | Arthropoda | 1                 |
| <i>Philoscia muscorum</i>           | Common striped woodlouse  | Arthropoda | 7                 |
| <i>Phylidorea ferruginea</i>        | Cranefly                  | Arthropoda | 1                 |
| <i>Physa acuta</i>                  | Common bladder snail      | Mollusca   | 2                 |
| <i>Physa fontinalis</i>             | Common bladder snail      | Mollusca   | 2                 |
| <i>Phytophthora bilorbang</i>       | Oomycete                  | Gyrsta     | 2                 |
| <i>Phytophthora cryptogea</i>       | Oomycete                  | Gyrsta     | 2                 |
| <i>Phytophthora gallica</i>         | Oomycete                  | Gyrsta     | 5                 |
| <i>Phytophthora infestans</i>       | Potato late blight fungus | Oomycota   | 1                 |
| <i>Phytophthora inundata</i>        | Oomycete                  | Gyrsta     | 6                 |
| <i>Phytophthora lacustris</i>       | Oomycete                  | Gyrsta     | 9                 |
| <i>Phytophthora plurivora</i>       | Oomycete                  | Gyrsta     | 3                 |
| <i>Phytophthora sp.</i>             | Oomycete                  | Gyrsta     | 5                 |
| <i>Phytophthora syringae</i>        | Oomycete                  | Gyrsta     | 1                 |
| <i>Phytophthora taxon</i>           | Oomycete                  | Gyrsta     | 1                 |
| <i>Phytophthora thermophila</i>     | Oomycete                  | Gyrsta     | 4                 |
| <i>Pipunculus cf. omissinervis</i>  | Big headed fly            | Arthropod  | 1                 |
| <i>Planorbis planorbis</i>          | Margined Ram's horn snail | Mollusca   | 1                 |
| <i>Plateumaris sericea</i>          | Beetle                    | Arthropoda | 1                 |
| <i>Platynothrus peltifer</i>        | Mite                      | Arthropoda | 1                 |
| <i>Pleuroptya ruralis</i>           | Mother of pearl           | Arthropoda | 1                 |
| <i>Plumatella fungosa</i>           | Bryzoan                   | Bryozoa    | 1                 |
| <i>Podura aquatica</i>              | water springtail          | Arthropoda | 1                 |
| <i>Pogonognathellus longicornis</i> | Springtail                | Arthropoda | 2                 |
| <i>Polyarthra sp.</i>               | Crustacea                 | Arthropoda | 1                 |
| <i>Polydora cornuta</i>             | Worm                      | Annelida   | 1                 |
| <i>Populicerus confusus</i>         | Cicadellidae              | Arthropoda | 1                 |
| <i>Potamotheix bavaricus</i>        | Worm                      | Annelida   | 2                 |
| <i>Pristina aequiseta</i>           | Sludge worm               | Annelida   | 4                 |
| <i>Proasellus meridianus</i>        | One-spotted waterlouse    | Crustacean | 4                 |
| <i>Prodiamesa olivacea</i>          | Chironomidae              | Arthropoda | 1                 |
| <i>Psallus falleni</i>              | Plant bug                 | Arthropoda | 1                 |
| <i>Psammotettix bavaricus</i>       | True bug                  | Arthropoda | 1                 |
| <i>Psammotettix sp.</i>             | True bug                  | Arthropoda | 1                 |

| Species                              | Common name/type         | Phylum     | Number of samples |
|--------------------------------------|--------------------------|------------|-------------------|
| <i>Pseudochattonella verruculosa</i> | Heterokont flagellate    | Gyrista    | 1                 |
| <i>Pseudolyciella pallidiventris</i> | Fly                      | Arthropoda | 1                 |
| <i>Pseudoparamoeba pagei</i>         | Amoeba                   | Amoebozoa  | 1                 |
| <i>Pseudoparamoeba sp.</i>           | Amoeba                   | Amoebozoa  | 2                 |
| <i>Pseudosinella alba</i>            | Springtail               | Arthropoda | 1                 |
| <i>Psocoptera sp.</i>                | Barklice                 | Arthropoda | 2                 |
| <i>Ptychoptera albimana</i>          | Crane fly                | Arthropoda | 2                 |
| <i>Ptychoptera lacustris</i>         | Crane fly                | Arthropoda | 1                 |
| <i>Pythium adhaerens</i>             | Oomycete                 | Gyrista    | 1                 |
| <i>Pythium aff. pachycaule</i>       | Oomycete                 | Gyrista    | 3                 |
| <i>Pythium cystogenes</i>            | Oomycete                 | Gyrista    | 2                 |
| <i>Pythium oligandrum</i>            | Oomycete                 | Gyrista    | 2                 |
| <i>Pythium periplocum</i>            | Oomycete                 | Gyrista    | 1                 |
| <i>Pythium phragmitis</i>            | Oomycete                 | Gyrista    | 2                 |
| <i>Pythium sp.</i>                   | Oomycete                 | Gyrista    | 10                |
| <i>Pythium torulosum</i>             | Oomycete                 | Gyrista    | 3                 |
| <i>Rhamphomyia simplex</i>           | Dance fly                | Arthropoda | 1                 |
| <i>Rheotanytarsus curtistylus</i>    | Non-biting midge         | Arthropoda | 3                 |
| <i>Rhyacodrilus coccineus</i>        | Naidid worm              | Annelida   | 2                 |
| <i>Rotaria rotatoria</i>             | Rotifer                  | Rotifera   | 2                 |
| <i>Rotaria sp.</i>                   | Rotifer                  | Rotifera   | 1                 |
| <i>Salisapilia tartarea</i>          | Oomycete                 | Gyrista    | 1                 |
| <i>Salpingus planirostris</i>        | Beetle                   | Arthropod  | 1                 |
| <i>Saprolegnia ferax</i>             | Water mould              | Gyrista    | 2                 |
| <i>Satchelliella trivialis</i>       | Moth fly                 | Arthropoda | 1                 |
| <i>Sheathia arcuata</i>              | Freshwater algae         | Gyrista    | 1                 |
| <i>Simocephalus exspinosus</i>       | Water flea               | Arthropoda | 1                 |
| <i>Sminthurus viridis</i>            | Clover springtail        | Arthropoda | 2                 |
| <i>Smittia sp.</i>                   | Non-biting midge         | Arthropoda | 2                 |
| <i>Stempellina bausei</i>            | Non-biting midge         | Arthropoda | 1                 |
| <i>Stigmatogaster subterranea</i>    | Western yellow centipede | Arthropoda | 1                 |
| <i>Streblospio benedicti</i>         | Ram's horn worm          | Annelida   | 2                 |
| <i>Strigamia acuminata</i>           | Centipede                | Arthropoda | 1                 |
| <i>Stylaria lacustris</i>            | Detritus worm            | Annelida   | 1                 |
| <i>Stylodrilus heringianus</i>       | Worm                     | Annelida   | 3                 |
| <i>Succinea sp.</i>                  | Amber snail              | Mollusca   | 1                 |
| <i>Succineidae sp.</i>               | Land snail               | Mollusca   | 3                 |
| <i>Sus scrofa domesticus</i>         | Pig                      | Mammalia   | 2                 |
| <i>Sympetrum sanguineum</i>          | Ruddy Darer              | Arthropoda | 1                 |
| <i>Synchaeta cecilia</i>             | Rotifer                  | Rotifera   | 2                 |
| <i>Synchaeta pectinata</i>           | Rotifer                  | Rotifera   | 1                 |
| <i>Tachidius discipes</i>            | Copepod                  | Arthropoda | 1                 |

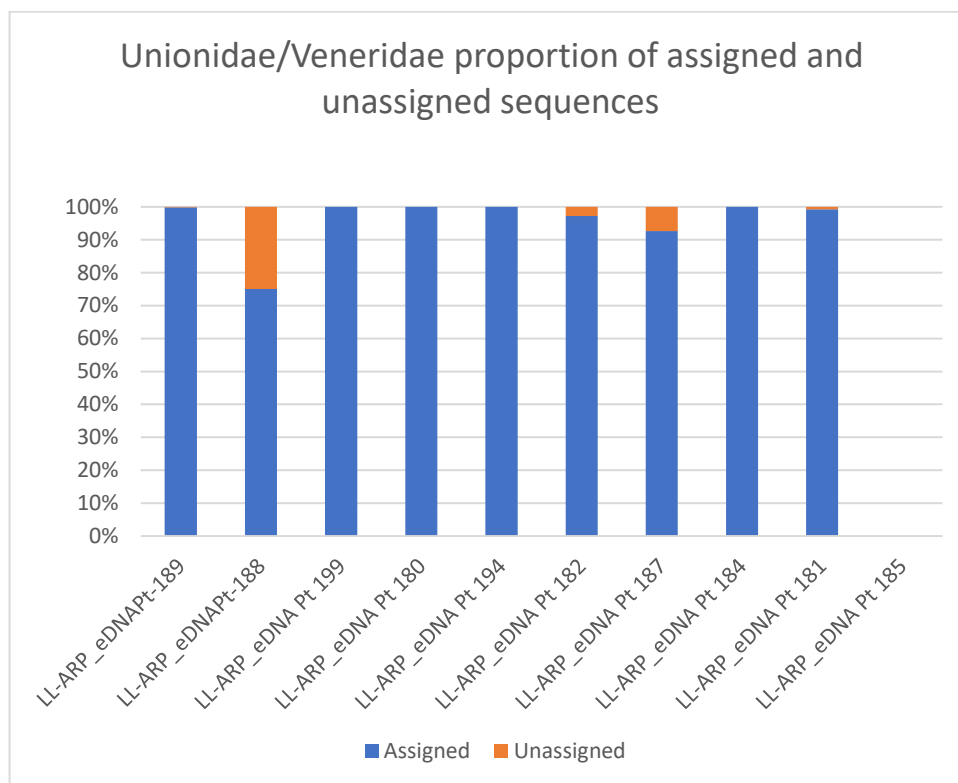
| Species                              | Common name/type             | Phylum         | Number of samples |
|--------------------------------------|------------------------------|----------------|-------------------|
| <i>Tachypodoiulus niger</i>          | White-legged snake millipede | Annelida       | 3                 |
| <i>Tanytarsus heusdensis</i>         | Non-biting midge             | Arthropoda     | 1                 |
| <i>Thalassiosira nordenskioeldii</i> | Diatom                       | Acillariophyra | 1                 |
| <i>Tipula lateralis</i>              | Cranefly                     | Arthropoda     | 1                 |
| <i>Tipula maxima</i>                 | Cranefly                     | Arthropoda     | 1                 |
| <i>Tipula oleracea</i>               | cranefly                     | Arthropoda     | 1                 |
| <i>Tipula paludosa</i>               | Cranefly                     | Arthropoda     | 2                 |
| <i>Tomocerus minor</i>               | Springtail                   | Arthropoda     | 2                 |
| <i>Tomocerus vulgaris</i>            | Springtail                   | Arthropoda     | 2                 |
| <i>Trichobranchidae sp.</i>          | Worm                         | Annelida       | 1                 |
| <i>Trichoniscus pusillus</i>         | Common pygmy woodlouse       | Arthropoda     | 4                 |
| <i>Trichoptera sp.</i>               | Caddisfly                    | Arthropoda     | 1                 |
| <i>Trioza urticae</i>                | Jumping plant louse          | Arthropoda     | 1                 |
| <i>Trochulus hispidus</i>            | Hairy snail                  | Mollusca       | 1                 |
| <i>Tubifex ignotus</i>               | Sludge worm                  | Annelida       | 1                 |
| <i>Tubifex tubifex</i>               | Sludge worm                  | Annelida       | 4                 |
| <i>Tubificinae sp.</i>               | Worm                         | Annelida       | 1                 |
| <i>Tubificoides benedii</i>          | Sludge worm                  | Annelida       | 2                 |
| <i>Tubificoides fraseri</i>          | Segmented worm               | Annelida       | 1                 |
| <i>Tubificoides pseudogaster</i>     | Segmented worm               | Annelida       | 2                 |
| <i>Tydeidae sp.</i>                  | Mite                         | Arthropoda     | 3                 |
| <i>Typhlocybiniae sp.</i>            | Leafhopper                   | Arthropoda     | 1                 |
| <i>Unclutured fungus</i>             | Fungi                        | Gyrsta         | 1                 |
| <i>Valenzuela flavidus</i>           | Barkfly                      | Arthropoda     | 2                 |
| <i>Willowsia nigromaculata</i>       | Springtail                   | Arthropoda     | 4                 |
| <i>Xantholinus longiventris</i>      | Rove beetle                  | Arthropoda     | 1                 |
| <i>Xenopelopia nigricans</i>         | Non-biting midge             | Arthropoda     | 1                 |
| <i>Xenylla humiolca</i>              | Springtail                   | Arthropoda     | 2                 |
| <i>Xenylla szeptyckii</i>            | Springtail                   | Arthropoda     | 2                 |
| <i>Zavrelia pentatoma</i>            | Non-biting midge             | Arthropoda     | 1                 |

### 3.2.3 Unionidae and Veneridae metabarcoding

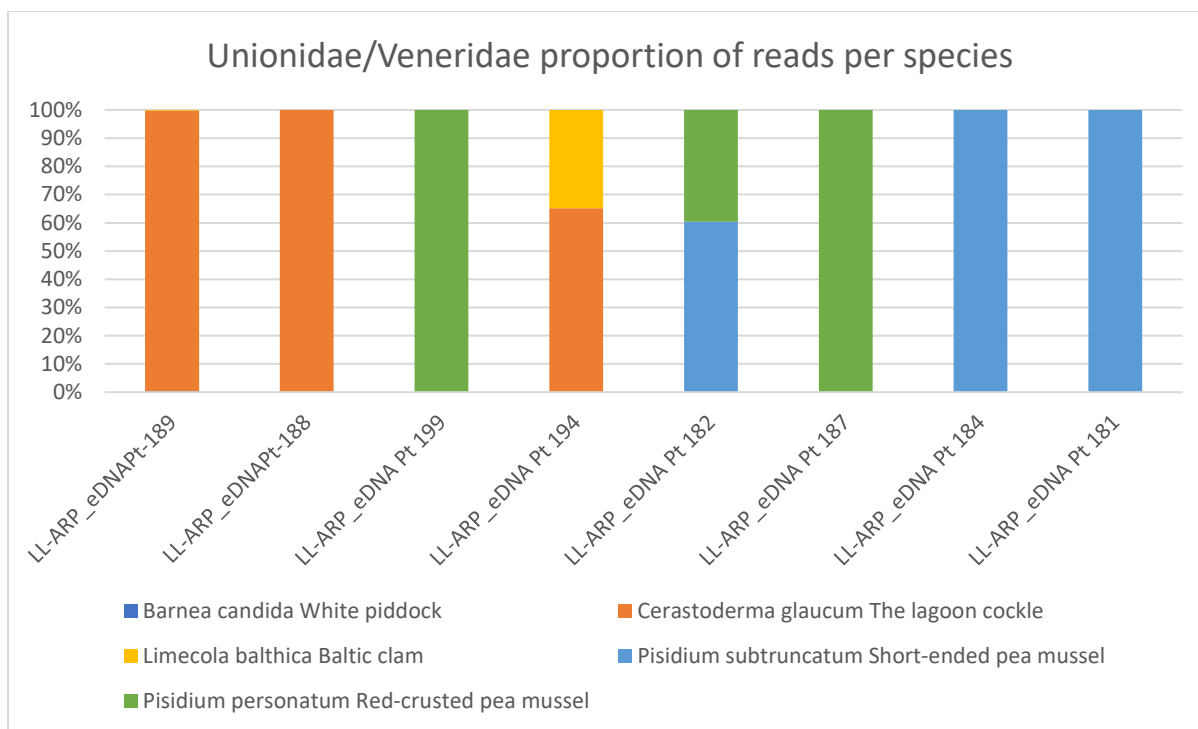
For the Unionidae and Veneridae analysis, of the sequences that passed all filters and went onto taxonomic assignment a total of 13k sequences (~4% of sequences) were assigned a taxonomic identification which represented five species including White piddock (*Barnea candida*), Lagoon cockle (*Cerastoderma glaucum*), and Baltic clam (*Limecola althica*). All three of these species are marine species which can be found in tidal estuaries, which fits with the sampling locations where they were found. A total of 329k sequences (~96% of sequences) were unassigned. The percentage of assigned and unassigned sequence reads per sample containing Unionidae/Veneridae are shown in Figure 9. Results per sample containing Unionidae/Veneridae are shown in Table 5, Figure 10 and Appendix 4. Samples contained between zero and four species of vertebrate with an average of one species per

sample. For the full list of Unionidae and Veneridae metabarcoding results, please see Appendix 4.

**Figure 9 Unionidae and Veneridae proportion of assigned and unassigned sequence reads.**



**Figure 10 Unionidae and Veneridae proportion of reads per species.**



**Table 5 Number of samples containing species (Unionidae or Veneridae) using Unionidae and Veneridae primers.**

| Species                      | Common name            | Number of samples |
|------------------------------|------------------------|-------------------|
| <i>Barnea candida</i>        | White piddock          | 1                 |
| <i>Cerastoderma glaucum</i>  | The lagoon cockle      | 3                 |
| <i>Limecola balthica</i>     | Baltic clam            | 2                 |
| <i>Pisidium subtruncatum</i> | Short-ended pea mussel | 2                 |
| <i>Pisidium personatum</i>   | Red-cruled pea mussel  | 2                 |

## 4 FACTORS FOR CONSIDERATION

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It is possible that species will have been missed during the metabarcoding process. This can happen for a number of reasons:

1. DNA from certain species may be misrepresented in the pool of eDNA. This can be either DNA from species that are much smaller in size than others within the sample pool, or DNA from species present in much smaller numbers than the dominant species. Therefore, the sequence read number does not necessarily correlate with species abundance.
2. DNA may have been inefficiently extracted from different species and/or there was differential degradation of the DNA. The DNA of some individuals may also have degraded more than that of others due to different rates of degradation. DNA is liable to degradation by factors such as nucleases, UV light, microbial action and the temperature and humidity of storage conditions all of which will affect DNA quality after sample collection.
3. The primers that are used in the initial PCR may have missed some species due to biases and/or the primers used may simply not work efficiently for some species. The success of metabarcoding is dependent upon the primer set chosen for use and its target loci and can be informed by in silico analysis. Ideally primers should target a hypervariable region (for high resolution taxonomic discrimination) and thus will determine the efficiency and accuracy of species detection and identification. DNA extracted from community samples may be subject to potential amplification bias where different species' DNA is in competition to bind to the universal primers. This can, therefore, prevent the capture of all species present in a given sample as more common template DNAs are likely to be amplified. Large numbers of human sequence reads in the samples are a potential example of primer bias/species masking.
4. There are 'gaps' in the current publicly available sequence databases i.e. not all species have been sequenced and it is possible that many of the unassigned reads will be from these species.

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## APPENDIX 1

### eDNA Survey Protocol

Kit contents: 2 sterile Whirl-Pak bags; 1 pair of sterile gloves; 1 sterile sampling ladle; a 50ml syringe, 2x 0.45  $\mu$ M filter and sealing caps; and a 10ml syringe containing preservative.

Roughly plan where you will collect the water sub-samples from. The aim is to spread the samples out evenly across the river/stream but at a minimum (for narrow streams) you should aim to collect sub-samples from three locations i.e. near bank, middle or river, and far bank. **Samples should be collected across a transect of the river/stream whilst moving upstream.** The samples should be taken from both open water and vegetated areas if present and if possible should avoid water that is less than 10 cm deep. Existing data shows that eDNA can be patchy depending on where the animals have been. Sampling in many areas considerably increases the chance of collecting their eDNA successfully.

**It is important not to collect sediment as this may cause inhibition of the PCR analysis which could lead to an inconclusive result** (please see examples of different sediment levels within sampling tubes at <http://www.adas.uk/Service/edna-analysis-for-great-crested-newt>).

### Sample Collection

- Open your kit and put on a pair of gloves.
- Open one of the sterile Whirl-Pak bag by tearing off the clear plastic strip along the perforated line, then pull the tabs.

Collect samples of water from across a transect of the river/stream (walking diagonally upstream whilst taking the samples, Figure 1.) using the sampling ladle and empty each sample into the Whirl-Pak bag (Figure 2A). You may need to take multiple scoops at each location to collect enough water e.g. if doing the minimum of three sub-sample locations from near bank, middle or river, and far bank you will need to take around 10 scoops in each location as you will be filtering up to 1L of water.

Repeat this process walking back across the river (again diagonally upstream) collecting the water into the second Whirl-Pak bag. This creates two independent water samples.

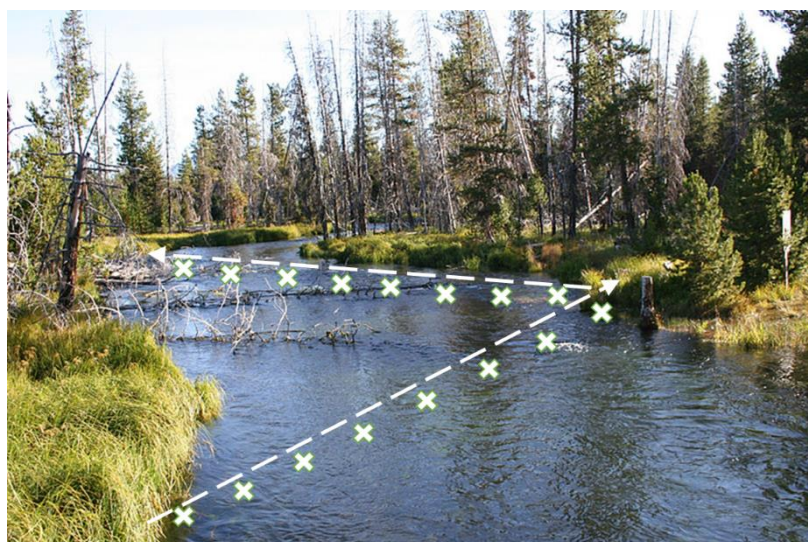


Figure 1. Left: arrows show direction of travel, X's mark potential sampling sites. Right: scoop full of water.

## Sample Preservation

1. When you have collected your samples, close the bags securely using the top tabs (fold over several times and bend tabs over) and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
2. Draw up water from the first bag into the 50 mL syringe (Figure 2B) and then attach the syringe to the filter unit using the luer lock (Figure 2C). Filter the water through the filter (Figure 2D). Keep repeating this process until the filter clogs i.e. you can no longer push water through it (between 500mls and 1 litre). **If there is a high sediment load allow the sediment to settle prior to filtration.**



Figure 2.

- A. sample collection
  - B. drawing up water from the bag
  - C. fitting the syringe to the filter
  - D. pushing the water through the filter
  - E. adding preservation solution
  - F. completed filter with caps on either end
3. Once water has been filtered (volume to be recorded on sample record sheet), flush any excess water out of the filter unit using the empty 50 mL syringe as follows: Draw up air into the syringe and attach to the filter unit and push the air through, this will force residual water out of the filter. This will not remove all the water, so tap the residual water out onto some tissue.
  4. Using the 10mL syringe filled with preservation solution attach the syringe to the filter unit and **very gently** push into the filter until it starts to come out of the top (Figure 2E). **Note: you will need to retain half of this for the second filter so do not use more than 3mls.**
  5. Screw/push caps onto each end of the filter unit (Figure 2F) and label the filter unit accordingly with a permanent marker. You need to label after applying the preservation solution as the preservation solution could wash off the marker pen. Place the filter into the grip seal bag that the black cap came in and label the bag with the filter name etc. for return to laboratory.
  6. Empty the remaining water from the whirl-Pack bag back into the river.

7. Repeat steps 2-6 with the second bag of water using the second filter and the same 50ml syringe.
8. Place all used gloves, pipettes, rubbish into the sampling bag and dispose.
9. Fill out the sample record form (scan the QR code to do this), recording: pond name; volume of water filtered; sample location; sampler; date and time sample taken, sample condition; site condition; and storage prior to return.

**If storage of samples is necessary prior to their return please store refrigerated (2-4°C) or in a cool place.**

## APPENDIX 2 12S METABARCODING RESULTS

| Species Name                               | Common Name          | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|--------------------------------------------|----------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Accipiter nisus</i>                     | Eurasian sparrowhawk |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 384                                             |
| <i>Aegithalos caudatus</i>                 | Long tailed tit      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 6                                                           |                                                             |                                                 |
| <i>Alburnus alburnus</i>                   | Bleak                |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 23                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Anas platyrhynchos</i>                  | Mallard duck         | 46                              |                                         |                                         |                                           |                                               | 1523                                         |                                                         | 48                                                           |                                           |                                           |                                                 | 10619                                                     |                                          |                                       |                                                             | 857                                                         |                                                 |
| <i>Anguilla anguilla</i>                   | Freshwater eel       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1788                                      | 36                                        | 872                                             |                                                           |                                          |                                       | 234                                                         |                                                             |                                                 |
| <i>Anguilla anguilla</i>                   | European eel         |                                 | 200                                     |                                         | 1899                                      |                                               |                                              | 586                                                     |                                                              |                                           |                                           |                                                 | 5962                                                      | 5149                                     |                                       |                                                             |                                                             |                                                 |
| <i>Anser anser</i>                         | Common goose         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 307                                       |                                           | 1053                                            | 28                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Apodemus sylvaticus</i>                 | European woodmouse   |                                 |                                         | 5                                       | 6                                         |                                               |                                              | 23                                                      |                                                              |                                           |                                           |                                                 |                                                           | 14                                       |                                       |                                                             |                                                             |                                                 |
| <i>Arvicola amphibius</i>                  | European water vole  | 355                             | 380                                     | 168                                     | 22                                        | 468                                           |                                              | 99                                                      | 20                                                           | 308                                       |                                           | 245                                             | 115                                                       | 35                                       | 1023                                  | 1254                                                        | 1577                                                        | 1331                                            |
| <i>Barbatula barbatula</i>                 | Stone loach          |                                 | 2186                                    | 3675                                    |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bos taurus</i>                          | Cow                  | 17                              |                                         | 22517                                   |                                           |                                               |                                              |                                                         | 5                                                            |                                           | 11                                        | 146                                             | 2046                                                      |                                          |                                       |                                                             | 63                                                          | 17                                              |
| <i>Botaurus stellaris</i>                  | Eurasian bittern     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 217                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bufo bufo</i>                           | Common toad          |                                 |                                         | 82                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 2023                                  |                                                             |                                                             | 586                                             |
| <i>Canis lupus familiaris</i>              | Domestic dog         | 55                              | 62                                      | 33                                      | 62                                        |                                               | 64                                           | 333                                                     | 341                                                          |                                           |                                           |                                                 |                                                           | 19                                       |                                       |                                                             |                                                             | 169                                             |
| <i>Chelon labrosus</i>                     | Thicklip grey mullet |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1014                                      | 477                                       |                                                 |                                                           | 9426                                     |                                       |                                                             |                                                             |                                                 |
| Chordate environmental sample <sup>2</sup> |                      |                                 |                                         | 5                                       |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 7                                                         |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chroicocephalus ridibundus</i>          | Black-headed gull    |                                 |                                         | 167                                     |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Columba livia</i>                       | Rock dove            |                                 | 211                                     | 1255                                    | 3885                                      |                                               | 16225                                        | 2377                                                    | 222                                                          |                                           |                                           |                                                 | 8                                                         |                                          |                                       |                                                             |                                                             | 84                                              |
| <i>Columba oenas</i>                       | Stock dove           |                                 |                                         |                                         | 9                                         |                                               | 17606                                        |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Corvus splendens</i>                    | House crow           |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 181                                             |
| <i>Cygnus columbianus bewickii</i>         | Bewick's swan        |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 470                                      |                                       |                                                             |                                                             |                                                 |
| <i>Dama dama</i>                           | European fallow deer |                                 |                                         | 1111                                    |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Dicentrarchus labrax</i>                | European seabass     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1000                                      | 315                                       |                                                 |                                                           | 1959                                     |                                       |                                                             |                                                             |                                                 |

<sup>2</sup> Unknown species from the phylum Chordata.

| Species Name                    | Common Name              | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|---------------------------------|--------------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Emberiza citrinella</i>      | Yellowhammer             |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 42                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Equus caballus</i>           | Domesticated horse       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 9                                                           |                                                             |                                                 |
| <i>Erithacus rubecula</i>       | European robin           |                                 |                                         | 29                                      |                                           |                                               |                                              |                                                         | 878                                                          |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 138                                             |
| <i>Fringilla montifringilla</i> | Brambling                |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 254                                             |
| <i>Gallinula chloropus</i>      | Common moorhen           |                                 | 370                                     |                                         |                                           |                                               |                                              |                                                         |                                                              | 228                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Gallus domesticus</i>        | Chicken                  |                                 | 335                                     | 273                                     |                                           |                                               |                                              | 127                                                     | 1804                                                         |                                           | 16                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 585                                             |
| <i>Gasterosteus aculeatus</i>   | Three-spined stickleback | 45498                           | 36735                                   | 12836                                   | 46285                                     | 201                                           | 107                                          | 23491                                                   |                                                              | 8362                                      | 9                                         | 1220                                            | 6777                                                      | 33424                                    | 64912                                 | 3728                                                        | 597                                                         | 32347                                           |
| <i>Gobio gobio</i>              | Gudgeon                  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 263                                       |                                           |                                                 | 16516                                                     |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lissotriton vulgaris</i>     | Smooth newt              | 754                             |                                         |                                         |                                           |                                               |                                              |                                                         | 191                                                          |                                           |                                           | 13466                                           |                                                           |                                          |                                       | 813                                                         | 6448                                                        |                                                 |
| <i>Liza aurata</i>              | Golden grey mullet       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 24                                       |                                       |                                                             |                                                             |                                                 |
| <i>Lutra lutra</i>              | Otter                    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 579                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Meles meles</i>              | European badger          |                                 |                                         | 51                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Micromys minutus</i>         | Eurasian harvest mouse   |                                 |                                         |                                         |                                           | 838                                           |                                              |                                                         | 383                                                          | 211                                       |                                           |                                                 | 250                                                       |                                          |                                       |                                                             |                                                             | 32                                              |
| <i>Microtus agrestis</i>        | Field vole               |                                 |                                         | 5                                       | 204                                       | 102                                           |                                              | 111                                                     |                                                              |                                           |                                           | 161                                             | 711                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Muntiacus reevesi</i>        | Reeves's muntjac         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 141                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Myodes glareolus</i>         | Bank vole                |                                 | 28                                      | 156                                     | 165                                       |                                               |                                              | 81                                                      | 50                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 36                                              |
| <i>Neomys fodiens</i>           | Eurasian water shrew     |                                 | 16                                      |                                         |                                           | 837                                           |                                              |                                                         |                                                              |                                           |                                           |                                                 | 93                                                        |                                          |                                       |                                                             | 229                                                         | 90                                              |
| <i>Oryctolagus cuniculus</i>    | European rabbit          |                                 |                                         | 11                                      | 1701                                      | 460                                           |                                              | 291                                                     |                                                              |                                           |                                           |                                                 | 173                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Ovis aries</i>               | Sheep                    |                                 | 12                                      | 7                                       |                                           | 101                                           |                                              |                                                         | 6                                                            |                                           |                                           |                                                 | 268                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Parus major</i>              | Great tit                |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 286                                                          |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 471                                             |
| <i>Passer montanus</i>          | Eurasian tree sparrow    |                                 |                                         | 192                                     |                                           |                                               |                                              | 536                                                     |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pelecus cultratus</i>        | Ziege                    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 162                                                          |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phalacrocorax carbo</i>      | Great cormorant          |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 47                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phasianus colchicus</i>      | Common pheasant          |                                 | 523                                     |                                         | 3632                                      | 5191                                          | 2967                                         | 6554                                                    | 795                                                          |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 533                                                         |                                                 |
| <i>Phylloscopus collybita</i>   | Eurasian chiffchaff      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 18                                                          |                                                             |                                                 |
| <i>Pica pica</i>                | Eurasian magpie          |                                 | 1451                                    | 84                                      |                                           |                                               |                                              |                                                         | 276                                                          |                                           | 8                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 4002                                            |
| <i>Pleuronectes platessa</i>    | European plaice          |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1797                                      | 45                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pomatoschistus microps</i>   | Common goby              |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 5752                                      | 578                                       |                                                 | 2865                                                      | 8054                                     |                                       |                                                             |                                                             |                                                 |

| Species Name                       | Common Name             | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------------------------|-------------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Pungitius pungitius</i>         | Nine-spined stickleback | 11085                           | 6686                                    | 1430                                    | 5446                                      | 45692                                         | 33249                                        | 12381                                                   |                                                              | 1789                                      |                                           | 15662                                           | 2301                                                      | 6                                        | 2351                                  | 15127                                                       | 53209                                                       | 3934                                            |
| <i>Pyrrhula pyrrhula</i>           | Eurasian bullfinch      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 590                                                       |                                          |                                       |                                                             |                                                             | 22                                              |
| <i>Rallus aquaticus</i>            | Water rail              |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 509                                       |                                           |                                                 | 55787                                                     |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Rana temporaria</i>             | Common frog             |                                 |                                         |                                         |                                           | 2738                                          |                                              | 338                                                     |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 55                                                          |                                                             |                                                 |
| <i>Rattus norvegicus</i>           | Brown rat               | 108                             | 83                                      | 127                                     | 420                                       |                                               |                                              | 166                                                     | 13                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Rutilus rutilus</i>             | Roach                   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1614                                      |                                           | 198                                             |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Salmo salar</i>                 | salmon                  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 5                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Salmo trutta</i>                | Brown trout             |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 7                                                           |                                                             |                                                 |
| <i>Scardinius erythrophthalmus</i> | Rudd                    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 545                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Sciurus carolinensis</i>        | Eastern grey squirrel   |                                 |                                         |                                         | 139                                       |                                               | 509                                          |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 5                                                           |                                                             | 9                                               |
| <i>Sorex araneus</i>               | Common shrew            |                                 |                                         | 102                                     |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Sus scrofa domesticus</i>       | Pig                     | 53                              |                                         | 1395                                    |                                           |                                               |                                              | 451                                                     | 1196                                                         | 1720                                      | 5480                                      | 7208                                            |                                                           | 468                                      |                                       |                                                             |                                                             | 83                                              |
| <i>Tinca tinca</i>                 | Tench                   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 6436                                                        |                                                             | 50                                              |
| <i>Turdus merula</i>               | Blackbird               |                                 |                                         | 125                                     |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Unknown ungulate</i>            |                         |                                 |                                         |                                         | 60                                        | 2078                                          |                                              |                                                         | 12                                                           |                                           |                                           |                                                 | 195                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Vulpes vulpes</i>               | Red fox                 |                                 |                                         | 59                                      |                                           |                                               |                                              | 70                                                      |                                                              | 237                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             | 106                                                         |                                                 |
| Total read count                   |                         | 72836                           | 75033                                   | 64663                                   | 72040                                     | 83270                                         | 84296                                        | 85310                                                   | 6730                                                         | 61244                                     | 17986                                     | 49172                                           | 105575                                                    | 59048                                    | 87581                                 | 27948                                                       | 63890                                                       | 71224                                           |

Each species present in an individual sample is shown as the number of sequence reads, where there is no entry the species was not present in the sample.

## APPENDIX 3 COI METABARCODING RESULTS

| Species Name                          | Common Name/Type               | Phyla          | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|---------------------------------------|--------------------------------|----------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Acalles misellus</i>               | Weevil                         | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 9                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Acanthamoeba hatchetti</i>         | Amoebae                        | Amoebozoa      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 8                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Acanthocyclops robustus</i>        | Copepod                        | Arthropoda     | 23                              |                                         |                                         |                                           | 12                                            |                                              |                                                         | 5                                                            |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Acartia tonsa</i>                  | Copepod                        | Arthropoda     |                                 | 1465                                    |                                         |                                           |                                               | 4896                                         |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 12                                       |                                       |                                                             |                                                             |                                                 |
| <i>Achlya ambisexualis</i>            | Water mould                    | Gyrsta         |                                 |                                         |                                         |                                           |                                               |                                              | 6                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Acrochaetium sp.</i>               | Seaweed                        | Algae          |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 450                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Acrotona fungi</i>                 | Beetle                         | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 6                                                           |                                                 |
| <i>Acrotrichis sp.</i>                | Beetle                         | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 15                                              |                                                           |                                          |                                       |                                                             | 29                                                          |                                                 |
| <i>Adalia decempunctata</i>           | Ten-spotted ladybird           | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 5                                         |                                           |                                                 |                                                           |                                          | 13                                    | 89                                                          |                                                             |                                                 |
| <i>Agabus bipustulatus</i>            | Diving beetle                  | Arthropoda     |                                 |                                         |                                         | 5                                         |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Agabus sturmii</i>                 | Diving beetle                  | Arthropoda     |                                 |                                         | 7                                       |                                           | 52                                            |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Agonum thoreyi</i>                 | Black ground beetle            | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 12                                                          |                                                             |                                                 |
| <i>Agraylea multipunctata</i>         | Salt and pepper microcaddisfly | Arthropoda     |                                 |                                         | 77                                      |                                           |                                               |                                              | 168                                                     |                                                              |                                           |                                           | 41                                              |                                                           |                                          | 82                                    | 10                                                          |                                                             |                                                 |
| <i>Albugo candida</i>                 | White rust                     | Gyrsta         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 8                                     |                                                             |                                                             |                                                 |
| <i>Allacma fusca</i>                  | Springtail                     | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Allolobophora chlorotica</i>       | Earthworm                      | Annelida       |                                 |                                         |                                         |                                           |                                               |                                              | 104                                                     |                                                              |                                           | 135                                       | 101                                             |                                                           |                                          |                                       |                                                             | 326                                                         |                                                 |
| <i>Alonella excisa</i>                | Water flea                     | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 123                                             |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Alphaproteobacteria</i>            | Bacteria                       | Pseudomonadota |                                 |                                         |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Amoebozoa sp.</i>                  | Amoebae                        | Amoebozoa      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 6                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Anania hortulata</i>               | Small magpie                   | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 9                                     |                                                             |                                                             |                                                 |
| <i>Anopheles claviger</i>             | Mosquito                       | Arthropoda     | 9                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Aporrectodea caliginosa</i>        | Earthworm                      | Annelida       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 27                                        | 81                                              |                                                           |                                          | 29                                    |                                                             | 21                                                          |                                                 |
| <i>Aporrectodea longa</i>             | Black-headed worm              | Annelida       | 22                              |                                         |                                         |                                           |                                               |                                              | 85                                                      |                                                              |                                           |                                           | 72                                              |                                                           |                                          | 8                                     |                                                             | 51                                                          |                                                 |
| <i>Aporrectodea rosea</i>             | rosy tipped worm               | Annelida       | 6                               |                                         |                                         |                                           |                                               |                                              | 17                                                      |                                                              |                                           | 26                                        | 10                                              |                                                           |                                          |                                       |                                                             | 56                                                          |                                                 |
| <i>Apsectrotanypus trifascipennis</i> | Midge                          | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 10                                              |                                                           |                                          | 8                                     |                                                             |                                                             |                                                 |
| <i>Arenicola marina</i>               | Sandworm                       | Annelida       |                                 | 929                                     |                                         |                                           |                                               | 35                                           |                                                         |                                                              | 994                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                           | Common Name/Type          | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|----------------------------------------|---------------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Arion sp.</i>                       | Roundback slug            | Mollusca   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Arrenurus sp.</i>                   | Arachnid                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 345                                                         |                                                             |                                                 |
| <i>Arthropoda environmental sample</i> | N/A                       | Arthropoda |                                 |                                         |                                         | 18                                        |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Asellus aquaticus</i>               | 2 spotted water hog-louse | Arthropoda | 610                             |                                         | 32                                      |                                           | 5                                             |                                              | 39                                                      |                                                              |                                           |                                           | 9                                               |                                                           |                                          | 6                                     | 38                                                          |                                                             |                                                 |
| <i>Assiminea grayana</i>               | Dun sentinel              | Mollusca   |                                 | 15                                      |                                         |                                           |                                               | 8                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 37                                       |                                       |                                                             |                                                             |                                                 |
| <i>Athripsodes cinereus</i>            | Caddisfly                 | Arthropoda |                                 |                                         | 12                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Atrichopogon hirtidorsum</i>        | Biting midge              | Arthropoda |                                 |                                         |                                         |                                           | 5                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Atrichopogon winnertzi</i>          | Biting midge              | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 20                                       |                                       |                                                             |                                                             |                                                 |
| <i>Aurelia sp.</i>                     | Moon jelly                | Cnidaria   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 7                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Baetis rhodani</i>                  | Large dark olive mayfly   | Arthropoda |                                 |                                         | 7                                       |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 13                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Baetis vernus</i>                   | Blue-winged olive         | Arthropoda |                                 |                                         | 17                                      |                                           | 11                                            |                                              | 63                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Baltidrilus costatus</i>            | Worm                      | Annelida   |                                 | 7                                       |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bezzia fuliginata</i>               | Biting midge              | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 6                                        |                                       |                                                             |                                                             |                                                 |
| <i>Bimastos eiseni</i>                 | Lumbricid worm            | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 17                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bithynia tentaculata</i>            | Common faucet snail       | Mollusca   |                                 |                                         |                                         |                                           | 22                                            |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bradysia trivittata</i>             | Fungus gnat               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 8                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Brillia bifida</i>                  | Non-biting midge          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 51                                              |                                                           |                                          | 36                                    |                                                             |                                                             |                                                 |
| <i>Bruchus rufimanus</i>               | Broadbean weevil          | Arthropoda |                                 | 8                                       |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Bryophaenocladus sp.</i>            | Non-biting midge          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 34                                       |                                       |                                                             |                                                             |                                                 |
| <i>Campsicnemus picticornis</i>        | Fly                       | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 23                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Candona candida</i>                 | Ostracod                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 29842                                                     |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Candonopsis kingsleii</i>           | Ostracod                  | Arthropoda | 1086                            |                                         |                                         |                                           | 13936                                         |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cantharis cryptica</i>              | Soldier beetle            | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 25                                                          |                                                             |                                                 |
| <i>Canthocamptidae sp.</i>             | Copepod                   | Arthropoda | 340                             |                                         |                                         |                                           | 67                                            |                                              | 97                                                      |                                                              |                                           |                                           | 20                                              |                                                           |                                          | 38                                    | 837                                                         | 55                                                          | 1194                                            |
| <i>Carcinus maenas</i>                 | European green crab       | Arthropoda |                                 | 5                                       |                                         |                                           |                                               | 10                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cernosvitoviella sp.</i>            | Worm                      | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 15                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cerobasis guestfalica</i>           | Psocoptera                | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 24                                              |                                                           |                                          | 8                                     |                                                             |                                                             |                                                 |
| <i>Chaetoceros socialis</i>            | Diatom                    | Gyrsta     |                                 | 8                                       |                                         |                                           |                                               |                                              |                                                         |                                                              | 17                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                          | Common Name/Type | Phyla       | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|---------------------------------------|------------------|-------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Chaetogaster diastrophus</i>       | Detritus worm    | Annelida    | 960                             |                                         | 32                                      | 50                                        | 439                                           | 5                                            | 108                                                     | 42                                                           |                                           |                                           | 17                                              |                                                           | 32                                       |                                       |                                                             |                                                             |                                                 |
| <i>Chironomidae sp.</i>               | Non-biting midge | Arthropoda  | 78                              |                                         | 234                                     | 1137                                      | 614                                           | 94                                           | 30                                                      | 62                                                           |                                           |                                           | 12                                              | 659                                                       | 32                                       | 69                                    | 752                                                         |                                                             |                                                 |
| <i>Chironomus annularius</i>          | Non-biting midge | Arthropoda  |                                 |                                         |                                         |                                           |                                               | 5                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chironomus aprilinus</i>           | Non biting midge | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 13                                       |                                       |                                                             |                                                             |                                                 |
| <i>Chironomus luridus</i>             | Non biting midge | Arthropoda  |                                 |                                         |                                         | 137                                       |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chironomus nr. piger</i>           | Non biting midge | Arthropoda  |                                 |                                         |                                         | 855                                       |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chironomus pseudothummi</i>        | Non biting midge | Arthropoda  |                                 |                                         |                                         | 556                                       |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 | 24                                                        | 24                                       | 21                                    |                                                             |                                                             |                                                 |
| <i>Chironomus salinarius</i>          | Non-biting midge | Arthropoda  |                                 |                                         |                                         |                                           |                                               | 10                                           |                                                         |                                                              | 59                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Choleva angustata</i>              | Beetle           | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 14                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chydorus brevilabris</i>           | Water flea       | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 324                                                          |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Chydorus sphaericus</i>            | Water flea       | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 9                                                            |                                           |                                           |                                                 | 431                                                       |                                          |                                       | 45                                                          |                                                             |                                                 |
| <i>Cladosporium bruhnei</i>           | Mould            | Gyrsta      |                                 | 10                                      | 5                                       |                                           |                                               |                                              | 11                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 34                                    | 22                                                          |                                                             |                                                 |
| <i>Cladosporium sp.</i>               | Mould            | Gyrsta      | 5                               | 17                                      |                                         |                                           |                                               |                                              | 20                                                      |                                                              | 44                                        | 5                                         | 25                                              |                                                           |                                          | 585                                   | 38                                                          | 16                                                          | 12                                              |
| <i>Cladosporium tenuissimum</i>       | Pigmented mould  | Gyrsta      |                                 | 6                                       |                                         |                                           |                                               |                                              | 17                                                      |                                                              | 7                                         | 8                                         |                                                 | 5                                                         |                                          | 189                                   | 47                                                          | 11                                                          |                                                 |
| <i>Cloeon dipterum</i>                | Mayfly           | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 7                                         |                                                 | 10                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Clydonella sawyeri</i>             | Amoeba           | Amoebazoa   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 53                                        |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Coniopteryx tineiformis</i>        | Waxfly           | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 11                                    |                                                             |                                                             |                                                 |
| <i>Conosanus obsoletus</i>            | Leafhopper       | Arthropoda  | 92                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Corophium volutator</i>            | Mud scud         | Arthropoda  |                                 | 5                                       |                                         |                                           |                                               | 6                                            |                                                         |                                                              | 69                                        |                                           |                                                 |                                                           | 10                                       |                                       |                                                             |                                                             |                                                 |
| <i>Corynoneura coronata</i>           | Non-biting midge | Arthropoda  |                                 |                                         | 27534                                   |                                           | 1279                                          |                                              | 50                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 16                                                          |                                                             |                                                 |
| <i>Corynoneura fittkau</i>            | Non biting midge | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              | 90                                                      |                                                              |                                           |                                           | 40                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Corynoneura lobata v</i>           | Non biting midge | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              | 13                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Corynoneura scutellata</i>         | Non-biting midge | Arthropoda  | 401                             |                                         |                                         | 88                                        |                                               |                                              |                                                         | 31                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Corynoptera furcata</i>            | Fly              | Arthropoda  |                                 |                                         |                                         |                                           |                                               | 5                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cricotopus sp.</i>                 | Chironomidae     | Arthropoda  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 8                                     | 21                                                          |                                                             |                                                 |
| <i>Cryptochironomus albofasciatus</i> | Chironomid       | Arthropoda  |                                 |                                         | 13                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cryptomonas curvata</i>            | Photolithotroph  | Cryptophyta |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 1147                                                      |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                   | Common Name/Type            | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|--------------------------------|-----------------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Culex pipiens</i>           | Southern house mosquito     | Arthropoda |                                 |                                         |                                         |                                           | 48                                            | 13                                           |                                                         |                                                              |                                           |                                           | 7                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Culicoides newsteadi</i>    | Biting midge                | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Culiseta annulata</i>       | Mosquito                    | Arthropoda |                                 |                                         |                                         |                                           | 157                                           |                                              | 9                                                       |                                                              |                                           |                                           |                                                 | 384                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cunea russae</i>            | Amoeba                      | Amoebozoa  |                                 |                                         |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 37                                       |                                       |                                                             |                                                             |                                                 |
| <i>Cyclopidae sp.</i>          | Copepods                    | Arthropoda | 54                              |                                         |                                         | 2804                                      |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cyclops strenuus</i>        | Copepod                     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 17                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       | 61                                                          | 27                                                          |                                                 |
| <i>Cyphoderia ampulla</i>      | Cyphoderia                  | Cercozoa   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 8                                     |                                                             |                                                             |                                                 |
| <i>Cyphon coarctatus</i>       | Beetle                      | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 72                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cyphon laevipennis</i>      | Leaf beetle                 | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 8                                                         |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Delphacinae sp.</i>         | Plant hopper                | Arthropoda | 45                              |                                         |                                         |                                           | 22                                            |                                              | 7                                                       |                                                              | 49                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Dendrobaena octaedra</i>    | Earthworm                   | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 104                                   |                                                             |                                                             |                                                 |
| <i>Deroceras reticulatum</i>   | Garden slug                 | Mollusca   | 6                               |                                         |                                         |                                           |                                               |                                              | 30                                                      |                                                              | 24                                        | 20                                        | 141                                             |                                                           | 30                                       | 206                                   |                                                             | 41                                                          | 7                                               |
| <i>Deroceras sp.</i>           | Slug                        | Mollusca   |                                 |                                         |                                         |                                           |                                               |                                              | 10                                                      |                                                              |                                           |                                           | 9                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Desoria trispinata</i>      | Collembola                  | Arthropoda |                                 |                                         |                                         |                                           | 5                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Dicranota bimaculata</i>    | Crane fly                   | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 5                                     |                                                             |                                                             |                                                 |
| <i>Dicyphus epilobii</i>       | Miridae                     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 15                                    |                                                             |                                                             |                                                 |
| <i>Dicyrtoma fusca</i>         | Springtail                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 10                                        | 5                                               |                                                           |                                          |                                       |                                                             | 10                                                          |                                                 |
| <i>Diplocladius cultriger</i>  | Chironamid-non biting midge | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 110                                             |
| <i>Diptera sp.</i>             | Fly                         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 41                                                          |                                                             |                                                 |
| <i>Dolichopus austriacus</i>   | Long legged fly             | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 21                                       |                                       |                                                             |                                                             |                                                 |
| <i>Drosophila suzukii</i>      | Spotted wing drosophila     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 37                                                      |                                                              | 5                                         |                                           | 124                                             |                                                           |                                          | 19                                    |                                                             | 32                                                          |                                                 |
| <i>Ectocarpus subulatus</i>    | Brown algae                 | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 67                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Ectopsocus briggsi</i>      | Psocoptera                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          | 23                                    |                                                             |                                                             |                                                 |
| <i>Ectopsocus californicus</i> | Barklouse                   | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 5                                     |                                                             |                                                             |                                                 |
| <i>Eiseniella tetraedra</i>    | Red worm                    | Annelida   |                                 |                                         | 365                                     | 7                                         |                                               |                                              |                                                         |                                                              |                                           | 8                                         | 48                                              |                                                           | 31                                       | 157                                   |                                                             | 71                                                          |                                                 |
| <i>Elasmucha grisea</i>        | Parent bug                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 19                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 19                                    |                                                             |                                                             |                                                 |
| <i>Enchytraeidae sp.</i>       | Pot worm                    | Annelida   | 6                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 214                                       | 34                                              |                                                           |                                          | 5                                     |                                                             | 15                                                          |                                                 |
| <i>Enchytraeus albidus</i>     | White worm                  | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 47                                       |                                       |                                                             |                                                             |                                                 |
| <i>Enchytraeus bulbosus</i>    | White worm                  | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 34                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

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|-----------------------------------|--------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Enochrus testaceus</i>         | Beetle             | Arthropoda |                                 |                                         |                                         |                                           | 5                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Entomobrya intermedia</i>      | Springtail         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Entomobrya nicoleti</i>        | Springtail         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 5                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Epistrophe eligans</i>         | Hoverfly           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 6                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Episyrphus balteatus</i>       | Marmalade hoverfly | Arthropoda |                                 |                                         | 11                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Eristalis tenax</i>            | Drone fly          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 13                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Erythroneurini sp.</i>         | Leaf hopper        | Arthropoda | 28                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Eucheilota maculata</i>        | Hydrozoa           | Cnidaria   |                                 | 14                                      |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Euchlanis dilatata</i>         | Rotifer            | Rotifera   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 287                                                         |                                                             |                                                 |
| <i>Eucyclops serrulatus</i>       | Copepod            | Arthropoda |                                 |                                         |                                         | 26592                                     | 490                                           | 7                                            | 7                                                       | 7                                                            |                                           |                                           |                                                 | 1110                                                      |                                          | 36                                    | 1350                                                        |                                                             |                                                 |
| <i>Eucypris viren</i>             | Ostracod           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 953                                             |
| <i>Eunotia sp.</i>                | Diatom             | Gyrsta     |                                 |                                         |                                         | 11                                        |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Eupteryx calcarata</i>         | Leafhopper         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 34                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Euscelis sordida</i>           | Leafhopper         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 40                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Euzetes globulus</i>           | Mite               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 8                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Fagocyba douglasi</i>          | Short-horned bug   | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 10                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Floscularia melicerta</i>      | Rotifer            | Rotifera   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         | 53                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Folsomia quadrioculata</i>     | Springtail         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 9                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Forficula aff. auricularia</i> | Common earwig      | Arthropoda |                                 |                                         |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Fridericia galba</i>           | Pot worm           | Annelida   |                                 |                                         |                                         |                                           | 21                                            |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 79                                                          |                                                 |
| <i>Fridericia parathalassia</i>   | Pot worm           | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 2509                                     |                                       |                                                             |                                                             |                                                 |
| <i>Fridericia perrieri</i>        | Pot worm           | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 35                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Fusarium diversisporum</i>     | Fungi              | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 6                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Fusarium oxysporum</i>         | Fungi              | Ascomycota |                                 |                                         | 9                                       |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Gammarus duebeni</i>           | Crustacean         | Arthropoda |                                 |                                         |                                         |                                           |                                               | 60                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 121                                      |                                       |                                                             |                                                             |                                                 |
| <i>Gammarus pulex</i>             | Crustacean         | Arthropoda |                                 |                                         | 38                                      |                                           |                                               |                                              | 17                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 38                                    |                                                             |                                                             |                                                 |
| <i>Globulidrilus riparius</i>     | Segmented worm     | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 37                                                        |                                          |                                       |                                                             | 15                                                          |                                                 |
| <i>Glyptothorax jacobsi</i>       | Grasshopper        | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 16                                    |                                                             |                                                             |                                                 |

| Species Name                             | Common Name/Type              | Phyla        | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------------------------------|-------------------------------|--------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Glyptotendipes cauliginellus</i>      | Non-biting midge              | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 6                                                           |                                                             |                                                 |
| <i>Glyptotendipes sp.</i>                | Non-biting midge              | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 3355                                                        |                                                             |                                                 |
| <i>Gomphonema parvulum</i>               | Diatom                        | Gyrsta       |                                 |                                         |                                         | 11                                        |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Gonocerus acuteangulatus</i>          | Box bug                       | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 22                                    |                                                             |                                                             |                                                 |
| <i>Gonothyraea loveni</i>                | Cnidaria                      | Cnidaria     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 79                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Graphopsocus cruciatus</i>            | Barkfly                       | Annelida     |                                 |                                         |                                         |                                           |                                               |                                              | 80                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 12                                                          |                                                 |
| <i>Haplophthalmus danicus</i>            | Spurred ridgeback isopod      | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 176                                             |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Harpacticoida sp.</i>                 | Copepod                       | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 14                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Hebecnema vespertina</i>              | Fly                           | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 9                                               |
| <i>Hediste diversicolor</i>              | Ragworm                       | Annelida     |                                 | 7550                                    |                                         |                                           |                                               | 9015                                         |                                                         |                                                              | 93                                        |                                           |                                                 |                                                           | 2947                                     |                                       |                                                             |                                                             |                                                 |
| <i>Helophilus pendulus</i>               | The footballer                | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 7                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Henlea nasuta</i>                     | Worm                          | Annelida     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 22                                       |                                       |                                                             |                                                             |                                                 |
| <i>Henlea perpusilla</i>                 | Worm                          | Annelida     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 26                                        | 57                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Heterocapsa rotundata</i>             | Dinoflagellate                | Myzozoa      |                                 |                                         |                                         |                                           |                                               | 13                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Humerobatidae sp.</i>                 | Mite                          | Arthropoda   |                                 |                                         | 249                                     |                                           |                                               |                                              | 6                                                       |                                                              |                                           |                                           | 15                                              |                                                           |                                          | 40                                    |                                                             |                                                             |                                                 |
| <i>Hydra circumcincta</i>                | Freshwater hydra              | Cnidaria     | 103                             |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Hydra viridissima</i>                 | Green hydra fresh-water polyp | Cnidaria     |                                 |                                         |                                         |                                           | 10                                            |                                              | 5                                                       | 76                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Hydrophilus piceus</i>                | Great silver water beetle     | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 48                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Hydrozetidae sp.</i>                  | Oribatids                     | Arthropoda   | 6                               |                                         |                                         |                                           | 8                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 | 524                                                       |                                          |                                       | 129                                                         |                                                             |                                                 |
| <i>Hypogastrura sp.</i>                  | Springtail                    | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Hypogastrura viatica</i>              | Springtail                    | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 28                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Idotea chelipes</i>                   | Myriapod                      | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 60                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Ilybius ater</i>                      | Water beetle                  | Arthropoda   | 5                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Invertebrate environmental sample</i> | N/A                           | Invertebrate | 40                              |                                         | 103                                     |                                           | 22                                            |                                              | 14                                                      | 19                                                           |                                           |                                           |                                                 | 48                                                        |                                          | 106                                   |                                                             |                                                             |                                                 |
| <i>Isotoma riparia</i>                   | Springtail                    | Arthropoda   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 13                                       |                                       |                                                             |                                                             |                                                 |
| <i>Isotoma viridis</i>                   | Springtail                    | Arthropoda   |                                 |                                         |                                         |                                           |                                               | 13                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Isotomurus fucicola</i>               | Springtail                    | Arthropoda   | 11                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 258                                       | 48                                              |                                                           |                                          |                                       |                                                             | 141                                                         |                                                 |

| Species Name                      | Common Name/Type       | Phyla         | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|-----------------------------------|------------------------|---------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Isotomurus unifasciatus</i>    | Springtail             | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 6                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Jaminalia sp.</i>              | Yeast                  | Basidiomycota | 18                              |                                         |                                         |                                           |                                               |                                              | 11                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 17                                    |                                                             |                                                             |                                                 |
| <i>Javesella dubia</i>            | Plant Hopper           | Arthropoda    | 9                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 20                                                          |                                                 |
| <i>Javesella forcipata</i>        | plant hopper           | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 15                                       |                                       |                                                             |                                                             |                                                 |
| <i>Kleidocerys ericae</i>         | Birch catkin bug       | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 36                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Korotnevella heteracantha</i>  | Amoeba                 | Amoebozoa     | 6                               |                                         | 73                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 31                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Korotnevella leshevi</i>       | Amoeba                 | Amoebozoa     | 9                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Korotnevella sp.</i>           | Amoeba                 | Amoebozoa     | 640                             |                                         | 63                                      |                                           | 83                                            |                                              | 62                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 75                                    |                                                             |                                                             | 5                                               |
| <i>Korotnevella stella</i>        | Amoeba                 | Amoebozoa     | 213                             |                                         | 1108                                    |                                           |                                               |                                              | 24                                                      | 49                                                           |                                           |                                           |                                                 |                                                           |                                          | 92                                    |                                                             |                                                             | 11                                              |
| <i>Krenopelopia sp.</i>           | Non-biting midge       | Arthropoda    | 59                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Leathesia diffformis</i>       | Sea cauliflower        | Gyrsta        |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Lecane closterocerca</i>       | Rotifer                | Rotifera      |                                 |                                         |                                         |                                           | 17                                            |                                              |                                                         |                                                              |                                           |                                           |                                                 | 11                                                        |                                          | 120                                   |                                                             |                                                             |                                                 |
| <i>Lehmannia marginata</i>        | Tree slug              | Mollusca      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 5                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Leistus spinibarbis</i>        | Ground beetle          | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              | 6                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lekanesphaera sp.</i>          | Sea slater             | Arthropoda    |                                 |                                         |                                         |                                           |                                               | 5                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Leptolegnia sp.</i>            | Oomycete               | Gyrsta        |                                 |                                         |                                         |                                           |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Limapontia depressa</i>        | Sea slug               | Mollusca      |                                 | 5                                       |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Limnodrilus hoffmeisteri</i>   | Red worm               | Annelida      | 25                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 7                                         |                                                 |                                                           |                                          | 33                                    |                                                             |                                                             |                                                 |
| <i>Limnophyes pentaplastus</i>    | Non-biting midge       | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 24                                    |                                                             |                                                             |                                                 |
| <i>Limonia macrostigma</i>        | Crane fly              | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lineus viridis</i>             | Ribbon worm            | Annelida      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 14                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lithobius microps</i>          | Stone centipede        | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 16                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lonchoptera uniseta</i>        | Spear-winged fly       | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 18                                                          |                                                             |                                                 |
| <i>Lucilia ampullacea</i>         | Streakless greenbottle | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lucilia caesar</i>             | blow fly               | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lucilia illustris</i>          | Green bottle fly       | Arthropoda    |                                 |                                         |                                         |                                           |                                               |                                              | 51                                                      |                                                              |                                           |                                           | 6                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lumbricidae sp.</i>            | Earthworm              | Annelida      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 12                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lumbricillus pagenstecheri</i> | Worm                   | Annelida      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 36                                       |                                       |                                                             |                                                             |                                                 |
| <i>Lumbriculus variegatus</i>     | Black worm             | Annelida      | 279                             |                                         |                                         | 47                                        |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                         | Common Name/Type | Phyla            | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|--------------------------------------|------------------|------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Lumbricus castaneus</i>           | Chestnut worm    | Annelida         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 8                                     |                                                             |                                                             |                                                 |
| <i>Lumbricus rivalis</i>             | Earthworm        | Annelida         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 15                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Lumbricus rubellus</i>            | Red earthworm    | Annelida         |                                 |                                         |                                         |                                           |                                               |                                              | 43                                                      |                                                              |                                           |                                           | 13                                              |                                                           | 57                                       | 38                                    |                                                             |                                                             |                                                 |
| <i>Lumbricus terrestris</i>          | Common earthworm | Annelida         |                                 |                                         |                                         |                                           |                                               |                                              | 105                                                     |                                                              |                                           |                                           | 32                                              |                                                           |                                          |                                       |                                                             | 13                                                          |                                                 |
| <i>Lype phaeopa</i>                  | Caddisfly        | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 9                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Macropelopia sp.</i>              | Non-biting midge | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 7                                     |                                                             |                                                             |                                                 |
| <i>Macropsis fuscula</i>             | Rubus leafhopper | Arthropoda       |                                 |                                         | 8                                       |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Marionina spicula</i>             | Pot worm         | Annelida         |                                 |                                         |                                         |                                           |                                               | 13                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Maso sundevalli</i>               | Dwarf spider     | Arthropod        |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 423                                             |
| <i>Maxillopoda sp.</i>               | Crustacean       | Arthropoda       | 4753                            |                                         |                                         |                                           | 5601                                          |                                              |                                                         | 723                                                          |                                           |                                           |                                                 |                                                           |                                          | 7                                     |                                                             |                                                             |                                                 |
| <i>Melampsora magnusiana</i>         | Rust fungi       | Basidiomycota    |                                 | 43                                      | 26                                      | 166                                       |                                               | 34                                           | 381                                                     |                                                              | 36                                        | 485                                       | 133                                             | 5                                                         |                                          | 91                                    |                                                             | 325                                                         | 14                                              |
| <i>Melosira varians</i>              | Diatom           | Gyrista          |                                 |                                         | 5                                       | 1534                                      |                                               | 8                                            | 15                                                      |                                                              |                                           | 66                                        | 238                                             |                                                           |                                          | 23                                    |                                                             | 22                                                          | 111                                             |
| <i>Mesocyclops leuckarti</i>         | Copepod          | Arthropoda       |                                 |                                         |                                         |                                           |                                               | 132                                          |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 244                                             |
| <i>Metriocnemus albolineatus</i>     | Chironomid       | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 7                                                           |                                                 |
| <i>Metriocnemus eurynotus</i>        | Non-biting midge | Arthropoda       | 5                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Micropsectra contracta</i>        | Non-biting midge | Arthropoda       |                                 |                                         | 28                                      | 35                                        |                                               |                                              | 219                                                     |                                                              |                                           |                                           | 32                                              |                                                           |                                          | 83                                    |                                                             |                                                             |                                                 |
| <i>Micropsectra notescens</i>        | Chironomidae     | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              | 47                                                      |                                                              |                                           | 17                                        | 9                                               |                                                           |                                          | 168                                   |                                                             |                                                             |                                                 |
| <i>Micropsectra pallidula</i>        | Chironomidae     | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 6                                     |                                                             |                                                             |                                                 |
| <i>Microscolex phosphoreus</i>       | Earthworm        | Annelida         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 9                                        |                                       |                                                             |                                                             |                                                 |
| <i>Minutocellus polymorphus</i>      | Diatom           | Heterokontophyta |                                 | 21                                      |                                         |                                           |                                               | 22                                           |                                                         |                                                              | 1313                                      |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Mollusca environmental sample</i> | N/A              | Mollusca         |                                 |                                         |                                         |                                           |                                               |                                              | 37                                                      |                                                              |                                           |                                           | 9                                               |                                                           | 11                                       |                                       | 238                                                         |                                                             |                                                 |
| <i>Monocorophium insidiosum</i>      | Crustacean       | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 100                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Monopelopia tenuicalcar</i>       | Non-biting midge | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 162                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Mytilina ventralis</i>            | Rotifer          | Rotifera         |                                 |                                         |                                         |                                           | 8                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Nais sp.</i>                      | Detritus worm    | Annelida         | 393                             |                                         |                                         | 3357                                      | 36                                            |                                              | 17                                                      | 19                                                           |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Natarsia punctata</i>             | Non-biting midge | Arthropoda       | 29                              |                                         |                                         |                                           |                                               |                                              | 392                                                     |                                                              |                                           |                                           |                                                 |                                                           |                                          | 5                                     |                                                             |                                                             |                                                 |
| <i>Neanura muscorum</i>              | Springtail       | Arthropoda       |                                 |                                         |                                         |                                           |                                               |                                              | 392                                                     |                                                              |                                           | 39                                        | 69                                              |                                                           | 55                                       |                                       |                                                             |                                                             | 29                                              |

| Species Name                       | Common Name/Type | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------------------------|------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Neonectria hubeiensis</i>       | Fungi            | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              | 6                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 8                                                           |                                                 |
| <i>Nesovitrea sp.</i>              | Snail            | Mollusca   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 8                                                           |                                                 |
| <i>Nitzschia cf. fonticola</i>     | Diatom           | Gyrsta     | 6                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Nitzschia longissima</i>        | Braown algae     | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 6                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Nothophytophthora intricata</i> | Oomycete         | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Nothophytophthora sp.</i>       | Oomycete         | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Octolasion tyrtaeum</i>         | Earthworm        | Anneilda   |                                 |                                         |                                         |                                           |                                               |                                              | 29                                                      |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Odacantha melanura</i>          | Ground beetle    | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 25                                                          |                                                             |                                                 |
| <i>Orchesella villosa</i>          | Springtail       | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 7                                               |                                                           |                                          |                                       |                                                             | 16                                                          |                                                 |
| <i>Ormosia hederiae</i>            | Hexapod          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Oscinella sp.</i>               | Fly              | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 31                                                          |                                                 |
| <i>Oxypoda elongatula</i>          | Beetle           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 13                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pantilius tunicatus</i>         | Beetle           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 25                                                      |                                                              |                                           |                                           | 8                                               |                                                           |                                          | 47                                    |                                                             |                                                             |                                                 |
| <i>Paracyclops fimbriatus</i>      | Copepod          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 8                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Paradromius linearis</i>        | Ground beetle    | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 182                                                         |                                                             |                                                 |
| <i>Paralemanea annulata</i>        | Red algae        | Rhodophyta |                                 |                                         | 170                                     |                                           |                                               |                                              | 3432                                                    |                                                              |                                           | 18                                        | 396                                             |                                                           |                                          | 165                                   |                                                             | 5                                                           | 30                                              |
| <i>Paramormia ustulata</i>         | Moth fly         | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 34                                       |                                       |                                                             |                                                             |                                                 |
| <i>Paranaïs botniensis</i>         | Worm             | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 99                                       |                                       |                                                             |                                                             |                                                 |
| <i>Paranaïs litoralis</i>          | Worm             | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 23                                       |                                       |                                                             |                                                             |                                                 |
| <i>Paranthomyza nitida</i>         | Diptera          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 7                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Paraphaenocladus impensus</i>   | Non-biting midge | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 14                                       | 8                                     |                                                             |                                                             |                                                 |
| <i>Paratanytarsus austriacus</i>   | Non-biting midge | Arthropoda |                                 |                                         |                                         | 125                                       |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Paratanytarsus grimmii</i>      | Non-biting midge | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 8                                                         |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Paratanytarsus intricatus</i>   | Non-biting midge | Arthropoda |                                 |                                         |                                         |                                           | 24                                            |                                              |                                                         | 10                                                           |                                           |                                           |                                                 | 288                                                       |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Paratanytarsus lauterborni</i>  | Non-biting midge | Arthropoda | 47                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 50                                        |                                                 |                                                           |                                          | 7                                     |                                                             |                                                             |                                                 |
| <i>Parisotoma notabilis</i>        | Springtail       | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 67                                        | 28                                              |                                                           |                                          |                                       |                                                             | 36                                                          |                                                 |
| <i>Pedospumella encystans</i>      | Golden algae     | Gyrsta     |                                 |                                         |                                         | 5                                         |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 18                                       |                                       | 12                                                          |                                                             |                                                 |

| Species Name                       | Common Name/Type          | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------------------------|---------------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Pedospumella sp.</i>            | Golden algae              | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 12                                              |                                                           | 22                                       |                                       |                                                             |                                                             |                                                 |
| <i>Pentatoma rufipes</i>           | Forest bug                | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phaenopsectra flavipes</i>      | Non-biting midge          | Arthropoda | 8                               |                                         | 12                                      |                                           |                                               |                                              | 22                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          | 12                                    |                                                             |                                                             |                                                 |
| <i>Phaenopsectra punctipes</i>     | Midge                     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 20                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phaeocystis globosa</i>         | Algae                     | Gyrista    |                                 | 16                                      |                                         |                                           |                                               |                                              |                                                         |                                                              | 5                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Philonthus politus</i>          | Rove beetle               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 24                                       |                                       |                                                             |                                                             |                                                 |
| <i>Philoscia muscorum</i>          | Common striped woodlouse  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 5                                         | 32                                        | 206                                             |                                                           | 50                                       | 55                                    | 39                                                          | 25                                                          |                                                 |
| <i>Phylidorea ferruginea</i>       | Cranefly                  | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Physa acuta</i>                 | Common bladder snail      | Mollusca   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 13                                              |                                                           |                                          |                                       | 31                                                          |                                                             |                                                 |
| <i>Physa fontinalis</i>            | Common bladder snail      | Mollusca   | 6                               |                                         | 23                                      |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phytophthora bilorbang</i>      | Oomycete                  | Gyrista    |                                 |                                         |                                         | 136                                       |                                               |                                              | 91                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Phytophthora cryptogea</i>      | Oomycete                  | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 59                                              |                                                           |                                          |                                       |                                                             | 28                                                          |                                                 |
| <i>Phytophthora gallica</i>        | Oomycete                  | Gyrista    |                                 |                                         |                                         | 15                                        |                                               |                                              | 43                                                      |                                                              |                                           |                                           | 5                                               |                                                           |                                          | 84                                    |                                                             | 20                                                          |                                                 |
| <i>Phytophthora infestans</i>      | Potato late blight fungus | Oomycota   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 17                                                          |                                                             |                                                 |
| <i>Phytophthora inundata</i>       | Oomycete                  | Gyrista    | 6                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 9                                         | 38                                              |                                                           | 24                                       |                                       | 5                                                           | 25                                                          |                                                 |
| <i>Phytophthora lacustris</i>      | Oomycete                  | Gyrista    |                                 |                                         | 104                                     | 65                                        | 10                                            |                                              | 540                                                     | 12                                                           |                                           | 28                                        | 366                                             |                                                           |                                          | 267                                   |                                                             | 37                                                          |                                                 |
| <i>Phytophthora plurivora</i>      | Oomycete                  | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              | 14                                                      |                                                              |                                           |                                           | 12                                              |                                                           |                                          | 15                                    |                                                             |                                                             |                                                 |
| <i>Phytophthora sp.</i>            | Oomycete                  | Gyrista    |                                 |                                         | 52                                      |                                           |                                               |                                              |                                                         | 13                                                           |                                           |                                           | 20                                              |                                                           |                                          | 95                                    |                                                             | 5                                                           |                                                 |
| <i>Phytophthora syringae</i>       | Oomycete                  | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 6                                                           |                                                 |
| <i>Phytophthora taxon</i>          | Oomycete                  | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 17                                    |                                                             |                                                             |                                                 |
| <i>Phytophthora thermophila</i>    | Oomycete                  | Gyrista    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 7                                               |                                                           |                                          | 10                                    |                                                             | 6                                                           | 7                                               |
| <i>Pipunculus cf. omissinervis</i> | Big headed fly            | Arthropod  |                                 |                                         |                                         |                                           |                                               |                                              | 20                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Planorbis planorbis</i>         | Margined Ram's horn snail | Mollusca   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 6                                                           |                                                             |                                                 |
| <i>Plateumaris sericea</i>         | Beetle                    | Arthropoda |                                 |                                         |                                         |                                           | 5                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Platynothrus peltifer</i>       | Mite                      | Arthropoda | 6                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pleuroptya ruralis</i>          | Mother of pearl           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 69                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Plumatella fungosa</i>          | Bryzoan                   | Bryozoa    |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 70                                                          |                                                             |                                                 |
| <i>Podura aquatica</i>             | water springtail          | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                         | Common Name/Type       | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|--------------------------------------|------------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Pogonognathellus longicornis</i>  | Springtail             | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             | 8                                                           |                                                 |
| <i>Polyarthra sp.</i>                | Crustacea              | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 40                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Polydora cornuta</i>              | Worm                   | Annelida   |                                 |                                         |                                         |                                           |                                               | 106                                          |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Populicerus confusus</i>          | Cicadellidae           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 24                                    |                                                             |                                                             |                                                 |
| <i>Potamothrix bavaricus</i>         | Worm                   | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 42                                                          |                                                             |                                                 |
| <i>Pristina aequiseta</i>            | Sludge worm            | Annelida   | 783                             |                                         |                                         |                                           | 2901                                          |                                              |                                                         |                                                              |                                           | 18                                        |                                                 |                                                           |                                          |                                       | 113                                                         |                                                             |                                                 |
| <i>Proasellus meridianus</i>         | One-spotted waterlouse | Crustacean |                                 |                                         |                                         |                                           |                                               |                                              | 32                                                      |                                                              |                                           | 11                                        |                                                 |                                                           | 13                                       | 62                                    |                                                             |                                                             |                                                 |
| <i>Prodiamesa olivacea</i>           | Chironomidae           | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 28                                    |                                                             |                                                             |                                                 |
| <i>Psallus falleni</i>               | Plant bug              | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 14                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Psammotettix bavaricus</i>        | True bug               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 6                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Psammotettix sp.</i>              | True bug               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             | 6                                                           |                                                 |
| <i>Pseudochattonella verruculosa</i> | Heterokont flagellate  | Gyrsta     |                                 |                                         |                                         |                                           |                                               | 5                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pseudolyciella pallidiventris</i> | Fly                    | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pseudoparamoeba pagei</i>         | Amoeba                 | Amoebazoa  |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 11                                       |                                       |                                                             |                                                             |                                                 |
| <i>Pseudoparamoeba sp.</i>           | Amoeba                 | Amoebozoa  |                                 | 5                                       |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 15                                       |                                       |                                                             |                                                             |                                                 |
| <i>Pseudosinella alba</i>            | Springtail             | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Psocoptera sp.</i>                | Barklice               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 6                                         |                                                 |                                                           |                                          | 14                                    |                                                             |                                                             |                                                 |
| <i>Ptychoptera albimana</i>          | Cranefly               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 19                                              |                                                           |                                          | 45                                    |                                                             |                                                             |                                                 |
| <i>Ptychoptera lacustris</i>         | Cranefly               | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pythium adhaerens</i>             | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pythium aff. pachycaule</i>       | Oomycete               | Gyrsta     | 73                              |                                         |                                         |                                           |                                               |                                              | 57                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 5                                               |
| <i>Pythium cystogenes</i>            | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              | 14                                                      |                                                              |                                           |                                           | 18                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pythium oligandrum</i>            | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 21                                              |                                                           |                                          |                                       |                                                             | 10                                                          |                                                 |
| <i>Pythium periplocum</i>            | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 6                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pythium phragmitis</i>            | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 8                                                         |                                          |                                       | 6                                                           |                                                             |                                                 |
| <i>Pythium sp.</i>                   | Oomycete               | Gyrsta     | 11                              |                                         | 54                                      |                                           | 5                                             |                                              | 736                                                     |                                                              | 8                                         | 35                                        | 1221                                            |                                                           |                                          | 251                                   |                                                             | 64                                                          | 51                                              |
| <i>Pythium torulosum</i>             | Oomycete               | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 29                                                        | 19                                       |                                       | 124                                                         |                                                             |                                                 |

| Species Name                      | Common Name/Type             | Phyla      | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|-----------------------------------|------------------------------|------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Rhamphomyia simplex</i>        | Dance fly                    | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 10                                       |                                       |                                                             |                                                             |                                                 |
| <i>Rheotanytarsus curtistylus</i> | Non-biting midge             | Arthropoda |                                 |                                         | 334                                     |                                           |                                               |                                              | 570                                                     |                                                              |                                           |                                           | 124                                             |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Rhyacodrilus coccineus</i>     | Naidid worm                  | Annelida   | 43                              |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 7                                     |                                                             |                                                             |                                                 |
| <i>Rotaria rotatoria</i>          | Rotifer                      | Rotifera   |                                 |                                         |                                         | 3226                                      |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 5                                                           |                                                             |                                                 |
| <i>Rotaria sp.</i>                | Rotifer                      | Rotifera   |                                 |                                         |                                         | 259                                       |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Salisapilia tartarea</i>       | Oomycete                     | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 12                                       |                                       |                                                             |                                                             |                                                 |
| <i>Salpingus planirostris</i>     | Beetle                       | Arthropod  |                                 |                                         |                                         |                                           |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Saprolegnia ferax</i>          | Water mould                  | Gyrsta     |                                 |                                         |                                         | 35                                        |                                               |                                              | 11                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Satchelliella trivialis</i>    | Moth fly                     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 5                                     |                                                             |                                                             |                                                 |
| <i>Sheathia arcuata</i>           | Freshwater algae             | Gyrsta     |                                 |                                         |                                         |                                           |                                               |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Simocephalus exspinosus</i>    | Water flea                   | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 10                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Sminthurus viridis</i>         | Clover springtail            | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 15                                                          |                                                             |                                                 |
| <i>Smittia sp.</i>                | Non-biting midge             | Arthropoda |                                 |                                         |                                         | 29                                        |                                               |                                              | 54                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Stempellina bausei</i>         | Non-biting midge             | Arthropoda |                                 |                                         | 8                                       |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Stigmatogaster subterranea</i> | Western yellow centipede     | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 5                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Streblospio benedicti</i>      | Ram's horn worm              | Annelida   |                                 | 30                                      |                                         |                                           |                                               | 82                                           |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Strigamia acuminata</i>        | Centipede                    | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Stylaria lacustris</i>         | Detritus worm                | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 55                                                        |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Stylodrilus heringianus</i>    | Worm                         | Annelida   | 5                               |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 10                                        |                                                 |                                                           |                                          |                                       | 63                                                          |                                                             |                                                 |
| <i>Succinea sp.</i>               | Amber snail                  | Mollusca   |                                 |                                         |                                         |                                           | 5                                             |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Succineidae sp.</i>            | Land snail                   | Mollusca   | 10                              |                                         |                                         |                                           | 5                                             |                                              | 5                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Sus scrofa domesticus</i>      | Pig                          | Mammalia   |                                 | 5                                       |                                         |                                           |                                               |                                              |                                                         |                                                              | 5                                         |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Sympetrum sanguineum</i>       | Ruddy Darter                 | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 6                                     |                                                             |                                                             |                                                 |
| <i>Synchaeta cecilia</i>          | Rotifer                      | Rotifera   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 1300                                      |                                           |                                                 |                                                           | 570                                      |                                       |                                                             |                                                             |                                                 |
| <i>Synchaeta pectinata</i>        | Rotifer                      | Rotifera   |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 11                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tachidius discipes</i>         | Copepod                      | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 850                                      |                                       |                                                             |                                                             |                                                 |
| <i>Tachypodoiulus niger</i>       | White-legged snake millipede | Annelida   |                                 |                                         |                                         |                                           |                                               |                                              | 19                                                      |                                                              |                                           |                                           | 8                                               |                                                           |                                          | 5                                     |                                                             |                                                             |                                                 |
| <i>Tanytarsus heusdensis</i>      | Non-biting midge             | Arthropoda |                                 |                                         |                                         |                                           |                                               |                                              | 9                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name                        | Common Name/Type       | Phyla          | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|-------------------------------------|------------------------|----------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Thalassiosira nordenskiöldii</i> | Diatom                 | Acillariophyra |                                 | 127                                     |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tipula lateralis</i>             | Crane fly              | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 5                                               |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tipula maxima</i>                | Crane fly              | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 24                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tipula oleracea</i>              | crane fly              | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 18                                       |                                       |                                                             |                                                             |                                                 |
| <i>Tipula paludosa</i>              | Crane fly              | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              | 7                                                       |                                                              |                                           |                                           |                                                 |                                                           |                                          | 17                                    |                                                             |                                                             |                                                 |
| <i>Tomocerus minor</i>              | Springtail             | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           | 27                                              |                                                           |                                          |                                       |                                                             | 14                                                          |                                                 |
| <i>Tomocerus vulgaris</i>           | Springtail             | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 15                                        |                                                 |                                                           |                                          |                                       |                                                             | 20                                                          |                                                 |
| <i>Trichobrychidae sp.</i>          | Worm                   | Annelida       |                                 |                                         |                                         |                                           |                                               | 6                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Trichoniscus pusillus</i>        | Common pygmy woodlouse | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 28                                        | 111                                             |                                                           | 5                                        |                                       |                                                             | 12                                                          |                                                 |
| <i>Trichoptera sp.</i>              | Caddisfly              | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 10                                    |                                                             |                                                             |                                                 |
| <i>Trioza urticae</i>               | Jumping plant louse    | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       | 19                                                          |                                                             |                                                 |
| <i>Trochulus hispidus</i>           | Hairy snail            | Mollusca       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 14                                    |                                                             |                                                             |                                                 |
| <i>Tubifex ignotus</i>              | Sludge worm            | Annelida       |                                 |                                         | 110                                     |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tubifex tubifex</i>              | Sludge worm            | Annelida       |                                 |                                         |                                         |                                           |                                               |                                              | 26                                                      |                                                              |                                           |                                           | 24                                              |                                                           |                                          | 39                                    |                                                             | 130                                                         |                                                 |
| <i>Tubificinae sp.</i>              | Worm                   | Annelida       |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 17                                    |                                                             |                                                             |                                                 |
| <i>Tubificoides benedii</i>         | Sludge worm            | Annelida       |                                 |                                         |                                         |                                           |                                               | 12                                           |                                                         |                                                              | 53                                        |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tubificoides fraseri</i>         | Segmented worm         | Annelida       |                                 |                                         |                                         |                                           |                                               | 7                                            |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tubificoides pseudogaster</i>    | Segmented worm         | Annelida       |                                 |                                         |                                         |                                           |                                               | 5                                            |                                                         |                                                              | 108                                       |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Tydeidae sp.</i>                 | Mite                   | Arthropoda     |                                 |                                         |                                         |                                           | 11                                            |                                              |                                                         |                                                              |                                           |                                           | 42                                              |                                                           |                                          |                                       | 18                                                          |                                                             |                                                 |
| <i>Typhlocybinae sp.</i>            | Leafhopper             | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              | 13                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Unclutred fungus</i>             | Fungi                  | Gyrsta         |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          | 16                                    |                                                             |                                                             |                                                 |
| <i>Valenzuela flavidus</i>          | Bark fly               | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              | 19                                                      |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 438                                             |
| <i>Willowsia nigromaculata</i>      | Springtail             | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              | 320                                                     |                                                              |                                           | 63                                        | 54                                              |                                                           |                                          |                                       |                                                             | 298                                                         |                                                 |
| <i>Xantholinus longiventris</i>     | Rove beetle            | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 5                                         |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Xenopelopia nigricans</i>        | Non-biting midge       | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 6                                                         |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Xenylla humiolca</i>             | Springtail             | Arthropoda     |                                 | 27                                      |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           | 5                                        |                                       |                                                             |                                                             |                                                 |
| <i>Xenylla szeptyckii</i>           | Springtail             | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 27                                        | 40                                              |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Zavrelia pentatoma</i>           | Non-biting midge       | Arthropoda     |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           |                                           |                                                 | 12                                                        |                                          |                                       |                                                             |                                                             |                                                 |

| Species Name     | Common Name/Type | Phyla |  | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt-188 (River Blythe Point 1) | LL-ARP_eDNA Pt-189 (River Blythe Point 2) | LL-ARP_eDNA Pt-192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------|------------------|-------|--|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| Total read count |                  |       |  | 11378                           | 10318                                   | 30923                                   | 41305                                     | 25941                                         | 14656                                        | 9154                                                    | 1471                                                         | 5061                                      | 1928                                      | 5204                                            | 35119                                                     | 8042                                     | 4125                                  | 8574                                                        | 2219                                                        | 3653                                            |

Each species present in an individual sample is shown as the number of sequence reads, where there is no entry the species was not present in the sample.

# APPENDIX 4 UNIONIDAE AND VENERIDAE METABARCODING RESULTS

| Species Name                 | Common Name            | LL-ARP_eDNA Pt 180 (Buss Creek) | LL-ARP_eDNA Pt 181 (River Wang Point 1) | LL-ARP_eDNA Pt 182 (River Wang Point 2) | LL-ARP_eDNA Pt 183 (River Wang Tributary) | LL-ARP_eDNA Pt 184 (River Wang ditch network) | LL-ARP_eDNA Pt 185 (River Wang feeder ditch) | LL-ARP_eDNA Pt 186 (River Wang Tributary ditch network) | LL-ARP_eDNA Pt 187 (River Wang Tributary at New Valley Farm) | LL-ARP_eDNA Pt 188 (River Blythe Point 1) | LL-ARP_eDNA Pt 189 (River Blythe Point 2) | LL-ARP_eDNA Pt 192 (River Blythe ditch network) | LL-ARP_eDNA Pt 193 (River Blythe Tributary ditch network) | LL-ARP_eDNA Pt 194 (Dunwich River Tidal) | LL-ARP_eDNA Pt 196 (Minsmere New Cut) | LL-ARP_eDNA Pt 197 (Minsmere New Cut ditch network Point 1) | LL-ARP_eDNA Pt 198 (Minsmere New Cut ditch network Point 2) | LL-ARP_eDNA Pt 199 (Minsmere New Cut Tributary) |
|------------------------------|------------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|-----------------------------------------------|----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|-----------------------------------------------------------|------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| <i>Barnea candida</i>        | White piddock          |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 31                                        |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Cerastoderma glaucum</i>  | The lagoon cockle      |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              | 33                                        | 10225                                     |                                                 |                                                           | 30                                       |                                       |                                                             |                                                             |                                                 |
| <i>Limecola balthica</i>     | Baltic clam            |                                 |                                         |                                         |                                           |                                               |                                              |                                                         |                                                              |                                           | 31                                        |                                                 |                                                           | 16                                       |                                       |                                                             |                                                             |                                                 |
| <i>Pisidium subtruncatum</i> | Short-ended pea mussel |                                 | 524                                     | 93                                      |                                           | 10                                            |                                              |                                                         |                                                              |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             |                                                 |
| <i>Pisidium personatum</i>   | Red-crusted pea mussel |                                 |                                         | 61                                      |                                           |                                               |                                              |                                                         | 4465                                                         |                                           |                                           |                                                 |                                                           |                                          |                                       |                                                             |                                                             | 8                                               |

Each species present in an individual sample is shown as the number of sequence reads, where there is no entry the species was not present in the sample.

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