# Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods

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**Abstract**

Organisms continuously release DNA into their environments via shed cells, excreta, gametes and decaying material. Analysis of this "environmental DNA" (eDNA) is revolutionising biodiversity monitoring. eDNA outperforms many established survey methods for targeted detection of single species, but few studies have investigated how well eDNA reflects whole communities of organisms in natural environments. We investigated whether eDNA can recover accurate qualitative and quantitative information about fish communities in large lakes, by comparison to the most comprehensive long-term gill-net dataset available in the UK. Seventy eight 2L water samples were collected along depth profile transects, gill-net sites and from the shoreline in three large, deep lakes (Windermere, Bassenthwaite Lake and Derwent Water) in the English Lake District. Water samples were assayed by eDNA metabarcoding of the mitochondrial 12S and cytochrome *b* regions. Fourteen of the 16 species historically recorded in Windermere were detected using eDNA, compared to four species in the most recent gill-net survey, demonstrating eDNA is extremely sensitive for detecting species. A key question for biodiversity monitoring is whether eDNA can accurately estimate abundance. To test this, we used the number of sequence reads per species and the proportion of sampling sites in which a species was detected with eDNA (i.e. site occupancy) as proxies for abundance. eDNA abundance data consistently correlated with rank abundance estimates from established surveys. These results demonstrate that eDNA metabarcoding can describe fish communities in large lakes, both qualitatively and quantitatively, and has great potential as a complementary tool to established monitoring methods.

## INTRODUCTION

Rapid monitoring of changes in biodiversity in response to climate change or other anthropogenic pressures is imperative, but the time and resources required to generate the necessary data are a major constraint in conservation management and ecological research. This is particularly relevant in large lake ecosystems, where for a number of taxa, established methods currently struggle to deliver the required data to fulfil legislative obligations such as the EC Water Framework (European_Communities 2000) and corresponding legislation elsewhere in the word. This difficulty is particularly marked for fish, for which all established sampling methods have various forms of bias (e.g. (Kubečka *et al.* 2009) and for which biological sampling is typically laborious and destructive (e.g. (Argillier *et al.* 2013). Arguably the biggest recent development in biodiversity monitoring is analysis of environmental DNA (eDNA), which refers to DNA released by organisms into their environment for example in the form of shed cells, excreta or decaying matter. eDNA has great potential for biodiversity monitoring since it is non-invasive, can detect rare or elusive species that are difficult to detect using established methods, and can distinguish cryptic species or juvenile stages that are difficult to identify taxonomically (as reviewed in (Bohmann *et al.* 2014; Lawson Handley 2015; Rees *et al.* 2015). Aquatic environments are particularly suited to eDNA analysis as DNA disperses rapidly in the water column and is more homogeneously distributed than in soil or other sediments.

The application of eDNA has so far largely focused on targeted detection of one or a few species using standard or quantitative Polymerase Chain Reaction (qPCR). Such targeted eDNA assays have proven highly successful for detecting individual species from a wide range of taxonomic groups in aquatic environments (see Table 1 in (Lawson Handley 2015) for a summary). For example, a recent eDNA study targeting great crested newts, *Triturus cristatus,* demonstrated high repeatability and substantially higher detection rates for eDNA compared to established survey methods (Biggs *et al.* 2015). The characterisation of entire communities is not feasible using such species-specific approaches due to the complexity of most ecosystems. An alternative approach is to simultaneously screen whole communities of organisms using eDNA metabarcoding. Here, community DNA is PCR-amplified using broad range primers, and sequenced on a High Throughput Sequencing (HTS) platform (reviewed by Lawson Handley 2015). Direct

metabarcoding of homogenized community samples is revolutionising our understanding of the diversity of microscopic eukaryotes (Bik *et al.* 2012) in environments that are notoriously difficult to study, such as soil (Creer *et al.* 2010), and the deep sea (Fonseca *et al.* 2010). Metabarcoding of macrobial eDNA is still in its infancy, but the field is moving forward at a fast pace. The first studies focussed on describing fish communities in tanks or aquaria (Evans *et al.* 2015; Kelly *et al.* 2014; Mahon *et al.* 2014; Miya *et al.* 2015) or on a small scale in natural settings (Thomsen *et al.* 2012a; Thomsen *et al.* 2012b). Recent refinements of the method, including more rigorous testing in aquaria (Miya *et al.* 2015) and in marine (Miya *et al.* 2015; Valentini *et al.* 2015), and freshwater habitats (Valentini *et al.* 2015) have confirmed the method is extremely sensitive for detecting rare species, and describing presence/absence. Important questions remain though about the efficacy of eDNA metabarcoding for obtaining accurate estimates of species abundance and biomass. Obtaining quantitative estimates from eDNA is challenging because of the large number of factors that influence DNA dynamics in the environment (reviewed by (Barnes *et al.* 2014; Lawson Handley 2015) and because of the many opportunities for bias during laboratory steps (sampling, DNA extraction, PCR), sequencing and bioinformatics stages (Ficetola *et al.* 2015; Yu *et al.* 2012). In metabarcoding studies, in principle, the number of sequences per taxon (or "operational taxonomic unit") could be taken as an estimator of species biomass, but unfortunately in practice, this relationship is not a simple one. For example, (Kelly *et al.* 2014) demonstrated a perfect correlation between rank abundance of eDNA sequences representing four fish genera and rank biomass in a large aquarium, but the actual number of sequence reads was not correlated to biomass. Similarly, Evans *et al.* (2015) found only a modest positive relationship between the number of sequence reads and abundance of eight fish and one amphibian species in mesocosm experiments. A second approach that may be more promising for estimating abundance is to carry out comprehensive spatial and temporal sampling of a given environment and calculate the proportion of sites in which a species is detected with eDNA. Such "site occupancy" data is often collected in ecological studies and can be used as a proxy for abundance (MacKenzie & Nichols 2004; MacKenzie *et al.* 2002). Recent studies indicate this approach could be very promising for analysing eDNA data from both targeted assays (Hunter *et al.* 2015; Pilliod *et al.* 2013; Schmidt *et al.* 2013), and metabarcoding data (Valentini *et al.* 2015).

How well eDNA metabarcoding performs compared to established survey methods for generating both qualitative (presence/absence) and quantitative (abundance/biomass) data remains a key question in the development of the technology for biodiversity monitoring. Here, we addressed this question by comparing eDNA metabarcoding data to the most comprehensive long-term data available for lake fish populations in the UK. We carried out rigorous spatial sampling in three large, deep lakes (Windermere, Bassenthwaite Lake and Derwent Water) in the English Lake District, which are the best-studied lakes in the UK in terms of their fish fauna. Firstly, we developed a workflow for lake fish eDNA metabarcoding, which included building an appropriate reference database of mitochondrial 12S and cytochrome *b* (CytB) genes, testing primer combinations, and developing pipelines for eDNA analyses from sampling to bioinformatics. Second, we carried out water sampling along depth-profile transects, at gill-net survey sites and at shoreline locations within the lakes. Finally we compared the qualitative and quantitative results from eDNA metabarcoding with long-term and recent gill-net survey datasets to investigate the performance of eDNA against established methods.

## MATERIAL AND METHODS

### *Sampling*

Sampling was carried out in three natural lakes (Bassenthwaite Lake, Derwent Water and Windermere) in the English Lake District, UK, that have been intensively studied in terms of their fish populations, physio-chemical and other biological properties for many years (Maberly *et al.* 2011, Fig. 1). Fish populations in these three lakes have been monitored since the early 1990s (Bassenthwaite Lake and Derwent Water, e.g. (Winfield *et al.* 2012a; Winfield *et al.* 2015b) or early1940s (Windermere, e.g. (Winfield *et al.* 2008a; Winfield *et al.* 2015b). This monitoring has been performed using gill netting, trapping, hydroacoustics or analysis of recreational anglers' catches and constitutes the best long-term lake fish datasets in the UK. Windermere, England's largest natural lake (surface area 1480 ha, maximum depth 64 m), is composed of two distinct basins with different physical, chemical and ecological characteristics (North Basin: surface area of 810 ha, maximum depth 64 m, mesotrophic; South Basin: surface area 670 ha, maximum depth 44 m, eutrophic). Bassenthwaite Lake (surface area 528 ha, maximum depth 19 m, eutrophic) and

147 Derwent Water (surface area 535 ha, maximum depth 22 m, mesotrophic) are also among
148 the largest lakes in England and are linked by the River Derwent.

149

150 In total 30 offshore samples were collected from each of the two Windermere basins.
151 Additionally, six samples were collected opportunistically from a small area of the
152 shoreline at the Northern end of the South Basin. Water samples were collected from
153 Windermere during 28$^{th}$ – 30$^{th}$ January 2015. Most offshore samples were collected along
154 three transects with approximately 1 km sampling interval between sites. Transects 1, 2
155 and 3 run along the 5m, 20m depth contour and the lake midline respectively (Fig. 1). The
156 sampling depth for transect 1, 2 and 3 was 2 m, 10 m and 20 m respectively. This sampling
157 scheme covered 7 of the 10 sites that are used for annual gill net surveys (Winfield *et al.*
158 2015b). Water samples were also collected at the 3 remaining gill net sites (Fig. 1). At the
159 deepest point along the midline transect in both North (approximate depth 64 m) and South
160 Basin (approximate depth 44 m) a depth profile was collected. The North Basin depth
161 transect was collected at 0-10-20-30-40-50-60 m depth and the South Basin depth transect
162 was collected at 0-10-20-30-40 m. (Fig. 1). Water samples were also collected at 5 gill net
163 sites (Winfield *et al.* 2015a) and one shore site per lake at both Bassenthwaite Lake and
164 Derwent Water (Fig. 1) on 10$^{th}$ February 2015. The total number of samples (excluding
165 blanks) was therefore $N$=78.

166

167 Offshore water sampling was carried out by boat using a Friedinger (Windermere) or
168 Ruttner (Bassenthwaite Lake and Derwent Water) sampler (Fig. S1) deployed at a
169 specified depth. For each 2 L water sample, five 400 ml subsamples were collected in
170 proximity of 100 m around the sampling point, and pooled in a sterile plastic bottle (Fig.
171 S1). The GPS location was recorded at the sampling midpoint (Appendix 1 and 2).
172 Between samples, sampling equipment was sterilised by washing in 10% of a commercial
173 bleach solution (containing <3% sodium hypochlorite) followed by 10% microsol
174 detergent (Anachem, UK) and rinsed with purified water (Fig. S1). The sampler was then
175 rinsed again in lake water at the next sampling location. 2 L of purified water was rinsed
176 through the sampler following decontamination after every 5 samples, and the water
177 retained as a sampling blank to allow us to check for contamination during sampling.

Shoreline samples were collected by immersing a sterile 2 L plastic bottle by hand. For each sample, five 400 ml samples were collected from within a 100 m stretch of shoreline and pooled. All samples were stored in an insulated box at approximately 4 °C until filtration.

***eDNA capture, extraction, amplification, library preparation and sequencing***

The full 2 L of each sample was filtered through sterile 0.45 µm cellulose nitrate membrane filters and pads (47 mm diameter; Whatman, GE Healthcare, UK) using Nalgene filtration units in combination with a vacuum pump (Fig. S1). Most samples required one filter and filtered in less than an hour. For more turbid and thus slow to filter samples, a second filter was used. Filtration equipment was sterilized in 10% commercial bleach solution for 10 minutes then rinsed with 10% microsol and purified water after each filtration. Filtration blanks (2 L purified water) were run before the first filtration and then approximately after every sixth sample, in order to test for contamination at the filtration stage. Windermere samples were filtered within 8 hours of collection in a lakeside laboratory (within the facilities of the Freshwater Biological Association, Windermere) that is not used for handling fish or DNA and was decontaminated before use by bleaching floors and surfaces. Samples from Bassenthwaite Lake and Derwent Water were filtered in a dedicated eDNA facility at the University of Hull within 12 hours of collection. Detailed operating procedures are in place in our eDNA laboratory which are aimed at avoiding contamination and access to the laboratory is strictly limited to staff who are familiar with these procedures. DNA was extracted from filters using the PowerWater DNA Isolation Kit (MoBio Laboratories, Inc. Carlsbad, USA) using the manufacturer's instructions.

Full details of the steps involved in reference database construction, *in silico* and *in vitro* primer testing, including PCR conditions, are given in the Supplementary Text. Briefly, we compiled custom, phylogenetically curated reference databases (Supplementary Text and Fig. S2) for standard mitochondrial fish DNA barcoding genes (12S and cytochrome *b*) for 67 freshwater fish species including all those recorded in the UK and additional non-native species that could potentially be present (Table S1). A number of published primers (Table S2) were evaluated against these databases *in silico* for conservation of primer binding

209 sites and species resolution of the resulting PCR amplicons (Table S3) using the program
210 EcoPCR (Ficetola *et al.* 2010). Two previously published primer pairs, which amplify
211 fragments of contrasting length, from two different mtDNA regions, were selected for
212 metabarcoding, since no single primer pair resolved all species (Table S3). The primer pair
213 12S_F1 and 12S_R1 (Table S2) amplifies a ~106 bp fragment of the mitochondrial 12S
214 gene. These primers were designed and tested *in silico* (Riaz *et al.* (2011) and used in a
215 large marine mesocosm eDNA metabarcoding study of bony fish communities (Kelly *et al.*
216 2014). The second selected primer pair, CytB_L14841 and CytB_H15149 (Table S2)
217 amplifies a 460bp fragment of the cytochrome *b* gene (CytB) gene and has been used
218 commonly for standard DNA barcoding of fishes (Kocher *et al.* 1989). Selected primer
219 pairs were then tested *in vitro* on 22 species, firstly in individual reactions (Fig. S3) to
220 check consistency of amplification across taxa, and secondly in 10 mock communities to
221 evaluate whether all species amplified in competitive mixed assemblages. Mock
222 communities were generated from spectrophotometer-quantified DNA extractions of same
223 22 species (Supplementary Text and Table S4) and community samples were sequenced
224 via metabarcoding as detailed below.

225

226 Samples for metabarcoding were PCR amplified with a one-step library preparation
227 protocol using, for each locus, 8 individually tagged forward primers and 12 individually
228 tagged reverse primers allowing for 96 uniquely dual-indexed combinations (Kozich *et al.*
229 2013). All collection and extraction blanks were included in PCRs and contamination
230 during PCR was evaluated by "amplifying" all 96 combinations of tagged primers with
231 purified water and checking on ethidium bromide-stained agarose gels. PCRs were
232 replicated three times for each sample, and pooled in order to minimise bias in individual
233 PCR reactions (see Supplementary Text for full PCR conditions). Each library was
234 normalised to approximately 1–2 ng/µl PCR product per sample using the SequalPrep
235 Normalization Plate Kit (Invitrogen, Life Technologies) and samples subsequently pooled.
236 Libraries were then quantified by qPCR (average of three replicate quantifications) using
237 the KAPA Illumina Library Quantification Kit on a Roche LightCycler Real-Time PCR
238 machine using manufacturers guidelines. Libraries were run at a 6 pM concentration on an
239 Illumina MiSeq using the 2 x 300 bp V3 chemistry. In order improve clustering during the
240 initial sequencing cycles 10% of PhiX genomic library was added.

241

242 ***Bioinformatics and data analysis***

243 The program Trimmomatic 0.32 (Bolger *et al.* 2014) was used for quality trimming and
244 removal of adapter sequences from the raw Illumina reads. Average read quality was
245 assessed in 5 bp sliding windows starting from the 3'-end of the read and reads were
246 clipped until the average quality per window was above phred 30. All reads shorter than a
247 defined minimum read length (12S - 90bp; CytB - 100bp) were discarded. Sequence pairs
248 were subsequently merged into single high quality reads using the program FLASH 1.2.11
249 (Magoč & Salzberg 2011). The remaining reads were screened for chimeric sequences
250 against the curated reference databases using the 'uchime_ref' function implemented in
251 vsearch 1.1 (https://github.com/torognes/vsearch). To remove redundancy, sequences were
252 clustered at 100% identity using vsearch 1.1 (https://github.com/torognes/vsearch).
253 Clusters represented by less than 3 sequences were considered sequencing error and were
254 omitted from further analyses. Non-redundant sets of query sequences were then compared
255 to the respective curated non-redundant reference database using BLAST (Zhang *et al.*
256 2000). BLAST output was interpreted using a custom python function, which implements a
257 lowest common ancestor (LCA) approach for taxonomic assignment similar to the strategy
258 used by MEGAN (Huson *et al.* 2007). In brief, after the BLAST search we recorded the
259 most significant matches to the reference database (yielding the top 10% bit-scores) for
260 each of the query sequences. If only a single taxon was present in the top 10%, the query
261 was assigned directly to this taxon. If more than one reference taxon was present in the top
262 10%, the query was assigned to the lowest taxonomic level that was shared by all taxa in
263 the list of most significant hits for this query. Sequences for which the best BLAST hit had
264 a bit score below 80 or had less than 100% / 95% identity (12S / CytB) to any sequence in
265 the curated database, were considered non-target sequences. The custom bioinformatics
266 pipeline used for data processing is available on Github (https://github.com/HullUni-
267 bioinformatics/metaBEAT). To assure full reproducibility of our analyses we have
268 deposited the entire workflow in an additional dedicated Github repository
269 (https://github.com/HullUni-bioinformatics/Haenfling_et_al_2016). In order to obtain a
270 qualitative assessment of the taxonomic diversity, non-target sequences were pooled across
271 all lake samples and subjected to a separate BLAST search against NCBI's complete

nucleotide (nt) database. Taxonomic assignment for non-target sequences was obtained using MEGAN 5.10.6 (Huson *et al.* 2007).

Filtered data were summarised in two ways for downstream analyses: 1) the number of sequence reads per species at each site (hereon referred to as read counts) and 2) the proportion of sampling sites in which a given species was detected (hereon referred to as the site occupancy). To reduce the possibility of false positives, we only regarded a species as present at a given site if its sequence frequency exceeded a certain threshold level (proportion of all sequence reads in the sample). The choice of threshold level was guided by the analysis of sequence data from the mock communities and is explained in full in the Supplementary Text (and corresponding Tables S4, S5 and Figs S5 and S6). This analysis revealed that threshold levels of 0.3% and 1% were required for 12S and CytB respectively to omit all false positives in the mock communities (hereon referred to as Th100, Tables S4, S5 and Fig. S5). At Th100 sequences of rare expected species were also lost from the mock community data (Tables S4 and S5) and the lake samples (Fig. S6). We therefore decided to apply slightly less conservative values of 0.1% and 0.2% for 12S and CytB respectively, at which over 90% of false positives were omitted in the mock communities to the main analysis of lake samples (Th90). We also investigated the potential extent of contamination from tag jumping in our libraries by exploring the distribution of PhiX assigned to target samples (see Supplementary Text and Fig. S7 for full details). The level of PhiX contamination in our samples also indicated that our thresholds were appropriate to eliminate most of false positives created during the sequencing process. In 95% of the 12S and CytB libraries the proportion of PhiX did not exceed 0.0015 and 0.001 respectively (with a corresponding maximum of 0.0023 and 0.0201).

All downstream analyses were performed in R v.3.1.3. (RCoreTeam 2015). Before investigating species detection and abundance estimation with eDNA, we first evaluated whether 12S and CytB datasets produced consistent results by calculating the Pearson product-moment correlation coefficient for both read count and site occupancy in R v.3.1.3. (RCoreTeam 2015).

A flow chart summarising of our analytical pipeline, from reference database compilation to data analyses is provided in Appendix 5 of the Supplementary Online Material.

***Species detection using eDNA***

In order to maintain a balanced sampling design, the Windermere shore sites which were only collected in a small area of the South basin, were excluded from all comparisons of species presence and abundance comparisons across basins.

First, we evaluated the performance of eDNA to detect species previously recorded in our four lake basins. Second, we used site occupancy data to investigate the spatial distribution of eDNA records within Windermere. It should be noted that full site occupancy modelling requires temporal replication to estimate the detection probability and the true proportion of occupied sites (MacKenzie *et al.* 2002). This was not possible during the current study, so our estimates of site occupancy are simply based on presence/absence, and should be treated as preliminary. We explored whether there were differences in eDNA distribution between transects, between offshore and shoreline samples, along depth profiles, and between Windermere North and South Basins. A persistent difference in species composition between the two Windermere basins has been extensively described by established sampling methods and is linked to their contrasting trophic status (Winfield *et al.* 2008a; Winfield *et al.* 2012b; Winfield *et al.* 2008b). eDNA records from species with no preference for trophic state are consequently expected to be distributed throughout the lake, whereas eDNA from eutrophic-favouring species will be more predominant in the south than north basin and eDNA from species that prefer less eutrophic conditions will be more predominant in the north than south basin. Finally, we used sample-based rarefaction (Gotelli & Colwell 2010) to determine the number of samples needed to detect species present, focussing on Windermere, where sampling was spatially comprehensive. Rarefaction was performed with 499 randomisations in the R package Vegan (Oksanen *et al.* 2015) for CytB and 12S for the North and South Basins of Windermere combined. Only sequences corresponding to the 16 species previously recorded in Windermere were included in these analyses.

***Comparison of data from eDNA and established survey methods***

Summaries of fish community composition and abundance were produced for each of the four lake basins using a combination of data collected at six sites in each of our four lake basins in September 2014 using standardised survey gill-netting techniques (described in

detail by (Winfield *et al.* 2015a) and (Winfield *et al.* 2015b). Gill-net survey data alone are not sufficient to describe the whole fish community since this technique under-samples or even fails to record some species, even when they are locally abundant (e.g. those with an extremely shallow distribution such as bullhead, *Cottus gobio*, or elongate morphology such as eel, *Anguilla anguilla*). Gill-net data were therefore supplemented with published information (Maberly *et al.* 2011; Pickering 2001; Winfield *et al.* 2012a; Winfield *et al.* 1996; Winfield & Durie 2004; Winfield *et al.* 2010; Winfield *et al.* 2008b) to summarise fish community compositions. This information and IJW's expert opinion developed during 25 years of sampling the four lake basins was then used to assign each recorded species to an abundance rank, with a rank of 1 given to the most abundant species by numbers. The ranking produced in this way is likely to be very robust for the most abundant species which consistently appeared in the catches of the survey gill nets, but is likely to be less so for a few species which anglers' catches indicate are present in small numbers in each lake but which are very rarely or never recorded by scientific sampling. This entire expert opinion ranking process was undertaken prior to the eDNA analysis and therefore with no knowledge of the corresponding rankings. Further details of the results from established surveys are provided in the Supplementary Text and Table S5.

A series of correlations was performed to compare the fish abundance data generated from established surveys and eDNA metabarcoding. Specifically, the relationship between eDNA data (read count and site occupancy) and data from established surveys (rank abundance or biomass based on long term expert opinion or actual numbers from September 2014 gill-net surveys) was investigated by calculating Spearman's Rho (for rank correlations) and Pearson's Product-moment correlation coefficient (for actual numbers, when data was normally distributed) in R v3.1.3 (R Core team 2015). The analyses were repeated for both loci and all four sampled basins.

A work flow diagram of our entire approach is available as electronic Appendix 5.

## RESULTS

The *in silico* testing of primer pairs showed that both of the chosen 12S and CytB fragments could unambiguously distinguish all species which could potentially occur at the

study sites (Table S1 and S3). However, across the wider reference database a number of taxa could not be identified to the species level. *Lampetra planeri* and *L. fluviatilis,* which are probably not reproductively isolated, could not be resolved by either fragment. Additionally, 12S did not distinguish species of the genera S*alvelinus* and *Coregonus*, three species of non-native Asian carp *(Hypophthalmichthys nobilis, H. molitrix, Ctenopharyngodon idella)* and two species of the family Percidae (*Perca fluviatilis* and *Sander lucioperca).* However, given that Percidae and the genera *Coregonus* and *Salvelinus* are represented only a single species each (*Perca fluviatilis, Salvelinus alpinus and Coregonus albula* respectively) in the study area we have attributed sequence counts for the higher taxonomic levels to these individual species for further downstream analysis. This was also confirmed by the CytB data which showed that no other members of these taxonomic groups were present. Both loci amplified consistently well across 22 target species in *in vitro* testing in single species amplifications (Fig. S3). All 22 species were detected in the 12S mock communities (Table S4, Fig. S4 a), whereas three species were not detected in the CytB mock community data (Table S5, Fig. S4 b and Supplementary Text for full details). Observed and expected number of sequence reads were not significantly different for either locus (12S $\chi^2 = 0.224$, df = 21, $P > 0.05$; CytB $\chi^2 = 0.367$, df = 21, $P > 0.05$ Fig. S4). Moreover, there was a significant correlation between the number of sequence reads/ng PCR template DNA for 12S and CytB (Pearson's $r = 0.599$, df = 20, $P = 0.01$, Fig. S4 c),

Clear PCR bands were obtained for all 78 eDNA samples at both loci. In contrast no target-sized bands were observed in the PCR negatives, collection or filtration blanks and we therefore decided not to sequence these. The total sequence read count passing quality control per library, before removal of chimeric sequences, was 6,306,326 for 12S and 4,793,108 for CytB (average read count per sample 71663 and 54467 respectively). After chimera removal, the 12S and CytB libraries contained 2,698,144 and 3,161,608 sequences respectively. This means that 43% of the raw dataset was non chimeric sequences for 12S, and 66% for CytB. The final libraries, after removal of redundant sequences, contained 2,562,183 sequences for 12S and 3,012,249 sequences for CytB, with average read counts per sample of 29,116 and 34,230 respectively. The proportion of target (fish) sequences ranging from 3.4-88.3% (average 23.5%) and 0-100% (average 49.0%) for 12S and CytB

respectively. Most of the target sequence assignments in the lake samples were to species level with the exceptions mentioned above. The assignments to higher taxonomic levels were taken into account for calculation of total sequences read number per sample but otherwise not considered for further downstream analysis. For the CytB data of the mock communities some genus level sequence assignments were interpreted as belonging to specific species (for full details see Supplementary text and Table S5). The full sequence count data for each primer pair are available in the Supplementary Material Appendix 1 and 2).

High consistency was found between CytB and 12S in terms of both site occupancy (SO) and average read count (RC) (Fig. S8). Data from the two loci were significantly correlated (Pearson's $r$ consistently $P < 0.05$) for all basins, for both SO and RC (Fig. S8). Consistent significant correlations were also found between SO and RC for each basin and locus (Fig. S9), therefore only the results for site occupancy are presented in the following main text. All results based on read count data are provided in the Supplementary Material.

***Species detection using eDNA***

The gill-net survey of September 2014 detected 25% (4/16) of the previously recorded species in Windermere. By contrast, 14 of the 16 previously recorded species (i.e. 88%) were detected using 12S and 75% (12/16) using CytB across the entire lake. Within each Windermere basin 13 previously-recorded species were detected with 12S whereas 12 and 11 species were detected for the North and South Basins respectively with CytB (Fig. 2 a, b; Fig. S10). A number of additional species were also detected in Windermere, including *C. carpio*, *Gymnocephalus cernuus*, *Leucaspius delineatus*, *O. mykiss*, *Osmerus eperlanus* (12S), *Platichthys flesus* and *Pseudorasbora parva* (CytB). Two species that have been recorded in Windermere but are not present in the sequence data are the two lamprey species *L. fluviatilis* and *Petromyzon marinus*. In the 12S data set the majority of potential false positives were found in a single sample from Windermere North Basin which was consequently omitted from all further analysis (sample W14). Gill-net sampling detected 60% (6/10) of the species known to be present in Bassenthwaite Lake whereas 90% (9/10) of species were detected using 12S and 70% (7/10) with CytB (Fig. 2 c; Fig. S10).

Additional species not previously recorded in Bassenthwaite included *Abramis brama* (CytB), and *Barbatula barbatula*, *G. aculeatus,* and *S. erythrophthalmus* (12S, Fig. 2 c). In Derwent Water, gill-net sampling in September 2014 detected 77% (7/9) recorded species, whereas 88% (8/9) of species were detected with 12S and 67% (6/9) with CytB (Fig. 2 d; Fig. S10). The 12S assay detected an additional four species previously unrecorded, including *B. barbatula*, *G. aculeatus*, *Pungitius pungitius* and *S. erythrophthalmus*.

Sample-based rarefaction analyses on the combined Windermere data set indicated that approximately 10-25 samples captures the majority (~85%) of the taxa present in the entire sample although the number of samples required to achieve the same taxon coverage is higher for CytB (Fig. 3).

***Estimating abundance with eDNA***

There was a consistent, negative relationship between eDNA site occupancy and long-term rank (where rank abundance decreases from 1-16) and this correlation is highly significant for Windermere North and South Basins, for both loci (Fig. 4 a, b, e, f). Similar trends were found for Bassenthwaite Lake and Derwent Water but correlations were not significant (Fig. 4 c, d, g, h). The number of sequence reads was also significantly correlated with long-term rank in Windermere North and South Basins, for both loci (Fig. S11 a, b, e, f). Again similar trends were seen for Derwent Water and Bassenthwaite Lake but only the correlation for Derwent Water at 12S is significant (Fig. S11 c, d).

Site occupancy and number of sequence reads were also compared against actual numbers sampled in the September 2014 gill-net surveys for all four basins (Figs S12 and S13 respectively). There was a consistent positive relationship between abundance data from the recent gill-net surveys and eDNA (both read count and occupancy, and both loci), in spite of the small number of species (4-6) detected in the gill net surveys and hence low statistical power in the analyses. However only the correlations for CytB read count were consistently significant in all basins (Fig. S13 e-h), and this result may be driven by the high abundance and read count for *P. fluviatilis*.

458

459 ***Spatial distribution of eDNA records within Windermere***

460 Comparing the distribution of eDNA data by transect indicates a slight trend for more
461 species to be detected at inshore versus deeper mid-lake regions (Fig. 5). With 12S, 13
462 species were detected in samples from the 5 m transect compared to 10 from the mid-line.
463 Twelve species were detected in the 6 geographically-close shore samples. A similar trend
464 was found for CytB, with 11 species detected in both 5 m transect and shore samples,
465 compared to 8 in the mid-line (Fig. 5). Depth profiles in the North and South Basins
466 revealed that eDNA from the majority of detected species was distributed throughout the
467 water column (Fig. S14). Within the depth profiles, *A. anguilla* and *S. alpinus* were only
468 detected in deep water in the North Basin (≥60 m and 30 m respectively, Fig. S14 a and c).
469 Similarly, in the South Basin depth profile *P. phoxinus* and *S. salar* were only detected at
470 the deepest sampling point (40 m) (Fig. S14 b and c).

471

472 Site occupancy data based on 12S sequences were used to investigate the spatial
473 distribution of each species recorded at more than two sites around Windermere (Fig. S15).
474 The general pattern emerging from this analysis is that species-specific eDNA was not
475 evenly distributed around the lake. Although some species such as *P. fluviatilis*, *R. rutilus*,
476 *E. lucius* and *S. trutta*, are recorded almost ubiquitously within the lake, eDNA from other
477 species is predominantly found in one of the two basins. *S. alpinus*, *P. phoxinus* and *G.*
478 *aculeatus* eDNA was common in the North Basin but very rare in the South Basin, whereas
479 *A. brama* and *A. anguilla* eDNA is more common in South Basin (Fig. S15). Overall the
480 relative proportion of sequence read counts for different species across sample sites was
481 significantly different between Windermere North and South Basins ($\chi^2 = 47817$; df = 13;
482 $P < 0.001$ and $\chi^2 = 134750$; df = 11; $P < 0.001$ for 12S and CytB respectively, Fig. 6 a, b).
483 A similar pattern was observed for the relative proportion of sites occupied ($\chi^2 = 61.43$; df
484 = 13; $P < 0.001$ and $\chi^2 = 48.65$; df = 11; $P < 0.001$ for 12S and CytB respectively Fig. 6 c,
485 d). Distribution of eDNA reflected in the two Windermere Basins reflected the expected
486 association between species and ecological condition. eDNA from species associated with
487 eutrophic conditions (*R. rutilus*, *T. tinca, S. erythrophthalmus*, *A. brama,* and *A. anguilla*)
488 was more abundant in the South than North Basin, while eDNA from species that prefer

489 less eutrophic conditions (*S. salar, S. trutta, S. alpinus, P. phoxinus,* and *C. gobio*) was
490 more abundant in the North than South Basin (Fig. 6).

491

492 ***Non-fish sequences***

493 A large proportion of both 12S and CytB sequences could not be assigned to UK
494 freshwater fish from the custom database, and were compared to the NCBI database using
495 BLAST. Non-fish sequences included a wide range of species directly associated with
496 aquatic habitats including mammals such as otter, *Lutra lutra* and birds, including
497 moorhen, *Gallinula chloropus*; cormorant, *Phalacrocorax carbo* and various duck and
498 geese species found within the UK. The list also included many other vertebrate species
499 potentially occurring in the wider catchment area (Table S6) including domesticated farm
500 animals such as cow, *Bos taurus*; sheep, *Ovis aries* and chicken, *Gallus gallus domesticus*,
501 and wild vertebrates such as red deer, *Cervus elaphus*; red squirrel, *Sciurus vulgaris*; red
502 fox, *Vulpes vulpes* and tawny owl, *Strix aluco*. Sequences assigned to *Homo sapiens* were
503 also abundant, likely present as genuine eDNA found in lake water due to the high degree
504 of human interaction with the lakes through water sports, angling and waste water, or
505 present as a laboratory contaminant. The primers appear to be largely vertebrate specific,
506 except for low-level amplification of bacterial 16S detected in the 12S dataset. No
507 invertebrate sequences were identified.

508

509

## DISCUSSION

In this study we used high-throughput sequencing of eDNA from the mitochondrial 12S and CytB genes to characterise the fish community composition in three large lakes (Lake Windermere, Derwent Water and Bassenthwaite Lake) in the UK. eDNA data was compared to comprehensive long-term data on fish distribution and abundance from established survey methods. eDNA outperformed established methods in terms of species detection. More surprisingly, eDNA data accurately reflected the rank abundance of species within the lake fish community, suggesting eDNA methods may be more quantitative than previously thought.

### *Comparison of of eDNA and established methods for species detection*

eDNA metabarcoding was effective in detecting fish species when compared against decades of data from established sampling techniques and other sources (as described most recently by Winfield *et al.* 2015a and Winfield *et al.* 2015b). In Windermere, 60 offshore (30 for each basin) and 6 shoreline samples were analysed and 14 of the 16 previously-recorded species were detected. The two rarest species, river lamprey, *L. fluviatilis* and sea lamprey, *P. marinus,* were not detected in the eDNA data, but these species were unlikely to be present in the lakes at the time of sampling and temporally replicated sampling is required to address this issue. Other rare species such as tench, *T. tinca* and rudd, *S. erythropthalmus* were detected at low levels with 12S in the North and South Basins respectively. The results of the rarefaction analysis on the Windermere data indicate that a detection probability of over 85% can be achieved with a substantially lower number of samples; approximately 10 for 12S and 25 for CytB. In contrast, only the four most common species were detected in the gill net survey from 2014, which is typical of surveys (4-5 species have been typically sampled each year since 2011, Winfield *et al.* 2012c; Winfield *et al.* 2013; Winfield *et al.* 2014).

The eDNA results from Bassenthwaite Lake and Derwent Water were also remarkably concordant with the fish community based on long-term gill-netting (Thackeray *et al.* 2006) given that only six samples were collected per lake. All but the rarest species were detected in Derwent Water and Bassenthwaite (dace, *L. leuciscus*, and vendace, *C. albula*

541 respectively) using 12S. Dace was however detected in Bassenthwaite, and vendace in
542 Derwent Water with 12S, while neither species was detected with CytB. Dace has been
543 recorded intermittently and in low numbers in Derwent Water within the last decade
544 (Thackeray *et al.* 2006) but was not detected by gill netting in 2014 (Winfield *et al.*
545 2015a). Vendace is known to occur only in a restricted deep area of Bassenthwaite Lake
546 and only three individuals have been recorded in gill-net surveys since 2000 (Winfield *et*
547 *al.* in press). In these cases DNA concentration might fall below the detection threshold of
548 the PCR assay or those which were set for the bioinformatics analysis in order to reduce
549 the possibility of "false positives". Roach, *R. rutilus*, on the other hand, is a common
550 species in all four basins, but was not detected with CytB in Bassenthwaite and Derwent
551 Water. This species was also detected in the CytB mock community at lower than expected
552 frequency, suggesting that the CytB primers may not amplify this species well in
553 competitive reactions.

554

555 Overall, eDNA metabarcoding data produced a more comprehensive species list than gill
556 net surveys with a similar effort. The under-representation of species in gill-netting surveys
557 is an acknowledged sampling artefact which has a number of causes including fish
558 morphology (e.g. eel species are not susceptible to retention in gill nets), fine-scale spatial
559 distribution (e.g. three-spined stickleback may be limited to the extreme inshore where nets
560 cannot be deployed) or movement patterns (e.g. bullhead may be unlikely to be sampled by
561 gill nets due to their relatively limited movements). This corroborates results from
562 Thomsen *et al.* (2012a) and Valentini *et al.* (2015) who showed that eDNA metabarcoding
563 data detected more species of marine fish than alternative surveying techniques.

564

565 ***Detection of previously unrecorded species with eDNA***

566 Eight previously unrecorded species were detected in Lake Windermere, four in
567 Bassenthwaite Lake and four in Derwent Water. In most cases these eDNA records were at
568 very low occupancy (1 or 2 sites) and read counts (0.1%-1.0%), just above our threshold
569 for accepting a positive record. These records could be either genuine detections of species
570 that have been missed with established methods, false positives from sequencing error
571 (barcode misassignment, Deakin *et al.* 2014; or "tag jumps" Schnell *et al.* 2015),

572 laboratory or environmental contamination (i.e. the presence of DNA in the environment
573 from, for example, the wider watershed, bird faeces, waste water or fishing bait). The
574 unexpected records likely originate from a combination of factors, discussed below.

575

576 Only one of the eight previously unrecorded Windermere species, ruffe, *G. cernua,* was
577 detected at high frequencies with eDNA. 12S sequences were present in 27% of the sites in
578 the South Basin and 38% of the sites in the North Basin although the species was not
579 detected with CytB. This species has been recently introduced to a number of Cumbrian
580 lakes (Winfield *et al.* 2010), and is present in Rydal Water approximately 3 km upstream
581 of Windermere. It is therefore possible that *G. cernua* has colonised Windermere and is
582 present at very low abundance (below the detection limits of gill-netting programme), or
583 that eDNA has been transported from the *G. cernua* populations upstream. Three
584 kilometres is well within the range of eDNA transport distances that have previously been
585 recorded (Deiner and Altermatt 2015). Absence of positive records with the long CytB
586 fragment also suggests that only relatively degraded *G. cernua* DNA was present in the
587 lake, lending further support to this hypothesis. Although this species was present in the
588 mock communities, the high frequency of occurrence means it is unlikely that this result
589 can be explained by sequencing errors such as barcode misassignment.

590

591 The other seven previously-unrecorded Windermere species (common carp, *C. carpio;*
592 sunbleak, *L. delineates*; topmouth gudgeon, *P. parva*; rainbow trout, *O. mykiss*; smelt, *O.*
593 *eperlanus*; flounder, *P. flesus* and mudminnow, *U. pygmea*) were detected at very low
594 levels. The actual presence of *U. pygmea*, *L. delineates* and *P. parva*, in Windermere
595 seems extremely unlikely since their known distribution does not overlap with the
596 Windermere catchment. Given that all three species were included in the mock
597 communities these records are most likely explained by low level laboratory contamination
598 or sequencing barcode misassignment from the mock communities into the samples
599 (Deakin *et al.* 2014). *O. mykiss, O. eperlanus* and *P. flesus*, do occur in the catchment and
600 the former two species are also a very popular dead bait used by pike anglers. Since none
601 of these species have been handled in the laboratory and pike anglers were active during
602 the water sampling, it seems that such dead baiting or eDNA transport from other parts of

603 the catchment are likely sources of eDNA for these species in the lake. *C. carpio,* was
604 recorded with both CytB and 12S at one of the shore sites. The fact that both markers were
605 recorded at the same site indicates that common carp DNA and individuals might have
606 been present in the lake water but highly localised and undetected by established sampling
607 techniques. However this species was also present in the mock communities and therefore
608 laboratory contamination or "tag jumping" cannot be excluded.

609

610 Four previously-unrecorded species were detected in each of the Bassenthwaite and
611 Derwent Water basins. Again most of these records were based on low sequence reads and
612 site occupancy. The records for some species (common bream, *A. brama* in Bassenthwaite
613 Lake, nine-spined stickleback, *P. pungitius* in Derwent Water) are most likely explained by
614 barcode misassignment because they have never been recorded in the catchment but are
615 present in the mock communities. The presence of the remaining species (stone loach, *B.*
616 *barbatula*; three-spined stickleback, *G. aculeatus*; and rudd, *S. cephalus*) in the lakes or in
617 the catchment cannot be so easily excluded. These records therefore could either represent
618 environmental contamination or indicate that the species are present at low numbers and
619 have not been detected by previous long-term gill-netting (summarised by Winfield *et al.*
620 2012a).

621

622 We quantified the level of background contamination using sequence information from
623 mock communities and the level of PhiX contamination in target samples, which enabled
624 us to choose a suitable threshold level for filtering the data for false positives without
625 losing more information than necessary. Ultimately though, it is not possible to distinguish
626 between false positives and true positives if they occur at the same frequency, and some
627 rare species are likely to be lost with a threshold approach. Using consistency across
628 technical replicates as recently used by Port *et al.* (2016) might be a more suitable
629 approach to control for false positive if rare species are of particular interest.

630

631 ***Use of eDNA for assessing relative abundance of lake fish***

This study attempted to assess the relative abundance of individual species by using their sequence read counts or site occupancy as proxies. Using read count data is a valid approach under the assumption that no significant bias is introduced during sampling, subsequent PCR or sequencing. However, this assumption is unrealistic, and previous studies have demonstrated that the relationship between abundance and read count is complex (e.g. Ficetola *et al.* 2015; Yu *et al.* 2012; Evans *et al.* 2015; Kelly *et al.* 2014). Site occupancy models have been developed to cope with multiple levels of bias and uncertainty (e.g. imperfect detection, MacKenzie *et al.* 2002) and are therefore highly promising for eDNA (Schmidt *et al.* 2013). As discussed in the Methods, full site occupancy modelling requires estimation of detection probability from temporal sampling, which was beyond the scope of the present study. Our site occupancy estimates should therefore be treated as preliminary. Encouragingly though, read count and site occupancy data were correlated for each basin and each locus, suggesting that both measures of abundance are informative. As we discuss below though, and not surprisingly, site occupancy relies on comprehensive spatial sampling to obtain sufficient power for estimating abundance.

We found a consistent significant relationship between rank abundance and read count or occupancy data for both basins of Lake Windermere. This indicates both read count and occupancy are equally effective at estimating relative abundance under comprehensive spatial sampling. In Derwent Water and Bassenthwaite Lake, correlations with both abundance measures are weak and not significant with one exception (number of 12S sequence reads for Derwent Water). We suggest this is related to low statistical power from analysing only six samples per lake. There was also a consistent trend between eDNA and gill-net data, but the results are less conclusive due to low statistical power from the small number of species sampled in the gill-net survey. Although these results are generally encouraging, further work is critically needed to determine how robust eDNA is for estimating abundance. Increased spatial coverage of Bassenthwaite Lake and Derwent Water, together with temporal sampling to allow estimation of detection probability and site occupancy modelling in all basins, are critical next steps.

663 ***Spatial distribution of eDNA in Lake Windermere***

664 We investigated the spatial distribution of eDNA in Lake Windermere by comparing 1) off
665 shore and shoreline samples, 2) three depth profile transects and 3) North and South
666 Basins, which differ in their trophic status. Firstly, more species were detected in shallower
667 than in deep water, with 13 species detected along the 5 m contour, compared to 9 in the
668 mid-line transect. Interestingly, 12 of the 16 previously-recorded species were detected in
669 the 6 shore samples, which were collected in close proximity to one another. This suggests
670 eDNA could accumulate on the shoreline, and that shoreline sampling could be adequate
671 for detection of most species. More rigorous sampling along the lake shore is needed to
672 investigate this further. Second, we expected little difference along depth profile transects
673 since our sampling was carried out in the winter, when water stratification has broken
674 down. As predicted, within the depth transects the majority of species were detected
675 throughout the water column but some, including the typically deep water species Arctic
676 charr, *S. alpinus,* were only detected at the deepest sampling points, indicating that surface
677 water sampling might be ineffective in deeper lakes. Given the small scale of this
678 experiment the results regarding vertical sampling should be regarded as preliminary.
679 Thirdly, we hypothesized that eDNA from species associated with less eutrophic (i.e.
680 mesotrophic) conditions would be more abundant in the North Basin, while eDNA from
681 species associated with more eutrophic conditions should be more abundant in the South
682 Basin, and species with no preference should be detected throughout the lake. We observed
683 clear differences in the spatial distribution of eDNA, consistent with this hypothesis. These
684 results are consistent with long-term datasets from trapping, gill-netting and recreational
685 anglers' catches (Winfield *et al.* 2008a; Winfield *et al.* 2008b; Winfield *et al.* 2011; Craig
686 *et al.* 2015; Winfield *et al.* 2015b). For example, established methods have found perch, *P.*
687 *fluviatilis* and pike, *E. lucius* consistently in both basins (Craig *et al.* 2015; Winfield *et al.*
688 2008a respectively) while *S. alpinus* is much more abundant in the North than in the South
689 Basin (Winfield *et al.* 2008b; Winfield *et al.* 2015b) and *A. brama*, although a relatively
690 minor component of the Windermere fish community, is consistently more abundant in the
691 South than in the North Basin (Winfield *et al.* 2011).

692

693 ***Technical approach and the use of 12S or CytB as a marker***

In the present study we chose to validate the assays by sequencing mock communities, constructed from 22 species of fish, on the same flow cell as the eDNA samples. Although this allows for the success of the assay to be assessed within the same sequencing library as the samples, this approach may cause problems due to the low level miss-assignment of sequences from the mock community to the samples. For future studies we would recommend not including mock communities in the same library, or only including species that have no chance of being found in the eDNA samples and to sequence all negative controls and blanks.

Both markers were generally consistent in terms of the number of read counts and occupancy data generated, although clear advantages and disadvantages were associated with each marker. All species were detected in the mock communities with 12S whereas three were undetected with CytB. In the eDNA samples, site occupancy was higher, and more species were detected with 12S than CytB, as discussed earlier. Differences in amplification success could be due to fragment size (~100bp for 12S and 460bp for CytB), mismatches in primer binding sites or both. Given that eDNA degrades rapidly in the environment (Barnes *et al.* 2014; Rees *et al.* 2014), the difference in detection is probably a result of longer persistence of the shorter 12S fragment in lake water. This may allow for dispersion of eDNA across a larger geographical scale, increasing the probability of detection at any site. Consequently, it may be that detection of the longer CytB fragment indicates the species is present closer to where the water sample was taken, while 12S fragments may have originated from some distance away either within the lake or even up its tributaries. Using a longer fragment may be useful for pinpointing the exact location of species, but using a shorter fragment might be more useful for simply detecting the presence of a species anywhere in the water body using a limited number of subsamples. An additional aspect to consider is the persistence of eDNA in sediments, which has been shown to be considerably longer when compared to the water column (Turner *et al.* 2014). Differential persistence of the different sized fragments, and resuspension of eDNA during rain events could account for historical eDNA being detected. However, differences in primer specificity and efficiency between the two genes prevent conclusive answers to these issues, and this issue warrants further systematic exploration through experimental approaches and analysing a wider range of eDNA fragment lengths.

726

727 ***Use of eDNA to survey non-fish vertebrates***

728 This study also offers some insights into the feasibility of eDNA techniques for the wider
729 assessment of non-fish vertebrates associated with lakes and their immediate catchments.
730 The majority of the 12S and CytB sequences generated did not match the comprehensive
731 UK fish reference database used and non-fish sequences could be assigned to a wide range
732 of vertebrate species including mammals, birds, amphibians and some marine fish species
733 (known to be used in the lakes as dead bait by anglers) which were not included in our
734 reference data base. Moreover, the primers used appear to be largely vertebrate-specific
735 since no invertebrate sequences were identified, although many such species are present.
736 Consequently, the eDNA approach employed in this study may have further applications in
737 the qualitative but extensive high-level survey of non-fish vertebrate taxa occurring in lake
738 catchments.

739

740 **Conclusions**

741 The present investigation was driven primarily by the need to develop robust and cost-
742 effective lake fish assessments to meet the requirements of the EC Water Framework
743 Directive and other international and national environmental legislation. It is universally
744 agreed that there is no single sampling method that can produce all of the kinds of
745 information needed to make such assessments, but even the use of a combination of
746 methods from the range of established techniques still presents an incomplete picture with
747 varying degrees of bias and incomplete coverage (Kubečka *et al.* 2009). The findings of
748 the present study indicated that eDNA approaches can make a very significant contribution
749 to this challenging task. The results were consistent with our understanding of the fish
750 communities of three large, deep lakes based on long-term monitoring using established
751 techniques. Moreover, this work moved beyond a simple presence/absence analysis to
752 produce indications of the relative abundance of species, which were again consistent with
753 earlier assessments and ecological interpretations. Although the eDNA approach cannot
754 produce information on individual condition or population characteristics such as growth
755 curves, it proved to be very effective at producing robust data at the community level
756 which is undoubtedly the most challenging task for established sampling methods.

757 eDNA is arguably one of the most rapidly expanding areas of research in molecular
758 ecology but there is much to learn before methods such as the one described here can be
759 deployed for biological monitoring; particularly under legislative or sensitive
760 circumstances. Temporal sampling is an essential next step from the current study, to
761 account for imperfect detection and fully test the site occupancy modelling approach, and
762 to investigate the effects of water stratification on the spatial distribution of eDNA. More
763 generally, there is a pressing need to develop and demonstrate the wider applicability of
764 eDNA to a greater range of water bodies (such as those with varied chemical and physical
765 properties) as well as other animal and plant communities.

766

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915

916

**Data accessibility:** All de novo sequences generated through Sanger sequencing made available directed through our archived analysis pipeline on Github (see below). Accession numbers and taxon affiliations of all curated sequences are available as electronic Appendices. Raw Illumina read data has been submitted to NCBI (BioProject: PRJNA313432; BioSample accessions: SAMN04530423-SAMN04530510; SRA accessions: SRR3359939-SRR3360124). To assure full reproducibility of our analyses we have deposited the entire bioinformatics workflow in a dedicated Github repository, which also contains the curated reference databases and further supplementary data, such as taxon specific read counts for each sample as tables (https://github.com/HullUni-bioinformatics/Haenfling_et_al_2016; the repository is permanently archived with Zenodo (DOI 10.5281/zenodo.49823). Our custom data processing pipeline is available on Github (https://github.com/HullUni-bioinformatics/metaBEAT).

**Author contributions:** B.H., L.L.H. and I.J.W., conceived the study; B.H., L.L.H., I.J.W., J.L. and R.B.; carried out the field work. I.J.W. prepared fish abundance data from established method surveys. P.N., J.L and R.B. carried out all pre-sequencing laboratory work. D.R. assisted in the design of the molecular assays and carried out Illumina sequencing and the initial steps of the raw data analysis; A.O. assisted with the Illumina sequencing; C.H. assembled the bioinformatics pipeline and reference data base and wrote the relevant sections of the manuscript. B.H., and L.L.H. performed the statistical analyses. B.H., L.L.H., I.J.W. and D.R. wrote the paper; all authors commented on the final draft.

939 **Tables**

940 **Table 1:** Species previously recorded in the study lakes or recorded with eDNA. Full scientific,
941 common names and three letter codes used in figures are given.

942

| Scientific Name | Common Name | Code | Previously recorded in study lakes |
|---|---|---|---|
| *Abramis brama* | Common bream | BRE | Yes |
| *Anguilla anguilla* | European eel | EEL | Yes |
| *Barbatula barbatula* | Stone loach | LOA | Yes |
| *Coregonus albula* | Vendace | VEN | Yes |
| *Cottus gobio* | Bullhead | BUL | Yes |
| *Cyprinus carpio* | Common carp | CAR | No |
| *Esox lucius* | Pike | PIK | Yes |
| *Gasterosteus aculeatus* | Three-spined stickleback | 3SS | Yes |
| *Gymnocephalus cernua (=cernuus)* | Ruffe | RUF | Yes |
| *Lampetra fluviatilis* | River lamprey | RLA | Yes |
| *Leucaspius deliniatus* | Sunbleak | SUN | No |
| *Leuciscus leuciscus* | Dace | DAC | Yes |
| *Oncorhynchus mykiss* | Rainbow trout | RTR | No |
| *Osmerus eperlanus* | Smelt | SME | No |
| *Perca fluviatilis* | Perch | PER | Yes |
| *Petromyzon marinus* | Sea lamprey | SLA | Yes |

| | | | |
|---|---|---|---|
| *Phoxinus phoxinus* | Minnow | MIN | Yes |
| *Platichthys flesus* | Flounder | FLO | No |
| *Pseudorasbora parva* | Topmouth gudgeon | TMG | No |
| *Pungitius pungitius* | Nine-spined stickleback | 9SS | No |
| *Rutilus rutilus* | Roach | ROA | Yes |
| *Salmo salar* | Atlantic salmon | SAL | Yes |
| *Salmo trutta* | Brown trout | BTR | Yes |
| *Salvelinus alpinus* | Arctic charr | CHA | Yes |
| *Scardinius erythrophthalmus* | Rudd | RUD | Yes |
| *Squalius cephalus (=Leuciscus cephalus)* | Chub | CHU | Yes |
| *Tinca tinca* | Tench | TEN | Yes |
| *Umbra pygmaea* | Mudminnow | MUD | No |

943

944

945 **Figure legends**

946 **Figure 1:** Sampling sites in the three study lakes a) Bassenthwaite Lake, b) Derwent
947 Water, and c) Windermere in the English Lake District (UK). Samples were collected from
948 gill net sites (orange circles) and single shoreline sites (yellow circles) in Bassenthwaite
949 Lake and Derwent Water. In Windermere, samples were collected along transects
950 following the 5 m (red circles), 20 m (green circles) and mid line (blue circles) depth
951 profiles, as well as additional gill net and shoreline sites.

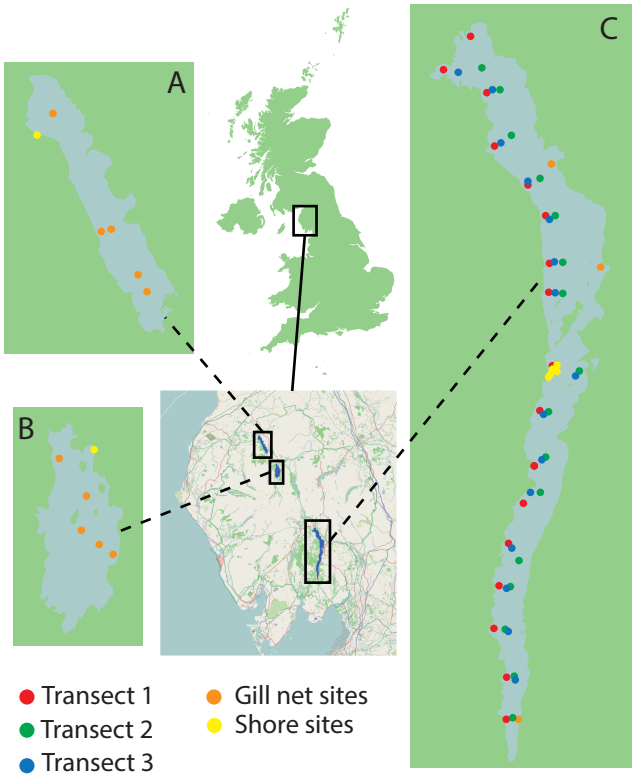
952 **Figure 2:** Site occupancy for 12S and CytB data from a) offshore sites Windermere North
953 Basin, b) offshore sites Windermere South Basin, c) Bassenthwaite Lake and d) Derwent
954 Water. All species recorded previously are included. Previously-recorded species are
955 ordered according to their rank abundance within basin from established survey methods.
956 Species that have not been recorded previously are indicated with an asterisk and are
957 ordered alphabetically. Full species names are given in Table 1.

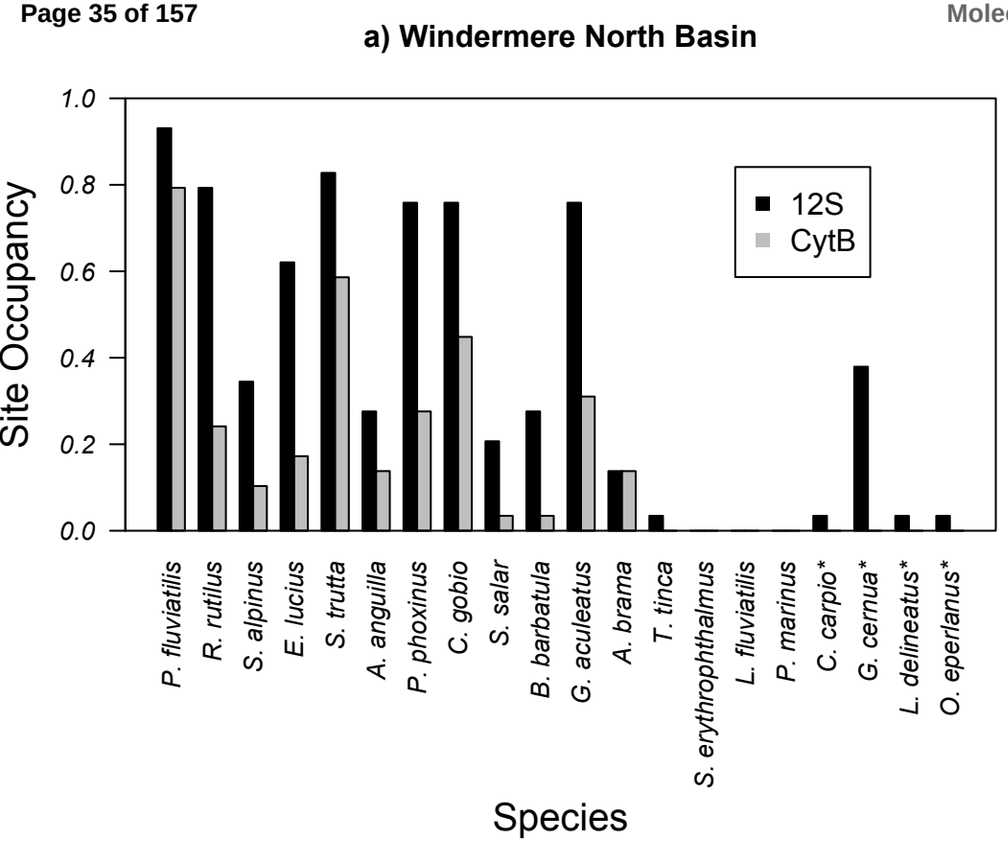
958 **Figure 3:** Sample based rarefaction analyses for Lake Windermere. Only offshore samples
959 and species recorded previously in Lake Windermere are included in the analyses.

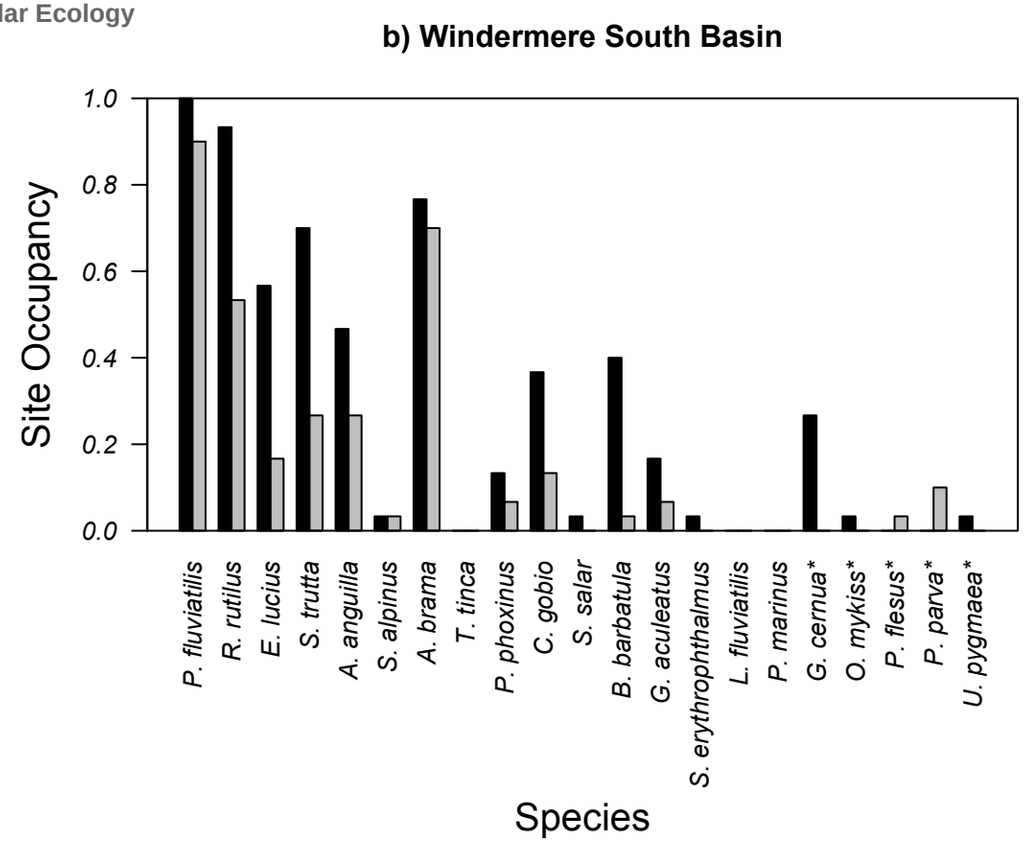
960 **Figure 4:** Correlations between site occupancy data and long-term rank based on
961 established surveys and expert opinion for all four basins and both 12S (a-d) and CytB (e-
962 h), where 1 is the highest and 16 the lowest rank abundance. Species three letter codes are
963 given in Table 1.

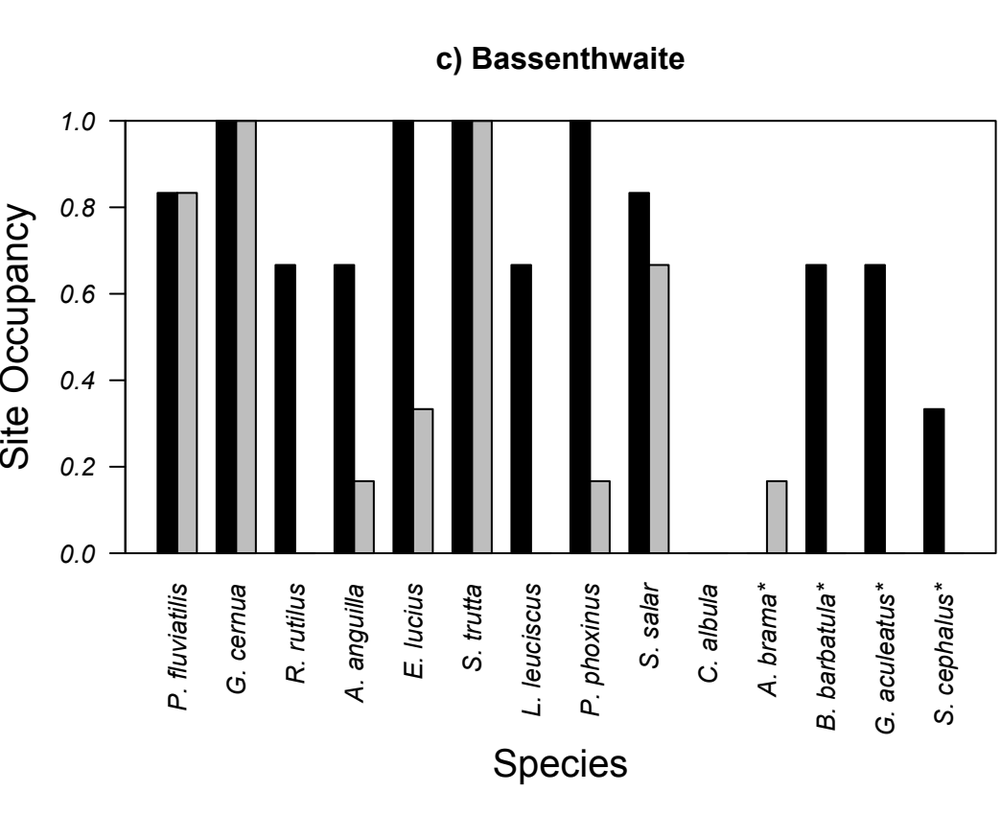
964 **Figure 5:** Average number of sequence reads obtained per transect for Lake Windermere
965 North Basin (a,b,) and South Basin (c,d) for both 12S (a,c) and CytB (b,d). Only species
966 that have been recorded previously are included. Species are ordered according to their
967 rank abundance within basin from established survey methods.

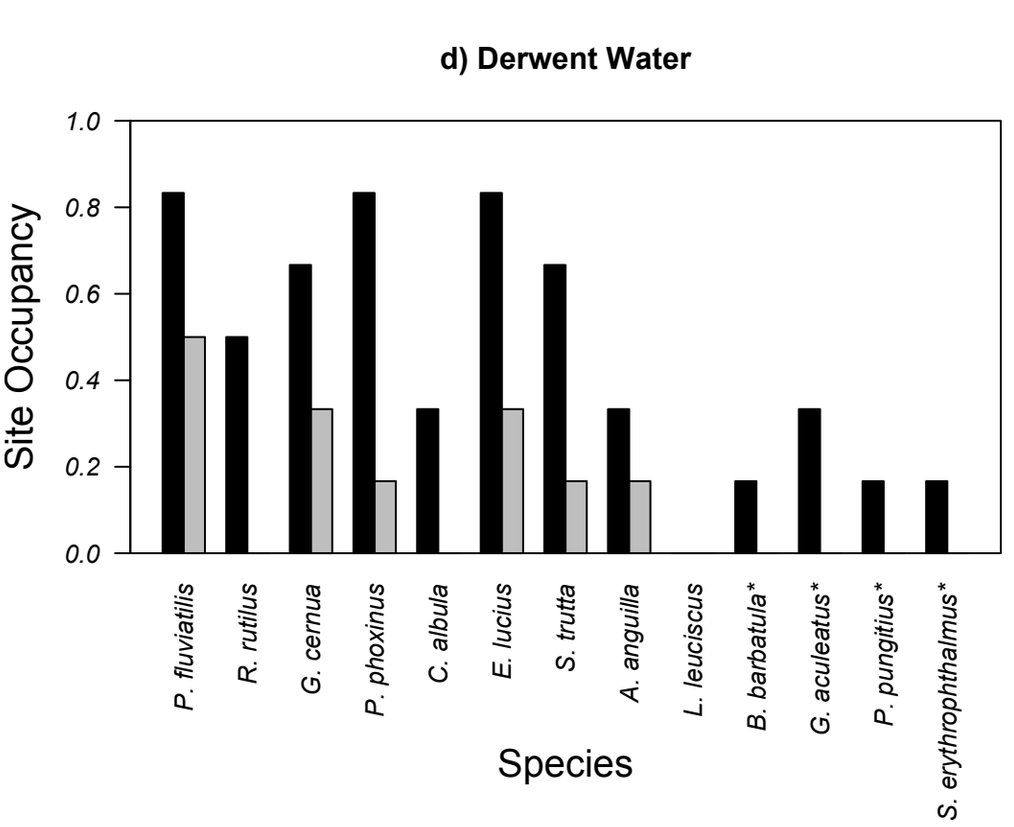
968 **Figure 6:** Relative distribution of fish species and their ecological preferences in
969 Windermere North Basin (mesotrophic) and South Basin (eutrophic) based on the
970 proportion from the total number of sequence reads (a, b) and the relative proportion of
971 sites occupied (c,d) reflecting the trophic status of the two basin**.**

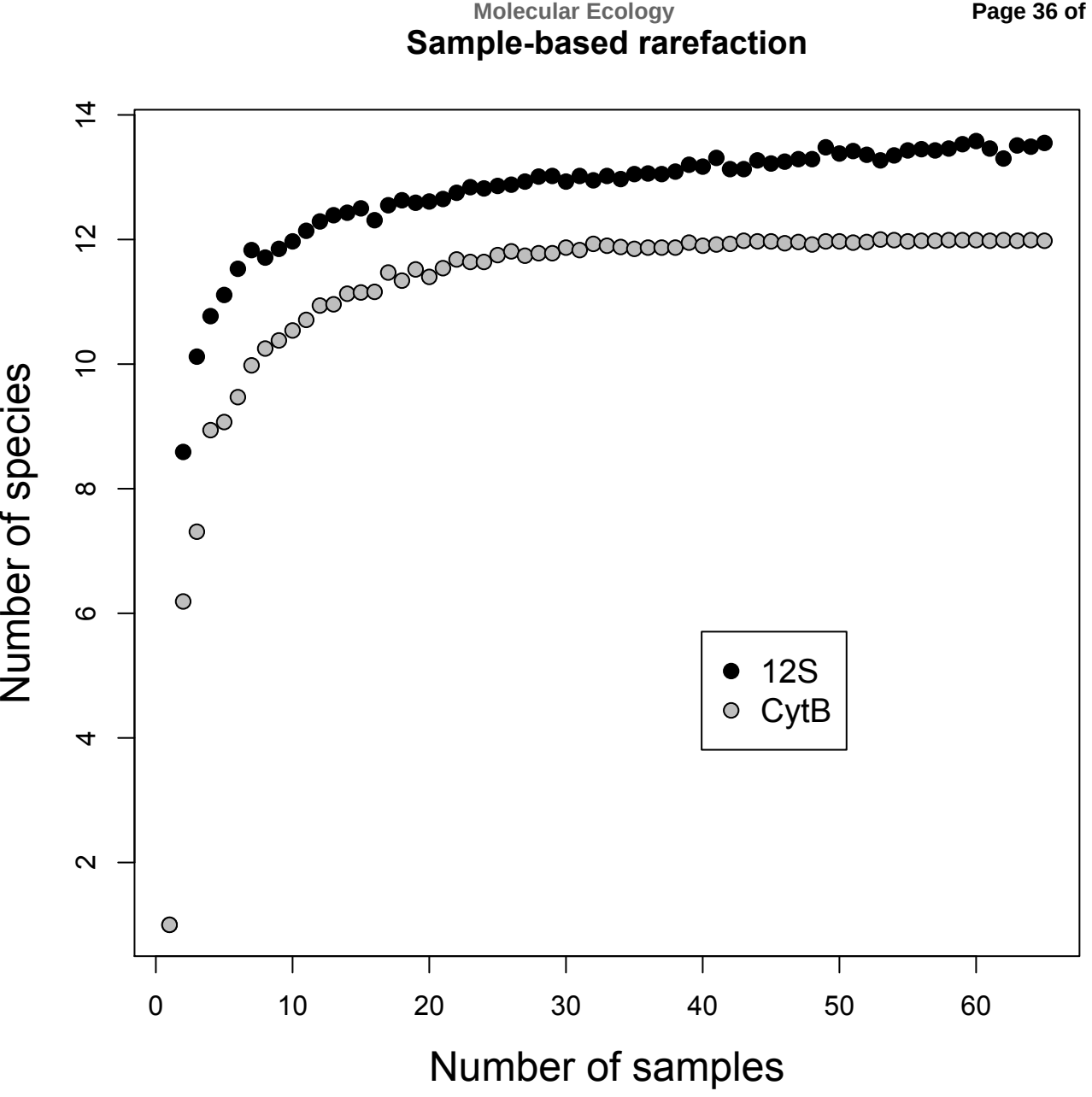

A
B
C
Transect 1
Transect 2
Transect 3
Gill net sites
Shore sites

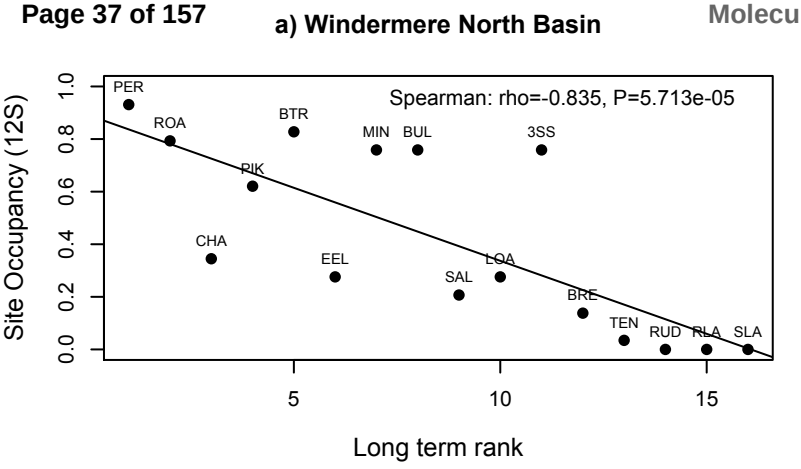

a) Windermere North Basin
Site Occupancy
1.0
0.8
0.6
0.4
0.2
0.0
12S
CytB
P. fluviatilis
R. rutilus
S. alpinus
E. lucius
S. trutta
A. anguilla
P. phoxinus
C. gobio
S. salar
B. barbatula
G. aculeatus
A. brama
T. tinca
S. erythrophthalmus
L. fluviatilis
P. marinus
C. carpio*
G. cernua*
L. delineatus*
O. eperlanus*
Species

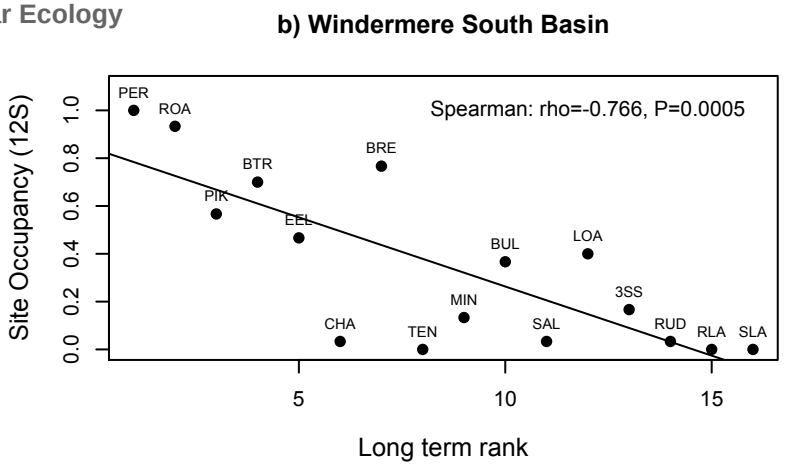

b) Windermere South Basin
Site Occupancy
1.0
0.8
0.6
0.4
0.2
0.0
P. fluviatilis
R. rutilus
E. lucius
S. trutta
A. anguilla
S. alpinus
A. brama
T. tinca
P. phoxinus
C. gobio
S. salar
B. barbatula
G. aculeatus
S. erythrophthalmus
L. fluviatilis
P. marinus
G. cernua*
O. mykiss*
P. flesus*
P. parva*
U. pygmaea*
Species

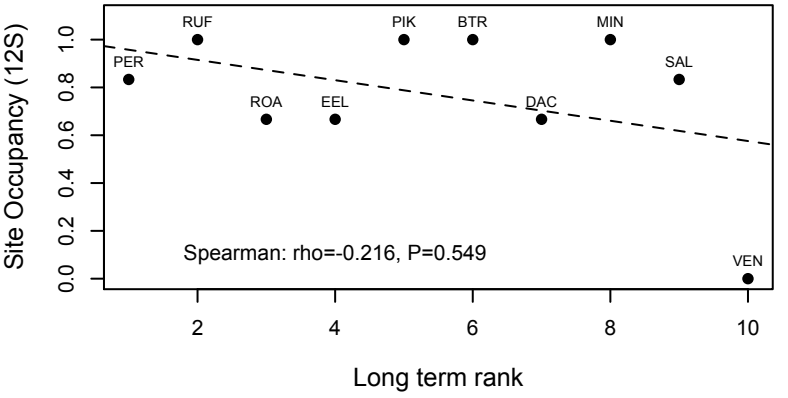

c) Bassenthwaite
Site Occupancy
1.0
0.8
0.6
0.4
0.2
0.0
P. fluviatilis
G. cernua
R. rutilus
A. anguilla
E. lucius
S. trutta
L. leuciscus
P. phoxinus
S. salar
C. albula
A. brama*
B. barbatula*
G. aculeatus*
S. cephalus*
Species

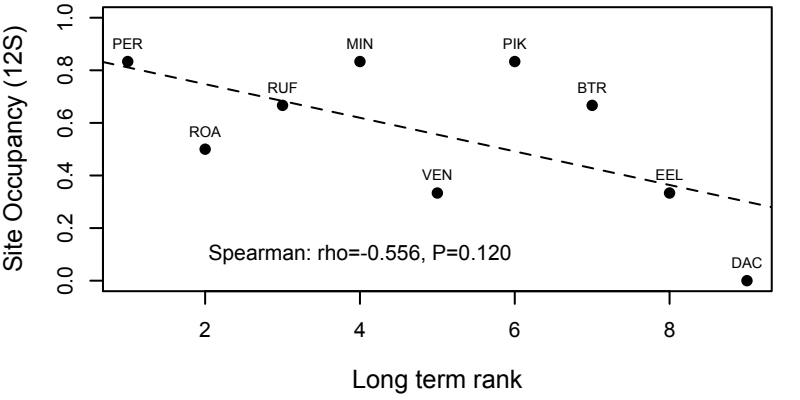

d) Derwent Water
Site Occupancy
1.0
0.8
0.6
0.4
0.2
0.0
P. fluviatilis
R. rutilus
G. cernua
P. phoxinus
C. albula
E. lucius
S. trutta
A. anguilla
L. leuciscus
B. barbatula*
G. aculeatus*
P. pungitius*
S. erythrophthalmus*
Species

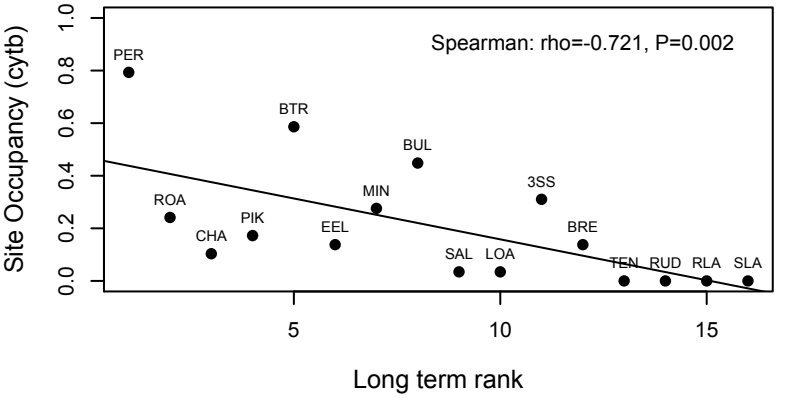

Sample-based rarefaction
Number of species
Number of samples
2
4
6
8
10
12
14
0
10
20
30
40
50
60
12S
CytB

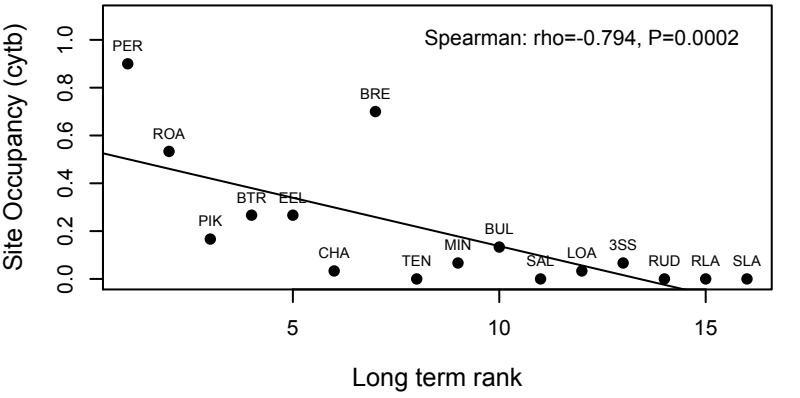

a) Windermere North Basin
Spearman: rho=-0.835, P=5.713e-05
Site Occupancy (12S)
Long term rank
PER
ROA
BTR
MIN
BUL
3SS
PIK
CHA
EEL
LOA
SAL
BRE
TEN
RUD
RLA
SLA

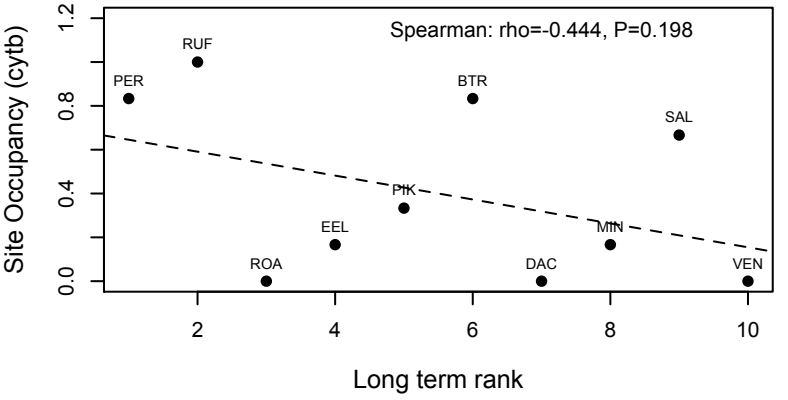

b) Windermere South Basin
Spearman: rho=-0.766, P=0.0005
Site Occupancy (12S)
Long term rank
PER
ROA
BRE
BTR
PIK
EEL
BUL
LOA
3SS
MIN
CHA
TEN
SAL
RUD
RLA
SLA

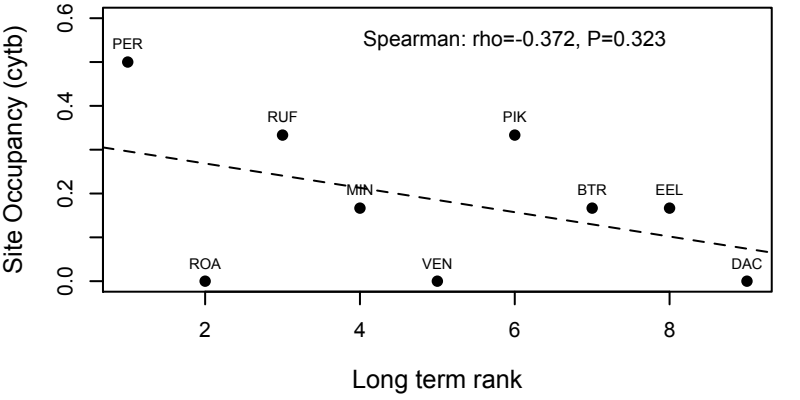

c) Bassenthwaite
Spearman: rho=-0.216, P=0.549
Site Occupancy (12S)
Long term rank
RUF
PIK
BTR
MIN
PER
SAL
ROA
EEL
DAC
VEN

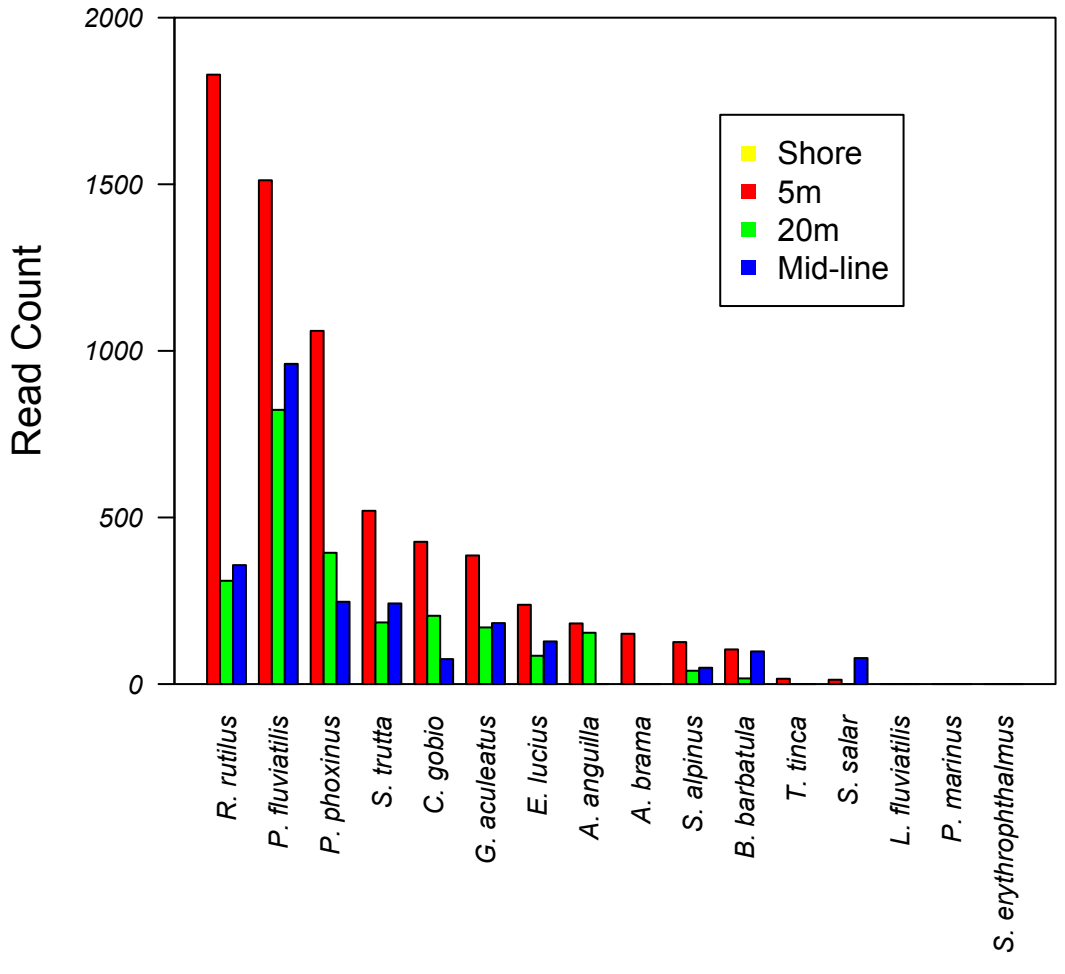

d) Derwent Water
Spearman: rho=-0.556, P=0.120
Site Occupancy (12S)
Long term rank
PER
MIN
PIK
RUF
BTR
ROA
VEN
EEL
DAC

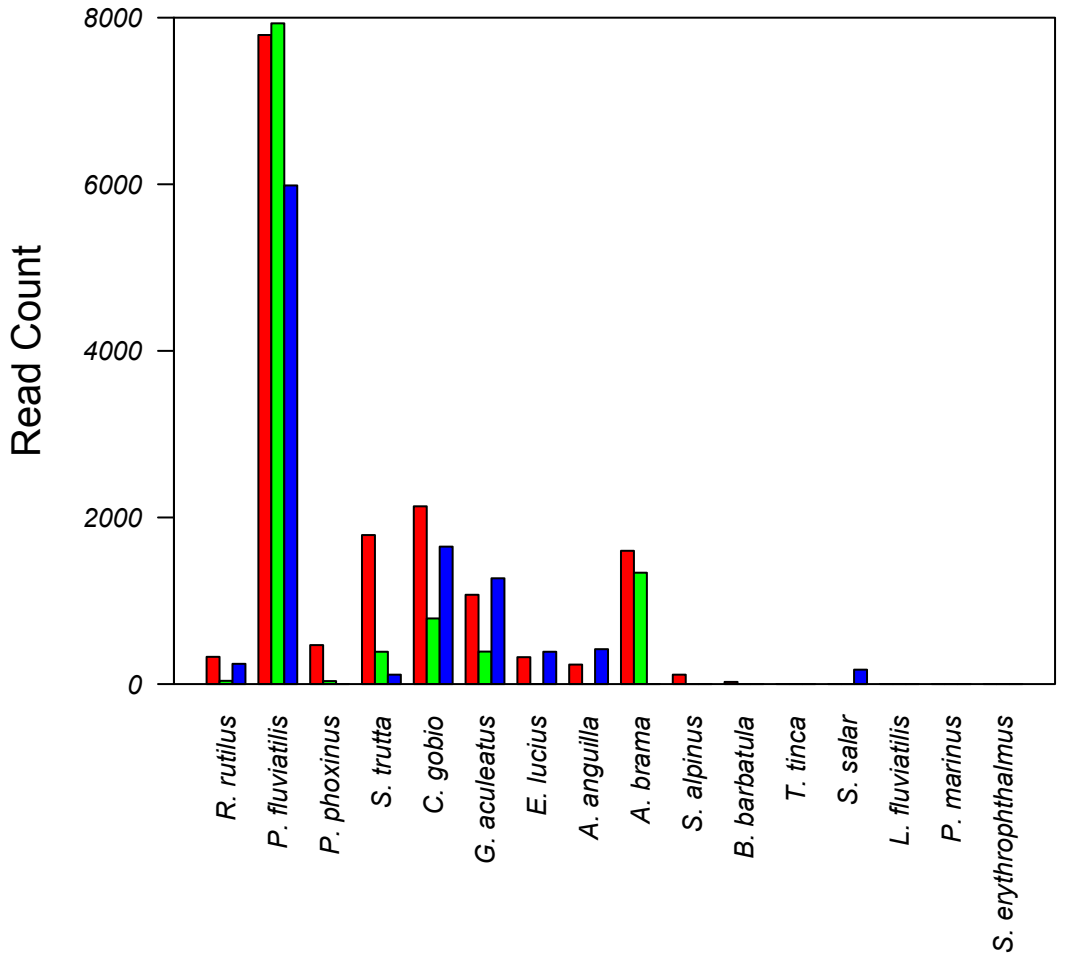

e) Windermere North Basin
Spearman: rho=-0.721, P=0.002
Site Occupancy (cytb)
Long term rank
PER
BTR
BUL
ROA
MIN
3SS
PIK
CHA
EEL
BRE
SAL
LOA
TEN
RUD
RLA
SLA

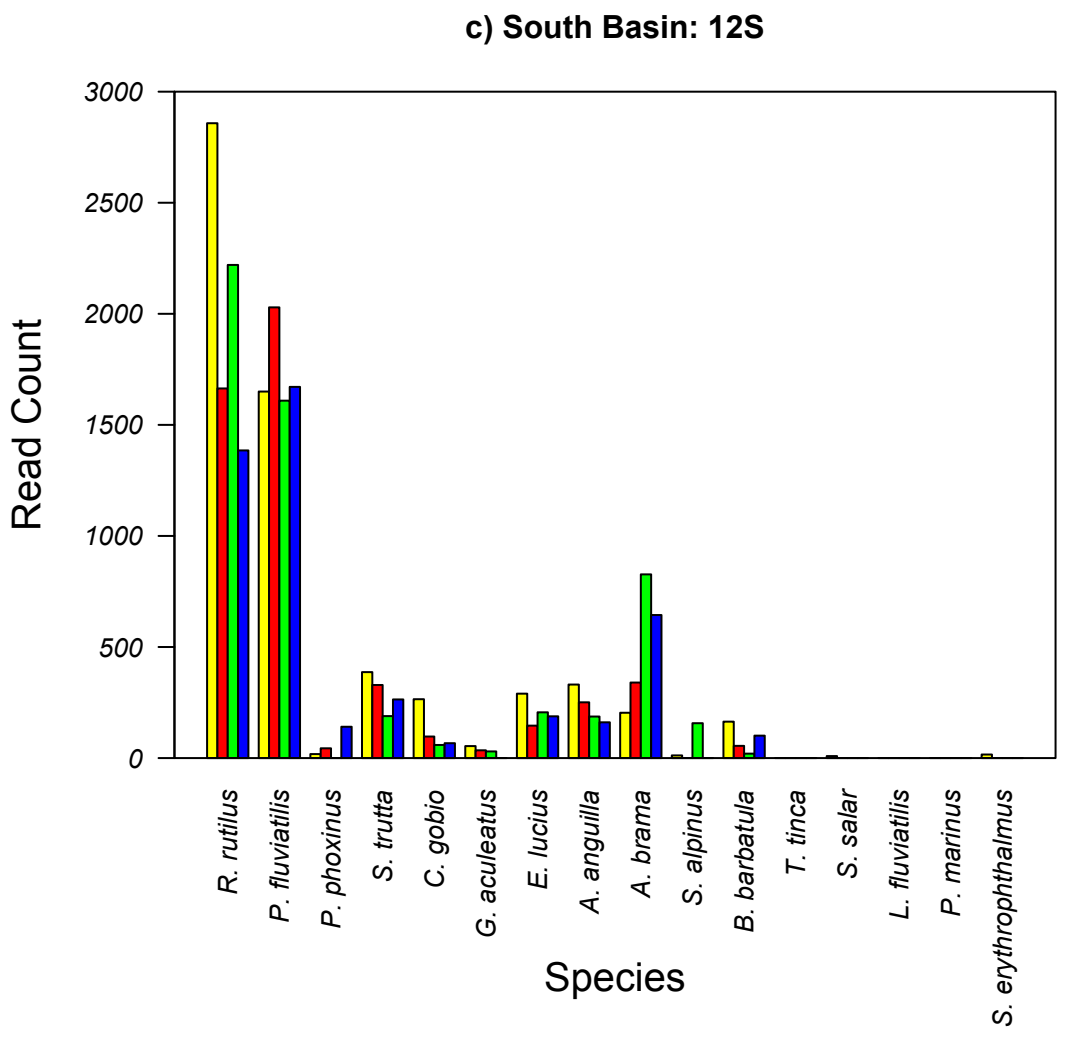

f) Windermere South Basin
Spearman: rho=-0.794, P=0.0002
Site Occupancy (cytb)
Long term rank
PER
BRE
ROA
BTR
EEL
PIK
BUL
CHA
MIN
TEN
SAL
LOA
3SS
RUD
RLA
SLA

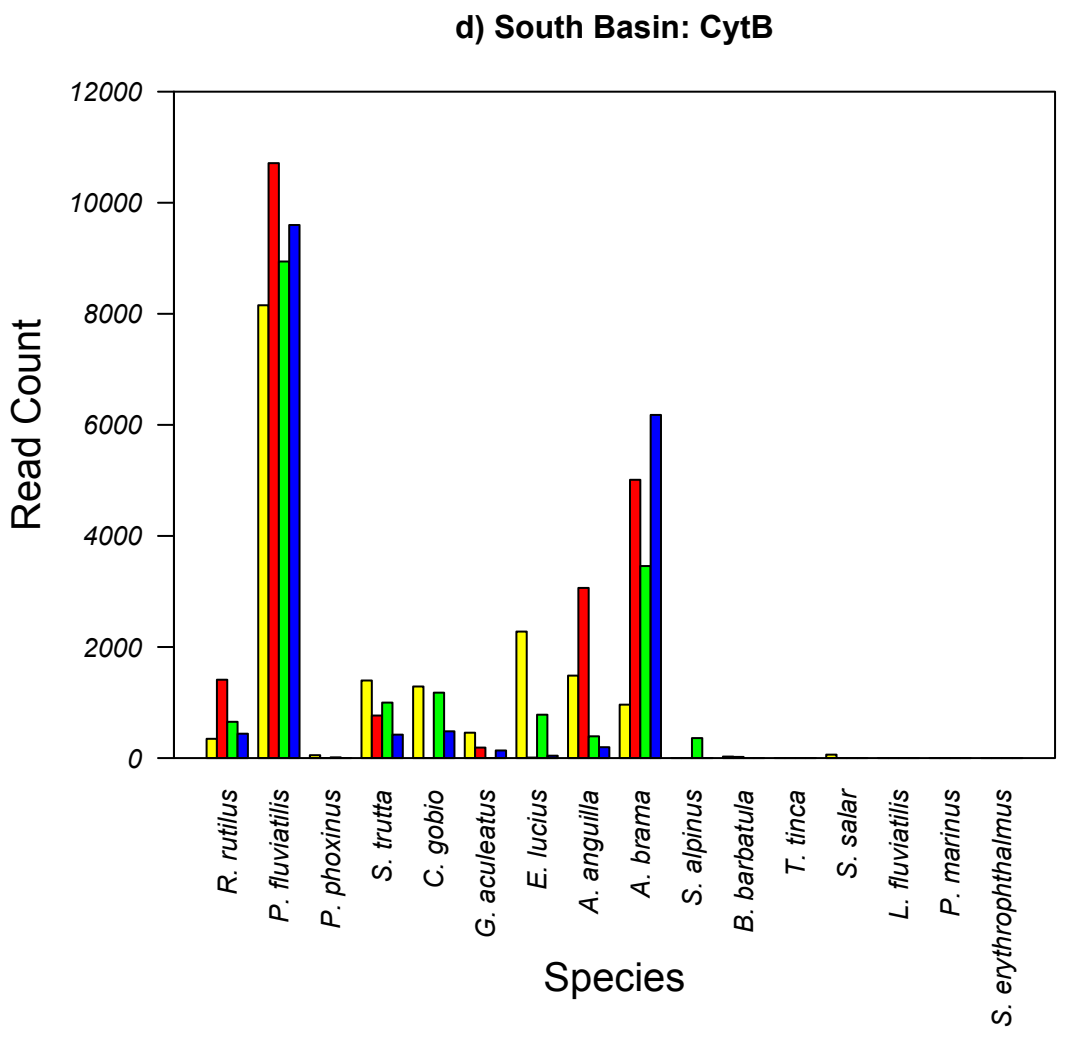

g) Bassenthwaite
Spearman: rho=-0.444, P=0.198
Site Occupancy (cytb)
Long term rank
RUF
PER
BTR
SAL
PIK
EEL
MIN
ROA
DAC
VEN

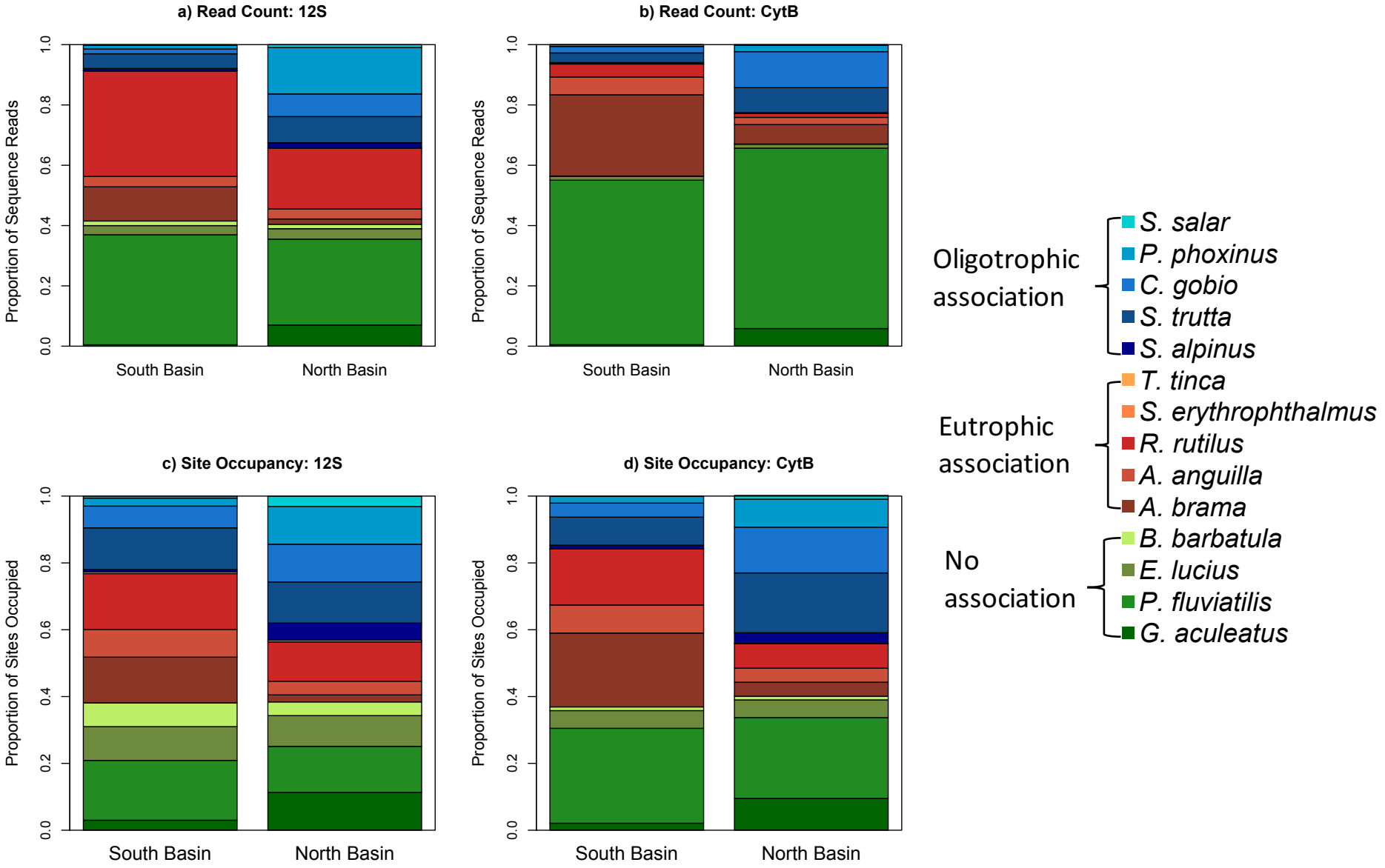

h) Derwent Water
Spearman: rho=-0.372, P=0.323
Site Occupancy (cytb)
Long term rank
PER
RUF
PIK
MIN
BTR
EEL
ROA
VEN
DAC


a) North Basin: 12S
Read Count
2000
1500
1000
500
0
Shore
5m
20m
Mid-line
R. rutilus
P. fluviatilis
P. phoxinus
S. trutta
C. gobio
G. aculeatus
E. lucius
A. anguilla
A. brama
S. alpinus
B. barbatula
T. tinca
S. salar
L. fluviatilis
P. marinus
S. erythrophthalmus


b) North Basin: CytB
Read Count
8000
6000
4000
2000
0
R. rutilus
P. fluviatilis
P. phoxinus
S. trutta
C. gobio
G. aculeatus
E. lucius
A. anguilla
A. brama
S. alpinus
B. barbatula
T. tinca
S. salar
L. fluviatilis
P. marinus
S. erythrophthalmus


c) South Basin: 12S
Read Count
3000
2500
2000
1500
1000
500
0
R. rutilus
P. fluviatilis
P. phoxinus
S. trutta
C. gobio
G. aculeatus
E. lucius
A. anguilla
A. brama
S. alpinus
B. barbatula
T. tinca
S. salar
L. fluviatilis
P. marinus
S. erythrophthalmus
Species


d) South Basin: CytB
Read Count
12000
10000
8000
6000
4000
2000
0
R. rutilus
P. fluviatilis
P. phoxinus
S. trutta
C. gobio
G. aculeatus
E. lucius
A. anguilla
A. brama
S. alpinus
B. barbatula
T. tinca
S. salar
L. fluviatilis
P. marinus
S. erythrophthalmus
Species


a) Read Count: 12S
b) Read Count: CytB
c) Site Occupancy: 12S
d) Site Occupancy: CytB
Proportion of Sequence Reads
Proportion of Sites Occupied
0.0
0.2
0.4
0.6
0.8
1.0
South Basin
North Basin
Oligotrophic association
S. salar
P. phoxinus
C. gobio
S. trutta
S. alpinus
Eutrophic association
T. tinca
S. erythrophthalmus
R. rutilus
A. anguilla
A. brama
No association
B. barbatula
E. lucius
P. fluviatilis
G. aculeatus

# Supplementary Online Material

## Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods

Bernd Hänfling[1*], Lori Lawson Handley[1*], Daniel S. Read[2], Christoph Hahn[1], Jianlong Li[1], Paul Nichols[1], Rosetta C. Blackman[1], Anna Oliver[2] and Ian J. Winfield[3].

# Supplementary Text: Methods & Results

## *Compilation of reference databases*

We compiled a reference database of 67 freshwater fish species including all those recorded in the UK and additional non-native species that could potentially be present, but have not yet been confirmed, in order to be able to confidently identify species from their DNA barcodes and facilitate bioinformatics steps. We targeted two regions of mtDNA: 12S and CytB, in order to compare species delimitation properties of the markers. Reference sequences were retrieved from Genbank for CytB for all 67 fish species and for 12S for 57 species (Table S1) using E-utilities (Sayers 2008).

Fresh tissue samples were collected for the 24 species which were used to generate additional reference sequences for 12S (21 species) and/or were used as positive controls (Table S1). Tissues were sourced from the existing collection at the University of Hull or specifically collected for this project. Fish DNA was extracted from fin clips and muscle tissues using a DNeasy Blood & Tissue kit (Qiagen, UK). In order to generate reference sequences of the entire 12S region a set of novel primers was designed from an alignment of whole mitochondrial fish genomes (12S_30F: CACTGAAGMTGYTAAGAYG and 12S_1380R: CTKGCTAAATCATGATGC). Polymerase chain reactions (PCRs) were performed in 25 µl volumes containing: 1x $NH_4$ Buffer, 2 mM $MgCl_2$, 1 mM total dNTPs, 0.8 µM of each primer, 1 U BIOTAQ polymerase (Bioline, London, UK), and ~10 ng DNA template. PCRs were performed on an Applied Biosystems Veriti Thermal Cycler with the following profile: 95 °C for 2 min, 30 cycles of 95 °C for 30 sec, 50 °C for 30 sec and 72 °C for 50 sec, followed by a final elongation step at 72 °C for 10 min. Purified PCR products were Sanger sequenced directly (Macrogen Inc., Republic of Korea) in both directions using the PCR primers. Sequences were edited using CodonCode Aligner (CodonCode Corporation, Centerville, MA, USA).

Reference sequences were further processed in the ReproPhylo environment (Szitenberg *et al.* 2015). Sequences were extracted in FASTA format and clustered at 100% identity to remove redundancy using CD-hit-est (Li & Godzik 2006). As a final quality control we inferred phylogenetic trees from the non-redundant sets of reference sequences for each marker gene in ReproPhylo, as follows: Sequences shorter than 400 bp were removed and the remaining

sequences were aligned using MAFFT (Katoh & Standley 2013). For CytB records, nucleotide sequences were translated to protein sequences prior to alignment and aligned protein sequences were converted back to nucleotide sequences using Pal2Nal (Suyama *et al.* 2006). Alignments were trimmed using trimAl (Capella-Gutiérrez *et al.* 2009). Maximum likelihood trees were inferred with RAxML 8.0.2 (Stamatakis 2006) using the GTR+gamma model of substitutions. Resulting trees were manually investigated to identify any sequence records that were obviously misplaced in the phylogenetic trees, i.e. records which were likely mislabelled (Fig. S2). Such sequences were removed from the database as they would likely cause conflicts in downstream analyses. The remaining sequences, i.e. the curated non-redundant reference databases, were used in all downstream analyses.

The complete reference database initially included a total of 775, and 4,813 sequences (partial or complete) for the two markers 12S, and CytB, respectively and covered all 67 target species for CytB and 60 species for 12S. Sequences for seven species were unobtainable for 12S (*Aspius aspius, Coregonus autumnalis, Lampetra planeri, Misgurnus fossilis, Neogobius melanostomus, Proterorhinus semilunaris, Vimba vimba*). Of these seven, only *C. autumnalis* and *L. planeri* have been confirmed in the UK, but they have not been recorded in our target lakes. After curating, i.e. removing redundant (i.e. identical haplotypes) and likely mislabelled records (based on phylogenetic tree inference Fig. S2 a and b), the database contained 272 and 2,155 sequences for 12S, and CytB, respectively. The complete list of retained reference sequences for 12S and CytB is provided in an excel spreadsheet (see Supplementary Appendix 1). The complete reference databases compiled in Genbank format have been deposited in the dedicated Github repository for this study: https://github.com/HullUni-bioinformatics/Haenfling_et_al_2016 .

### In silico *and* In vitro *testing of metabarcoding primers*

To test the suitability of primers for eDNA based metabarcoding of freshwater fish communities we carried out *in silico* experiments with a number of published primer pairs which amplify fragments of the 12S and CytB region using the curated non-redundant sets of reference sequences (Table S2). The program EcoPCR (Ficetola *et al.* 2010) was used to test whether the variability of the amplified region for 12S is high enough to distinguish the all target species. This approach could not be applied to the Kocher *et al.* (1989) CytB primers since a large proportion of the sequences downloaded from Genbank did not cover the location of the forward

primer. To evaluate this primer pair we therefore cropped the alignment to the 413 bp Kocher amplicon (excluding primers), clustered the resulting sequences at 100% identity using CD-hit-est (Li & Godzik 2006) and subsequently checked specificity of the fragments based on these clusters (see Table S3).

A subset of 22 (33%) of the species from Table S1 was chosen to test the consistency of PCR amplification across taxa *in vitro* in single amplifications DNA extractions from a single individual from each species were used for these tests after DNA concentrations were normalised to 5 ng/µl using a Nanodrop. PCR reagent concentrations were identical to those given above. Thermal cycling conditions consisted of an initial denaturation at 95°C for 2 minutes followed by 30 cycles with 15 sec at 95°C, 15 sec at the annealing temperature (48°C-56°C) and 20 sec at 72°C, and a final extension step of 5 min at 72°C. All species amplified in single reactions for both loci (Fig. S3). Normalised DNA from these species were also used to create 10 mock communities with different concentrations of DNA (Tables S4 and S5), which were sequenced together with the lake samples.

### *Library preparation*

PCRs were performed in 25 µl volumes with Q5 High-Fidelity PCR Kit (New England Biolabs, UK) containing: 1X Master Mix, 0.5 µM of each tagged primer and 2.5 µl template DNA (equivalent to approximately 10 ng). PCRs were performed on an Applied Biosystems Veriti Thermal Cycler with the following profile: 98 °C for 30 min, 40 cycles of 98 °C for 10 sec, 50 °C for 15 sec and 72 °C for 20 sec, followed by a final elongation step at 72 °C for 5 min. PCR products were checked on ethidium bromide-stained agarose gels. PCRs were performed in triplicate and replicates pooled for sequencing.

### *Mock communities*

All species were detected in the mock communities with the exception of *P. pungitius* at CytB (Tables S4 and S5). Two other species were represented by very low number of sequence reads: *Lepomis gibbosus* for 12S (125 reads), and *G. gobio* (32 reads) for CytB. On each occasion when the species *A. nebulosus, Coregonus albula, Leuciscus leuciscus* and *Salmo trutta* were present in a CytB mock community there was also a significant genus level assignment to *Almeirerus, Coregonus, Leuciscus* and *Salmo* respectively. We therefore interpreted both the species and the

genus level assignments as belonging to the individual species (see Table S5). On the whole though there was reasonable consistency between observed and expected number of sequence reads in the mock communities for both loci (12S $\chi^2 = 0.224$, df = 21, $P > 0.05$; CytB $\chi^2 = 0.367$, df = 21, $P > 0.05$, Fig. S4). Remaining variation between expected and observed sequence reads could be caused either by PCR bias or unequal template DNA quality. Standardised sequences reads (i.e. per ng DNA in the PCR template) were highly correlated between the two data sets suggesting that a significant proportion of this bias might be attributed to variation in DNA quality and/or inaccurate DNA quantification (Fig. S4c). Further stochastic variation might have been introduced because only one individual per species was used and the age of the tissue extract varied between 1 week and > 10 years.

### *Determining a threshold for defining the presence of species at individual sites*

False positive records (defined by the false detection of species where eDNA is not present) can arise from a variety of sources, including carryover of eDNA on sampling and filtering equipment, laboratory contamination or barcode missassignment. Evidence is accumulating that the latter is the norm rather than the exception in metabarcoding studies but usually results in records of low frequency. The number of low frequency false positives can be reduced by applying a threshold value, i.e. a minimum frequency in the sample from which a record is accepted as positive. We used sequence data from the mock communities to inform a decision regarding a suitable threshold level for analysis of the lake fish data. On average eight false positives (i.e sequences of species not added to the mock community) were found in each of the ten 12S mock communities (Table S4) and five in the CytB mock communities (Table S5). These were largely species present in the other mock communities. Most of these were present at a very low frequency and the number of false positives dropped quickly when increasing the threshold level (Fig. S5). At a threshold of 0.001 and 0.002 for 12S and Cytb respectively over 90% of false positives were omitted from the data (Th90) and a threshold of 0.003 and 0.01 was required in order eliminate all false positives from the data (Th100). We also tested the impact of these different thresholds on detection of species in Windermere (Fig. S6). We provisionally identified 12S sequences from 20 species in Windermere, 10 species in Bassenthwaite Lake and 12 species in Derwent Water but a number of species were represented by only a few sequences per site. False positive results from sequencing error or low level cross contamination pre or post PCR could explain these rare sequences. A very similar picture emerged using CytB sequence

data, although fewer species were identified, with a total of 16 species in Windermere, 11 species in Bassenthwaite Lake and 6 species in Derwent Water. We therefore empirically investigated a range of threshold values for detection for both loci. At Th90 the number of species detected in Windermere decreased from 21 to 19 for 12S and from 16 to 15 for CytB. For both markers the species "lost" at this threshold level were species not previously recorded (i.e. potential false negatives). At Th100 the species count decreased to 16 for 12S and 14 for CytB. Again only species not previously not recorded were lost from the 12S data set but the species lost from the CytB data set, *B. barbatula,* was previously recorded in Windermere and is likely to represent a true positive. These results were similar for Derwent Water, where two previously recorded species were lost at Th100, and Bassenthwaite Lake where one previously recorded species was lost (data not shown). We quanitifed PhiX contamination in the raw sequence reads for each sample as a proxy for the extent of tag jumping in the respective library using the MITObim pipeline (Hahn et al. 2013). PhiX phage genomic DNA is added routinely to Illumina metabarcoding runs in order to increase sequence diversity, but since it does not contain universal identifier tags it can only be assigned to target samples through "tag" jumping during the sequencing process. MITObim 1.8 was run for a single iteration using the nucleotide sequence of PhiX (Gebank accession: J02482) as bait. For each sample/library we recorded the proportion of sequences mapping to the PhiX reference, with a maximum number of mismatches smaller than 15% of read length. The data show that PhiX contamination was on average lower in the CytB library (median = 0.00002) compared to the 12S library (median 0.0007) but the maximum value was higher in CytB (0.0210) compared to 12S (0.0023, Fig. S7).

### *Fish abundance and distribution estimated from established method surveys*

In September 2014, the gill-netting survey produced a total of 191 individuals at Bassenthwaite Lake, 202 individuals at Derwent Water, 627 individuals at Windermere North Basin and 525 individuals at Windermere South Basin (Table S6). Note that while *S. alpinus* was not recorded in this survey of September 2015, probably because of the relatively low sampling effort in the context of this rare species, they were recorded using more intensive but non-destructive specialised gill netting on a spawning ground in Windermere North Basin during the following autumn of 2014 as described by Winfield et al. (2015).

The total fish species lists for Bassenthwaite Lake, Derwent Water and Windermere comprised 10, 9 and 16 species, respectively, as shown together with the expert opinion abundance score for each species (presented separately for the two basins of Windermere in Table S6).

In addition to the above species known to be present as native or introduced populations, a number of further species have been recorded at each lake being used as live-bait prior to the local banning of the use of freshwater fish as live- or dead-bait in 2002 as described by Winfield and Durie (2004). It is possible that some of these species have subsequently established small populations yet to be detected by survey gill nets or other forms of biological sampling. At Bassenthwaite Lake these potential populations comprise *Cyprinus carpio*, *Oncorhynchus mykiss* and *Scardinius erythrophthalmus*, while at Windermere they comprise *Carassius carassius*, *Leuciscus leuciscus*, *Thymallus thymallus* and *O. mykiss*. Furthermore *Gasterosteus aculeatus* has been recorded at many Cumbrian lakes and although they have not been caught during routine surveys in Bassenthwaite Lake and Derwent Water they are likely to be present there.

# Supplementary Tables

## Table S1: List of species included in Reference database

| Scientific Name | Common Name | Code | Previously recorded in study lakes | Species number in Fig. S2 | 12S sequenced during current project |
|---|---|---|---|---|---|
| *Abramis brama* | Common bream | BRE | Yes | 5 | Yes |
| *Acipenser sturio* | Common sturgeon | STU | | | |
| *Alburnoides bipunctatus* | Schneider | SCH | | | |
| *Alburnus alburnus* | Bleak | BLE | | 20 | Yes |
| *Alosa alosa* | Allis shad | ASH | | | |
| *Alosa fallax* | Twaite shad | TSH | | | |
| *Ambloplites rupestris* | Rock bass | RBA | | | |
| *Ameiurus melas* | Black bullhead | BLB | | | |
| *Ameiurus nebulosus* | Brown bullhead | BRB | | 17 | Yes |
| *Anguilla anguilla* | European eel | EEL | Yes | | |
| *Aspius aspius* | Asp | | | | |
| *Barbatula barbatula* | Stone loach | LOA | Yes | | Yes |
| *Barbus barbus* | Barbel | BAR | | 21 | Yes |
| *Blicca bjoerkna (=Abramis bjorkna)* | | | | | |
| *Carassius auratus* | Goldfish | GOL | | 18 | |
| *Carassius carassius* | Crucian carp | CRU | | | |
| *Chondrostoma nasus* | Nase | NAS | | | |
| *Cobitis taenia* | Spined loach | SLO | | | |
| *Coregonus albula* | Vendace | VEN | Yes | 4 | Yes |

| | | | | | |
|---|---|---|---|---|---|
| *Coregonus autumnalis* | Pollan | POL | | | Yes |
| *Coregonus lavaretus* | Whitefish | WHI | | | |
| *Coregonus oxyrinchus* | Houting | HOU | | | |
| *Cottus gobio* | Bullhead | BUL | Yes | 23 | Yes |
| *Ctenopharyngodon idella* | Grass carp | GCA | | | |
| *Cyprinus carpio* | Common carp | CAR | No | 10 | Yes |
| *Esox lucius* | Pike | PIK | Yes | 1 | Yes |
| *Gasterosteus aculeatus* | Three-spined stickleback | 3SS | Yes | | |
| *Gobio gobio* | Gudgeon | GUD | | 19 | Yes |
| *Gymnocephalus cernuus* | Ruffe | RUF | Yes | 2 | Yes |
| *Hypophthalmichthys molitrix* | Silver carp | SCA | | | |
| *Hypophthalmichthys nobilis* | Bighead carp | BCA | | | |
| *Lampetra fluviatilis* | River lamprey | RLA | Yes | | |
| *Lampetra planeri* | Brook lamprey | BLA | | | |
| *Lepomis gibbosus* | Pumpkinseed | PUM | | 11 | Yes |
| *Leucaspius deliniatus* | Sunbleak | SUN | | 12 | |
| *Leuciscus idus* | Orfe | ORF | | | Yes |
| *Leuciscus leuciscus* | Dace | DAC | Yes | 22 | Yes |
| *Lota lota* | Burbot | BUR | | | |
| *Micropterus salmoides* | Largemouth bass | LBA | | | |
| *Misgurnus fossilis* | Weather loach | WLO | | | |
| *Neogobius kessleri* | Bighead goby | BGO | | | |
| *Neogobius melanostomus* | Round goby | RGO | | | |
| *Oncorhynchus gorbuscha* | Pink salmon | PSA | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | Rainbow trout | RTR | | | |
| *Osmerus eperlanus* | Smelt | SME | | | |
| *Perca fluviatilis* | Perch | PER | Yes | 3 | Yes |
| *Petromyzon marinus* | Sea lamprey | SLA | Yes | | |
| *Phoxinus phoxinus* | Minnow | MIN | Yes | 8 | Yes |
| *Pimephales promelas* | Fathead minnow | FMI | | | |
| *Platichthys flesus* | Flounder | FLO | | | Yes |
| *Proterorhinus semilunaris* | Western tubenose goby | WTG | | | |
| *Pseudorasbora parva* | Topmouth gudgeon | TMG | | 13 | Yes |
| *Pungitius pungitius* | Nine-spined stickleback | 9SS | | 14 | Yes |
| *Rhodeus sericeus* | Bitterling | BIT | | | |
| *Rutilus rutilus* | Roach | ROA | Yes | 6 | |
| *Salmo salar* | Atlantic salmon | SAL | Yes | | |
| *Salmo trutta* | Brown trout | BTR | Yes | 7 | Yes |
| *Salvelinus alpinus* | Arctic charr | CHA | Yes | | |
| *Salvelinus fontinalis* | Brook charr | BCH | | | |
| *Sander lucioperca* | Pikeperch (zander) | ZAN | | | |
| *Scardinius erythrophthalmus* | Rudd | RUD | Yes | 15 | Yes |
| *Siluris glanis* | Wels catfish | WCA | | | |
| *Squalius cephalus (=Leuciscus cephalus)* | Chub | CHU | Yes | | Yes |
| *Thymallus thymallus* | Grayling | GRA | | | |
| *Tinca tinca* | Tench | TEN | Yes | 9 | Yes |
| *Umbra pygmaea* | Mudminnow | MUD | | 16 | |
| *Vimba vimba* | Vimba bream | VBR | | | |

**Table S2:** Sequences and references of primers tested in this study

| Primer | Sequence 5'-3' | Reference |
|---|---|---|
| teleo_F | ACACCGCCCGTCACTCT | Valentini et al. 2015 |
| teleo_Rdeg | CTTCCGGTACACTTACCRTG | Valentini et al. 2015 |
| 12S_F1 | ACTGGGATTAGATACCCC | Kelly et al. 2014 |
| 12S_R1 | TAGAACAGGCTCCTCTAG | Kelly et al. 2014 |
| Fish2bCBR | GATGGCGTAGGCAAACAAGA | Thompson et al. 2012 |
| Fish2CBL | ACAACTTCACCCCTGCAAAC | Thompson et al. 2012 |
| Fish2degCBL | ACAACTTCACCCCTGCRAAY | Thompson et al. 2012 |
| Fish2CBR | GATGGCGTAGGCAAATAGGA | Thompson et al. 2012 |
| CytB_L14841 | AAAAACCACCGTTGTTATTCAACTA | Kocher et al. 1989 |
| CytB_15149R | GCDCCTCARAATGAYATTTGTCCTCA | Kocher et al. 1989 |

**Table S3:** Summary of *in silico* testing results

Unresolved species pairs: 1 = *Coregonus*; 2 = *Hypophthalmichthys nobilis, H. molitrix*; 3 = *Ctenopharyngodon idella*, *H. molitrix*; 4 = *Ameiurus melas, A. nebulosus*; 5 = *Leuciscus idus, L. leuciscus*; 6 = *Salvelinus alpinus, S. fontinalis*, 7 = *Alosa fallax, A. alosa*, 8 = *Perca fluviatilis, Sander lucioperca*: 9 = *Lampetra planeri, L. fluviatilis*

| Target region | Forward primer | Reverse primer | Length | % Species amplified | Unresolved species pairs | Reference |
|---|---|---|---|---|---|---|
| **12S** | teleo_F | teleo_R | ~70 bp | 74 | 1, 2, 4, 5, 6 | Valentini et al. 2015 |
| **12S** | 12S_F1 | 12S_R1 | ~106 bp | 77 | 1, 2, 3, 6, 8 | Kelly et al. 2014 |
| **CytB** | Fish2bCBR | Fish2CBL | 40 bp | 16 | 1, 2, 4, 6, 9 | Thompson et al. 2012 |
| **CytB** | Fish2degCBL | Fish2CBR | 40 bp | 23 | 1, 2, 4, 6, 9 | Thompson et al. 2012 |
| **CytB** | CytB_L14841 | CytB_15149R | 460 bp | 91 | 1, 2, 5, 9 | Kocher et al 1989 |

**Table S4:** Total sequence reads in individual 12S mock communities. Green colours indicate species which were added to the community and the amount of DNA added (see legend below). Orange and brown colours indicate false positives and their frequencies (see legend below)

| Species | MC01 | MC02 | MC03 | MC04 | MC05 | MC06 | MC07 | MC08 | MC09 | MC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. brama* | 8985 | 3 | 4736 | 5238 | 0 | 19987 | 12 | 1224 | 13454 | 4 |
| *A. alburnus* | 3629 | 2721 | 36 | 0 | 5076 | 399 | 506 | 50 | 0 | 6368 |
| *A. nebulosus* | 7913 | 0 | 13 | 14 | 6582 | 1618 | 0 | 29 | 0 | 1655 |
| *B. barbus* | 0 | 3611 | 4935 | 0 | 3 | 0 | 7686 | 1062 | 0 | 0 |
| *Coregonus spp* | 290 | 0 | 329 | 451 | 0 | 2475 | 0 | 63 | 38 | 3 |
| *C. gobio* | 3678 | 0 | 7 | 5 | 3022 | 10055 | 0 | 4 | 0 | 986 |
| *C. carpio* | 3284 | 4392 | 31 | 30 | 6250 | 692 | 848 | 101 | 33 | 8654 |
| *E. lucius* | 3541 | 9 | 3187 | 6142 | 0 | 409 | 14 | 4224 | 4588 | 0 |
| *G. gobio* | 0 | 2606 | 0 | 0 | 10 | 0 | 347 | 0 | 0 | 14 |
| *G. cernua* | 8 | 8172 | 6421 | 10379 | 0 | 59 | 9925 | 14600 | 1159 | 0 |
| *L. gibbosus* | 0 | 64 | 0 | 0 | 38 | 0 | 15 | 0 | 0 | 8 |
| *L. delineatus* | 0 | 4 | 11889 | 0 | 0 | 0 | 5 | 23635 | 0 | 0 |
| *L. leuciscus* | 5 | 7029 | 0 | 0 | 6297 | 3 | 1598 | 41 | 0 | 8380 |
| *P. fluviatilis* | 0 | 2847 | 0 | 3319 | 0 | 5 | 2504 | 0 | 4780 | 0 |
| *P. phoxinus* | 4 | 5 | 5279 | 6505 | 0 | 13 | 8 | 624 | 6746 | 3 |
| *P. parva* | 19 | 3447 | 15 | 33 | 3475 | 0 | 5731 | 29 | 34 | 342 |
| *P. pungitius* | 4 | 0 | 0 | 9 | 4128 | 4 | 0 | 11 | 3 | 882 |
| *R. rutilus* | 0 | 3124 | 3 | 2163 | 6 | 0 | 973 | 0 | 396 | 4 |
| *S. trutta* | 3 | 19 | 4219 | 5802 | 0 | 10 | 7 | 8005 | 6454 | 0 |
| *S. cephalus* | 5248 | 6 | 0 | 6354 | 7 | 9891 | 5 | 0 | 648 | 15 |
| *T. tinca* | 983 | 3 | 527 | 667 | 0 | 1094 | 3 | 125 | 77 | 0 |
| *U. pygmaea* | 1426 | 3 | 1793 | 6 | 0 | 461 | 3 | 2174 | 0 | 3 |
| *A. anguilla* | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. bjoerkna* | 3 | 0 | 3 | 0 | 0 | 4 | 0 | 0 | 7 | 0 |
| *H. molitrix* | 8 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| *L. idus* | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 4 |
| *S. erythrophthalmus* | 157 | 27 | 83 | 8 | 66 | 41 | 25 | 83 | 3 | 11 |
| nohit | 13948 | 23891 | 12393 | 10343 | 18476 | 9657 | 19320 | 7433 | 10847 | 11031 |
| Total | 53136 | 61983 | 55906 | 57468 | 53439 | 56883 | 49535 | 63517 | 49267 | 38367 |

10 ng DNA added to mock community
5 ng DNA added to mock community
0.5 ng DNA added to mock community
False positive < 0.001
False positive < 0.003

**Table S5:** Total sequence reads in individual CytB mock communities. Green colours indicate species which were added to the community and the amount of DNA added (see legend below). Orange and brown colours indicate false positives and their frequencies (see legend below).

| Species | MC01 | MC02 | MC03 | MC04 | MC05 | MC06 | MC07 | MC08 | MC09 | MC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. brama* | 8193 | 26 | 10769 | 15074 | 393 | 18219 | 14 | 4124 | 22141 | 36 |
| *A. alburnus* | 1860 | 765 | 0 | 0 | 2232 | 136 | 70 | 8 | 96 | 7615 |
| *Ameirurus total* | 12168 | 0 | 42 | 37 | 9492 | 1678 | 10 | 107 | 373 | 2850 |
| *B. barbus* | 0 | 2526 | 3533 | 0 | 0 | 3 | 5619 | 1486 | 0 | 0 |
| *Coregonus total* | 76 | 0 | 124 | 170 | 4 | 285 | 0 | 39 | 23 | 0 |
| *C. gobio* | 1864 | 0 | 5 | 0 | 4704 | 7118 | 0 | 4 | 63 | 779 |
| *C. carpio* | 7059 | 9349 | 80 | 180 | 16713 | 2874 | 4030 | 314 | 442 | 17813 |
| *E. lucius* | 2467 | 16 | 4642 | 9733 | 0 | 60 | 16 | 4784 | 6859 | 3 |
| *G. gobio* | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *G. cernua* | 3 | 2851 | 3123 | 5646 | 0 | 0 | 5306 | 7071 | 510 | 0 |
| *L. gibbosus* | 4 | 8828 | 59 | 0 | 5196 | 0 | 15178 | 100 | 4 | 1133 |
| *L. delineatus* | 0 | 10 | 8216 | 0 | 0 | 0 | 0 | 7624 | 0 | 3 |
| *Leuciscus total* | 0 | 368 | 0 | 0 | 106 | 0 | 34 | 0 | 0 | 621 |
| *P. fluviatilis* | 10 | 2197 | 0 | 3521 | 0 | 0 | 4792 | 0 | 3782 | 0 |
| *P. phoxinus* | 3 | 3 | 2307 | 2295 | 0 | 0 | 0 | 138 | 3130 | 0 |
| *P. parva* | 0 | 1340 | 0 | 0 | 923 | 0 | 2158 | 7 | 11 | 185 |
| *P. pungitius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *R. rutilus* | 0 | 149 | 0 | 155 | 0 | 0 | 23 | 0 | 13 | 0 |
| *Salmo total* | 6 | 10 | 2312 | 2383 | 0 | 0 | 7 | 4171 | 3460 | 0 |
| *S. cephalus* | 2046 | 0 | 0 | 2920 | 83 | 3898 | 0 | 0 | 400 | 7 |
| *Tinca tinca* | 273 | 0 | 345 | 296 | 0 | 55 | 0 | 33 | 37 | 4 |
| *U. pygmaea* | 1788 | 10 | 4827 | 13 | 26 | 860 | 13 | 12943 | 49 | 0 |
| *A. anguilla* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. erythrophthalmus* | 7 | 0 | 30 | 7 | 0 | 3 | 0 | 38 | 3 | 0 |
| Cyprinidae | 216 | 0 | 276 | 352 | 0 | 300 | 0 | 63 | 371 | 0 |
| Percidae | 0 | 57 | 46 | 119 | 0 | 0 | 196 | 157 | 18 | 0 |
| Salmonidae | 0 | 0 | 3 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clupeocephala | 0 | 0 | 0 | 0 | 135 | 0 | 0 | 9 | 0 | 0 |
| Percinae | 0 | 208 | 17 | 481 | 0 | 0 | 401 | 0 | 511 | 0 |
| nohit | 6921 | 4190 | 2638 | 5295 | 3285 | 5336 | 3519 | 3362 | 1981 | 3929 |
| Total | 44964 | 32938 | 43394 | 48683 | 43292 | 40825 | 41386 | 46582 | 44277 | 34978 |

10 ng DNA added to mock commumity
5 ng DNA added to mock commumity
0.5 ng DNA added to mock commumity
False positive < 0.002

False positive < 0.01

**Table S6:** Summary of species abundance data from established method survey for the four Cumbrian lake basins. Left, relative abundance rank based on long-term monitoring data; right (in brackets) number of individuals caught in a gill netting survey in September 2014.

| | **Bassenthwaite Lake** | | **Derwent Water** | | **Windermere North Basin** | | **Windermere South Basin** | |
|---|---|---|---|---|---|---|---|---|
| Arctic charr | | | | | 3 | | 6 | |
| Atlantic salmon | 9 | | | | 9 | | 11 | |
| Brown trout | 6 | (2) | 7 | (1) | 5 | (12) | 4 | (6) |
| Bullhead | | | | | 8 | | 10 | |
| Common bream | | | | | 12 | | 7 | |
| Dace | 7 | (2) | 9 | | | | | |
| Eel | 4 | | 8 | | 6 | | 5 | |
| Minnow | 8 | | 4 | | 7 | | 9 | |
| Perch | 1 | (78) | 1 | (132) | 1 | (595) | 1 | (477) |
| Pike | 5 | (1) | 6 | (1) | 4 | (5) | 3 | (4) |
| River lamprey | | | | | 15 | | 15 | |
| Roach | 3 | (38) | 2 | (30) | 2 | (15) | 2 | (38) |
| Rudd | | | | | 14 | | 14 | |
| Ruffe | 2 | (68) | 3 | (22) | | | | |
| Sea lamprey | | | | | 16 | | 16 | |
| Stone loach | | | | | 10 | | 12 | |
| Tench | | | | | 13 | | 8 | |
| Three-spined stickleback | | | | | 11 | | 13 | |
| Vendace | 10 | (2) | 5 | (16) | | | | |
| **Total number of species recorded** | **10** | **(7)** | **9** | **(5)** | **16** | **(4)** | **16** | **(4)** |

**Table S7:** Overview of families detected in the 12S and CytB data set respectively.

| Domain | Family | 12S | CytB |
|---|---|---|---|
| Bacteria | Acidobacteriaceae | yes | no |
| Bacteria | Clostridiales Family XVII. | yes | no |
| Bacteria | Coriobacteriaceae | yes | no |
| Bacteria | Opitutaceae | yes | no |
| Bacteria | Peptococcaceae | yes | no |
| Bacteria | Planctomycetaceae | yes | no |
| Bacteria | Prochlorococcaceae | yes | no |
| Bacteria | Sphingomonadaceae | yes | no |
| Bacteria | Verrucomicrobiaceae | yes | no |
| Eukaryota | Anatidae | yes | no |
| Eukaryota | Anguillidae | yes | yes |
| Eukaryota | Balitoridae | yes | no |
| Eukaryota | Bovidae | yes | yes |
| Eukaryota | Canidae | yes | no |
| Eukaryota | Cervidae | yes | yes |
| Eukaryota | Cichlidae | yes | no |
| Eukaryota | Clupeidae | yes | no |
| Eukaryota | Columbidae | yes | no |
| Eukaryota | Cricetidae | yes | yes |
| Eukaryota | Cyprinidae | yes | yes |
| Eukaryota | Esocidae | no | yes |
| Eukaryota | Felidae | yes | no |
| Eukaryota | Gadidae | yes | no |
| Eukaryota | Gasterosteidae | no | yes |
| Eukaryota | Hominidae | yes | yes |
| Eukaryota | Leporidae | yes | no |
| Eukaryota | Moronidae | no | yes |
| Eukaryota | Muridae | yes | yes |
| Eukaryota | Mustelidae | yes | no |
| Eukaryota | Nemacheilidae | yes | no |
| Eukaryota | Percidae | yes | yes |
| Eukaryota | Phalacrocoracidae | yes | no |
| Eukaryota | Phasianidae | yes | no |
| Eukaryota | Rallidae | yes | no |
| Eukaryota | Ranidae | yes | no |
| Eukaryota | Salamandridae | yes | no |
| Eukaryota | Salmonidae | yes | yes |
| Eukaryota | Sciuridae | yes | no |
| Eukaryota | Scolopacidae | yes | no |
| Eukaryota | Scombridae | yes | no |
| Eukaryota | Soricidae | yes | no |
| Eukaryota | Strigidae | yes | no |

# Supplementary Figures

## Fig. S1: Sampling

a) boat and b) Friedinger sampler attached to winch (used for sampling on Windermere); c) pooling subsamples in sterile 2 L plastic bottles; d) sterile collection bottles; e) treatment of equipment with 10% bleach; f) water filtration units at Freshwater Biological Association laboratory, Far Sawrey, Windermere, UK. All photographs taken by the authors.

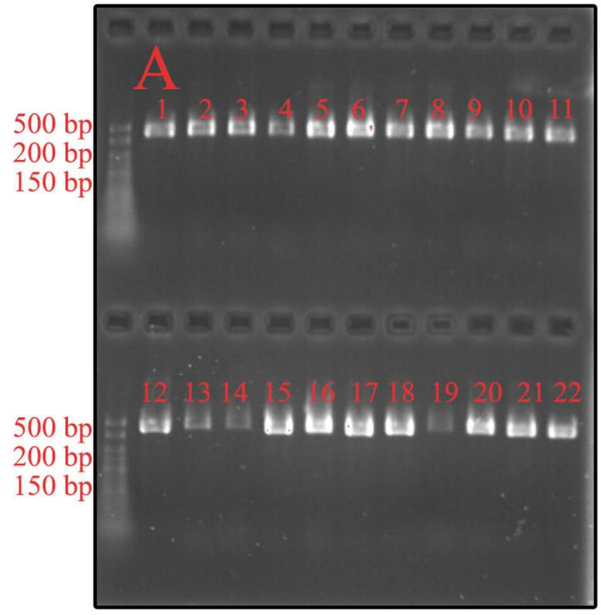
**Fig. S2:** Maximum Likelihood Phylogenies used to evaluate utility of loci for species resolution in *in silico* testing.

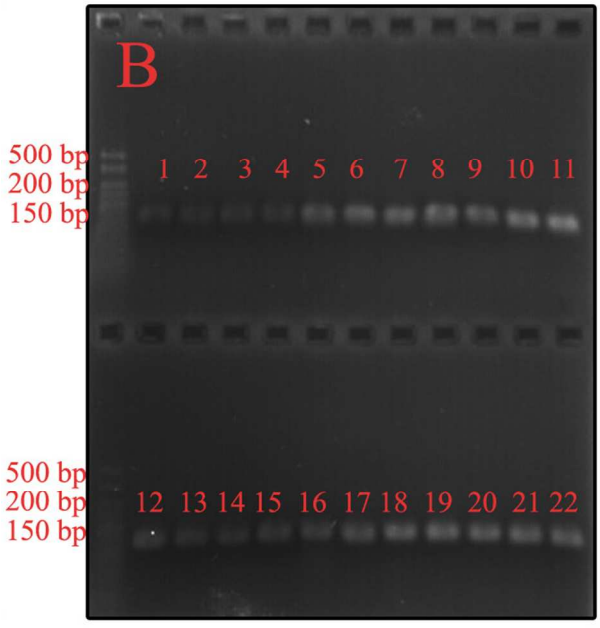
**a)** ML tree of the all 12S sequences from the reference data base (supplied in a separate file)

**b)** ML tree of the all Cytb sequences from the reference data base (supplied in a separate file)

**Fig S3**: Results of *in vitro* tests (single species amplifications) of the two chosen primer combinations A) Cytb Kocher *et al.* (1989), B) 12S Kelly *et al.* (2014).

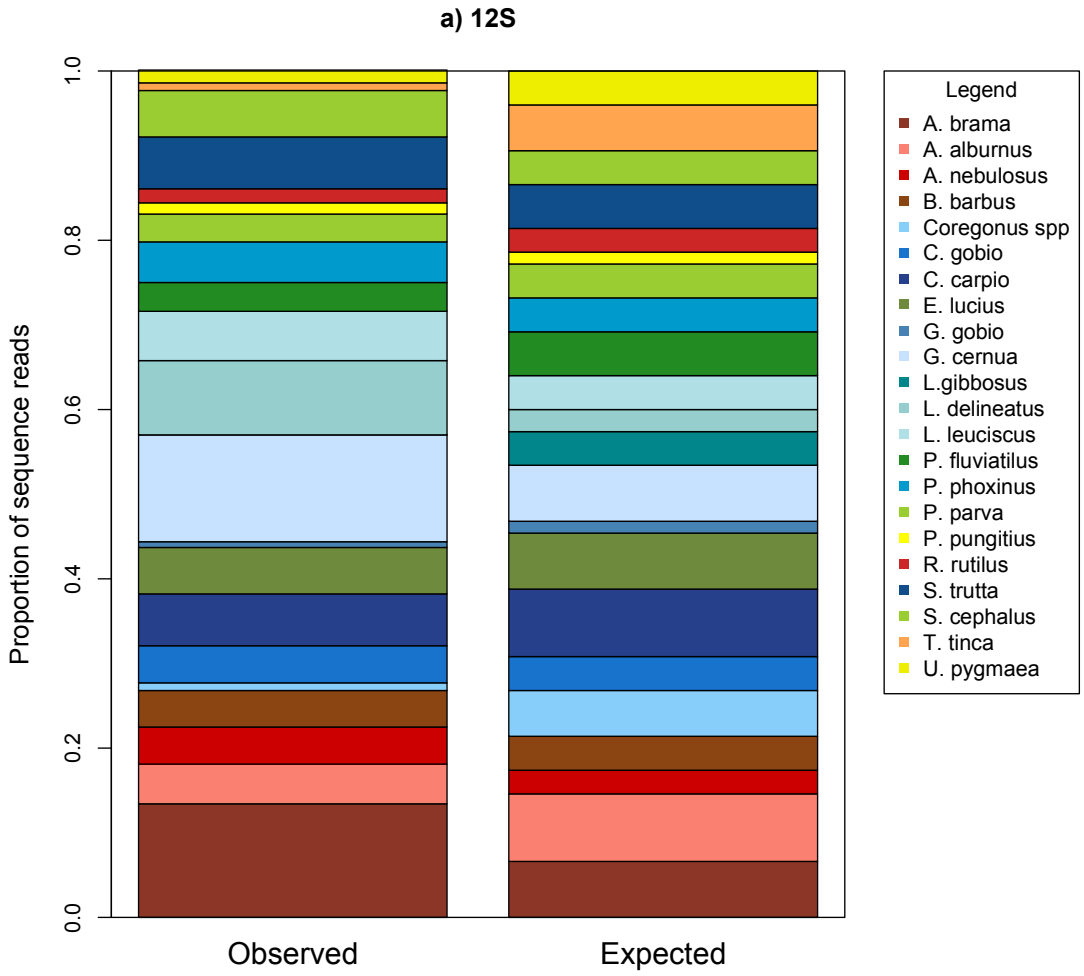
PCR products were run on 2.5% agarose gels, and stained with ethidium bromide. Numbers indicate different species and correspond to those in Table S1

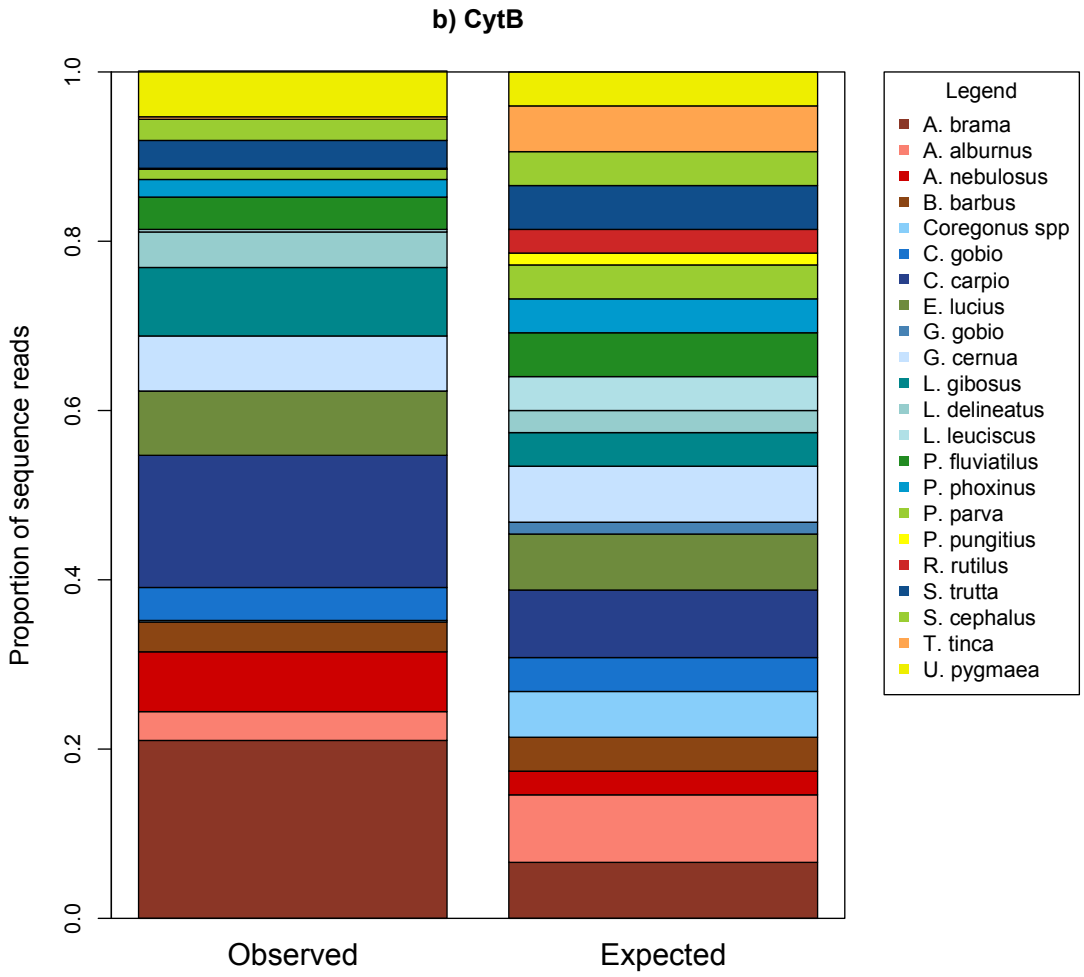


## Fig. S4: Mock community results

A) 12S and B) CytB mock community data. Bar plots show comparison of observed (number of sequence reads) and expected (DNA concentrations) data for combined mock communities. C) Correlation between read count per nanogram of DNA for 12S and CytB in the combined mock communities.




b) CytB
Proportion of sequence reads
0.0
0.2
0.4
0.6
0.8
1.0
Observed
Expected
Legend
A. brama
A. alburnus
A. nebulosus
B. barbus
Coregonus spp
C. gobio
C. carpio
E. lucius
G. gobio
G. cernua
L. gibosus
L. delineatus
L. leuciscus
P. fluviatilus
P. phoxinus
P. parva
P. pungitius
R. rutilus
S. trutta
S. cephalus
T. tinca
U. pygmaea

## C) Comparison of 12S and CytB for mock communities

## (Number of sequence reads per ng DNA)

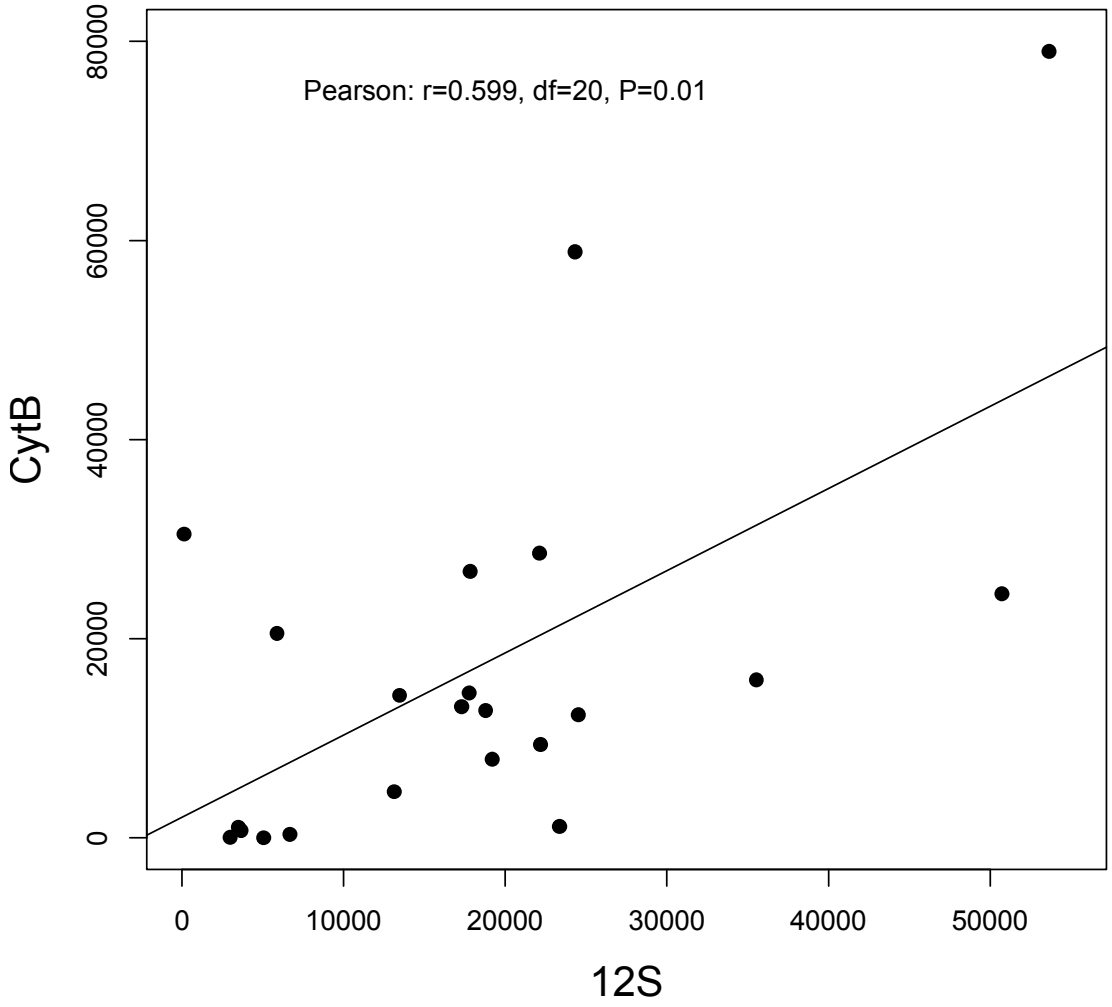
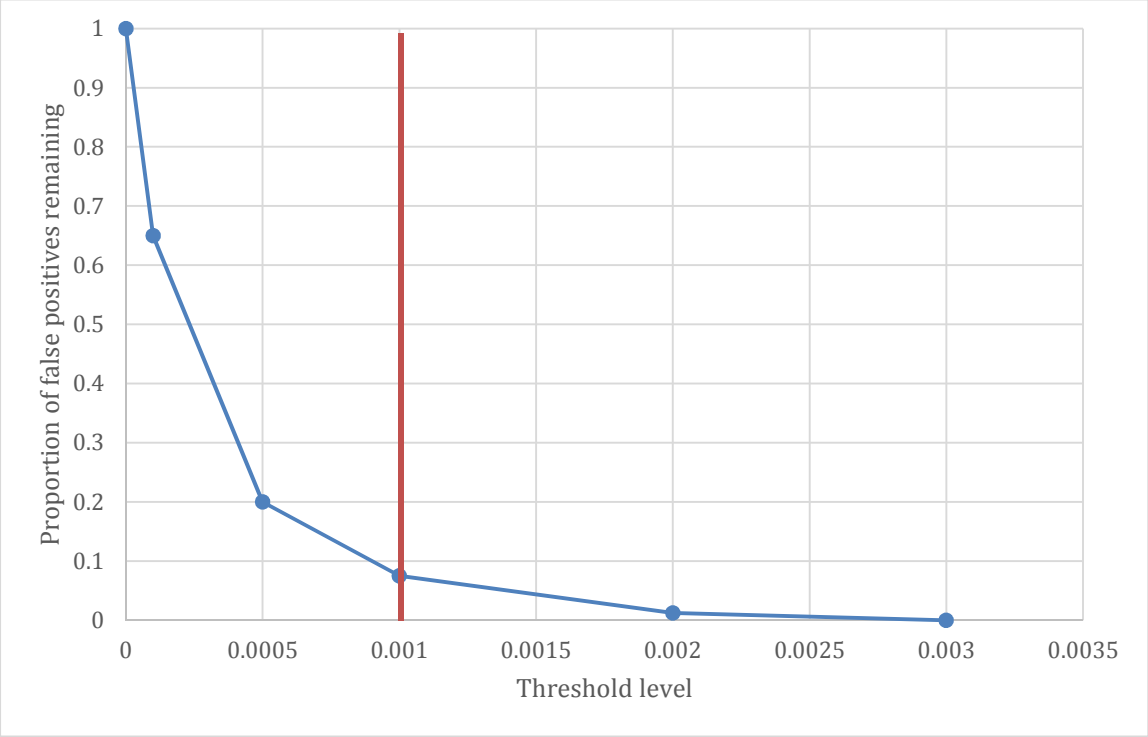


**Fig. S5:** Proportion of total false positives retained at different threshold levels for the 12S (a) and CytB (b) mock community data. The red vertical line indicates the threshold level used to analyse the lake samples



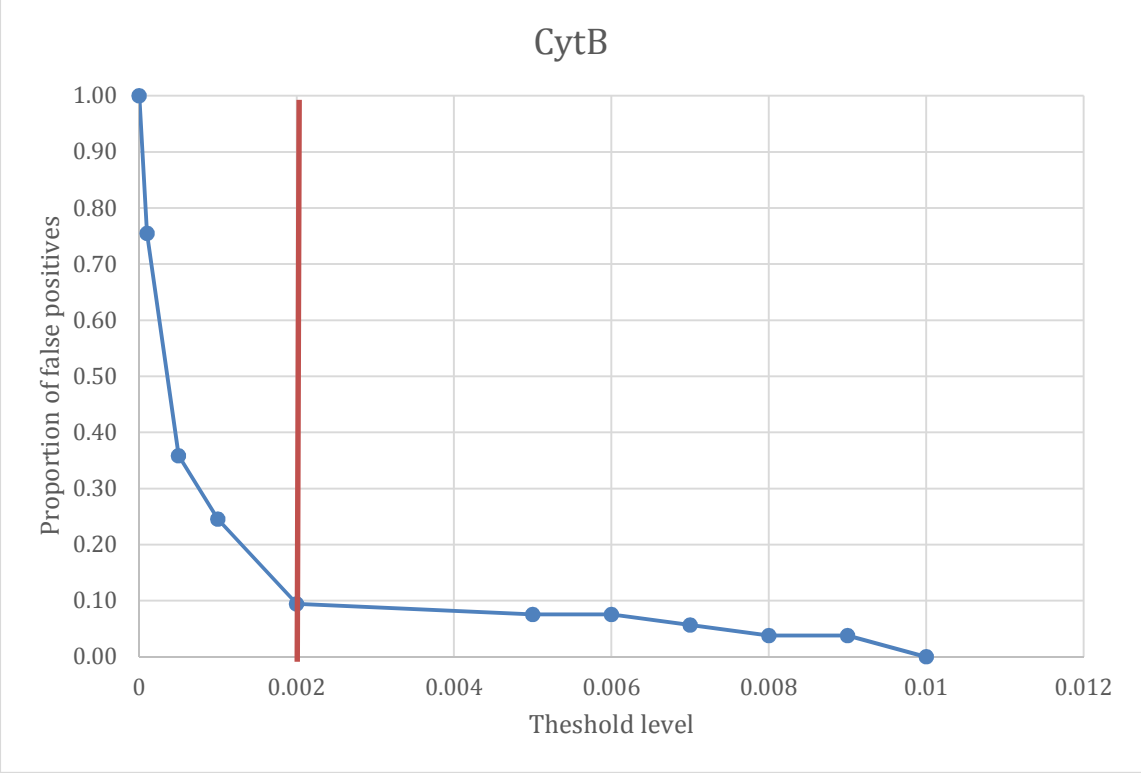



**Fig. S6:** Site occupancy across 29 sites in Windermere based on 12S (a) and CytB (b) using three different thresholds for defining "presence". In a) and b) dark blue corresponds to the site occupancy data when no detection threshold is used. Medium blue corresponds to the thresholds used in the main analyses (0.001 for 12S and 0.002 for CytB). Light blue corresponds to the threshold needed to eradicate false positives in the mock communities (0.003 in 12S and 0.01 for CytB). All species which can potentially occur in the study lakes and all species which were included in the mock communities (identified by an asterisk) are represented in the figure.

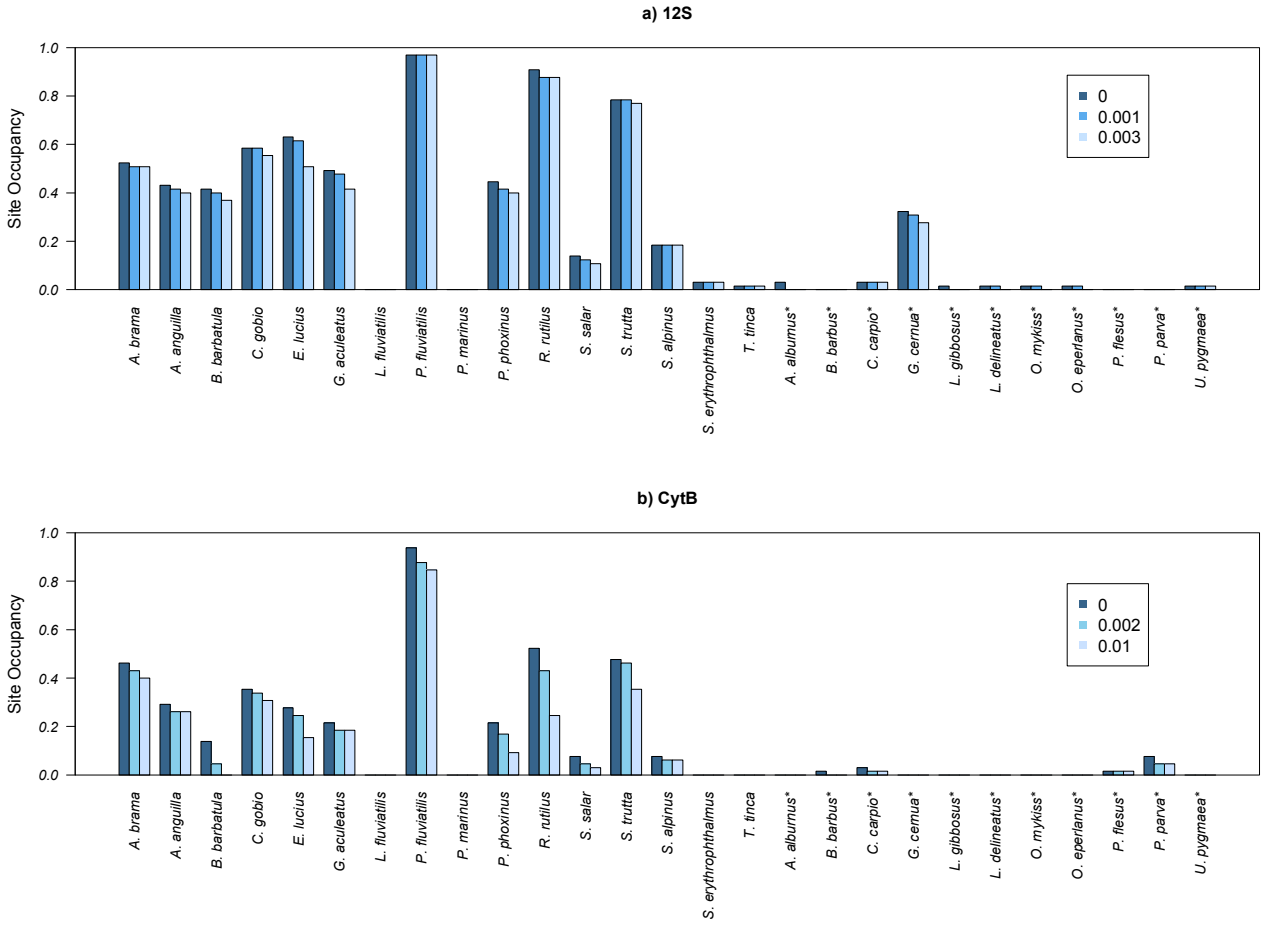


**Fig. S7:** Cumulative frequency distribution of relative PhiX read counts in the raw sequence data of the 12S libraries (a) and CytB libraries (b). Note that the y-axis ranged was capped at to 0.005 so the maximum value for CytB (0.0201) is not shown in figure (b)

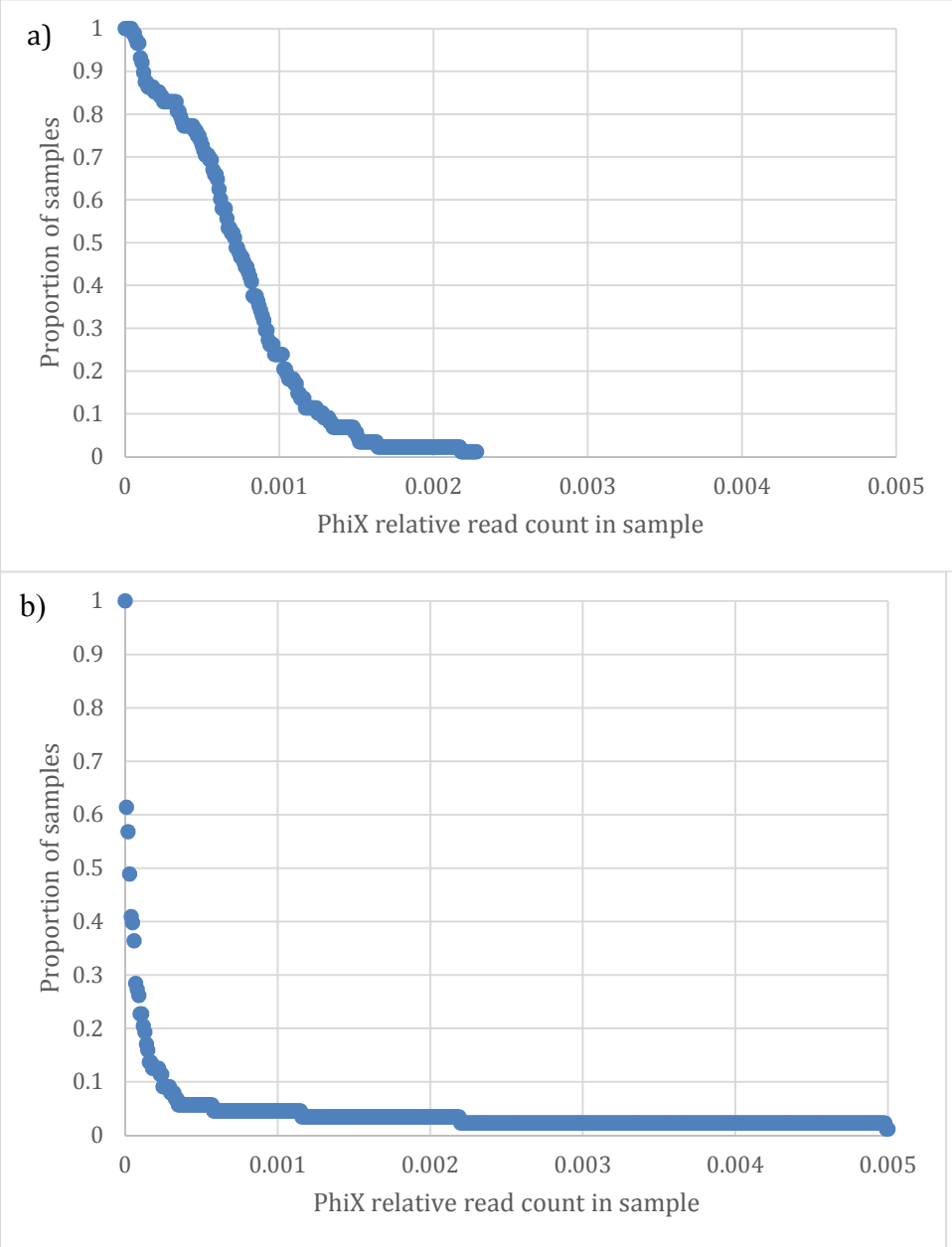


**Fig. S8:** Correlations between 12S and Cytb in terms of site occupancy ("SO", a-d) and read count ("RC", e-h) data. Abbreviations adjacent to scatter points correspond to species, and are explained in the List of Abbreviations in Table S1.

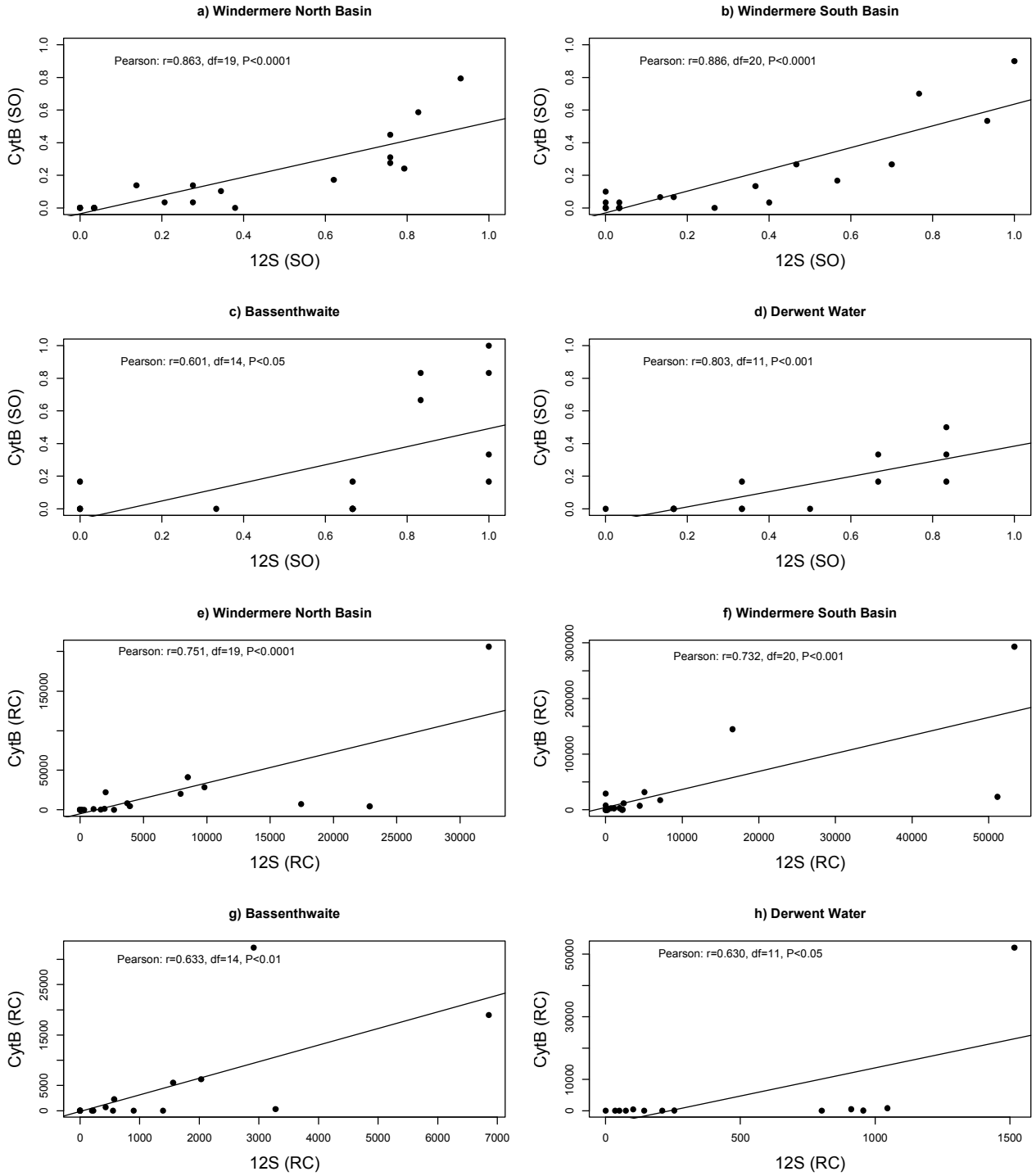


**Fig. S9:** Correlations between site occupancy and read count data per basin for 12S (a-d) and CytB (e-h)

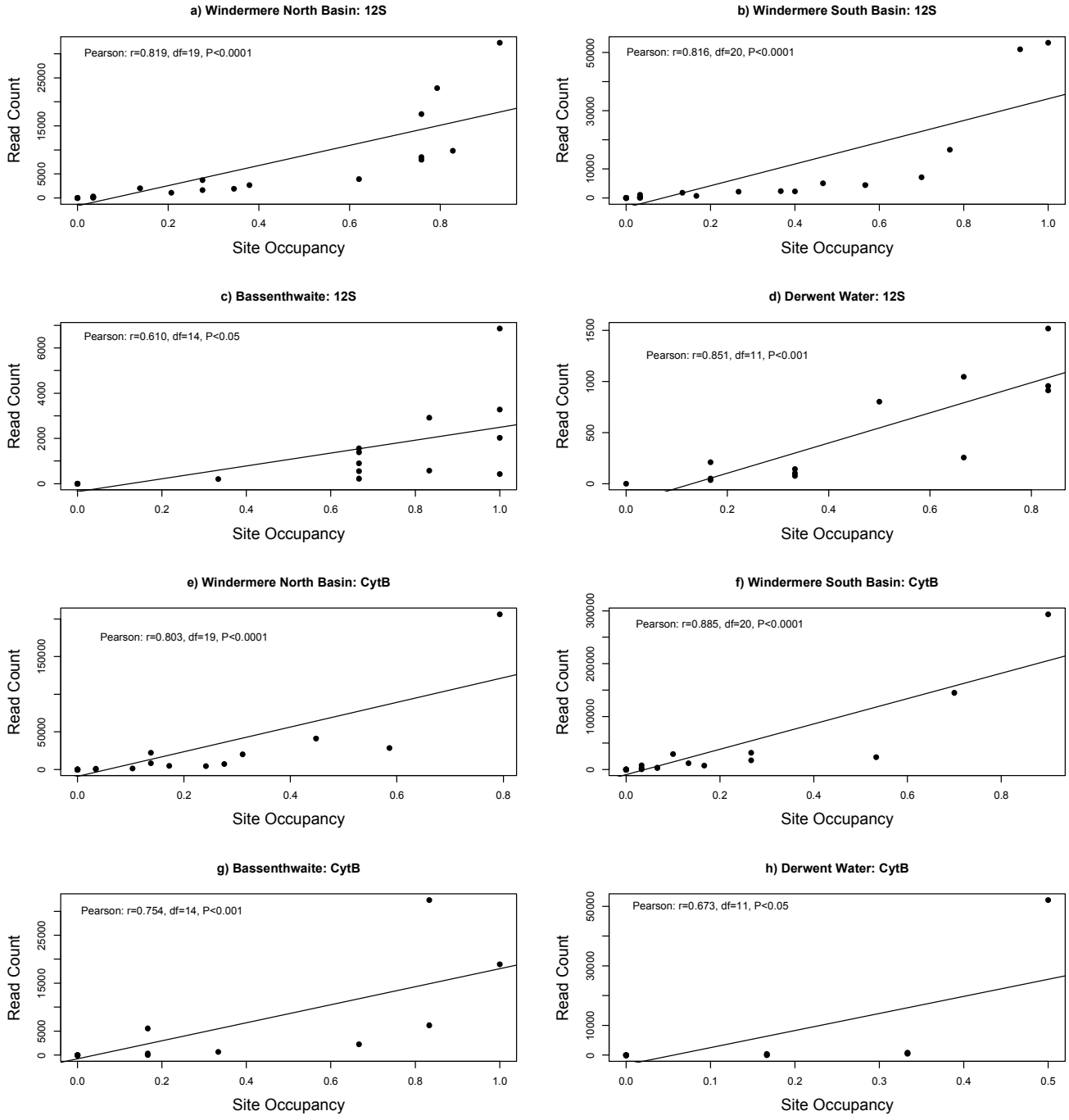
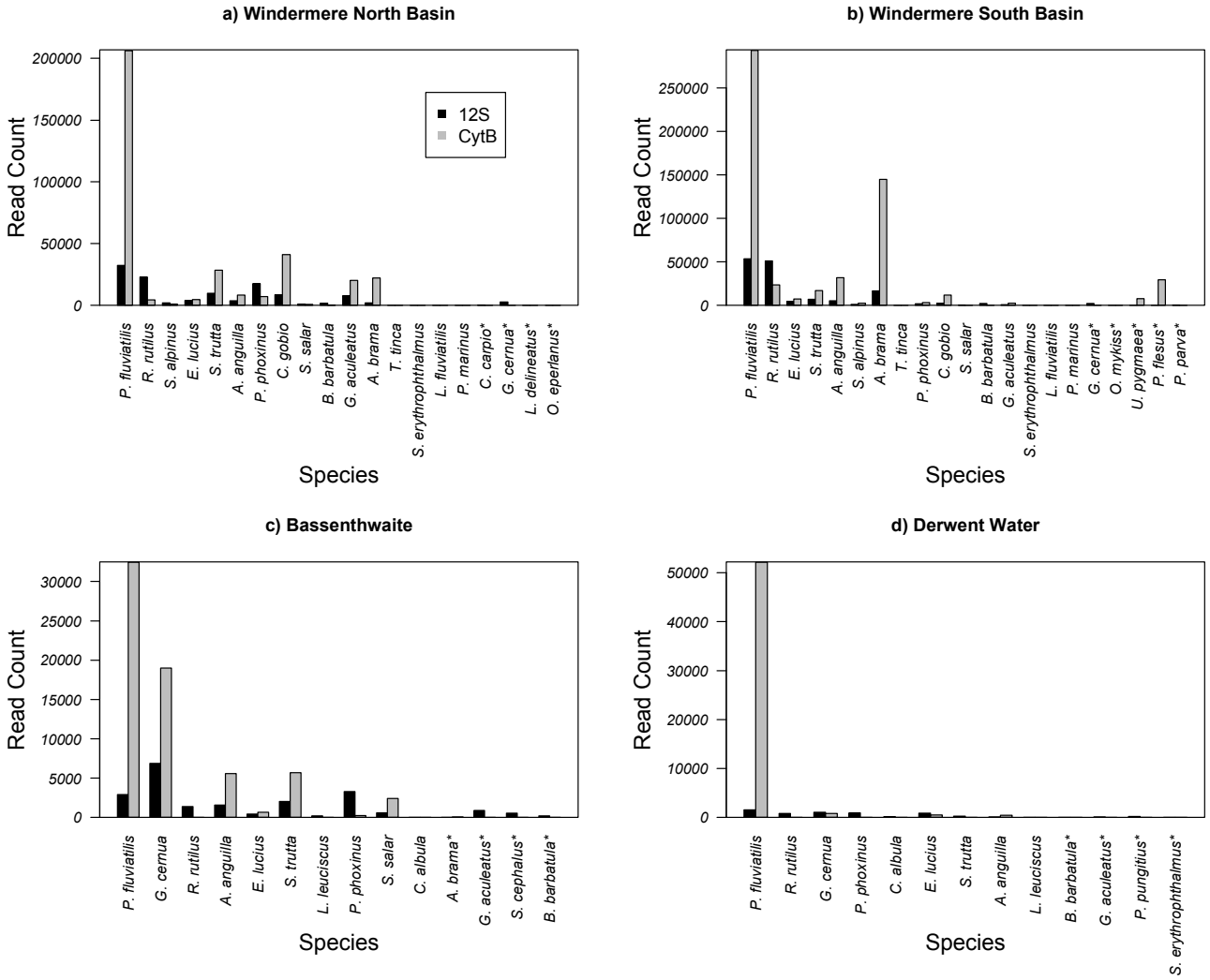


**Fig. S10:** a) Read count and b) proportion of sequence reads by lake basin. Species highlighted with an asterisk have not been previously recorded in the lake basin. Previously-recorded species are ordered according to their long term rank.

a)



**b)**

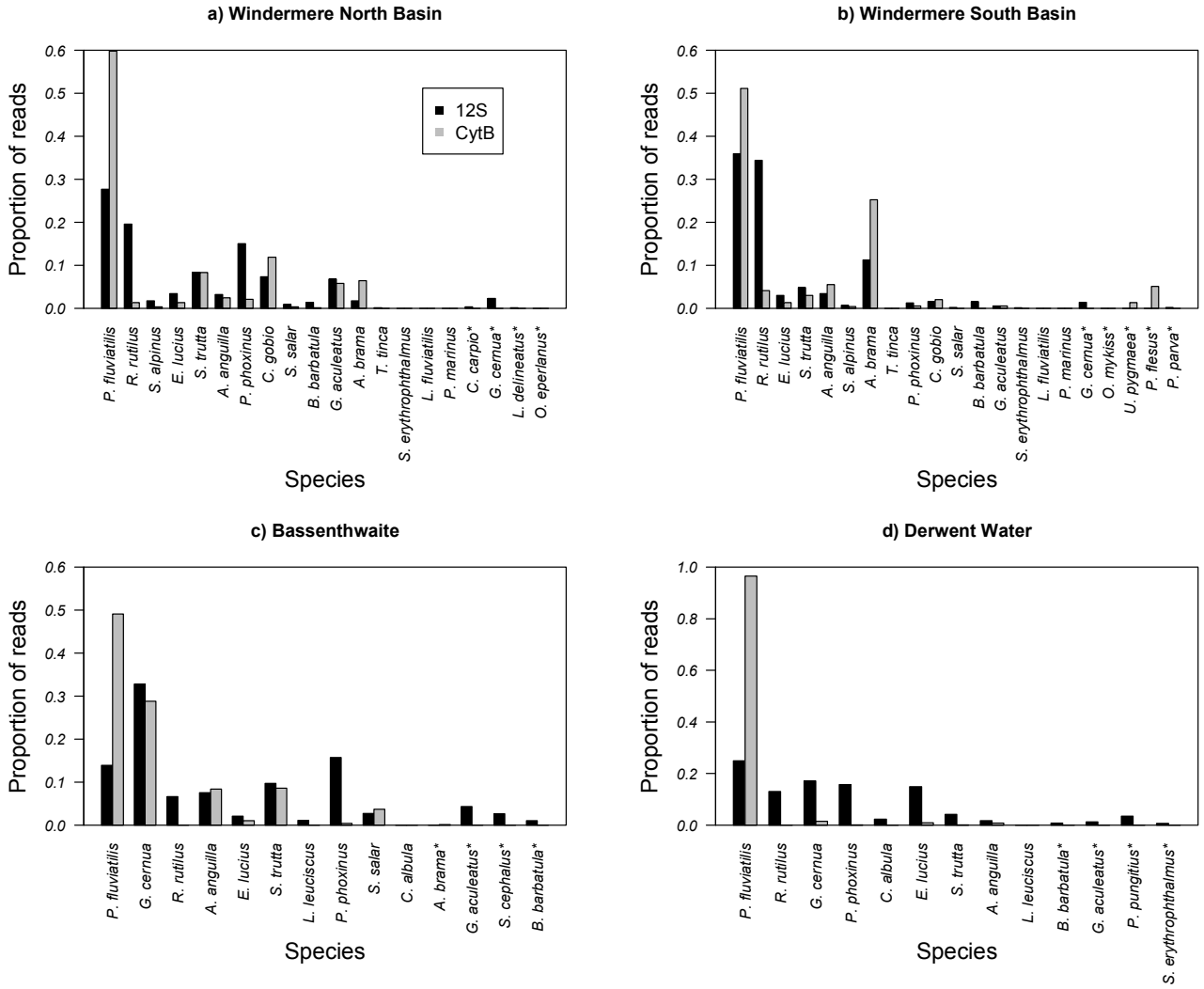

a) Windermere North Basin
b) Windermere South Basin
c) Bassenthwaite
d) Derwent Water
Proportion of reads
Species
12S
CytB
P. fluviatilis
R. rutilus
S. alpinus
E. lucius
S. trutta
A. anguilla
P. phoxinus
C. gobio
S. salar
B. barbatula
G. aculeatus
A. brama
T. tinca
S. erythrophthalmus
L. fluviatilis
P. marinus
C. carpio*
G. cernua*
L. delineatus*
O. eperlanus*
O. mykiss*
U. pygmaea*
P. flesus*
P. parva*
G. cernua
C. albula
A. brama*
S. cephalus*
B. barbatula*
L. leuciscus
P. pungitius*
S. erythrophthalmus*

**Fig. S11:** Correlations between read count ("number of sequence reads") and long term rank (with "1" corresponding to the most abundant species). Dashed lines indicate non significant trends. Three letter codes correspond to species and are listed in Table S1.

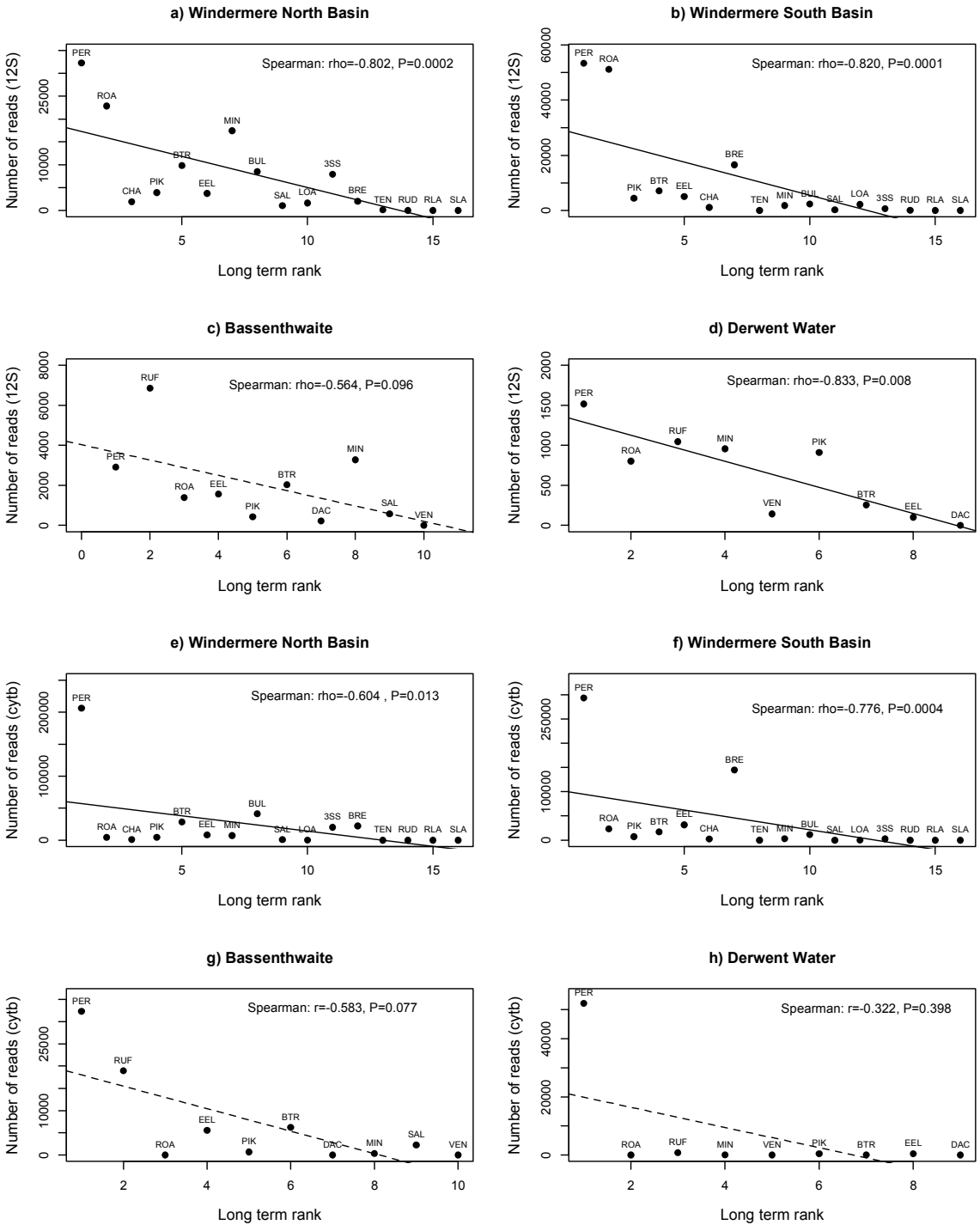


**Fig. S12:** Correlations between site occupancy and 2014 gill net numbers. Dashed lines indicate non significant trends. Three letter codes correspond to species and are listed in Table S1.

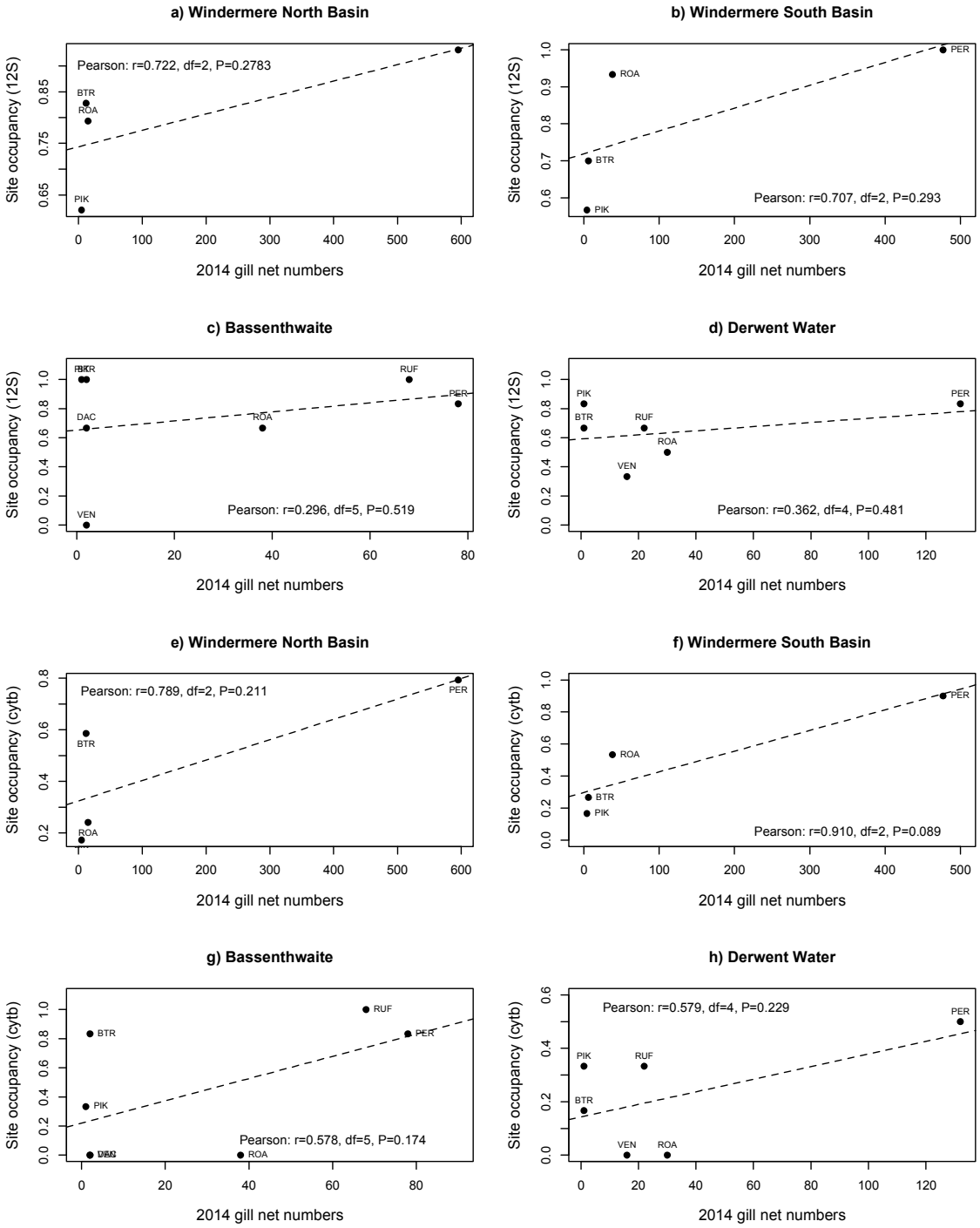


**Fig. S13:** Correlations between read count ("number of sequence reads") and 2014 gill net numbers. Dashed lines indicate non significant trends. Three letter codes correspond to species and are listed in Table S1.

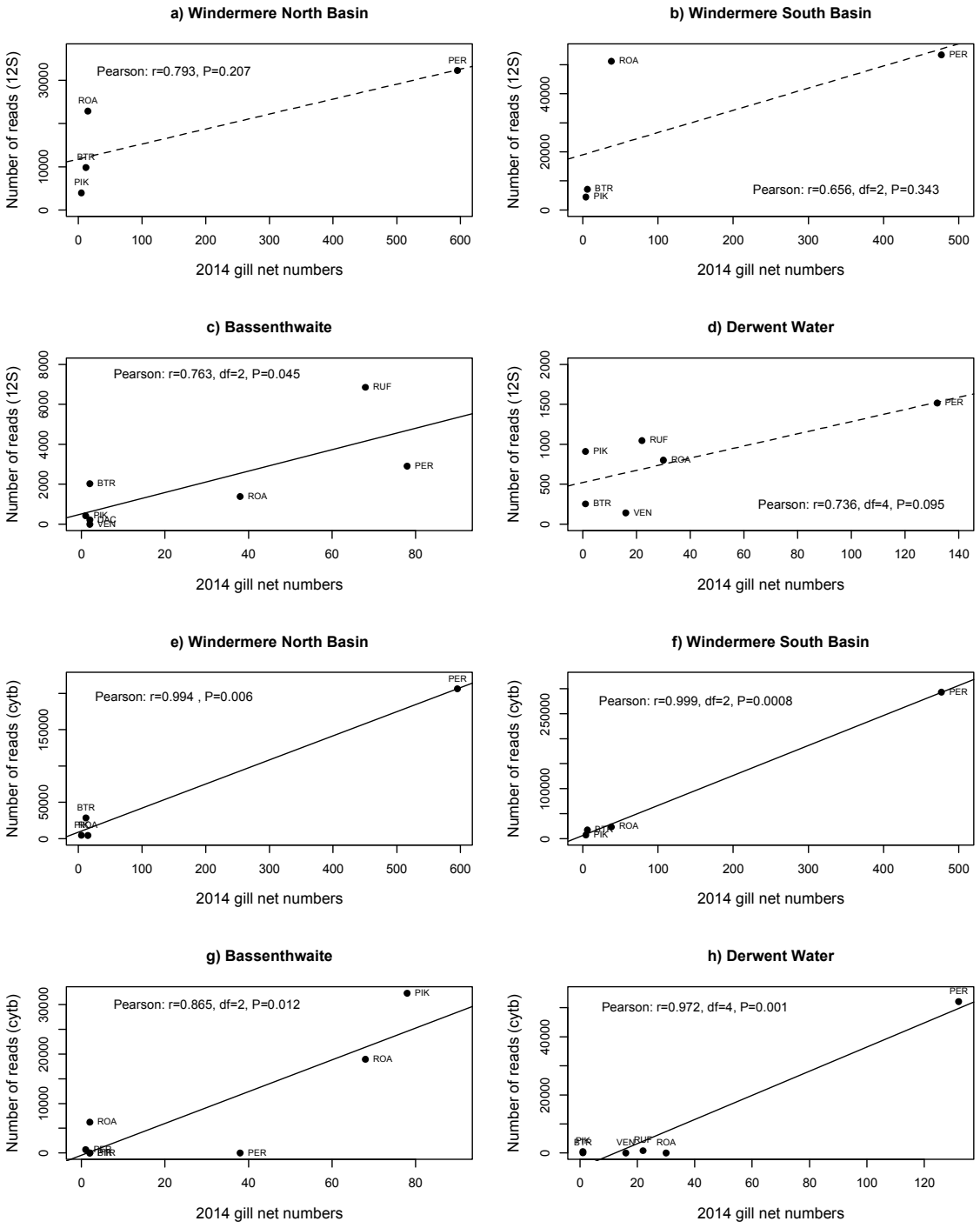


**Fig. S14:** Distribution of eDNA sequence reads in each of the depth profile transects

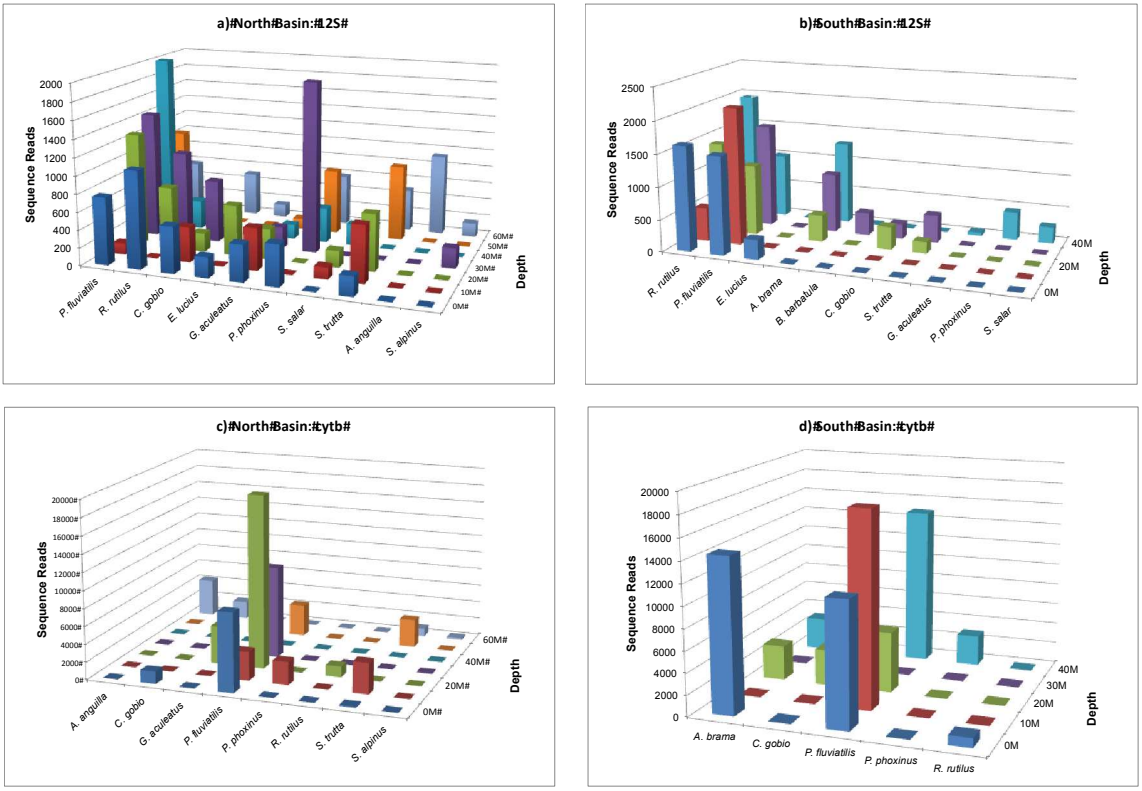
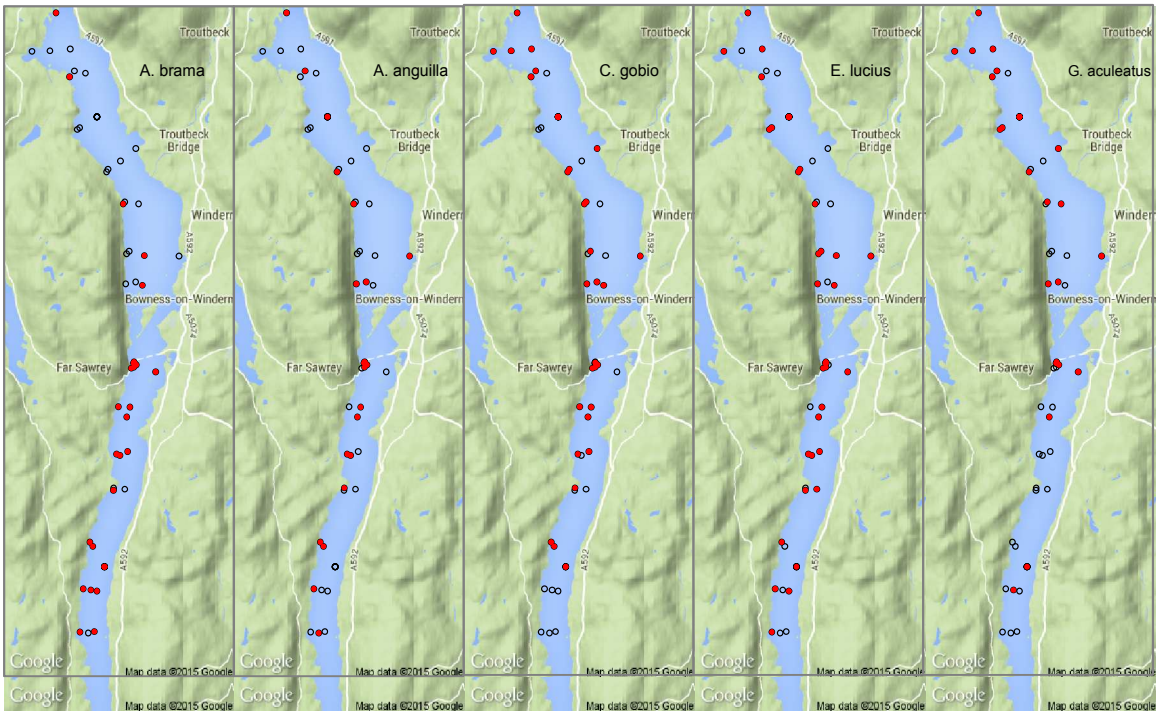


**Fig. S15:** Spatial Distribution of species recorded in Windermere North and South Basins at more than 2 sites (Site Occupancy) using 12S data



## Additional file information:

Appendix 1-4 Complete list of retained sequences for the curated non-redundant (nr) reference databases (provided in an excel spreadsheet)

Appendix 1: Sequence count data for the 12S primer data set

Appendix 2: Sequence count data for the CytB primer data set

Appendix 3: Genbank accession numbers and taxon affiliations of curated 12S sequences

Appendix 4: Genbank accession numbers and taxon affiliations of curated CytB sequences

Appendix 5: Flow chart of steps taken during the method development and full analytical pipeline stages.

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Winfield IJ, Durie NC (2004) Fish introductions and their management in the English Lake District. *Fisheries Management and Ecology* **11**, 195-201.

Winfield IJ, Fletcher JM, James JB (2015) *Monitoring the fish populations of Windermere, 2014.* Report to United Utilities. LA/NEC05364/2. 66pp.

| species | accession |
|---|---|
| Abramis brama | AP009305.1 |
| Abramis brama | KC894466.1 |
| Abramis brama | denovo17 |
| Abramis brama | denovo18 |
| Acipenser sturio | AF004980.1 |
| Acipenser sturio | AY544145.1 |
| Acipenser sturio | FN256366.1 |
| Acipenser sturio | Y12663.1 |
| Alburnoides bipunctatus | Y12665.1 |
| Alburnus alburnus | AB239593.1 |
| Alburnus alburnus | AJ002629.1 |
| Alburnus alburnus | denovo16 |
| Alosa alosa | AP009131.1 |
| Alosa fallax | EU552656.1 |
| Ambloplites rupestris | KM273799.1 |
| Ambloplites rupestris | KM282394.1 |
| Ameiurus melas | DQ421854.1 |
| Ameiurus melas | DQ421855.1 |
| Ameiurus melas | JN015532.1 |
| Ameiurus nebulosus | AY430252.1 |
| Ameiurus nebulosus | JX899750.1 |
| Ameiurus nebulosus | denovo28 |
| Anguilla anguilla | AF266494.1 |
| Anguilla anguilla | AF266495.1 |
| Anguilla anguilla | AF454706.1 |
| Anguilla anguilla | AP007233.1 |
| Anguilla anguilla | FJ612585.1 |
| Anguilla anguilla | KJ564227.1 |
| Anguilla anguilla | KJ564228.1 |
| Anguilla anguilla | KJ564230.1 |
| Anguilla anguilla | KJ564239.1 |
| Anguilla anguilla | KJ564240.1 |
| Anguilla anguilla | KJ564245.1 |
| Anguilla anguilla | KJ564247.1 |
| Anguilla anguilla | KJ564250.1 |
| Anguilla anguilla | KJ564253.1 |
| Anguilla anguilla | KJ564254.1 |
| Anguilla anguilla | KJ564255.1 |
| Anguilla anguilla | KJ564257.1 |
| Anguilla anguilla | KJ564268.1 |
| Anguilla anguilla | KJ564269.1 |
| Barbatula barbatula | denovo13 |
| Barbatula barbatula | denovo14 |
| Barbatula barbatula | denovo15 |
| Barbatula barbatula | denovo29 |
| Barbus barbus | AB238965.1 |

| | |
|---|---|
| Barbus barbus | Y12666.1 |
| Barbus barbus | denovo12 |
| Blicca bjoerkna | AF038468.1 |
| Blicca bjoerkna | AP009304.1 |
| Carassius auratus | AB006953.1 |
| Carassius auratus | AB111951.1 |
| Carassius auratus | AB379915.1 |
| Carassius auratus | FJ817301.1 |
| Carassius auratus | FJ817319.1 |
| Carassius auratus | GU086395.1 |
| Carassius auratus | GU086397.1 |
| Carassius auratus | HQ875340.1 |
| Carassius auratus | KF147851.1 |
| Carassius auratus | KJ476998.1 |
| Carassius auratus | KM657132.1 |
| Carassius auratus | KM657133.1 |
| Carassius carassius | JQ911695.1 |
| Chondrostoma nasus | DQ447667.1 |
| Chondrostoma nasus | DQ455047.1 |
| Cobitis taenia | AJ001795.1 |
| Cobitis taenia | AJ001797.1 |
| Cobitis taenia | AJ001798.1 |
| Coregonus albula | denovo27 |
| Coregonus lavaretus | JQ661382.1 |
| Coregonus lavaretus | JQ661390.1 |
| Coregonus lavaretus | JQ661446.1 |
| Coregonus lavaretus | JQ661474.1 |
| Coregonus lavaretus | JQ661479.1 |
| Coregonus lavaretus | JQ661480.1 |
| Coregonus oxyrinchus | JQ661401.1 |
| Cottus gobio | AB188189.1 |
| Cottus gobio | denovo26 |
| Ctenopharyngodon idella | AY897013.1 |
| Ctenopharyngodon idella | EU391390.1 |
| Ctenopharyngodon idella | HQ891005.1 |
| Ctenopharyngodon idella | JQ231115.1 |
| Ctenopharyngodon idella | KC292921.1 |
| Ctenopharyngodon idella | KC292922.1 |
| Cyprinus carpio | AP009047.1 |
| Cyprinus carpio | JN105352.1 |
| Cyprinus carpio | JN105357.1 |
| Cyprinus carpio | JX188253.1 |
| Cyprinus carpio | JX188254.1 |
| Cyprinus carpio | KC292935.1 |
| Cyprinus carpio | KF856964.1 |
| Cyprinus carpio | KF856965.1 |
| Cyprinus carpio | NC_001606.1 |

| | |
|---|---|
| Cyprinus carpio | denovo11 |
| Esox lucius | AP004103.1 |
| Esox lucius | AY430273.1 |
| Esox lucius | FJ425098.1 |
| Esox lucius | HM177472.1 |
| Esox lucius | HM177473.1 |
| Esox lucius | HM177474.1 |
| Esox lucius | HM177475.1 |
| Esox lucius | denovo10 |
| Esox lucius | denovo31 |
| Esox lucius | denovo9 |
| Gasterosteus aculeatus | AP002944.1 |
| Gasterosteus aculeatus | AY283316.1 |
| Gasterosteus aculeatus | AY283317.1 |
| Gasterosteus aculeatus | AY283318.1 |
| Gasterosteus aculeatus | KM273828.1 |
| Gasterosteus aculeatus | KM282418.1 |
| Gobio gobio | AB239596.1 |
| Gobio gobio | denovo7 |
| Gobio gobio | denovo8 |
| Gymnocephalus cernuus | AY141373.1 |
| Gymnocephalus cernuus | KM978956.1 |
| Gymnocephalus cernuus | denovo25 |
| Hypophthalmichthys mol | AM778105.1 |
| Hypophthalmichthys mol | EU315941.1 |
| Hypophthalmichthys mol | JQ231114.1 |
| Hypophthalmichthys mol | KC292923.1 |
| Hypophthalmichthys mol | KC292927.1 |
| Hypophthalmichthys mol | KC292930.1 |
| Hypophthalmichthys mol | KC292931.1 |
| Hypophthalmichthys mol | KC292934.1 |
| Hypophthalmichthys mol | KF384055.1 |
| Hypophthalmichthys mol | KF384062.1 |
| Hypophthalmichthys mol | KF384065.1 |
| Hypophthalmichthys mol | KF384066.1 |
| Hypophthalmichthys mol | KF384070.1 |
| Hypophthalmichthys mol | KF384076.1 |
| Hypophthalmichthys mol | KF384088.1 |
| Hypophthalmichthys mol | KF384091.1 |
| Hypophthalmichthys mol | KF384093.1 |
| Hypophthalmichthys mol | KJ671449.1 |
| Hypophthalmichthys mol | KJ671450.1 |
| Hypophthalmichthys mol | KJ746953.1 |
| Hypophthalmichthys mol | KJ746964.1 |
| Hypophthalmichthys mol | KJ746965.1 |
| Hypophthalmichthys nob | AY050550.1 |
| Hypophthalmichthys nob | EU343733.1 |

| | |
|---|---|
| Hypophthalmichthys nob | HM162839.1 |
| Hypophthalmichthys nob | KC292939.1 |
| Hypophthalmichthys nob | KC292942.1 |
| Hypophthalmichthys nob | KC292943.1 |
| Hypophthalmichthys nob | KJ679504.1 |
| Hypophthalmichthys nob | KJ729077.1 |
| Hypophthalmichthys nob | KJ729078.1 |
| Hypophthalmichthys nob | KJ756343.1 |
| Lampetra fluviatilis | NC_001131.1 |
| Lepomis gibbosus | JN655528.1 |
| Lepomis gibbosus | KM273834.1 |
| Lepomis gibbosus | KM282424.1 |
| Lepomis gibbosus | denovo24 |
| Leucaspius delineatus | AP009307.1 |
| Leuciscus cephalus | denovo22 |
| Leuciscus cephalus | denovo23 |
| Leuciscus idus | AJ002632.1 |
| Leuciscus idus | KF913024.1 |
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| Leuciscus leuciscus | denovo21 |
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| Ponticola kessleri | KM583832.1 |
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| Rhodeus sericeus | KM052222.1 |
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| Pungitius pungitius | JF798919.1 |
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| Pungitius pungitius | JF798921.1 |
| Pungitius pungitius | JF798922.1 |
| Pungitius pungitius | JF798923.1 |
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| Salmo trutta | JN007717.1 |
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| Thymallus thymallus | FJ853655.1 |
| Thymallus thymallus | JX960868.1 |
| Thymallus thymallus | JX960869.1 |
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| Umbra pygmaea | AP013049.1 |
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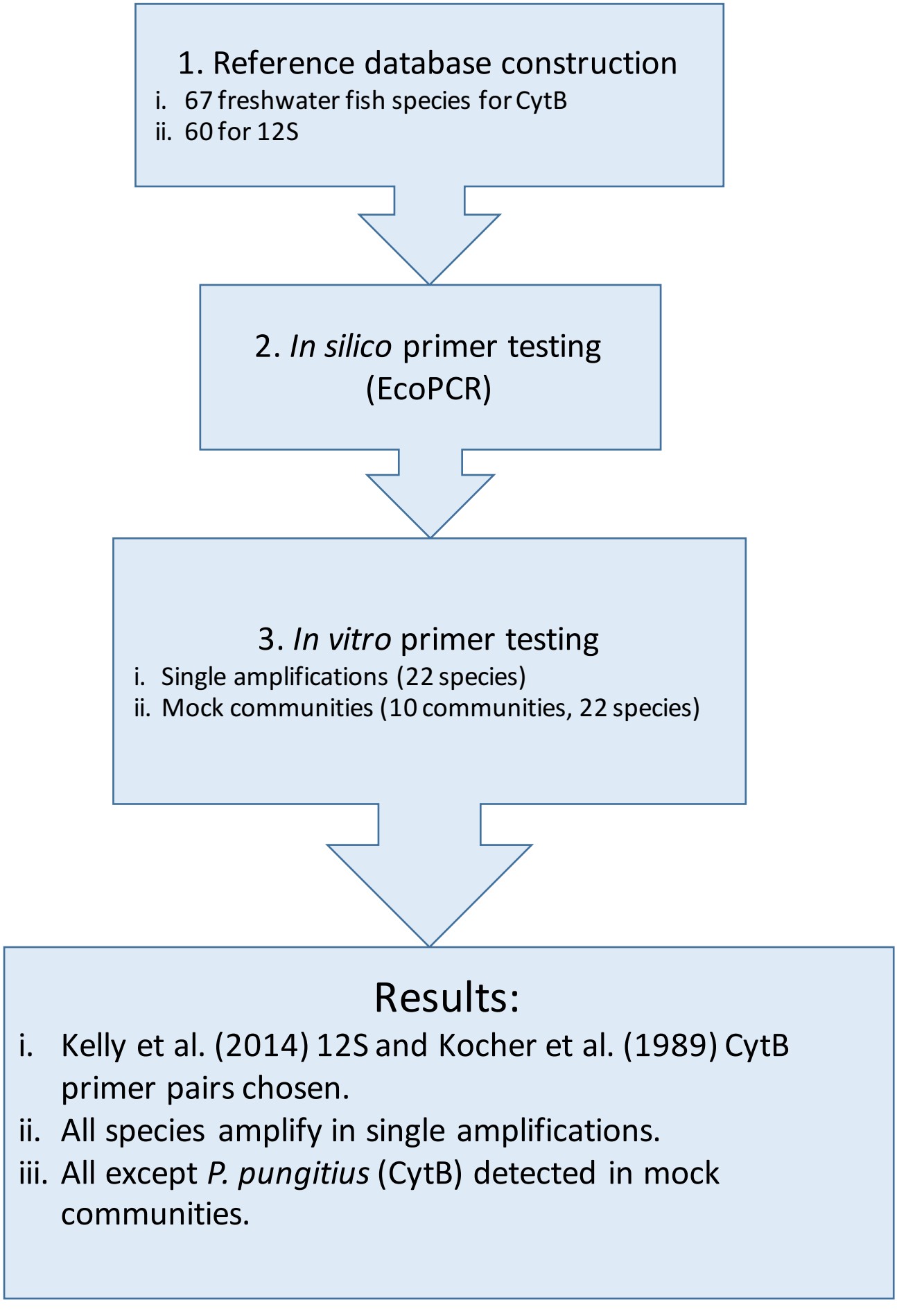
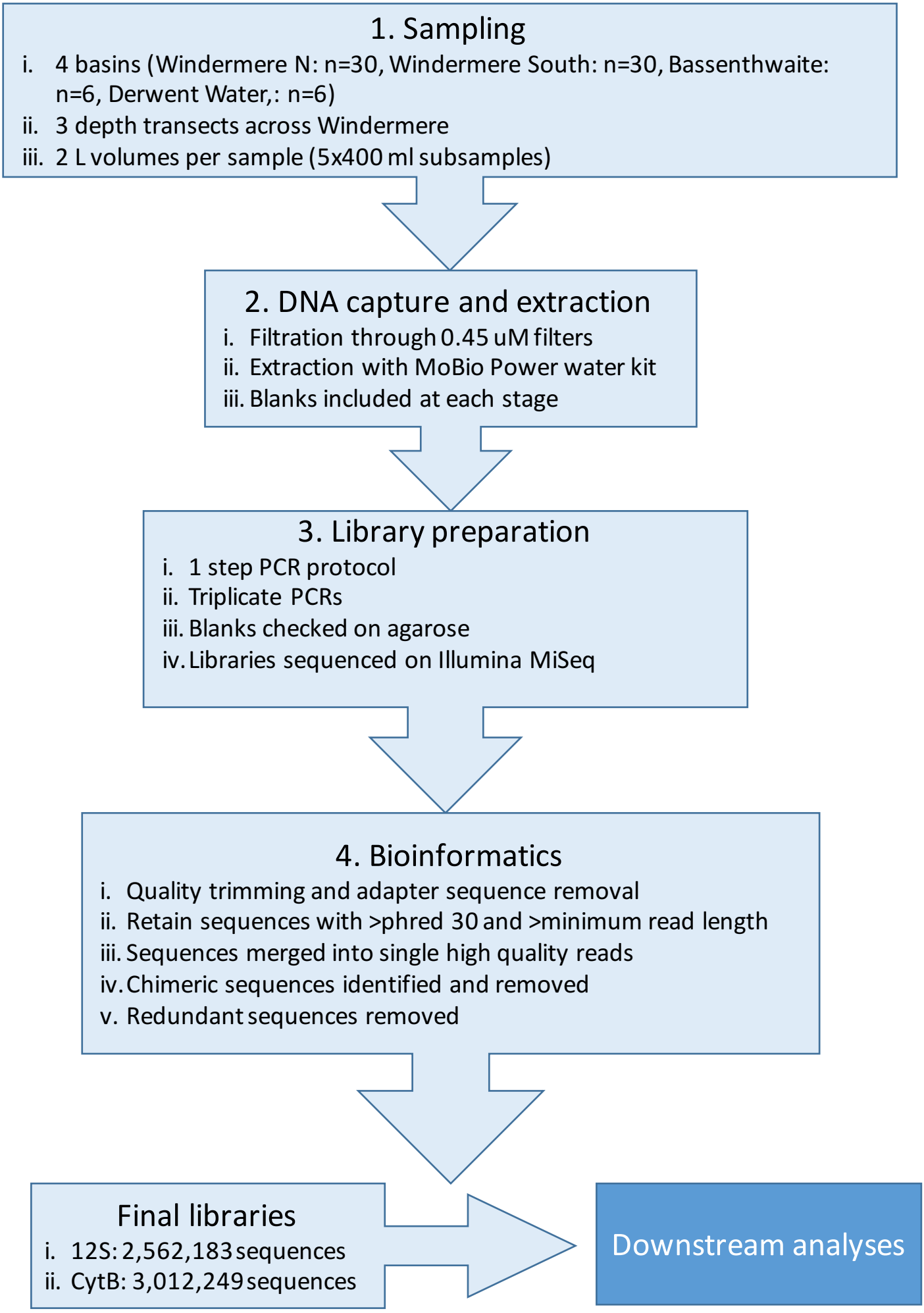
| Vimba vimba | HM560237.1 |

# Method Development pipeline



# Analytical Pipeline



| Sample site | Basin | latitude | longitude | Total raw reads |
|---|---|---|---|---|
| B1 | Bassenthwaite | 54.6713 | -3.23252 | 43078 |
| B2 | Bassenthwaite | 54.6473 | -3.21827 | 22844 |
| B3 | Bassenthwaite | 54.648167 | -3.21515 | 37448 |
| B4 | Bassenthwaite | 54.642367 | -3.20968 | 28584 |
| B5 | Bassenthwaite | 54.638783 | -3.20388 | 57644 |
| B-shore | Bassenthwaite | 54.666551 | -3.23678 | 49988 |
| D1 | Derwent water | 54.59285 | -3.1525 | 22728 |
| D2 | Derwent water | 54.585017 | -3.14308 | 22682 |
| D3 | Derwent water | 54.578967 | -3.14525 | 12018 |
| D4 | Derwent water | 54.575783 | -3.14055 | 17462 |
| D5 | Derwent water | 54.572717 | -3.13373 | 28356 |
| D-shore | Derwent water | 54.59481 | -3.14061 | 27886 |
| W01 | Windermere N basin | 54.419115 | -2.96878 | 69070 |
| W02 | Windermere N basin | 54.411788 | -2.97764 | 67800 |
| W03 | Windermere N basin | 54.406964 | -2.96369 | 96786 |
| W04 | Windermere N basin | 54.39691 | -2.96085 | 67420 |
| W05 | Windermere N basin | 54.388842 | -2.95005 | 56014 |
| W06 | Windermere N basin | 54.382744 | -2.94389 | 95464 |
| W07 | Windermere N basin | 54.373274 | -2.94258 | 56904 |
| W08 | Windermere N basin | 54.367531 | -2.94293 | 51892 |
| W09 | Windermere N basin | 54.412253 | -2.96348 | 49898 |
| W10 | Windermere N basin | 54.407645 | -2.95784 | 130504 |
| W11 0 m | Windermere N basin | 54.399328 | -2.9536 | 90008 |
| W11 10 m | Windermere N basin | 54.399328 | -2.9536 | 52906 |
| W11 20 m | Windermere N basin | 54.399328 | -2.9536 | 89026 |
| W11 30 m | Windermere N basin | 54.399328 | -2.9536 | 97578 |
| W11 40 m | Windermere N basin | 54.399328 | -2.9536 | 66142 |
| W11 50 m | Windermere N basin | 54.399328 | -2.9536 | 70642 |
| W11 60 m | Windermere N basin | 54.399328 | -2.9536 | 65262 |
| W12 | Windermere N basin | 54.390919 | -2.94485 | 41332 |
| W13 | Windermere N basin | 54.38277 | -2.93814 | 60754 |
| W14 | Windermere N basin | 54.3729 | -2.93601 | 72010 |
| W15 | Windermere N basin | 54.367248 | -2.93675 | 59686 |
| W16 | Windermere S basin | 54.352419 | -2.94006 | 99902 |
| W17 | Windermere S basin | 54.344103 | -2.94563 | 85608 |
| W18 | Windermere S basin | 54.335065 | -2.94632 | 87870 |
| W19 | Windermere S basin | 54.328632 | -2.9474 | 42514 |
| W20 | Windermere S basin | 54.318287 | -2.95622 | 53126 |
| W21 | Windermere S basin | 54.309392 | -2.95867 | 63958 |
| W22 | Windermere S basin | 54.301149 | -2.95988 | 52028 |
| W23 | Windermere S basin | 54.291392 | -2.95637 | 72760 |
| W24 | Windermere S basin | 54.282824 | -2.95591 | 79590 |
| W25 | Windermere S basin | 54.344027 | -2.94134 | 71292 |
| W26 | Windermere S basin | 54.335562 | -2.94221 | 81664 |
| W27 | Windermere S basin | 54.328396 | -2.94326 | 68908 |
| W28 0 m | Windermere S basin | 54.313594 | -2.95079 | 98364 |

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|---|---|---|---|---|
| W28 10 m | Windermere S basin | 54.313594 | -2.95079 | 96174 |
| W28 20 m | Windermere S basin | 54.313594 | -2.95079 | 91722 |
| W28 30 m | Windermere S basin | 54.313594 | -2.95079 | 92752 |
| W28 40 m | Windermere S basin | 54.313594 | -2.95079 | 88994 |
| W29 | Windermere S basin | 54.308946 | -2.95359 | 101974 |
| W30 | Windermere S basin | 54.301234 | -2.95457 | 62234 |
| W31 | Windermere S basin | 54.291735 | -2.95343 | 72822 |
| W32 | Windermere S basin | 54.282891 | -2.95388 | 93770 |
| W33 | Windermere S basin | 54.283275 | -2.95135 | 83472 |
| W34 | Windermere S basin | 54.291419 | -2.95474 | 91254 |
| W35 | Windermere S basin | 54.300954 | -2.95683 | 81000 |
| W36 | Windermere S basin | 54.309169 | -2.95583 | 60774 |
| W37 | Windermere S basin | 54.317485 | -2.9551 | 61136 |
| W38 | Windermere S basin | 54.328189 | -2.94747 | 49048 |
| W39 | Windermere S basin | 54.334825 | -2.94507 | 48802 |
| W40 | Windermere S basin | 54.342145 | -2.94255 | 54504 |
| W41 | Windermere S basin | 54.350749 | -2.93181 | 60314 |
| W42 | Windermere N basin | 54.372798 | -2.92319 | 58896 |
| W43 | Windermere N basin | 54.393287 | -2.93916 | 46228 |
| W44 | Windermere N basin | 54.411895 | -2.97101 | 71636 |
| W45 | Windermere N basin | 54.408059 | -2.96192 | 52808 |
| W46 | Windermere N basin | 54.397257 | -2.95996 | 44854 |
| W47 | Windermere N basin | 54.389364 | -2.94947 | 89084 |
| W48 | Windermere N basin | 54.383115 | -2.94328 | 50968 |
| W49 | Windermere N basin | 54.373741 | -2.94167 | 43892 |
| W50 | Windermere N basin | 54.367881 | -2.93924 | 49710 |
| Winshore_01 | Windermere S basin | 54.352641 | -2.93986 | 45074 |
| Winshore_02 | Windermere S basin | 54.352551 | -2.93985 | 50362 |
| Winshore_03 | Windermere S basin | 54.352083 | -2.93897 | 33670 |
| Winshore_04 | Windermere S basin | 54.351639 | -2.93992 | 64056 |
| Winshore_05 | Windermere S basin | 54.351444 | -2.94089 | 71258 |
| Winshore_06 | Windermere S basin | 54.352387 | -2.93976 | 79970 |
| MC01 | Mock community | not applicable | not applica | 147912 |
| MC02 | Mock community | not applicable | not applica | 146060 |
| MC03 | Mock community | not applicable | not applica | 138688 |
| MC04 | Mock community | not applicable | not applica | 137102 |
| MC05 | Mock community | not applicable | not applica | 133380 |
| MC06 | Mock community | not applicable | not applica | 143298 |
| MC07 | Mock community | not applicable | not applica | 117776 |
| MC08 | Mock community | not applicable | not applica | 155108 |
| MC09 | Mock community | not applicable | not applica | 117994 |
| MC10 | Mock community | not applicable | not applica | 90396 |

| Trimmed-total | Post-merging | Post-chimera-filter | Cluster_thres | Clusters_total | Clusters_min_cov |
|---|---|---|---|---|---|
| 34773 | 18381 | 18350 | 1 | 1635 | 3 |
| 19087 | 10083 | 10006 | 1 | 1090 | 3 |
| 27790 | 15286 | 15154 | 1 | 1577 | 3 |
| 21355 | 11493 | 11345 | 1 | 1312 | 3 |
| 41804 | 22696 | 22424 | 1 | 2349 | 3 |
| 40594 | 21423 | 21105 | 1 | 1964 | 3 |
| 17571 | 9441 | 9285 | 1 | 844 | 3 |
| 14500 | 8358 | 8358 | 1 | 893 | 3 |
| 7638 | 4242 | 4227 | 1 | 322 | 3 |
| 13344 | 7235 | 7149 | 1 | 835 | 3 |
| 19748 | 10595 | 10546 | 1 | 1074 | 3 |
| 24665 | 12813 | 12755 | 1 | 863 | 3 |
| 50112 | 30239 | 29936 | 1 | 1838 | 3 |
| 50065 | 28914 | 28697 | 1 | 2683 | 3 |
| 69821 | 40153 | 39657 | 1 | 2432 | 3 |
| 51391 | 29190 | 28898 | 1 | 1865 | 3 |
| 38493 | 24562 | 24442 | 1 | 2835 | 3 |
| 75713 | 43813 | 43540 | 1 | 2095 | 3 |
| 38650 | 25570 | 25477 | 1 | 1753 | 3 |
| 35125 | 22113 | 21994 | 1 | 1573 | 3 |
| 39343 | 22927 | 22802 | 1 | 1557 | 3 |
| 100914 | 53961 | 53926 | 1 | 2831 | 3 |
| 71927 | 39963 | 39912 | 1 | 2200 | 3 |
| 37265 | 23593 | 23544 | 1 | 1645 | 3 |
| 65612 | 38802 | 38712 | 1 | 2621 | 3 |
| 67128 | 41833 | 41748 | 1 | 2142 | 3 |
| 50388 | 29426 | 29393 | 1 | 1934 | 3 |
| 52884 | 30571 | 30494 | 1 | 2034 | 3 |
| 45510 | 29425 | 29321 | 1 | 1672 | 3 |
| 28859 | 18236 | 18197 | 1 | 1549 | 3 |
| 42807 | 26390 | 26342 | 1 | 1473 | 3 |
| 48354 | 31186 | 31059 | 1 | 1708 | 3 |
| 42314 | 27536 | 27336 | 1 | 1521 | 3 |
| 71699 | 44016 | 43716 | 1 | 2449 | 3 |
| 58987 | 37238 | 36749 | 1 | 1866 | 3 |
| 56693 | 39391 | 39184 | 1 | 1619 | 3 |
| 28843 | 19077 | 18938 | 1 | 1291 | 3 |
| 35235 | 23288 | 22996 | 1 | 1800 | 3 |
| 42661 | 28651 | 28596 | 1 | 1703 | 3 |
| 32740 | 23536 | 23446 | 1 | 1589 | 3 |
| 43477 | 31313 | 31200 | 1 | 1962 | 3 |
| 51166 | 34859 | 34670 | 1 | 1905 | 3 |
| 54981 | 32123 | 31633 | 1 | 1963 | 3 |
| 54110 | 36049 | 35808 | 1 | 1352 | 3 |
| 42974 | 30793 | 30758 | 1 | 1404 | 3 |
| 59910 | 43909 | 43829 | 1 | 1668 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 63331 | 44177 | 44112 | 1 | 1501 | 3 |
| 55191 | 41363 | 41283 | 1 | 1465 | 3 |
| 58488 | 42651 | 42527 | 1 | 1524 | 3 |
| 54667 | 40744 | 40669 | 1 | 1490 | 3 |
| 57964 | 44265 | 44216 | 1 | 1529 | 3 |
| 33215 | 23476 | 23385 | 1 | 1421 | 3 |
| 39228 | 26772 | 26717 | 1 | 1612 | 3 |
| 56898 | 35045 | 34911 | 1 | 1483 | 3 |
| 52343 | 33166 | 33077 | 1 | 1759 | 3 |
| 49859 | 33563 | 33511 | 1 | 1817 | 3 |
| 48276 | 30787 | 30637 | 1 | 1834 | 3 |
| 34893 | 22871 | 22770 | 1 | 1366 | 3 |
| 44718 | 27736 | 27576 | 1 | 1411 | 3 |
| 35220 | 22999 | 22904 | 1 | 1005 | 3 |
| 36303 | 22173 | 21975 | 1 | 1251 | 3 |
| 43905 | 25478 | 25199 | 1 | 1434 | 3 |
| 45347 | 27019 | 26657 | 1 | 1305 | 3 |
| 46505 | 26897 | 26687 | 1 | 1561 | 3 |
| 31884 | 20125 | 20054 | 1 | 1122 | 3 |
| 52149 | 29562 | 29559 | 1 | 2204 | 3 |
| 36465 | 22766 | 22717 | 1 | 2013 | 3 |
| 32860 | 19059 | 19047 | 1 | 1796 | 3 |
| 68029 | 39071 | 38989 | 1 | 2070 | 3 |
| 36514 | 21539 | 21497 | 1 | 1916 | 3 |
| 27828 | 17968 | 17922 | 1 | 1812 | 3 |
| 33870 | 21231 | 21113 | 1 | 2233 | 3 |
| 36544 | 20726 | 20463 | 1 | 1274 | 3 |
| 43282 | 23403 | 23027 | 1 | 1416 | 3 |
| 27148 | 15519 | 15265 | 1 | 1290 | 3 |
| 49513 | 28410 | 28025 | 1 | 1813 | 3 |
| 55060 | 31573 | 31050 | 1 | 1797 | 3 |
| 64089 | 35901 | 35265 | 1 | 2051 | 3 |
| 126511 | 69072 | 55397 | 1 | 2445 | 3 |
| 131623 | 68382 | 64076 | 1 | 2241 | 3 |
| 124525 | 64622 | 58376 | 1 | 2593 | 3 |
| 123444 | 64097 | 59470 | 1 | 2094 | 3 |
| 115638 | 62130 | 55229 | 1 | 1904 | 3 |
| 116450 | 66998 | 58335 | 1 | 1521 | 3 |
| 106573 | 55289 | 51232 | 1 | 1806 | 3 |
| 140554 | 72888 | 65176 | 1 | 1860 | 3 |
| 107083 | 55411 | 50871 | 1 | 1736 | 3 |
| 80262 | 42400 | 39522 | 1 | 1232 | 3 |

| Cluster_above_thres | Queries | Abramis b | Alburnus a | Ameiurus | Anguilla a | Barbatula | Barbus ba | Blicca bjoe |
|---|---|---|---|---|---|---|---|---|
| 363 | 16864 | 0 | 0 | 0 | 33 | 0 | 0 | 0 |
| 256 | 9050 | 0 | 0 | 0 | 18 | 0 | 0 | 0 |
| 343 | 13737 | 0 | 0 | 0 | 0 | 21 | 0 | 0 |
| 282 | 10157 | 0 | 0 | 0 | 66 | 82 | 0 | 0 |
| 542 | 20335 | 0 | 0 | 0 | 6 | 772 | 0 | 0 |
| 429 | 19364 | 0 | 0 | 0 | 1444 | 23 | 0 | 0 |
| 191 | 8539 | 0 | 0 | 0 | 0 | 51 | 0 | 0 |
| 203 | 7570 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 79 | 3955 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 186 | 6417 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 245 | 9609 | 0 | 0 | 0 | 43 | 0 | 0 | 0 |
| 162 | 11951 | 0 | 0 | 0 | 59 | 0 | 0 | 0 |
| 406 | 28320 | 342 | 0 | 0 | 327 | 0 | 0 | 0 |
| 572 | 26306 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 539 | 37477 | 435 | 0 | 0 | 0 | 238 | 0 | 0 |
| 415 | 27228 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 518 | 21828 | 0 | 0 | 0 | 295 | 0 | 0 | 0 |
| 467 | 41703 | 434 | 0 | 0 | 705 | 333 | 0 | 0 |
| 351 | 23879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 341 | 20599 | 0 | 0 | 0 | 128 | 262 | 0 | 0 |
| 320 | 21384 | 0 | 0 | 0 | 0 | 201 | 0 | 0 |
| 664 | 51432 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 483 | 37935 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 352 | 22086 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 570 | 36343 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 426 | 39784 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 446 | 27704 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 446 | 28705 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 364 | 27822 | 0 | 0 | 0 | 914 | 0 | 0 | 0 |
| 315 | 16783 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 278 | 24970 | 0 | 0 | 0 | 0 | 291 | 0 | 0 |
| 352 | 29487 | 187 | 11 | 0 | 0 | 0 | 0 | 0 |
| 311 | 25932 | 802 | 0 | 0 | 0 | 95 | 0 | 0 |
| 557 | 41527 | 279 | 0 | 0 | 259 | 0 | 0 | 0 |
| 426 | 35058 | 338 | 0 | 0 | 0 | 97 | 0 | 0 |
| 363 | 37729 | 928 | 0 | 0 | 446 | 172 | 0 | 0 |
| 286 | 17785 | 0 | 0 | 0 | 342 | 113 | 0 | 0 |
| 362 | 21348 | 419 | 0 | 0 | 330 | 116 | 0 | 0 |
| 400 | 27095 | 322 | 0 | 0 | 377 | 0 | 0 | 0 |
| 363 | 22024 | 257 | 0 | 0 | 0 | 0 | 0 | 0 |
| 440 | 29455 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 400 | 32917 | 518 | 0 | 0 | 508 | 0 | 0 | 0 |
| 413 | 29858 | 448 | 13 | 0 | 172 | 154 | 0 | 0 |
| 305 | 34544 | 1621 | 0 | 0 | 0 | 0 | 0 | 0 |
| 318 | 29477 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 390 | 42329 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 345 | 42742 | 0 | 0 | 0 | 0 | 6 | 0 | 0 |
| 360 | 39980 | 415 | 0 | 0 | 0 | 0 | 0 | 0 |
| 398 | 41201 | 931 | 0 | 0 | 0 | 352 | 0 | 0 |
| 341 | 39336 | 1322 | 0 | 0 | 0 | 0 | 0 | 0 |
| 417 | 42915 | 1003 | 0 | 0 | 0 | 0 | 0 | 0 |
| 307 | 22094 | 596 | 0 | 0 | 0 | 211 | 0 | 0 |
| 349 | 25254 | 870 | 0 | 0 | 330 | 498 | 0 | 0 |
| 287 | 33538 | 0 | 0 | 0 | 794 | 0 | 0 | 0 |
| 358 | 31467 | 191 | 0 | 0 | 191 | 156 | 0 | 0 |
| 378 | 31850 | 951 | 0 | 0 | 0 | 0 | 0 | 0 |
| 374 | 28961 | 0 | 0 | 0 | 437 | 0 | 0 | 0 |
| 282 | 21529 | 329 | 0 | 0 | 0 | 207 | 0 | 0 |
| 294 | 26299 | 226 | 0 | 0 | 656 | 0 | 0 | 0 |
| 196 | 21964 | 1032 | 0 | 0 | 0 | 0 | 0 | 0 |
| 260 | 20835 | 2503 | 0 | 0 | 62 | 89 | 0 | 0 |
| 300 | 23896 | 560 | 0 | 0 | 155 | 54 | 0 | 0 |
| 260 | 25428 | 517 | 0 | 0 | 0 | 0 | 0 | 0 |
| 357 | 25309 | 0 | 0 | 0 | 277 | 95 | 0 | 0 |
| 242 | 19059 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 474 | 27579 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 404 | 20902 | 0 | 0 | 0 | 286 | 0 | 0 | 0 |
| 397 | 17447 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 434 | 37122 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 405 | 19769 | 0 | 0 | 0 | 0 | 122 | 0 | 0 |
| 371 | 16282 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 420 | 19073 | 0 | 0 | 0 | 793 | 0 | 0 | 0 |
| 274 | 19315 | 114 | 4 | 0 | 606 | 60 | 0 | 0 |
| 309 | 21762 | 89 | 0 | 0 | 252 | 176 | 0 | 0 |
| 238 | 14064 | 213 | 0 | 0 | 520 | 63 | 0 | 0 |
| 408 | 26435 | 239 | 0 | 0 | 215 | 85 | 0 | 0 |
| 407 | 29464 | 400 | 0 | 0 | 0 | 236 | 0 | 0 |
| 444 | 33410 | 171 | 0 | 0 | 391 | 366 | 0 | 0 |
| 539 | 53136 | 8985 | 3629 | 7913 | 0 | 0 | 0 | 3 |
| 485 | 61983 | 3 | 2721 | 0 | 0 | 0 | 3611 | 0 |
| 500 | 55906 | 4736 | 36 | 13 | 7 | 0 | 4935 | 3 |
| 402 | 57468 | 5238 | 0 | 14 | 0 | 0 | 0 | 0 |
| 398 | 53439 | 0 | 5076 | 6582 | 0 | 0 | 3 | 0 |
| 339 | 56883 | 19987 | 399 | 1618 | 0 | 0 | 0 | 4 |
| 398 | 49535 | 12 | 506 | 0 | 0 | 0 | 7686 | 0 |
| 478 | 63517 | 1224 | 50 | 29 | 0 | 0 | 1062 | 0 |
| 376 | 49267 | 13454 | 0 | 0 | 0 | 0 | 0 | 7 |
| 273 | 38367 | 4 | 6368 | 1655 | 0 | 0 | 0 | 0 |

| Cottus gol | Cyprinus c | Esox luciu | Gasteroste | Gobio gob | Gymnocep | Hypophth | Lepomis g | Leucaspiu | Leuciscus |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 26 | 0 | 0 | 244 | 0 | 0 | 0 | 0 |
| 0 | 0 | 52 | 0 | 0 | 818 | 0 | 0 | 0 | 0 |
| 0 | 0 | 70 | 246 | 0 | 1937 | 0 | 0 | 0 | 0 |
| 0 | 0 | 31 | 32 | 0 | 1740 | 0 | 0 | 0 | 0 |
| 0 | 0 | 91 | 251 | 0 | 965 | 0 | 0 | 0 | 0 |
| 0 | 0 | 159 | 24 | 0 | 1153 | 0 | 0 | 0 | 0 |
| 0 | 0 | 215 | 58 | 0 | 90 | 0 | 0 | 0 | 0 |
| 0 | 0 | 454 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 193 | 0 | 0 | 0 | 0 |
| 0 | 0 | 103 | 17 | 0 | 625 | 0 | 0 | 0 | 0 |
| 0 | 0 | 85 | 0 | 0 | 138 | 0 | 0 | 0 | 0 |
| 0 | 0 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 522 | 0 | 140 | 689 | 0 | 0 | 0 | 0 | 0 | 0 |
| 525 | 0 | 35 | 1038 | 0 | 0 | 0 | 0 | 0 | 0 |
| 412 | 0 | 98 | 576 | 0 | 0 | 0 | 4 | 0 | 0 |
| 0 | 320 | 546 | 257 | 0 | 0 | 0 | 0 | 0 | 0 |
| 695 | 0 | 85 | 143 | 0 | 235 | 0 | 0 | 0 | 0 |
| 1058 | 0 | 537 | 0 | 0 | 442 | 0 | 0 | 0 | 0 |
| 0 | 0 | 376 | 0 | 0 | 128 | 0 | 0 | 0 | 0 |
| 200 | 0 | 84 | 384 | 0 | 160 | 0 | 0 | 0 | 0 |
| 164 | 0 | 67 | 458 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 39 | 0 | 0 | 76 | 0 | 0 | 0 | 0 |
| 522 | 0 | 232 | 416 | 0 | 0 | 0 | 0 | 0 | 0 |
| 397 | 0 | 0 | 491 | 0 | 164 | 0 | 0 | 0 | 0 |
| 210 | 0 | 572 | 347 | 0 | 0 | 0 | 0 | 0 | 0 |
| 706 | 0 | 0 | 248 | 0 | 0 | 0 | 0 | 0 | 0 |
| 86 | 0 | 83 | 163 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 119 | 0 | 0 | 0 | 0 | 82 | 0 |
| 505 | 0 | 150 | 194 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 63 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 110 | 0 | 644 | 0 | 0 | 0 | 0 |
| 0 | 0 | 534 | 0 | 0 | 0 | 0 | 0 | 374 | 0 |
| 208 | 0 | 292 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 231 | 314 | 0 | 127 | 0 | 0 | 0 | 0 |
| 272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 107 | 0 | 97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 85 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 |
| 242 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 543 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 116 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 168 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 363 | 0 | 72 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 119 | 0 | 371 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 201 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 304 | 0 | 0 | 464 | 0 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 344 | 0 | 0 | 0 | 0 |
| 350 | 0 | 0 | 0 | 0 | 464 | 0 | 0 | 0 | 0 |
| 232 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 47 | 0 | 380 | 0 | 0 | 0 | 0 |
| 0 | 0 | 519 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 0 |
| 0 | 0 | 227 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 742 | 0 | 0 | 190 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 107 | 0 | 0 | 0 | 0 | 0 | 0 |
| 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 276 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 154 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 119 | 154 | 0 | 9 | 0 | 0 | 0 | 0 |
| 0 | 0 | 151 | 55 | 0 | 0 | 0 | 0 | 0 | 0 |
| 357 | 0 | 40 | 820 | 0 | 0 | 0 | 0 | 0 | 0 |
| 503 | 0 | 0 | 286 | 0 | 0 | 0 | 0 | 0 | 0 |
| 165 | 0 | 0 | 240 | 0 | 0 | 0 | 0 | 0 | 0 |
| 192 | 0 | 0 | 530 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 138 | 78 | 0 | 0 | 0 | 0 | 0 | 0 |
| 334 | 0 | 336 | 0 | 0 | 260 | 0 | 0 | 0 | 0 |
| 94 | 0 | 0 | 84 | 0 | 156 | 0 | 0 | 0 | 0 |
| 259 | 0 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 392 | 0 | 0 | 257 | 0 | 351 | 0 | 0 | 0 | 0 |
| 0 | 97 | 523 | 101 | 0 | 0 | 0 | 0 | 0 | 0 |
| 63 | 0 | 184 | 61 | 0 | 0 | 0 | 0 | 0 | 0 |
| 264 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| 167 | 0 | 143 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 292 | 0 | 262 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 802 | 0 | 628 | 136 | 0 | 53 | 0 | 0 | 0 | 0 |
| 3678 | 3284 | 3541 | 0 | 0 | 8 | 8 | 0 | 0 | 0 |
| 0 | 4392 | 9 | 0 | 2606 | 8172 | 0 | 64 | 4 | 0 |
| 7 | 31 | 3187 | 0 | 0 | 6421 | 0 | 0 | 11889 | 0 |
| 5 | 30 | 6142 | 0 | 0 | 10379 | 0 | 0 | 0 | 0 |
| 3022 | 6250 | 0 | 0 | 10 | 0 | 0 | 38 | 0 | 3 |
| 10055 | 692 | 409 | 0 | 0 | 59 | 6 | 0 | 0 | 0 |
| 0 | 848 | 14 | 0 | 347 | 9925 | 0 | 15 | 5 | 0 |
| 4 | 101 | 4224 | 0 | 0 | 14600 | 0 | 0 | 23635 | 0 |
| 0 | 33 | 4588 | 0 | 0 | 1159 | 0 | 0 | 0 | 0 |
| 986 | 8654 | 0 | 0 | 14 | 0 | 0 | 8 | 0 | 4 |

| Leuciscus | Oncorhyn | Osmerus e | Phoxinus | Pseudoras | Pungitius | Rutilus rut | Salmo sal | Salmo tru | Scardinius |
|---|---|---|---|---|---|---|---|---|---|
| 38 | 0 | 0 | 127 | 0 | 0 | 0 | 52 | 170 | 0 |
| 78 | 0 | 0 | 241 | 0 | 0 | 170 | 84 | 60 | 0 |
| 0 | 0 | 0 | 533 | 0 | 0 | 887 | 111 | 437 | 0 |
| 0 | 0 | 0 | 229 | 0 | 0 | 123 | 177 | 251 | 0 |
| 82 | 0 | 0 | 1153 | 0 | 0 | 0 | 149 | 549 | 0 |
| 24 | 0 | 0 | 996 | 0 | 0 | 210 | 0 | 564 | 0 |
| 0 | 0 | 0 | 140 | 0 | 210 | 368 | 0 | 113 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 83 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 57 | 0 | 0 | 179 | 0 | 88 | 35 |
| 0 | 0 | 0 | 506 | 0 | 0 | 255 | 0 | 22 | 0 |
| 0 | 0 | 0 | 170 | 0 | 0 | 0 | 0 | 32 | 0 |
| 0 | 0 | 0 | 1368 | 0 | 0 | 2656 | 3 | 139 | 0 |
| 0 | 0 | 38 | 1501 | 0 | 0 | 218 | 0 | 1320 | 0 |
| 0 | 0 | 0 | 1824 | 0 | 0 | 7413 | 0 | 734 | 0 |
| 0 | 0 | 0 | 734 | 0 | 0 | 1302 | 0 | 354 | 0 |
| 0 | 0 | 0 | 237 | 0 | 0 | 290 | 0 | 154 | 0 |
| 0 | 0 | 0 | 2191 | 0 | 0 | 950 | 0 | 713 | 0 |
| 0 | 0 | 0 | 625 | 0 | 0 | 694 | 106 | 491 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1108 | 0 | 254 | 0 |
| 0 | 0 | 0 | 701 | 0 | 0 | 472 | 0 | 369 | 0 |
| 0 | 0 | 0 | 468 | 0 | 0 | 236 | 0 | 0 | 0 |
| 0 | 0 | 0 | 464 | 0 | 0 | 1115 | 0 | 222 | 0 |
| 0 | 0 | 0 | 4 | 0 | 0 | 0 | 132 | 638 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 701 | 188 | 644 | 0 |
| 0 | 0 | 0 | 1927 | 0 | 0 | 1002 | 0 | 0 | 0 |
| 0 | 0 | 0 | 400 | 0 | 0 | 332 | 246 | 0 | 0 |
| 0 | 0 | 0 | 745 | 0 | 0 | 0 | 0 | 873 | 0 |
| 0 | 0 | 0 | 581 | 0 | 0 | 0 | 0 | 489 | 0 |
| 0 | 0 | 0 | 66 | 0 | 0 | 374 | 0 | 195 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 201 | 0 | 0 |
| 988 | 0 | 0 | 0 | 0 | 0 | 446 | 0 | 299 | 386 |
| 0 | 0 | 0 | 208 | 0 | 0 | 872 | 0 | 257 | 0 |
| 0 | 0 | 0 | 301 | 0 | 0 | 2284 | 0 | 856 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 4222 | 0 | 323 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2738 | 0 | 362 | 0 |
| 0 | 0 | 0 | 98 | 0 | 0 | 1845 | 0 | 217 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1698 | 0 | 135 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1194 | 0 | 203 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 265 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 991 | 0 | 303 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 293 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 4856 | 0 | 99 | 93 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3201 | 0 | 568 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1145 | 0 | 361 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1663 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 521 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1411 | 0 | 181 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1398 | 0 | 427 | 0 |
| 0 | 0 | 0 | 434 | 0 | 0 | 1945 | 256 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1822 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 327 | 0 | 148 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 387 | 0 | 0 | 0 |
| 0 | 0 | 0 | 986 | 0 | 0 | 1405 | 0 | 589 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 226 | 0 | 458 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 943 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1678 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 325 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1908 | 0 | 140 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2013 | 0 | 448 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2164 | 0 | 359 | 0 |
| 0 | 47 | 0 | 0 | 0 | 0 | 3598 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3237 | 0 | 376 | 0 |
| 0 | 0 | 0 | 648 | 0 | 0 | 461 | 194 | 201 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 491 | 0 | 478 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 512 | 0 | 116 | 0 |
| 0 | 0 | 0 | 377 | 0 | 0 | 252 | 0 | 0 | 0 |
| 0 | 0 | 0 | 41 | 0 | 0 | 216 | 0 | 44 | 0 |
| 0 | 0 | 0 | 1730 | 0 | 0 | 3 | 0 | 300 | 0 |
| 0 | 0 | 0 | 160 | 0 | 0 | 0 | 0 | 283 | 0 |
| 0 | 0 | 0 | 452 | 0 | 0 | 162 | 0 | 100 | 0 |
| 0 | 0 | 0 | 5 | 0 | 0 | 1028 | 0 | 454 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1362 | 0 | 332 | 98 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1603 | 56 | 269 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2347 | 0 | 249 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3644 | 0 | 457 | 0 |
| 0 | 0 | 0 | 108 | 0 | 0 | 4593 | 0 | 837 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3599 | 0 | 177 | 0 |
| 5 | 0 | 0 | 4 | 19 | 4 | 0 | 0 | 3 | 157 |
| 7029 | 0 | 0 | 5 | 3447 | 0 | 3124 | 0 | 19 | 27 |
| 0 | 0 | 0 | 5279 | 15 | 0 | 3 | 0 | 4219 | 83 |
| 0 | 0 | 0 | 6505 | 33 | 9 | 2163 | 0 | 5802 | 8 |
| 6297 | 0 | 0 | 0 | 3475 | 4128 | 6 | 0 | 0 | 66 |
| 3 | 0 | 0 | 13 | 0 | 4 | 0 | 0 | 10 | 41 |
| 1598 | 0 | 0 | 8 | 5731 | 0 | 973 | 0 | 7 | 25 |
| 41 | 0 | 0 | 624 | 29 | 11 | 0 | 0 | 8005 | 83 |
| 0 | 0 | 0 | 6746 | 34 | 3 | 396 | 0 | 6454 | 3 |
| 8380 | 0 | 0 | 3 | 342 | 882 | 4 | 0 | 0 | 11 |

| Squalius c | Tinca tinc | Umbra py | Coregonus | Salvelinus | Cyprinidae | Percidae | Salmonida | nohit | Total |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 322 | 0 | 15852 | 16864 |
| 16 | 0 | 0 | 0 | 0 | 0 | 263 | 0 | 7250 | 9050 |
| 0 | 0 | 0 | 0 | 0 | 0 | 207 | 0 | 9288 | 13737 |
| 0 | 0 | 0 | 0 | 0 | 0 | 484 | 0 | 6942 | 10157 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 16313 | 20335 |
| 185 | 0 | 0 | 0 | 0 | 0 | 1635 | 0 | 12947 | 19364 |
| 0 | 0 | 0 | 78 | 0 | 0 | 875 | 0 | 6341 | 8539 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7112 | 7570 |
| 0 | 0 | 0 | 0 | 0 | 0 | 192 | 0 | 3487 | 3955 |
| 0 | 0 | 0 | 65 | 0 | 0 | 99 | 0 | 5149 | 6417 |
| 0 | 0 | 0 | 0 | 0 | 0 | 206 | 0 | 8354 | 9609 |
| 8 | 0 | 0 | 0 | 0 | 0 | 145 | 0 | 11483 | 11951 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1825 | 0 | 20309 | 28320 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21631 | 26306 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1392 | 0 | 24351 | 37477 |
| 0 | 129 | 0 | 0 | 191 | 0 | 886 | 0 | 22505 | 27228 |
| 0 | 0 | 0 | 0 | 119 | 0 | 828 | 0 | 18747 | 21828 |
| 0 | 0 | 0 | 0 | 310 | 0 | 3007 | 0 | 31023 | 41703 |
| 0 | 0 | 0 | 0 | 214 | 0 | 2350 | 0 | 18895 | 23879 |
| 0 | 0 | 0 | 0 | 173 | 0 | 1809 | 0 | 16037 | 20599 |
| 0 | 0 | 0 | 0 | 90 | 0 | 1098 | 0 | 17764 | 21384 |
| 0 | 0 | 0 | 0 | 0 | 0 | 950 | 0 | 49663 | 51432 |
| 0 | 0 | 0 | 0 | 0 | 0 | 761 | 0 | 34203 | 37935 |
| 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0 | 20135 | 22086 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1277 | 0 | 32404 | 36343 |
| 0 | 0 | 0 | 0 | 230 | 0 | 1430 | 0 | 34241 | 39784 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2000 | 0 | 24394 | 27704 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1049 | 0 | 25837 | 28705 |
| 0 | 0 | 0 | 0 | 160 | 0 | 572 | 0 | 24257 | 27822 |
| 0 | 0 | 0 | 0 | 0 | 0 | 700 | 0 | 15385 | 16783 |
| 0 | 0 | 0 | 0 | 156 | 0 | 781 | 0 | 22787 | 24970 |
| 0 | 0 | 0 | 256 | 0 | 0 | 1701 | 0 | 24305 | 29487 |
| 0 | 0 | 0 | 0 | 0 | 11 | 1681 | 0 | 21506 | 25932 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2297 | 0 | 34579 | 41527 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1877 | 0 | 27929 | 35058 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1814 | 0 | 31065 | 37729 |
| 0 | 0 | 0 | 0 | 0 | 0 | 903 | 0 | 14093 | 17785 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1624 | 0 | 16728 | 21348 |
| 0 | 0 | 0 | 0 | 0 | 0 | 842 | 0 | 23614 | 27095 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1055 | 0 | 20314 | 22024 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2029 | 0 | 25860 | 29455 |
| 0 | 0 | 0 | 0 | 0 | 0 | 5816 | 0 | 25614 | 32917 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2393 | 0 | 21186 | 29858 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1965 | 0 | 26699 | 34544 |
| 0 | 0 | 0 | 0 | 0 | 0 | 721 | 0 | 27049 | 29477 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1509 | 0 | 38389 | 42329 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 2099 | 0 | 39772 | 42742 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1098 | 0 | 36061 | 39980 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1601 | 0 | 36260 | 41201 |
| 0 | 0 | 0 | 0 | 0 | 0 | 988 | 0 | 33964 | 39336 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1197 | 0 | 38374 | 42915 |
| 0 | 0 | 229 | 0 | 0 | 0 | 1143 | 0 | 19365 | 22094 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1635 | 0 | 21307 | 25254 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3939 | 3 | 25822 | 33538 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1913 | 0 | 28332 | 31467 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1881 | 0 | 27143 | 31850 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3147 | 0 | 23699 | 28961 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1637 | 0 | 18924 | 21529 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1287 | 0 | 21768 | 26299 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1219 | 0 | 16976 | 21964 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1211 | 3 | 14290 | 20835 |
| 0 | 0 | 0 | 0 | 0 | 4 | 1057 | 0 | 18039 | 23896 |
| 0 | 0 | 0 | 0 | 1096 | 0 | 1460 | 5 | 18531 | 25428 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1361 | 0 | 20855 | 25309 |
| 0 | 0 | 0 | 0 | 0 | 0 | 625 | 0 | 16676 | 19059 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26546 | 27579 |
| 0 | 0 | 0 | 0 | 0 | 0 | 344 | 0 | 18921 | 20902 |
| 0 | 0 | 0 | 0 | 0 | 0 | 437 | 0 | 16493 | 17447 |
| 0 | 0 | 0 | 0 | 0 | 0 | 2362 | 0 | 31793 | 37122 |
| 0 | 0 | 0 | 0 | 0 | 0 | 469 | 0 | 18401 | 19769 |
| 0 | 0 | 0 | 0 | 280 | 0 | 747 | 0 | 14162 | 16282 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1401 | 0 | 14392 | 19073 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1738 | 0 | 14280 | 19315 |
| 0 | 0 | 0 | 0 | 72 | 0 | 1190 | 3 | 17744 | 21762 |
| 0 | 0 | 0 | 0 | 0 | 0 | 379 | 0 | 10006 | 14064 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1913 | 0 | 19572 | 26435 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1582 | 3 | 21144 | 29457 |
| 0 | 0 | 0 | 0 | 0 | 0 | 3098 | 0 | 23989 | 33410 |
| 5248 | 983 | 1426 | 290 | 0 | 0 | 0 | 0 | 13948 | 53136 |
| 6 | 3 | 3 | 0 | 0 | 0 | 2847 | 0 | 23891 | 61983 |
| 0 | 527 | 1793 | 329 | 0 | 0 | 0 | 0 | 12393 | 55906 |
| 6354 | 667 | 6 | 451 | 0 | 0 | 3319 | 0 | 10343 | 57468 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18476 | 53439 |
| 9891 | 1094 | 461 | 2475 | 0 | 0 | 5 | 0 | 9657 | 56883 |
| 5 | 3 | 3 | 0 | 0 | 0 | 2504 | 0 | 19320 | 49535 |
| 0 | 125 | 2174 | 63 | 0 | 0 | 0 | 0 | 7433 | 63517 |
| 648 | 77 | 0 | 38 | 0 | 0 | 4780 | 0 | 10847 | 49267 |
| 15 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 11031 | 38367 |

| Sample site | Basin | Lattitude | Longitude | Total raw reads |
|---|---|---|---|---|
| B1 | Bassenthwaite | 54.6713 | -3.232517 | 15756 |
| B2 | Bassenthwaite | 54.6473 | -3.218267 | 7844 |
| B3 | Bassenthwaite | 54.648167 | -3.21515 | 36180 |
| B4 | Bassenthwaite | 54.642367 | -3.209683 | 17638 |
| B5 | Bassenthwaite | 54.638783 | -3.203883 | 60480 |
| B-shore | Bassenthwaite | 54.666551 | -3.236783 | 43218 |
| D1 | Derwent water | 54.59285 | -3.1525 | 40982 |
| D2 | Derwent water | 54.585017 | -3.143083 | 1052 |
| D3 | Derwent water | 54.578967 | -3.14525 | 41356 |
| D4 | Derwent water | 54.575783 | -3.14055 | 22932 |
| D5 | Derwent water | 54.572717 | -3.133733 | 39498 |
| D-shore | Derwent water | 54.59481 | -3.140609 | 7688 |
| W01 | Windermere N basin | 54.419115 | -2.968784 | 78050 |
| W02 | Windermere N basin | 54.411788 | -2.977643 | 72898 |
| W03 | Windermere N basin | 54.406964 | -2.963693 | 38054 |
| W04 | Windermere N basin | 54.39691 | -2.960845 | 43938 |
| W05 | Windermere N basin | 54.388842 | -2.95005 | 52578 |
| W06 | Windermere N basin | 54.382744 | -2.943889 | 41950 |
| W07 | Windermere N basin | 54.373274 | -2.942581 | 3462 |
| W08 | Windermere N basin | 54.367531 | -2.942934 | 43426 |
| W09 | Windermere N basin | 54.412253 | -2.963476 | 58590 |
| W10 | Windermere N basin | 54.407645 | -2.957843 | 81852 |
| W11 0 m | Windermere N basin | 54.399328 | -2.953599 | 94870 |
| W11 10 m | Windermere N basin | 54.399328 | -2.953599 | 67994 |
| W11 20 m | Windermere N basin | 54.399328 | -2.953599 | 98508 |
| W11 30 m | Windermere N basin | 54.399328 | -2.953599 | 17818 |
| W11 40 m | Windermere N basin | 54.399328 | -2.953599 | 83384 |
| W11 50 m | Windermere N basin | 54.399328 | -2.953599 | 82260 |
| W11 60 m | Windermere N basin | 54.399328 | -2.953599 | 29312 |
| W12 | Windermere N basin | 54.390919 | -2.944854 | 64006 |
| W13 | Windermere N basin | 54.38277 | -2.938143 | 61554 |
| W14 | excluded Windermere N basin | 54.3729 | -2.93601 | 63644 |
| W15 | Windermere N basin | 54.367248 | -2.936747 | 42464 |
| W16 | Windermere S basin | 54.352419 | -2.940061 | 62730 |
| W17 | Windermere S basin | 54.344103 | -2.945634 | 79044 |
| W18 | Windermere S basin | 54.335065 | -2.946318 | 57032 |
| W19 | Windermere S basin | 54.328632 | -2.947401 | 28800 |
| W20 | Windermere S basin | 54.318287 | -2.956222 | 61970 |
| W21 | Windermere S basin | 54.309392 | -2.958669 | 53360 |
| W22 | Windermere S basin | 54.301149 | -2.959883 | 20864 |
| W23 | Windermere S basin | 54.291392 | -2.956366 | 92040 |
| W24 | Windermere S basin | 54.282824 | -2.95591 | 63442 |
| W25 | Windermere S basin | 54.344027 | -2.941337 | 56856 |
| W26 | Windermere S basin | 54.335562 | -2.942206 | 50300 |
| W27 | Windermere S basin | 54.328396 | -2.943259 | 74326 |
| W28 0 m | Windermere S basin | 54.313594 | -2.950786 | 62538 |

| | | | | |
|---|---|---|---|---|
| W28 10 m | Windermere S basin | 54.313594 | -2.950786 | 69550 |
| W28 20 m | Windermere S basin | 54.313594 | -2.950786 | 53484 |
| W28 30 m | Windermere S basin | 54.313594 | -2.950786 | 39668 |
| W28 40 m | Windermere S basin | 54.313594 | -2.950786 | 65004 |
| W29 | Windermere S basin | 54.308946 | -2.953592 | 86172 |
| W30 | Windermere S basin | 54.301234 | -2.954574 | 54398 |
| W31 | Windermere S basin | 54.291735 | -2.95343 | 59980 |
| W32 | Windermere S basin | 54.282891 | -2.953878 | 69254 |
| W33 | Windermere S basin | 54.283275 | -2.951353 | 20856 |
| W34 | Windermere S basin | 54.291419 | -2.954738 | 67032 |
| W35 | Windermere S basin | 54.300954 | -2.95683 | 75240 |
| W36 | Windermere S basin | 54.309169 | -2.955833 | 62938 |
| W37 | Windermere S basin | 54.317485 | -2.955099 | 18258 |
| W38 | Windermere S basin | 54.328189 | -2.947473 | 43632 |
| W39 | Windermere S basin | 54.334825 | -2.94507 | 38210 |
| W40 | Windermere S basin | 54.342145 | -2.942546 | 39728 |
| W41 | Windermere S basin | 54.350749 | -2.931811 | 35004 |
| W42 | Windermere N basin | 54.372798 | -2.923187 | 51538 |
| W43 | Windermere N basin | 54.393287 | -2.939156 | 51738 |
| W44 | Windermere N basin | 54.411895 | -2.971011 | 46256 |
| W45 | Windermere N basin | 54.408059 | -2.961924 | 45876 |
| W46 | Windermere N basin | 54.397257 | -2.959958 | 45500 |
| W47 | Windermere N basin | 54.389364 | -2.94947 | 1192 |
| W48 | Windermere N basin | 54.383115 | -2.943279 | 64660 |
| W49 | Windermere N basin | 54.373741 | -2.941665 | 45306 |
| W50 | Windermere N basin | 54.367881 | -2.939239 | 82234 |
| Winshore_01 | Windermere S basin | 54.352641 | -2.939863 | 55270 |
| Winshore_02 | Windermere S basin | 54.352551 | -2.939846 | 60134 |
| Winshore_03 | Windermere S basin | 54.352083 | -2.938972 | 9140 |
| Winshore_04 | Windermere S basin | 54.351639 | -2.939917 | 59608 |
| Winshore_05 | Windermere S basin | 54.351444 | -2.940889 | 58090 |
| Winshore_06 | Windermere S basin | 54.352387 | -2.939755 | 33114 |
| MC01 | Mock community | not applicable | not applicable | 95154 |
| MC02 | Mock community | not applicable | not applicable | 74520 |
| MC03 | Mock community | not applicable | not applicable | 87184 |
| MC04 | Mock community | not applicable | not applicable | 95040 |
| MC05 | Mock community | not applicable | not applicable | 89242 |
| MC06 | Mock community | not applicable | not applicable | 84336 |
| MC07 | Mock community | not applicable | not applicable | 93726 |
| MC08 | Mock community | not applicable | not applicable | 83718 |
| MC09 | Mock community | not applicable | not applicable | 91180 |
| MC10 | Mock community | not applicable | not applicable | 72406 |

| Trimmed-total | Post-merging | Post-chimera-filter | Cluster_thres | Clusters_total | Clusters_min_cov |
|---|---|---|---|---|---|
| 9352 | 5951 | 5951 | 1 | 362 | 3 |
| 6839 | 5086 | 5072 | 1 | 362 | 3 |
| 31177 | 23991 | 23985 | 1 | 1360 | 3 |
| 14449 | 10932 | 10931 | 1 | 742 | 3 |
| 51443 | 40110 | 40062 | 1 | 2039 | 3 |
| 36964 | 27901 | 27714 | 1 | 1429 | 3 |
| 34865 | 27851 | 27848 | 1 | 1059 | 3 |
| 887 | 747 | 747 | 1 | 71 | 3 |
| 36271 | 27643 | 27641 | 1 | 853 | 3 |
| 18791 | 13180 | 13180 | 1 | 629 | 3 |
| 32413 | 30505 | 30498 | 1 | 1303 | 3 |
| 6656 | 5097 | 5097 | 1 | 278 | 3 |
| 67527 | 51654 | 51593 | 1 | 2572 | 3 |
| 64649 | 47457 | 47423 | 1 | 2149 | 3 |
| 34282 | 25331 | 25298 | 1 | 1431 | 3 |
| 38568 | 31056 | 31043 | 1 | 1497 | 3 |
| 44622 | 34827 | 34825 | 1 | 1784 | 3 |
| 36741 | 29769 | 29760 | 1 | 1510 | 3 |
| 3144 | 2295 | 2287 | 1 | 191 | 3 |
| 38439 | 30612 | 30285 | 1 | 1876 | 3 |
| 51724 | 40556 | 40526 | 1 | 2209 | 3 |
| 67540 | 63104 | 63103 | 1 | 1585 | 3 |
| 77856 | 69531 | 69531 | 1 | 2177 | 3 |
| 56402 | 46563 | 46556 | 1 | 2249 | 3 |
| 83653 | 67967 | 67956 | 1 | 2781 | 3 |
| 15078 | 11370 | 11368 | 1 | 542 | 3 |
| 67461 | 61370 | 61370 | 1 | 1974 | 3 |
| 70409 | 55138 | 55134 | 1 | 2544 | 3 |
| 26027 | 19171 | 19171 | 1 | 1095 | 3 |
| 56025 | 47097 | 47080 | 1 | 2103 | 3 |
| 53916 | 46966 | 46966 | 1 | 1939 | 3 |
| 56299 | 45504 | 45504 | 1 | 2012 | 3 |
| 38187 | 28375 | 28373 | 1 | 1225 | 3 |
| 55521 | 43030 | 43024 | 1 | 2013 | 3 |
| 71167 | 52158 | 52130 | 1 | 2031 | 3 |
| 50753 | 38990 | 38988 | 1 | 1705 | 3 |
| 25337 | 20032 | 20031 | 1 | 944 | 3 |
| 54048 | 43789 | 43789 | 1 | 1971 | 3 |
| 45750 | 37840 | 37806 | 1 | 1670 | 3 |
| 18282 | 13351 | 13351 | 1 | 755 | 3 |
| 69083 | 55625 | 55622 | 1 | 2115 | 3 |
| 56130 | 42822 | 42758 | 1 | 1764 | 3 |
| 49333 | 40663 | 40581 | 1 | 1987 | 3 |
| 42846 | 33965 | 33952 | 1 | 1829 | 3 |
| 63613 | 51621 | 51595 | 1 | 2055 | 3 |
| 53782 | 42026 | 41986 | 1 | 1803 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 58871 | 46488 | 46487 | 1 | 1786 | 3 |
| 45554 | 35707 | 35518 | 1 | 2066 | 3 |
| 32768 | 28826 | 28826 | 1 | 1422 | 3 |
| 55595 | 43208 | 43112 | 1 | 2059 | 3 |
| 74505 | 56740 | 56736 | 1 | 2216 | 3 |
| 46182 | 41287 | 41285 | 1 | 1502 | 3 |
| 52254 | 45158 | 45157 | 1 | 1459 | 3 |
| 59921 | 50164 | 50163 | 1 | 1902 | 3 |
| 18828 | 13776 | 13775 | 1 | 725 | 3 |
| 58152 | 48153 | 48143 | 1 | 1947 | 3 |
| 67172 | 52717 | 52712 | 1 | 2141 | 3 |
| 56690 | 40982 | 40974 | 1 | 1876 | 3 |
| 15542 | 12535 | 12533 | 1 | 749 | 3 |
| 37068 | 33523 | 33522 | 1 | 1453 | 3 |
| 31587 | 25984 | 25974 | 1 | 1294 | 3 |
| 34753 | 28998 | 28947 | 1 | 1503 | 3 |
| 30617 | 24834 | 24771 | 1 | 1507 | 3 |
| 45136 | 36435 | 36421 | 1 | 1858 | 3 |
| 44811 | 38183 | 38182 | 1 | 1462 | 3 |
| 37668 | 33193 | 33192 | 1 | 1298 | 3 |
| 39750 | 31113 | 31113 | 1 | 1256 | 3 |
| 38130 | 32935 | 32935 | 1 | 1277 | 3 |
| 745 | 729 | 728 | 1 | 49 | 3 |
| 54598 | 44852 | 44848 | 1 | 1849 | 3 |
| 38118 | 30486 | 30484 | 1 | 1345 | 3 |
| 60086 | 48272 | 48261 | 1 | 1739 | 3 |
| 48285 | 38025 | 37902 | 1 | 2148 | 3 |
| 51665 | 42168 | 42146 | 1 | 2107 | 3 |
| 7808 | 6554 | 6468 | 1 | 592 | 3 |
| 52538 | 42950 | 42865 | 1 | 2335 | 3 |
| 51394 | 41495 | 41401 | 1 | 2104 | 3 |
| 29554 | 22641 | 22472 | 1 | 1459 | 3 |
| 78443 | 59764 | 49029 | 1 | 4298 | 3 |
| 53831 | 42141 | 35934 | 1 | 3193 | 3 |
| 77472 | 57146 | 47533 | 1 | 4377 | 3 |
| 85132 | 62296 | 52563 | 1 | 4214 | 3 |
| 65057 | 51927 | 46212 | 1 | 3082 | 3 |
| 74227 | 53187 | 43828 | 1 | 3248 | 3 |
| 63229 | 51102 | 44459 | 1 | 3358 | 3 |
| 71484 | 56607 | 50013 | 1 | 3599 | 3 |
| 81223 | 57234 | 47107 | 1 | 3487 | 3 |
| 59604 | 44086 | 37386 | 1 | 2573 | 3 |

| Cluster_above_thres | Queries | Abramis br | Alburnus a | Ameiurus r | Anguilla an | Barbatula b | Barbus bar |
|---|---|---|---|---|---|---|---|
| 68 | 5634 | 0 | 0 | 0 | 0 | 0 | 0 |
| 71 | 4752 | 41 | 0 | 0 | 0 | 0 | 0 |
| 224 | 22674 | 0 | 0 | 0 | 0 | 0 | 0 |
| 166 | 10290 | 0 | 0 | 0 | 0 | 0 | 0 |
| 348 | 38101 | 46 | 0 | 0 | 0 | 0 | 0 |
| 255 | 26320 | 0 | 15 | 8 | 5568 | 0 | 0 |
| 281 | 26850 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 695 | 0 | 0 | 0 | 0 | 0 | 0 |
| 233 | 26808 | 0 | 0 | 0 | 0 | 0 | 0 |
| 146 | 12597 | 0 | 0 | 0 | 0 | 0 | 0 |
| 291 | 29264 | 21 | 0 | 0 | 0 | 0 | 7 |
| 45 | 4844 | 0 | 0 | 0 | 430 | 0 | 0 |
| 416 | 49033 | 6445 | 0 | 0 | 0 | 0 | 0 |
| 570 | 45514 | 0 | 0 | 0 | 0 | 0 | 0 |
| 185 | 23878 | 6354 | 0 | 0 | 1548 | 7 | 0 |
| 239 | 29593 | 0 | 0 | 0 | 0 | 0 | 0 |
| 293 | 33120 | 0 | 0 | 0 | 0 | 0 | 0 |
| 247 | 28338 | 0 | 0 | 0 | 324 | 13 | 0 |
| 33 | 2119 | 0 | 0 | 0 | 0 | 0 | 0 |
| 293 | 28473 | 0 | 0 | 0 | 3 | 207 | 0 |
| 348 | 38318 | 0 | 0 | 0 | 2094 | 0 | 0 |
| 518 | 61780 | 0 | 0 | 0 | 0 | 0 | 0 |
| 556 | 67527 | 0 | 0 | 0 | 0 | 0 | 0 |
| 387 | 44351 | 0 | 0 | 0 | 0 | 0 | 0 |
| 551 | 65187 | 0 | 0 | 0 | 0 | 0 | 0 |
| 84 | 10800 | 0 | 0 | 0 | 0 | 0 | 0 |
| 543 | 59604 | 0 | 0 | 0 | 0 | 0 | 0 |
| 470 | 52671 | 0 | 0 | 0 | 0 | 0 | 0 |
| 147 | 18103 | 0 | 0 | 0 | 4278 | 0 | 0 |
| 382 | 45051 | 0 | 0 | 0 | 0 | 0 | 0 |
| 407 | 45147 | 0 | 0 | 0 | 0 | 0 | 3 |
| 369 | 43512 | 3799 | 0 | 0 | 0 | 0 | 0 |
| 241 | 27183 | 8 | 0 | 0 | 0 | 0 | 0 |
| 342 | 41012 | 0 | 0 | 0 | 1046 | 186 | 0 |
| 460 | 50199 | 27820 | 0 | 0 | 0 | 0 | 0 |
| 336 | 37351 | 988 | 0 | 0 | 1315 | 0 | 0 |
| 161 | 19130 | 1891 | 0 | 0 | 4779 | 0 | 0 |
| 384 | 41888 | 2903 | 0 | 0 | 0 | 0 | 0 |
| 278 | 36162 | 8524 | 0 | 0 | 22 | 0 | 0 |
| 118 | 12620 | 61 | 0 | 0 | 0 | 0 | 0 |
| 409 | 53488 | 0 | 0 | 0 | 19114 | 0 | 0 |
| 427 | 41120 | 2911 | 0 | 0 | 1319 | 66 | 0 |
| 436 | 38741 | 3843 | 0 | 0 | 0 | 65 | 0 |
| 278 | 32216 | 3903 | 0 | 0 | 1372 | 0 | 0 |
| 397 | 49564 | 12439 | 0 | 0 | 0 | 0 | 0 |
| 333 | 40179 | 14894 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 387 | 44733 | 0 | 0 | 0 | 0 | 0 | 0 |
| 322 | 33528 | 3625 | 0 | 0 | 0 | 0 | 0 |
| 268 | 27490 | 0 | 0 | 0 | 0 | 0 | 0 |
| 361 | 41050 | 3388 | 0 | 0 | 0 | 0 | 0 |
| 475 | 54568 | 23060 | 0 | 0 | 0 | 0 | 0 |
| 321 | 39849 | 223 | 0 | 0 | 0 | 0 | 0 |
| 348 | 43727 | 5 | 0 | 0 | 0 | 0 | 0 |
| 392 | 48325 | 0 | 0 | 0 | 0 | 0 | 0 |
| 76 | 13010 | 5449 | 0 | 0 | 0 | 0 | 0 |
| 445 | 46298 | 0 | 0 | 0 | 0 | 0 | 0 |
| 409 | 50614 | 10583 | 0 | 0 | 1704 | 0 | 0 |
| 349 | 39137 | 10145 | 0 | 0 | 0 | 0 | 0 |
| 125 | 11856 | 2171 | 0 | 0 | 0 | 0 | 0 |
| 294 | 32162 | 0 | 0 | 0 | 1038 | 0 | 0 |
| 228 | 24749 | 6600 | 0 | 0 | 0 | 0 | 0 |
| 252 | 27527 | 2575 | 0 | 0 | 0 | 0 | 0 |
| 238 | 23348 | 2275 | 0 | 0 | 0 | 0 | 0 |
| 256 | 34539 | 0 | 0 | 0 | 0 | 0 | 0 |
| 288 | 36799 | 0 | 0 | 0 | 0 | 0 | 0 |
| 296 | 32005 | 0 | 0 | 0 | 0 | 0 | 0 |
| 259 | 29874 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 31693 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 693 | 10 | 0 | 0 | 0 | 0 | 0 |
| 333 | 43008 | 0 | 0 | 0 | 0 | 0 | 0 |
| 235 | 29149 | 9350 | 0 | 0 | 0 | 0 | 0 |
| 464 | 46711 | 0 | 0 | 0 | 0 | 0 | 0 |
| 334 | 35819 | 599 | 0 | 0 | 3801 | 11 | 0 |
| 373 | 40133 | 0 | 0 | 0 | 1549 | 3 | 0 |
| 139 | 5970 | 0 | 0 | 0 | 245 | 0 | 0 |
| 405 | 40617 | 5173 | 0 | 0 | 0 | 159 | 0 |
| 392 | 39402 | 0 | 0 | 0 | 925 | 0 | 0 |
| 247 | 21101 | 0 | 0 | 0 | 2388 | 0 | 0 |
| 865 | 44969 | 8193 | 1860 | 5805 | 0 | 0 | 0 |
| 688 | 32938 | 26 | 765 | 0 | 3 | 0 | 2526 |
| 833 | 43394 | 10769 | 0 | 42 | 0 | 0 | 3533 |
| 975 | 48683 | 15074 | 0 | 10 | 0 | 0 | 0 |
| 673 | 43298 | 393 | 2232 | 5613 | 0 | 0 | 0 |
| 728 | 40825 | 18219 | 136 | 1401 | 0 | 0 | 3 |
| 768 | 41386 | 14 | 70 | 7 | 0 | 0 | 5619 |
| 746 | 46582 | 4124 | 8 | 100 | 0 | 0 | 1486 |
| 825 | 43911 | 22141 | 96 | 337 | 0 | 0 | 0 |
| 598 | 34978 | 36 | 7615 | 2237 | 0 | 0 | 0 |

| Coregonus | Cottus gob | Cyprinus ca | Esox lucius | Gasteroste | Gobio gobi | Gymnocep | Lepomis gi | Leucaspius |
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| 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| 0 | 8 | 5 | 0 | 0 | 0 | 1689 | 0 | 0 |
| 0 | 0 | 0 | 165 | 35 | 0 | 5980 | 0 | 0 |
| 0 | 0 | 0 | 4 | 0 | 0 | 2288 | 0 | 0 |
| 0 | 0 | 7 | 10 | 0 | 0 | 3817 | 0 | 0 |
| 0 | 0 | 51 | 511 | 0 | 0 | 5160 | 4 | 0 |
| 0 | 0 | 0 | 58 | 0 | 0 | 783 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 3 | 0 | 0 | 6 | 0 | 10 |
| 0 | 0 | 0 | 427 | 0 | 0 | 12 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1195 | 0 | 0 | 0 | 0 |
| 0 | 3113 | 0 | 0 | 2066 | 0 | 0 | 0 | 0 |
| 0 | 875 | 0 | 56 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 921 | 51 | 0 | 0 | 0 | 0 |
| 0 | 6130 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 4341 | 0 | 1605 | 656 | 0 | 0 | 0 | 0 |
| 0 | 434 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 2178 | 0 | 0 | 4662 | 0 | 0 | 0 | 0 |
| 0 | 8248 | 0 | 0 | 2005 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 |
| 0 | 1392 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 4267 | 0 | 0 | 0 | 0 |
| 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1307 | 0 | 0 | 0 | 0 |
| 0 | 2005 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1940 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 144 | 389 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 |
| 0 | 7 | 0 | 84 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1698 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1407 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 303 | 957 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 5379 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1611 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1151 | 0 | 86 | 0 | 0 | 0 | 0 | 0 |
| 0 | 5481 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 6919 | 0 | 0 | 1275 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 4379 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1137 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 2733 | 0 | 0 | 0 | 0 |
| 0 | 327 | 1740 | 5979 | 2740 | 0 | 0 | 0 | 0 |
| 0 | 3400 | 0 | 1534 | 0 | 0 | 0 | 0 | 0 |
| 0 | 438 | 0 | 286 | 0 | 0 | 0 | 0 | 0 |
| 0 | 2298 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 3790 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1270 | 0 | 2076 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1864 | 7059 | 2467 | 0 | 0 | 3 | 4 | 0 |
| 0 | 0 | 9349 | 16 | 0 | 32 | 2851 | 8828 | 10 |
| 0 | 5 | 80 | 4642 | 0 | 0 | 3123 | 59 | 8216 |
| 0 | 0 | 180 | 9733 | 0 | 0 | 5646 | 0 | 0 |
| 4 | 4704 | 16713 | 0 | 0 | 0 | 0 | 5196 | 0 |
| 0 | 7118 | 2874 | 60 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 4030 | 16 | 0 | 0 | 5306 | 15178 | 0 |
| 0 | 4 | 314 | 4784 | 0 | 0 | 7071 | 100 | 7624 |
| 8 | 63 | 442 | 6859 | 0 | 0 | 510 | 4 | 0 |
| 0 | 779 | 17813 | 3 | 0 | 0 | 0 | 1133 | 3 |

| Leuciscus l | Perca fluvi | Phoxinus p | Platichthys | Pseudoras | Rutilus ruti | Salmo sala | Salmo truti | Salvelinus a |
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| 0 | 0 | 0 | 0 | 0 | 0 | 482 | 27 | 0 |
| 0 | 1466 | 0 | 0 | 0 | 0 | 24 | 9 | 0 |
| 0 | 6754 | 0 | 0 | 0 | 0 | 638 | 2256 | 0 |
| 0 | 2073 | 7 | 0 | 0 | 0 | 1116 | 56 | 0 |
| 0 | 9969 | 328 | 0 | 0 | 0 | 0 | 3276 | 0 |
| 0 | 12066 | 25 | 0 | 0 | 0 | 0 | 611 | 0 |
| 0 | 21686 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 26592 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 4 | 0 | 0 | 0 | 0 | 0 | 54 | 0 |
| 0 | 17 | 47 | 0 | 0 | 0 | 0 | 3 | 0 |
| 0 | 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 25700 | 0 | 0 | 0 | 359 | 19 | 1481 | 0 |
| 0 | 0 | 166 | 0 | 0 | 0 | 0 | 2150 | 0 |
| 0 | 7938 | 249 | 0 | 0 | 2153 | 0 | 349 | 0 |
| 0 | 7564 | 0 | 0 | 0 | 0 | 0 | 4111 | 602 |
| 0 | 5120 | 0 | 0 | 0 | 0 | 0 | 2887 | 0 |
| 0 | 6698 | 29 | 0 | 0 | 105 | 0 | 276 | 298 |
| 0 | 867 | 508 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 8449 | 2823 | 0 | 0 | 0 | 0 | 3059 | 0 |
| 0 | 6 | 46 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 4 | 0 | 0 | 0 | 15 | 0 | 563 | 0 |
| 0 | 9079 | 0 | 0 | 0 | 0 | 0 | 140 | 0 |
| 0 | 3248 | 2601 | 0 | 0 | 0 | 0 | 2868 | 0 |
| 0 | 20083 | 0 | 0 | 0 | 1216 | 0 | 0 | 0 |
| 0 | 10488 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 |
| 0 | 3698 | 0 | 0 | 0 | 0 | 0 | 3270 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 31 | 756 | 212 |
| 0 | 5487 | 0 | 0 | 0 | 0 | 866 | 0 | 0 |
| 0 | 4362 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 2302 | 0 | 0 | 0 | 7 | 0 | 3 | 0 |
| 0 | 22317 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 14132 | 0 | 0 | 0 | 6927 | 0 | 0 | 0 |
| 0 | 10492 | 0 | 0 | 1838 | 3022 | 0 | 140 | 0 |
| 0 | 4423 | 0 | 0 | 0 | 247 | 0 | 0 | 0 |
| 0 | 5169 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 17145 | 0 | 7553 | 0 | 89 | 0 | 1819 | 0 |
| 0 | 3150 | 0 | 0 | 0 | 2366 | 0 | 0 | 0 |
| 0 | 4878 | 0 | 0 | 0 | 51 | 0 | 4118 | 0 |
| 0 | 10269 | 0 | 0 | 0 | 16 | 0 | 0 | 0 |
| 0 | 26761 | 0 | 0 | 0 | 0 | 0 | 814 | 0 |
| 0 | 15563 | 0 | 0 | 0 | 1387 | 0 | 320 | 0 |
| 0 | 5407 | 0 | 0 | 0 | 665 | 0 | 2955 | 0 |
| 0 | 13720 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 11691 | 0 | 0 | 0 | 870 | 0 | 0 | 0 |

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| 0 | 18683 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 5620 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 15391 | 2985 | 0 | 8756 | 0 | 0 | 0 | 0 |
| 0 | 6 | 0 | 0 | 6 | 3 | 0 | 0 | 0 |
| 0 | 11130 | 0 | 0 | 0 | 2411 | 0 | 0 | 0 |
| 0 | 20703 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 10615 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 7247 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 23364 | 0 | 0 | 0 | 2085 | 0 | 0 | 0 |
| 0 | 21754 | 0 | 0 | 5 | 56 | 0 | 6756 | 0 |
| 0 | 5805 | 0 | 0 | 18577 | 694 | 0 | 0 | 0 |
| 0 | 3064 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1963 | 0 | 0 | 0 | 109 | 0 | 0 | 0 |
| 0 | 2400 | 89 | 0 | 0 | 394 | 0 | 0 | 0 |
| 0 | 6605 | 0 | 0 | 0 | 1865 | 0 | 0 | 0 |
| 0 | 3446 | 0 | 0 | 0 | 121 | 0 | 238 | 2518 |
| 0 | 9469 | 0 | 0 | 0 | 262 | 0 | 317 | 0 |
| 0 | 102 | 519 | 0 | 0 | 0 | 0 | 3552 | 0 |
| 0 | 6316 | 41 | 0 | 0 | 82 | 0 | 1096 | 12 |
| 0 | 0 | 149 | 0 | 0 | 194 | 0 | 94 | 0 |
| 0 | 9567 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1027 | 105 | 0 | 0 | 0 | 0 | 1524 | 0 |
| 0 | 5434 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 33176 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 7425 | 314 | 0 | 0 | 19 | 266 | 58 | 0 |
| 0 | 13293 | 0 | 0 | 0 | 298 | 0 | 3513 | 0 |
| 0 | 1015 | 0 | 0 | 0 | 39 | 104 | 339 | 0 |
| 0 | 3953 | 0 | 0 | 0 | 728 | 0 | 2846 | 0 |
| 0 | 12861 | 0 | 0 | 0 | 794 | 0 | 1068 | 0 |
| 0 | 10376 | 0 | 0 | 0 | 222 | 0 | 618 | 0 |
| 0 | 10 | 3 | 0 | 0 | 0 | 0 | 6 | 0 |
| 322 | 2197 | 3 | 0 | 1340 | 149 | 0 | 10 | 0 |
| 0 | 0 | 2307 | 0 | 0 | 0 | 0 | 1919 | 0 |
| 0 | 3521 | 2295 | 0 | 0 | 155 | 0 | 1653 | 0 |
| 93 | 0 | 0 | 0 | 923 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 4792 | 0 | 0 | 2158 | 23 | 0 | 7 | 0 |
| 0 | 0 | 138 | 0 | 7 | 0 | 0 | 3744 | 0 |
| 0 | 3782 | 3130 | 0 | 11 | 13 | 0 | 2566 | 0 |
| 472 | 0 | 0 | 0 | 185 | 0 | 0 | 0 | 0 |

| Scardinius | Squalius ce | Tinca tin | Umbra p | Ameiuru | Coregon | Leuciscu | Salmo | Salvelinu | Cyprinid | Gasteros |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 620 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 417 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 207 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 149 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 272 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 329 | 0 | 0 | 3 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 696 | 20 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 682 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 45 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 176 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 38 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1963 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 659 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1057 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 646 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 634 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 234 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2098 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 482 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 531 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 68 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1477 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 226 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 364 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 179 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 321 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 182 | 0 | 332 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 189 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 2046 | 273 | 1788 | 6363 | 76 | 0 | 0 | 0 | 216 | 0 |
| 0 | 0 | 0 | 10 | 0 | 0 | 46 | 0 | 0 | 0 | 0 |
| 30 | 0 | 345 | 4827 | 0 | 124 | 0 | 393 | 0 | 276 | 0 |
| 7 | 2920 | 296 | 13 | 27 | 170 | 0 | 730 | 0 | 352 | 0 |
| 0 | 83 | 0 | 26 | 3879 | 0 | 13 | 0 | 0 | 0 | 0 |
| 3 | 3898 | 55 | 860 | 277 | 285 | 0 | 0 | 0 | 300 | 0 |
| 0 | 0 | 0 | 13 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | 0 | 33 | 12943 | 7 | 39 | 0 | 427 | 0 | 63 | 0 |
| 3 | 400 | 37 | 49 | 36 | 15 | 0 | 894 | 0 | 371 | 0 |
| 0 | 7 | 4 | 0 | 613 | 0 | 149 | 0 | 0 | 0 | 0 |

| Percidae | Salmonic | Percinae | Clupeoce | nohit | total |
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| 0 | 0 | 0 | 0 | 5011 | 5634 |
| 0 | 0 | 22 | 0 | 1488 | 4752 |
| 93 | 0 | 149 | 0 | 5984 | 22674 |
| 40 | 0 | 0 | 0 | 4289 | 10290 |
| 3 | 0 | 161 | 0 | 20277 | 38101 |
| 22 | 0 | 1404 | 0 | 726 | 26320 |
| 16 | 0 | 616 | 0 | 3691 | 26850 |
| 0 | 0 | 0 | 0 | 695 | 695 |
| 116 | 0 | 100 | 0 | 0 | 26808 |
| 0 | 0 | 0 | 0 | 12531 | 12597 |
| 0 | 0 | 0 | 0 | 29144 | 29264 |
| 7 | 0 | 24 | 0 | 105 | 4844 |
| 70 | 0 | 2006 | 0 | 11486 | 49033 |
| 0 | 0 | 0 | 0 | 37687 | 45514 |
| 108 | 0 | 42 | 0 | 4107 | 23878 |
| 0 | 0 | 176 | 0 | 15452 | 29593 |
| 25 | 0 | 76 | 0 | 18200 | 33120 |
| 0 | 0 | 69 | 0 | 13860 | 28338 |
| 0 | 0 | 0 | 0 | 310 | 2119 |
| 82 | 0 | 34 | 0 | 6800 | 28473 |
| 0 | 0 | 0 | 0 | 25919 | 38318 |
| 0 | 0 | 0 | 0 | 61082 | 61780 |
| 70 | 0 | 37 | 0 | 56798 | 67527 |
| 14 | 0 | 116 | 0 | 33541 | 44351 |
| 34 | 0 | 239 | 0 | 39348 | 65187 |
| 62 | 0 | 151 | 0 | 71 | 10800 |
| 0 | 0 | 0 | 0 | 59579 | 59604 |
| 0 | 0 | 20 | 0 | 43717 | 52671 |
| 0 | 0 | 0 | 0 | 10794 | 18103 |
| 50 | 0 | 0 | 0 | 35651 | 45051 |
| 7 | 0 | 65 | 0 | 40710 | 45147 |
| 0 | 0 | 55 | 0 | 36813 | 43512 |
| 68 | 0 | 328 | 0 | 4373 | 27183 |
| 0 | 0 | 419 | 0 | 18211 | 41012 |
| 34 | 0 | 1307 | 0 | 5542 | 50199 |
| 0 | 0 | 0 | 0 | 28680 | 37351 |
| 90 | 0 | 466 | 0 | 6715 | 19130 |
| 20 | 0 | 165 | 0 | 11536 | 41888 |
| 27 | 0 | 13 | 0 | 22057 | 36162 |
| 0 | 0 | 263 | 0 | 2615 | 12620 |
| 0 | 0 | 151 | 0 | 23938 | 53488 |
| 181 | 0 | 3206 | 0 | 5628 | 41120 |
| 86 | 0 | 259 | 0 | 15784 | 38741 |
| 12 | 0 | 104 | 0 | 14440 | 32216 |
| 0 | 0 | 0 | 0 | 23405 | 49564 |
| 24 | 0 | 557 | 0 | 12139 | 40179 |

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|---|---|---|---|---|---|
| 138 | 0 | 4 | 0 | 25908 | 44733 |
| 44 | 0 | 267 | 0 | 20601 | 33528 |
| 0 | 0 | 0 | 0 | 27479 | 27490 |
| 20 | 0 | 75 | 0 | 10435 | 41050 |
| 0 | 0 | 0 | 0 | 31490 | 54568 |
| 14 | 0 | 227 | 0 | 25844 | 39849 |
| 21 | 0 | 168 | 0 | 22830 | 43727 |
| 27 | 0 | 197 | 0 | 37477 | 48325 |
| 82 | 0 | 99 | 0 | 24 | 13010 |
| 115 | 0 | 169 | 0 | 15186 | 46298 |
| 96 | 0 | 2818 | 0 | 6357 | 50614 |
| 0 | 0 | 123 | 0 | 3786 | 39137 |
| 43 | 0 | 0 | 0 | 4967 | 11856 |
| 4 | 0 | 34 | 0 | 29014 | 32162 |
| 0 | 0 | 74 | 0 | 15192 | 24749 |
| 91 | 0 | 0 | 0 | 15154 | 27527 |
| 0 | 0 | 0 | 0 | 8702 | 23340 |
| 41 | 0 | 141 | 0 | 16047 | 34539 |
| 0 | 0 | 0 | 0 | 31149 | 36799 |
| 63 | 0 | 737 | 0 | 23432 | 32005 |
| 0 | 0 | 0 | 0 | 29437 | 29874 |
| 101 | 0 | 57 | 0 | 21968 | 31693 |
| 0 | 0 | 0 | 0 | 677 | 693 |
| 13 | 0 | 0 | 0 | 35596 | 43008 |
| 47 | 0 | 14 | 0 | 12988 | 29149 |
| 93 | 0 | 519 | 0 | 10190 | 46711 |
| 34 | 0 | 28 | 0 | 12449 | 35819 |
| 136 | 0 | 276 | 0 | 15810 | 40133 |
| 0 | 0 | 45 | 0 | 3437 | 5970 |
| 0 | 0 | 71 | 0 | 24875 | 40617 |
| 105 | 0 | 312 | 0 | 19355 | 39402 |
| 23 | 0 | 587 | 0 | 3541 | 21101 |
| 0 | 0 | 0 | 0 | 6921 | 44964 |
| 57 | 0 | 208 | 0 | 4190 | 32938 |
| 46 | 3 | 17 | 0 | 2638 | 43394 |
| 119 | 6 | 481 | 0 | 5295 | 48683 |
| 0 | 0 | 0 | 135 | 3285 | 43292 |
| 0 | 0 | 0 | 0 | 5336 | 40825 |
| 196 | 0 | 401 | 0 | 3519 | 41386 |
| 157 | 0 | 0 | 9 | 3362 | 46582 |
| 18 | 0 | 511 | 0 | 1615 | 43911 |
| 0 | 0 | 0 | 0 | 3929 | 34978 |