1   **Contrasting strengths of eDNA and electrofishing compared to historic**

2   **records for assessing fish community diversity and composition**

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19   **Abstract**

20   In times of rapid environmental changes, baseline biodiversity data are crucial for

21   management. In freshwaters, fish inventories are commonly based on the capture and

22   morphological identification of specimens. The sampling of environmental DNA (eDNA)

23   provides an alternative to assess diversity across large catchments. Here, we used extensive

24   historic data of fish communities collected across 89 river sites in all major catchments of

25   Switzerland and compared their diversity and community composition to a single campaign

26   of eDNA and electrofishing, respectively. Locally, we found that eDNA provided diversity

27   estimates similar to the integrated historic richness, while the electrofishing campaign

28   captured a significantly lower local richness. Fish species locally recorded by electrofishing

29   were nested (Jaccard's dissimilarity index) within the respective eDNA community for most

30   sites. Finally, eDNA sequence reads positively correlated with the overall electrofishing

31   biomass. Despite the congruences, the eDNA data did not correlate well with the

32   electrofishing water quality index. Overall, eDNA was more accurately assessing overall

33   diversity than a simultaneous electrofishing campaign, but yet cannot be directly used to

34   calculate fish-based water quality indices.

35

36   **Keywords**: biomonitoring, fish, eDNA, rivers, indicators

Introduction

Fish are a highly diverse group of freshwater vertebrates. The increased pressure on freshwater bodies (e.g., damming, settlements, chemical pollution, or nutrient loading) is degrading natural habitats and consequently negatively affecting the associated fauna (Dudgeon et al. 2021), in particular fish. Often, the severity of this loss for various spatial (local, regional, global) and temporal (months to decades) scales cannot be quantified robustly, because crucial baseline data of community composition and structure is lacking. Inventories of fish species in freshwaters are commonly conducted by capturing specimens, followed by morphological identification of the catch. Sampling can be done based on gillnet, hoop, fyke or trap nets or (anecdotic) rod fishing, but for a more standardized and comprehensive approach, electrofishing is the most widely accepted approach (Barbour et al., 1999, Champ et al., 2009). However, electrofishing is logistically challenging, can only be applied to wadable rivers up to a certain size, and may not be representative of all fish species. The method is also invasive, having an adverse effect on the sampled specimens and populations, which is especially impactful for rare or threatened species and small populations (Snyder et al., 2003, Panel & Densmore, 2011). Like any method, inherent methodological biases can lead to false absences (Richter et al. 2022), such as failing to detect rare species, and species with certain life history traits (e.g., benthic fish are underrepresented). Further, body size of fish (Millar et al. 2016) and local population structure (Glover et al. 2019) as well as habitat characteristics (e.g., structural complexity) (Korman et al. 2011) can interfere with capture efficiency of fish and lead to underestimated fish diversity. These limitations lead to an incomplete assessment of local fish communities and affect the accuracy of assessments of anthropogenic impacts on rivers. Overall,

60   electrofishing provides a spatially and taxonomically constrained inventory of fish species

61   diversity (Peterson et al. 2004, Rosenberger & Dunham, 2006, Lyon et al. 2014).

62   As a novel approach, environmental DNA (eDNA) based monitoring is increasingly

63   propagated to solve some of the limitations of the routine monitoring of aquatic life

64   (Taberlet et al. 2012, Pawlowski et al., 2018, Pawlowski et al., 2020, Deiner et al., 2021), yet

65   hitherto relatively small spatial scales were considered (e.g., catchment level) and there is a

66   high variability in matching data from eDNA with classic sampling (Keck et a. 2022). While

67   the sampling of eDNA, as any approach, has its own limitations (Goldberg et al. 2016, Evans

68   & Lamberti, 2018, Stauffer et al. 2021), it has been shown to be adequate for fish

69   community assessment in parallel sampling assays (Hänfling et al. 2016, Keck et al. 2022,

70   Pont et al. 2018). In particular, the sampling of eDNA as a monitoring method comes with

71   the benefit of being non-invasive, time-efficient, and spatially integrative over a larger area

72   (Deiner et al. 2017). The eDNA sampling approach can be applied to all freshwater systems,

73   including lentic (Harper et al., 2019; Hänfling et al., 2016) and lotic systems (Hallam et al.

74   2021, Pont et al. 2018, Blackman et al., 2021). In contrast to the commonly applied

75   methodologies for fish capture (e.g., electrofishing), eDNA sampling can be performed also

76   in large rivers that are not wadable, it does not rely on electrical equipment (anodes) and is

77   harmful for the sampled organisms. As eDNA relies purely on genetic traces, it not only has

78   a high sensitivity of detecting local species, but also detects species that are not physically

79   present at the sampled spot but where the signal is transported from upstream (Deiner &

80   Altermatt 2013, Carraro et al. 2020). This holds true especially for highly mobile organisms,

81   such as fish, that use various habitats within a stream and leave genetic material suspended

82   in the water column. Applied to a community context, eDNA sampling can provide

83   biodiversity information at a larger spatial scale (catchment level) for a variety of fish

84   species when coupled with a metabarcoding approach.

85   Most past work, however, has either only compared eDNA and electrofishing at relatively

86   small scales, often single reach systems, or has generally not been put into a historic

87   context, that is, assessing how the fish community assessed at a single campaign fit into the

88   regionally established species pool. Here, we used extensive historic data, electrofishing and

89   eDNA sampling to characterize fish communities sampled in 89 rivers across all of

90   Switzerland. We thereby integrated large spatial and temporal dimensions, by including

91   rivers with catchment basins ranging between 25 km^2 to 3,345 km^2 and compared a single

92   standardized sampling campaign (including both eDNA and electrofishing) to integrated

93   decadal long-term data. Our study addressed four key points:

94   i)   How well do electrofishing and eDNA assessments capture the historically known

95   fish diversity in a sub-catchment (i.e., time-integrated information), and how well

96   correspond the two approaches to each other?

97   ii)   Are the fish taxa detected by electrofishing a nested subset of the eDNA results,

98   as expected based on first principles of signal detection/transport?

99   iii) How well do quantitative estimates from eDNA metabarcoding correspond to the

100   biomass catch by electrofishing?

101 iv)   What is the potential of using eDNA based fish-community predictions for

102   calculating biotic indices on water quality and how well does it match currently

103   used biotic indices derived from electrofishing?

Methods

Fish community data

We base our analysis on 89 sampling sites distributed across all major rivers in Switzerland

(Figure 1). From all of these sites we have extensive historic data and contemporary eDNA

samples, and for a subset of 57 also contemporary electrofishing data. Historic data on fish

communities encompass all cumulative data on fish from the Swiss data center on faunistic

data (InfoSpecies, Neuchâtel, Switzerland). We retrieved data on all fish species recorded

between 1990 and 2022 within a 5 x 5 km of our sampling site from the database

InfoSpecies (part of GBIF, accessed 15.08.2022). Species that were recorded before 1990,

that had a single occurrence, or that were recently reassigned to another species due to a

wrong taxonomic classification (e.g., *Phoxinus phoxinus*) were excluded from the

downstream analysis (Table S2). We also pooled records that contain morpho-types that are

not distinguishable genetically on the barcode-region used (12S) due to their young

evolutionary age (in particular, species within *Salmo* spp., *Coregonus* spp.). The database is a

positive record database only, that is, all data on fish reported are aggregated, including

systematic and also unsystematic sampling campaigns. Based on the 5x5 km area covered,

reflecting part of the subcatchment, and the time-integration across the 32 years period,

the list of species can be assumed to be near complete or complete of all fish species

possibly occurring at the 89 study sites. We note, however, due to the integration across a

large area and time scale, not all of these species may have been found ever together, yet

reflect the pool of species to be possibly found at a given site. These historic data are used

as our comprehensive inventory ("perfect knowledge" scenario) to which we compared the

respective eDNA and electrofishing data.

Field Sampling

128

129   All 89 river sites were sampled for eDNA in 2019 and a subset of the sites (n = 57)

130   was also assessed with electrofishing at the same time. Sampling with both methods was

131   conducted as part of the regular NAWA sampling campaign, which is a Swiss wide

132   monitoring of fish communities conducted by the Swiss Federal Office for the Environment

133   (BAFU, 2013; BAFU, 2016). For the eDNA sampling, at each site, a total of 2 L of water was

134   sampled in spring 2019, and filtered across 4 Sterivex filters (0.22 μm pore size, Merck

135   Millipore, Merck KgaA, Darmstadt, Germany). Two of the samples (equivalent to 2 x 500 mL)

136   were taken at the right and two were taken at the left riverbank. In addition to the eDNA

137   samples, each sampling campaign included negative controls for which each field team

138   filtered 4 replicates of 500 mL of ddH_2O water (2 at the beginning, 2 at the end of the

139   campaign). The filters were sealed with Luer caps and placed in a cool box for transport to

140   the lab where samples were stored at −20 °C until further processing.

141   The electrofishing was conducted following the standard method for surface water

142   monitoring (Schager & Armin, 2004). At each site, fish were immobilized using electrical

143   anodes (mobile field team or stationary anode) along the river stretch sampling in a multi-

144   pass sampling (three passes per stretch). For each pass, the community composition, fish

145   specimen size, abundance, and biomass were documented. The fish specimens were

146   released back into the river stretch. For each site and each fish species, the average biomass

147   of individual species was calculated by dividing the total biomass collected of this species by

148   the number of individuals. From the three passes, the population size (total number of

149   individuals per site) and total biomass (sum of all specimens' biomass) were estimated.

150

151   *eDNA extraction and processing*

7

152   The DNA from the Sterivex filters was extracted in a cleanroom laboratory at Eawag,

153   Switzerland (as described by Deiner et al., 2015). For this, the Qiagen PowerWater Sterivex

154   Extraction Kit was used (Qiagen, Hilden, Germany). All samples and negative controls from

155   all sites were extracted in batches following the manufacturer's protocol. In the end, the

156   DNA was eluted into 100 µl of elution buffer and stored until further processing at −20

157   °C. Negative controls, positive controls (tissue DNA extract from Atlantic Cod, *Gadus*

158   *morhua*), and randomized samples were distributed over each 96-well PCR plate and were

159   prepared for sequencing in a two-step library preparation. First, the 12S rRNA barcode

160   region was amplified (size: 163–185 bp) for all the samples using MiFish-U-F/R primer pair—

161   forward primer sequence: 5'-GTCGGTAAAACTCGTGCCAGC-3' and reverse primer sequence:

162   5'-CATAGTGGGGTATCTAATCCCAGTTTG-3' (Miya et al., 2015).

163   The PCR reaction contained 0.5 µM of the forward and reverse primer each, 0.4

164   mg/mL BSA, 12.5 µL Q5 High Fidelity 2X Master Mix (New England Biolabs) and 2 µL

165   template DNA in a final volume of 25 µl. The reaction followed the protocol of: initial

166   denaturation of 98 °C for 5 min, followed by 35 cycles of 98 °C for 10 s, 65 °C for 20 s, and 72

167   °C for 30 s, and a final extension step of 72 °C for 7 min. The first PCR was carried out in four

168   replicates that were pooled and cleaned up with SPRI beads (Applied Biological Materials

169   Inc.) in 0.7x volume ratio and then used as a template for the second PCR. The second PCR

170   consisted of 15 µl of clean product from the first PCR, 25 µl of the KAPA HIFI MasterMix and

171   5 µl of each adaptor from the Nextera XT Index Kit v2 (Illumina), following the subsequent

172   PCR profile: initial denaturation 95 °C for 3 min, then 10 cycles of 95 °C for 30 s, 58 °C for 30

173   s, and 72 °C for 30 s, and a final extension step of 72 °C for 5 mins. All PCR products were

174   checked on the QiAxcel Advanced System with the Screening Cartridge (Qiagen, Hilden,

175   Germany) and the products were cleaned using 0.7 x SPRI beads. The DNA was then

176 quantified using the Spark 10M Multimode Microplate Reader (Tecan Group Ltd.) by using

177 the Qubit dsDNA BR assay (Thermo Fisher) and a standard calibration curve. All samples

178 were pooled at equimolar concentrations. The final pool was cleaned up 2 times with SPRI

179 beads and a 0.7x ratio and the final product was verified on the Agilent TapeStation. and

180 loaded onto a flow cell 17.6 pM concentration, with 10 % PhiX control. A paired-end 300

181 cycle (2 × 150 nt) sequencing was performed on an Illumina MiSeq (MiSeq Reagent Kit v3)

182 following the manufacturer's run protocols (Illumina).

183

184 *Bioinformatic Analysis*

185 We use here the pipeline and bioinformatic analysis described in detail in Blackman et al.

186 (2023) to process the raw data and used the same standardized workflow with optimized

187 parameters for this library. In short, the raw reads were first filtered to remove low

188 complexity reads and PhiX sequences. In the next step, the low-quality 3'-end were trimmed

189 to improve read merging. We used an in-silico PCR approach to remove the primer site from

190 the merged reads (amplicons). Subsequently, the amplicons were subjected to a quality-

191 and a size-range filter. The cleaned amplicons were de-replicated prior to clustering with

192 UNOISE (Edgar, 2016a). UNOISE3 includes error correction, zero-radius clustering and

193 chimera removal. We used an abundance threshold of 10 reads to remove artificially

194 produced and therefore untrustworthy singletons and rare zero-radius Operational

195 Taxonomic Units (in brief: zOTUs). The cleaned reads were then mapped against a reference

196 database.

197

198 *Reference database*

199 The raw sequencing data were analysed using a customized database build from fish

200 specimens collected in a Swiss wide fish monitoring (see Alexander & Seehausen, 2021) to

201 yield robust taxonomic assignments and improve the species level resolution. For this

202 reference database, a total of 912 specimens, covering 106 of all species of the 124 fish

203 known in Switzerland, were sequenced for their 12S barcode, generating a virtually

204 complete database (Blackman et al. 2023). These clustered into 116 unique Operational

205 Sequence Units (OSUs) and were assembled into a closed taxonomic database for mapping

206 against the eDNA data with an identity of 97 % and a query coverage of 100 %. Additionally,

207 sequences from other groups (arthropods, invertebrates, bacteria) were included in the

208 database as outgroups to avoid forced taxonomic assignments. A more detailed report of

209 the assembly and the utility of this customized database is described in Blackman et al.

210 (2023).

211

212 *Data analysis and visualization*

213 All analyses were performed in R (version 4.1.2; Core team, 2021). The raw data was

214 imported into R and quality filtered based on the number of reads in negative and positive

215 samples. In detail, we defined an OTU-specific minimal abundance threshold for samples

216 based on their presence in the positive and negative controls. Subsequently, we removed

217 taxa that were not assigned to the class of Actinopterygii. For a more detailed list of the

218 number of reads processed during the bioinformatic and quality filtering workflow, see

219 Table S1. The four filter samples per site were then merged for further analysis. For the

220 analysis using reads and not presence-absence data, we performed a rarefaction to account

221 for stochasticity during the PCR step and different sequencing depths (after rarefaction:

222 31,365 reads per sample site, 1 zOTU dropped).

223   The maps of waterbodies in Switzerland were generated using the Swissriverplot package

224   (Alther & Altermatt, 2020). The community analysis was done with the phyloseq package

225   (McMurdie & Holmes, 2013) and the vegan package (Oksanen et al., 2020). Differences in

226   the species richness between the historic versus eDNA data and the electrofishing versus

227   eDNA data at a site level were evaluated with a paired t-test. Using the rarefied data to

228   control for different sequencing depths, we also looked at Shannon and Simpson diversity

229   indices, to see if and how dominant and rare species influence the comparison between

230   eDNA and electrofishing. In order to compare not only the species richness at a site, but

231   also the composition, the overlap between species lists based on eDNA, electrofishing, and

232   historic records at a site level were evaluated using the BioVenn package (Hulsen et al.,

233   2021).

234   To test if the eDNA-based assessment reflects a subset of the historic records, and if

235   electrofishing assessments are more constrained to a local river stretch, we tested for a

236   non-random nestedness based on the Jaccard's dissimilarity index between the two

237   methods (historic vs. eDNA and eDNA vs. electrofishing) by using the betapart package

238   (Baselga et al., 2021) (see Figure S1 for details). First, we generated a null model of the

239   communities at each sampling site using the "randomizeMatrix" function in the picante

240   package (Kembel et al., 2010). For this, randomized eDNA community matrices (compared

241   to historic data) or randomized electrofishing community matrices (compared to eDNA)

242   were generated by applying the "trialswap" algorithm (Mikals and Podani, 2004). Second,

243   the observed nestedness value calculated from the observed data was then mapped against

244   the null distribution from the randomized community dissimilarities to infer if the observed

245   value was significantly different from the null distribution.

246 Further, the simultaneous monitoring of fish communities with eDNA and electrofishing

247 enabled us to evaluate the potential of eDNA sampling as a quantitative method for the

248 total catch (biomass in kg) by electrofishing. For this, the proportions of a given species

249 accounted for in the data sets (total reads vs. biomass) was compared using the ggalluvial

250 package (Brunson and Read, 2002). The significance of the relationship was tested by a

251 linear model (formula: biomass ~ reads) on the log-transformed variables. Further, we also

252 investigated the relationship at the local scale, using rarefied reads for eDNA and biomass

253 per species and site in a mixed effect model in the lmer package, using site as a random

254 effect (formula: biomass ~ reads + 1|site).

255 Lastly, we linked the eDNA community data to the biotic index as the two sampling

256 campaigns (eDNA and electrofishing) were part of an applied ecological assessment (NAWA)

257 and the implemented index was purely based on electrofishing. Here, the eDNA based fish

258 data was linked to the biotic index (MSK, for further details on the index see Schager et al.,

259 2004) in order to test if eDNA can be used as an alternative method to electrofishing in the

260 assessment. Based on electrofishing, the biotic index (MSK) is composed of 4 parameters: 1.

261 diversity and dominance, 2. population structure of indicator species, 3. fish density, 4.

262 deformations and anomalies. The numerical score (0 to 20) is classified into an ecological

263 state ranging between "poor" to "very good". The eDNA-based fish community was

264 implemented in a random forest model in the ranger package (Wright and Ziegler, 2017) as

265 previously done for diatoms (for implementation see also Cordier et al., 2018, for more

266 details on the R-Code of the random forest modeling see the Supplementary File II). For

267 every site, a regression model with the observed index of the site (based on electrofishing

268 data) as a response variable and the community data (based on eDNA) were used as

269 predictive variables (formula: MSK observed index ~ eDNA community, number of trees =

270   400, min.node.size = 3, mtry = n/3). The model was trained iteratively by using a part of the

271   data as a training data set, to perform a regression between the observed community and

272   the associated index score. Subsequently, the trained model was used to predict the index

273   for the eDNA data of a given site, that the model was not trained on. This process was

274   iterative to predict the index score of every site. We tested for a difference in the mean of

275   the MSK predictions for the distinct classifications ("poor" to "very good") with an Analysis

276   of Variance (Anova).

Results

Historic inventory and raw data from the simultaneous sampling campaign

In the historic dataset, integrating all data collected between 1990 and 2022, 65 of the in total 124 fish species known from Switzerland (105 native and 19 non-native species) were detected across the 89 5 x 5 km squares surrounding the 89 sampling sites (Table S2). For the field sampling in 2019, the eDNA metabarcoding library for all these 89 sampling sites generated a total of 14.76 million raw reads mapped to 101 OSUs. From those, 92 were assigned to the targeted class of Actinopterygii (the others being assigned to Mammalia, Aves and Amphibia, and thus excluded). These 92 OSUs were partially assigned to the same taxonomic fish species, and clustered into 51 taxonomic species. However, for some taxonomic groups, the genetic barcode does not allow for a distinction between species, which were subsequently aggregated and correspond to 45 distinct species (Table S2). Interestingly, the eDNA sampling campaign detected 3 species that had not been registered locally in the historic inventory (Figure S2). In contrast, all the species detected captured with electrofishing were also documented in the historic records. Overall, the electrofishing captured 33 fish species (aggregated) with an overall catch of 81,789 specimens and 10,383 kg across all 57 sampling sites.

Alpha diversity compared between historic, eDNA and electrofishing data

The mean local species richness detected by the eDNA approach (mean = 15.3 ± 4.7) corresponded closely to the species richness established by the historic inventory of fish species covering a 5 x 5 km square around each sampling site (mean = 15.8 ± 9.2), and there was no significant difference between these two approaches (t = 0.5, df = 130, p > 0.6) (Figure 2). Focusing on the species that were recorded historically and were also detected

14

301   with eDNA sampling, the overlap of detected species locally decreased with the number of

302   overall site detections (detected at fewer sites) (Figure 3A). Species that were common in

303   the eDNA dataset (many site detections) were also common in the historic inventory.

304   Species that were detected at fewer sites (less than 30 % of sites) were predominantly

305   detected by one or the other method (Figure 3B).

306   Contrastingly, local richness estimates were significantly different between the

307   simultaneous monitoring of the sites with eDNA and electrofishing. The sampling of eDNA

308   detected a significantly higher local species richness (mean = 15.3 ± 4.7) compared to the

309   electrofishing approach (mean = 6.5 ± 4) (t = 10.6, df = 108, p < 0.001; Figure 2). Similarly,

310   we also compared the evenness and dominance of individual species, and on average, eDNA

311   showed a higher index value for eDNA measures (see Figure S3). A subset of species was

312   commonly detected by both methods, but locally eDNA uniquely detected a surplus

313   detection (mean 9.4 ± 4 species uniquely detected by eDNA at a site) by the eDNA sampling

314   (Figure 3C). At the site level, the electrofishing captured only a few species (mean 1.8 ± 2

315   species per site) that were not detected by eDNA sampling at that specific site. Similarly, the

316   comparison of the historic data with eDNA species that were common (detected at many

317   sites, e.g., *Oncorhynchus mykiss, Gobio gobio,* or *Phoxinus* sp.*)* showed a better congruence

318   in detection between the eDNA and the electrofishing method (Figure 3D).

319

320   *Quantitative estimates of fish as biomass and reads respectively*

321   Contrasting the quantitative estimates of fish abundances from the simultaneous sampling

322   campaigns (compared as the biomass in kg to the number of sequences in reads) ranked

323   different species as the most abundant across all sites (Figure 4A). Yet, overall, the more

324   diverse families were ranking the highest with both methods. Thus, the number of reads for

325   a species was significantly correlated with the biomass from the electrofishing catch

326   (formula: biomass ~ reads, F = 37.1, df = 42, p < 0.001, R^2 = 0.44, Figure 4B) , similar to the

327   presence-absence (number of detections) that was significantly correlated with higher

328   biomass (see Figure S4). However, when looking at the same relationship of reads versus

329   biomass on a site level, the reads did not correspond with the catch of a given species

330   (formula: biomass ~ reads + 1|site, see examples in Figure S5). With the electrofishing

331   approach, the Cyprinidae accounted for the highest portion of biomass across all sites (> 60

332   %) (Figure 3B), especially the widely abundant species *Phoxinus* sp. captured at 33 of 57

333   sites with > 30,000 individuals. In the eDNA sampling, the family of Salmonidae made up the

334   highest proportion (> 25 %) (the species aggregate *Salmo* sp. accounted for > 2 million

335   reads).

336

337   *Nestedness of community patterns based on Jaccard's dissimilarity between approaches*

338   The eDNA approach reported a highly similar local species richness estimate compared to

339   the historic inventory, yet the composition of the local community (i.e., the identity of the

340   species) differed. The mean observed dissimilarity at a site between communities based on

341   historic and eDNA records was 0.8 ± 0.01 for the Jaccard index ranging from 0 (total overlap)

342   to 1 (complete replacement) indicating a difference in community assessment across the

343   sampling sites. Dissimilarities (measured as Jaccard's nestedness component) between the

344   communities recorded historically and the ones detected with eDNA resulted in a

345   dissimilarity value for each sampled site (Figure 5A). When observed dissimilarity values

346   were compared to the null model, 61 % of the sites showed a non-random nestedness of

347   the eDNA data within the historic records. A site was significant, when the significance the

348   observed nestedness was different than compared to the dissimilarities for 999 random

349   communities (Figure 5B) leading to a significance level of 0.05.

350   Generally, species detected by electrofishing were also detected by eDNA locally (yet with

351   the latter method also reporting additional species), resulting in a nested structure of the

352   electrofishing data in the eDNA based community. Calculating the dissimilarity between

353   communities from the eDNA and the electrofishing at a site level resulted in a mean

354   Jaccard's dissimilarity of 0.6 ± 0.17. The observed dissimilarity, as nestedness of

355   electrofishing in eDNA, was significantly different from a random distribution for 31 out of

356   the 57 sites (55 %). For these sites, the probability to detect the observed nestedness was

357   below 0.05 when compared to the null distribution (Figure 5C). Similar calculations were

358   also established for the total dissimilarity and the turnover component of the Jaccard's

359   index (Figure S6).

360

361   *Limitations of species level taxonomic assignments*

362   Across the diversity of fish that are monitored for the routine assessments of water quality,

363   some groups cannot be fully taxonomically resolved to species level. These cryptic groups

364   represent a hindrance for eDNA sampling and electrofishing, as species can be either

365   morphologically or molecularly indistinguishable, both of which occurred in our dataset .

366   Morphology is constrained by distinct features for species assignment, resulting in some

367   species being grouped into morpho-species (e.g., *Salmo trutta, Salmo marmoratus,* and

368   *Salmo labrax* are all lumped together as *Salmo* sp.). Similarly, a group of lake whitefish, here

369   only detected by eDNA (Figure 3A) probably due to DNA transport from upstream lakes,

370   underwent a relatively recent radiation and have been reclassified morphologically, as they

371   show a high diversity across Swiss lakes (Alexander & Seehausen, 2021). Interestingly, also

372   on a genetic level, these two groups contain species that were not distinguishable with the

373   12S barcode used here, and thus were united at the genus level (e.g., *Salmo* sp*., Coregonus*

374   sp.). Additionally, there are strong geographic boundaries that divide sister taxa North and

375   the South of the Alps (Table S2), that cannot be genetically distinguished based on the

376   barcoding region. For example, the species *Esox lucius* is present in the North of Switzerland

377   (Rhein catchment, Figure 1A) and the *Esox cisalpinus* confined to the South of the Alps

378   (Ticino catchment, Figure 1A). Given these hindrances for the identification, some groups

379   (such as *Coregonus* sp., *Esox* sp., *Salmo* sp., *Alburnus* sp.) are not resolved to species level

380   constrained by morphology or by genetic similarity.

381

382   *Methodological limitations of eDNA in the framework of the biotic index*

383   The classification of the river's ecological status, routinely calculated from the electrofishing

384   sampling (BAFU 2016), was inferred from the eDNA based communities using a data-driven

385   random forest approach that did not lead to congruent classifications of the sampled sites

386   (Figure S7). In the framework of the biotic index (MSK), the eDNA monitoring data did not

387   provide information for all of the parameters that are reported from the electrofishing

388   sampling such as species abundance, local population structure, parasite loading and

389   deformation of specimens. The original calculation was therefore hindered by the lack of

390   this information from the eDNA sampling, here resulting in a machine learning

391   implementation for the prediction of the index. The ecological classification of river sites

392   based on this index– based on variables given by the electrofishing monitoring – could not

393   be calculated given the eDNA data, the predicted means for the index was not significantly

394   different between the observed classifications (MSK observed ~ eDNA community , F =

395   0.001, df = 178, p-value = 0.8).

Discussion

eDNA as a monitoring tool is more integrative than electrofishing

Despite the increased need for scalable, efficient, and standardized assessment methods,

establishing baseline patterns for aquatic diversity remains challenging. Here, we used

extensive historic records on integrated fish diversity and compared it with a recent

standard biomonitoring to characterize fish communities across a large spatial scale. The

historic records are based on a combination of long-term standardized sampling efforts and

individual observations covering >30 years, and served as a baseline for the comparison

between two contemporary sampling (eDNA and electrofishing) methods in terms of their

efficacy to detect diversity and composition of fish communities. We did so to evaluate if

and how the sampling of eDNA may add novel approaches in the monitoring of aquatic

biodiversity and ecological assessments, for example water quality monitoring. In this

context, established methods (here: electrofishing) are compared with eDNA-based

approaches and assessed by a site-by-site comparison of the methods. This, however, thus

not consider sampling errors inherent to any method, and thus a comparison to an

integrated historic inventory may provide a better baseline for method comparability (see

also Keck et al. 2022). Having a historic inventory of species recorded around the sampling

site as an independent source of information also allows to better understand possible

discrepancies between simultaneous sampling methods, as it provides information on a

regional (and even time-integrated) community that is then hypothetically sampled by point

estimates.

Null model identifies significant nestedness at some sampling sites

419   The sampling of eDNA has shown fit for purpose for the detection of fish communities in

420   different aquatic systems (Harper et al., 2019; Hänfling et al., 2016, Pont et al., 2018,

421   Blackman et al., 2021). Here, it was applied to a routine ecological assessment based on the

422   monitoring of fish to see how the method compared to electrofishing as an established

423   standard method (Barbour et al., 1999, Champ et al., 2009). Given certain technical

424   constrains (Goldberg et al. 2016, Evans & Lamberti, 2018, Stauffer et al. 2021), the richness

425   estimates, and locally also species composition were similar between eDNA and the historic

426   species inventory. A dissimilarity between taxa lists from various methods is expected,

427   stemming from different detection efficiencies for the sampled species, as well as inherent

428   sampling variability. When calculating dissimilarities in community composition, differences

429   in alpha-and gamma-diversity between methods can drive variations in communities and

430   therefore patterns in dissimilarity indices (Chase et al., 2011). These effects can be

431   addressed by using a null model approach that accounts for methodological dissimilarities

432   and allows to disentangle methodological from ecological dissimilarities (Gotelli and Grave,

433   1996). By using the Jaccard index as a measure of dissimilarity, the significant nestedness of

434   the contemporary eDNA sampling in the historic inventory and the local electrofishing in the

435   DNA signals indicates how congruent the methods are with respect to detecting specific

436   communities. Sites with a significant nestedness of the eDNA sampling campaign in the

437   historic inventory shows that the eDNA sampling reflected the community composition

438   locally detected over an integrated time span. The eDNA sampling gives valid results

439   compared to historic records but still provides an incomplete species list, leading to a high

440   turnover of species between the methods, shown here as non-significant nestedness for

441   almost a third of sampling sites.

442   The eDNA approach detected locally a similar richness like the historic inventory

443   despite the difference in sampling effort (a time span from 1990 to 2022 for the historic

444   inventory, and one sampling campaign in 2019 for eDNA). The significant nestedness of the

445   electrofishing sampling in the eDNA data likely reflects the spatial integration of DNA

446   fragments at a catchment scale: with eDNA we not only found the species that were locally

447   occurring (and documented by electrofishing) but also got an insight into species occurring

448   in habitats that are under-sampled with electrofishing (benthos) or upstream habitats. This

449   result supports that eDNA captures species from upstream in addition to species that can be

450   locally detected (Keck et al., 2022), resulting in the integration of genetic signals within a

451   catchment into an eDNA sample (Deiner et al., 2016, Carraro et al., 2020). Previous studies

452   have shown that DNA fragments can be transported from a few (Deiner & Altermatt, 2013;

453   Thalinger et al., 2020) to dozens of kilometers (Pont et al., 2018). Here, a prime example for

454   this transport is the detection of some lake fish species (*Coregonus* sp*., Salvelinus*

455   *profundus, Salvelinus namaycush*) that do not occur in riverine habitats and were not

456   captured by electrofishing yet showed up in the eDNA signal.

457   The historic records, reflecting a "perfect knowledge scenario" was overall more

458   comprehensive than the eDNA sampling (i.e., recorded more distinct species). This could be

459   due to the inventory not actually reflecting co-existing species but pooled communities. The

460   detection of rare species that are missed with the eDNA approach could be increased by the

461   consideration of technical aspects, such as increasing the volume of water filtered (Stauffer

462   et al., 2021, Altermatt et al., 2023), improving the specificity of the primer pairs (Miya et al.,

463   2012) or increasing the number of PCR replicates. Further, the length of the amplified

464   fragment is relatively short and can hinder the identification to species resolution (as here

465   observed for several species (*Salmo* sp., *Esox* sp., *Phoxinus* sp., *Alburnus* sp.). Another

466   common limitation to metabarcoding studies, namely the lack of curated sequences in the

467   reference database (Zhang et al., 2020) was overcome in our study by using a customized

468   reference database that covered barcode sequences of all Swiss fish species (see Alexander

469   & Seehausen, 2021) and that significantly improved the assignments of sequences to taxa

470   when compared with public databases (Blackman et al., 2023).

471

472   *eDNA as quantitative estimate for biomass only on a gamma level*

473   A positive relationship between local abundance of a species and eDNA reads has been

474   reported previously for rivers (Pont et al., 2018) and other aquatic systems such as marine

475   environments, ponds, lakes (Rouke et al., 2022). For the monitoring campaign in 2019, we

476   found a high congruence in the common species which was reflected in the correlation of

477   the quantitative estimates (biomass and reads) based on electrofishing or eDNA sampling

478   across all sites. The most widespread groups made up for the most reads and also

479   accounted for the most biomass. At a site level, however, the relationship between the

480   number of reads assigned to a species did not correspond to the biomass of this species.

481   Both methods have some inherent biases. Consequently, we cannot directly infer the

482   accuracy of the local biomass or abundance by a discrepancy of the local quantitative

483   estimates, as such a discrepancy can be due to either's approach bias, or due to stochastic

484   components. As discussed for diversity estimates of fish species from electrofishing, the

485   capture efficiency is impacted by the life cycle, behavior, and body size of species, and by

486   the local environmental conditions at the sampling site. For eDNA, it has been recognized

487   that fish species shed DNA at different rates that scale allometrically (accounting for surface

488   area, and physiological excretion), resulting in interspecific variation in the signal strength

489   (DNA reads) and the abundance of organisms (Yates et al., 2022). Additionally, the

490   laboratory processing, especially the stochasticity introduced during the PCR introduces

491   further biases to the relationship. This is influenced by the differences in the primer binding

492   efficiency (Taberlet et al., 2018) and amplification efficiency of different DNA templates of

493   fish species as shown using an internal standard (Ushido et al., 2018). Furthermore,

494   different spatial integration between methods, systematic non-detections with one or the

495   other method, and abiotic factors like degradation and transport can break the linkage

496   between local biomass and DNA fragments shed into the water (Yates, Cristescu & Derry,

497   2021).

498

499   *The eDNA based information shows limited resolution for ecological classification*

500   Overall, we showed that eDNA sampling for monitoring results in robust diversity and

501   community composition in comparison to electrofishing, as suggested by previous studies in

502   different aquatic systems (Blackman et al., 2021, Hallam et al., 2021, Pont et al., 2018, Pont

503   et al., 2021, Yao et al., 2022). However, the implementation of the eDNA derived fish

504   community data into the established biotic index for the water quality assessment did not

505   success and an accurate classification was not possible. Most likely, this is because the

506   calculation of ecological indices is not just based on diversity and composition of species,

507   but also on local population age structure, fish density and physical characteristics of the

508   specimens. Such information implemented in the calculation of the index go beyond what

509   can be established from eDNA sampling. As biotic indices are often developed from

510   established standard sampling methods, they can complicate the implementation of eDNA

511   based monitoring for ecological assessments based on the calculation of biotic indices.

512   However, eDNA samples can inform about multiple organismal groups with one sampling

513   campaign and despite eDNA not being congruent with the established index based on

514   electrofishing data, biotic indices can further be developed and adapted to include a

515   different type of information (Pawlowski et al., 2018). We conclude that eDNA is fit for

516   purpose as a useful, fast, and efficient monitoring tool to assess local species richness of

517   fish, and to some level also community composition, but cannot resolve ecological status

518   based on established indices that too strongly rely on morphological estimates and

519   abundance counts.

530   **Author contributions**

531   FA and JB designed the study, analyzed the data, and wrote the manuscript. Laboratory

532   work was carried out by JB.

538

539   **Data Availability Statement**

540   All the raw sequencing data will be made available upon publication in the DRYAD archive

541   accessible at https://doi.org/10.5061/dryad.1g1jwsv15.

542

543   **Conflict of Interest**

544   The authors declare no conflict of interest.

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Figures & Legends
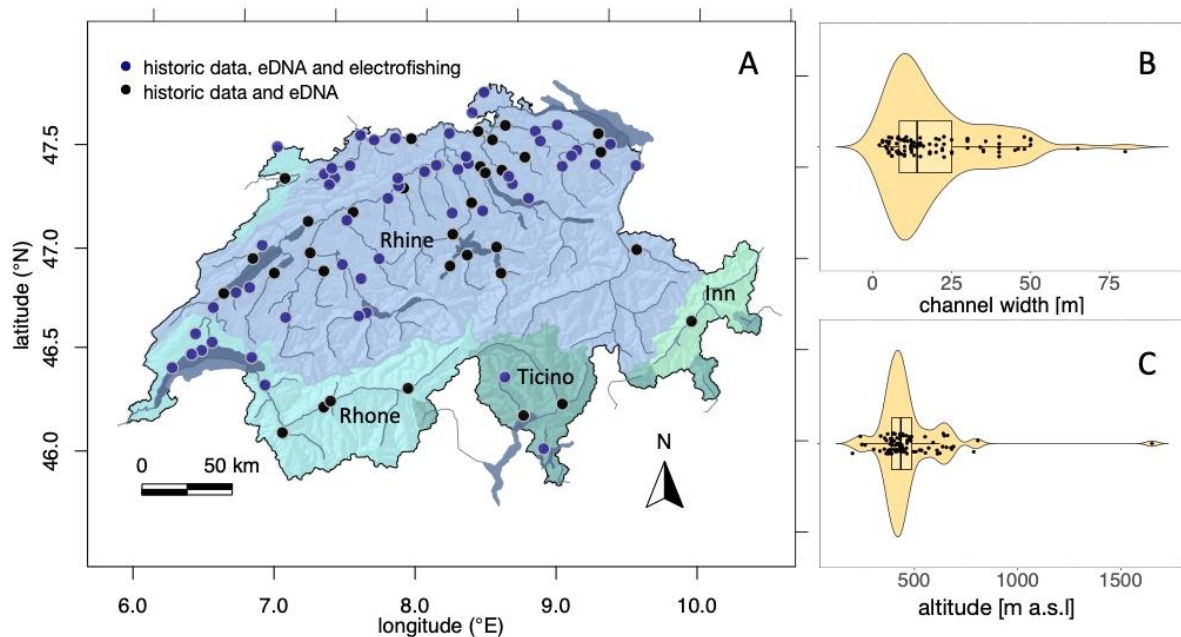
768 Figure 1: Spatial setup of the monitoring of fish communities in Switzerland. A) The map of

769 Switzerland shows the sampling sites (n = 89) across the four major Swiss catchments

770 (Rhine, Rhone, Ticino, and Inn) indicated with different color shades. For all sampling sites

771 data on historic fish occurrence (in the respective subcatchment) as well as recent fish

772 occurrence measured locally by eDNA were available. For a subset of 57 sites data (blue

773 dots), recent fish occurrences assessed locally by electrofishing were also available. The

774 sampled river systems range from intermediate to large rivers as shown by B) the

775 distribution of their channel widths and C) span an altitudinal gradient with most sampling

776 sites being situated in lowland rivers.

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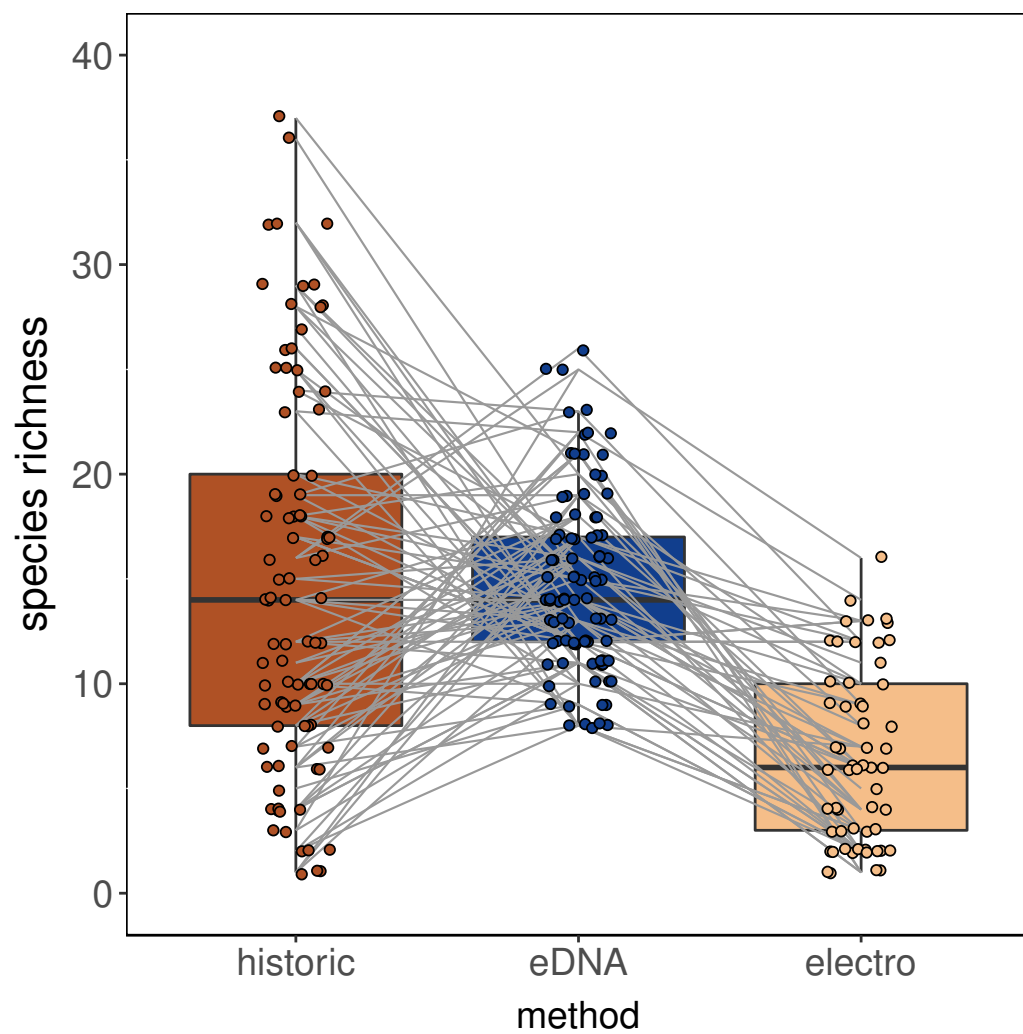
Figure 2: Comparison of local fish richness at 89 sites in Swiss rivers comparing historic

species richness detected at the sub catchment scale (5 x 5 km squares) between 1990–

2022 versus the respective local species richness detected by eDNA and (for a subset of 57

sites) electrofishing in one single monitoring campaign in 2019.
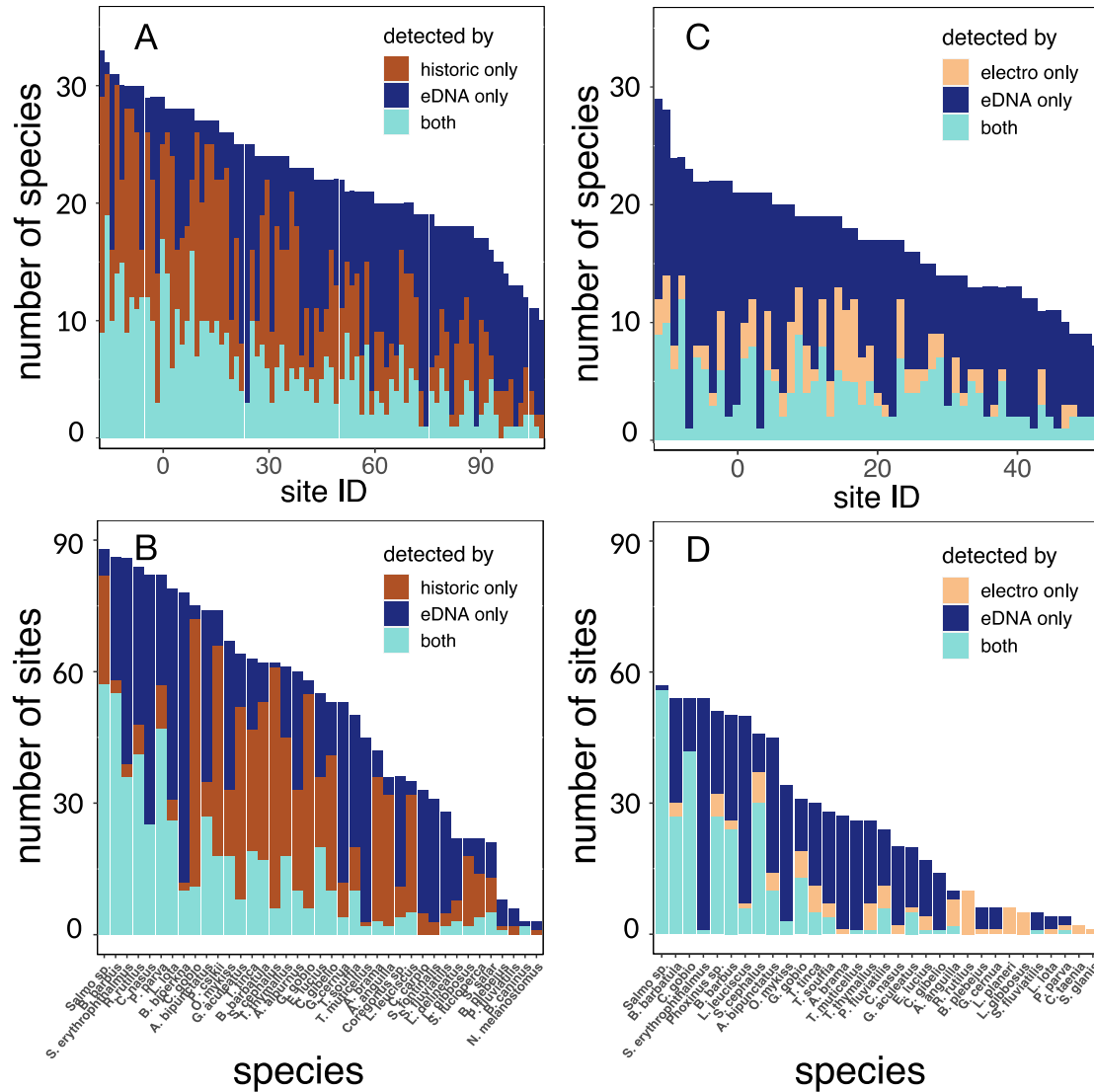
Figure 3: A) Site-richness curves giving species richness per site and (B) number of sites where a species was found, resolving what part of the richness is depicted by historic data only, by eDNA only, or by both. Overall, there was no systematic difference in the species richness detected between the two methods, yet the overlap of the respective species detected was relatively small with a high proportion only recorded in the historic data but not captured with eDNA. C) Site-richness curves giving species richness per site and D) number of sites where a species was found, resolving what part of the richness is depicted by eDNA only, by electrofishing only, or by both. With electrofishing a significantly lower number of species was detected compared to the richness estimates from eDNA samples.

792   Note that the number of sites and species covered is lower for the comparison with

793   electrofishing (57 out of 89), as this method was only applied to a subset of all sites.

794
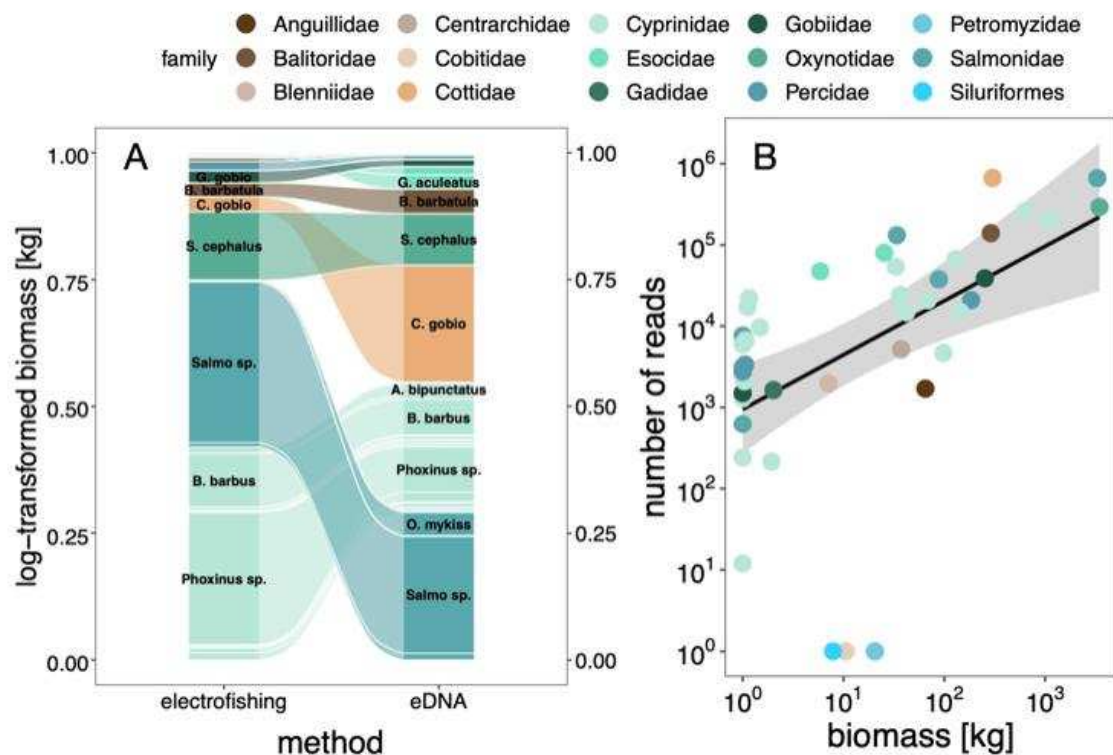
795

796 Figure 4: Overall quantitative estimates of fish based on electrofishing and eDNA sampling.

797 A) The proportions of species overall sites are shown as stacked bar plots that give the

798 proportions of biomass (kg) for electrofishing and of sequences (number of reads) for eDNA,

799 respectively. The flows connecting the bars indicate the association to families, and the

800 most common taxa are named. B) Over all sites, there is a significant relationship between

801 the number of reads and the biomass of a species (formula: biomass ~ reads, F = 37.1, df =

802 42, $p < 0.001$, R^2 = 0.44), as indicated by the linear regression (black line). There is a high

803 variability (se = grey area) across different species. The colors reflect the association of

804 species to their taxonomic family.

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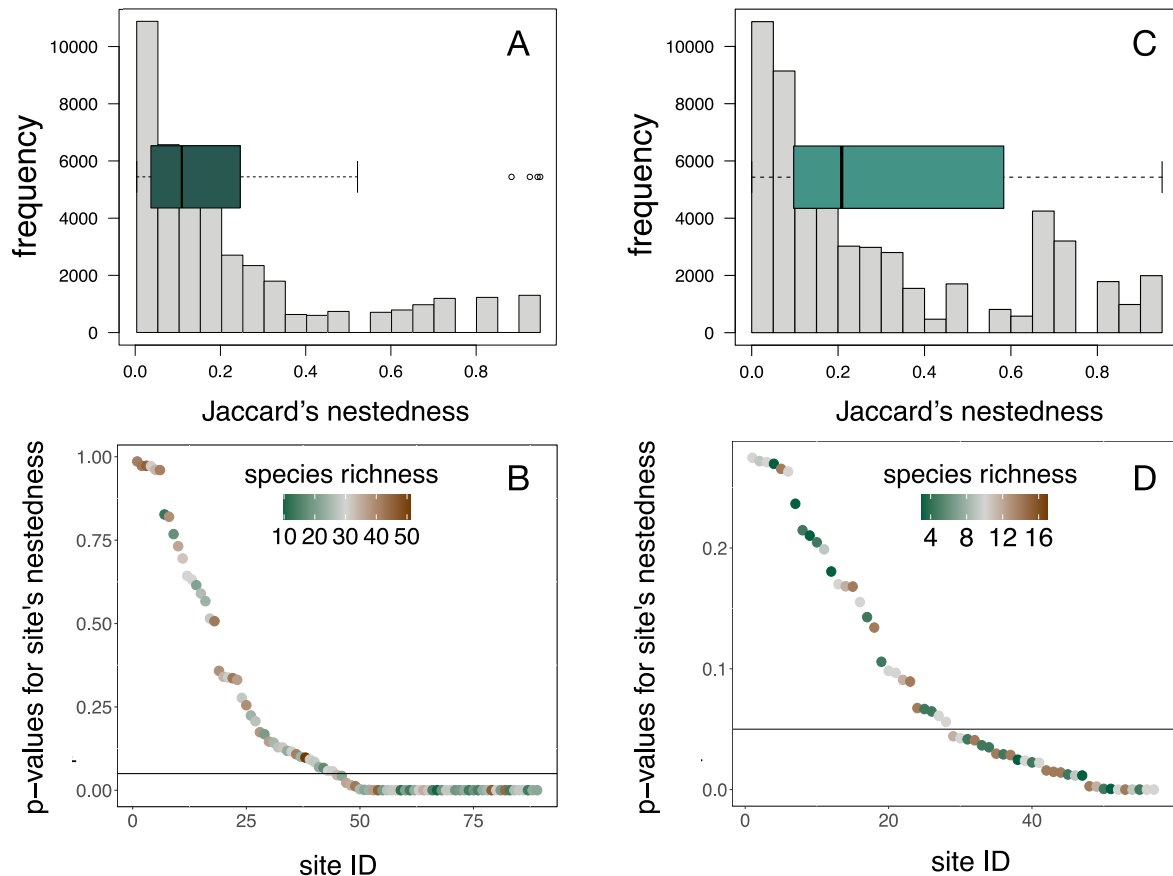
806 Figure 5: Nestedness of communities observed with eDNA (A and B) and electrofishing (C

807 and D) based on Jaccard's dissimilarity between sites. The dissimilarities of randomized

808 community matrices (n = 1000) were used as a random frequency distribution (grey bars) of

809 nestedness values under the assumption of random species assembly in the eDNA (A) within

810 the historic data, and the electrofishing within the eDNA data (C). The actual observed

811 nestedness values of all sites are indicated as boxplots. For every site, the probability of the

812 observed value was calculated based on the null model distribution. The p-values indicate

813 the probability of a site's community being significantly nested (B) for eDNA in the historic

814 inventory, and (D) for the electrofishing in the eDNA community data. The black line

815 corresponds to a significance level of 0.05. The effect of nestedness was not based on

816 overall species richness, as the color gradient of the points shows.

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818 **Supplementary Material:**

819

820 **Contrasting strengths of eDNA and electrofishing compared to historic records**

821 **for assessing fish community diversity and composition**

822

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849 **Table S1**: List of the number of reads at each step during the data processing and the number
850 of reads assigned to non-target taxa. The taxonomic assignment was performed using a
851 customized reference database of Swiss fish, with some eukaryotic outgroups. Non-
852 eukaryotic and unassigned reads were filtered after the assignment (approx. 1 Mio reads).
853 Also, we excluded reads that were not fitting the length of the targeted gene-sequence for
854 fish.

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Step	mio of reads
raw data	14.76
QF: > Q30	11.68
bioinformatics	8.88
tax-assigned (eukarya)	7.88
QF (based on controls)	7.12
taxonomy	**number reads to**
human	33958
cow	99672
pig	45865
chamois	1474
star	780
trush	610
alpine newt	84
Actinopterygii	**6933141**

868　　**Table S2**: List of species detected in the NAWA eDNA and electrofishing sampling campaign

869　　sampling, and extracted from the Swiss Info Fauna database (infospecies.ch, recorded in the

870　　5 x 5 km square around the sampling sites) in 2019. The first column describes the species

871　　subset: common species at the top of the table are species detected with eDNA, electrofishing

872　　and in the historic inventory, below are species additionally detected in 2019 with eDNA, with

873　　electrofishing and at the bottom species only detected in the historic inventory. The columns

874　　with a "tax:"-prefix describe the taxonomic ranks of assignments; the common name is the

875　　name known in Switzerland. The "tax: resolution"-column describes the taxonomic resolution

876　　used in this analysis, as some species were genetically or morphologically not distinguishable.

877　　Also, for the species detected with eDNA, some are represented by multiple OSUs clustering

878　　into the same taxonomic species as indicated in the column "Nr. of OSUs". For the species

879　　only recorded in the historic inventories, we excluded species that were detected rarely (< 5)

880　　or before 1990. Furthermore, some species detected with the electrofishing would be

881　　morphologically or genetically indistinguishable, but a distinction can be made by geographic

882　　boundaries of their distribution (North/South of the Alps). The last three columns give the

883　　presence-absence (1-0) of species with each method.

| subset	tax:class	tax:order	tax:family	tax:species	common name	tax:resolution	Comments	Last observed	Geographic morphs	eDNA	electro	historic
Common Fish Species							Nr. Of OSUs					
	c__Actinopterygii	o__Anguilliformes	f__Anguillidae	*Anguilla anguilla*	Aal	Species	1	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Balitoridae	*Barbatula barbatula*	Schmerle	Species	6	2019		1	1	1
	c__Actinopterygii	o__Perciformes	f__Blenniidae	*Salaria fluviatilis*	Cagnetta	Species	3	2019	South CH	1	1	1
	c__Actinopterygii	o__Perciformes	f__Centrarchidae	*Lepomis gibbosus*	Sonnenbarsch	Species	1	2019		1	1	1
	c__Actinopterygii	o__Scorpaeniformes	f__Cottidae	*Cottus gobio*	Groppe	Species	3	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Alburnoides bipunctatus*	Schneider	Species	2	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Barbus barbus*	Barbe	Species	3	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Barbus plebejus*	Suedbarbe	Species	2	2019	South CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Blicca bjoerkna*	Guester	Species	1	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Carassius gibelio*	Giebel	Species	1	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Chondrostoma nasus*	Nase	Species	2	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Cyprinus carpio*	Karpfen	Species	1	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Leuciscus leuciscus*	Hasel	Species	3	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Phoxinus csikii*	Elritze	Genus	5	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Phoxinus lumaireul*	Elritze	Genus	2	2019	South CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Phoxinus septimaniae*	Elritze	Genus	1	2019	West CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Pseudorasbora parva*	Blaubandbaerbling	Species	1	2019		1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Rutilus rutilus*	Rotauge	Species	2	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Scardinius erythrophthalmus*	Rotfeder	Species	3	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Scardinius hesperidicus*	Schwarzfeder	Species	2	2019	South CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Telestes muticellus*	Strigione	Species	1	2019	South CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Telestes souffia*	Stroemer	Species	1	2019	North CH	1	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Tinca tinca*	Schleie	Species	1	2019		1	1	1
	c__Actinopterygii	o__Esociformes	f__Esocidae	*Esox cisalpinus*	Italienischer Hecht	Genus	2	2019	South CH	1	1	1
	c__Actinopterygii	o__Esociformes	f__Esocidae	*Esox lucius*	Hecht	Genus	2	2019	North CH	1	1	1
	c__Actinopterygii	o__Esociformes	f__Esocidae	*Gasterosteus aculeatus*	Stichling	Species	1	2019		1	1	1
	c__Actinopterygii	o__Gadiformes	f__Gadidae	*Lota lota*	Truesche	Species	1	2019		1	1	1
	c__Actinopterygii	o__Perciformes	f__Gobiidae	*Gobio gobio*	Gruendling	Species	1	2019		1	1	1
	c__Actinopterygii	o__Perciformes	f__Gobiidae	*Gobio obtusirostris*	Gruendling	Species	2	2019		1	1	1
	c__Actinopterygii	o__Gobiiformes	f__Gobiidae	*Neogobius melanostomus*	Schwarmundgrundel	Species	1	2019		1	1	1
	c__Actinopterygii	o__Squaliformes	f__Oxynotidae	*Squalius cephalus*	Alet	Species	1	2019	North CH	1	1	1
	c__Actinopterygii	o__Perciformes	f__Percidae	*Gymnocephalus cernua*	Kaulbarsch	Species	2	2019		1	1	1
	c__Actinopterygii	o__Perciformes	f__Percidae	*Perca fluviatilis*	Egli	Species	1	2019		1	1	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Oncorhynchus mykiss*	Regenbogenforelle	Species	2	2019		1	1	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salmo carpio*	Forelle	Genus	7	2019		1	1	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Thymallus thymallus*	Aesche	Species	3	2019		1	1	1
Species additionally detected in NAWA sampling 2019 with eDNA												
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Chondrostoma soetta*	Savetta	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Leucaspius delineatus*	Moderlieschen	Species	1	2019		1	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Rutilus aula*	Triotto	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Squaliformes	f__Oxynotidae	*Squalius squalus*	Cavedano	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Perciformes	f__Percidae	*Sander lucioperca*	Zander	Species	1	2019		1	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Coregonus albelus*	Felche	Genus	4	2019		1	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salmo salar*	Atlantischer Lachs	Species	1	2019		1	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salvelinus fontinalis*	Bachsaibling	Species	1	2019		1	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Chondrostoma soetta*	Savetta	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Leucaspius delineatus*	Moderlieschen	Species	1	2019		1	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Rutilus aula*	Triotto	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Squaliformes	f__Oxynotidae	*Squalius squalus*	Cavedano	Species	1	2019	South CH	1	0	1
	c__Actinopterygii	o__Perciformes	f__Percidae	*Sander lucioperca*	Zander	Species	1	2019		1	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Coregonus albelus*	Felche	Genus	4	2019		1	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salmo salar*	Atlantischer Lachs	Species	1	2019		1	0	1
Species additionally detected in NAWA sampling 2019 with electro												
	c__Actinopterygii	o__Cypriniformes	f__Cobitidae	*Cobitus taenia*	Steinbeisser	Species		2019		0	1	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Carassius carassius*	Karausche/Schusterkarpfen	Species		2019		0	1	1
	c__Actinopterygii	o__Petromyzontiformes	f__Petromyzontidae	*Lampetra planeri*	Bachneunauge	Species		2019		0	1	1
	c__Actinopterygii	o__Siluriformes	f__Siluridae	*Silurus glanis*	Wels	Species		2019		0	1	1
Additional species in the historic inventory (CSCF database)							Nr. of observations					
	c__Actinopterygii	o__Perciformes	f__Centrarchidae	*Micropterus salmoides*	Forellenbarsch	Species	3	1997	excluded	0	0	1
	c__Actinopterygii	o__Perciformes	f__Cobitidae	*Misgurnus fossils*	Europäischer Schlammpeitzger	Species	1	1906	excluded	0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Aspius aspius*	Rapfen	Species	1	2003	excluded	0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Carassius auratus*	Oranda	Species	18	2018		0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Coregonus alpinus*	Felche	Genus	13	2011		0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Coregonus fatioi*	Felche	Genus	1	1895		0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Coregonus steinmanni*	Felche	Genus	4	2002		0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Ctenopharyngodon idella*	Graskarpfen	Species	3	2005	excluded	0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Parachondrostoma toxostoma*	Südwesteuropäsche Nase	Species	1	2021	excluded	0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Phoxinus parva*	Elritze	Genus	2	2009	excluded	0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Rutilus amarus*	Bitterling	Species	14	2018		0	0	1
	c__Actinopterygii	o__Cypriniformes	f__Cyprinidae	*Rutilus pigus*	Frauennerfling	Species	1	1987	excluded	0	0	1
	c__Actinopterygii	o__Gobiiformes	f__Gobiidae	*Padogobius bonelli*	Padanische Grundel	Species	1	2005	excluded	0	0	1
	c__Actinopterygii	o__Siluriformes	f__Ictaluridae	*Ameiurus melas*	Schwarzer Zwergwels	Species	1	2012	excluded	0	0	1
	c__Actinopterygii	o__Perciformes	f__Percidae	*Zingel asper*	Rhone-Streber	Species	1	2006	excluded	0	0	1
	c__Actinopterygii	o__Petromyzontiformes	f__Petromyzontidae	*Lampetra fluviatilis*	Flussneunauge	Species	1	1894	excluded	0	0	1
	c__Actinopterygii	o__Petromyzontiformes	f__Petromyzontidae	*Lampetra zanandreai*	Lombardey-Neunauge	Species	1	1871	excluded	0	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salmo rhodanensis*	Rhone-Forelle	Genus	10	2006		0	0	1
	c__Actinopterygii	o__Salmoniformes	f__Salmonidae	*Salvelinus umbla*	Seesaibling	Species	3	1997	excluded	0	0	1

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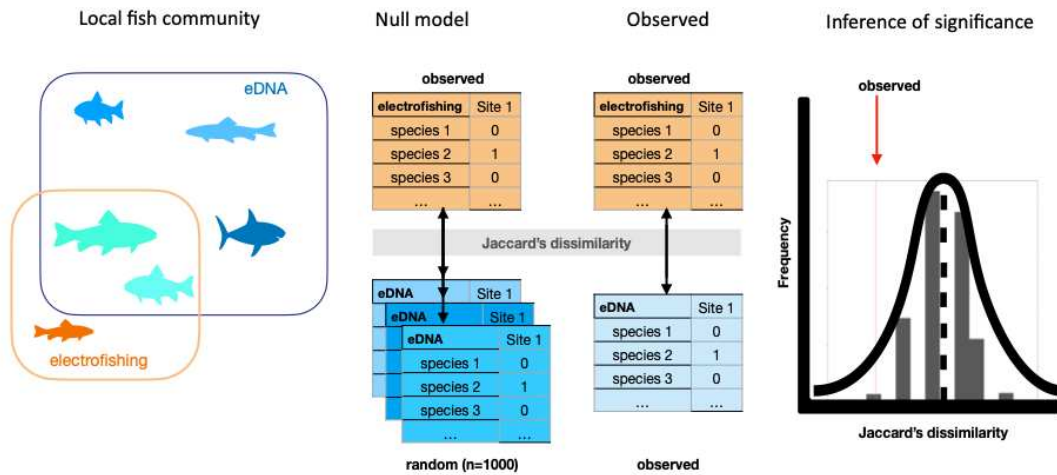
891 **Figure S1**: Conceptual workflow for the Null model of community dissimilarity. The baseline

892 data are the observed community data per site based on eDNA sampling and the

893 electrofishing approach. To test for a non-significant dissimilarity between the methods, we

894 first calculated the Jaccard's dissimilarity (i.e., total, turnover and nestedness components)

895 and between the observed electrofishing community at any given site and the corresponding

896 eDNA based community composition. To establish a Null Model, the eDNA based community

897 was then randomized according to the algorithm of Miklòs & Podani (2004) under the

898 constrains to keep the species frequency across all sites and to keep the species richness for

899 each site constant. From the randomized community composition of a site, the dissimilarity

900 was calculated similar to the observed values. By repeating this randomization 1000 times,

901 the probability of observing the real dissimilarity was then calculated directly from the

902 distribution of dissimilarity values. The workflow for the calculations of the eDNA vs. historic

903 records followed the same workflow.

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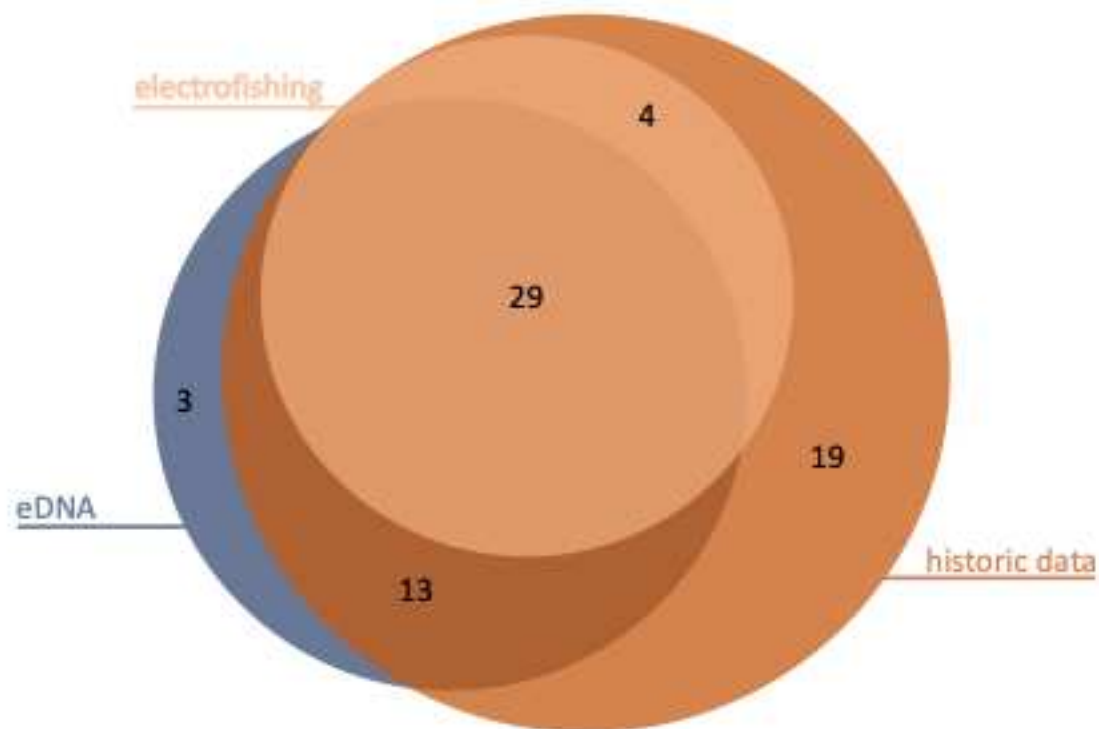
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907 **Figure S2:** Gamma diversity showing the number of fish species detected by historic records,

908 electrofishing and eDNA sampling. 29 species were detected by all three approaches. The

909 historic data contained 19 species that were not detected in either of the contemporary

910 sampling campaign. The electrofishing campaign detected 33 species that were completely

911 contained in the historic records. The eDNA method detected 3 species that were uniquely

912 found by eDNA (*Salvelinus profundus, Barbatual quinardii*, *Salvenilus namaycush*). The species

913 *S. profundus* is a lake species living in the deep of Lake Constance and detected in the adjacent

914 Rhine catchment. It was reported extinct from the Lake, but recently rediscovered in the

915 deeper layer. Also *S. namaycush* is a lake species that is originally from Northern America and

916 kept in fishing ponds in Switzerland which have an outlet into rivers. The species *B. quinardi*

917 is a small (average 7 cm) benthic fish mainly occurring in Southern Europe, however it is also

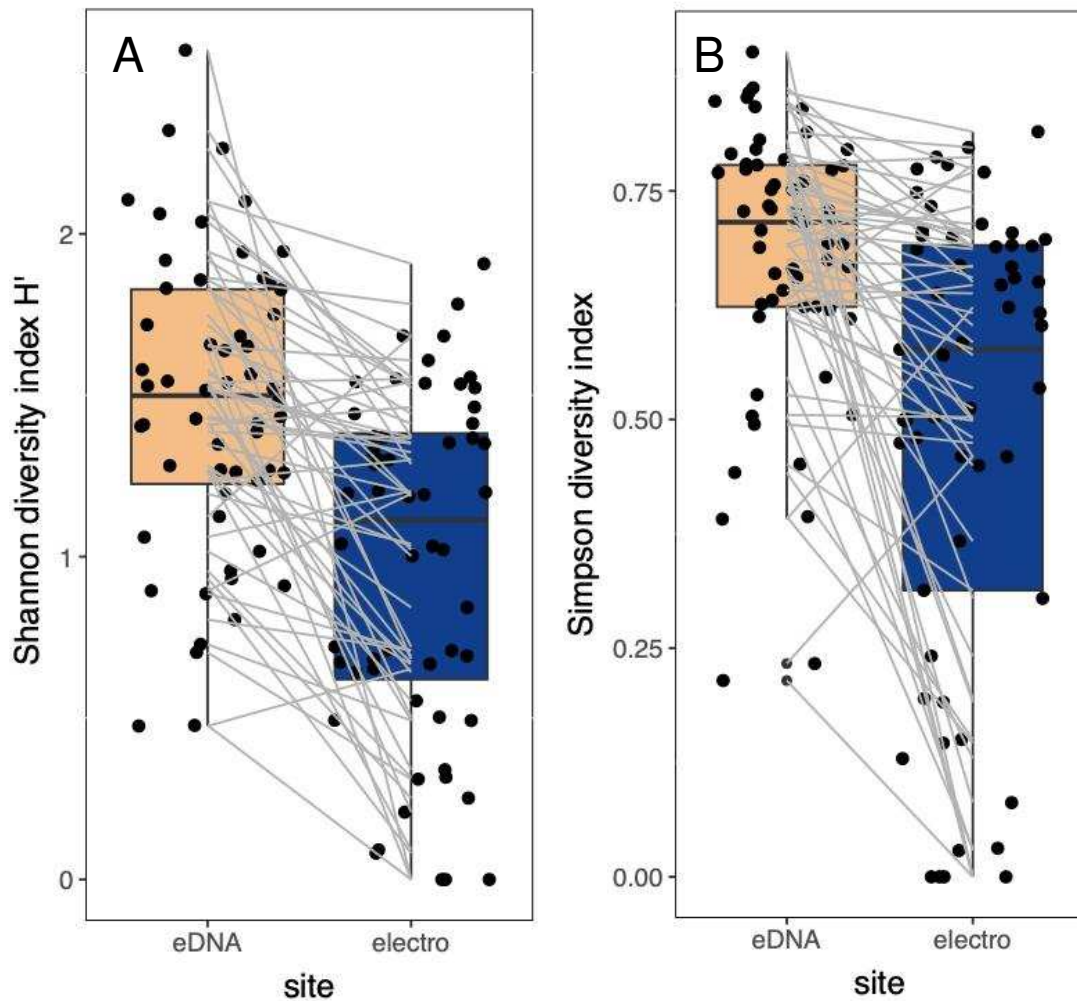918 recorded at the border to the Rhone catchment.

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926   **Figure S3:** Local diversity estimates, namely A) the Shannon diversity index and B) the Simpson

927   diversity index compared between eDNA (orange) and electrofishing (blue) that consider

928   relative abundances of species. The grey lines connect the two diversity values corresponding

929   to one sampling site. As for the observed richness, the sampling of eDNA vs electrofishing

930   (1.47 vs 0.98) was significantly different for the Shannon Index (t = 5.23, df = 110, p-value <

931   0.001) and for the Simpson Index (t = 4.69, df = 112, p-value < 0.001) with a mean value of

932   0.68 for eDNA, and a mean of 0.5 for electrofishing data.

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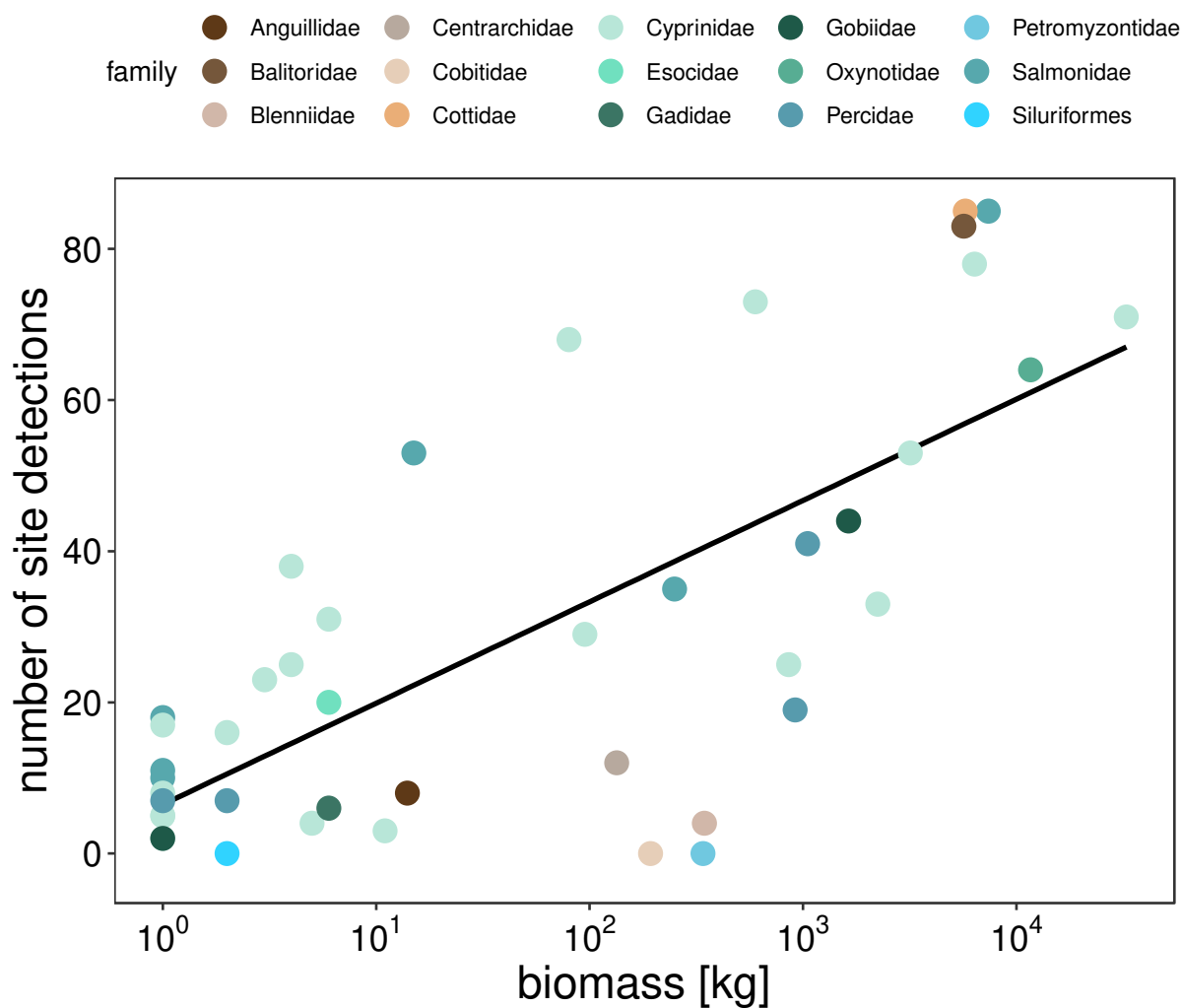
Figure S4: The presence-absence (number of detections) for a species over the overall biomass of this species. The biomass from the electrofishing catch was significantly correlated with the number of detections with eDNA for a species (formula: biomass ~ reads, F = 23.14, df = 41, R^2 = 0.27, p < 0.001).
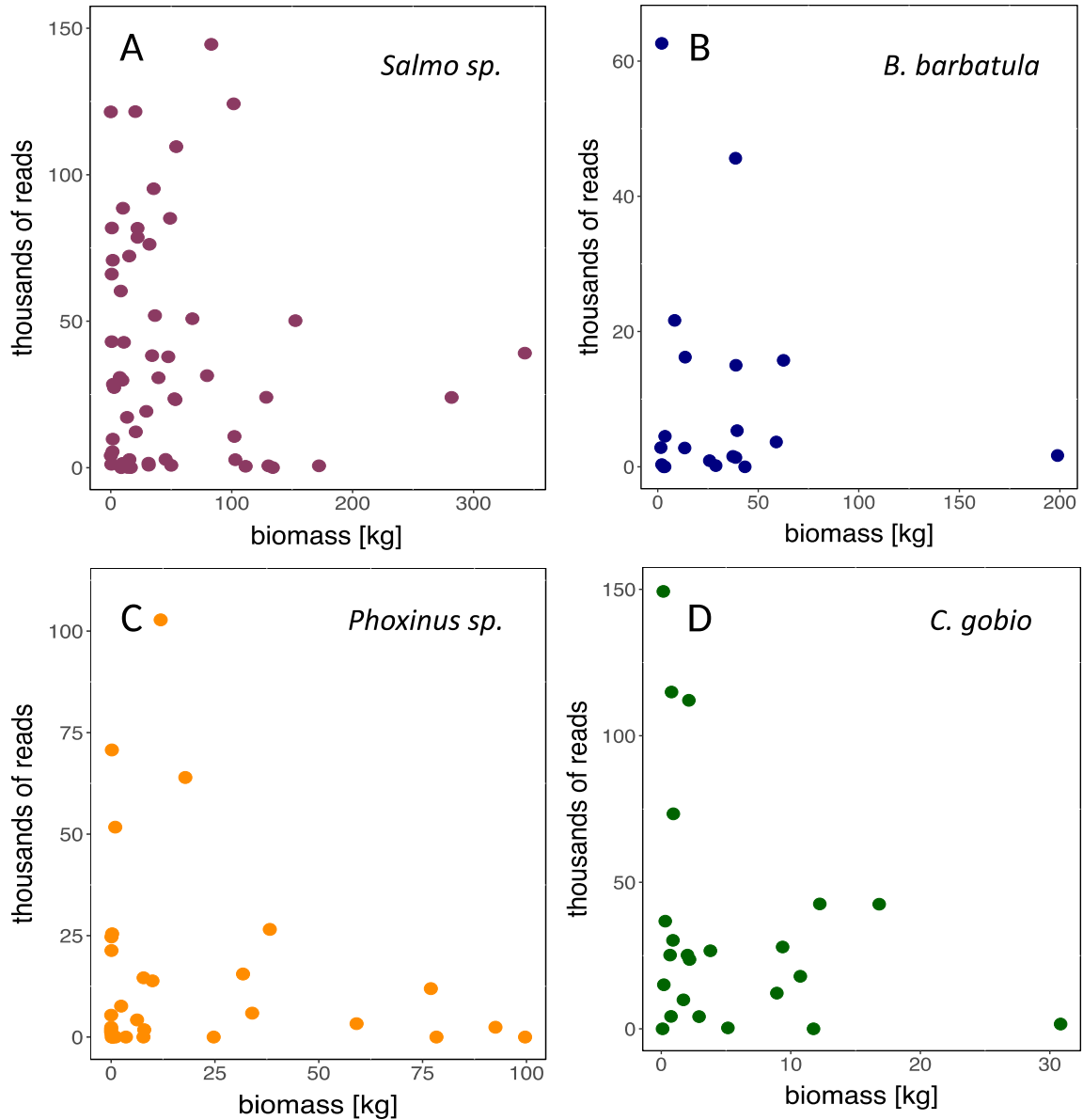
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Figure S5: These four examples of taxa showcase the relationship between the number of reads to the biomass on a site level (each point represents a site) for A) *Salmo* sp*.,* B) *B. barbatula*, C) *Phoxinus* sp*.,* and D) *C. gobio*. These species were commonly detected with both methods, electrofishing and eDNA, but when comparing the quantitative estimate at every site (biomass and reads, respectively), there was no significant relationship. The four examples here represent common species on the gamma diversity level, but even if a species showed similar proportions of biomass to reads on the gamma diversity level, like *B. barbatula*, the relationship did not hold up on the local level. A mixed effect model across all species showed that there was no correlation between reads and biomass (formula: biomass ~ reads + 1|site, F = 3.6, df = 182, R^2 = 0.13, p < 0.62).
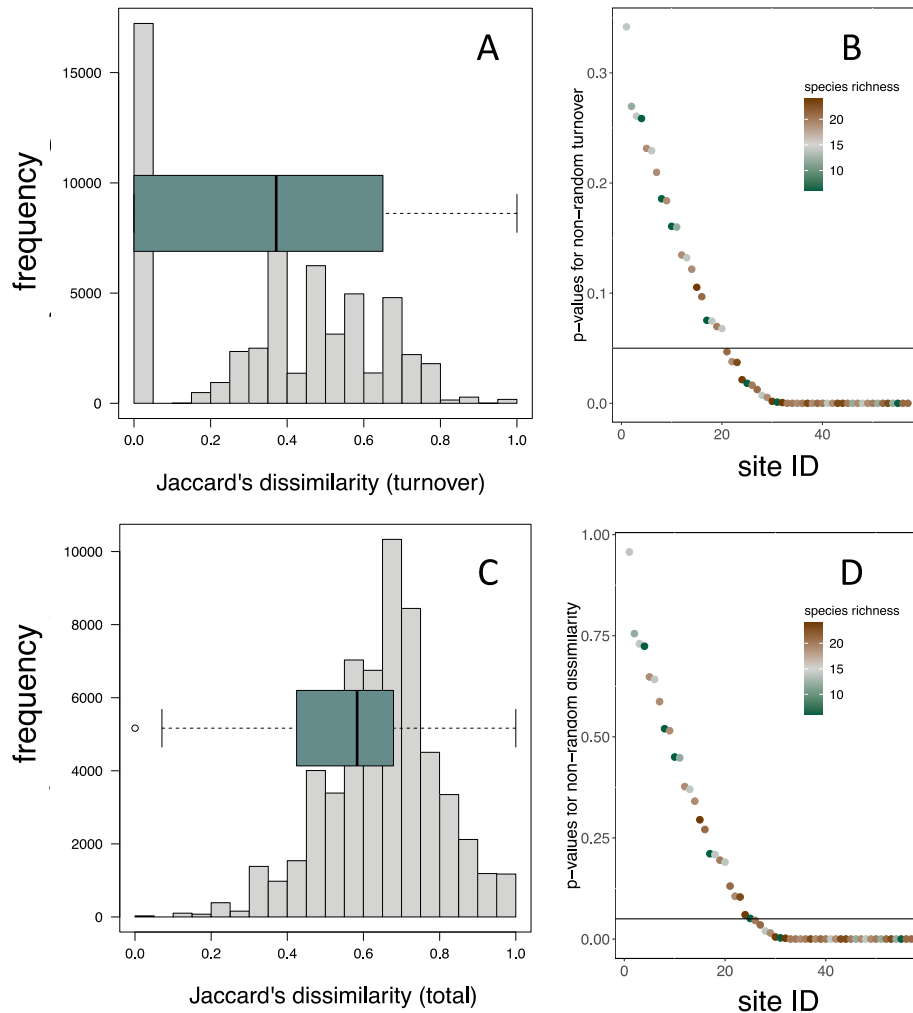
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Figure S6: Non-random dissimilarity between the monitoring data between electrofishing and eDNA based data. A) The distribution of random turnover values indicated in gray bars. The observed values range are indicated by the boxplot. B) The p-value distribution of all sites for the comparison of observed turnover vs. randomised values. One point shows the p-value of one site, coloured by the local richness detected by both methods. C) The distribution of random total dissimilarity based on Jaccard's index. D) The p-value distribution of all sites for the comparison of the total dissimilarity vs. randomized values.
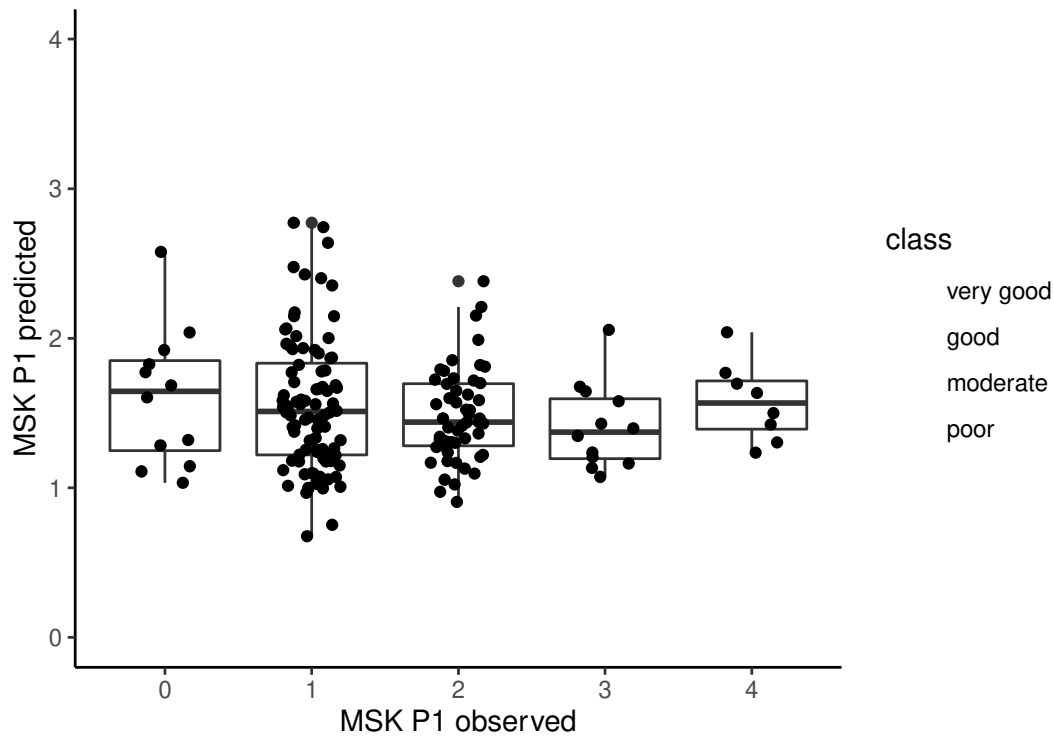
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Figure S7: Boxplots showing the biotic index scores predicted using a Random Forest Model from eDNA derived fish community data (on the y-axis) against the observed score of the biotic index (MSK 19) based on electrofishing data for all sampling sites. The predictions showed no significant differences between the classes (formula: predicted index ~ observed index, F = 0.001, df = 178, p-value = 0.85). The colored boxes indicate the different classes of the water quality at a river site. The index is composed of 4 parameters, where only P1 is based on the diversity of the local community therefore eDNA data that was implemented here only cover 1 out of 4 parameters, and the model gives poor predictions based on the 46 sites and 45 fish species available to us.