

1 **Environmental DNA metabarcoding provides enhanced detection of**  
2 **the European eel *Anguilla anguilla* and fish community structure in**  
3 **pumped river catchments**

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## 28 **Abstract**

29 The European eel *Anguilla anguilla* (eel hereafter) is critically endangered and has a  
30 catadromous lifecycle, which means adult eels that live in pumped catchments must  
31 pass through pumps during their downstream spawning migration. We are currently  
32 lacking detailed site-by-site eel distribution information in order to estimate the overall  
33 impact of individual pumping stations on eel escapement, and as such lack the data  
34 to enable informed prioritisation of pumping station management and targeted  
35 mitigation. In this study, we investigated whether environmental DNA (eDNA)  
36 metabarcoding can provide increased detection sensitivity for eel and fish community  
37 structure in highly regulated pumped catchments, when compared directly to current  
38 standard practice fish survey protocols (seine netting/electric fishing). Eels were  
39 detected in 14/17 sites (82.4%) using eDNA metabarcoding in contrast to 3/17 (17.6%)  
40 using traditional catch methods. Additionally, when using eDNA monitoring species  
41 richness was higher in 16/17 sites (94.1%) and site occupancy  $\geq$  traditional methods  
42 for 23/26 of the fish species detected (88.5%). While eDNA methods presented  
43 significantly higher average species richness and species site occupancy overall,  
44 eDNA and Catch methods were positively correlated in terms of species richness and  
45 site occupancy. We therefore found that eDNA metabarcoding was a high sensitivity  
46 method for detecting eels in pumped catchments, while also increasing the detection  
47 of overall fish community structure compared to traditional catch methods. In addition,  
48 we highlight how eDNA monitoring is especially suited to increased detection of  
49 particular species, with traditional methods sufficient for others. This high sensitivity,  
50 coupled with the ability to sample multiple sites in a short time frame suggests eDNA  
51 metabarcoding could be an invaluable tool when prioritising pumping station  
52 management.

53 **KEYWORDS**

54 Catadromous migration, Fish community, Habitat fragmentation, Lowland rivers,  
55 Prioritisation, Pumping stations

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## 71 1 | INTRODUCTION

72 The European eel (*Anguilla anguilla*) is a critically endangered catadromous fish  
73 species which has faced significant declines in recent decades (Bilotta *et al.*, 2011;  
74 Jacoby & Gollock, 2014; Podgorniak *et al.*, 2016; Correia *et al.*, 2018). This marked  
75 decline has resulted in specific EU legislation, requiring member states to adopt eel  
76 management plans (The EC Eel Regulation (1100/2007)). These regulations aim to  
77 promote recovery by allowing >40% of the historic eel biomass prior to anthropogenic  
78 impacts passage from inland waters to the sea to facilitate spawning activity (Aalto *et*  
79 *al.*, 2016). Despite these measures, The International Council for the Exploration of  
80 the Sea (ICES) Working Group on Eels (WGEEL) reports that current *A. anguilla*  
81 recruitment remains consistently <2% in recent years, with recruitment at 1.9% and  
82 1.4% in 2018 and 2019 respectively (ICES, 2019). Such declines, at least in part, are  
83 a consequence of anthropogenic impacts on rivers - the focus here being societal  
84 reliance on land-drainage pumping stations for water level management (Solomon &  
85 Wright, 2012; Buysse *et al.*, 2014, 2015; Bolland *et al.*, 2019). These structures  
86 operate by pumping water against the natural gradient to a higher downstream river  
87 elevation, regulating river levels in the upstream catchment. This is a requirement in  
88 many areas across the world to enable flood management, agricultural water supply,  
89 and navigation (Solomon & Wright, 2012; Bolland *et al.*, 2019; ICES, 2019). The  
90 overall ecological impacts of operating pumps are not fully understood however, and  
91 only recently have concerns regarding their adverse impacts on eels and whole fish  
92 communities been highlighted (Solomon & Wright, 2012; Buysse *et al.*, 2014, 2015;  
93 Bolland *et al.*, 2019).

94 The ecology of *A. anguilla* makes this species particularly vulnerable to adverse  
95 impacts at pumping stations. As a catadromous species, *A. anguilla* must undertake  
96 two transatlantic migrations between their European inland/estuarine occupancy  
97 range and spawning grounds located in the Sargasso sea (Bonhommeau *et al.*, 2008;  
98 Podgorniak *et al.*, 2016; Correia *et al.*, 2018). This life cycle means that in pumped  
99 catchments, mature eels must pass through pumps in order to achieve escapement  
100 and spawn. It is the necessity to pass through pumps, in addition to their elongated  
101 morphology that makes *A. anguilla* especially susceptible to entrainment at pumping  
102 stations (Buysse *et al.*, 2014; Bolland *et al.*, 2019). Buysse *et al.* (2014) found that  
103 mortality rates were  $97 \pm 5\%$  for a propeller pump,  $17 \pm 7\%$  for a large Archimedes  
104 screw pump, and  $19 \pm 11\%$  for a small Archimedes screw pump respectively -  
105 indicating that mortality rates differ between pump types. However, Bolland *et al.*  
106 (2019) highlighted the importance of accounting for indirect impacts such as reduced  
107 fitness, delayed migration and increased predation when coming into contact with  
108 pumps, which may prevent successful spawning. ICES (2019) provided a first estimate  
109 of the total loss of eel biomass attributed to hydropower and land-drainage pumps,  
110 estimated at 444.4 tonnes per year in the UK alone, with a 'minimum estimate' of  
111 1625.8 tonnes across Europe. While these figures suggest clear adverse impacts of  
112 pumping, given the increased likelihood of flood events predicted under future climate  
113 change scenarios (Team *et al.*, 2014), we will likely be increasingly reliant on these  
114 pumps for land drainage in the coming years. To mitigate this, highly efficient, non-  
115 delayed and safe downstream eel passage routes must be provided for pumped  
116 catchments that contain *A. anguilla*.

117 Pumping stations regulate rivers where *A. anguilla* were undoubtedly once present but  
118 flood risk management infrastructure (flood banks / levees, pipework and pumps) can

119 present a complete barrier to upstream migrating eels. We are currently lacking  
120 detailed site-by-site fish community information required to estimate the overall impact  
121 of individual structures on eel spawning escapement, and as such lack the data to  
122 enable informed prioritisation of pumping station management and perform targeted  
123 mitigation (ICES, 2019). Knowledge of the eel distribution and fish community present  
124 at these sites is therefore valuable to water managers (Solomon & Wright, 2012), yet  
125 due to sampling difficulties the probability of detecting rare and elusive species using  
126 traditional methods is low - particularly in large river systems (Pont *et al.*, 2018).  
127 However, numerous studies in freshwater habitats have demonstrated that  
128 environmental DNA (eDNA) monitoring methods can achieve higher detection  
129 sensitivity than traditional monitoring techniques (Hänfling *et al.*, 2016; Pont *et al.*,  
130 2018; Strickland & Roberts, 2018; Itakura *et al.*, 2019; McDevitt *et al.*, 2019). These  
131 molecular monitoring methods are ideal for detecting species with patchy distributions  
132 or low abundances, often overlooked by traditional catch methods (Turner *et al.*,  
133 2015). Recent developments mean that PCR-based metabarcoding of eDNA is now  
134 considered a powerful tool for monitoring entire ecological communities (Deiner *et al.*  
135 2017; Hering *et al.* 2018), enabling vast amounts of data acquisition from a single  
136 sampling visit. In addition, it has been reported that eDNA is applicable to river  
137 systems, yielding higher detection rates, less sensitivity to sampling conditions and  
138 increased efficiency (Pont *et al.*, 2018; Strickland & Roberts, 2018). This suggests  
139 eDNA could be a useful tool for screening species composition in pumped catchments  
140 – enabling multiple sites to be screened in a single survey.

141 In this study, we investigated whether eDNA metabarcoding (Hänfling *et al.*, 2016;  
142 Bylemans *et al.*, 2018; Pont *et al.*, 2018; Li *et al.*, 2019) can be used as a tool to monitor  
143 eel and fish presence in pumped catchments. While the application of species-specific

144 qPCR methods may reduce the likelihood of false negatives by avoiding “species  
145 masking” effects (Harper *et al.* 2018), the holistic understanding that is provided by  
146 eDNA metabarcoding is integral to better inform management decisions (Ruppert *et*  
147 *al.* 2019). If successful, eDNA metabarcoding could be applied to enable evidence-  
148 based management of pumping stations, facilitating the attainment of policy-based  
149 objectives and conservation targets going forwards. However, it is important that this  
150 method is validated in such fragmented lotic systems with highly regulated catchments  
151 and flows. Therefore here, eDNA metabarcoding data are directly compared to data  
152 from standard practice traditional fish capture methods (seine netting/electrofishing)  
153 gathered from the same sites in the same year. We hypothesised that eDNA  
154 metabarcoding will enable increased detection sensitivity for our target species *A.*  
155 *anguilla*, while also increasing our coverage of whole fish communities (indicated by  
156 species richness and species site occupancy). Furthermore, we expected eDNA and  
157 Catch methods would be positively correlated for species richness and species site  
158 occupancy, indicating agreement between the methods, and thus continuity in regard  
159 to decision making.

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## 166 **2 | MATERIALS AND METHODS**

### 167 **2.1 | Study sites**

168 This study was carried out in a low lying and heavily pumped section of the Fens, UK  
169 (Figure 1). Our study system 'The Middle Level' is a complex network of heavily  
170 pumped waterways and drainage ditches. Due to peat shrinkage caused by historic  
171 land drainage, ground levels have continued to sink meaning that there is no longer a  
172 possibility for gravity drainage at the 70+ pumping stations required to drain the area  
173 (Solomon and Wright 2012). Thus, all water transfer from this catchment to the sea is  
174 pumped, often passing through multiple pumping stations as it is moved through the  
175 system. There is little/no natural flow in the absence of pumping, meaning this system  
176 transitions between a lentic and lotic state temporally. The 17 study sites had a routine  
177 fish survey (performed by the Environment Agency between 07/04/2017 and  
178 25/07/2017; seine netting = 15, electric fishing = 2 (Table 2, Supporting information))  
179 and an eDNA survey (collected between 23/10/2017 and 28/11/2017; 5 water samples  
180 at each site (plus field blank)) carried out at identical points, allowing a direct  
181 comparison between methods that same year. The eDNA sampling was carried out  
182 later to enable the catchment to return to baseline conditions following the traditional  
183 survey season, and to ensure eDNA detection was not biased by an influx of glass eel  
184 recruitment which peaks between March and June in the region (Kroes *et al.* 2020).

185

### 186 **2.2 | Water sampling**

187 Five 2L surface water samples were taken at each site using sterile Gosselin™ HDPE  
188 plastic bottles (Fisher Scientific UK Ltd, UK). Each 2L sample consisted of 5 x 400ml



189 sub-samples taken a few metres apart to account for the stochastic distribution of  
190 eDNA. Samples were taken by hand from a small inflatable boat, sterile gloves were  
191 worn by the sampler and changed between samples, the boat, oars, and waders were  
192 cleaned with bleach (10%), rinsed, then sprayed with Virkon (Antec International)  
193 between sites in order to prevent cross-site contamination. Samples were taken  
194 starting at the downstream end, then working upstream in 150m intervals with the mid-  
195 point being the national grid reference where traditional catch surveys were  
196 conducted. For each site, a 2L field blank (purified water) was included and handled  
197 alongside eDNA water samples to monitor for contamination.

198

199 Upon collection, water samples were stored on ice in a bleach-sterilised cool box  
200 during transit and taken back to our dedicated eDNA facility at the University of Hull  
201 for filtration. All samples and blanks were vacuum-filtered within 24 hours of collection.  
202 All surfaces and equipment were sterilised using 10% v/v chlorine-based commercial  
203 bleach solution (Elliott Hygiene Ltd, UK). Filtration equipment was immersed in 10%  
204 bleach for 10 minutes, soaked in 5% v/v MicroSol detergent (Anachem, UK) for an  
205 additional 10 minutes, then rinsed thoroughly with purified water between filtration  
206 runs. Whenever possible, the full 2L of water was vacuum-filtered through sterile  
207 0.45µm cellulose nitrate membrane filters with pads (47mm diameter; Whatman, GE  
208 Healthcare, UK) using Nalgene filtration units - with 2 filters per sample to reduce filter  
209 clogging. Filters were then removed from units using sterile tweezers, placed in sterile  
210 50mm petri dishes (Fisher Scientific UK Ltd, UK), sealed with parafilm (Sigma-  
211 Aldrich®, UK), and stored at -20°C until extraction.

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### 213 **2.3 | DNA extraction**

214 DNA was extracted from filters at the University of Hull dedicated eDNA facility in a  
215 designated sterile extraction area using the DNeasy PowerWater Kit (QIAGEN,  
216 Germany) following the manufacturer's protocol. The duplicate filters from each  
217 sample were co-extracted by placing both filters back-to-back in a single tube for bead  
218 milling. Following extraction, the eluted DNA extracts (100µL) were quantified on a  
219 Qubit™ 3.0 fluorometer using a Qubit™ dsDNA HS Assay Kit (Invitrogen, UK) to  
220 confirm DNA was successfully isolated, then stored at -20°C.

221

### 222 **2.4 | eDNA metabarcoding**

223 The eDNA library preparation and metabarcoding workflow applied here follows that  
224 outlined in Harper *et al.* (2019), but with the following modifications: the first PCR used  
225 2µL of template DNA, 7µL of ddH<sub>2</sub>O, and 0.5µL of BSA (Q5 2x High Fidelity Master  
226 Mix and primer volumes remained unchanged). The second PCR used 4µL of template  
227 DNA and 15µL of ddH<sub>2</sub>O (Q5 2x High Fidelity Master Mix and primer volumes  
228 remained unchanged). The second PCR thermocycling profile was also adapted as  
229 follows: 95°C for 3 mins, 10 cycles of 98°C for 20s and 72°C for 1 min, followed by a  
230 final elongation step at 72°C for 5 mins. The workflow is summarised below:

231 Nested metabarcoding using a two-step PCR protocol was performed, using multiplex  
232 identification (MID) tags in the first and second PCR step to enable sample  
233 identification as described in Kitson *et al.* (2019). The first PCR was performed in  
234 triplicate (i.e. 3x PCR replicates), amplifying a 106bp fragment using published 12S  
235 ribosomal RNA (rRNA) primers 12S-V5-F (5'-ACTGGGATTAGATACCCC-3') and

236 12S-V5-R (5'-TAGAACAGGCTCCTCTAG-3') (Kelly *et al.* 2014; Riaz *et al.* 2011).  
237 These selected primers have been previously validated, in silico, in vitro and in situ for  
238 UK freshwater fish species showing that all UK freshwater species can be detected  
239 reliably with the exceptions of distinctions between: *Lampetra planeri* / *Lampetra*  
240 *fluviatilis*, *Perca fluviatilis* / *Sander lucioperca*, three species of Asian carp  
241 (*Hypophthalmichthys nobilis*, *H. molitrix*, *Ctenopharyngodon idella*), and species  
242 within the genera *Salvelinus* and *Coregonus* (Hänfling *et al.* 2016). In our study,  
243 Lamprey were therefore assigned only to genus level, and Percidae assumed to be *P.*  
244 *fluviatilis*. PCR negative controls (MGW) were used throughout, as were positive  
245 controls using DNA (0.05ng/ $\mu$ L) from the non-native cichlid *Maylandia zebra*. The three  
246 replicates from the first PCR were pooled to create sub-libraries and purified with  
247 MagBIND® RxnPure Plus magnetic beads (Omega Bio-tek Inc., GA, USA), following  
248 a double size selection protocol (Quail *et al.*, 2009). Based on the ratios outlined in  
249 Harper *et al.* (2019), ratios of 0.9 $\times$  and 0.15 $\times$  magnetic beads to 100 $\mu$ L of amplified  
250 DNA from each sub-library were used. Following this, a second shuttle PCR was  
251 performed on the cleaned product to bind Illumina adapters to the sub-libraries. A  
252 second purification was then carried out on the PCR products with Mag-BIND®  
253 RxnPure Plus magnetic beads (Omega Bio-tek Inc., GA, USA). Ratios of 0.7 $\times$  and  
254 0.15 $\times$  magnetic beads to 50 $\mu$ L of each sub-library were used. Eluted DNA was then  
255 refrigerated at 4°C until quantification and normalisation. Once pooled, the final library  
256 was then purified again (following the same protocol as the second clean-up),  
257 quantified by qPCR using the NEBNext® Library Quant Kit for Illumina® (New England  
258 Biolabs® Inc., MA, USA), and verified for fragment size (318bp) and purity using an  
259 Agilent 2200 TapeStation with High Sensitivity D1000 ScreenTape (Agilent  
260 Technologies, CA, USA). Once verified, the library was loaded (mixed with 10% PhiX)

261 and sequenced on an Illumina MiSeq® using a MiSeq Reagent Kit v3 (600-cycle)  
262 (Illumina, Inc., CA, USA) at the University of Hull. Raw sequence output was  
263 demultiplexed using a custom Python script, and our in-house bioinformatics pipeline  
264 metaBEAT v0.97.13 (<https://github.com/HullUni-bioinformatics/metaBEAT>) was used  
265 for quality trimming, merging, chimera removal, clustering, and taxonomic assignment  
266 of sequences against our curated UK fish reference database (Hänfling *et al.* 2016).  
267 Taxonomic assignment here used a lowest common ancestor approach based on  
268 BLAST matches that matched our reference database with minimum identity set at  
269 98%.

270

## 271 **2.5 | Data analysis**

272 During downstream analysis, data were analysed and visualised using R Version 3.6.3  
273 (R Core Team, 2020). Reads assigned to family and genera containing only a single  
274 UK species were manually reassigned and merged with that species. In order to  
275 reduce the likelihood of eDNA false positives, blanks were used throughout and a low-  
276 read frequency threshold applied. All field/filtration blanks, PCR negative, and PCR  
277 positive controls were negative for *A. anguilla*, and the threshold applied at 0.001 to  
278 remove any reads making up less than 0.1% of total reads as previously applied with  
279 this 12S marker (Hänfling *et al.* 2016; Handley *et al.* 2019).

280 Samples of interest for this study (N=85) were then subset, and the mean number of  
281 reads for each site (N=17) calculated based on the five samples per site. Initially, data  
282 from traditional catch surveys were converted into 'percentage catch' for each species  
283 at each site. This enabled direct visual comparisons of relative abundance between  
284 methods, based on 'percentage reads' and 'percentage catch', visualised as bubble

285 plots using ggplot2 3.2.0 (Wickham, 2016). Differences between survey methods were  
286 then compared statistically, based on the species richness and species occupancy  
287 obtained. Richness and Occupancy data were screened for normality, in order to meet  
288 assumptions a paired t-test was applied to species richness data, and a paired  
289 Wilcoxon signed rank test to species site occupancy data to test for significant  
290 differences between survey methods (McDonald, 2014). Correlations between eDNA  
291 and traditional catch methods were then tested for using Pearson's and Spearman's  
292 tests, for richness and site occupancy respectively (McDonald, 2014), and visualised  
293 using ggpubr (Kassambara, 2020).

294

## 295 **3 | RESULTS**

### 296 **3.1 | Eel distribution**

297 The total average eDNA reads across all study sites was 842,159, of which 8,830  
298 (1.05%) were assigned to *A. anguilla*. The total number of individual fish caught using  
299 traditional catch methods across study sites was 14,102, of which 3 (0.02%) were *A.*  
300 *anguilla*. This comparison of survey methods shows that eDNA metabarcoding yielded  
301 an overall higher detection rate for *A. anguilla* than traditional catch methods. The  
302 eDNA metabarcoding approach detected *A. anguilla* in 14/17 (82.4%) of the sites  
303 surveyed, whereas traditional methods captured *A. anguilla* in 3/17 (17.6%) of sites  
304 (Figure 1). Both approaches tested positive for *A. anguilla* in three sites (7, 21 and 23),  
305 and both negative for another three sites (2, 3, and 17); agreement between methods  
306 in 6/17 sites (35.3%). The other 11/17 (64.7%) sites however did not agree, with  
307 traditional methods capturing no *A. anguilla* in sites where eDNA surveys tested  
308 positive.

### 309 **3.2 | Species composition**

310 Overall, 26 fish species were detected in this study; eDNA metabarcoding detected  
311 25/26 fish species (96.2%) across the 17 study sites, whereas traditional methods  
312 captured 16/26 fish species (61.5%). Of the 26 species detected, 15 (57.7%) were  
313 detected in both eDNA and traditional catch methods, whereas 10 (38.5%) were  
314 detected only using eDNA and one (3.85%) species was detected only using  
315 traditional capture methods (Figure 2). There were visual consistencies between  
316 species with higher % Reads (eDNA) and % Catch (traditional methods), including  
317 *Abramis brama*, *Esox lucius*, *Perca fluviatilis* and *Rutilus rutilus* (Figure 2).

318

### 319 **3.3 | Species richness**

320 eDNA metabarcoding yielded a higher species richness than traditional catch methods  
321 in 16/17 sites, with the exception being site 1. Additionally, eDNA (M=11.06) reported  
322 significantly higher mean species richness than traditional methods (M=7.47) (paired  
323 t-test;  $t = -5.0355$ ,  $df = 16$ ,  $p = 0.0001$ ) (Figure 3a). While a Pearson's product-moment  
324 correlation test showed the two methods were significantly correlated ( $R = 0.6$ ,  $p =$   
325  $0.011$ ) (Figure 3b).

326

### 327 **3.4 | Species site occupancy**

328 eDNA metabarcoding reported a site occupancy  $\geq$  traditional catch methods for 23/26  
329 of the fish species detected, with *Alburnus alburnus*, *Rhodeus amarus* and *Gobio*  
330 *gobio* being the exceptions (Table 1). Additionally, eDNA (M=7.23) reported

331 significantly higher mean site occupancy than traditional methods ( $M=4.88$ ) (paired  
332 Wilcoxon test;  $V = 29.5$ ,  $p = 0.001607$ ) (Figure 4a). While a Spearman's rank  
333 correlation test showed the two methods were significantly correlated ( $R = 0.76$ ,  $p =$   
334  $5.762e-06$ ) (Figure 4b).

335

## 336 **4 | DISCUSSION**

337 To our knowledge, this study is the first to validate eDNA metabarcoding of fish  
338 communities specifically within heavily regulated pumped river catchments. We found  
339 that eDNA metabarcoding consistently outperformed standard practice methods, in  
340 terms of increased detection for our target species *A. anguilla* and enhancing fish  
341 community structure knowledge at our study sites, while revealing presence of  
342 additional elusive species. Here, we discuss these findings and what they mean for  
343 eDNA as a tool for stakeholders to inform management decisions in pumped river  
344 catchments.

345

### 346 **4.1 | Detecting *A. anguilla* in pumped catchments**

347 When considering pumping station management *A. anguilla* is a key species given  
348 they are critically endangered and have specific legislation to protect them from  
349 human-mediated activities (Council Regulation (EC) No. 1100/2007) (Buysse *et al.*,  
350 2014, 2015; Bolland *et al.*, 2019). In this study, all three sites where traditional methods  
351 captured *A. anguilla* had positive eDNA signals, while the three eDNA negative sites  
352 were also negative for traditional catch methods. Most notably, eDNA metabarcoding  
353 detected *A. anguilla* at 11 additional sites where traditional capture methods did not.

354 We therefore conclude that eDNA metabarcoding was more sensitive for detecting *A.*  
355 *anguilla* in managed pumped catchments than traditional methods. This was not  
356 unexpected, given the documented challenges in sampling eels from aquatic  
357 environments using seine netting and electric fishing (Naismith & Knights, 1990;  
358 Degerman *et al.*, 2019). Similarly, a recent study by Itakura *et al.* (2019) found that  
359 single species qPCR based eDNA monitoring had a greater detection sensitivity for  
360 the Japanese eel *Anguilla japonica* than electrofishing in rivers in Japan. One  
361 consideration in our study however, is that silver eel migration generally begins in  
362 autumn when water temperature decreases (Acou *et al.* 2008), this corresponds with  
363 our eDNA sampling and could potentially enhance detection as these mature eels  
364 migrate downstream. Based on our results, we recommend eDNA metabarcoding as  
365 a complimentary/alternative method to traditional catch when eel presence/absence  
366 data is required for informing management decisions in pumped catchments.

#### 367 **4.2 | Fish community detection in pumped catchments**

368 While the priority species for this study was *A. anguilla*, the composition and structure  
369 of the fish community as a whole remains an important factor in management decision  
370 making and is of interest to wider stakeholders (Solomon & Wright, 2012; Pont *et al.*,  
371 2019). It is therefore important that eDNA monitoring is able to produce data on these  
372 should it be implemented as a standardised monitoring framework.

373 We observed that eDNA methods were able to detect significantly more species in  
374 pumped catchments than standard practice catch methods, as indicated by the total  
375 number of species detected (25 and 16, respectively) and increased species richness  
376 at individual sites. Previous studies have clearly shown that eDNA metabarcoding is a  
377 highly sensitive method for detection of freshwater fish which outperforms traditional



378 survey techniques in lentic environments (Hänfling *et al.*, 2016; Handley *et al.*, 2019;  
379 Li *et al.*, 2019). This study adds to the mounting evidence that this is also true in lotic  
380 environments with unregulated (Bylemans *et al.*, 2018; Pont *et al.*, 2018) and regulated  
381 flows (McDevitt *et al.*, 2019). Despite the underestimation of true species richness in  
382 traditional surveys, when compared to eDNA metabarcoding there was a strong  
383 positive correlation between both methods, and thus the relative importance of sites  
384 based on species richness for both methods were related.

385 Of course, not all species are equally weighted when it comes to making management  
386 decisions (Solomon & Wright, 2012; Nunn *et al.*, 2014; Beng & Corlett, 2020;  
387 Sepulveda *et al.*, 2020), and so it is important to consider the potential for any method  
388 biases (preferential detections or underrepresented species). We found that overall  
389 eDNA had significantly higher mean site occupancy than traditional methods, and thus  
390 was more sensitive to individual species detections, while site occupancy was  
391 positively correlated between methods. Here we observed that species missed by  
392 catch methods were low in percentage reads and site occupancy using eDNA methods  
393 (Figure 2), suggesting low abundance species may be overlooked by catch methods,  
394 whereas agreement between methods is higher for abundant species (Figure 4b). This  
395 increased sensitivity could enable a more targeted focus to conservation species  
396 management, enabling increased detection of priority species such as *Cobitis Taenia*  
397 notable in our study (Nunn *et al.*, 2014). However, while eDNA site occupancy was  $\geq$   
398 traditional methods for 23/26 of the fish species detected. Two species *A. alburnus*  
399 and *R. amarus* had a higher detection rate using traditional methods, while *G. gobio*  
400 was not detected using eDNA metabarcoding at all, despite being identified on two  
401 occasions using traditional methods. While this could be due to the morphological  
402 identification bias of traditional surveys (Li *et al.*, 2019), the influences of reference

403 databases and species ecology should be considered with eDNA metabarcoding in  
404 such instances (Bylemans *et al.*, 2018). In our case primers and reference databases  
405 had been previously validated showing that this species can be reliably detected  
406 (Hänfling *et al.* 2016). Furthermore, while *G.gobio* was not detected in this study, it  
407 was present in other samples in this workflow which were not part of this study. This  
408 suggests species ecology, sampling conditions, or misidentification from catch surveys  
409 as possible explanations. Previous comparisons of detection rates from eDNA and  
410 traditional surveys have found that species detectability increases for both methods  
411 based on the density of target organisms (Hering *et al.* 2018). However, it must be  
412 considered that detectability remains imperfect for both methods. While the detection  
413 rate in our study was on average higher for eDNA this is not true for all species. We  
414 observed increased stochasticity for rare species with lower detection rates across  
415 methods, meaning that some species may genuinely have higher detection rates with  
416 conventional survey designs, or may be overlooked by both methods. It has been  
417 noted in the literature for example, that traditional surveys are prone to over-estimation  
418 of sub-surface species, and under-estimation of benthic and rare species when  
419 compared to eDNA (Pont *et al.* 2019). This could explain why eDNA was more  
420 sensitive for *A.anguilla* in our study, while traditional catch methods yielded higher  
421 detection for *A.alburnus*. Here, we highlight the importance of understanding potential  
422 limitations or biases of species detectability, prior to using any type of monitoring data  
423 to inform management decisions.

424

425 In order to enable direct comparisons between methods we used naïve occupancy,  
426 which does not account for imperfect detection (Ficetola *et al.* 2015). This can lead to

427 underestimation of species distribution if replication is low (Sutter and Kinziger 2019),  
428 yet risk false positives if replication is large and false positive rate is high. While a  
429 conservative approach to occupancy can reduce false positives, many true positives  
430 are also discarded (Ficetola *et al.* 2015). Our study therefore applied a low-frequency  
431 threshold to reduce eDNA false positive rates, rather than use a conservative  
432 approach to occupancy and risk overlooking rare and elusive species. Based on this  
433 and our blanks, the potential for unfiltered false positives in our data is reduced.  
434 However, false positives due to environmental contamination remain a potential  
435 source of error which could be reflected in species detected in single sites. In our study  
436 these included *Carassius auratus*, *Leuciscus idus*, and *Oncorhynchus mykiss*; the  
437 species with only a single positive eDNA sample. These species are all considered  
438 non-native but introduced to the UK (Table 1), but this does not mean we can rule out  
439 environmental contamination, and as such decision-support schematics should  
440 account for such scenarios based on local management plans (Sepulveda *et al.* 2020).

441

### 442 **4.3 | Conclusions**

443 While eDNA has been well validated in lentic systems (Hänfling *et al.*, 2016; Handley  
444 *et al.*, 2019; Li *et al.*, 2019), it is acknowledged that downstream transportation of  
445 eDNA and fluctuations in river flow can influence eDNA detection and spatial  
446 resolution in lotic systems (Turner *et al.*, 2015; Bylemans *et al.*, 2018; Pont *et al.*, 2018;  
447 Itakura *et al.*, 2019; Milhau *et al.*, 2019; Laporte *et al.*, 2020). Pumped catchments are  
448 prone to binary fluctuations in flow, changing from a lentic to a lotic water body with  
449 pump operation to regulate river level (Solomon & Wright, 2012; Buysse *et al.*, 2014;  
450 Bolland *et al.*, 2019). As pump operation, in most instances, is influenced by rainfall,

451 there are likely seasonal trends to consider as well as smaller scale temporal variation  
452 in pumping regimes with currently unknown influences on species detectability. It may  
453 be that pump operation acts as a conveyor belt for eDNA diversity, as described in  
454 (Deiner *et al.*, 2016), and sampling at a pumping station after a pumping event can  
455 reduce eDNA stochasticity and reduce the number of spatial replicates required.  
456 Alternatively, small pumped catchments may be diluted by heavy rainfall events and  
457 pump operation flushing the system, reducing the detectability of prevailing fish. A  
458 study by Shogren *et al.* (2017) however, investigated the impact of eDNA transport in  
459 controlled streams - highlighting the complexities of applying predictive models to  
460 variable environments. In respect to our highly variable study site, any eel detected  
461 upstream of a sampling point would eventually have to pass through downstream  
462 pumps in order to achieve seaward migration, and so such effects would be negligible  
463 on the interpretation of results from a management perspective. These factors should  
464 however be considered in regard to study/sampling design, and complexity of the  
465 study system when making additional inferences from results in pumped catchments.  
466 As such, future research into the influence of seasonal and daily variation in pump  
467 activity on eDNA performance in pumped catchments is recommended.

468 While further consideration may be required before applying this method as a  
469 standardised tool in management frameworks, this study demonstrates successful  
470 application of eDNA as a high sensitivity tool to screen for eel and fish in pumped  
471 catchments. In addition to increased detection sensitivity, significant correlations  
472 between eDNA and Catch methods further evidence increased confidence in eDNA  
473 based decision making (Jerde, 2019). Validating eDNA metabarcoding as a non-  
474 invasive method with decreased sampling effort and higher detection rates of target  
475 species. Sepulveda *et al.* (2020) suggests that the reliability of eDNA methods is not

476 a barrier, the problem is that we often lack the tools to integrate inherent uncertainty  
477 into decision-making frameworks. These molecular methods could be applied  
478 methodically to work with such frameworks, but should be integrated in a manner  
479 which could fill existing knowledge gaps while accounting for related uncertainty. We  
480 therefore conclude that this workflow could be optimised as a method to inform  
481 management for pumped catchments in Europe and beyond.

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499 fieldwork, and L. R. Harper, C. Di Muri, G. Sellers and M. Benucci for their support  
500 with bioinformatics and the handling of outputs.

## 501 **Contributions**

502 N.P.G carried out bioinformatics, data analysis and manuscript preparation. L.A.M  
503 coordinated fieldwork and sample collection. R.K.D and H.V.W carried out lab work.  
504 J.B, R.W, and B.H conceived the study, acquired funding and supported with  
505 manuscript preparation/ideas.

506

## 507 **Data accessibility**

508 Raw sequence reads have been archived on the NCBI Sequence Read Archive (SRA)  
509 under BioProject: PRJNA646357; BioSamples: SAMN15541168 - SAMN15541275;  
510 SRA accessions: SRR12232432 - SRR12232539. R scripts, Jupyter notebooks and  
511 corresponding data have been made available in a dedicated GitHub repository, which  
512 is permanently archived at (<https://doi.org/10.5281/zenodo.3951418>).

## 513 **Supporting Information**

514

515 **Table 2.** An overview of Environment Agency Catch methods applied at each site,  
516 including Date, Area fished, Survey method, Survey strategy and Number of runs.  
517 This is tabulated and available as a .csv file.

518

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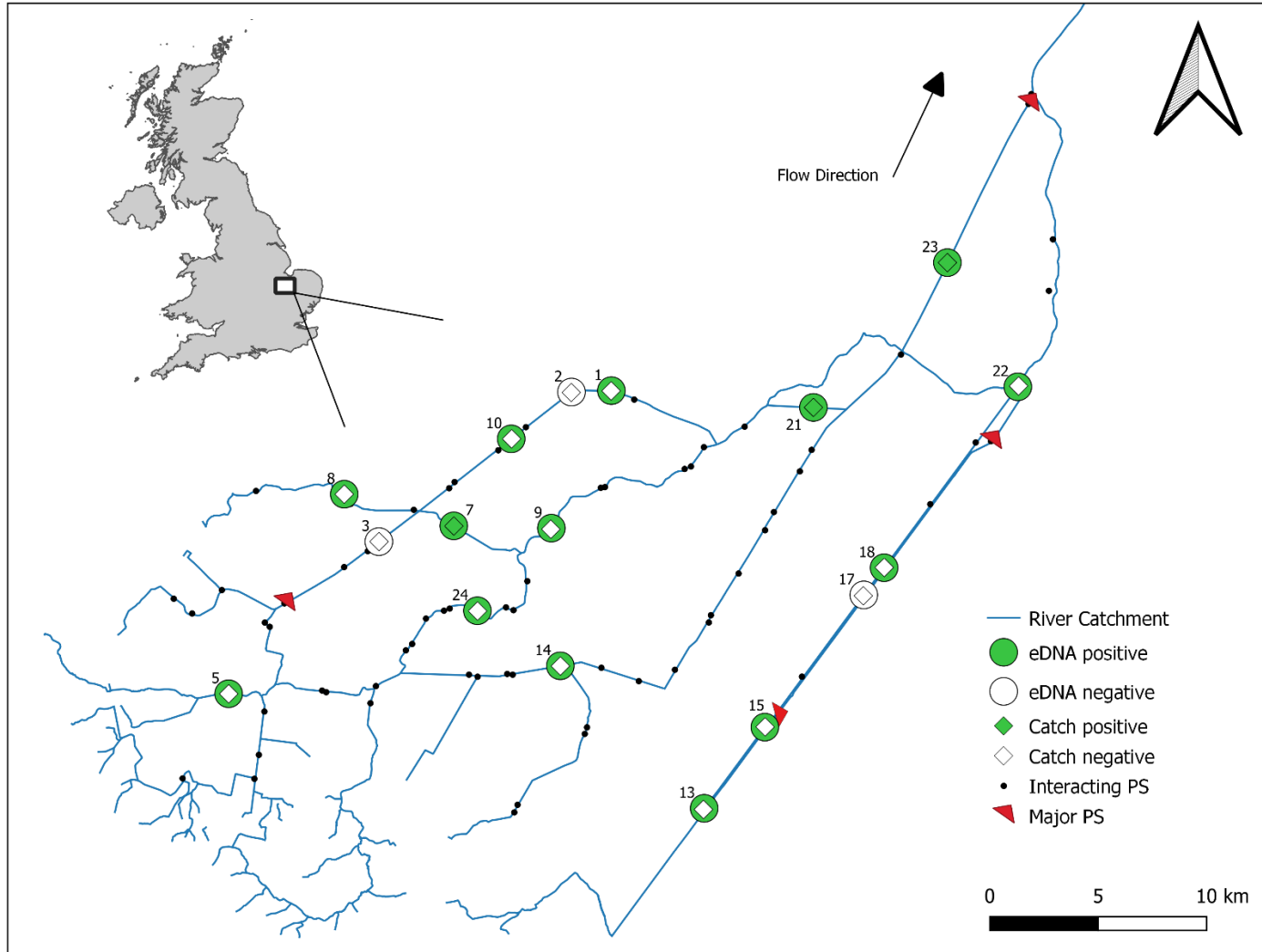
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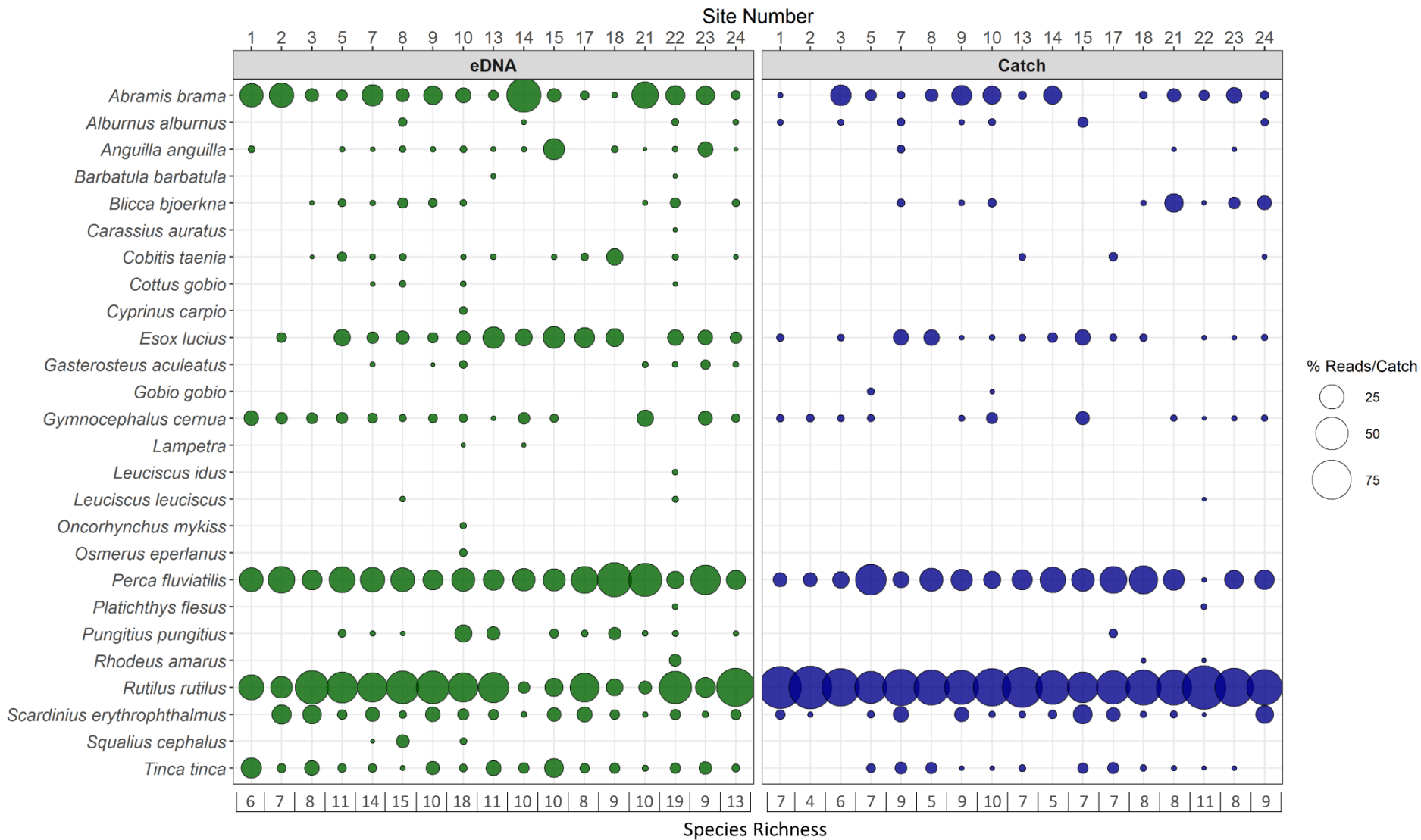
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**Table 1.** A comparison of the species site occupancy obtained from eDNA metabarcoding and traditional catch methods. In the species column, UK BAP (Biodiversity action plan) species (+) and non-native/introduced species (\*) are indicated.

<b>Species</b>	<b>eDNA</b>	<b>Catch</b>
<i>Abramis brama</i>	17	14
<i>Alburnus alburnus</i>	4	7
<i>Anguilla anguilla</i> +	14	3
<i>Barbatula barbatula</i>	2	0
<i>Blicca bjoerkna</i>	9	8
<i>Carassius auratus</i> *	1	0
<i>Cobitis taenia</i> +	11	3
<i>Cottus gobio</i>	4	0
<i>Cyprinus carpio</i> *	1	0
<i>Esox lucius</i>	14	14
<i>Gasterosteus aculeatus</i>	7	0
<i>Gobio gobio</i>	0	2
<i>Gymnocephalus cernua</i>	14	11
<i>Lampetra</i> +	2	0
<i>Leuciscus idus</i> *	1	0
<i>Leuciscus leuciscus</i>	2	1
<i>Oncorhynchus mykiss</i> *	1	0
<i>Osmerus eperlanus</i> +	1	0
<i>Perca fluviatilis</i>	17	17
<i>Platichthys flesus</i>	1	1
<i>Pungitius pungitius</i>	11	1
<i>Rhodeus amarus</i> *	1	2
<i>Rutilus rutilus</i>	17	17
<i>Scardinius erythrophthalmus</i>	16	14
<i>Squalius cephalus</i>	3	0
<i>Tinca tinca</i>	17	12



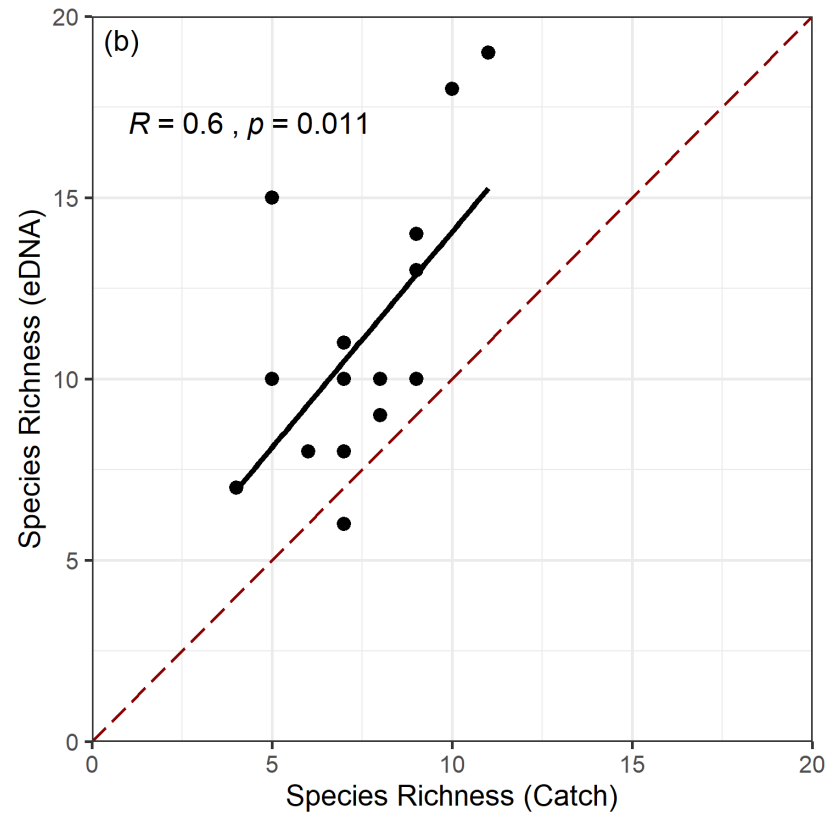
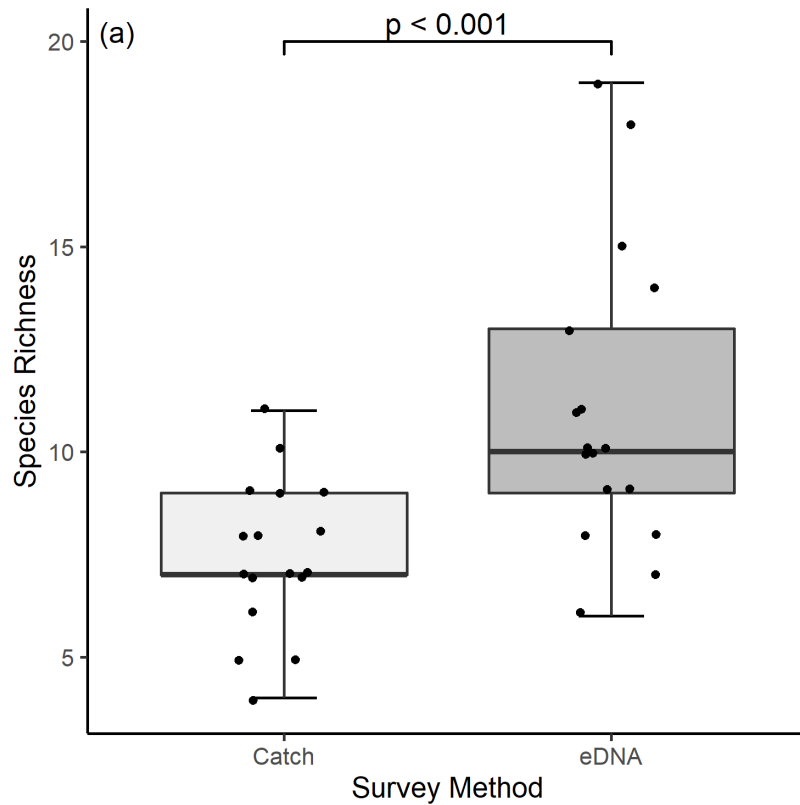
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 2 **Figure 1.** A map depicting the presence/absence and overall distribution of *Anguilla anguilla* across study sites based on eDNA surveys (circle) and traditional  
 3 catch surveys (diamond). Net flow direction is indicated by the black arrow, while the pumping direction of major pumping stations (PS) is indicated by the  
 4 direction of red triangles. Interacting pumping stations are also highlighted here (black dot), these pumps are connected to the main channel but pump drainage  
 5 ditches into the river catchment (blue line), and thus are unlikely to entrain species present at our survey sites.



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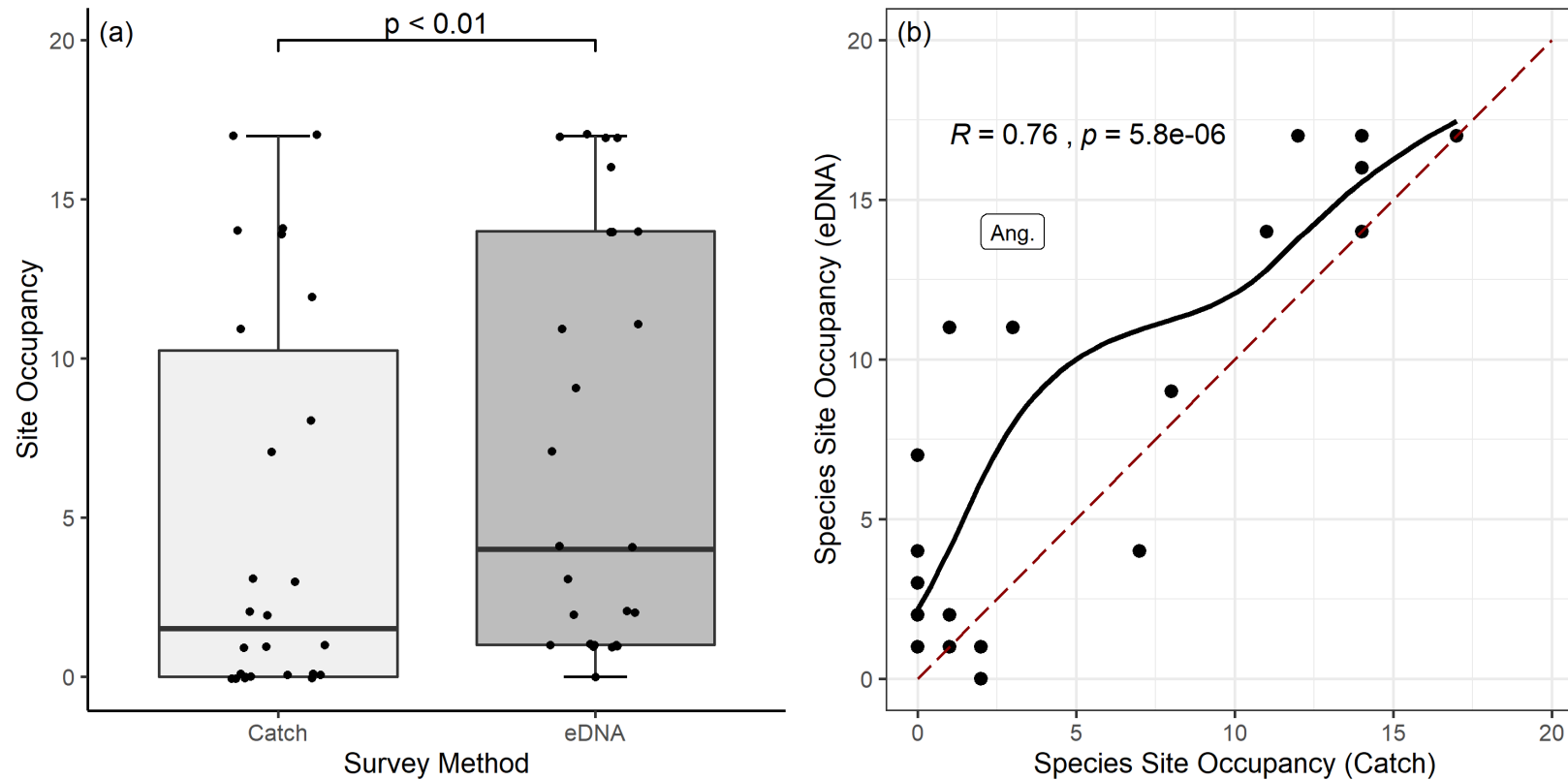
7 **Figure 2.** Bubble plot depicting % Reads/Catch (circle size) of each species detected at each site using eDNA metabarcoding (green fill, left) and traditional  
 8 catch (blue fill, right). Presenting a visual comparison of relative abundance between methods, with total species richness at each site on the bottom row.





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**Figure 3.** Box plot (a) of species richness at each site for each survey method, including p-value (paired t-test). Scatter plot (b) comparing site species richness between eDNA and traditional methods, including the Pearson correlation test output and associated regression line to visualise correlations between methods. The dashed red line indicates species richness equilibrium, where points above the line had a higher species richness using eDNA, while points below had higher Catch species richness.



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**Figure 4.** Box plot (a) of species site occupancy at each site for each survey method, including p-value (paired Wilcoxon test). Scatter plot (b) comparing species-specific site occupancy between eDNA and traditional methods, including the Spearman's rank correlation test output, and a smooth curve (loess) to visualise associations. The dashed red line indicates site occupancy equilibrium, where points above the line indicate a species was detected at more sites using eDNA and points below the line indicate a species was captured at more sites using traditional methods (text label indicates our target species *A. anguilla*).