

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.

212

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 ddH₂O, 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 ddH₂O (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/ μ 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 μ 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 μ 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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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.

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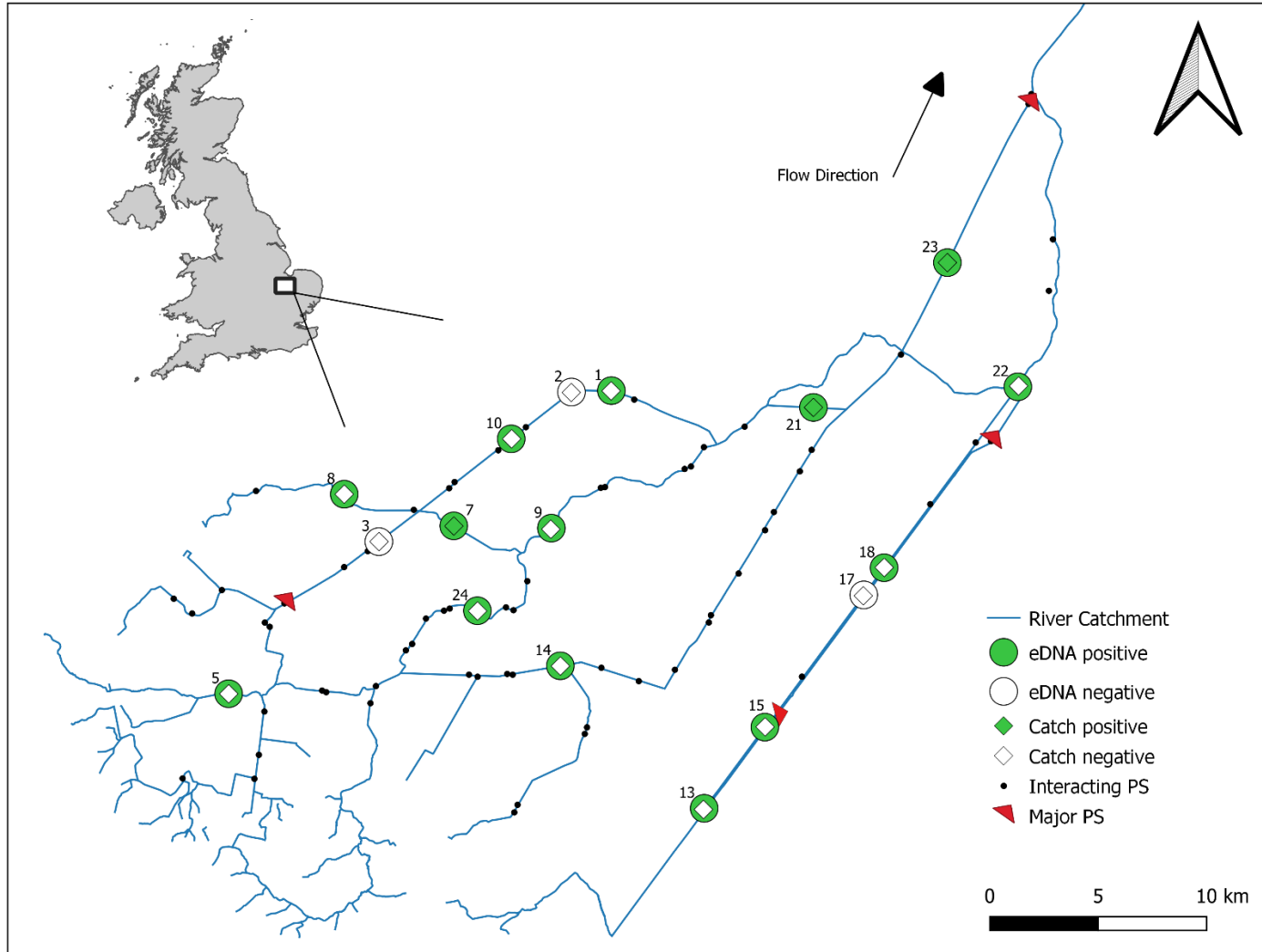
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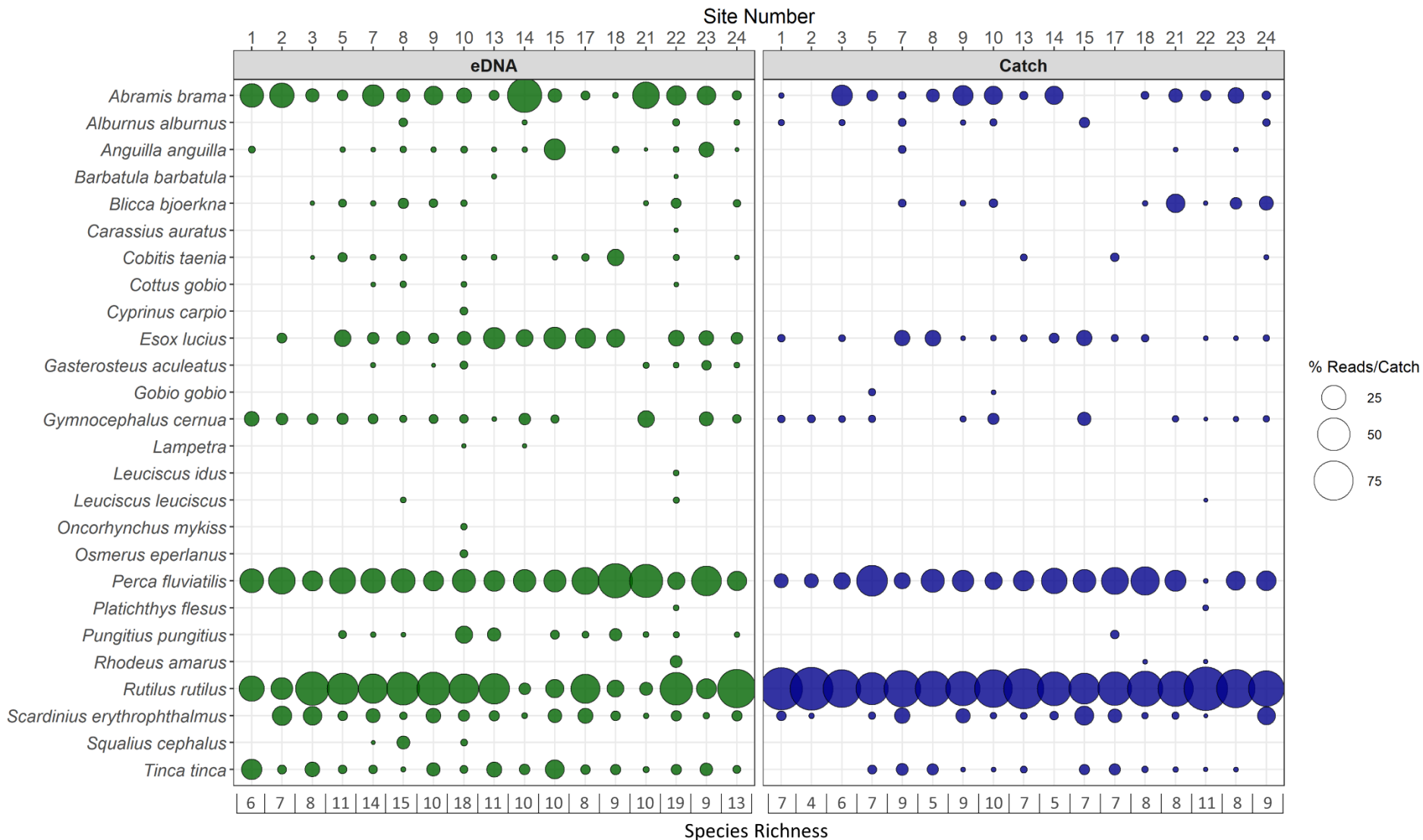
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Table 1. A comparison of the species site occupancy obtained from eDNA metabarcoding and traditional catch methods. In the species column, UK BAP (Biodiversity action plan) species (+) and non-native/introduced species (*) are indicated.

Species	eDNA	Catch
<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

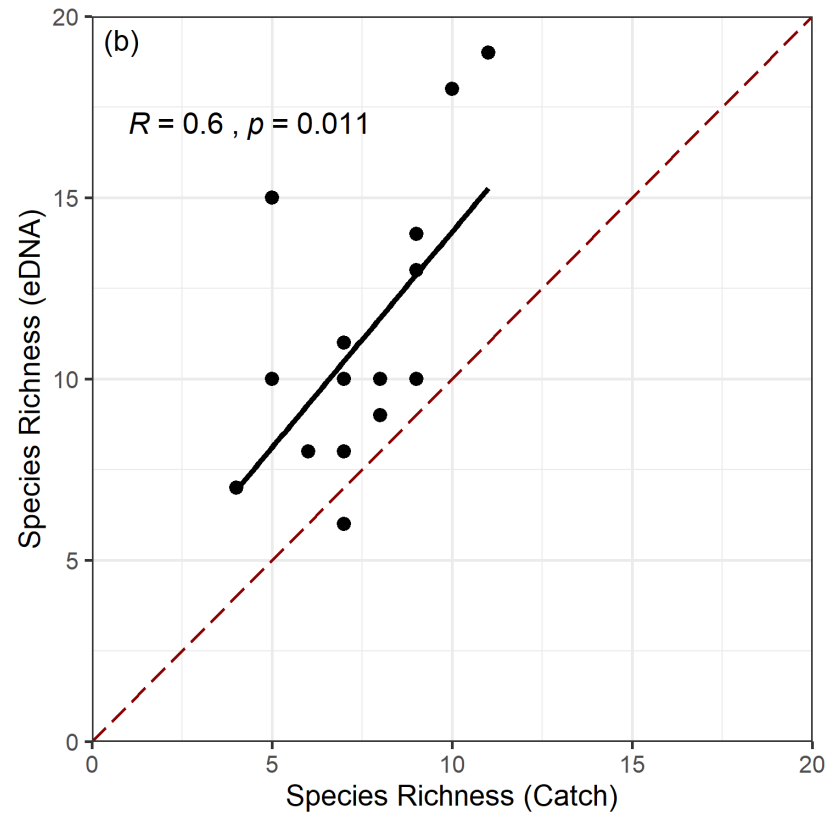
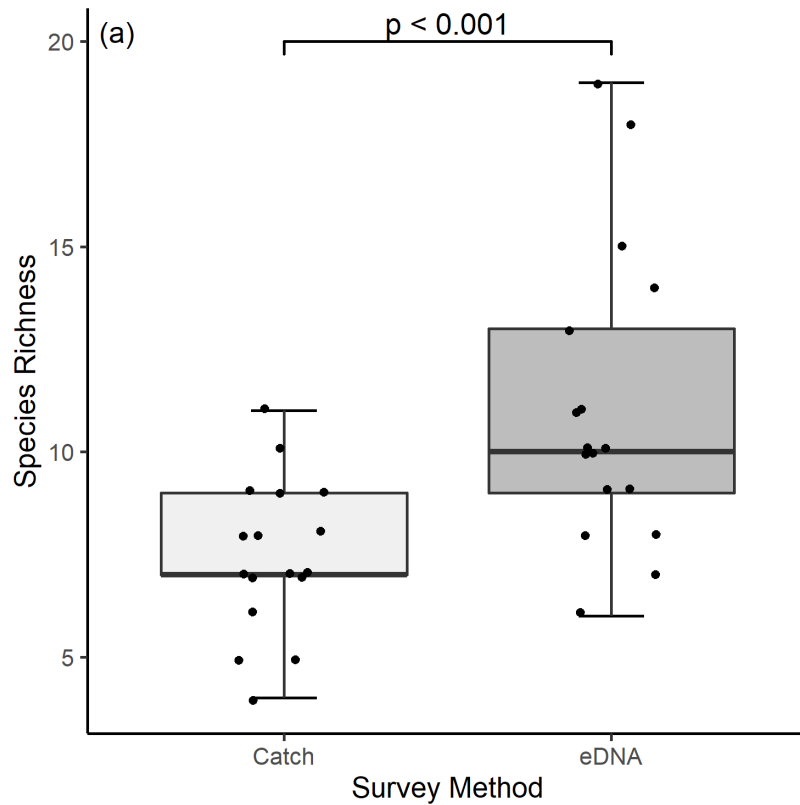


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 2 **Figure 1.** A map depicting the presence/absence and overall distribution of *Anguilla anguilla* across study sites based on eDNA surveys (circle) and traditional
 3 catch surveys (diamond). Net flow direction is indicated by the black arrow, 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.



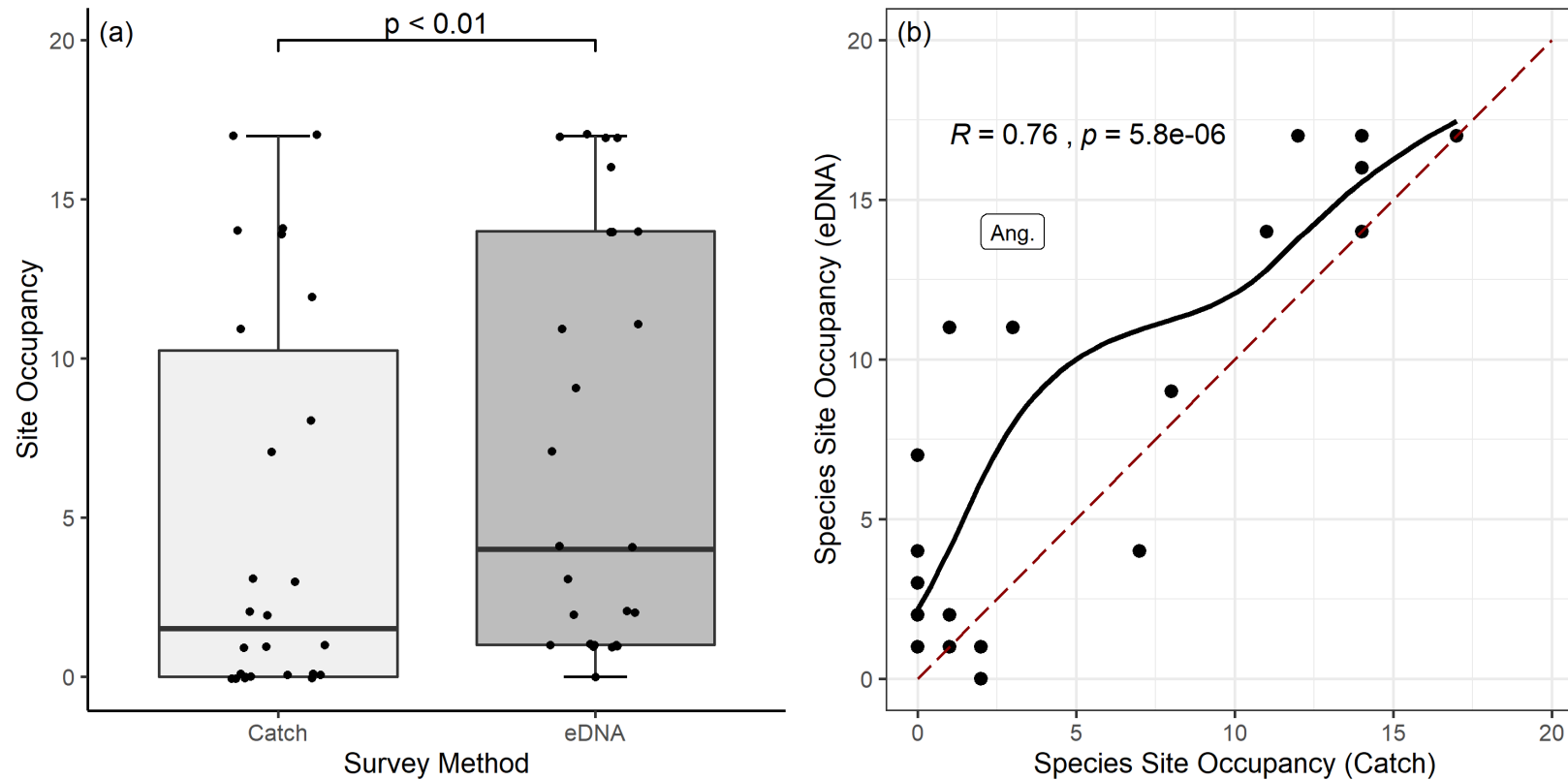
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7 **Figure 2.** Bubble plot depicting % Reads/Catch (circle size) of each species detected at each site using eDNA metabarcoding (green fill, left) and traditional
 8 catch (blue fill, right). Presenting a visual comparison of relative abundance between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