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1 **Monitoring freshwater fish communities in large rivers using environmental DNA**
2 **(eDNA) and a long-term electrofishing survey (1990-2018)**

3

4

5 Aurélie Goutte^{1,2}, Noëlie Molbert¹, Sabrina Guérin³, Robin Richoux³, Vincent Rocher³

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8 ¹ Sorbonne Université, CNRS, EPHE, UMR METIS, F-75005, Paris, France

9 ² EPHE, PSL Research University, F-75005, Paris, France

10 ³ Syndicat Interdépartemental pour l'Assainissement de l'Agglomération Parisienne
11 (SIAAP), Colombes, France.

12

13 Correspondance: Aurélie Goutte, aurelie.goutte@ephe.psl.eu

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15

16 **Abstract**

17 Monitoring freshwater fish communities in a large human-impacted river represents a
18 challenging task. The structure of fish assemblages has been monitored yearly in the Marne
19 and the Seine Rivers, across the Paris conurbation, France, using traditional electrofishing
20 (EF) surveys since 1990, in accordance with the European Water Framework Directive. In
21 addition, metabarcoding of DNA extracted from environmental samples (eDNA) was
22 concomitantly conducted in 9 sampling sites in 2017 and in 2018, to compare estimates of
23 species richness and relative abundance among three methods: annual, long-term EF
24 monitoring, and eDNA. The present study confirms better detection of fish species using
25 eDNA compared to annual EF. eDNA metabarcoding was also more efficient for species
26 detection than 3- to 6-year EF survey, but was similar or less efficient than long-term EF
27 survey of 14 years of monitoring. In addition, the numbers of reads per species relative to the
28 total number of reads significantly increased with (1) increasing relative abundance (relative
29 % of individuals caught per species) and (2) increasing number of years that a fish species
30 was detected during the 2000-2018 period. These results suggest that eDNA could reflect
31 local population persistence.

32

33 **Keywords:** eDNA, electrofishing, fish species assemblage, Seine River, long-term
34 monitoring, population persistence

35

36 **Introduction**

37 Although fresh water occupies less than 1% of the Earth's surface and represents
38 approximately 0.01% of the World's water (Gleick, 1996), these habitats support
39 exceptionally rich biodiversity, with over 10000 freshwater fish species, corresponding to
40 40% of global fish diversity (Lundberg et al., 2000). Fresh water ecosystems are threatened by
41 intensive and widespread human activities, such as overexploitation of resources, acute and
42 chronic release of pollutants, habitat modification and fragmentation, hydrological regime
43 modification, and introduction of invasive exotic species (Dudgeon et al., 2006, Vörösmarty
44 et al., 2010). Several freshwater fish populations are declining or at risk of extinction,
45 especially large, long-lived, or potamodromous fish species (Liermann et al., 2012).

46 Freshwater ecosystem quality, as established by the Water Framework Directive
47 (WFD, 2000/60/CE) and its transposing legislation in France (2006-1772 of 30/12/06) is
48 evaluated using a combination of several bio-indicators. Four biological quality elements -
49 diatoms, macrophytes, benthic macro-invertebrates and fish- are monitored at regular
50 intervals (EEA 2012). Traditional sampling (gillnet, electrofishing) is an effective tool for
51 determining fish abundance, population structure and species composition (Bonar et al.,
52 2009). Sampled organisms are identified using morphological criteria and a metric index is
53 computed based on taxonomic richness and abundance. Ecological representativeness of
54 sampling methods is a critical task, especially in large rivers. Indeed, electrofishing (EF) may
55 fail to detect rare species, may exclude some species, due to low size or to the use of
56 microhabitats and may underestimate population size at greater river widths, because of
57 increasing depth and flow velocity in the open water zone (Kennedy & Strange, 1981; Evans
58 and Lamberti, 2017; Zajicek and Wolter, 2018). Moreover, EF is costly, time consuming,
59 requires teams of skilled and qualified workers and can induce harmful effect for fish and
60 eggs (Dwyer, et al., 1993; Snyder, 2003; Goldberg et al., 2016; Shaw et al., 2016).

61 During the last decade, environmental DNA (eDNA) has emerged as a rapid, reliable
62 and non-invasive tool for inventorying fish richness (Rees et al., 2014; Pawlowski et al.,
63 2018). Tracing eDNA allows a better species detection compared to traditional EF methods
64 (Bohmann et al., 2014; Valentini et al., 2016), reduces sampling effort and cost (Evans et al.,
65 2017). However a major constraint of the eDNA method is its limited ability for the
66 quantitative estimation of fish assemblages. Indeed the detectability and concentration of
67 eDNA depends on several parameters: (i) production rates from organisms (faeces, saliva,
68 urine, skin, mucus, dead cells, Taberlet et al., 2012), according to the individual's abundance
69 and metabolic rate (Lacoursière- Roussel et al., 2016), (ii) water dispersion (dilution,
70 deposition, downstream transport, Wilcox et al., 2016) and (iii) environmental degradation in
71 the environment (UV light, microbial activity, temperature, pH, Barnes et al., 2014, Thomsen
72 et al., 2012, but see Lacoursiere-Roussel et al., 2016). It should be noted that eDNA
73 degradation rate under laboratory conditions (20°C, 12-h light) is around 10% reduction per
74 hour, so that more than 90% of eDNA is degraded after one day (Maruyama et al., 2014). A
75 challenging task is to shift from presence-absence monitoring to an estimation of relative
76 abundance of individuals. In mesocosms, the number of eDNA copies increased with biomass
77 of freshwater fish and amphibians (Evans et al., 2015) or marine fish (Kelly et al., 2014), but
78 correlations are weak and non-linear. Promising correlations were found between sequence
79 read counts and fish relative abundance in field studies along the Rhône River (Pont et al.,
80 2018) and in large lakes in England (Hanfling et al., 2016). In that regards, Hering et al.
81 (2018) have explored the suitability of DNA-based identification into ecological status
82 assessment under the European Water Framework Directive and concluded that eDNA
83 method is particularly efficient for fish.

84 The interdepartmental association for sewage disposal in Paris conurbation (SIAAP)
85 has monitored the structure and composition of fish communities in the Marne and the Seine

86 Rivers, France, with traditional EF surveys since 1990. Previous results have highlighted an
87 increase in fish species diversity from 1990 to 2013 in eight stations of the Seine River across
88 the Paris conurbation (Azimi and Rocher, 2016). Annual EF surveys yielded 21 species in
89 2013 vs 14 in 1990, and the cumulative number of species increased from 14 species in 1990
90 to 32 in 2013 (Azimi and Rocher, 2016). Fish assemblages also changed, with a relative
91 decline of limnophilic and omnivorous species, and an increase of limnophilic and
92 carnivorous species and the presence of rheophilic and carnivorous species, primary after
93 2009 (Azimi and Rocher, 2016). This change has been attributed to recent achievements in
94 wastewater treatment in the greater Paris area and that have considerably improved the water
95 quality, especially dissolved oxygen, organic matter and ammonia nitrogen in the Seine River
96 (Azimi and Rocher, 2016). At a larger spatial and temporal scale, a recent study showed that
97 amphidromous species declined for the past 150 years on 29 river sections of the Seine River
98 basin, where non-native species colonized (Belliard et al., 2018).

99 During two consecutive years (2017 and 2018), eDNA was tracked in nine sampling
100 sites, the same week than traditional EF inventories, to compare their species richness
101 estimates using eDNA metabarcoding, EF and long-term electrofishing survey (LTES). We
102 expected better species detection from eDNA, compared to EF. In addition, we tested whether
103 difference in species detection between LTES and eDNA increased with the LTES period
104 (from 3 to 29 years). Lastly, we investigated the potential of eDNA to estimate relative
105 abundance, by testing the correlation between the number of individuals caught per species
106 and the numbers of reads per species relative to the total number of reads.

107

108 **Materials and methods**

109

110 *Study area*

111 Electrofishing and eDNA surveys were conducted in summer 2017 and 2018 at nine sampling
112 sites distributed from upstream (Gournay-sur-Marne (GM), Bonneuil-sur-Marne (BM) and
113 Maisons-Alfort (MA) in the Marne River, and Choisy-le-Roi (CR) in the Seine River) to
114 downstream (Paris (P), Levallois-Perret (LP), Colombes (C), Herblay (H) and Triel-sur-Seine
115 (TS) in the Seine River) of the Paris conurbation (Fig. 1). The distance between adjacent
116 sampling sites was: 20.0 km (GM-BM), 6.2 km (BM-MA), 14.6 km (MA-P), 19.3 km (CR-P),
117 14.5 km (P-LP), 15 km (LP-C), 27.5 km (C-H), 18.1 km (H-TS).

118

119 *Electrofishing survey*

120 EF surveys started in 1990 for P and LP, with sampling twice a year, from 1990 to 1999, then
121 once a year, from year 2000 onwards. Three additional sampling sites were monitored from
122 2000: GM, MAI and TS. BM was added in 2005, CR in 2013, C and H in 2016. Information
123 for LTES periods and EF surveys conducted in July 2017 and 2018 is given in Table 1.
124 Single-pass electrofishing was performed according to a European standardized protocol
125 (CEN 14011), using a portable unit which generated up to 200 V and 3 A pulsed D.C. in an
126 upstream direction (Azimi and Rocher, 2016). Sampling method changed in 2005 from the
127 per-habitats fishing method (1990-2004) to Abundance Grab Sampling (AGS) method (from
128 2005 onwards), without significantly affecting diversity and composition of fish assemblages
129 (Azimi and Rocher, 2016). Captured fish were sorted by species and counted.

130

131 *eDNA sampling and metabarcoding*

132 Two filtrations (i.e. 2 samples) per site were conducted, lasting 30 minutes each and
133 corresponding to a water volume of 30 L, which is considered sufficient to detect more than
134 95% of the local species richness (Valentini et al. 2016). For one site (Colombes), only one
135 water sample was collected in 2017, because of a technical problem. A total of 35 water
136 samples were collected in the nine sampling sites from July 26 to August 2 2017 and from
137 July 18 to 20 2018 (Table 1). All devices were provided by SPYGEN (le Bourget du Lac,
138 France). Water samples were collected on the left bank or on the right bank and filtered using
139 a peristaltic pump ($1.1 \text{ L}\cdot\text{min}^{-1}$), a VigiDNA® 0.45- μM cross flow filtration capsule and a
140 disposable sterile tubing for each sample. After emptying the capsule at the end of each
141 filtration, CL1 Conservation buffer (80 mL, SPYGEN, le Bourget du Lac, France) was added
142 and filters were stored at room temperature. DNA extraction, amplification using teleo
143 primers (Valentini et al., 2016), high-throughput sequencing, sequence analysis, and taxon
144 assignments were conducted by SPYGEN (le Bourget du Lac, France), as described in Pont et
145 al. (2018) and Milhau et al. (2019). Library preparation and sequencing were performed using
146 the FASTERIS MetaFast protocol ([www.fasteris.com/dna/?q=content/metafast-protocol-](http://www.fasteris.com/dna/?q=content/metafast-protocol-ampliconmetagenomic-analysis)
147 [ampliconmetagenomic-analysis](http://www.fasteris.com/dna/?q=content/metafast-protocol-ampliconmetagenomic-analysis)). Paired-end sequencing ($2 \times 125\text{bp}$) was carried out on an
148 Illumina HiSeq2500 sequencer (www.illumina.com) with the HiSeq SBS Kit v4 (Illumina)
149 following the manufacturer's instructions. Taxonomic assignment of molecular operational
150 taxonomic units (MOTU) was performed using the program ecotag with the local reference
151 database Teleostei (Valentini et al., 2016). MOTUs showing <98% similarity to the local
152 reference database were removed. All sequences with an occurrence frequency < 0.001 per
153 taxon and per library were discarded. After the bioinformatic analysis, taxa present in only
154 one PCR replicate and in only one field replicate were discarded (Ficetola et al., 2015).

155 We excluded six species that had not been detected in the Seine and the Marne Rivers, since
156 they are consumed by humans and their DNA are likely released in the environment from
157 treatment plants: *Dicentrarchus labra* L. (2006 reads in total across all samples), *Liza ramada*
158 (Risso, 1826), 295 reads in total across all samples), *Pangasianodon hypophthalmus*
159 (Sauvage, 1878) (740 reads in total across all samples), *Salmo salar* L. (4385 reads in total
160 across all samples), *Sparidae* sp. (375 reads in total across all samples), *Trachurus* sp. (1947
161 reads in total across all samples).

162 The following fish species were not differentiated according to molecular marker used
163 in this study: (1) the vairone *Telestes souffia* (Risso 1827), the common nase *Chondrostoma*
164 *nasus* L. and the south-west European nase *Parachondrostoma toxostoma* (Vallot 1837), (2)
165 the grass carp *Ctenopharyngodon idella* (Valenciennes 1844) and the silver carp
166 *Hypophthalmichthys molitrix* (Valenciennes 1844), (3) black bullhead *Ameiurus melas*
167 (Rafinesque 1820) and brown bullhead *Ameiurus nebulosus* (LeSueur 1819), (4) ide
168 *Leuciscus idus* L. and common dace *Leuciscus leuciscus* L., (5) crucian carp *Carassius*
169 *carassius* L., goldfish *Carassius auratus* L. and Prussian carp *Carassius gibelio* (Bloch 1782),
170 (6) *Cottus* species: *C. aturi* (Freyhof, Kottelat and Nolte, 2005), *C. duranii* (Freyhof, Kottelat
171 and Nolte, 2005), *C. gobio* L., *C. hispaniolensis* (Bacescu & Bacescu-Mester 1964), *C.*
172 *perifretum* (Freyhof, Kottelat and Nolte, 2005) and *C. petiti* (Bacescu & Bacescu-Mester
173 1964), (7) gudgeon *Gobio gobio* L., *G. lozanoi* (Doadrio & Madeira 2004) and *G. occitaniae*
174 (Kottelat & Persat 2005).

175

176 *Statistical analyses*

177 Species richness was first compared between eDNA and annual EF methods in 2017 and in
178 2018 separately, and then between two-year eDNA metabarcoding and LTES, using

179 Wilcoxon signed-rank tests for paired data. The correlation between difference in the number
180 of detected fish species between LTES and eDNA (2017 and 2018 data sets) methods and the
181 LTES period (from 3 to 29 years) was tested using a Spearman's rank-order correlation test.

182 All eDNA samples were resampled using R package MASS (function sample without
183 replacement; www.r-project.org) to randomly select 45,950 reads per sample, i.e. the smallest
184 number of reads in one sample. All taxa that were detected from the initial dataset were still
185 found after resampling. Correlations between the number of reads per species relative to the
186 total number of reads and the number of individuals caught per species were tested for each
187 using Spearman's rank-order correlation tests. Links between the number of reads per species
188 relative to the total number of reads and the number of years a fish species detected during the
189 2000-2018 period (19 years) were tested using Spearman's rank-order correlation tests in
190 three historical sampling stations: the most upstream site (GM), within the metropolis (P) and
191 the most downstream point (TS).

192

193 **Results**

194 *Qualitative pattern*

195 A total of 8,072,888 reads were assigned to 31 fish taxa. Whatever the method used, the total
196 number of species or groups of species did not significantly differ between the two
197 consecutive years 2017 and 2018 (eDNA : $W = 18$, $p = 0.551$; EF: $W = 13$, $p = 0.932$; LTES
198 : $W = 0$, $p = 0.089$).

199 A greater number of species or groups of species per site was detected through eDNA
200 metabarcoding (min-max: 18-24 in 2017 and 16-23 in 2018) compared to EF inventory (10-17

201 in 2017 and 10-19 in 2018, Wilcoxon signed-rank tests for paired data, $W=45$, p -value =
202 0.009 for both years, Table 2, Fig. 2).

203 Total number of fish species that were sampled at least once during LTES from 1990
204 at the earliest to 2018 (19 to 30 species per site) did not differ significantly from species
205 richness detected during the two-year eDNA survey (compiled data from the 2017 and 2018
206 sampling sessions, 20 to 26 species or groups of species per site, Wilcoxon signed-rank tests
207 for paired data, $W = 22$, p -value = 0.203, Fig. 3). Moreover, difference in the number of
208 detected fish species between LTES and two-year eDNA methods was positively correlated to
209 the LTES period (Spearman's rank-order correlation test, $r = 0.957$, $p < 0.001$, Fig. 4). This
210 was also true for one of the years of eDNA sampling (2017, $r = 0.953$, $p < 0.001$; 2018, $r =$
211 0.798, p -value = 0.010).

212

213 *Semi-quantitative pattern*

214 Relative abundance was calculated as the abundance of fish individual of each species relative
215 to the total number of fish caught by electrofishing in one sampling site in 2017 or in 2018.

216 Relative number of reads increased with increasing relative abundance, but with some
217 variations among the 9 studied sites and between the two years (Table 3, Figure 5).

218 At last, the number of reads per species relative to the total number of reads in 2018
219 increased with increasing number of years that a fish species was detected during the 2000-
220 2018 period (Figure 6, Spearman's rank-order correlation test, $p < 0.001$ for all sites,
221 Gournay-sur-Marne: $r = 0.778$; Paris : $r = 0.837$; Triel-sur-Seine: $r = 0.806$).

222

223 **Discussion**

224 This study confirms the higher detection capability of fish species using eDNA
225 metabarcoding compared to traditional electrofishing method, as previously demonstrated
226 (e.g. Civade et al., 2016; Valentini et al., 2016). New fish species were detected during the
227 studied period though either EF, either eDNA or both methods: sunbleak *Leucaspius*
228 *delineatus* was first sampled by EF in 2017 (Paris), spiralin *Alburnoides bipunctatus* by EF in
229 2018 (Gournay-sur-Marne), largemouth bass *Micropterus salmoides* by eDNA and EF in
230 2018 (Gournay-sur-Marne), and asp *Aspius aspius* by eDNA in 2018 (Maison-Alfort).

231 Some species were detected by eDNA but not caught during EF survey at one site, and
232 the relative number of reads was very low (mean \pm se: 1.57% \pm 0.48%). These species were
233 either solitary, either rheophilic with good swimming ability in streams, being more prone to
234 avoid the boat and fishing capture, such as the barbel *Barbus barbus* L., the ruffe
235 *Gymnocephalus cernua* L., the pike *Esox Lucius* L., the pike-perch *Sander lucioperca* L.. On
236 the other hand, some species (tench *Tinca tinca* L., rudd *Scardinius erythrophthalmus* L.,
237 pike, pumpkinseed sunfish *Lepomis gibbosus* L., common nase, common carp *Cyprinus*
238 *carpio* L., European bitterling *Rhodeus amarus* (Bloch, 1782)) were not detected by eDNA at
239 one site but were caught during EF survey at a relatively low abundance (0.64% \pm 0.15%).

240 The detection of a species using eDNA could result from the presence of individuals
241 upstream, because of downstream transport from few meters up to several dozens of
242 kilometers (Laramie et al., 2015; Jane et al., 2015; Civade et al., 2016; Shogren et al., 2017;
243 Pont et al., 2018). In our data sets, many fish species were detected using eDNA and EF in
244 one sampling site, but not at the station(s) downstream: the spined loach (*Cobitis taenia* L.)
245 was recorded in Gournay-sur-Marne only, in 2017 and in 2018 ; the three-spined stickleback
246 *Gasterosteus aculeatus* L. in Paris in 2017 and in Maison-Alfort in 2018 ; the largemouth bass
247 in Gournay-sur-Marne in 2018. Hence, our results suggest that downstream transport in the
248 Marne and the Seine River did not reach 14.5 km, likely because of low velocities within

249 these rivers at low altitude and high eDNA degradation in summer. Mean flows and
250 temperature in the Seine River during eDNA sampling were 113 m³/s and 22°C in 2017 and
251 150 m³/s and 25.2°C in 2018.

252 Two-year eDNA monitoring produced similar results than long-term electrofishing
253 survey that was conducted yearly since up to 1990. Consistently with a 10-year study on the
254 Rhône River (2006–2016, Pont et al., 2018), our results suggest that eDNA metabarcoding is
255 a good proxy of fish assemblage during the last decade. Specifically, an electrofishing survey
256 of 3 to 6 years was less efficient than eDNA to detect fish species, an electrofishing inventory
257 of 14 to 19 years was equivalent or slightly better (4 more caught species) than eDNA and the
258 29-year survey allow inventorying 6 to 7 more species than the two-year eDNA survey. As
259 previously shown (Azimi and Rocher, 2016), the number of caught species through EF
260 increased from 1990 to 2000, and then remained nearly constant, as a potential consequence
261 of river quality's improvement. Some species were caught occasionally (1 to 4 specimen)
262 during the 29-year survey, but were not detected by eDNA metabarcoding at these sites (GM,
263 P, TS): sunbleak, spirilin, rudd, ninespine stickleback *Pungitius pungitius* L., three-spined
264 stickleback *Gasterosteus aculeatus* L., Brook Lamprey *Lampetra planeri* (Bloch, 1784),
265 Topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846). Their occasional
266 detection through LTES but not eDNA did not reflect establishment of these fish population
267 and could be attributed to morphometric misidentification (Jerde, 2019) and to exceptional
268 exchange with the connecting tributaries of the Seine River and the Marne River.

269 The relative number of eDNA reads per species is a good proxy of the relative
270 abundance of caught individuals. Correlations were strong and variabilities among sampling
271 sites and between the two years were weak, which is a promising result for the use of eDNA
272 as an estimate of quantitative patterns of fish biodiversity in large rivers. However, it worth
273 noting that the vast majority of fish species had finished their reproduction and fry were

274 growing, when EF and eDNA sampling were conducted, in late July, early August. Whereas
275 only adults above a given size only are caught by EF, eDNA is released from different
276 individuals at different stages, including gametes and eggs (Bylemans, et al., 2017). The
277 eDNA release rate of fish is higher in adults than in juveniles, but the opposite pattern is
278 observed when corrected by fish body weight (Maruyama et al., 2014). This would entail
279 considering the timing of water sampling for the interpretation of eDNA-based data (Shaw et
280 al., 2016), according to freshwater fish phenology (the timing of migration, spawning,
281 hatching). Phenological trends are species-specific and vary from year to year, because of
282 environmental changes (Shuter et al., 2012; Krabbenhoft et al., 2014), which makes the
283 interpretation more complex.

284 Production rates of eDNA should be higher in resident species compared to transitory
285 fish or newly established populations, because of a relatively higher abundance and biomass
286 of individuals, wider spatial distribution and temporal persistence, and higher breeding
287 probability. This study confirms this hypothesis, since the relative number of reads were
288 positively correlated to the number of years a fish species were detected during the last two
289 decades (2000-2018). Bleak *Alburnus alburnus* L., eel *Anguilla Anguilla* L., perch *Perca*
290 *fluviatilis* L., roach *Rutilus rutilus* L. and chub *Squalius cephalus* L. were caught by EF during
291 at least 10 years from 2000 to 2018 in the three historical sites (GM, P and TS). Mean relative
292 number of eDNA reads for these 5 species at the 3 sites was 0.10 ± 0.02 . Hence eDNA gives
293 accurate information on local fish population persistence.

294

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297 PIREN-Seine research program for financial support.

298

299 **Contributions**

300 AG, SG and VR conceived the idea and designed the methodology. A.G. and R.R. contributed
301 to field work. A.G. performed data analyses. A.G. and N.M. prepared the manuscript and
302 figures. All authors approved the final version of the manuscript.

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