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

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Abstract

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

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

Introduction

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

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

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

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

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

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

Materials and methods

Study area

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

Electrofishing survey

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

eDNA sampling and metabarcoding

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

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

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

Results

Qualitative pattern

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

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

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

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

Semi-quantitative pattern

Relative abundance was calculated as the abundance of fish individual of each species relative to the total number of fish caught by electrofishing in one sampling site in 2017 or in 2018. Relative number of reads increased with increasing relative abundance, but with some variations among the 9 studied sites and between the two years (Table 3, Figure 5).

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

Discussion

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

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

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

these rivers at low altitude and high eDNA degradation in summer. Mean flows and temperature in the Seine River during eDNA sampling were 113 m^3/s and 22°C in 2017 and 150 m^3/s and 25.2°C in 2018.

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

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

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

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

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Contributions

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

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