JOURNAL OF **FISH** BIOLOGY

Seasonal dynamics of riverine fish communities using eDNA

Tristan Milhau[1] | Alice Valentini[1] | Nicolas Poulet[2] | Nicolas Roset[3] |
Pauline Jean[1] | Coline Gaboriaud[1] | Tony Dejean[1]

[1]Savoie, SPYGEN, Le Bourget du Lac, France

[2]Haute Garonne, French National Agency for Biodiversity, Pôle Écohydraulique, Toulouse, France

[3]Rhône, French National Agency for Biodiversity, Bron, France

Correspondence
Tristan Milhau, Savoie, SPYGEN, 17 rue du Lac Saint-André, Savoie Technolac, 73370 Le Bourget du Lac, France.
Email: tristan.milhau@spygen.com

Funding information
This work was financially supported by SPYGEN and the 'Agence Française pour la Biodiversité' (AFB).

Abstract

As fish communities are a major concern in rivers ecosystems, we investigated if their environmental (e)DNA signals vary according to the sampling period or hydromorphological conditions. Three rivers were studied over a year using eDNA metabarcoding approach. The majority of the species (*c.* 80%) were detected all year round in two rivers having similar hydromorphological conditions, whereas in the river affected by an upstream lake waterflow, more species were detected sporadically (42%). For all the rivers, in more than 98% of the occasional detections, the reads abundance represented <0.4% of the total reads per site and per sampling session. Even if the majority of the fish communities remained similar over the year for each of the three rivers, specific seasonal patterns were observed. We studied if the waterflow or the reproduction period had an effect on the observed dynamics. Waterflow, which influences eDNA downstream transportation, had a global influence in taxonomic richness, while the fishes' reproductive period had only an influence on certain species. Our results may help selecting the best sampling strategy according to research objectives. To study fish communities at local scale, seasons of low waterflow periods are recommended. This particularly helps to restraint effects of external eDNA coming from connections with other aquatic environment (tributaries, lakes, wetlands, sewage effluents, *etc.*). To obtain a more integrative overview of the fish community living in a river basin, high waterflow or breeding seasons are preferable for enhancing species detection probability, especially for rare species.

KEYWORDS

eDNA metabarcoding, environmental DNA, riverine fish, seasonal dynamics

1 | INTRODUCTION

Environmental surveys are essential to evaluate the effects of global changes and human activities on biodiversity (Pimm *et al.*, 2014). In aquatic ecosystems, such as rivers and lakes, fish populations are of particular interest due to their key role in ecosystems interactions (Jeppesen *et al.*, 2010), their socioeconomic importance and their responsiveness to various environmental stressors. Traditional methods like electrofishing (EF) and gillnets have been used for years to investigate fish communities in rivers (Beaudou *et al.*, 2007). EF has

lower adverse effect on fish survival than gillnets (McMichael, 1993), but its efficiency is reduced in large and deep rivers (Zajicek & Wolter, 2018) and also varies between species. An innovative and alternative approach is the use of environmental (e)DNA, which is defined as the DNA that can be extracted from the environment without isolating any target organisms (Taberlet *et al.*, 2012). eDNA assets include non-invasive methods (Thomsen & Willerslev, 2015) and a high detection capacity in comparison with traditional inventories, particularly in large rivers (Pont *et al.*, 2018). Moreover, this approach is cost-effective compared with conventional surveys (Biggs *et al.*, 2015;

Dejean *et al.*, 2012; Miya *et al.*, 2015) and can be easily standardised (Leese *et al.*, 2016; Taberlet *et al.*, 2018). Coupled with high-throughput sequencing (eDNA metabarcoding), a high number of species can be simultaneously detected, thereby allowing complete and accurate biodiversity assessments (Cilleros *et al.*, 2019; Civade *et al.*, 2016; Hänfling *et al.*, 2016; Olds *et al.*, 2016; Pont *et al.*, 2018; Shaw *et al.*, 2016; Valentini *et al.*, 2016).

Despite potential limitations caused by a non-optimised sampling strategy or molecular analysis, species detection using eDNA metabarcoding depends on eDNA transportation and persistence in aquatic environments (Barnes & Turner, 2016). This is why several authors have investigated the dynamics of eDNA, especially among fish species and have demonstrated the capacity of eDNA to provide information on spatial dynamics of fish communities (Civade *et al.*, 2016; Pont *et al.*, 2018; Wilcox *et al.*, 2016). Wilcox *et al.* (2016) and Pont *et al.* (2018) hypothesised that eDNA may behave in the water column like fine particulate organic matter and that eDNA detection distance range is influenced by river depth and water velocity. On a temporal scale, some studies have investigated how the fish communities change over time and what are the factors that may influence those changes, with, for instance, evaluation of eDNA barcoding for monitoring the spawning activity of fish species (Antognazza *et al.*,

2019; Bylemans *et al.*, 2017; Tillotson *et al.*, 2018). In a case study, it has been proved that the seasonal patterns detected with eDNA for marine fishes in an urban estuary were related to fish movements (Stoeckle *et al.*, 2017), whereas, for lake fish species, they were due to the physical characteristics of the water column (*i.e.*, water stratification, Handley *et al.*, 2019).

We investigated the seasonal variations on fish eDNA signals in three rivers: a major river, one of its affluent and a small stream that is the unique outlet of a large lake. We then examined the role played by the hydrology and the reproductive cycle, from the liberation of gametes to egg hatching (Keith *et al.*, 2011).

2 | MATERIALS AND METHODS

2.1 | Study area and context

The three sampling sites were located in eastern France (Rhone River basin; Figure 1) and presented different hydromorphological patterns. The first sampling site was located in the Rhone River, 18 km upstream of Lyon and 7.5 km downstream of the confluence with the Ain River (Figure 1a). It is situated immediately upstream from a
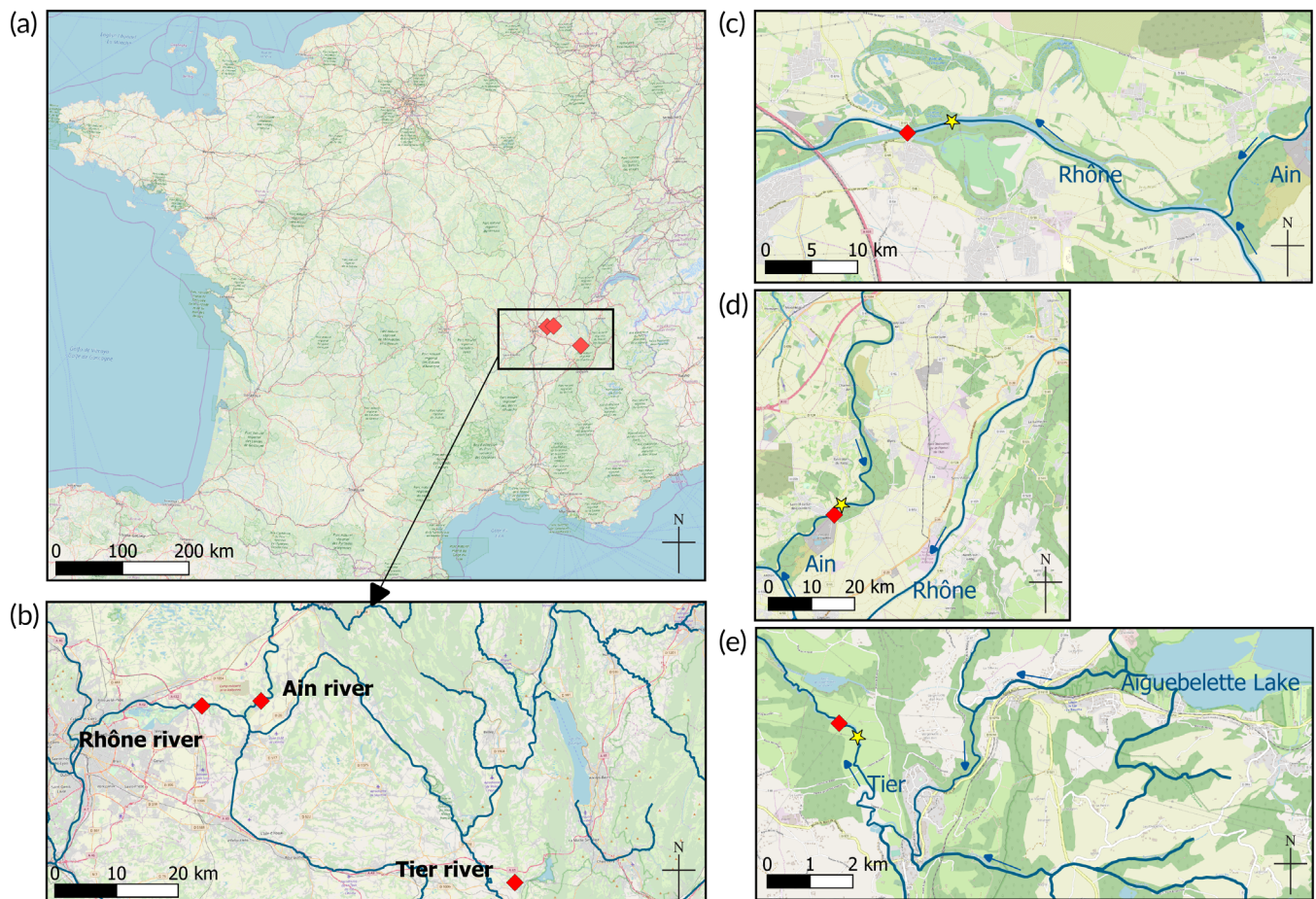

FIGURE 1 (a) Locations of rivers sampled within France and (b) the Rhone Basin: (c) the Rhone River; (d) the Ain River; (e) the Tier River. ←, Direction of river flow. (Charts from QGIS 3.6 Noosa). (☆) Electrofishing station and (◆) eDNA station

hydroelectric dam. The Rhone River is the 3rd longest river in France (812 km) and at the sampling point is 150 m wide and > 2 m deep. The second sampling site was located in the Ain River, 4.5 km upstream of the confluence with the Rhone River (Figure 1b). The Ain River is 190 km long and 80 m wide and 1 m deep at low flow. The third sampling site was located in the Tier River, 6.9 km downstream of Aiguebelette Lake that has a surface area of 545 ha (Figure 1c). The Tier River is a small stream, 13 km long and 10 m wide, with a mean depth of <0.5 m during low flow. This stream is the unique outlet of the lake. A dam is situated 1.6 km downstream from the lake outlet and the first 7 km below this dam are characterised by a regulated and residual flow (Civade et al., 2016).

Existing hydrometric stations provide information on the average daily waterflow of the Rhone (source: Compagnie Nationale du Rhone) and the Ain rivers (N° V2942010; source: Direction régionale – Ministère de l'Environnement), from which we calculated an average waterflow over 3 days: the sampling day and 2 days before. For the Tier River, a hydrometric station is located at the outlet, but no information on the waterflow was available for the sampling period.

2.2 | Electrofishing surveys

Fish communities on the Rhone and the Ain River have been monitored since 1995 by EF. Since 2007, they have been sampled using a point abundance sampling strategy (100 points for the Rhone River, 75 points for the Ain River) that was recently adapted for the Water Framework Directive monitoring (EC, 2000). This protocol consists in fishing partially in a river at various selected locations according to hydrological characteristics (substratum, water velocity, depth) and relative fish abundance on a river section (Tomanova et al., 2013). To compare fish communities inventories made by EF with our results from eDNA surveys, we chose to extract data for the 2 years corresponding to our surveys: 2015 and 2016 (September for the Rhone River, July for the Ain River; http://www.naiades.eaufrance.fr/acces-donnees#/hydrobiologie). EF data for the Tier River refer to the station R2 fished in April 2014 in the study of Civade et al. (2016), with a removal multi-pass strategy that consists in fishing entirely a short and representative section of the river (Vehanen et al., 2013).

2.3 | Environmental DNA metabarcoding analysis

Each of the three stations was sampled at an interval of 2 months from October 2015 to August 2016. eDNA sampling was performed using a filtration device (SPYGEN VigiBOAT; www.spygen.com; nominal flow of 1.1 l min^{-1}), a VigiDNA 0.45 µM filtration capsule (SPYGEN) and a disposable sterile tubing for each sample. For each station and at every sampling session, water filtration was performed on the left bank, on the right bank and in the middle of the watercourse for 30 min with volume of 30 l; 54 water samples were collected. At the end of each filtration, the water inside the capsule was emptied and the capsule was filled with 80 ml of CL1 Conservation buffer (SPYGEN) and stored at room temperature.

DNA extraction, amplification using teleo primers (Valentini et al., 2016), high-throughput sequencing and bioinformatic analysis were performed following the protocol described in Pont et al. (2018). Library preparation and sequencing were performed at Fasteris (www.fasteris.com). The libraries were prepared using the Fasteris MetaFast protocol (www.fasteris.com/dna/?q=content/ metafast-protocol-ampliconmetagenomic-analysis) and paired-end sequencing (2 × 125 bp) was carried out on an Illumina HiSeq 2500 sequencer (www.illumina.com) with the HiSeq SBS Kit v4 (Illumina) following the manufacturer's instructions. Nine libraries were sequenced across seven HiSeq runs. To monitor possible contaminants, six negative extraction controls and seven negative PCR controls (ultrapure water) were amplified (12 replicates for each control) and sequenced in parallel to the samples.

Sequence reads were analysed using programs implemented in the OBITools package (http://metabarcoding.org/obitools; Boyer et al., 2016) following a protocol already described in Valentini et al. (2016). The forward and reverse reads were assembled using the illuminapairedend program using a minimum score of 40 and by retrieving only joined sequence. The reads were then assigned to each sample using the ngsfilter program. A separate data set was created for each sample by splitting the original data set in several files using obisplit. After this step, each PCR replicate was analysed individually before merging the taxon list for final ecological analysis. Strictly identical sequences were clustered together using obiuniq. Sequences shorter than 20 bp, or with occurrence lower than 10 reads were excluded using the obigrep program. The obiclean program was then run within a PCR replicate. All sequences labelled internal, that most likely correspond to PCR substitutions and indel errors, were discarded. 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. Finally, considering the bad assignment of a few sequences to the wrong sample due to tag-jumps (Schnell et al., 2015), all sequences with an occurrence frequency < 0.001 per taxon and per library were discarded. These thresholds were set empirically in our global data production procedure (Barba et al., 2014). After the bioinformatic analysis, taxa present in only one PCR replicate and in only one field replicate were discarded (Ficetola et al., 2015).

As the total number of DNA reads varied among samples and HiSeq runs, it was standardised to ensure that the numbers of reads per taxon were comparable among sites and sampling sessions and thus could be interpreted in terms of relative abundance (Stæhr et al., 2016). All eDNA samples were resampled to randomly select 241,353 reads per sample (R package MASS, function sample without replacement; www.r-project.org), which was the smallest total number of reads found in one sample. All taxa detected from the initial dataset were still found after resampling and the proportions of reads per species and per samples were identical before and after

resampling. For each month, a mean value was calculated from the number of taxa detected from the three filtered water samples per river.

The molecular marker used in this study did not discriminate species between the vairone *Telestes souffia* (Risso 1827), the common nase *Chondrostoma nasus* (L. 1758) and the south-west European nase *Parachondrostoma toxostoma* (Vallot 1837) (identified as a group: Cho-Tel), or between the grass carp *Ctenopharyngodon idella* (Valenciennes 1844) and the silver carp *Hypophthalmichthys molitrix* (Valenciennes 1844) (identified as a group: Cte-Hyp). Within some genera, the following species were not differentiated: black bullhead *Ameiurus melas* (Rafinesque 1820) and brown bullhead *Ameiurus nebulosus* (LeSueur 1819) (*Ameiurus* spp.); arctic char *Salvelinus alpinus* (L. 1758) and brook trout *Salvelinus fontinalis* (Mitchill 1814) (*i.e.*, *Salvelinus* spp.); ide *Leuciscus idus* (L. 1758) and common dace *Leuciscus leuciscus* (L. 1758) (*i.e.*, *Leuciscus* spp.); crucian carp *Carassius carassius* (L. 1758), goldfish *Carassius auratus* (L. 1758) and Prussian carp *Carassius gibelio* (Bloch 1782) (*i.e.*, *Carassius* spp.); twaite shad *Alosa fallax* (Lacepède 1803) and allis shad *Alosa alosa* L. (*i.e.*, *Alosa* spp.); all the French *Cottus* species (*Cottus* spp.); European brook lamprey *Lampetra planeri* (Bloch 1784) and river lamprey *Lampetra fluviatilis* (L. 1758) (*i.e.*, *Lampetra* spp.). One MOTU was identified as *Barbus* spp. even though molecular marker can differentiate the barbel *Barbus barbus* (L. 1758) from the Mediterranean barbel *Barbus meridionalis* Risso 1827 and one taxon was identified as Cyprinidae, which represents <1% of the total number of reads. As suggested by Stoeckle *et al.* (2017), Atlantic salmon *Salmo salar* L. 1758 (30 standardised reads in total across all samples) was removed, since this species has never been observed in the Rhone Basin and the detected DNA is probably linked to human consumption.

For each sampling session, all the three sampling points (left bank, right bank, middle of the watercourse) taken in a river were associated. To focus on the differences in fish species assemblages over time, using the number of reads per taxon, a between-class PCA (BCA; Dolédec & Chessel, 1987) among sampling months was performed on each river to identify the fish assemblages characterising each session. Differences between months were tested using a Monte Carlo test with 1000 permutations. A co-inertia analysis (Dray *et al.*, 2003) was used to investigate the co-structure of temporal patterns in fish assemblage between sampling sites: the R_V coefficient, a multivariate extension of the Pearson correlation coefficient, was calculated to measure the overall similarity between each pair of sites. A Monte-Carlo test with 1000 permutations test was conducted on the R_V coefficient to investigate the statistical significance. Multivariate analyses were performed using the ade4 1.7–13 package in R 3.5.2 (www.r-project.org). ANOVA test was executed to assess differences between annual taxonomic richness per river. Pearson's correlation analysis was performed for each species per sampling session to examine the correlations between taxon abundance (*i.e.*, the number of reads per taxon as described above) and waterflow variation. The fish taxonomic richness and the number of reads per taxon for each sampling session across the three rivers were visualised using the package ggplots2 3.1.0 in R 3.5.2.

3 | RESULTS

3.1 | Taxa recorded

In total, 39,426,801 reads (46% of the initial number of reads before filtering) were assigned to 40 fish taxa. The annual taxonomic richness recorded using eDNA metabarcoding, which is the average number of fish taxa recorded over the year, was significantly lower for the Tier River (17.6 taxa) than for the two other rivers (33.1 and 27.7 for the Rhone and the Ain River, respectively; ANOVA *P* < 0.001). Among the 40 detected taxa in the three rivers, the fish communities have 23 taxa in common (Table 1). Few taxa were site specific: European eel *Anguilla anguilla* (L. 1758) (August), *B. meridionalis* (February and August) and burbot *Lota lota* (L. 1758) (February) were only found in the Rhone River and largemouth bass *Micropterus salmoides* (Lacépède 1802) (August) was only found in the Ain River. According to the EF data, the highest species richness over 2015 and 2016 was recorded in the Rhone River, with 22 species detected, followed by the Ain River, with 18 species and finally the Tier River, with 14 species recorded in 2014 (Table 1).

3.2 | Seasonal dynamics

The majority of the species were detected all year round for the Rhone River and the Ain River (respectively 83% and 79%). For the Tier River, 58% of the species were detected in every sampling session (Figure 2a). The minimum taxonomic richness was recorded in December for the Rhone River (30.3 mean taxa) and in August for the Ain River (23.7 mean taxa), while the maximum taxonomic richness was observed in February for both rivers (35.3 and 32.3 mean taxa, respectively). In the Tier River, the lowest mean number of taxa per sampling session was obtained in October (13.7) and this value reached its maximum value in June, with a mean taxon count of 20.3 (Figure 2b). A higher seasonal variability was observed for the Ain and the Tier River (average deviation from the mean: 2.6 and 2.3, respectively) than for the Rhone River (average deviation from the mean: 1.1). Some taxa were only detected during certain periods of the year (Figure 2a): *Ameiurus* spp., *Carassius* spp., European whitefish *Coregonus lavaretus* (L. 1758) (Rhone, Ain and Tier River); *Salvelinus* spp. (Rhone and Ain River); *C. Idella–H. molitrix* (Rhone and Tier River); rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Ain and Tier River). Those taxa, considered as sporadic, were detected in less than 0.4% of the total reads per site and per sampling session, with the exception of *O. mykiss*, which was detected in 7.5% of the reads of the samples collected in the Ain River in February.

For all species, the abundance of reads varies between species, river, or period of the year (Figure 2a). Some taxa display large variations such as the group *T. souffia– C. nasus– P. toxostoma* in the Ain and the Rhone River (more than 300,000 standardised reads in April) and some taxa correspond to the majority of the yearly total number of reads for a river, like *Barbus* spp. for the Rhone River; *Barbus spp.*, *Barbus barbus* and the Eurasian minnow *Phoxinus phoxinus* (L. 1758)

TABLE 1 Fish communities recorded (x) at the three sampling sites for the electro-fishing (EF) and eDNA surveys

Fish taxa	Taxa code	Rhône River		Ain River		Tier River	
		eDNA	EF	eDNA	EF	eDNA	EF
Abramis brama	Abb	×	×	×			
Alburnoides bipunctatus	Alb	×	×	×	×	×	×
Alburnus alburnus	Ala	×	×	×	×	×	
Ameiurus spp.	Ame	×		×		×	
Anguilla anguilla	Ana	×					
Barbatula barbatula	Bab	×	×	×	×	×	×
Barbus spp.	Bar	×					
Barbus barbus	Ba	×	×	×	×	×	×
Barbus meridionalis	Bam	×					
Blicca bjoerkna	Blb	×	×	×	×		
Carassius spp.	Car	×		×		×	
Carassius auratus	Caa		×				
Chondrostoma nasus	Cho-Tel	x	×	x		×	
Parachondrostoma toxostoma							
Telestes souffia							×
Coregonus lavaretus	Col	×		×		×	
Cottus spp.	Cot	×		×		×	
Cottus gobio	Cog		×		×		
Ctenopharyngodon idella	Cte-Hyp	×				×	
Hypophthalmichthys molitrix							
Cyprinus carpio	Cyc	×		×		×	
Esox lucius	Esl	×	×	×	×	×	
Gasterosteus aculeatus	Gaa	×		×		×	
Gobio gobio	Gog	×	×	×	×	×	×
Gymnocephalus cernua	Gyc	×		×			
Lampetra spp.	Lam	×		×		×	×
Lepomis gibbosus	Leg	×	×	×	×	×	×
Leuciscus spp.	Leu	×		×		×	
Leuciscus leuciscus	Lel		×		×		×
Lota lota	Lol	×					
Micropterus salmoides	Mis			×			
Oncorhynchus mykiss	Onm	×		×		×	×
Perca fluviatilis	Pef	×	×	×	×		
Phoxinus phoxinus	Pho	×	×	×	×	×	×
Pseudorasbora parva	Psp	×	×	×			
Rhodeus amarus	Ram	×	×	×	×		
Rutilus rutilus	Rur	×	×	×	×	×	×
Salaria fluviatilis	Saf	×				×	
Salmo trutta	Sat	×		×	×	×	×
Salvelinus spp.	Sav	×		×			
Sander lucioperca	Sal	×		×			
Scardinius erythrophthalmus	Sce	×	×	×	×	×	×
Silurus glanis	Sig	×	×	×			
Squalius cephalus	Sqc	×	×	×	×	×	×

(Continues)

TABLE 1 (Continued)

Fish taxa	Taxa code	Rhône River		Ain River		Tier River	
		eDNA	EF	eDNA	EF	eDNA	EF
Thymallus thymallus	Tht	×		×			
Tinca tinca	Tit	×	×	×	×	×	
Nb. of taxa detected with eDNA		39		34		25	
Nb. of taxa only detected with eDNA		16		16		11	
Nb. of taxa detected with EF			22	18			14
Nb. of taxa only detected with EF			0	0			0
Nb. of taxa detected with both methods		22		18		14	

for the Ain River and the sea trout *Salmo trutta* L. 1758, *Cottus* spp. and the chub *Squalius cephalus* (L. 1758) for the Tier River. For all the other taxa, the number of standardised reads per sample period is fewer than 50,000 (Figure 2a).

Within every site, the BCA showed that the fish communities were significantly different between months (Rhone, Ain and Tier rivers: Monte Carlo test: $P < 0.001$). In the BCA for the Rhone River, the first axis explained 34.5% of the variance and discriminated February from April (Figure 3a). April month was significantly characterised by a greater detection of the complex *P. toxostoma–C. nasus–T. souffia*, pike-perch *Sander lucioperca* (L. 1758) and schneider *Alburnoides bipunctatus* (Bloch 1782), whereas the detection of many taxa occurred in February, including the European perch *Perca fluviatilis* L. 1758, *O. mykiss*, the tench *Tinca tinca* (L. 1758), *Cottus* spp. *and L. lota*. The second axis explained 22.2% of the variance and mainly separated August, with a higher detection of the *B. barbus*, the freshwater blenny *Salaria fluviatilis* (Asso y del Rio 1801) and the complex *C. Idella–H. molitrix* (Figure 3a). The first axis of the BCA for the Ain River explained 46.2% of the variance. It mainly discriminated February from the other periods (Figure 3b). February was characterised by a higher detection of several taxa, especially *O. mykiss*, *P. fluviatilis*., freshwater bream *Abramis brama* (L. 1758), *Salvelinus* spp. and the roach *Rutilus rutilus* (L. 1758). The second axis explained 20.8% of the variance and discriminated June from April and December. June was mainly characterised by the detection of *Ameiurus* spp. and the stone loach *Barbatula barbatula* (L. 1758), whereas the other months displayed a higher detection of *Cottus* spp., the complex *P. toxostoma–C. nasus–T. souffia* and *Leuciscus* spp. (Figure 3b). For the Tier River, the first axis of the BCA explained 38.5% of the variance and discriminated June from December and February (Figure 3c). June was mainly characterised by a higher detection of *Gobio* sp., *S. cephalus*, *A. bipunctatus* and the rudd *Scardinius erythrophthalmus* (L. 1758), while December and February showed a higher detection of brown trout *S. trutta* and *Cottus* spp. The second axis explained 24.2% of the variance and mainly discriminated April from the other months, with a higher detection of *B. barbatula*, *O. mykiss*, *Leuciscus* spp. and *S. fluviatilis* (Figure 3c). The co-intertia analysis showed a significant co-structure among the Rhone et the Ain rivers ($R_V = 0.90$, $P < 0.01$): similar assemblages of species

according to seasons were found among the Rhone and the Ain rivers. However, this was not the case for the Tier River (Tier–Rhone, $R_V = 0.85$, $P > 0.05$; Tier–Ain, $R_V = 0.75$, $P > 0.05$).

3.3 | Hydrology and eDNA

Maximum waterflow in the Rhone River was recorded in February 2016 (1030 m^3 s^{-1}) and in June 2016 (1157 m^3 s^{-1}). The Ain River showed a maximum waterflow during the same period of time, with 346 m^3 s^{-1} in February 2016 and 177 m^3 s^{-1} in June 2016 (Figure 2c). The Pearson's correlation coefficient between monthly taxonomic richness and waterflow was not significant for the Rhone River ($r = 0.599$, $P > 0.05$) but was significant for the Ain River ($r = 0.885$, $P < 0.05$; Table 2). Concerning the relation between the number of reads per taxon and the hydrological variables, the Rhone River showed a high Pearson's correlation coefficient for *A. brama*, *B. barbatula*, *C. lavaretus* and the ruffe *Gymnocephalus cernua* (L. 1758) ($r > 0.8$, $P < 0.05$). The Ain River showed a high Pearson's correlation coefficient for *A. brama*, *C. lavaretus* and *G. cernua*, Cyprinidae, common carp *Cyprinus carpio* L. 1758, *Gobio* spp., *O. mykiss*, *P. fluviatilis*, *Salvelinus* spp., *S. lucioperca* and the *T. tinca* ($r > 0.8$, $P < 0.05$; Table 2).

4 | DISCUSSION

Comparing eDNA and EF results on fish communities composition reveals similar fish assemblages, with some taxa detected only by eDNA. Traditional inventories detect species at the local scale, while eDNA surveys integrate signals of fish taxa on a larger scale (Cilleros *et al.*, 2019; Civade *et al.*, 2016; Deiner & Altermatt, 2014; Pont *et al.*, 2018). Several studies have also demonstrated that eDNA surveys have a higher detection capacity than conventional survey methods (Civade *et al.*, 2016; Hänfling *et al.*, 2016; Miya *et al.*, 2015; O'Donnell *et al.*, 2017; Port *et al.*, 2016; Thomsen *et al.*, 2012; Valentini *et al.*, 2016; Yamamoto *et al.*, 2016). This is especially true in large river systems, such as the Rhone River, where it was possible to detect all the species captured by EF within 10 years of inventories with only one eDNA sampling session (Pont *et al.*, 2018). In our study, for the

FIGURE 2 (a) Taxon abundance in terms of number of reads per sampling site, (b) mean fish taxonomic richness per sampling site (*n* = 3) and (c) mean of daily waterflow on the day of sampling and 2 days before sampling per site. Colours represent reproductive periods according to Keith *et al.* (2011) for each species; NA, taxa for which information on the reproductive period is not available. Abbreviations refer to the codes used for taxa in Table 1. Reproduction: (·) NA, (·) No, and (·) Yes. Nb. reads: (·) 1e+03, (·) 1e+04, (•) 5e+04, and (●) 3e+05
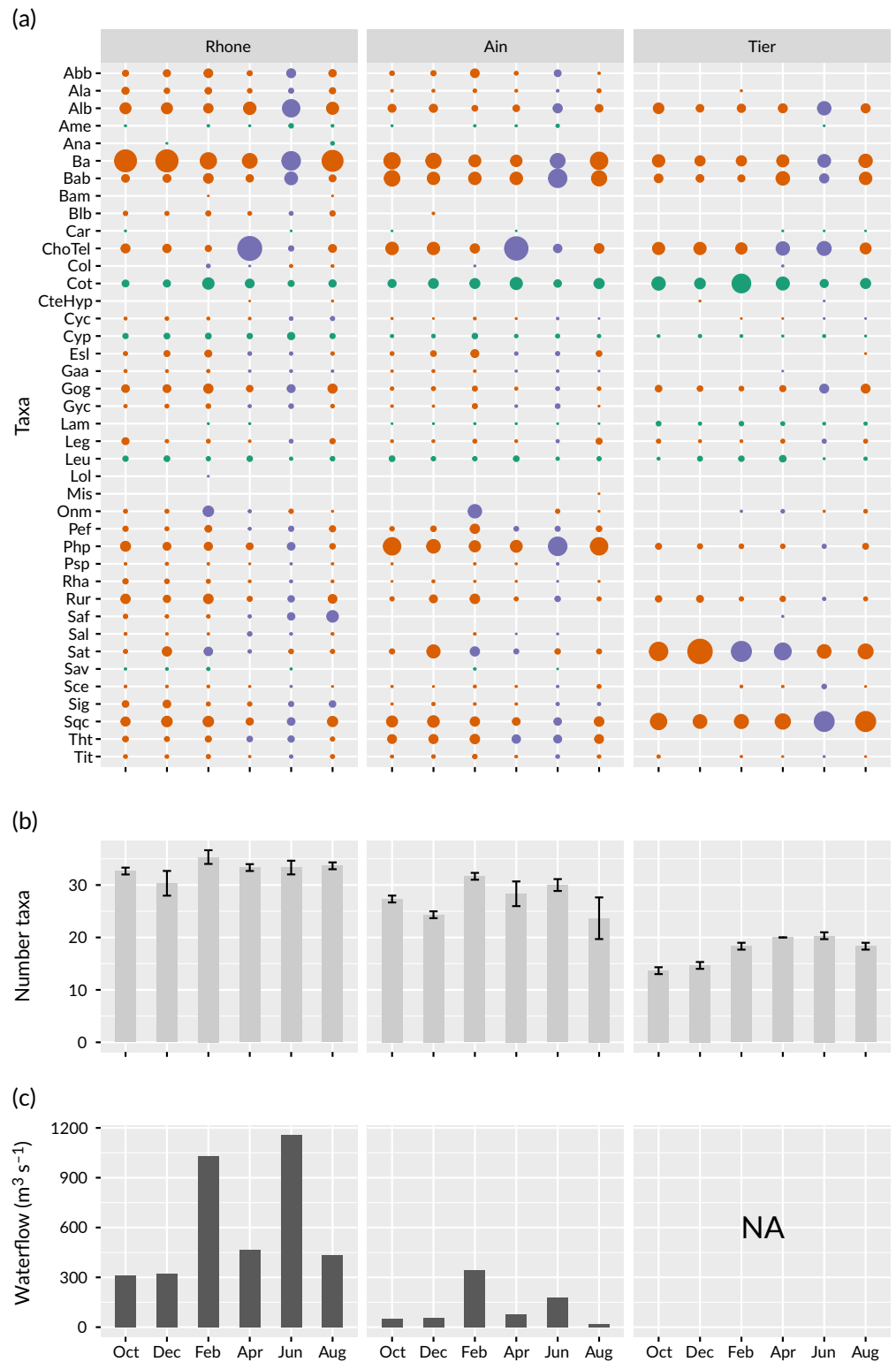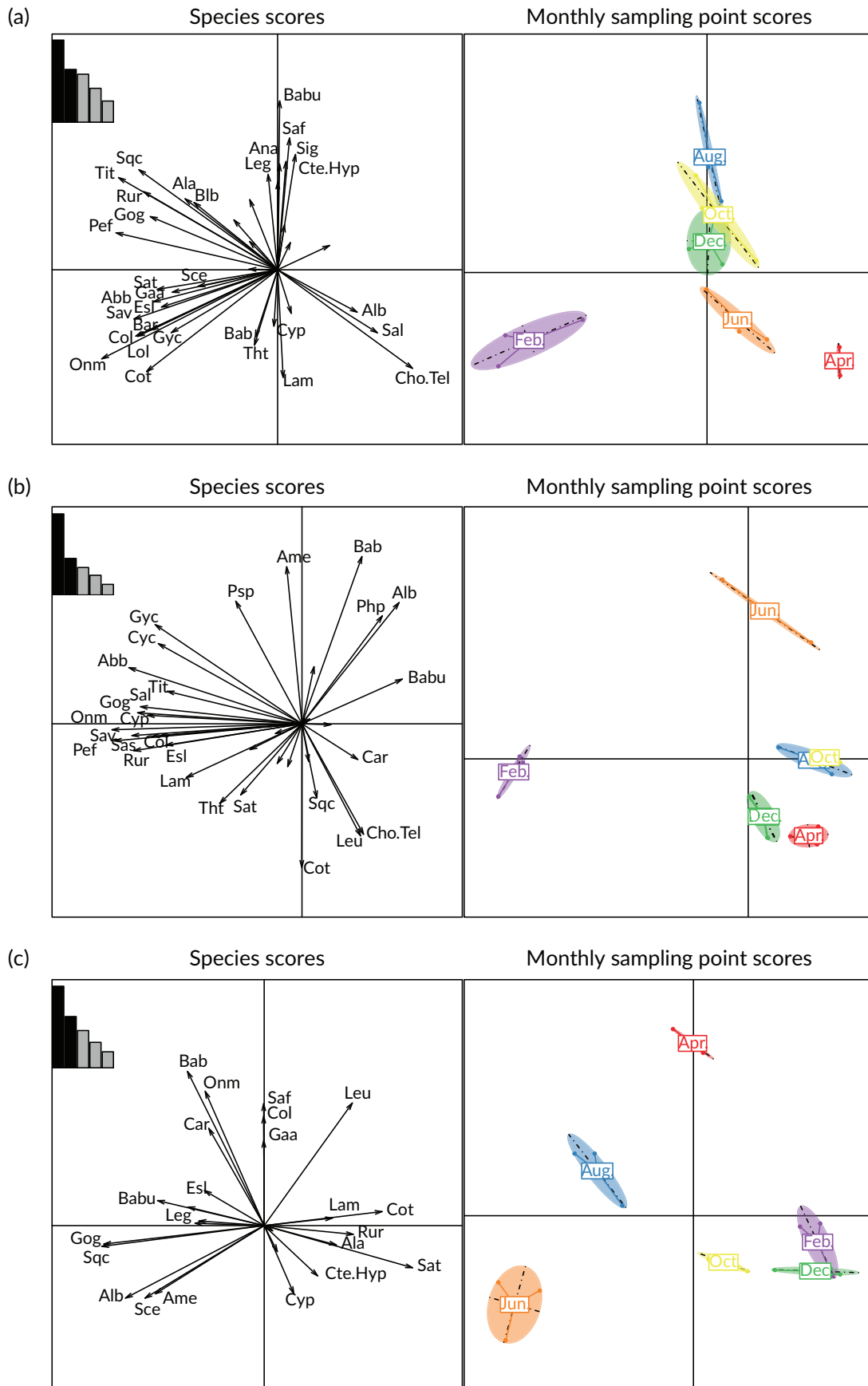
FIGURE 3 Between-class analysis on centre-normed PCA of fish communities (number of reads per taxon per sampling point) for the (a) Rhone River, the (b) Ain River, and the (c) Tier River. Abbreviations refer to the codes used for taxa in Table 1; to ease the reading, the species with the lowest scores were removed. ■, Eigen values for species scores and □, for monthly sampling point scores

TABLE 2 Pearson's correlations between waterflow and taxonomic richness and taxon at the Rhone River and the Ain River

Fish taxa	Rhône		Ain	
	r	P	r	P
Abramis brama	0.863	<0.05	0.979	<0.001
Alburnoides bipunctatus	0.531	>0.05	−0.389	>0.05
Alburnus alburnus	−0.142	>0.05	0.119	>0.05
Ameiurus spp.	0.749	>0.05	0.398	>0.05
Anguilla anguilla	−0.250	>0.05	–	>0.05
Barbatula barbatula	0.874	<0.05	−0.075	>0.05
Barbus barbus	−0.545	>0.05	−0.639	>0.05
Barbus meridionalis	−0.093	>0.05	–	–
Barbus spp.	0.538	>0.05	–	–
Blicca bjoerkna	−0.003	>0.05	−0.256	>0.05
Carassius spp.	−0.130	>0.05	−0.370	>0.05
Chondrostoma nasus–Parachondrostoma toxostoma–Telestes souffia	−0.281	>0.05	−0.271	>0.05
Coregonus lavaretus	0.846	<0.05	0.897	<0.05
Cottus spp.	0.354	>0.05	−0.112	>0.05
Ctenopharyngodon idella–Hypophthalmichthys molitrix	−0.258	>0.05	–	–
Cyprinidae	0.791	>0.05	0.963	<0.01
Cyprinus carpio	−0.044	>0.05	0.957	<0.01
Esox lucius	0.222	>0.05	0.730	>0.05
Gasterosteus aculeatus	0.293	>0.05	0.190	>0.05
Gobio gobio	0.342	>0.05	0.878	<0.05
Gymnocephalus cernua	0.911	<0.05	0.949	<0.01
Lampetra spp.	0.152	>0.05	0.771	>0.05
Lepomis gibbosus	−0.390	>0.05	−0.198	>0.05
Leuciscus spp.	−0.868	<0.05	−0.344	>0.05
Lota lota	0.538	>0.05	–	>0.05
Micropterus salmoides	–	–	−0.409	>0.05
Oncorhynchus mykiss	0.573	>0.05	0.918	<0.01
Perca fluviatilis	0.476	>0.05	0.864	<0.05
Phoxinus phoxinus	−0.093	>0.05	−0.398	>0.05
Pseudorasbora parva	−0.345	>0.05	0.697	>0.05
Rhodeus sericeus	−0.401	>0.05	−0.077	>0.05
Rutilus rutilus	−0.025	>0.05	0.769	>0.05
Salaria fluviatilis	−0.042	>0.05	–	>0.05
Salmo trutta	0.064	>0.05	0.102	>0.05
Salvelinus spp.	0.392	>0.05	0.903	<0.05
Sander lucioperca	−0.271	>0.05	0.969	<0.001
Scardinius erythrophthalmus	0.003	>0.05	−0.147	>0.05
Silurus glanis	−0.681	>0.05	−0.112	>0.05
Squalius cephalus	−0.207	>0.05	−0.336	>0.05
Thymallus thymallus	0.744	>0.05	0.305	>0.05
Tinca tinca	0.158	>0.05	0.847	<0.05
Taxonomic richness	0.599	>0.05	0.885	<0.05

species only detected by eDNA, the read abundance was generally low, with only two exceptions; *S. fluviatilis* highly detected by eDNA in August and *Cottus* spp., detected in the Tier River in April. The absence of *S. fluviatilis* in EF surveys may be explained by the difficulty to catch such a small benthic species in a large river (Pont *et al*., 2018). The presence of *Cottus* spp. in the Tier River was confirmed by historical EF data (Civade *et al*., 2016).

All the species detected in the present study for the Rhone River were also found by eDNA previous survey, as well as in the historical EF data (Pont *et al*., 2018), except *B. meridionalis*, *O. mykiss* and *Salvelinus* spp. The latter two species are commonly used in fish farming in the Rhone tributaries and kept for angling purposes. *Barbus meridionalis* has never been detected in the Rhone River (either using EF or any other traditional method), but we recorded a low eDNA signal for this taxon in February, when the waterflow was almost at its peak level. It may be possible that the signal detected was coming from a population inhabiting the upper Usses River (Syndicat Mixte d'Etude du Contrat de Rivières des Usses, 2010), a Rhone River tributary located 120 km upstream of the sampling station where *B. meridionalis* is present; a plausible distance considering eDNA downstream transportation (Pont *et al*., 2018). Elsewhere, *B. meridionalis* can hybridise with *B. barbus* (Berrebi *et al*., 1993) and a strong introgression in the *B. barbus* population was observed in southern France (Crespin *et al*., 1999). Therefore, we cannot exclude the possibility that the signal of *B. meridionalis* found in the Rhone River corresponds to the introgressed *B. barbus* population. For the Tier River, all species detected by Civade *et al*. (2016) were also found in this study. Eight detected taxa were not found in the previous eDNA study on the same station in the Tier River (R2 in Civade *et al*., 2016): *Ameiurus* spp., *Carassius* spp., *C. lavaretus*, *C. Idella–H. molitrix*, *C. carpio*, pike *Esox lucius* (L. 1758), three-spined stickleback *Gasterosteus aculeatus* (L. 1758) and *T. tinca*. Of those species, *Carassius* spp., *C. lavaretus*, *C. carpio*, *E. lucius* and *T. tinca* were detected in the lake samples (Civade *et al*., 2016) and their detection in the Tier River can be explained by eDNA transportation, probably due to a high waterflow but waterflow data on the Tier River are not available to test this hypothesis. The difference between the eDNA metabarcoding results in Civade *et al*. (2016) and our study's outcomes may also be due to sampling and DNA extraction protocols optimisation.

Our results highlight that the main structure of the fish communities does not strongly change over the year for the Rhone River and the Ain River, with respectively 83% and 79% of the whole fish community detected all around the year. Indeed, these rivers are environmentally and geographically close, with similar hydromorphological conditions. For the Tier River, 10 species out of the 24 were not found all around the year and were represented by only few reads in the samples (Figure 2a). These results may be explained by the stream hydrological specificities. The Tier River is marked by a regulated and residual flow due to the dam management. Indeed, out of the 10 sporadically detected species, seven belong to the upstream lake fish community (Civade *et al*., 2016).

Even if the majority of the fish community remains similar for the three rivers, seasonal differences were observed in the number of detected taxa and in the number of sequences reads, as a proxy for species abundance. These variations are associated with the species detected in only few sampling sessions and representing <0.4% of the read abundance per river and per sampling session for 98.3% of the cases. The seasonal dynamics may be explained by the hydrology, as a result of waterflow variations that may increase the eDNA detectability distance (Pont *et al*., 2018; Figure 2). Looking at the seasonal patterns clearly reveals that, for the Rhone and the Ain rivers, February month outstands from other seasons, with a higher taxonomic richness detected. This can be explained by hydrological conditions because of a particularly high waterflow that can contribute to the potential detection of species living upstream. *O. mykiss* presents the maximum number of reads in winter and it is the only sporadic species represented by more than 0.4% of reads per river per sampling session, but to our knowledge, it does not reproduce in these rivers. A high correlation between the number of reads and the waterflow was recorded for this river ($r = 0.918$ $P < 0.01$), so the eDNA signal for this species may potentially arise from the fish farms upstream.

The spawning period may also play a significant role in the variation in the number of reads, since the concentration of cells released in the water (gametes), as well as the density of individuals (larvae), increase during the reproductive period. Furthermore, during this period, adult metabolism might be accelerated, increasing excretion and cell release (Maruyama *et al*., 2014). The presence of *S. fluviatilis* DNA in the Rhone River may be due to the spawning activity since the read abundance peak values correspond to the breeding season. By comparing the Rhone River and the Ain River communities in February, it appears that some species are highly detected, which may be related to their winter activity, such as *L. lota*, *S. trutta* (0+ emergence), or *E. lucius* (spawning in lateral channels; Keith *et al*., 2011). For the Tier River, the fish communities in February and December are characterised by the strong detection of *S. trutta*, which could be associated with the species reproductive period. In the Rhone and the Ain rivers, the fish community in April was different from February, with April being the reproductive period for rheophilic Cyprinidae, such as *L. leuciscus*, *T. souffia*, *C. nasus* and *P. toxostoma* (Keith *et al*., 2011). Therefore, it is not clear if eDNA transport distance or spawning are responsible for this result.

Disentangling the effects of hydrology and spawning on seasonal detection remains difficult. In the Ain and the Rhone rivers, we show that fish communities in June and February were distinct (Figure 3), which seems correlated to the high waterflow during this period. However, this is also a very active period for a majority of species in terms of reproduction, which can explain the occurrence of particular communities in the Tier River as well as in the Rhone and the Ain rivers (reproduction, for example, of gudgeon *Gobio gobio* (L. 1758), *S. cephalus* and *S. erythrophthalmus*; Keith *et al*., 2011). Some species, such as *Barbus* spp. in the Rhone River; *B. barbatula* and the *P. phoxinus* in the Ain River and the Tier River, exhibited an increase in the number of reads independent from a high waterflow or a reproductive period. At this point and without any other information, this

abundance peak may be linked to the occurrence of local fish schools immediately upstream of the sampling zone.

In this study, we present the dynamics of eDNA seasonal detection in fish communities living in three French rivers. Hydrology *via* the water flow and ecology *via* the reproductive period, can explain some of these seasonal variations. It is difficult to find accurate explanations at the community scale, as both factors can influence seasonal dynamics. Nevertheless, our investigation clearly highlighted the role of rivers hydromorphogical variables in fish species detection, with notable differences associated with the river typology. In environments with low contribution from connections with other aquatic environment a better homogeneity in fish communities detected by eDNA over the year was observed. In contrast, when the eDNA signals is influenced by other aquatic environment (such as for the Tier River), the eDNA seasonal dynamics are more important and detection stochasticity is higher. Nevertheless, it is possible to exclude this background noise by setting a threshold for all the species represented by <0.4% of reads abundance, in order to have only the local fish species. Because eDNA metabarcoding approach is sensitive enough to highlight temporal variations in fish species detections, the sampling period and the locations should be carefully selected according to research objectives and river typology, in order to avoid signal interferences. For studying fish communities at the local scale, it is recommended to sample during low flow periods and far from connections with any other aquatic environment (*e.g.*, tributaries, lakes, wetlands, sewage effluents *etc.*). On the other hand, to obtain a more integrative overview of the fish community living in a river basin, a high waterflow period should be selected. Finally, as the species detection probability is increased by high waterflow and during reproductive periods, it is preferable to target these seasons to enhance the chances to detect rare, endangered or invasive species.

CONTRIBUTIONS
T.D. and N.P. designed the study. P.J. collected the eDNA samples in the field. C.G. conducted the laboratory analysis and A.V. conducted the bioinformatics analyses. T.M., A.V., N.P. and N.R. analysed the data, performed the statistical analysis and prepared the Figures. T.M., A.V., N.P., N.R. and T.D. wrote most of the manuscript, with significant contributions from all the authors.

COMPETING INTERESTS
Teleo primers and the use of the amplified fragment for identifying fish species from environmental samples are patented by the CNRS and the Université Grenoble Alpes. This patent only restricts commercial applications and has no implications for the use of this method by academic researchers. SPYGEN owns a licence for this patent. T.M., A.V., P.J., C.G. and T.D. are research scientists at a private company specialising in the use of eDNA for species detection.

ORCID
Tristan Milhau https://orcid.org/0000-0003-0120-1384
Alice Valentini https://orcid.org/0000-0001-5829-5479
Nicolas Poulet https://orcid.org/0000-0002-5611-0749

REFERENCES
Antognazza, C., Britton, R. J., Potter, C., Franklin, E., Hardouin, E. A., Roberts, C., ... Andreou, D. (2019). Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of European anadromous shads (*Alosa* spp.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 148–152.

Barba, D. M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, 14, 306–323.

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, 17, 1–17.

Beaudou, D., Richard, S., Roset, N., & Saint-Olympe, L. (2007). Réseau hydrobiologique et piscicole - bassin Rhone Méditerranée et Corse. In *Synthèse des données de 1995 à 2004* (p. 103). France: Office National de l'Eau et des Milieux Aquatiques - Délégations inter-régionales de Lyon, Dijon et Montpellier.

Berrebi, P., Cattaneo-Berrebi, G., & Brun, N. (1993). Natural hybridisation of two species of tetraploid barbels: *Barbus meridionalis* and *Barbus barbus* (Osteichtyes, Cyprinidae) in southern France. *Biological Journal of the Linnean Society*, 48, 319–333. https://doi.org/10.1111/j.1095-8312.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... Dunn, F. (2015). Using EDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, 183, 19–28.

Boyer, F., Mercier, C., Bonin, A., Bras, Y. L., Taberlet, P., & Coissac, E. (2016). Obitools: A Unix-inspired software package for DNA Metabarcoding. *Molecular Ecology Resources*, 16, 176–182.

Bylemans, J., Furlan, E. M., Hardy, C. M., McGuffie, P., Lintermans, M., & Gleeson, D. M. (2017). An environmental DNA-based method for monitoring spawning activity: A case study, using the endangered Macquarie perch (*Macquaria australasica*). *Methods in Ecology and Evolution*, 8, 646–655.

Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., ... Brosse, S. (2019). Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Molecular Ecology Resources*, 19, 27–46.

Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J.-C., Bonin, A., ... Pont, D. (2016). Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. *PLoS One*, 11, e0157366.

Crespin, L., Berrebi, P., & Lebreton, J. D. (1999). Asymmetrical introgression in a freshwater fish hybrid zone as revealed by a morphological index of hybridisation. *Biological Journal of the Linnean Society*, 67, 57–72. https://doi.org/10.1111/j.1095-8312.

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS One*, 9, e88786.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology*, 49, 953–959.

Dolédec, S., & Chessel, D. (1987). Rythmes saisonniers et composantes stationnaires en milieu aquatique I- Description d'un plan d'observations complet par projection de variables. *Acta Oecologica, Oecologia Generalis*, 8, 403–426.

Dray, S., Chessel, D., & Thioulouse, J. (2003). Co-inertia analysis and the linking of the ecological data tables. *Ecology*, *84*, 3078–3089.

EC (2000). Directive 2000/60/EC of the European Parliament and of the Council of October 23, 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L 327: 1–72. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:327:0001:0072:EN:PDF

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., Barba, M., et al. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA Metabarcoding data. *Molecular Ecology Resources*, *15*, 543–556.

Handley, L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., et al. (2019). Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. *Environmental DNA*, *1*, 26–39.

Hänfling, B., Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., … Winfield, I. J. (2016). Environmental DNA Metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*, 3101–3119.

Jeppesen, E., Meerhoff, M., Holmgren, K., González-Bergonzoni, I., Mello, F., Declerck, S. A., et al. (2010). Impacts of climate warming on lake fish community structure and potential effects on ecosystem function. *Hydrobiologia*, *646*, 73–90.

Keith, P., Persat, H., Feunteun, É., & Allardi, J. (2011). *Les Poissons d'eau douce de France*. France: Biotope.

Leese, F., Altermatt, F., Bouchez, A., Ekrem, T., Hering, D., Meissner, K., … Zimmermann, J. (2016). DNAqua-net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *Research Ideas and Outcomes*, *2*, e11321. https://doi.org/10.3897/rio.2.e11321

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PLoS One*, *9*, e114639.

McMichael, G. A. (1993). Examination of electrofishing injury and short-term mortality in hatchery rainbow trout. *North American Journal of Fisheries Management*, *13*, 229–233.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J., Sato, K., et al. (2015). MiFish, a set of universal PCR primers for Metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society of Open Science*, *2*, 150088. https://doi.org/10.1098/rsos.150088.

O'Donnell, J. L., Kelly, R. P., Shelton, A., Samhouri, J. F., Lowell, N. C., & Williams, G. D. (2017). Spatial distribution of environmental DNA in a nearshore marine habitat. *PeerJ*, *5*, e3044.

Olds, B. P., Jerde, C. L., Renshaw, M. A., Li, Y., Evans, N. T., Turner, C. R., … Lamberti, G. A. (2016). Estimating species richness using environmental DNA. *Ecology and Evolution*, *6*, 4214–4226.

Pimm, S., Jenkins, C., Abell, R., Brooks, T., Gittleman, J., Joppa, L., … Sexton, J. (2014). The biodiversity of species and their rates of extinction, Ddistribution and protection. *Science*, *344*, 1246752.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., … Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*, 10361.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., … Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology*, *25*, 527–541.

Schnell, I., Bohmann, K., & Gilbert, T. M. (2015). Tag jumps illuminated – reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, *15*, 1289–1303.

Shaw, J. L., Weyrich, L., & Cooper, A. (2016). Using environmental (e)DNA sequencing for aquatic biodiversity surveys: A beginner's guide. *Marine and Freshwater Research*, *68*, 20–33.

Stæhr, P. A., Santos, S., Hansen, L. H., Lundsteen, S., Haraguchi, L., Dahl, K., … Winding, A. (2016). *Comparison of eDNA and conventional techniques for monitoring species diversity of Boulder Reefs in Danish waters*. Denmark: *DCE - Danish Centre for Environment and Energy*. https://doi.org/10.13140/RG.2.2.27500.16005

Stoeckle, M. Y., Soboleva, L., & Charlop-Powers, Z. (2017). Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PLoS One*, *12*, e0175186.

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. France: Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*, 1789–1793.

Thomsen, P., Kielgast, J., Iversen, L., Møller, P., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One*, *7*, e41732.

Thomsen, P., & Willerslev, E. (2015). Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18.

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning Salmon abundance at fine spatial and temporal scales. *Biological Conservation*, *220*, 1–11.

Tomanova, S., Tedesco, P., Roset, N., dit Thomas, B. R., & Belliard, J. (2013). Systematic point sampling of fish communities in medium- and large-sized rivers: Sampling procedure and effort. *Fisheries Management and Ecology*, *20*, 533–543.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*, 929–942.

Vehanen, T., Sutela, T., Jounela, P., Huusko, A., & Mäki-Petäys, A. (2013). Assessing electric fishing sampling effort to estimate stream fish assemblage attributes. *Fisheries Management and Ecology*, *20*, 10–20.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., … Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216.

Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., Murakami, H., … Kondoh, M. (2016). Environmental DNA as a 'snapshot' of fish distribution: A case study of Japanese Jack mackerel in Maizuru Bay, sea of Japan. *PLoS One*, *11*, e0149786.

Zajicek, P., & Wolter, C. (2018). The gain of additional sampling methods for the fish-based assessment of large rivers. *Fisheries Research*, *197*, 15–24.