

# Analysis of a coastal North Sea fish community: Comparison of aquatic environmental DNA concentrations to fish catches

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## Abstract

Analyses of environmental DNA have the potential to become an integrated tool in fish research and management. We performed a pilot study during the spring migration of fishes from the North Sea into the Wadden Sea and present comparative results from daily fyke catches (20 mm mesh) in the Marsdiep tidal inlet on two locations and results from weekly fish eDNA analyses on three locations, all within 2 km distance.

Fish catches did not differ significantly between the two locations, whereas the eDNA composition showed a significant location effect. However, when eDNA analysis was restricted to species that were caught with the fykes, differences among locations became insignificant. Over ten weeks, from late April to early July, presence-absence calls of fishes based on weekly eDNA sampling significantly agreed with calls based on seven days of fyke fishing 1 km westwards. Fish eDNA compositions differed significantly among sample days and months but not between tides. Over the season, patterns in eDNA concentration (12S rRNA gene copies/L) inferred from quantitative PCR and Illumina HiSeq community composition corresponded to patterns in wet mass for the eight most abundant fish species in the fyke (>6 weeks present) despite changes in water temperature and changes in fish size class. Small sandeel and gobies, which are important prey for large fishes and birds, were typically missed with the fyke but contributed up to 25%–40% of the fish eDNA depending on the sample location.

## KEYWORDS

biodiversity, biological monitoring, biomass, DNA sequencing, fishes, marine ecology, methodology, seasonal variation

van Bleijswijk and Engelmann contributed equally to this work.

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## 1 | INTRODUCTION

The shallow Dutch Wadden Sea, part of Europe's largest estuarine area, is known as an important nursery and feeding area for bottom-dwelling and pelagic fishes (Bergman et al., 1989; Dickey-Collas, Bolle, Van Beek, & Erftemeijer, 2009; Zijlstra, 1972, 1983). Continual measurements of fish fauna are essential to track the use of the area and to detect changes over time. Therefore, fish fauna has been monitored (figure 1 in Jager et al., 2009) for nearly 60 years by various types of annual trawl (e.g., Bolle et al., 2009; Tulp, Bolle, & Rijnsdorp, 2008), acoustic surveys (e.g., Couperus et al., 2016), and a daily fyke net catch program in the Marsdiep, at the border of the coastal North Sea and the western Wadden Sea (van der Meer, Witte, & van der Veer, 1995; van der Veer et al., 2015). Each sampling strategy has its own specific bias. Combining all surveys provides the best indication of the fish fauna of the area. Although both demersal and pelagic fishes are caught with the fyke, we cannot exclude the possibility that some species or some life stages of species are not sampled or underrepresented.

An alternative or complementary approach might be the analyses of environmental DNA (eDNA). Surveys based on eDNA (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012) have already been applied for a variety of organisms (Pedersen et al., 2015). Environmental DNA is released from its source organism, via, for example, feces, slime, and skin flakes, and can be harvested by sampling the environmental media (e.g., fresh or marine water, sediment, soil, or air). For marine fishes, a pilot study in Danish coastal water (Thomsen, Kielgast, Iversen, Wiuf, et al., 2012) showed that small volumes (0.5 L) of seawater contained eDNA from a wide range of local fish species. Currently, over twenty field studies have been published on marine fish eDNA and it has been concluded that with informed insights into advantages and disadvantages, analyses of eDNA can become an integrated tool in fisheries assessment and management (Hansen, Bekkevold, Clausen, & Nielsen, 2018).

Interpretation of eDNA results is still hampered by a lack of knowledge on the processes that determine the rates of eDNA release and degradation. The rate of eDNA release varies with fish taxa (Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016), life stage (Jo, Murakami, Yamamoto, Masuda, & Minamoto, 2019; Maruyama, Nakamura, Yamanaka, Kondoh, & Minamoto, 2014), physiological stress (Pilliod, Goldberg, Arkle, & Waits, 2014; Thomsen, Kielgast, Iversen, Møller, et al., 2012), and metabolic rate (Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012). The rate of eDNA degradation is mainly dependent on environmental factors, of which the most important are probably temperature (Strickler, Fremier, & Goldberg, 2015; Tsuji, Ushio, Sakurai, Minamoto, & Yamanaka, 2017) and microbial phosphate limitation (Salter, 2018). However, it may already be possible for well-characterized systems to predict fish species abundance from eDNA surveys using multivariate models incorporating biotic and abiotic variables (Tillotson et al., 2018).

Here, we test whether eDNA sampled from the NIOZ jetty can provide an easy and complete "fingerprint" of the fish community

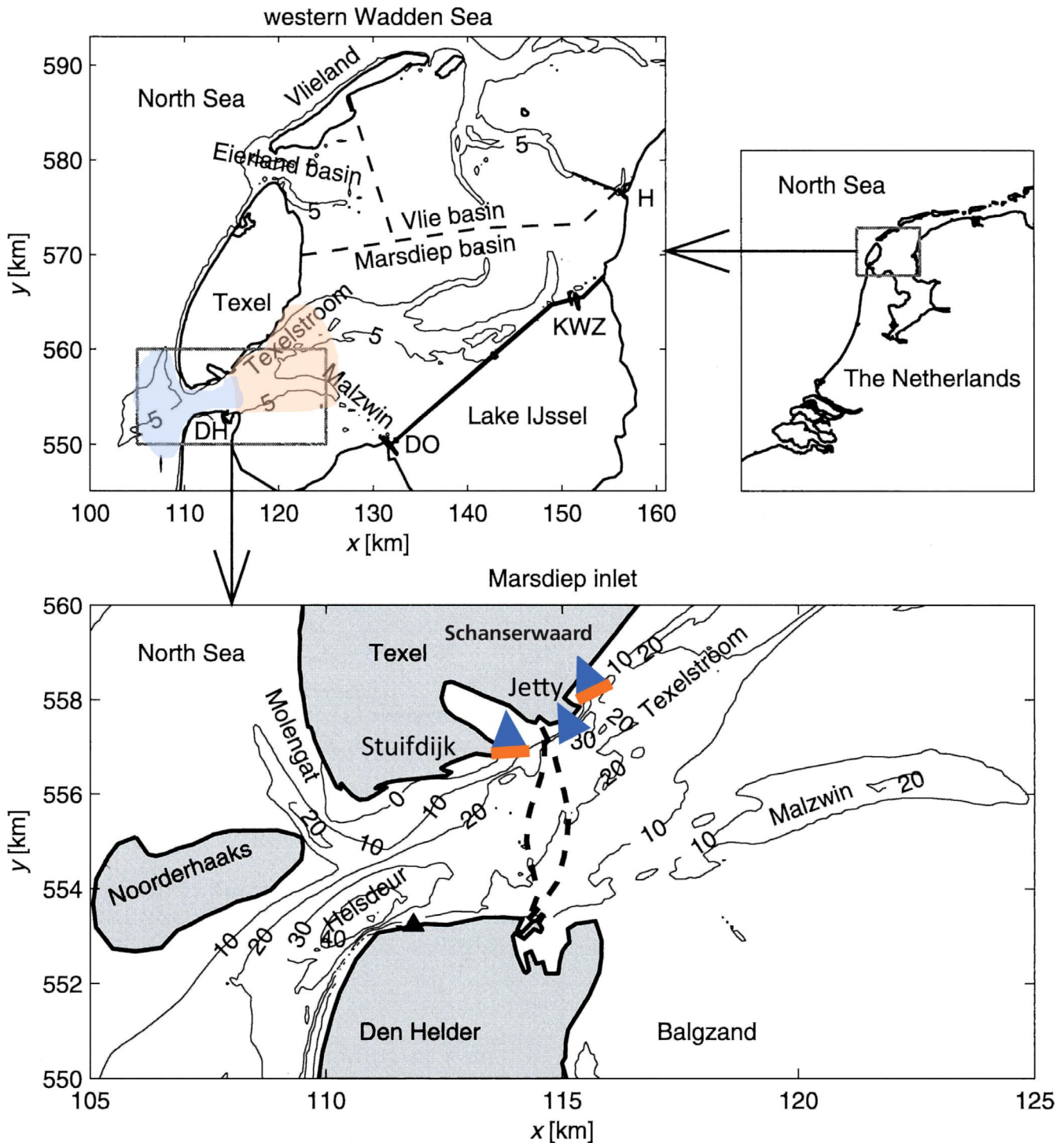
in the highly dynamic coastal North Sea–Wadden Sea area by comparing eDNA results with daily fyke catches. Environmental DNA was sampled weekly from surface water off the NIOZ jetty at high tide and at low tide over 18 weeks, from April to September 2015. For comparison, eDNA samples were also taken 1 km west and 1 km northeast of the jetty. Community compositions, including bony fishes as well as cartilaginous fishes, were inferred from Illumina HiSeq sequence analyses of 12S rRNA gene amplicons. The methodology was tested on an artificial (mock) community with 30 bony fish species, two cartilaginous fish species, and eight marine invertebrate species. Quantitative PCR, in many studies used for eDNA quantification of single species (e.g., Doi et al., 2015; Fernandez et al., 2018; Harper et al., 2018; Knudsen et al., 2019; Nevers et al., 2018; Weltz et al., 2017), was here used to determine the *in situ* eDNA concentration (copies/L seawater) of the entire vertebrate community (Ushio et al., 2018). Species-specific eDNA concentrations of 39 fishes were inferred from the total eDNA concentration and the relative read abundance.

The aims of this study were to test the applicability of fish eDNA analyses by (a) comparing the presence–absence calls of fish species based on eDNA to fish catches; (b) evaluating the effects of eDNA sampling time, with respect to tide, on presence–absence calls; (c) investigating relationships between *in situ* eDNA concentration (copies per liter Marsdiep water) and fish mass or fish counts extracted from the fyke results; and (d) comparing seasonal patterns in eDNA abundance of the dominant fish species to the patterns found in the fyke catches.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites

Aquatic eDNA was sampled from the Marsdiep tidal inlet between the Dutch coastal North Sea and the western Wadden Sea off the NIOZ jetty (53°00'06.57"N, 04°47'20.43"E) and for comparison on five days also at Stuifdijk (52°59'48.85"N, 04°46'25.43"E) and Schanserwaard (53°00'28.42"N, 04°47'51.42"E) (Figure 1). The inlet is about 4 km wide and has a maximum depth of 28 m. At the eDNA sampling locations, the water depth varies between 2 and 4 m. The physical characteristics of the area have been described in detail (Ridderinkhof, 1988; Sassi, Gerkema, Duran-Matute, & Nauw, 2016 and references therein). In the Marsdiep inlet, tides constitute up to 81% of the total variance of water levels measured at Den Helder and 98% of the water transport and current velocities (Buijsman & Ridderinkhof, 2007a; 2007b). The semidiurnal tide has a tidal range between 1 and 2 m and near-surface currents between 1 and 1.8 m/s with a net outflow toward the North Sea (Buijsman & Ridderinkhof, 2007b). The currents change direction 1–2 hr after the tidal maximum or minimum water level (Ijseldijk, Camphuysen, Nauw, & Aarts, 2015). The potential source area of fresh eDNA, released within 6 hr prior to sampling at the NIOZ jetty, was estimated based on maps of the surface currents (hydrographical service



**FIGURE 1** Sample locations in the Marsdiep tidal inlet between the Dutch coastal North Sea and the western Wadden Sea of environmental DNA (blue triangle) and fyke fishing (orange bar) at Stuifdijk, NIOZ jetty, and Schanserwaard. The potential source area for aquatic eDNA sampled off the jetty is indicated in blue for the high tide sample and in orange for the low tide sample. Figure is based on original by Buijsman and Ridderinkhof (2007b)

HP33D maps). Sampling off the NIOZ jetty at high tide (HW) will mainly target sources from the west, from fishes that are present in the coastal North Sea, whereas sampling at low tide will target sources from the east, from fishes that are present in the Wadden Sea (Figure 1). The Stuifdijk fyke ( $52^{\circ}59'48.85''\text{N}$ ,  $04^{\circ}46'25.43''\text{E}$ ) is located 1 km west of the NIOZ jetty. A second fyke ( $53^{\circ}00'28.42''\text{N}$ ,

$04^{\circ}47'51.42''\text{E}$ ) at Schanserwaard is located 1 km northeast of the jetty. Results of daily Stuifdijk catches over almost 60 years are available (van der Veer et al., 2015), and for the majority of the fish species, yearly trends were similar among kom fykes at three locations including Stuifdijk and Schanserwaard (van der Meer et al., 1995; van der Veer et al., 1992).

## 2.2 | Sampling procedures

Samples were taken off the NIOZ jetty from the Marsdiep surface water during high tide (30 min before predicted maximum in Den Helder; <https://www.rijkswaterstaat.nl/water/waterdata-en-water-berichtgeving/waterdata/getij/index.aspx>) as well as during low tide (30 min before predicted minimum) each Wednesday over 18 weeks, from 22 April till 9 September 2015. On 1 September, eDNA samples were taken from the jetty every hour over an entire tidal cycle (13 hr). To test for spatial heterogeneity, eDNA was also sampled next to the kom fykes located at the Stuifdijk and the Schanserwaard in the mornings at random tides on five days (22 April, 8 May, 20 May, 10 June, and 24 June).

Prior to eDNA sampling, all sample equipment was decontaminated by washing with hot water and soap, incubation in a 10% bleach solution, rinsing with tap water and 3x rinsing with demineralized water. Three consecutive samples of 10 L were taken from the surface water using the same precleaned bucket. In between consecutive sampling, the 10 L bucket was emptied completely and rinsed (to remove the former sample) with fresh surface water seconds before the sample was taken. Of each 10 L sample, a 1 L subsample was stored dark in a precleaned bottle in a cool box. In this way, three consecutive samples were taken in a period of 15–20 min. Samples were transported to a dedicated climate container (10°C) only used for eDNA filtration where each surface water sample was filtered over a 47-mm polycarbonate filter with 0.2 µm pore size, as recommended for quantitative eDNA work (Eichmiller, Miller, & Sorensen, 2016), until the filter was nearly clogged (the average filter volume was 180 ml ± 50 ml standard deviation for 108 samples). On two days (19 August and 2 September) both at high tide and at low tide, a larger volume (1,000–1,200 ml) was filtered in duplo over Sterivex columns in order to determine the effect on presence–absence calls. Laboratory gloves were worn during sampling and filtration of the water. Only samples from the same day and tide were processed simultaneously. Between different samples, all equipment was decontaminated as described above. Filters were folded and stored in 2-ml tubes at –80°C. Sterivexes were capped and also stored at –80°C.

The sampling procedure for the Stuifdijk fyke catches has already been described in detail (van der Veer et al., 2015). Catches based on 24-hr fyke fishing were collected daily over 10 weeks, from 16 April till 2 July 2015. Due to bad weather conditions or malfunctioning of the fyke, several catches ( $n = 9$ ) could not be retrieved. An overview of successful fyke sampling ( $n = 69$ ) and eDNA sampling ( $n = 10$ ) in the same period is presented in Figure S1.

## 2.3 | DNA extraction and PCR amplification

In a pre-PCR laboratory, filters were cut in small pieces with sterile scissors. DNA was extracted quantitatively using the MoBio Powersoil extraction kit (Eichmiller et al., 2016), according to the standard protocol but with identical volumes of the pipetting steps for all samples. Triplicate samples were extracted separately

as recommended by Lanzén, Lekang, Jonassen, Thompson, and Troedsson (2017). Only one tube was open at a time, filter tips were always used, and pre- and post-PCR activities, reagents, and plastic ware were strictly separated in space (in three different laboratories) in order to prevent contamination. With each PCR series, negative controls were taken along. These were all pooled and sent for NGS sequencing.

PCR primer sequences, originally designed for vertebrates (Riaz et al., 2011) and already used for marine fishes in aquaria (Kelly, Port, Yamahara, & Crowder, 2014), were evaluated using the ARB (Ludwig et al., 2004) probe search against a multiple alignment of 12S fish sequences extracted from GenBank. In order to target both bony and cartilaginous fishes with the same primer set, allowing relative abundance comparisons between these two groups, the forward primer was slightly adapted. The newly adapted primer pair (12S-F1niz: 5'-AACTGGGATTAGATACCC-3' and 12S-R1: 5'-TAGAACAGGCTCCTCTAG-3') was tested with a gradient PCR on four fish species and one invertebrate in 25 µl reactions containing 2.5 µM of each primer, 200 µM dNTP, 1 U Phusion Polymerase (Thermo Scientific Inc.), 1x Phusion HF buffer, and 2 µl DNA extract. Annealing at 55.5°C, 57°C, and 58°C produced equally strong bands for fish, whereas for the invertebrate species, no bands were visible. Annealing at 59.2°C reduced the yield of fish PCR product. The chosen cycling protocol included an initial incubation at 98°C for 30 s followed by 30 cycles of 98°C for 10 s, 55.5°C for 20 s, and 72°C for 30 s, with a final incubation at 72°C for seven minutes. Robustness of this protocol was confirmed with DNA extracts of 53 local fish species and 8 nonfish invertebrate species. PCR products of the fish species were sequenced (BaseClear; Leiden, the Netherlands), and these sequence data have been submitted to the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession numbers MN844079–MN844132. QPCR was done with SYBR Green added to the master mix. Reactions were run on a Bio-Rad CFX with a plate set up according to Svec, Tichopad, Novosadova, Pfaffl, and Kubista (2015) including the biological triplicates (different 10 L sample, different 1 L subsample, different extraction, and PCR) of each field sample, and a calibration curve based on plaice (*Pleuronectes platessa*) 12S copies (6 decades,  $10^6$ – $10^1$  copies/µl). The efficiency of the amplification was 96.3%. Differences in the slopes of the exponential phases of the S-curves among the 120 samples were small (<10%; Figure S2) indicating that the efficiency of the amplification was constant over the season. Fish-specific eDNA concentrations were calculated from the ratios of fish species-specific read counts over the total read count (including assigned and nonassigned reads) of a sample, multiplied by the total eDNA concentration measured with QPCR. An alternative procedure based on the ratio of fish-specific read counts over the assigned read count gave similar results.

Metabarcoding of eDNA from the field series was done in 50 µl reactions with 4 µl template and 37 PCR cycles as described above. However, the primers described above were each tagged with sample-specific 6 nt barcodes, such that amplicons from a specific sample had a unique 6-mer at the beginning and another unique 6-mer at the end, in order to avoid sequence-to-sample



misidentifications (Schnell, Bohmann, Thomas, & Gilbert, 2015). The three replicate samples from the same sample date and tide were labeled with identical barcode combinations. Negative controls were included in every PCR series, and all these were labeled with the same barcode combination. In a post-PCR laboratory, amplicons were run on agarose gels, excised, and purified using the QIAquick gel extraction kit. Amplicons of the triplicates were combined and quantified using a Qubit fluorometer. The average concentration of the field samples was 2.5 ng/μl (±0.5 SE). The concentration of the pooled negative controls was too low to be measured. Samples were pooled such that from each sample, there were 35 ng DNA (corresponding to  $2 \times 10^{11}$  amplicons) in the pool. For the negative control, we added the maximum volume that was used for a field sample in order to get a maximum value for the read abundance of potential contaminants. After a final concentration step with a Qiagen MinElute PCR purification column, the sample was sent to GATC Biotech for 2x 125 bp paired-end (PE) sequencing with Illumina HiSeq technology.

## 2.4 | Reference database construction and taxonomic resolution

For an initial rough taxonomic identification of the Illumina sequences, they were first processed in Galaxy (<http://usegalaxy.org>): Reads 1 and 2 were paired using PEAR with default settings (Zhang, Kobert, Flouri, & Stamatakis, 2014), demultiplexed (barcode-splitter), and subjected to quality filtering (cshl\_fastq\_quality\_filter/1.0.0 requiring a Quality score of 30 for a minimum of 97% of the nucleotides). Reads were aligned (mothur\_toolsuite/ mothur\_align\_seqs/ 1.19.0) and trimmed (mothur\_toolsuite/ mothur\_chop\_seqs/ 1.19.0) to 141 columns. Operational taxonomic unit (OTU) clustering (qiime1\_3\_0/pick\_otus/2.0.0) was done with a 0.98 identity cutoff value. OTU representatives (qiime1\_3\_0/pick\_rep\_set/2.0.0; rep\_set\_picking\_method based on most\_abundant) were blasted (Altschul et al., 1990) against the entire NCBI nucleotide (NT) database. Top blast hits included sequences of bony and cartilaginous fishes, but also of humans, cattle and pet species, marine mammals, and birds. The top blast hits were downloaded and complemented with newly derived 12S sequences of 53 local fish species that had been caught in the NIOZ fyke over the last 5 years, together forming our project-specific custom reference database. Over time, repeatedly and iteratively, the unassigned reads were checked against the NT database and missing reference sequences were added until we were confident that the remaining unclassified sequences were mainly sequence artifacts.

The resolution of the fish identification based on the 12S amplicon was evaluated using a multiple alignment (122 valid positions) of our custom-made reference database. The dendrogram (Figure S3) shows sufficient distance between most of the fish species and 39 species had unique amplicon sequences. *Chelon labrosus* (thick-lip grey mullet) and *Chelon auratus* (golden grey mullet) differed by one diagnostic nucleotide. This was also the case for *Ammodytes*

*tobianus* (small sandeel) and *Hyperoplus lanceolates* (great sandeel). In seven cases, identical amplicon sequences were shared between two or three closely related species, and therefore, seven unresolved groups were defined: 1. *Platichthys flesus*, *Limanda limanda*, *Pleuronectes platessa* (flounder, dab, plaice); 2. *Chelon labrosus*, *C. ramada* (grey mullet); 3. *Merlangius merlangus*, *Pollachius pollachius*, *P. virens* (whiting pollack, saithe); 4. *Trisopterus luscus*, *T. minutus* (pouting, poor cod); 5. *Pomatoschistus minutus*, *P. lozanoi* (sand goby, Lozano's goby); 6. *Clupea harengus*, *Sprattus sprattus* (herring, sprat); and 7. *Myoxocephalus scorpius*, *Taurulus bubalis* (sculpin, bullhead).

## 2.5 | 12S amplicon sequence data analysis

Once the reference database had been established, read pairs were merged with PEAR (Zhang et al., 2014), requiring a minimum overlap of 7 nt, a minimum individual read length of 20 nt after quality trimming, and a *p*-value threshold of 0.05 for the statistical test to decide whether to merge the reads. From the merged reads, containing a 6 nt barcode at the beginning and at the end, barcodes were extracted with the "barcode\_paired\_stitched" method of QIIME (Caporaso et al., 2010). The "split\_libraries\_fastq.py" script of QIIME was used to assign sequences to samples, accepting only reads with perfect barcode sequences and a minimum Phred score of 24 at all positions. Afterward, sequences that were too short (<134 nt) or too long (>154 nt), which are likely to represent PCR artifacts, were removed. The remaining merged reads were aligned against our custom database of 12S sequences of local fishes and common contaminants using MOTHUR v.1.34.4 (Schloss et al., 2009). Next, the PCR primers (18 nt at the beginning and end) were removed using "cutadapt" (version 1.15) and alignment characters were removed with "degapseq" from EMBOSS 6.6.0.0 (Rice, Longden, & Bleasby, 2000). Sequences shorter than 90 nt, resulting from poor alignment with the reference database, were discarded, and OTUs were generated with QIIME's trie method. For each OTU, the most abundant sequence was selected as representative sequence. Reference database matches were identified with blastn (BLAST 2.6.0+, Morgulis et al., 2008), requiring 98% sequence identity in the aligned part, and 98% query sequence coverage. The best database hit with respect to sequence identity was used to annotate the OTU. The OTU table was summarized on species level by summing counts assigned to the same species. The taxonomic assignment of the OTUs from the field samples was confirmed by calculating all representatives into a maximum likelihood tree along with the reference sequences. The most abundant field-OTU that was assigned to a species was always 100% similar to the reference sequence of that species (Figure S3) except for *Lipophrys pholis* (shanny), where the field-OTU differed by 1 nucleotide from the available reference sequence. The average number of accepted reads per field sample was 70,590 (±9,054 SE; *n* = 18), and these values were similar for high tide (64,548 ± 12,566 SE; *n* = 18) and low tide

(76,632  $\pm$  13,259 SE;  $n$  = 18) samples. The average percentage of assigned reads per total number of accepted reads per sample was 93% ( $\pm$ 1% SE). Large numbers of reads were assigned to humans or to livestock species, while the average percentage of fish reads per total assigned reads was 33% ( $\pm$ 4% SE; Figure S4). Accumulation curves (Figure S5) showed that the numbers of detected fish species started to become saturated above 700 reads. The mock community sample was represented with 168,000 reads and the negative control with 731 reads.

## 2.6 | Mock community and negative control

Equimolar amounts of genomic DNA extracts of 30 local bony fish species, two cartilaginous fish species, and eight invertebrate species (Table S1) were used to compose an artificial mock community. This sample and a negative control were processed along with the field samples to verify the data analysis workflow. The sequence library of the mock sample represented all of the 32 fish genotypes with an average amount of 5,254 ( $\pm$ 852 SE) reads per genotype. The invertebrate species that had been added to the mock community were not represented in the sequence library. Thirteen fish species were present as contaminant (detected in the sequence library although they were not part of the mock community) with an average amount of 9 ( $\pm$ 2 SE) reads per species, the most prominent being *Gadus morhua* (cod, 24 reads) and *A. tobianus* (small sandeel, 21 reads). Nonfish contamination (4–20 reads) came from human, pig, chicken, and sperm whale. The negative control contained 12 reads of *Salmo trutta* (sea trout) and 719 reads of Hominidae.

The highest number of contaminating fish reads was divided by the total number of reads in the mock sample to obtain a library-specific error, according to Thomsen et al. (2016). The resulting threshold of 0.014% was used for all samples as cutoff value for scoring presence/absence of a species. Two alternative higher thresholds of 0.028% and an absolute count of 50 reads for each fish genotype were also applied to check the robustness of the results.

## 2.7 | Statistics

Statistical significance of agreement between the detection of species with the fyke and eDNA sequencing was assessed with Cohen's kappa (Cohen, 1960). When both methods perfectly agree, a  $p$ -value cannot be calculated. The significance of effects of sampling day and tide on the fish eDNA community composition was assessed with a sequential PERMANOVA test (Anderson, 2001) using the PRIMER software v7 with PERMANOVA+ add-on (PRIMER-e Ltd.) with sampling day (random) as factor 1 and tide (fixed) as factor 2. ANOSIM was used to test whether fish communities differed among locations. Resemblance was either based on the Bray–Curtis similarity of fourth root-transformed data or based on S7 Jaccard with presence–absence data, using 9999 permutations.

## 3 | RESULTS

### 3.1 | Spatial variability in fish catches and eDNA presence–absence

Resemblance of fish catches and eDNA among different locations was measured on five days (22 April, 8 May, 20 May, 10 June, and 24 June; Figure 2). Fish catches with kom fykes at two different locations, Stuifdijk and Schanserwaard (about 2 km separated), showed a significant sample day effect (ANOSIM,  $p$  < 0.002) but no effect of location. The fish eDNA composition also showed a significant effect of sample day (ANOSIM,  $p$  < 0.0005), but in contrast to the fyke results, in addition a significant effect (ANOSIM,  $p$  < 0.05) of the sample location, with a higher proportion of small-sized fishes, for example, *A. tobianus* (small sandeel) and *Pholis gunnellus* (rock gunnel) at the jetty compared to Stuifdijk and Schanserwaard. When eDNA analyses were restricted to the 22 fish species that were also caught in the fykes, the day effect was still significant ( $p$  < 0.002; ANOSIM), whereas differences among locations were not significant.

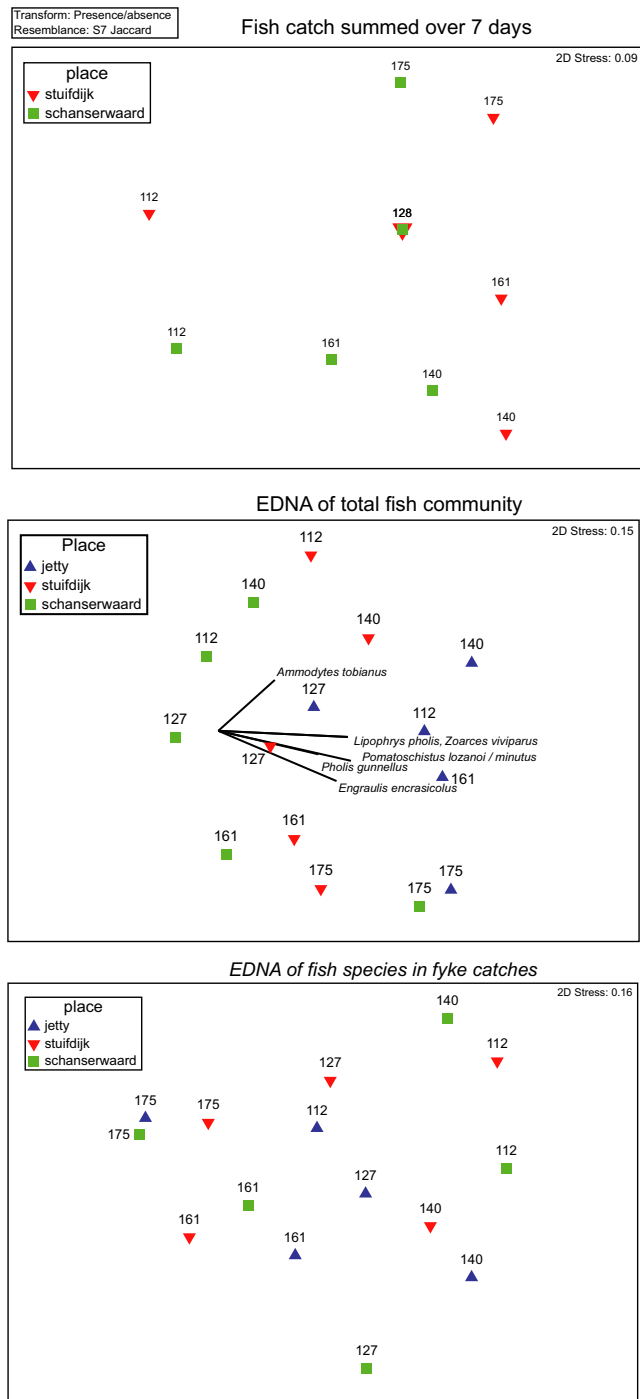
Testing resemblance between results of different methods (eDNA vs. catch; Figure S6) showed that single 24-hr fish catches and eDNA sampled within 3 m of the fykes located at Stuifdijk and Schanserwaard grouped significantly (ANOSIM,  $p$  < 0.05) according to sample day. Resemblance between catch and eDNA increased after pooling the fish catches over seven days preceding the eDNA sampling (ANOSIM,  $p$  < 0.0005) and only these pooled fyke results are shown in the next section.

### 3.2 | Comparison of eDNA sampled at jetty and fish catch at Stuifdijk

The results of fish eDNA sampled at the NIOZ jetty were compared to fish catches with a kom fyke at the Stuifdijk over ten weeks between late April and early July. On average, 24-hr catches contained 5.5 ( $\pm$ 0.3 SE;  $n$  = 69) fish species per sample and species detection was highly stochastic. Environmental DNA (the sum of a high tide and a low tide sample) contained a higher number of fish species per sample (16.4  $\pm$  0.8 SE;  $n$  = 10) and agreed best with fyke catches pooled over seven days (13.8 species per sample  $\pm$  1.0 SE;  $n$  = 10) also showing highly similar trends over time, with high species diversity on 20 May and 17 June (Figure S7).

In total, 39 fish species were detected of which 22 species were detected with both eDNA and the fyke during comparative sampling. In addition, twelve species were exclusively detected with eDNA, whereas only one species was detected with the fyke and not with eDNA.

The abundant core fishes (average mass > 956 g per week in the fyke catch), *P. flesus*, *L. limanda*, *P. platessa* (flounder, dab, plaice), *Dicentrarchus labrax* (seabass), *C. harengus*, *S. sprattus* (herring, sprat), and *C. auratus* (golden grey mullet), were always detected with both eDNA and fyke from April till July (10 detections; Figure 3). *Belone belone* (garfish), *C. labrosus* (thicklip grey mullet), and *Salmo trutta* (sea trout) were often found with both methods (7–8 detections;



**FIGURE 2** Resemblance of fish community compositions measured at two different locations with kom fykes and at three different locations with eDNA. Numbers next to symbols are day numbers: 112 = 22 April; 128 = 8 May; 140 = 20 May; 161 = 10 June; 175 = 24 June

>825 g average wet mass per week in the fyke). Less abundant core species, like *Callionymus lyra* (common dragonet) and *Arnoglossus laterna* (scaldfish), were found 1–2 times with the fyke and 1–2 times with eDNA. *Scophthalmus maximus* (turbot, 6 fyke detections; 22 g average wet mass) was found in the catch but not in eDNA sampled at the NIOZ jetty. However, this species was found with eDNA at

the other sample locations nearby (~1 km, at the Stuifdijk and the Schanserwaard). *Agonus cataphractus* (hooknose, 4 fyke detections, 26 g average wet mass), *Anguilla anguilla* (eel, 1 fyke detection, 135 g wet mass), and *Echiichthys vipera* (lesser weever, 1 fyke detection, 33 g wet mass) were not detected with eDNA during comparative sampling, but later in the season. *Mullus surmuletus* (mullet, 1 fyke detection, 142 g wet mass) was found in the catch but never in eDNA. Conversely, species with a small diameter, like *A. tobianus*, *P. gunnellus*, *Z. vivipares* (eelpout), and *L. pholis* (shanny), were exclusively found with eDNA (> 7 detections) and never in the fyke.

The presence–absence calls based on eDNA sampled at the jetty and pooled fish catches at the Stuifdijk matched in 68% of the cases, meaning that for 39 fish species detected in total, out of 390 calls over ten weeks at least 264 cases showed the presence or the absence of a species the same in both methods (Figure 3). For the 27 species that were found with the fyke, the match percentage was 71%, whereas for the eight most abundant fish species in the fyke (together 97% of the wet mass; each >4%), the presence–absence calls based on eDNA and fyke matched in 95% of the cases. The agreement between the detection of species with eDNA and fyke fishing was statistically significant as assessed with Cohen's kappa (Cohen, 1960) for all 27 fish species that were present in the catches ( $k = 0.478$ ;  $p = 4.22e^{-15}$ ).

### 3.3 | Effect of tide

On a diel scale, fish eDNA composition showed variability (Figure 4); however, no significant trend was found (ANOSIM with sample time as sequential factor). Samples taken during outgoing tide (Wadden Sea water) grouped relatively close together, whereas samples taken during incoming tide (North Sea water) were not tightly clustered, and the two groups of samples (incoming vs. outgoing tide) were not significantly different ( $p > 0.1$ , ANOSIM). Also, over the season, during 18 weeks of comparative sampling at high water and at low water, fish eDNA composition did not differ significantly between tides ( $p > 0.1$ , PERMANOVA test based on Bray–Curtis similarities, Figure 4). The species count per sample varied between 4 and 19 in low tide samples and between 5 and 18 in high tide samples, and presence calls were consistent between tides except for *Pomatoschistus microps* (common goby) and *Ciliata mustela* (five bearded rockling) that had seven detections more at low tide than at high tide. The average eDNA concentration was slightly higher and more variable in low tide samples (69,442 copies/L  $\pm$  35,963 SE;  $n = 18$ ) than in high tide samples (22,885 copies/L  $\pm$  4,543 SE;  $n = 18$ ).

### 3.4 | Seasonal trends in fish community composition

Fish eDNA community composition differed significantly ( $p < 0.0001$ , PERMANOVA) between sample days and between sample months (Figure 4). In April, the dominant fishes were *P. flesus*, *L. limanda*, *P. platessa* (17% of total fish eDNA), *D. labrax* (16%), *C. labrosus* (16%), and

Fish species	#Detections		Sample date									
	eDNA	fyke	22-Apr	29-Apr	7-May	13-May	20-May	27-May	10-Jun	17-Jun	24-Jun	8-Jul
<i>Platichthys, Limanda, Pleuronectes</i>	10	10	1	1	1	1	1	1	1	1	1	1
<i>Dicentrarchus labrax</i>	10	10	1	1	1	1	1	1	1	1	1	1
<i>Chelon labrosus, C. ramada</i>	10	7	1	D	1	1	1	1	1	D	1	D
<i>Ammodytes tobianus</i>	10	0	D	D	D	D	D	D	D	D	D	D
<i>Clupea harengus, Sprattus sprattus</i>	10	10	1	1	1	1	1	1	1	1	1	1
<i>Belone belone</i>	8	8	0	1	1	1	1	1	1	1	1	0
<i>Chelon auratus</i>	10	10	1	1	1	1	1	1	1	1	1	1
<i>Pholis gunnellus</i>	10	0	D	D	D	D	D	D	D	D	D	D
<i>Zoarces viviparus</i>	7	0	D	D	0	0	D	0	D	D	D	D
<i>Salmo trutta</i>	7	7	1	1	1	1	0	0	1	1	1	0
<i>Alosa fallax</i>	5	6	1	1	F	0	0	0	0	1	1	1
<i>Pomatoschistus minutus, P. lozanoi</i>	8	3	F	D	0	D	D	D	1	1	D	D
<i>Myoxocephalus scorpius, Taurulus bubalis</i>	7	2	D	0	D	1	1	D	D	D	0	0
<i>Engraulis encrasicolus</i>	5	0	0	0	0	0	D	D	D	D	D	0
<i>Solea solea</i>	5	1	1	0	D	D	D	D	0	0	0	0
<i>Trachurus trachurus</i>	3	2	0	0	0	0	0	F	0	1	D	D
<i>Sardina pilchardus</i>	3	2	D	F	D	0	1	0	0	0	0	0
<i>Atherina presbyter</i>	4	6	0	D	F	1	1	1	F	F	0	0
<i>Lipophrys pholis</i>	7	0	D	D	0	D	D	0	D	D	D	0
<i>Ciliata mustela</i>	6	7	1	F	1	1	F	F	1	D	0	D
<i>Pomatoschistus microps</i>	3	0	0	0	0	0	0	0	0	D	D	D
<i>Syngnathus acus</i>	1	6	F	1	F	F	0	0	0	F	F	0
<i>Hippoglossoides platessoides</i>	2	0	D	0	D	0	0	0	0	0	0	0
<i>Gasterosteus aculeatus</i>	1	5	F	D	F	F	F	0	0	F	0	0
<i>Merlangius, Pollachius</i>	2	8	1	0	F	F	F	0	F	F	1	F
<i>Trisopterus luscus</i>	2	5	0	0	0	0	F	0	F	1	1	F
<i>Arnoglossus laterna</i>	1	1	0	0	0	0	D	0	F	0	0	0
<i>Callionymus lyra</i>	2	2	0	0	F	1	D	0	0	0	0	0
<i>Hyperoplus lanceolatus</i>	1	0	0	0	0	0	D	0	0	0	0	0
<i>Neogobius melanostomus</i>	1	0	D	0	0	0	0	0	0	0	0	0
<i>Reinhardtius hippoglossoides</i>	1	0	0	0	D	0	0	0	0	0	0	0
<i>Chelidonichthys lucerna</i>	1	7	0	0	F	F	F	0	F	1	F	F
<i>Scomber scombrus</i>	1	0	0	0	0	0	0	0	0	0	D	0
<i>Anguilla anguilla</i>	0	1	0	0	0	F	0	0	0	0	0	0
<i>Echiichthys vipera</i>	0	1	0	0	0	0	0	0	0	F	0	0
<i>Syngnathus rostellatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agonus cataphractus</i>	0	4	0	0	0	0	F	F	0	F	F	0
<i>Mullus surmuletus</i>	0	1	0	0	0	0	F	0	0	0	0	0
<i>Scophthalmus maximus</i>	0	6	F	0	0	0	F	0	F	F	F	F

**FIGURE 3** Presence-absence calls based on eDNA and fyke fishing for 39 fish species. Gray color indicates matching calls for eDNA and fyke, with 1 indicating presence and 0 indicating absence; "D" indicates presence with only eDNA; "F" indicates presence with only fyke

small-sized species (19%) that were not caught in the fyke (Figure 5). In May, *P. flesus*, *L. limanda*, *P. platessa* eDNA concentrations were very high (>60% of total fish eDNA), whereas in June *C. harengus*, *S. sprattus* eDNA became high (28%), next to *C. labrosus* (20%) and *D. labrax* (15%). In July and August, high concentrations of *C. harengus*, *S. sprattus* eDNA persisted (32%–24%), next to high eDNA concentrations of small-sized species (38%–41%). Early September, small-sized species still dominated the eDNA pool (48%), next to flatfish *P. flesus*, *L. limanda*, *P. platessa* (17%), *D. labrax* (14%), *C. harengus*, *S. sprattus* (9%), and *Syngnathus rostellatus* (7%). Community composition based on fyke catches matched well with eDNA results in April and June, but did not show the large dominance of flatfishes in May.

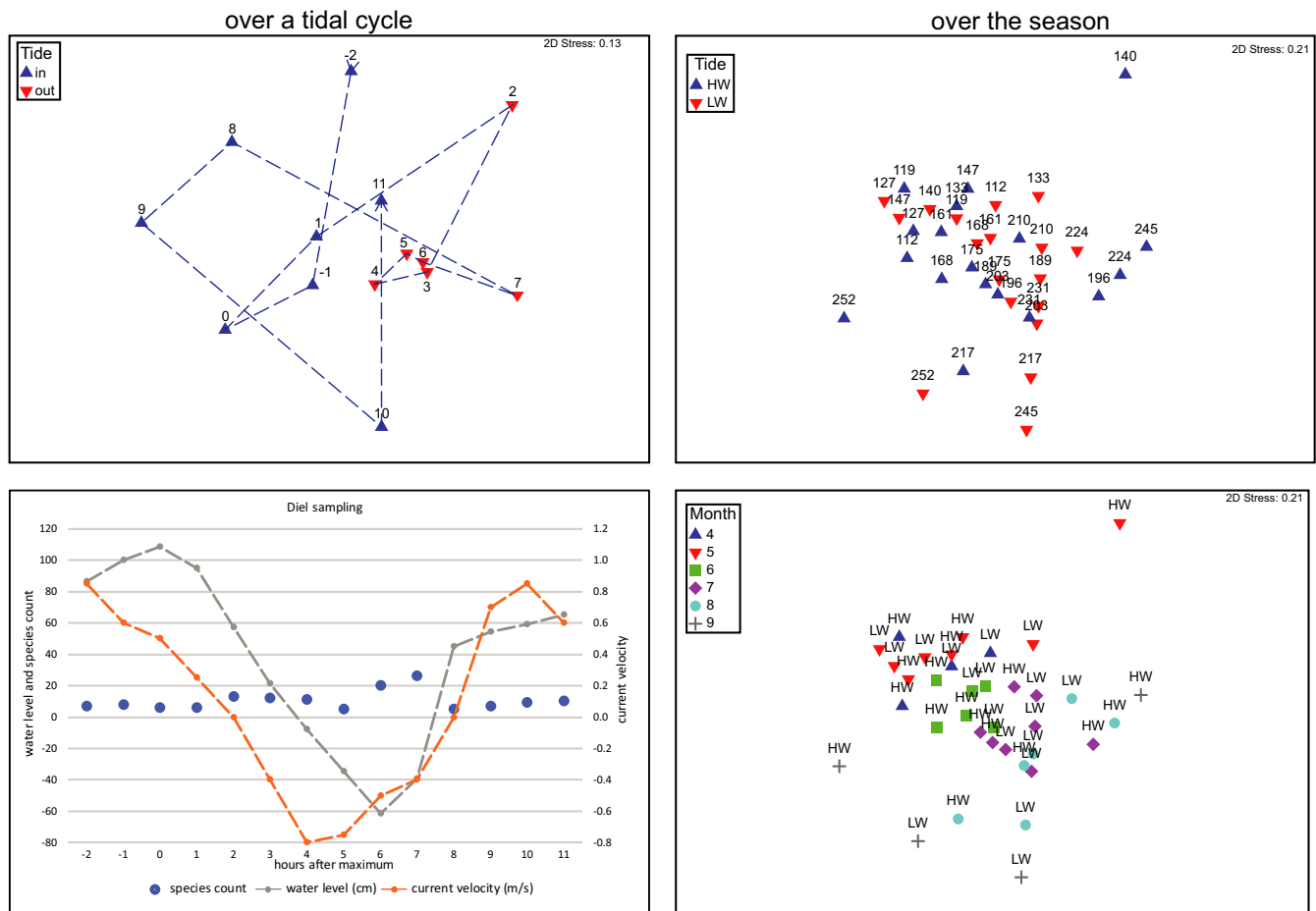
Focusing on the small-sized fish community (Figure 5), the dominant species in April was *Zoarces vivipares* with 9% of the total fish

eDNA, whereas in May and June, *A. tobianus* dominated, with 5%–10% of the total fish eDNA. In July, *Pomatoschistus minutus*, *P. lozanoi* were abundant (13%) next to *A. tobianus* (10%) and *P. gunnellus* (9%). Subsequently, in August, *P. microps* became dominant (18%) next to *P. minutus*, *P. lozanoi* (10%). Finally, in September, *P. microps* (21%) and *A. tobianus* (24%) still contributed large amounts of eDNA.

### 3.5 | eDNA concentration in relation to fish mass and fish counts

The bulk fish-specific eDNA concentration (the sum of the fish eDNA concentration in high tide and low water tide samples) increased from 57,000 copies/L at 22 April to a maximum of





**FIGURE 4** Resemblance of fish community over a tidal cycle at 1 September with indications of water height and current velocity (x-axis shows hours before maximum water level); and resemblance of fish community over the season with indication of day numbers and with different months presented in different colors

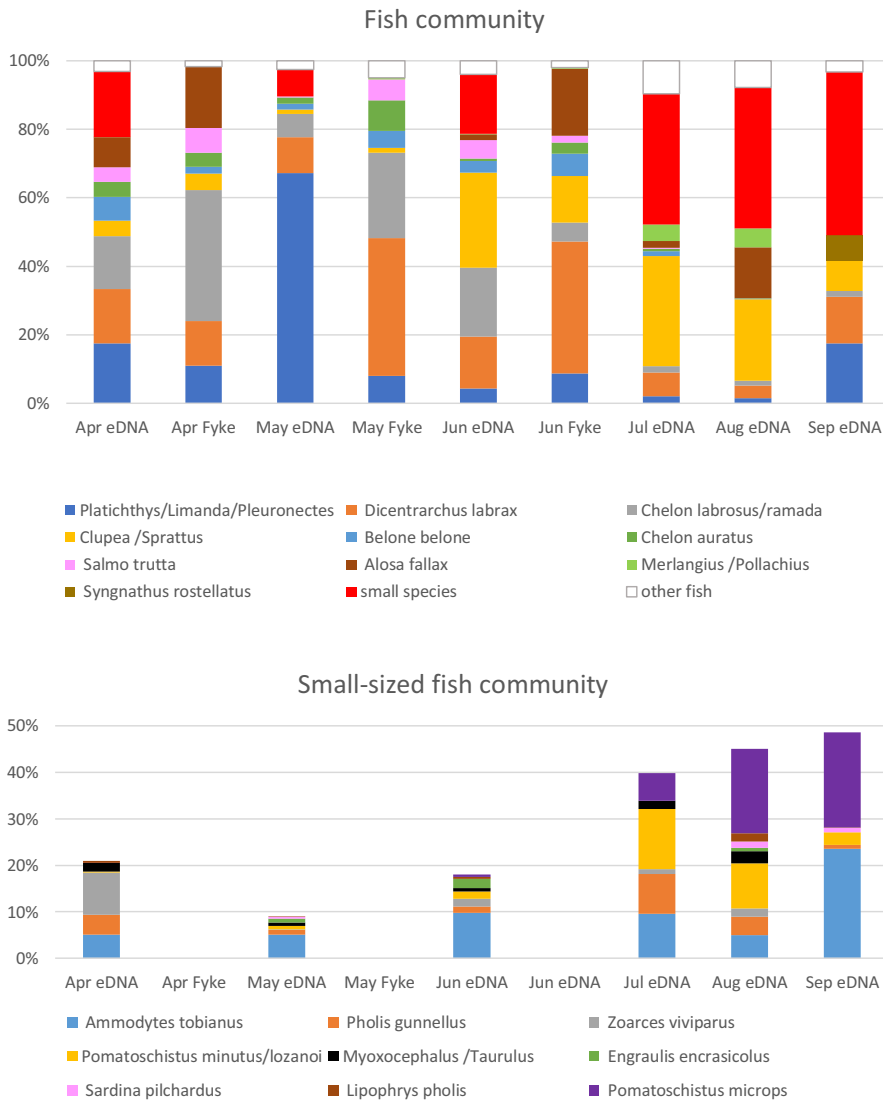
712,000 copies/L on 7 May (Figure 6). During this period, the average water temperature increased from 10.2 to 11.1°C. Between 13 May and 8 July, the fish eDNA concentration varied between 20,000 and 120,000 copies/L, while water temperatures increased steadily from 11 to 18°C. In July and August, eDNA concentrations were relatively stable (11,000–60,000 copies/L), while the temperature of the water stayed high (between 18 and 19°C). Fish wet mass per seven days of fyke catch before eDNA sampling was highest (22 kg) on 22 April (Figure 6), when 110 fish specimens were caught with an average size of 20 cm. A week later, wet mass was lower (10 kg) due to a smaller number of fishes in the catch. Subsequently, between 6 and 20 May, wet mass was 16–18 kg, corresponding to an increased average size (23–26 cm) of the catch. On 27 May, both the numbers and the average size class of the fish catch were relatively low, resulting in a minimum wet mass of 5 kg per seven days of fishing. After 27 May, wet mass increased, while numbers of small juvenile fishes increased exponentially.

Fish eDNA concentrations were significantly correlated with the fish wet mass in pooled 24-hr catches over the seven days preceding eDNA sampling (85 observations,  $p < 0.0001$ ; Figure S8). Fish eDNA concentrations were not related to the numbers of fishes in the fyke catches.

### 3.6 | Species-specific eDNA concentrations related to wet mass and size class

The seasonal patterns in eDNA from the eight most abundant fish species in the fyke corresponded well to the patterns in their wet mass (Figure 7). *Platichthys*, *Limanda*, *Pleuronectes* eDNA showed a first peak on 7 May and a second peak on 10 June. The first peak was not recorded with the fyke. Catches in April–early May contained mainly *L. limanda* (dab, 11–26 cm) and *P. flesus* (European flounder, 7–27 cm), whereas around 10 June, both *P. flesus* (9–30 cm) and juvenile *P. platessa* (European plaice, 5–10 cm) were most abundant (Figure S9).

*Clupea harengus*, *S. sprattus* eDNA was high on 29 April and 6 May, then decreased until 27 May, subsequently increased to high values in June, and maintained high values throughout July and August (Figure 7). The high eDNA concentration in the summer months co-occurred with high numbers of juvenile *C. harengus* (5–10 cm) in the fyke catch (Figure S9). Throughout the season, *C. harengus* was the dominant Clupeinae species and relatively small amounts of *S. sprattus* were caught for only two weeks (Figure S9): around 17 June (19% of Clupeinae mass) and in early July (5% of Clupeinae



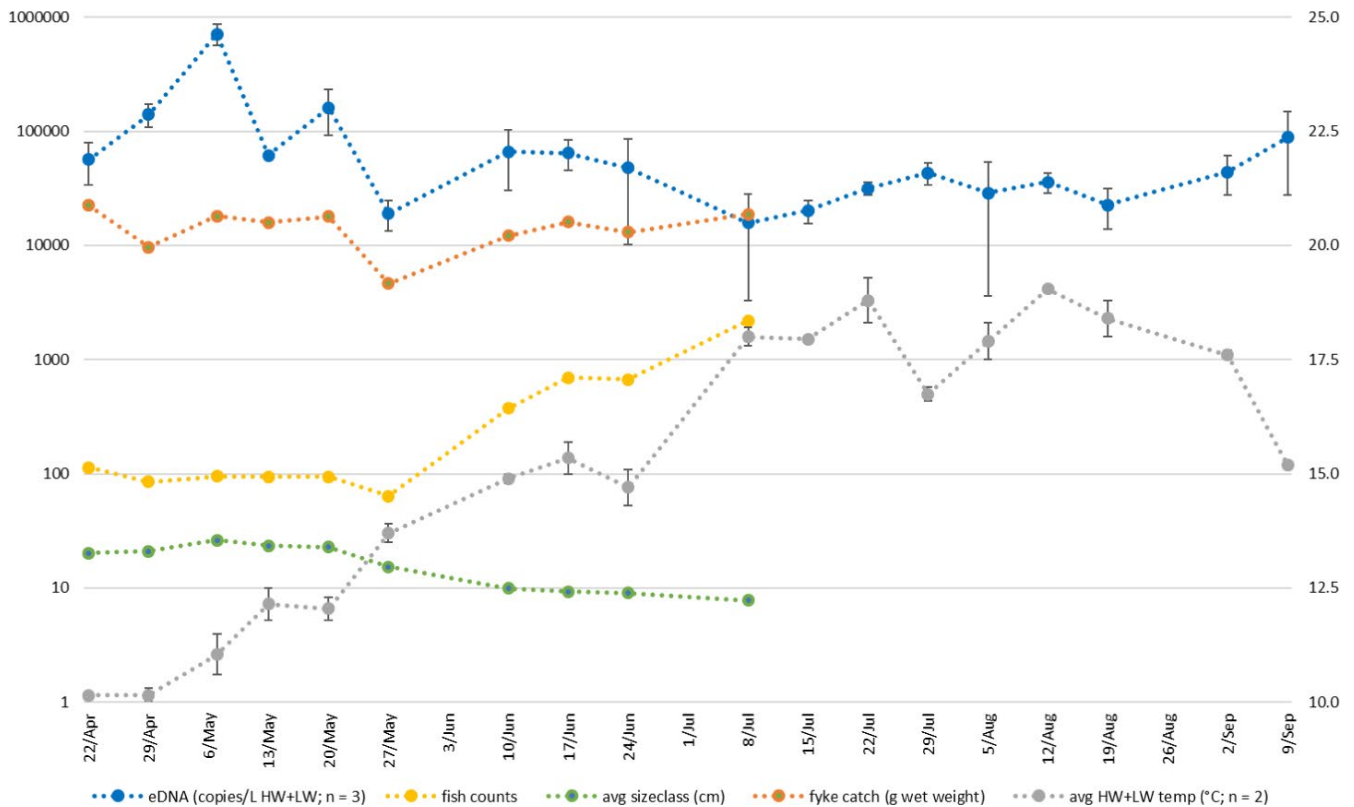
**FIGURE 5** Fish community composition per month for all fish species; and only for the small-sized fish species that are not caught in the fyke

mass). *Dicentrarchus labrax* eDNA concentrations were higher early in the season than after 17 June (Figure 7). Fyke results for this species showed consistently high mass values and large variation in size class (10–50 cm) of specimens (Figure S9). The relatively high eDNA concentrations on 29 April, 20 May, and 17 June seem to co-occur with the presence of large specimens (>50 cm) in the fyke during the week preceding eDNA sampling.

## 4 | DISCUSSION

Ongoing daily fish catches with a kom fyke at the Stuifdijk delivered almost 60 years of information on seasonal migration of fish from the North Sea into the Wadden Sea in spring and vice versa in autumn (van der Veer et al., 2015). These fyke catches at the Stuifdijk are considered to be representative for the area and showed similar trends as catches with fykes on other locations in the Marsdiep tidal inlet (van der Meer et al., 1995; van der Veer et al., 1992). Since fyke fishing is a laborious and invasive method,

we tested whether an alternative method based on eDNA sampling from the easily accessible NIOZ jetty in the Marsdiep would give similar results. The strong resemblance among catches with fykes at the Stuifdijk and the Schanserwaard indicates that the fish community composition is homogeneous in the sample area. Also, the eDNA community composition of fish species that were caught in the fykes was homogeneous among the three sample locations Stuifdijk, NIOZ jetty, and Schanserwaard. We cannot exclude the possibility that eDNA samples taken at the NIOZ jetty are slightly influenced by the presence of the fykes, since water passing the fykes may need only 20 min to reach the jetty assuming a straight trajectory, but we consider this insignificant because of the small biomass (on average 2 kg) in the fykes and the vast volume of water, about  $5 \times 10^4 \text{ m}^3/\text{s}$  (Nauw, Merckelbach, Ridderinkhof, & Van Aken, 2014) moving with the tide: Assuming an eDNA production of 10 million copies per gram fish per hour (Maruyama et al., 2014), the fishes in the fyke will contribute 0.11 copies/L seawater, whereas we measured values between 11.000 and 712.000 copies/L.



**FIGURE 6** Seasonality in total fish eDNA concentration and fish wet mass, fish counts, fish size class, and water temperature

Based on tidal models, we estimated that potential source areas of eDNA produced within 6 hr prior to water sampling are located within 15 km west and 15 km east of the jetty. When the main source of eDNA would rapidly sink out of the water column, for example, potentially in the case of feces, the eDNA distribution range would probably be much smaller. Indeed, limited distribution ranges of eDNA were measured by Kelly, Gallego, and Jacobs-Palmer (2018) for near-shore eDNA from benthic and planktonic taxa, and by Murakami et al. (2019) for eDNA of caged fish in a marine environment. Within our sample area, the eDNA community composition including small species that were not caught in the fykes showed significant differences among locations. At the jetty, within 10 m of a stony dike, and a water depth of 2–4 m, higher proportions of *P. gunnellus* (rock gunnel), *P. minutus*, *P. lozanoi* (gobies), *A. tobianus* (sand eel), and *Engraulis encrasicolus* (anchovy) were found compared to the intertidal sample locations, at least 100 m away from the coastline. This indicates that for our eDNA sample locations, there is a significant imprint of local (territorial) small-sized fishes, on top of the integrated signal from the larger (migratory) core fish species in the area.

#### 4.1 | eDNA compared to fish catch

Presence-absence calls of fish species based on eDNA sampled at the NIOZ jetty significantly agreed with fyke net catches at the Stuifdijk over 10 weeks of sampling between late April and early July 2015. All but one

of the fish species that were detected with the Stuifdijk fyke were also detected with eDNA sampled off the NIOZ jetty. This illustrates the potential of the eDNA approach for fish monitoring. *Agonus cataphractus* (hooknose) was detected four times with the fyke but not with eDNA during the same period. Later in the season, when the fyke was not operational, *A. cataphractus* was found with eDNA (1 detection on 29 July at Schanserwaard and 3 detections on 1 September at the jetty during diel sampling) indicating there is no primer bias for hooknose. It is possible that the low *Agonus* biomass (<26 g average for four fyke detections) in combination with low slime release by this armored species does not deliver enough eDNA to the water column for detection in three times 250 ml samples. Even when we applied a larger sample volume (two times 1,000 ml), the species was not found. The species was also underrepresented in an eDNA-based field study of Sigsgaard et al. (2017).

Eleven species were detected in surplus with eDNA: the most important (>7 detections) being *A. tobianus* (small sandeel), *P. gunnellus* (butterfish), *Z. vivipares* (eelpout), and *L. pholis* (shanny). These are all small-sized species, which likely pass the 20 mm fyke net. Other small-sized species, like *Pomatoschistus* sp. and *Myoxocephalus scorpius*, were detected with the fyke but much less often than with eDNA. This was also the case for sole *Solea solea* which may be underrepresented in the fyke catches because during the spawning period (April–June) adults are known to swim high up at the surface.

Presence-absence calls are affected by the choice of a threshold level. We used a library-specific relative error threshold (0.0143%) based on the maximum number of false fish reads in the mock community and negative control samples divided by the total number of



FIGURE 7 Seasonal patterns in eDNA concentration and wet mass for the most abundant fish species in the fyke

reads in these samples (Thomsen et al., 2016). Since back in 2015, like many others, we did not take along extraction negatives, that may contain more reads than PCR negatives, presence-absence was also called using two alternative higher threshold levels of 0.0286% and of 50 absolute read counts per species per sample. The first alternative (double the relative threshold that we applied) did not affect the total number of detected species but reduced the number of presence calls by 1 in four species and by 2 in one species. The second approach (an absolute threshold of 50 reads for all species) reduced the total number of detected species by 2, resulting in a loss of Greenland halibut (*Reinhardtius hippoglossoides*) and American plaice (*Hippoglossoides platessoides*), while simultaneously reducing the number of presence calls by 1 for twelve fish species and by 2 for two fish species. Applying these alternative threshold levels did not affect the statistical significance of the agreement between presence-absence calls with the fyke and eDNA sequencing ( $k = 0.471$ ;  $p = 1.04e^{-14}$  compared to  $k = 0.478$ ;  $p = 4.22e^{-15}$ ). In all cases, relative abundance, community composition, and seasonal trends were highly similar because only the results of low abundant species were affected by the choice of the threshold value.

We chose our methods based on information that was available when we started sampling in 2015. We used 12S primers and 0.2- $\mu$ m filters based on Kelly et al. (2014). Later, alternative 12S primers were designed (Miya et al., 2015) and 0.4 and 0.7  $\mu$ m filters were more often used (e.g., Ushio et al., 2018) because these allow a larger sample volume providing a higher sensitivity for the detection of less abundant or smaller fish species. For the eutrophic Marsdiep area, harvest of fish eDNA from 3x 200 ml water on 47-mm polycarbonate filters with a pore size of 0.2  $\mu$ m gave similar fish species counts as harvest from 2x 1,000 ml on a Sterivex filter with 0.2  $\mu$ m pore size, and resulted in presence-absence calls and biomass estimations that fitted well to fish catches. A practical argument in favor of 0.2- $\mu$ m filters is that DNA extracts can be used both for the analyses of vertebrate eDNA and for analyses of the microbial community.

The 12S rRNA primers used in our study allow presence-absence scoring of bony fishes as well as cartilaginous fishes. Tests on genomic DNA extracts of identified fish specimens and analyses of the mock community, containing two species of cartilaginous fish, showed that the primers amplified starry ray (*Amblyraja radiata*) and starry smoothhound (*Mustellus asterias*). During comparative sampling in the field, neither ray nor shark eDNA was detected, corresponding to their absence in the nearby fyke catches. Initially, we experienced false presence calls of starry ray because human genes with similarity to the ray 12S were not in our reference database, and sequences originating from these genes were assigned to starry ray. By adding the relevant human sequences to our reference database, we effectuated accurate annotations as confirmed with a maximum likelihood tree (Figure S3) of all OTU representatives and all reference sequences.

## 4.2 | Effect of tide (high tide vs. low tide samples)

Although we found considerable variability in the fish eDNA community composition over a tidal cycle at 1 September, there was no trend,

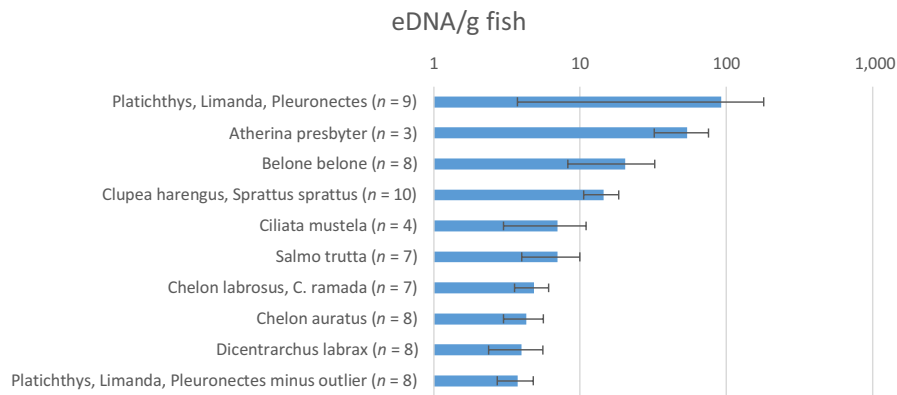
and differences between samples taken at incoming and outgoing tide were not significant. Also, comparison between samples taken at high tide and low tide over the season (18 weeks) did not reveal a significant difference between tides. This corresponds to biological knowledge on the distribution of fish species over the area. Within 15 km from the NIOZ jetty, the coastal North Sea and the western Wadden Sea represent relatively similar habitats (Jager et al., 2009) and no big differences between fish communities are expected. The average fish eDNA concentration was slightly higher in low tide samples than in high tide samples in spring and early summer (April-June), which may imply the presence of the same local fishes in a smaller water volume. Also, effects of cosampling resuspended sediment with the surface water, which is probably stronger at low water, cannot be excluded. In contrast to Buxton, Groombridge, and Griffiths (2018), who showed higher detection probabilities of eDNA from water than from sediment, it has been shown that fish eDNA can be more concentrated in sediments than in surface water (Turner, Uy, & Everhart, 2015). Thus, it is possible that cosampling of resuspended sediment had an effect on the yield of fish eDNA on our filters.

## 4.3 | Relationship between eDNA concentration and fish wet mass

Fish eDNA concentration in surface water in the Marsdiep tidal inlet was significantly correlated (on log scale) to fish wet mass in the catches nearby. Other field studies (Schmelzle & Kinziger, 2016; Thomsen et al., 2016; Yamamoto et al., 2017) already presented moderate relationships between eDNA and fish biomass, based on relative read distributions and relative mass, whereas we compared absolute eDNA concentrations (copies/L water) of fish species to their absolute wet mass (kg) in the fyke catches. Thomsen et al. (2016) also reported a significant correlation between eDNA and fish counts. This we did not find, presumably due to changes in relative abundance of juvenile fishes and changes in species composition over the season, whereas Thomsen et al. (2016) sampled over a short period.

The concentration of eDNA per total fish counts showed a similar trend as the average size class of the total fish population. This confirms previous observations (Jo et al., 2019; Maruyama et al., 2014) that larger fish specimens release more eDNA than smaller fish specimens. The concentration of eDNA per fish wet mass showed a different trend and was not always consistent with changes in fish size class: Between 22 April and 6 May, eDNA per mass increased when average size class increased, whereas between 27 May and 10 June, eDNA per mass increased when average size class decreased due to high numbers of juvenile fishes (especially plaice *P. flesus* and herring *C. harengus*). The latter observation is consistent with other studies: In aquarium settings, eDNA release rate per fish mass was about four times higher for small juvenile fishes than for adult fishes (Maruyama et al., 2014). It is likely that changes in species composition also affected overall eDNA per mass values. For example, we found that average eDNA/mass for different fish species varied between 4 and 92 copies  $L^{-1} g^{-1}$  (Figure 8) with high values for *A. tobianus* (sand smelt), *Belone*





**FIGURE 8** Average concentration of eDNA in the surface water per fish wet mass in the fyke net catch for different fish species

*belone* (garfish), and *C. harengus*, *S. sprattus*. The high average value for *Platichthys*, *Limanda*, *Pleuronectes* eDNA/mass may be biased because on 4, 6, and 7 May, the fyke was not operational, while a large peak in flatfish eDNA was measured exactly on 7 May. Calculations of the average flatfish eDNA/mass, excluding the data for 7 May, resulted in values that were more similar to values of other fishes, for example, *Salmo trutta*, *C. labrosus*, and *D. labrax*.

#### 4.4 | Effect of water temperature on eDNA concentration

Our field study was not designed to draw firm conclusions about the effect of water temperature on eDNA concentration, and we cannot differentiate between lower eDNA release and higher eDNA decay rates. However, since aquarium studies also have their limitations and biases (e.g., due to different stress levels of natural and aquarium populations, artificial light conditions, and potential different microbial communities from those in the field), we tried to provide useful clues/hypothesis for the effect of water temperature on the eDNA concentration and whether or not one should correct for changes in temperature when monitoring the fish community via eDNA. During the period of field sampling, the temperature of the water increased from 10°C on 22 April to 19°C on 8 July, remaining high (19–20°C) throughout July and August. As a result of the temperature increase, the half-life time of eDNA may have decreased 24x from 6 days in April to 6.3 hr in July and August (values based on measurements in aquarium settings; Maruyama et al., 2014; Thomsen, Kielgast, Iversen, Møller, et al., 2012). However, during our field study, eDNA values per wet mass of fish did not change a lot over the season: The average was  $9 \pm 3$  copies  $L^{-1} g^{-1}$  (value  $\pm$  SE;  $n = 10$ ) for all fishes, for example,  $4 \pm 2$  copies  $L^{-1} g^{-1}$  (value  $\pm$  SE;  $n = 8$ ) for *D. labrax*, and  $15 \pm 4$  copies  $L^{-1} g^{-1}$  (value  $\pm$  SE;  $n = 10$ ) for *C. harengus*, *S. sprattus*; Figure 8). Perhaps not only the degradation rate of eDNA but also the release rate of eDNA is higher at higher temperatures, due to propensity for species to live in warmer waters (Takahara et al., 2012), or due to higher metabolic rates of fish at higher temperatures (Pilliod et al., 2014). This hypothesis is in line with recent findings (Jo et al., 2019), showing higher eDNA release rates at higher temperatures in tank experiments with *Trachurus japonicus* (Japanese jack mackerels). Obviously, other factors that were not included in our study may have increased or decreased

the eDNA concentration and process studies that include local settings in the Marsdiep area are needed to better clarify the effect of physiology and environmental factors on eDNA release. In the Marsdiep tidal inlet, the correlation between fish wet mass and fish eDNA concentration persisted despite a water temperature increase of 9°C.

## 5 | CONCLUSIONS

Weekly eDNA-based assessments of coastal North Sea fishes are a sustainable alternative for daily fyke catches. Good correspondence was found between presence-absence calls with both methods. Also, total fish eDNA concentration was significantly correlated to total fish wet mass. Moreover, seasonal trends of eDNA concentration and wet mass were similar for the eight most abundant fish species in the fyke. Small-sized species (small sandeel *A. tobianus* and gobies *Pomatoschistus* sp.) that are typically missed with the fyke contributed up to 25% or 40% of the total fish eDNA pool depending on the sample location.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

JvB conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and tables, and revised the paper; JE performed bioinformatic and statistical analyses and constructed the final workflow, wrote parts of the paper, and reviewed drafts of the paper; LK conceived and designed the experiment, organized the eDNA sampling, performed laboratory work, and reviewed drafts of

the paper; HW performed bioinformatic and statistical analyses and performed laboratory work. IJW performed the fyke fishing and the fish length and biomass measurements and contributed local fish ecology information; HvdV conceived and designed the experiment, organized the eDNA sampling, and reviewed drafts of the paper.

## DATA AVAILABILITY STATEMENT

Sequence data are available from the European Nucleotide Archive under accession number PRJEB35813. Analysis code, processed data, and the arb file with reference sequences are available at the NIOZ data portal via <https://dataportal.nioz.nl/doi/10.25850/nioz/7b.b.w>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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