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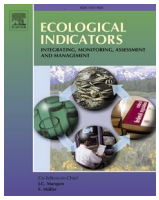

Original Articles

Fish diversity assessment and semi-quantitative biomass estimation through metabarcoding of environmental DNA

Yassine Kasmi [a,*], Ismael Núñez-Riboni [b], Tina Blancke [a], Benita Möckel [a], Matthias Bernreuther [b], Christoph Stransky [b], Reinhold Hanel [a]

[a] Thünen Institute of Fisheries Ecology, Herwigstraße 31, 27572 Bremerhaven, Germany
[b] Thünen Institute of Sea Fisheries, Herwigstraße 31, 27572 Bremerhaven, Germany

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ABSTRACT

A sustainable management of marine fisheries resources requires the continuous development and adaptation of monitoring and tracking techniques, both in terms of species diversity and abundance. In order to find alternatives to common invasive catch-based biodiversity assessments, we tested a fish-specific environmental DNA (eDNA) metabarcoding approach, targeting the mitochondrial 12S rRNA gene. Analysis of 64 seawater and sediment samples revealed a high detectability of fish DNA in the North Sea, with more reliable results in water compared to sediment samples. Species diversity assessments overlapped by 81 % between eDNA and bottom trawl catch analyses, with more than 32 % of the species detected only by eDNA compared to bottom trawling as reference value (100 %).

With regard to quantitative biomass estimates for common dab (*Limanda limanda)*, the eDNA-based estimates revealed similar results as compared to bottom-trawl catches. Modelling of trawl outputs as a function of read counts and sampling depth yielded up to 70% correlation between the model and the observed data for common dab.

The outcomes of this study again highlight the potential of eDNA for marine biodiversity monitoring, not only for presence/absence assignments of species, but also for biomass estimates, with a high degree of reliability as compared to reference methods. The model reveals the importance of including environmental data to correct the bias linked to the persistence of eDNA in seawater.

1. Introduction

Many marine ecosystems are outstanding in terms of species richness (Alidoost Salimi et al., 2021; McQuatters-Gollop et al., 2022). However, biodiversity assessments in the sea are often biased due to water column and sediment structure related habitat complexity as well as rapid temporal changes. Classical monitoring schemes therefore mostly cover only a fraction of the diversity in a given area (Browman et al., 2004; Crain et al., 2009; Fock et al., 2020; Williams, 2011). For a more comprehensive overview of the present state of marine resources, the development and adaptation of new monitoring and tracking methods is inevitable, especially when they are the basis for management decisions (Browman et al., 2004; Crain et al., 2009; Williams, 2011).

The application of environmental DNA (eDNA)-based methods in fisheries research started in the beginning of the last decade (Foote et al., 2012). The increasing attention to the implementation of eDNA-based assessments is mainly due to their non-invasive nature and facilitated by the relatively long half-life of eDNA of up to 48 h in seawater (Collins et al., 2018). Numerous studies have demonstrated the potential of eDNA to reveal species richness in freshwater and the seas. Mathon et al. (2022) detected 16 % more species by eDNA analyses at 1224 stations than in underwater visual surveys in the Central and Southwestern Pacific and the Western Indian Ocean. Similarly, Doorenspleet et al. (2021) found eDNA-based results well suited to assess marine biodiversity as compared to trawl catches. Various studies demonstrated the ability of Mifish primers targeting the mitochondrial 12S gene (Miya et al., 2015) to reveal fish diversity in marine and freshwater habitats (Baidouri et al., 2025; Bowen et al., 2024; Doi et al., 2023; Kasmi et al., 2024; Spens et al., 2017; Tibone et al., 2024). Other studies demonstrated the capacity of Mifish primers to also confirm presences of marine mammals (Valsecchi et al., 2021; Zhang et al., 2023).

While eDNA analyses of water samples are largely intended to reflect

* Corresponding author.
E-mail address: yassine.kasmi@thuenen.de (Y. Kasmi).

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a spatially and temporally confined overview of biodiversity, sediment samples are often considered as biodiversity archives, by accumulating biological information over potentially long periods of time, dependent on the physical and chemical conditions of the seafloor (Baltazar-Soares et al., 2022; Ogata et al., 2021; Sakata et al., 2020; Tagliabue et al., 2023).

Metabarcoding via high-throughput sequencing (HTS) generates millions to billions of sequence reads covering several giga to tera-bases in a single run. However, most metabarcoding studies only focus on qualitative (presence/absence) assignments of species and thereby largely ignore the quantitative information in the generated data, due to a lack of information on abiotic and biotic impacts on eDNA shedding, a lack of adequate computational tools and the error linked to multi-PCR steps during library preparation. Abiotic factors, like water temperature, pH, salinity and UV, largely determine persistence and degradation of eDNA in the aquatic ecosystems (Qian et al., 2022), highlighting also the influence of sampling season on eDNA-based biomass and abundance estimations (Doi et al., 2019; Doi et al., 2017a, Doi et al., 2017b).

While the potential of qPCR-based eDNA analyses for biomass estimation has repeatedly been demonstrated (Ai et al., 2025; George et al., 2023; Ledger et al., 2024, 2024; Rehill et al., 2024; Shelton et al., 2022; Urban et al., 2023; Yates et al., 2023), quantitative analyses of metabarcoding and next-generation sequencing (NGS) data are comparably scarce (Ershova et al., 2021; Handley et al., 2020; Merson et al., 2025; Yates et al., 2023). A recent study by Yates et al. (2023) shows a direct mismatch between eDNA reads and either numerical abundance or biomass. However, the application of an allometric scaling framework derived from Metabolic Ecology Theory improved the accuracy of the eDNA-derived data and provided a flexible tool for estimating population-level metrics across diverse aquatic ecosystems. The introduction of an allometric scaling framework also significantly improved the correlation between eDNA read numbers and biomass in Walleye pollock (*Gadus chalcogrammus*), Pacific cod (*Gadus macrocephalus*), and Arctic cod (*Boreogadus saida*) (Ledger et al., 2024).

The present study aimed at determining the effectivity of eDNA to assess marine fish diversity using high-throughput DNA sequencing in the North Sea. Furthermore, the possibility of extracting quantitative information from NGS outputs, as opposed to solely qualitative information, was tested in combination with modelling approaches comparing the number of reads obtained from HTS with bottom trawl fisheries data.

2. Method

2.1. Sampling

2.1.1. Water and sediments

Sampling was conducted from 8 July to 6 August 2019 during the scientific research survey WH428 aboard the German fisheries research vessel Walther Herwig III. A total of 16 International Bottom Trawl Survey (IBTS) monitoring stations covering a wide gradient of environmental conditions were sampled in the North Sea (Fig. 1).

Water: A total of 32 water samples (two replicates per station) were collected by 5 L Niskin bottles attached to a CTD (conductivity-temperature-depth) rosette at approximately four meters above the seafloor at each station to avoid sediment contamination while still matching the average bottom trawl height of around five meters. Each Niskin bottle was treated as an individual sample, and immediately filtered on board through Sartorius™ PES membranes (pore size: 0.45 μm, diameter: 47 mm) with a vacuum pump. After filtration, the filters were stored at −20 °C until DNA extraction. In addition, 16 negative samples of ultrapure water used to rinse the Niskin bottles prior to sampling were taken.

Contamination avoidance included the thorough rinsing of the CTD

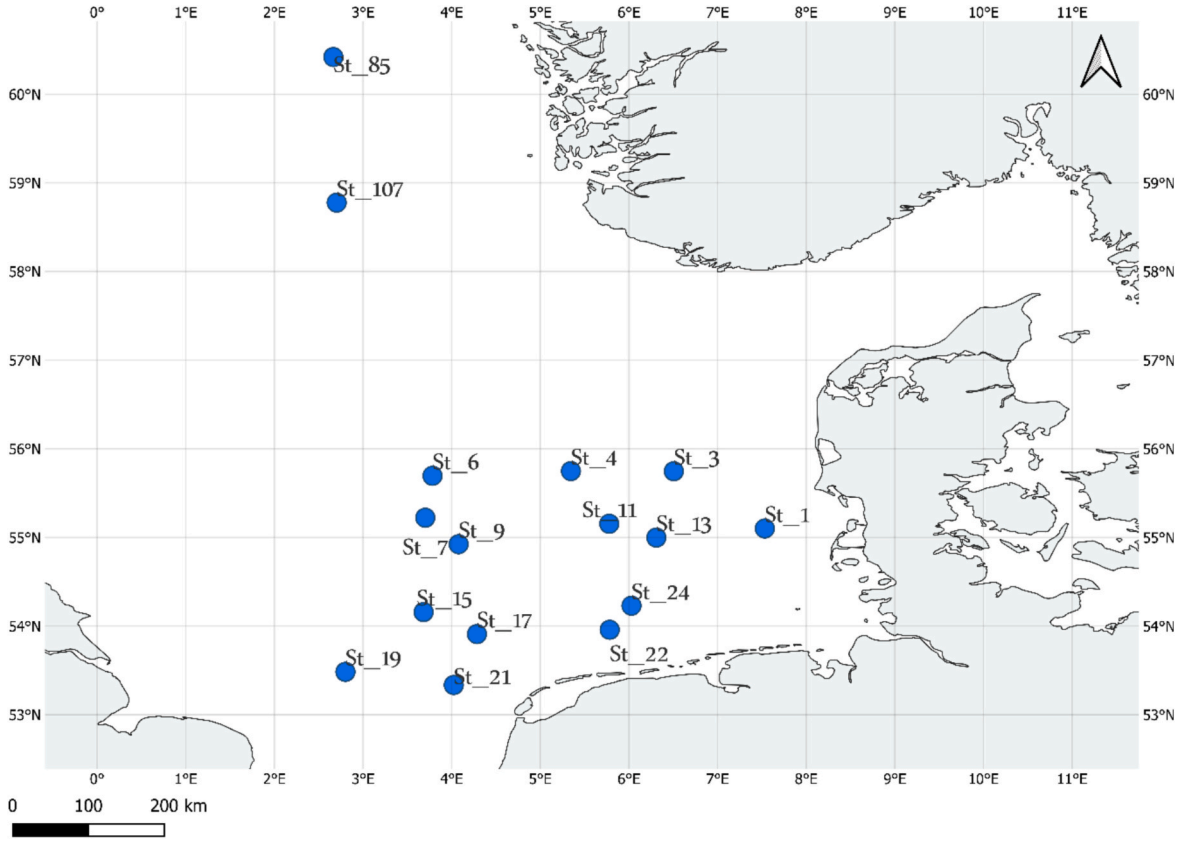

Fig. 1. Sampling map. Blue circles indicate sampling stations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rosette and the Niskin bottles with fresh water on deck immediately after recovery, followed by rinsing with ultrapure water. The on-board workbench area was covered with aluminium foil and rinsed with a 20 % commercial bleach solution followed by ultrapure water. Each lab bottle was rinsed three times with sample water and then filled with 2.5 L of ultrapure water, of which one bottle was subsequently used as negative control per station. All further processing of the samples took place in a sterile environment in a molecular biology lab on land. The negative controls have been extracted separately from water samples and sequenced in separate library.

***Sediments*:** In total, 32 superficial sediment samples were collected at the 16 sampling positions in duplicates (two per station) using a common Van Veen grab. The grab was rinsed with sterile water in between sampling. Approximately 50 g (≈ 40 ml) of the superficial strata of the sediment were taken with a plastic spoon (one per sample) and placed into a 100 ml sterile container filled with 60 ml of pure ethanol and stored at −20 °C until extraction.

Depth, salinity and temperature data were obtained in parallel with a CTD probe (CTD 48 M, Sea and Sun, Germany).

2.1.2. Trawl assessment

Demersal trawling was carried out according to the International Bottom Trawl Survey (IBTS) standards (ICES, 2020). Trawling was conducted for up to 30 min at a trawl speed of approximately 4 knots using the standard bottom trawl GOV (chalut à Grande Ouverture Verticale), immediately after water sampling at each station. Each catch was sorted by species. Weights of the total catch and of each sorted species were recorded together with length measurements of all specimens. For selected species, such as common dab (*Limanda limanda*), European sprat (*Sprattus sprattus*) and whiting (*Merlangius merlangus*) (ICES, 2020), single fish weight measurements, sex and maturity stage discrimination were performed according to IBTS standard species for subsequent laboratory age determination. In this study, catch per unit effort (CPUE) is defined as the number of fishes and weight (kg) of a certain species that are caught per hour of trawling.

2.2. DNA extraction

2.2.1. eDNA extraction from water samples

eDNA was extracted from water according to Renshaw et al. (2015) from 32 water samples and 16 negative controls. In brief, filtered and frozen PES membranes were placed in a 2 ml tube and then supplied with 700 µL of CTAB buffer (2 % CTAB (w/v), 1.4 M NaCl, 100 mM Tris, 20 mM EDTA) and incubated at 65 °C for 10 min with agitation at 650 rpm. Subsequently, 900 µL of PCI phenol/chloroform/isoamyl alcohol (25:24:1) were added and vortexed for 5 s, prior to centrifugation at 15,000 x g for 5 min. 700 µL of supernatant was transferred to a new 2 ml tube, prior to adding 700 µL of chloroform, followed by centrifugation at 15,000 x g for 5 min. After transferring 500 µL of the supernatant to a new 2 mL tube, the Monarch® cleaning kit (New England Biolabs, Frankfurt, Germany) was used by adding 1000 µL of binding buffer. The elution was performed into 20 µl of elution buffer. DNA extracts were stored at −20 °C until analysis. All DNA extraction steps were performed under a chemical hood in a dedicated eDNA area of a genetics laboratory. Negative control of extraction were included to each extraction batch and treated in the same way like water samples.

2.2.2. eDNA extraction from sediments

The extraction of eDNA from the sediments was performed by using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's instructions. Ultrapure water was used as negative extraction control and treated the same way as regular samples throughout the experiment. DNA extracts were stored at −20 °C until analysis. All DNA extraction steps were performed under a chemical hood in a dedicated eDNA area of the genetics laboratory. Negative control of extraction were included to each extraction batch and treated in the same way like the samples.

2.3. Sequencing and NGS

All libraries were prepared with MiFish primers (Table 1) targeting the 12S rRNA gene, according to the protocol of Miya et al. (2015). The library preparation followed two PCR steps. The first PCR cycle (1st PCR) aimed at specifically amplifying the 12S rRNA gene of fishes with adapter sequences. The second PCR cycle (2nd PCR) was performed using the diluted 1st PCR product and Illumina adapters for binding to the Illumina sequencer flow cell (MiSeq). NGS was performed with an Illumina MiSeq sequencer.

The 1st PCR was performed in a reaction volume of 12 µl containing 6.0 µl of 2 × KAPA HiFi HotStart ReadyMix (Roche, Germany), 2.8 µl of each primer (Primer Mix: MiFish-E-F/R-v2: MiFish-U-F/R: MiFish-U2-F/R = 0.125: 0.250: 0.125 µM), 1.2 µl of H_2O sterile distilled water, and 2.0 µl of eDNA. The primer pairs co-amplify a hypervariable region of the fish mitochondrial 12S rRNA gene (approximately 180 bp) and add primer binding sites (5′ ends of the five N forward sequences) for sequencing at both ends of the amplicon. The five random bases were used to improve cluster separation on flow cells during initial base-calling calibrations on the MiSeq platform. The thermal cycling profile after an initial 3 min denaturation at 95 °C was as follows (35 cycles): denaturation at 98 °C for 20 s; annealing at 65 °C for 15 s; and extension at 72 °C for 15 s, with a final extension at the at 72 °C for 5 min. Four replicates were performed for the 1st PCR, and replicates were pooled to minimise PCR dropouts into a single 1.5 ml tube. Then, the products were purified with a GeneRead Size Selection Kit (Qiagen, Germany) to remove all sequences less than 150 bp in length. The purified products were diluted to a concentration of 0.1 ng/µL after quantitative and qualitative verification by TapeSation4200. The 2nd PCR was performed in a reaction volume of 15 µl containing 7.5 µl of 2 × KAPA HiFi HotStart ReadyMix, 0.88 µl of each primer (5 µM), 3.88 µl of sterile distilled H2O and 1.86 µl of template. MiSeq primers included the dual index sequences (8 nucleotides indicated by X's) and flow cell binding sites for the MiSeq platform (5′ ends of sequences before eight X's) were used. The thermal cycling profile after an initial 3 min denaturation at 95 °C was as follows (12 cycles): denaturation at 98 °C for 20 s; combined annealing and extension at 72 °C for 15 s, with a final extension at 72 °C for 5 min. The concentration of each 2nd PCR product was measured by Qubit. Subsequently, the pooled 2nd PCR product was size-isolated to approximately 370 bp and the "fish band" was purified by the MinElute Gel Extraction Kit (Qiagen, Germany). The product was sequenced on the MiSeq platform using a MiSeq v2 Reagent Kit (2 × 150 bp) (Illumina, Inc.). Negative control of library preparation were included to each batch and treated in the same way as the samples.

2.4. Bioinformatics analysis for metabarcoding data

The quality of the reads was checked by using the FASTQC program (Babraham Bioinformatics). Subsequently, forward and reverse reads were assembled by vsearch via the *--fastq_mergepairs* option. Cutadapt was used to trim the reads and remove primers. Subsequently, Operational Taxonomic Unit (OTU) analysis was performed on sequences with a similarity of 98 % or more, while Amplicon Sequence Variant (ASV) analysis took place on those with a confidence percentage ≥ 75 %. For both methods, only sequences with a fragment length between 160 and 260 bp were selected. The processed reads were subjected to a BLASTN search of the complete NCBI (Macher et al., 2017) and MAREMAGE (Kasmi et al., 2023) databases. All sequences originating from organisms other than bony fish, sharks and rays or with lengths less than ≤ 150 bp were eliminated. All sequences with similarities of more than 97 % between the queries and the BLASTN were assigned to a distinct species. Sequences with a similarity below 97 % but higher than 92 % were assigned to a distinct genus, while all sequences with similarities less than 92 % but higher than 75 % were assigned to a family level (Xiong

Table 1
12S rRNA primers.

Name	Sequence (5′ – 3′)
MiFish-U-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGTCGGTAAAACTCGTGCCAGC
MiFish-U-R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCATAGTGGGGTATCTAATCCCAGTTTG
MiFish-E-F-v2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNRGTTGGTAAATCTCGTGCCAGC
MiFish-E-R-v2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNGCATAGTGGGGTATCTAATCCTAGTTTG
MiFish-U2-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGCCGGTAAAACTCGTGCCAGC
MiFish-U2-R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCATAGGAGGGTGTCTAATCCCCGTTTG

et al., 2022).

2.5. Computational modelling of metabarcoding data

Assuming that there is a possible quantitative relationship between the number of fish caught in the bottom trawl and the number of DNA reads from metabarcoding, we investigated the possibility of modelling the number of fish caught as a function of the number of reads. Data from common dab (*Limanda limanda*) were used for this purpose. This species was chosen because it was observed at high abundance at most stations and they represent distant taxonomic bony fish groups. The number of reads was modelled against the trawl results by univariate analysis using linear regression modelling methods, namely generalized least squares (GLS). In addition, we considered applying multivariate analysis using depth, salinity and water temperature as well as number of reads per litre as predictive descriptors and catch data as response value/model output. Pressure, salinity and temperature were chosen as parameters with potential effects on the persistence of eDNA in seawater.

To assess the relationship between biomass and eDNA counts from the metabarcoding data, two generalised linear models (GLM) were selected out of 150 models based on their correlation with biomass predictor, lower AIC values and a significant number of model terms. The GLM response in both models was catch per unit effort (CPUE) in kg/h and fish/h combined with log link functions.

eDNA degrade exponentially in time due influence of various abiotic factors such as temperature, filtration duration, etc (Jo et al., 2019; Strickler et al., 2015; Wood et al., 2020). To account for this degradation and standardize our eDNA measurements, we introduced a calculation for the expected initial eDNA concentration at the time of sampling, denoted as $eDNA_{expected(t0)}$ or eDNA0. This approach allows us to estimate the original eDNA concentration before degradation occurred, providing a more accurate basis for comparison across samples with varying processing and storage conditions (Jo et al., 2019).

Predictors in the models included the observed number of eDNA copies per litre of sampled water (referred to as $eDNA_{observed}$ in this paper) and the expected number of eDNA copies at the time of sampling ($eDNA_{expected(t0)}$ or eDNA0) (eq 01). Utilizing $eDNA_{expected(t0)}$ instead of $eDNA_{observed}$ allows us to standardize the initial amount of eDNA present at the time of sampling. This standardization helps to address and mitigate potential biases that arise due to variations in the timing of filtration and the different conservation methods used, ensuring a more accurate and consistent comparison across samples.

$$eDNA_{expected(t0)} = \frac{eDNA_{observed}}{e^{-(t1*r1+t2*r2)}} \quad (1)$$

with $eDNA_{observed}$ the number of eDNA reads observed in this data. $r1$ and $r2$ are the decay rate of eDNA at 24 °C equivalent to filtration temperature and 4 °C (conservation temperature), respectively according to Scriver et al., (2023), multiplied by the filtration and conservation time represented by $t1$ and $t2$ in this equation, respectively.

We also included environmental factors derived from the CTD data, mainly sampling depth as a proxy for correlated environmental factors and water temperature. To reduce multicollinearity, variables with more than 70 % correlation were removed, leaving only two key variables for the final analysis: 1. Sampling depth, expressed as a positive value starting from 0 at sea surface and 2. Temperature expressed in degrees Celsius. Given the high correlation between temperature and depth (approximately 70 %), only one environmental factor was included in each model to reduce the risk of false positive correlations.

To further explore the relation between the response variable and the model predictors, we constructed non-linear models based on the two chosen GLMs, by inserting a parameter into a logarithmic function (Eq. 2 and 3). The model parameters were estimated using the Nelder-Mead method (Nelder & Mead, 1965). Initial values of the non-linear model coefficients were estimated from the GLM model, with the coefficients of the environmental factors within the logarithm initially set to 1. The best two no-linear models were (eq. 02 and 03):

$$\log(\mathrm{CPUE}(eDNA0, \mathrm{Depth.})) = (\alpha + \beta \times (Depth)) + \gamma \times log(eDNA_{expected\ (t0)} - \delta \times \mathrm{Depth})) \quad (2)$$

$$\log(\mathrm{CPUE}(eDNA0, Tp)) = (\alpha \times \mathrm{Tp} + \beta \times (\mathrm{Tp}^2)) + \gamma \times log(eDNA_{expected\ (t0)} - \delta \times \mathrm{Tp}) \quad (3)$$

The non-linear model equations serve as a tool for estimating biomass levels based on a combination of eDNA and environmental factors. This model uses two interdependent complementary functions to elucidate different aspects of the relationship between eDNA, environmental conditions and biomass:

$\log(CPUE\ (eDNA_{expected(t0)},\ environmental\ factor)) = g(environmental\ factor) + f(eDNA_{expected(t0)},\ environmental\ factor))$.

The first function describes the influence of environmental factors on biomass, denoted *g (environmental factor),* and provides insight into how factors such as depth affect the overall biomass composition within a given ecosystem. The second function f($eDNA_{expected(t0)}$, *environmental factor*) explores the interplay between eDNA concentration ($eDNA_{expected(t0)}$), environment (e.g., eDNA degradation) and catch per unit effort (CPUE), revealing nuanced relationships between these variables. In this function, CPUE is modelled as a logarithmic function of both eDNA concentration and depth, recognizing the non-linear nature of these relationships. Depth is used as a proxy for other environmental factors that may not be directly measurable but are nonetheless influential. The coefficients within the equation, including α, β, γ and δ, represent the strength and direction of these relationships and provide insight into how changes in eDNA concentration and depth affect CPUE. Importantly, the inclusion of the parameter (δ) highlights the nuanced relationship between eDNA concentration and depth, suggesting that the influence of eDNA on CPUE may vary with depth in the water column.

All analyses and data visualizations were performed in R version 4.2.0. The GLM was implemented using the GLM package and the *glm* function, while the Nelder-Mead optimization was performed using the 'nelder mead' package.

Replicates from the same station were treated as independent samples. CPUE in a specific area was used as a basis for prediction. CPUE was defined as kg/h and number of fish/h.

eDNA copy numbers in the original seawater samples (X: copies/L) were calculated based on the volume of filtered seawater (a: 5 L), the re-

suspended volume of purified DNA (b: 20 μL), the amount of purified DNA added to each NGS-reaction volume (c: 2 μL/ reaction), and the number of reads in your NGS data (Y: reads/NGS reaction).

3. Results

3.1. Fish biodiversity

3.1.1. Results of eDNA

3.1.1.1. Sequencing performance and pre-processing. Metabarcoding of 34 water samples generated a total of 15,982,574 12S rRNA paired-end reads, in the MiSeq platform (Illumina), compared to 7,208,776 reads in the sediment samples (3,604,388 each for forward and reverse). All sample files were of high quality for reads of up to 260 bp. The reads were filtered for primers, chimeras, quality score and copy number. From all water and sediment libraries, 85 % of all reads were merged. The percentage of fish reads was between 40 % and 98 % per sample, with an average of 84 %. All negative control – water samples, –extraction and –library preparation were negative. For the sediments' negative controls 2 samples included less than 10 reads of European pilchard, therefore considered as negative as well.

3.1.1.2. eDNA sequencing from water samples. The species composition revealed from the water samples was dominated by bony fishes (Actinopteygii) with an average relative abundance of 92 % (48 species) (Fig. 2). Cartilaginous fishes (Elasmobranchii) were only represented by the thorny skate (*Amblyraja radiata)* and the porbeagle shark (*Lamna nasus)*. In addition, with the harbour porpoise (*Phocoena phocoena*) a marine mammal was recorded using MiFish primers at stations 6 and 7, in the central North Sea.

The most abundant bony fish orders in our results were the Clupeiformes, Pleuronectiformes, Scombriformes, Perciformes and Gadiformes, respectively, covering more than 75 % of the total number of species. At the genus level, an average of 27.5 % and 13.7 % of the reads were attributed to *Clupea* and *Limanda*, respectively. The fish species present in almost all stations within the study area were Atlantic herring (*Clupea harengus),* grey gurnard *(Eutrigla gurnardus),* common dab *(Limanda limanda),* whiting *(Merlangius merlangus),* starry flounder *(Platichthys stellatus),* European plaice *(Pleuronectes platessa),* Atlantic mackerel *(Scomber scombrus),* common sole *(Solea solea)*, and European sprat (*Sprattus sprattus)* (Fig. 2). 38 % of the reads couldn't be assigned to any fish genus or species, 3 % were assigned to human or mouse.

Amplicon sequence variant (ASV) analysis (based on confidence percentages ≥ 75 %) increased the number of assigned species to 53 compared to 45 from Operational Taxonomic Unit (OTU) analysis. Only sequences with a fragment length between 160 and 260 bp were assigned. ASV also reduced the number of unassigned reads from 41 % to 38 %.

Fish composition clearly differed between sampling sites, with a general increase in abundance and diversity with increasing latitude, as revealed from both, water and sediment eDNA analyses. Diversity increased significantly at the northernmost stations and along the northern Danish coasts (St 85).

Overall misidentification rate amounted to 19 %, with 6 of the falsely identified species (11 %) originating from the Pacific Ocean and 4 species (8 %) from the North Atlantic and Arctic. However, many of the obviously false records have closely related, often congeneric relatives in the North Sea, especially in the family Gadidae, with differences of only one to three (0.5–1.5 %) nucleotides in the target region of the 12S rRNA gene. The most common water-derived false positive hit was starry flounder (*Platichthys stellatus)*, for sediment-derived eDNA white perch (*Morone americana)*.

3.1.1.3. eDNA sequencing from sediment samples. The metabarcoding results of the sediments showed a heterogeneous diversity together with an uneven distribution of species between stations. Many southern stations showed low richness, in which 10 from 14 stations showing less than 5 species.

In total, 25 fish species were recorded in sediment samples, of which 18 (72 %) were detected only in station no. 85. The most common species detected in sediment eDNAs were European pilchard, European sprat, sand goby and common dab. (Fig. 3).

The fish composition in sediment samples was dominated by bony fish with an average relative abundance of 99.3 % (24 species) (Fig. 3), dominated by the orders Clupeiformes, Gadiformes and Pleuronectiformes, (Table S3). Furthermore, 69 % of the reads remained unassigned and 19 % were of human or mouse origin.

3.1.2. Trawl catches

3.1.2.1. Geographical distribution of fish species. Trawl data analysis confirmed the observed trend of an increase in fish diversity and abundance with increasing latitude (Fig. 4), with a maximum catch of 1385.77 kg/h in western Norway (station 107) and a minimum of 49.76 kg/h at station 13 in the southern North Sea. The most abundant species

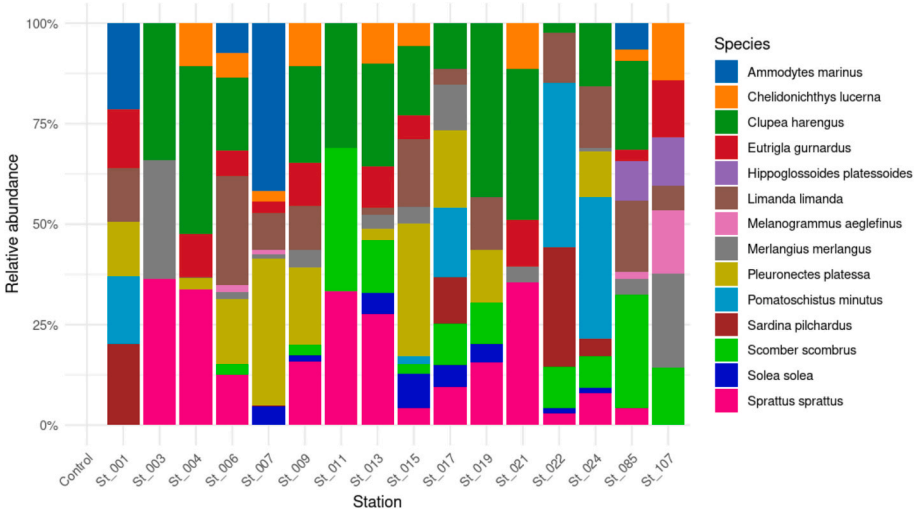

Fig. 2. The 14 most common fish species in water samples. The Y-axis represents the percentage of reads of each species per sample (relative abundance), while the X-axis presents the relative abundance in percentage. This figure is generated basing on ASV results.

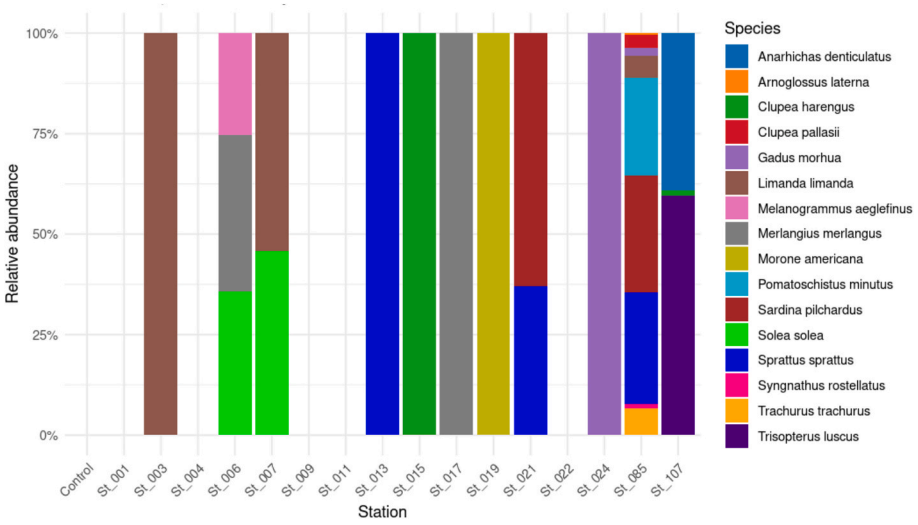

Fig. 3. Fish biodiversity in sediment samples. The Y-axis represents the percent of reads for each species into each sample (relative abundance), while the X-axis presents the relative abundance per stations. This figure is generated basing on ASV results.

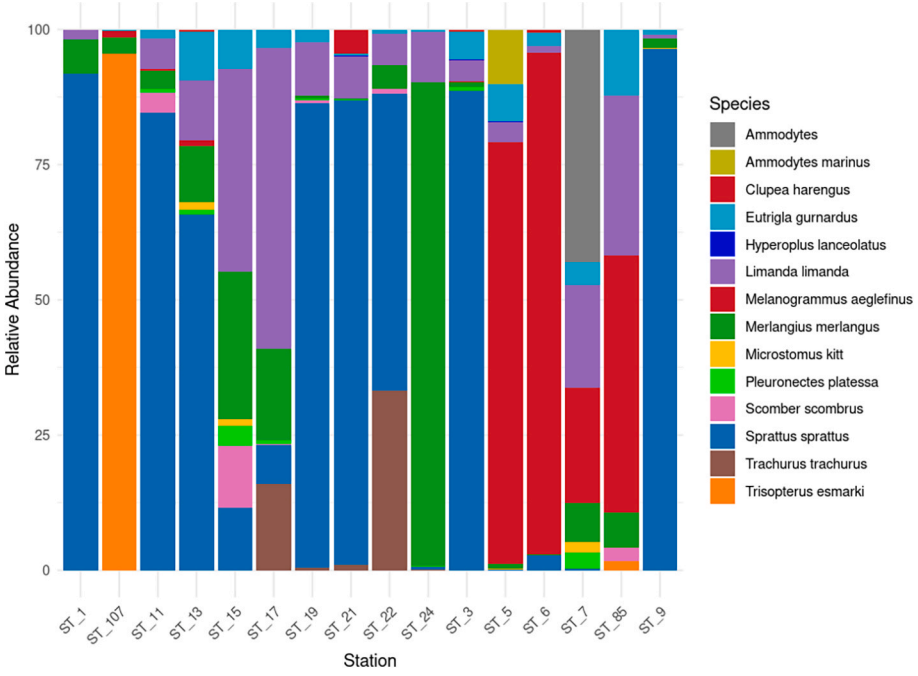

Fig. 4. Fish biodiversity as relative abundance (%) in the trawl samples (by station).

across all stations were European sprat, Norway pout, whiting and haddock.

3.2. Comparison between trawl and eDNA derived fish biodiversity assessment

81 % of the fish species caught by the bottom trawling were also detected via eDNA. However, eDNA data revealed species not found through bottom trawling.

Seven of the bottom trawl caught species were not detected in the respective eDNA analyses due to the unavailability of their 12S rRNA sequences in public genetic databases at the time of the study: solenette (*Buglossidium luteum*), lesser weever (*Echiichthys vipera*), common dragonet (*Callionymus lyra*), great sand eel (*Hyperoplus lanceolatus*), starry smooth-hound (*Mustelus asterias*), spotted ray (*Raja montagui*), and tub gurnard (*Chelidonichthys lucerna*). Another eight species were not detected by eDNA because their DNA was below the detection limit (1 to 3 individuals per catch): European anchovy (*Engraulis encrasicolus*), Atlantic halibut (*Hippoglossus hippoglossus*), striped red mullet (*Mullus surmuletus*), shorthorn sculpin (*Myoxocephalus scorpius*), turbot (*Scophthalmus maximus*), thornback ray (*Raja clavata*) and cuckoo ray (*Leucoraja naevus*).

3.3. Modelling biomass from eDNA reads compared to trawl catches

Table 2 and Fig. 5 summarize the results of our modeling approach, highlighting the explained variances for common dab. The univariate correlation between log-transformed eDNA read counts and fish biomass (CPUE) was initially 35 %. This value has been increased by including $eDNA_{expected(t0)}$. Furthermore, this correlation significantly improved when sampling depth was included as a predictor in a multivariate model. The best-performing non-linear model (Eq. 02) achieved a

Table 2
Comparison of the regression between the eDNA-based model outputs and the observed catch data.

Model		Common dab	
	Link	AIC	R2
Eq02	log	161	0,70
Eq03	log	196	0,31

correlation of up to 70 %, making it the most effective method for predicting fish biomass as a function of eDNA read counts per litre and sampling depth. For a second model (Eq. 03), which used temperature instead of depth as a predictor, the correlation between eDNA and CPUE reached 31 %. These results underscore the importance of including environmental variables like depth to improve model performance.

4. Discussion

A direct comparison of the results of an eDNA metabarcoding approach and standardized bottom trawl catches revealed the general suitability of non-invasive genetic methods for fish biodiversity assessments in the North Sea. In addition, a quantitative analysis based on the number of eDNA reads showed a positive correlation with fish biomass and density for common dab. Modelling approaches for fish biomass and abundance in relation to the number of eDNA reads and sampling depths based on ensemble learning techniques revealed good non-linear correlations for the common dab.

4.1. Biodiversity

Water-derived eDNA analyses much better reflected fish biodiversity compared to eDNA from sediment sources. In 10 from 16 stations, maximum one species has been detected by analysing eDNA sediments, whereas at all water-derived eDNA at least 3 species were detected. In contrast, the proportion of non-fish reads (including high numbers of phytoplankton and microbial) was significantly higher in sediments compared to water.

The primary reason for this disparity can be explained by a higher homogeneity of water compared to sediment. Sediments, being less homogeneous, provide a favourable niche for bacteria to form biofilms, resulting in 2–1000 times richer bacterial contents compared to water (Sander & Kalff, 1993). This disparity poses challenges in obtaining reliable information, and it needs to be tested, if an increase of the number of sediment samples per station would result in a more comprehensive understanding of fish composition. Sediments are reported as valuable archives that potentially enable to trace back the ecological history of marine regions and the determination of the impacts of biotic and abiotic factors on biodiversity over a period spanning more than two million years (Kjær et al., 2022; Ogata et al., 2021; Sakata et al., 2020; Turner et al., 2015). However, targeting ancient DNA requires alternative sequencing techniques, preferably the application of shotgun methods, such as whole mitochondrial metagenomics (Doane et al., 2018).

In contrast, water-derived eDNA results, though rather providing a snapshot picture, matched very well with the concomitant bottom trawl catches. Exceptions include species yet unrepresented for 12S rRNA sequences in genetic public databases and rather rare, mostly benthic species. However, eDNA results also revealed species missing in the bottom trawl, including various mostly pelagic bony fish but also sharks (porbeagle) and cetaceans (harbor porpoise). This is not surprising, considering net avoidance capabilities of fast swimming large pelagic species. The standard IBTS trawl (GOV) differs in its catchability for different fish species (Fraser et al., 2007). In addition, the trawl has a scare effect by e.g. the action of the otter doors and trawl sweeps on the seabed stirring up a sediment cloud, allowing some fish species to avoid the net (Kaartvedt et al., 2012), while other species are herded into the path of the net (Engås & Godø, 1989). Considering a half-life of eDNA in seawater of approximately 24 h (Barnes et al., 2014), it is plausible for fish passing the area minutes or hours before the net was deployed but still contributing to the eDNA data. Barnes et al. (2014) and Maruyama et al. (2014) demonstrated that eDNA from fish in ambient water has a relatively slow degradation rate of 10 % per hour and 90 % per day, allowing for a longer-term view on fish diversity compared to short-duration bottom trawl surveys. Govindarajan et al. (2021) also found that fish composition from eDNA remains stable for up to 16 h, making it a sensitive and relatively long-term monitoring tool. This highlights the advantage of eDNA in providing meaningful information and a comprehensive overview of species richness over a longer timeframe compared to fisheries-based reference methods.

Another advantage of eDNA, particularly from water samples, is its ability to identify all species regardless of fish size, unlike traditional methods that may be limited by mesh-size selectivity. This is in line with previous studies demonstrating eDNA to be compatible with trawl or other reference methods both in marine and freshwater environments (Andruszkiewicz et al., 2017; Baker et al., 2018; Ficetola et al., 2015; Hinlo et al., 2017; Sakata et al., 2020) and an explanation for eDNA

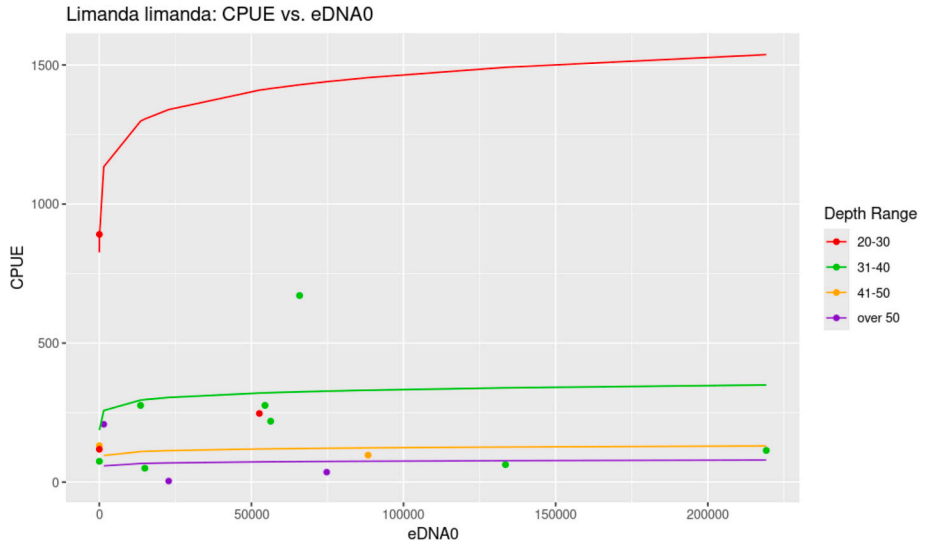

Fig. 5. Model simulation of biomass (CPUE) as a function of eDNA at time of sampling ($eDNA_{expected(t0)}$; X axes) and of depth (colour coding).

detecting more fish species than trawling at most stations in this study. Additionally, sampling water at different depths can provide a more comprehensive understanding of fish species richness, with qualitative results comparable to reference methods (Govindarajan et al., 2021). However, it is important to note that this aptitude to detect all sizes of species can also be perceived as a disadvantage. Indeed, knowledge of the length and/or life stage of fish can be crucial for fish stock assessments. Furthermore, eDNA cannot provide additional information on population structure, such as age, sex or size distribution, which is often key information for species management and conservation. It is also crucial to mention that the persistence time of eDNA in water can distort the results. The long persistence of eDNA in the water, after the organism has left the area, can lead to an overestimation of species abundance. Consequently, it is essential to take eDNA persistence into account when interpreting eDNA results.

The integration of different target genes can be beneficial to enhance the sensitivity of eDNA methods, potentially even allowing stock differentiation (Kumar et al., 2022). Furthermore, combining eDNA with other non-invasive monitoring methods like hydroacoustic and visual surveys in one model can offer a more comprehensive overview of fish biodiversity, distribution and biomass estimation.

Despite a generally high coherence between eDNA results and the known distribution of the respective species, some misidentifications occur, mostly based on high similarities of closely related species in the 12S rRNA region covered by the Mifish primers. While the total number of misidentified species is not significant (7 from 53 species), the total number of reads for some of them is high, suggesting the potential presence of undiscovered 12S rRNA haplotypes in the target species. In the same line Miya et al. (2020), Nester et al. (2020) and Takeuchi et al. (2019) mentioned the possibility to detect false positive species by Mifish primers in marine and freshwater eDNA due to various reasons and conclude on the inability of the primers to distinguish between closely related species. To solve this problem, primer modifications and target sequencing fragments of up to 1000 bp might be necessary to enhance the discriminative power as compared to current methods limited to 180–200 bp (Deiner et al., 2017; Doorenspleet et al., 2022; Koda et al., 2023; Nousias et al., 2024). However, new methods need to include solutions to also mitigate the high error rates associated with longer fragment sequencing. Another problem is the misannotation of available sequences in public databases, emphasizing the need for curated reference genomic databases. It is also recommended to increase the number of PCR replications and pooling them in order to reduce the risk of false negatives in eDNA metabarcoding studies (Miya et al., 2015, 2020).

One of the main limitations of the application of eDNA approaches remains the lack of genetic information for a multitude of fish species, reducing the percentage of overlapping between eDNA and trawling to 75 % (Duhamet et al., 2023; Hestetun et al., 2020; Kumar et al., 2022; Xiong et al., 2022). In the North Sea, for only 123 (56 %) out of 220 recorded fish species mitochondrial genetic information is publicly available. For others, only a single sequence is published, limiting insights into genetic variability and potential North Sea-specific haplotypes. This calls for greater efforts to integrate more species-specific DNA sequences in open access databases (Duhamet et al., 2023; Hestetun et al., 2020; Kasmi et al., 2023; Kumar et al., 2022; Xiong et al., 2022).

Nothing is yet known about species-specific detection limits, even though they may play a crucial role in presence/absence findings of metabarcoding outputs. The absence of some species in the eDNA results that were present in low abundance in single trawl catches may give a hint towards potential detection thresholds.

Data analysis clearly revealed the ASV method to be better suited than the OTU method for marine species, especially with 12S as target gene, as it is characterized by its high similarity between species, as previously shown by Antognazza et al. (2021) and Forster et al. (2019). OTU methods better fit to microbial studies, which often focus on genera or families, and thus less frequently touch the species or subspecies level. Since the ASV approach aims at assigning each read as a unique output and not as a cluster output, this increases its specificity.

From a biogeographic point of view, a clear pattern of an increase of fish diversity from the German and Danish coastal areas in the southeast to off the Norwegian coast in the northwest was observed, as previously reported by Bom et al. (2022).

4.2. Estimation of biomass from number of reads

Despite of the fact that NGS metabarcoding has long been used in marine science, its application remains primarily a qualitative rather than quantitative approach. Its quantitative value is still controversially discussed, because of claims that PCR amplifications during library preparation can negatively influence the final read counts or result in a lack of correlation between biomass and number of reads (Bik et al., 2012; Bucklin et al., 2016). Consequently, the millions of reads generated by NGS at each run are often not adequately used.

In order to overcome this issue, some studies have used internal standards or a corrective allometric scaling framework for biomass estimations (Bell et al., 2019; Handley et al., 2020; Ledger et al., 2024; Rehill et al., 2024; Zemb et al., 2020). However, none of these studies considered the potential impact of abiotic factors on eDNA integrity. Weak correlations were found between organism abundance and sequence reads using Pearson correlation methods (Bucklin et al., 2016; Ershova et al., 2021; Loos & Nijland, 2020; Mariac et al., 2022; Pont et al., 2018). In contrast, studies in the field of medical science have shown good correlations between the outputs of quantitative PCR (qPCR) and the number of reads (Flügge et al., 2023; Forster et al., 2019; Min et al., 2020; Robin et al., 2016; Song et al., 2021).

The results of this study show that biomass positively correlates with read numbers, under consideration of sampling depth and other ecological parameters (Table 2 and Fig. 5). By considering the influence of the abiotic variables on eDNA stability at the time of sampling through including an allometric scaling framework derived from the Metabolic Ecology Theory, the correlation between eDNA read numbers and abundance estimates significantly increased. Similar studies revealed correlations of around 70 and 90 % between metabarcoding-derived and reference data on species abundance (Handley et al., 2020; Yates et al., 2023).

Incorporating environmental variables such as temperature and sampling depth considerably improved the predictive accuracy of our models of fish biomass as function of eDNA copies. This is in agreement with recent research that has indicated that environmental factors can significantly affect eDNA degradation rates and, consequently, its detectability (Qian et al., 2022; Doi et al., 2019). For instance, water temperature is known to accelerate eDNA degradation, which could lead to underestimations of biomass if not accounted for. Moreover, depth and therefore pressure are factors that can impact eDNA solubility and diffusion, thus affecting the measured eDNA copies per litre (Duhamet et al., 2023). However, it is also important to highlight the limitation of this work linked to the small dataset used to fitting the model and absence of any cross-validation data.

While this study introduces a model for estimating fish biomass from eDNA, it is recognized that there are limitations that need to be addressed in future research studies to improve its application. A key question that needs to be addressed in future studies is the standardization of the sequencing data for ensuring accurate and comparable results across different studies and conditions. The current model does not account for sequencing depth and competitive amplification between different barcodes during the metabarcoding procedure. Moreover, the model does not take into account the variations introduced by the different sampling methods and types of net used as only one net has been used in to collect all fishing data. Future research should focus on integrating these variables to develop more comprehensive models. This includes not only the standardisation of sequencing data, but also taking

into account environmental and methodological variations. By addressing these limitations, it could be possible to improve the accuracy, reliability and applicability of eDNA metabarcoding as a quantitative ecological assessment tool.

5. Conclusion

The results obtained show that metabarcoding is an effective method for qualitatively determining marine fish diversity in the North Sea, with a relatively long detection time compared to bottom trawling as the reference method, which can be up to 24 h before sampling. However, it is necessary to choose the read assignment method very carefully, hence we recommend adopting ASV read assignment instead of the OTU method, because it allows to assign reads more precisely, eliminating the false assignment error due to the close similarity between species, especially in the 12S gene.

On the other hand, the results obtained in this study present evidence that NGS outputs could be exploited quantitatively, compatible with qPCR output or quantitative reference methods. This also reveals the presence of a non-linear relationship between the number of reads and biomass in CPUE, calling for more work on the modelling part and the development of quantitative and semi-quantitative models for estimating stock sizes.

6. Contribution

BM, TB: Filtration and DNA Extraction, Molecular Biology analysis.

INR &YK: Conceptualization, Bioinformatics and computational modelling.

BM, MB & TB: Sampling and fish data.

CS & RH: Methodology establishing, Supervision, Coordination and Correction of manuscript.

YK: Library preparation & First draft manuscript.

All authors proofed and correct the final version before submission.

Ethical

No ethical permission is required for this work.

CRediT authorship contribution statement

Yassine Kasmi: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ismael Núñez-Riboni:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Tina Blancke:** Writing – review & editing, Resources, Formal analysis, Data curation. **Benita Möckel:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Matthias Bernreuther:** Writing – review & editing, Resources, Formal analysis, Data curation. **Christoph Stransky:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Reinhold Hanel:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2025.113406.

Data availability

The FASTQ files are accessible at ENA (accession number: PRJEB86796). The scripts and sequences are currently available on Zendo 10.5281/zenodo.11242794.

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