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A study of feasibility and detection sensitivity of environmental DNA for fish biodiversity monitoring and stock assessment in the Black Sea

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Introduction: Human activities and their effects on ecosystems are threatening biodiversity and causing a decline in species, population, and genetic diversity. Environmental DNA (eDNA) metabarcoding represents a powerful instrument to assess marine biodiversity, enabling an efficient, cost-effective, and non-invasive approach. eDNA metabarcoding technology has significantly progressed in recent years and has been widely employed to assess marine fish populations.

Methods: This study assessed its potential, detection sensitivity, and effectiveness for an initial evaluation of fish biodiversity in the Black Sea. The efficacy of eDNA was evaluated by contrasting metabarcoding results with trawling data obtained from two sampling campaigns (summer and autumn 2022) across 16 selected locations along the Bulgarian Black Sea coast. Mitochondrial genes 12S and 16S were utilized as standard markers for metabarcoding given their high specificity and sensitivity in amplifying fish DNA, and the availability of large reference databases. A multi-model analytical approach utilizing Bayesian regression and Generalized Additive Models (GAMs) was implemented to assess the relationships between environmental DNA (eDNA) 12S metabarcoding data and abundance indices obtained from catch-per-unit-effort (CPUE). The method addresses significant challenges in predicting abundance from eDNA, including scarce, noisy datasets, excessive zeros, and nonlinear predictor-response relationships. Both frameworks reliably detected biologically meaningful associations between eDNA signal strength, environmental gradients, and trawl-derived abundance.

Results: Metabarcoding, employing the 12S mitochondrial gene with MiFish-U primers, exhibited superior sensitivity in identifying a broader array of fish species, highlighting its efficacy in detecting eDNA traces of rare and migratory species. During the autumn survey, environmental DNA (eDNA) analysis identified 23 fish species, compared to 15 species recorded through trawl sampling. In the summer expedition, 12 species were detected using molecular methods, while trawl surveys recorded 9 species. The Bayesian framework provided robust

uncertainty quantification and reliable inference with few samples, whereas the GAM framework effectively captured nonlinear environmental and spatial effects. The results indicate that eDNA data can replicate patterns observed in conventional trawl surveys. This supports the assumption that eDNA data may serve as a low-impact, scalable method for monitoring fish populations. Model uncertainty informs the need for more sampling or a redesign of covariates to improve the accuracy of eDNA-based abundance predictions.

Discussion: Our findings demonstrate that the eDNA 12S primer combination exhibited superior taxonomic diversity compared to trawling, while necessitating reduced sampling effort. Furthermore, the eDNA-based approach can identify species across diverse life stages and sizes, unlike traditional trawling, which predominantly captures adult specimens within particular size categories. Although eDNA metabarcoding has limits in providing estimates for absolute biomass and size distributions, it provides significant insights on the presence of numerous fish species within the studied ecosystem. The consistency of results from multiple statistical frameworks supports the validity of eDNA counts as an abundance measure, provided that analytical models effectively account for dispersion and environmental variability. The Bayesian regression approach enabled thorough uncertainty quantification and dependable parameter inference with constrained sample sizes, whereas Generalized Additive Models (GAMs) provided an advanced representation of potentially nonlinear relationships among environmental DNA, environmental variables, and abundance.

KEYWORDS

metabarcoding, fish diversity, biodiversity, hierarchical Bayesian model, marker sensitivity

Introduction

Marine biodiversity is rapidly declining as a result of human activities, including climate change, overfishing, the introduction of invasive species, and pollution. These factors expose a significant portion of marine life at risk of extinction (Brito-Morales et al., 2020; Yang et al., 2024). The Black Sea, a unique and highly vulnerable marine environment, is strongly affected by these challenges (Erdogan et al., 2009; Oguz, 2017). In recent years, selective commercial overfishing, amplified by illegal fishing practices and the use of non-selective fishing gears, has led to a rapid decline in the populations of economically important fish species, surpassing their regenerative capacity and resulting in a dramatic reduction in their overall abundance (Dai et al., 2022; Radulescu, 2023). This tendency represents significant threats to regional biodiversity economic stability, and human well-being. Hence, assessments of the marine living resources are essential for sustainable fisheries and ecosystem-based management (Trenkel et al., 2019).

The assessment of marine fish populations has traditionally depended on landings and catches, or on utilizing highly selective

and invasive techniques that target commercially important species in specific geographic areas with favorable conditions. Consequently, it is essential to reevaluate current fisheries research methodologies and integrate complementary, minimally invasive techniques alongside traditional trawl surveys. This integrative strategy would reduce ecological consequences from sampling while improving spatial resolution for monitoring fish migration patterns and developmental stages and broaden community-level observation (Hammerl et al., 2024; Westgaard et al., 2024). Furthermore, the risk of species misidentification is significantly magnifying these challenges (Thomsen et al., 2012). Traditional species identification techniques are becoming increasingly limited due to dependence on the shrinking pool of skilled taxonomists having specialized phenotaxonomic knowledge (Hopkins and Freckleton, 2002). This highlights the pressing necessity for more precise, efficient, and cost-effective fish survey methodologies such as environmental DNA (eDNA) that will address current gaps in biodiversity assessment and improve fisheries management effectiveness.

Environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for assessing marine biodiversity, allowing for the

identification of many taxa within a single sample (Rourke et al., 2022; Tsuji et al., 2023; Haderlé et al., 2024). The data collected through eDNA metabarcoding contain complex and comprehensive biodiversity information. This method has the potential to become a cutting-edge instrument for biodiversity study and aquatic ecosystem conservation (Sahu et al., 2023). Recent advancements in eDNA technology have led to its increasing application for monitoring marine fish populations (Miya et al., 2015; Zhang et al., 2020; Afzali et al., 2021; Stoeckle et al., 2021; Valsecchi et al., 2021; Dai et al., 2022; Maiello et al., 2022; Zhang et al., 2022; Capurso et al., 2023; Cote et al., 2023; He et al., 2023; Osathanunkul and Suwannapoom, 2023; Veron et al., 2023; Westgaard et al., 2024). Despite its growing application globally, eDNA metabarcoding remains underutilized for assessing fish diversity and community structure in the Black Sea.

The application of eDNA to assess abundance in aquatic ecosystems is presently most advanced in freshwater and controlled environments (Rourke et al., 2022). Nonetheless, the complex interplay of external biological, physical, and environmental factors makes direct quantitative assessments in marine ecosystems highly challenging (Iversen et al., 2015; Hansen et al., 2018; Gilbey et al., 2021).

Using environmental DNA (eDNA) to quantitatively assess fish abundance in marine environments presents several difficulties. Key issues include establishing a robust relationship between eDNA concentrations and actual fish abundance, mitigating false positives and false negatives, elucidating the processes of eDNA degradation and dispersal in marine systems, and quantifying uncertainty in abundance estimates. Furthermore, the development of comprehensive DNA reference databases and the adoption of standardized protocols remain essential (Baetscher et al., 2025; Nneji et al., 2025). Despite this, significant evidence supports the use of eDNA as a supplementary tool for assessing fish population abundance and/or biomass across various spatio-temporal scales, dependent on preliminary studies to identify species- and context-specific factors influencing the eDNA abundance/biomass relationship (Salter et al., 2019; Rourke et al., 2022; Baetscher et al., 2025).

Clarifying the efficacy of a quantitative eDNA methodology and understanding its potential as a complementary instrument in traditional stock assessments would signify substantial progress in developing a cost-effective strategy for monitoring regional fisheries of both local and migratory species (Gilbey et al., 2021; Rourke et al., 2022). Further investigation is necessary to improve this prospective method as a viable tool for monitoring catch composition, assessing stock spatial distribution, and systematically recording changes in fish ecosystems across marine ecosystems (Rourke et al., 2022; Maiello et al., 2024).

The present study aims to advance the ongoing discourse on the application of eDNA methodologies in the Black Sea, with a particular focus on evaluating their effectiveness in:

- a. improving fish biodiversity monitoring;
- b. detecting and quantifying species presence;

- c. providing robust scientific data to inform the conservation and sustainable management of marine fishery resources;
- d. evaluating the feasibility of integrating eDNA monitoring into existing fisheries management frameworks in the Black Sea;
- e. assessing the potential of eDNA metabarcoding counts to serve as a viable alternative to conventional trawl surveys;
- f. support decision-making by engaging relevant stakeholders (e.g., policymakers, conservationists, and fisheries managers) in the adoption of eDNA as a complementary or alternative monitoring tool.

Materials and methods

Study area

The study was conducted in the western Black Sea, within Bulgarian coastal and shelf waters between Cape Shabla (north) and Tsarevo (south). This region is ecologically and socio-economically important, influenced by seasonal hydrographic variability (summer stratification, autumn mixing) that shapes fish community composition. It supports diverse pelagic and demersal assemblages, and several locally rare or threatened species. The area also overlaps with national fisheries management zones and monitoring frameworks (Figure 1).

Trawl surveys

The trawl surveys were conducted under the National Fisheries Data Collection Program, which aims towards assessing the biomass of various species, including sprat, whiting, red mullet, and horse mackerel, with some species obtained as bycatch (Raykov et al., 2020). Sixteen randomly selected sites were surveyed to collect fisheries data, biological samples, and eDNA, with sampling carried out in July 2022 (summer) and November 2022 (autumn) as part of the National Data Collection Program (project № BG14MFOP001-3.003-0002-C02) (Figure 1; Supplementary Table S1).

A small-mesh bottom otter trawl designed for coastal and shelf surveys featured a four-panel body and a single codend of diamond mesh (63 mm stretched – bar \approx 31.3 mm), and selection factor (SF) of approximately 3, targeting a $L_{50} \approx 9.4$ cm was used during the surveys. This configuration ensured high retention for multi-species assemblages, while maintaining consistent selectivity baseline across species. Each tow lasted 30 to 40 minutes, with an average trawling speed of 2.7 knots, conducted solely during daylight hours according to the standard protocol, when target species were abundant in the deeper strata reaching the thermocline zone.

Each tow was treated as independent haul. The total catch was categorized to species through morphological identification, catch weight (kg)/numbers were recorded, length frequencies were processed to the nearest 0.5 cm (total length), and biological data

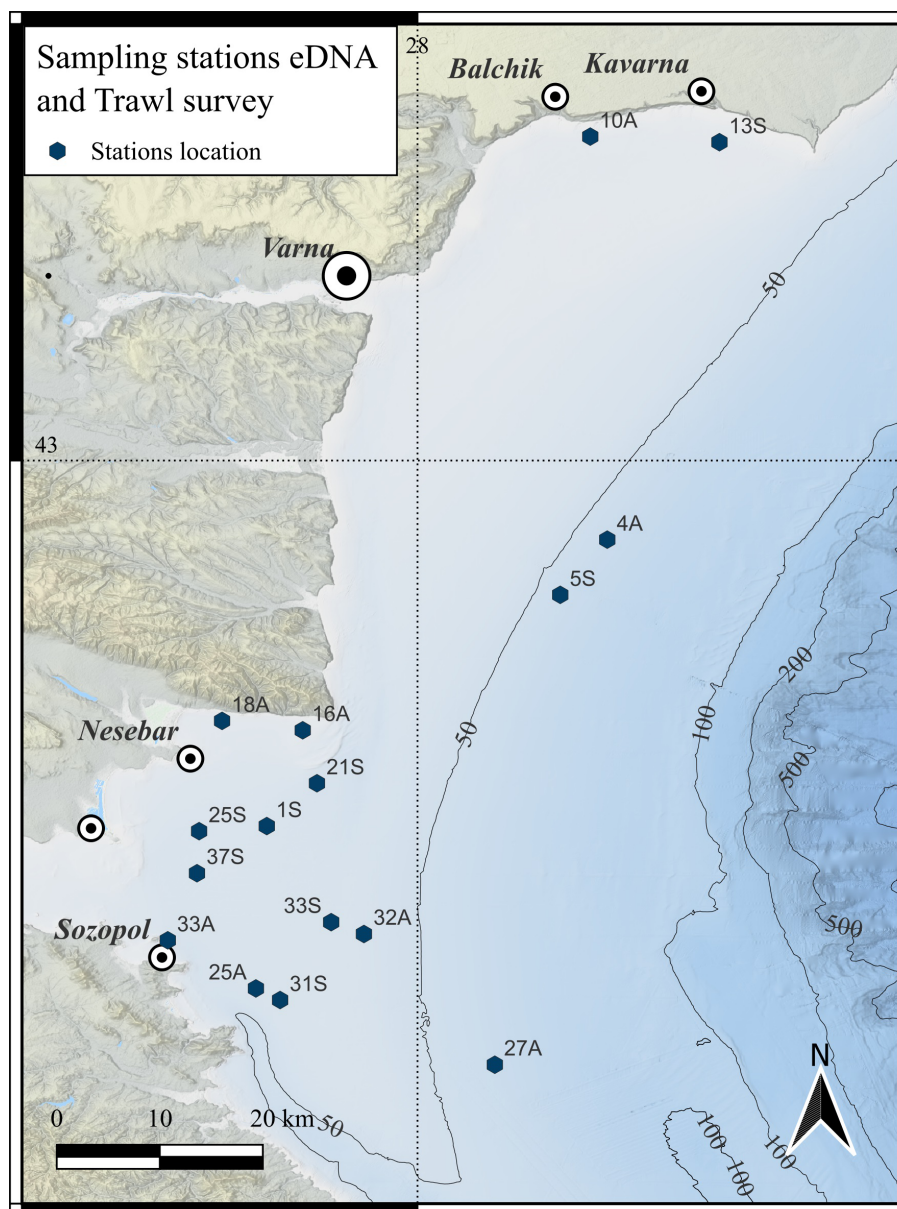


FIGURE 1

Map of sampling stations along the Bulgarian Black Sea coastline. The stations indicate the sites for eDNA sample that align with the endpoints of the corresponding trawling.

(sex, maturity) were collected in laboratory conditions following standard protocols. The length-frequency data (LFD) obtained during the surveys were used to compute the implied retention thresholds for the four key commercial species (Table 1). Given the higher retention rate of the fishing gear the immature fractions as a percentage were computed based on the species-specific logistic maturity ogives, parameterized by the species-specific L_{m50} (the length at which 50% of the population has reached sexual maturity). For each length class, the immature proportion $[1 - M(L)]$ ($M(L)$ represents the proportion of mature individuals) was applied to adjust the expected catch-at-length resulting in a catch-weighted

immature fraction. To produce mature-only indices, we calculated the catch-per-unit-effort CPUE based on the standardized biomass (kg) and abundance (the latter derived from biomass using mean weight-at-length values for Black Sea fish species where available; Supplementary Table S2), and subsequently multiplied each by the maturity proportion $M(L)$. The present study utilized abundance derived from maturity-adjusted CPUE.

Environmental covariates (temperature, salinity and pH) were logged at haul start and end.

Ethical approval was not required for this study, as no experimental animals were used.

TABLE 1 Estimation of retention lengths for key commercial species using single trawl codend diamond mesh, size 63mm stretched, targeting $L_{50} \approx 9.4$ cm and $SF \approx 3$.

Species	L_{25} (cm)	L_{50} (cm)	L_{75} (cm)	Size classes range presented in the LFD (cm)
<i>T. mediterraneus</i>	8.1	9.4	10.8	7.0 -17.5
<i>M. merlangus</i>	8.0	9.4	10.8	10.0-17.5
<i>M. barbatus</i>	8.2	9.4	10.6	7.5 - 16
<i>S. sprattus</i>	8.7	9.4	10.1	6.5 - 11.5

eDNA sampling

Seawater was collected at each sampling station from three distinct depths within the water column: surface/near-surface (0–5 m), mid-depth (10–20 m), and deeper layers (30–50 m), using 5 L Limnos water samplers (KC Denmark A/S), with the exception of station 13S, where only two depth layers were sampled due shallow water depth. Environmental parameters (temperature, salinity and pH) were logged at each depth for all stations using internal thermometer of the water sampler, a digital refractometer (MA886, Milwaukee Electronics Kft), and a pH meter (HI83141, Hanna Instruments Ltd). To avoid contamination, the sampling device was thoroughly washed before each use. Two liters of seawater from each sampling depth were filtered using a 0.22 μ m Millipore Sterivex filter with a peristaltic pump, labeled, and initially stored at -20°C onboard the research vessel, then transferred to -80°C in the laboratory until DNA extraction.

eDNA metabarcoding analyses

Genomic DNA was extracted from a total of 47 seawater samples (Sterivex Filter Units) using MO BIO PowerSoil[®] DNA Isolation Kit (QIAGEN). DNA concentration was quantified with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). A set of 12S (MiFish-U) and 16S primers targeting fish eDNA was used for PCR amplification (Berry et al., 2019; Stoeckle et al., 2021). The preparation of paired-end libraries and high-throughput sequencing on the Illumina Miseq 300 PE platform (Illumina, San Diego, CA, USA) were performed by Macrogen Inc. (Seoul, South Korea).

Raw demultiplexed sequencing reads were quality-checked using FASTQC v0.12.1 (Andrews, 2010), and primers were removed with Cutadapt v4.0 (Martin, 2011). Sequence processing was carried out with DADA2 pipeline v1.32.0 (Callahan et al., 2016), including quality trimming, error rate modeling, merging of paired-end reads, chimera removal, and generation of Amplicon Sequence Variants (ASVs). In the filterAndTrim step, reads were truncated at 160 nucleotides (12S), and 200 nucleotides (16S). Sequences with expected error rate (EE) higher than 2, or containing ambiguous bases were removed. For error rate modeling the default parameters were used with standard pooled processing for sample inference. Paired-end reads with fewer than

12 nucleotide overlaps were removed, with no mismatches allowed in the overlap region. Taxonomic assignment was performed with the BLAST + tool blastn: v2.16.0, using a Top Hit approach against custom-made 12S and 16S reference databases. These custom databases consist of complete or partial sequences obtained from the NCBI nucleotide database (<https://www.ncbi.nlm.nih.gov>), comprising 637 sequences (12S) and 769 sequences (16S), representing 117 and 121 fish species, respectively, known to occur in the Black Sea (Yankova et al., 2014).

Statistical analyses

Biodiversity indices, including species richness, Pielou evenness, Shannon index, Simpsons' and its inverse and unbiased forms (Margalef, 1958; Moreno and Halffter, 2000; Spellerberg and Fedor, 2003; Colwell, 2009; Bowman and Hacker, 2020), were calculated to quantify diversity patterns. Species accumulation curves were generated to illustrate the relationship between sampling effort and cumulative species detection (Moreno and Halffter, 2000; Bowman and Hacker, 2020).

To ascertain statistically significant correlations between eDNA sequence counts and trawl data, Spearman rank correlation (Simpson, 1949; Lehman, 2005) was applied. Additionally, Moran's I was calculated to assess spatial autocorrelation prior to data modeling; nevertheless, no significant values were identified, hence incorporation of spatial lag was not considered feasible.

Community-environment relationships were analyzed using Redundancy Analysis (RDA) (Legendre and Gallagher, 2001; Borcard et al., 2011). A representative model for examining community dynamics in relation to seasonal trends was:

$$Ab_h = \beta_0 + \sum_{k=1}^p \beta_k \text{Season}_{ik} + \varepsilon_{ij},$$

where: Ab_h is the Hellinger-transformed scaled abundance or sequence counts of species j at site i ; Env_{ik} represents the Season as categorical predictor throughout the sampling period and ε_{ij} indicates the residual error. Equivalent model formulations were employed for the examination of all continuous (including salinity, temperature, and pH) and categorical (sampling depth and season) environmental variables.

A Hierarchical Bayesian Logistic regression model was implemented using Stan, incorporating site and species-level random effects to account for uncertainty in species and site

variation, along with environmental covariates, to evaluate the detection sensitivity of the sampling methods (eDNA and trawl survey) and the species-specific detection sensitivity for each method. The model can be expressed as follows:

$$\log\left(\frac{p_{i,s}}{1-p_{i,s}}\right) = \beta_0 + \beta_1 M_i + \beta_2 Sp_i + \beta_3(M_i * Sp_i) + \beta_4 T + \beta_5 pH + \beta_6 S + u_{Sp_i} + u_{Site_i},$$

where: $p_{i,s}$ is the probability of detection of species Sp_i by sampling method M_i (eDNA12S, eDNA 16s or Trawl survey), assuming that the probability of observing species detection by M_i is a binary outcome with Bernoulli distribution: $y_i \sim \text{Bernoulli}(p)$; β_0 is the intercept; $\beta_{1,2,3,4,5,6}$ are fixed effects which capture the differences in the probability of detection of species Sp_i across different methods and covariates; u_{Sp_i} and u_{Site_i} represents random effect which accounts for species- and site-specific variability in detection.

Model performance was evaluated by partitioning the dataset into training (80%) and testing (20%) subsets. Prediction accuracy was assessed using Brier score (Brier, 1950; Stone, 1974; Yeung et al., 2005), overall model accuracy, the area under the ROC curve (AUC) (Fielding and Bell, 1997; Junge and Dettori, 2018), and true skill statistics (TSS) (Allouche et al., 2006), which are the most commonly used metrics for assessing classification model performance. The Brier score is a metric that evaluates the precision of probabilistic forecasts, ranging from 0 to 1, where 0 signifies perfect predictive accuracy and 1 denotes complete inaccuracy (Brier, 1950). AUC is a threshold-independent statistic that indicates a model's ability to differentiate between positive and negative occurrences, whereas TSS is a threshold-dependent and prevalence-independent metric, providing it particularly useful for evaluating classifier performance in imbalanced datasets. The latter were computed with the subsequent formulas:

$$\text{Brier score} = 1/N \sum_{i=1}^N (p_i - y_i)^2,$$

where: N denotes the total number of observations, p_i represents the predicted probability for observation i and y_i is the actual classification outcome (1 – detected; 0 – not detected);

$$\text{AUC} = \int_0^1 \text{TPR}(t) d(\text{FPR}(t))$$

where: TPR is the true positive rate (sensitivity), which represents the proportion of positive instances that were correctly classified by the model and FPR is the false positive rate, which is the proportion of positive instances that were incorrectly classified by the model. The ROC curve plots TPR against FPR across various probability thresholds t ;

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FN + FP}$$

where: TP and TN are respectively the true positive and the true negative predictions/classifications, whereas FN and FP represent the false positive and false negative predictions/classifications;

$$\text{TSS} = \text{TPR} + \text{TNR} - 1 = \text{TPR} - \text{FPR},$$

where: TPR is the true positive rate (sensitivity) and FPR is the false positive rate.

Four commonly detected species in both eDNA and trawl survey expeditions (*M. merlangus*, *M. barbatus*, *S. sprattus* and *S. maximus*) were chosen as a test case to model the relationship between the maturity-adjusted CPUE-derived abundance and the eDNA 12s counts along with environmental covariates.

Given the limited size of the empirical data (only 16 observations per species (Supplementary Table S3), which is insufficient for robust modeling using hierarchical or multivariate approaches, intended for methodological testing, sensitivity analyses and comparability across various model families, a synthetic dataset ($n = 100$) was generated. The synthetic data was constructed using the actual raw eDNA 12s sequence counts and CPUE-derived species abundance within a simulation framework, specifically designed to maintain the marginal distributions of each variable through empirical or parametric models, while preserving the dependence structure among the variables and between species. The framework addressed the overdispersion and zero-inflation of eDNA data resulting from known causes, including stochasticity, insufficient sampling, and detection thresholds (Deiner et al., 2017; Peixoto et al., 2023). Consequently, eDNA observations were analyzed using a Zero-Inflated Negative Binomial (ZINB) distribution (Warton, 2005; Hilbe, 2011), which accounts for both detection failure and variability in biological counts. Species abundances were modeled conditionally on simulated eDNA sequence counts and environmental covariates using Generalized Additive Models (GAMs) (Wood, 2017), to capture nonlinear ecological responses. This hybrid ZINB-GAM model was intended to resemble the hierarchical relationship among eDNA generation, persistence, and organismal abundance as outlined in empirical case studies (Deiner et al., 2017; Yates et al., 2019; Harrison et al., 2019).

Environmental variables which have been reported to influence eDNA persistence and fish dispersal, such as temperature, pH, salinity, and site locations, were included in the analysis (Barnes and Turner, 2016; Goldberg et al., 2016; Harrison et al., 2019). Each covariate was treated as a continuous random variable, with empirical marginal distributions derived from observed data and subsequently resampled using quantile functions. The latter maintains the natural variability and environmental covariate ranges commonly observed in aquatic ecosystems. A Gaussian copula was employed to model the combined dependency between species' eDNA and environmental variables, providing an adaptive multivariate framework that separates marginal distributions from their dependence structure (Nelsen, 2006). This is especially significant in ecology, where interactions among species as well as between species and their environment are nonlinear and non-Gaussian (Clark et al., 2017; Krupskii and Joe, 2013).

The result was a synthetic dataset including 100 observations, ensuring that all simulated variables maintain realistic interrelationships and conform to the actual covariance structure of the original sample data (Supplementary Figures S1-S3).

TABLE 2 Generalized additive models (GAMs) multi-model framework - summary.

Model	Distribution family	Link function	References
Negative Binomial Generalized Additive Model	Negative Binomial	Log	Hilbe (2011); Wood (2017)
Negative Binomial GAM with Tensor Interaction Term	Negative Binomial	Log	Wood (2017)
Reduced Negative Binomial GAM (reduced predictors set)	Negative Binomial	Log	Wood (2017)
Tweedie Generalized Additive Model	Tweedie (Compound Poisson–Gamma)	Log	Dunn and Smyth (2005); Shono (2008)
Gaussian GAM	Normal (Gaussian)	Log	McCullagh (1989)
Gaussian GAM	Normal (Gaussian)	Identity	McCullagh (1989)
Gaussian GAM	Normal (Gaussian)	Inverse	McCullagh (1989)
Two-Part Hurdle Model: Binomial GAM + Negative Binomial GAM	Binomial + Negative Binomial	Logit + Log	Mullahy (1986); Zuur et al. (2009)
Two-Part Hurdle Model: Binomial GAM + Tweedie GAM	Binomial + Tweedie	Logit + Log	Mullahy (1986); Dunn and Smyth (2005)
Zero-Inflated Poisson Location–Scale–Shape GAM	Zero-Inflated Poisson	Log (μ), Logit (π)	Lambert (1992); Rigby and Stasinopoulos (2005)
Tweedie Location–Scale–Shape GAM	Tweedie	Log (μ), Log (σ)	Rigby and Stasinopoulos (2005); Stasinopoulos et al. (2017)
Gaussian Location–Scale–Shape GAM	Gaussian (Normal)	Log (μ), Log (σ)	Rigby and Stasinopoulos (2005); Wood (2017)
Assumptions and data pre-processing – valid for all models	All predictors z-scored for comparability.		

Various modeling methodologies were employed to determine their effectiveness in modeling CPUE-derived fish species abundance through eDNA 12S counts, alongside environmental covariates. These included Generalized Additive Models (GAM), Bayesian Generalized Linear/Additive models (GLM/GAM), Bayesian hierarchical regression models, and ensemble machine learning (ML) models, the latter of which notably failed to achieve cross-validation due to insufficient data. The model selection inference focused on two robust multi-model approaches: a

Generalized Additive Models (GAMs) Multi-model framework utilizing bootstrap blocks as a reliable alternative to cross-validation methods like Leave-One-Out Cross-Validation (LOOCV) due to the limited sample size, and Bayesian Regression Multi-model framework employing species-specific configurations.

Generalized additive models multi-model framework for best model selection

A sampling size adaptive modeling approach, adequate for small and moderate datasets ($n < 25$ and $n > 25$) was adopted, featuring 11 Generalized Additive Models (GAMs) with penalized regression splines, recognized for their ecological validity in analyzing overdispersed and continuous zero-rich data, such as abundance counts (Table 2). The latter was utilized to predict maturity-adjusted CPUE abundance for the test case species, integrating predictors including eDNA 12S sequence counts, environmental covariates (temperature, salinity, and pH), and spatial structure (geographic coordinates of the sampling sites), while applying a variety of response distributions. The framework was intended to facilitate the comparison of results and the selection of the most suitable model - “Best model”, given the expectation that the species may display diverse statistical distributions, including skewness, zero-inflation, over- and underdispersion, and nonlinear relationships with the response variable.

Bayesian regression multi-model framework for best model selection

Furthermore, a Bayesian regression multi-model framework was employed, beginning with a simple hierarchical pipeline that included only eDNA sequence counts as predictors, focusing on a small dataset, and then expanding to include temperature in an attempt to conform with ecological plausibility. The latter evolved to the final version which is well suited to working with both small and moderate datasets, and provides distinct per-species models, allowing for species-specific flexibility and model comparison. A similar model selection approach to the GAM framework was adopted, featuring four possible models (Table 3).

A set of metrics was used to evaluate the resulting models, including Out-of-fold (OOF) Pearson correlation to assess the relationship between observed and predicted values (in the context of models’ ability to capture overall variability and accurately reproduce the linear trend in abundance), and OOF Spearman rank correlation, which provided an insight into how the model preserves sample ordering. Furthermore, the Root Mean Squared (RMSE) and Root Mean Squared Logarithmic Errors (RMSLE), as well as the Mean Absolute Error, were calculated to allow for effective comparison of predictive error magnitudes among models and to provide a metric for model predictive accuracy. The best model was chosen based on LOOIC (Leave-one-out Information Criterion), WAIC (Watanabe-Akaike

TABLE 3 Bayesian regression multi-model framework - summary.

Best model	Distribution family	Link function	Priors (distribution) can be further improved	Model terms
Bayesian Negative Binomial model	Negative Binomial	Log	Priors on intercept, slopes, dispersion	eDNA, Temp, pH, Salinity, Latitude, Longitude, Interaction (<i>eDNA</i> × <i>Temp</i>)
Bayesian Zero-Inflated Negative Binomial (ZINB)	Zero-Inflated Negative Binomial	Log (count); Logit (zero prob)	Priors on both count and zero submodels	eDNA, Temp, pH, Salinity, Latitude, Longitude, Interaction (<i>eDNA</i> × <i>Temp</i>) + zero process covariates
Bayesian Hurdle Negative Binomial	Hurdle Negative Binomial	Log (count>0); Logit (hurdle prob)	Priors on presence and abundance components	eDNA, Temp, pH, Salinity, Latitude, Longitude, Interaction (<i>eDNA</i> × <i>Temp</i>) + hurdle covariates
Bayesian Negative Binomial	Negative Binomial with spline	Log	Priors on spline standard deviations and all coefficients	eDNA (smooth), Temp, pH, Salinity, Latitude, Longitude
Assumptions and data pre-processing – valid for all models	All predictors z-scored for comparability. Scale-dependent priors, assuming incomparable predictors' scales.			

Information Criterion) for the multi-model Bayesian framework, and RMSE or RMSLE values for best model selection in the multi-model GAM framework. ANOVA (analysis of deviance) was used to examine the significance of models' terms and quantitative evaluation of deviance explained by the "Best model", as well as the R^2_{adj} (GAMs) and Bayesian R^2_{adj} value for measuring Bayesian regression models' overall significance.

All analyses and the accompanying graphical outputs were carried out in the R programming environment (R Core Team, 2021) utilizing R packages 'brms' (Bürkner, 2017a, 2017) with Stan (Carpenter et al., 2017), 'rstan' (Guo et al., 2020); 'ggplot2' (Wickham, 2016); 'vegan' (Oksanen et al., 2013), 'mgcv' (Wood

and Wood, 2015; Wood, 2001), 'copula' (Hofert et al., 2014) and all related dependencies.

Results

Fish diversity via eDNA metabarcoding and trawling

The eDNA analysis using the 12S marker demonstrated seasonal fluctuations in the number of detected fish species (demersal and pelagic), totaling 23 species. A higher number of

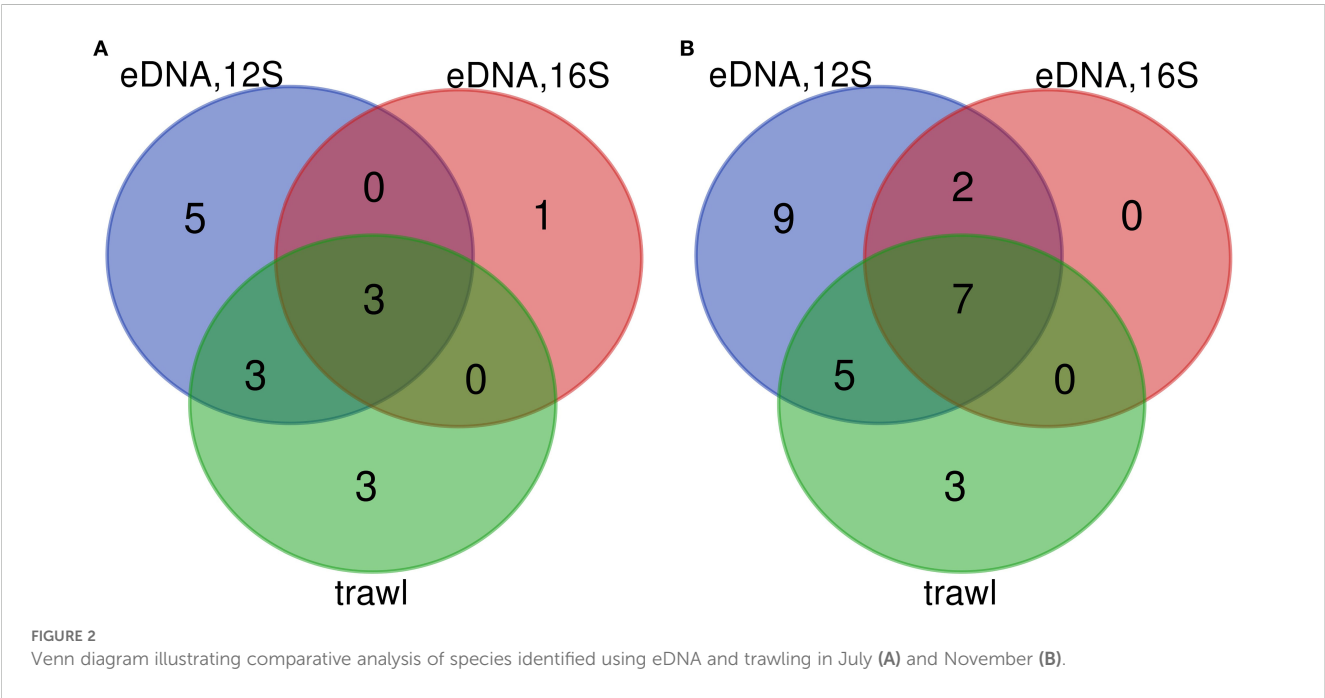


TABLE 4 Species identified via eDNA (12S and 16S markers) and parallel trawl surveys throughout seasonal samplings in 2022.

Species	Trawl		eDNA, 12S		eDNA, 16S	
	Summer	Autumn	Summer	Autumn	Summer	Autumn
<i>Acipenser stellatus</i> (P, A, E)	+	–	–	–	–	–
<i>Alosa</i> sp. (P, A)	+	+	+	+	–	+
<i>Alosa immaculata</i> (P, A)	–	+	–	–	–	–
<i>Aphia minuta</i> (P)	–	–	+	+	–	–
<i>Belone belone</i> (D, M)	–	–	–	+	–	–
<i>Chelidonichthys lucerna</i> (D)	–	+	–	–	–	–
<i>Chelon saliens</i> (P)	–	–	–	+	–	–
<i>Engraulis encrasicolus</i> (P, M)	+	–	+	+	–	–
<i>Euthynnus alletteratus</i> (P, M, R)	–	–	–	+	–	–
<i>Gobius niger</i> (D)	–	–	+	+	–	–
<i>Merlangius merlangus</i> (P)	+	+	+	+	+	+
<i>Mullus barbatus</i> (D, M)	+	+	+	+	+	+
<i>Neogobius melanostomus</i> (D)	+	+	–	+	–	–
<i>Platichthys flesus</i> (D)	+	+	–	+	–	–
<i>Pomatomus saltatrix</i> (P, M)	–	+	–	+	–	+
<i>Pomatoschistus minutus</i> (D)	–	–	+	+	–	–
<i>Raja clavata</i> (D)	–	–	–	+	–	+
<i>Scophthalmus maximus</i> (D)	+	+	+	+	–	+
<i>Scomber japonicus</i> (P, M, R)	–	–	–	+	–	–
<i>Sprattus sprattus</i> (P)	+	+	+	+	+	+
<i>Sparus aurata</i> (P, R)	–	+	–	+	–	–
<i>Squalus acanthias</i> (D)	–	+	+	+	–	–
<i>Thunnus thynnus</i> (P, M, R)	–	–	–	+	–	–
<i>Trachinus draco</i> (D)	–	+	–	+	–	–
<i>Trachurus mediterraneus</i> (P, M)	–	+	+	+	–	+
<i>Trachurus trachurus</i> (P, M)	–	–	–	+	+	+
<i>Uranoscopus scaber</i> (D)	–	+	–	–	–	–

Species are classified by habitat type (D – demersal and P – pelagic) and behavior (A – anadromous and M – migratory), with additional designations for conservation status (E – endangered, I – invasive and R – rare). Species printed in bold are commercially important for the Black Sea.

species was detected in eDNA samples during autumn (23 species) compared to summer (12 species). Correspondingly, trawl catches revealed a greater diversity of fish species in autumn (15 species) than in summer (9 species), reflecting expected seasonal variations in species assemblages (Figure 2; Table 4).

Species diversity was more than twofold lower in both seasons when using the 16S primers, yielding only 9 species. The species identified using the 16S marker coincided with those detected using the 12S marker (Figure 2; Table 4). Furthermore, six species were identified in summer and twelve in autumn using both methods (eDNA with 12S and trawling) (Table 4). Species such as turbot (*Scophthalmus maximus* Linnaeus, 1758), whiting (*Merlangius*

merlangus, Linnaeus, 1758), *S. sprattus*, *M. barbatus*, flounder (*Platichthys flesus*, Linnaeus, 1758), round goby (*Neogobius melanostomus* Pallas, 1814), and *Alosa* sp. were documented in trawl surveys during both summer and autumn, while migratory species like *T. mediterraneus*, anchovy (*Engraulis encrasicolus* Linnaeus, 1758), and bluefish (*Pomatomus saltatrix* Linnaeus, 1766) were found only in the specific seasons corresponding to their migratory patterns. The disparity in the autumn sampling survey was mainly attributable to eleven species (*Alosa* sp., transparent goby (*Aphia minuta* Risso, 1810), garfish *Belone belone* Linnaeus, 1761), leaping mullet (*Chelon saliens* Risso, 1810), little tunny (*Euthynnus alletteratus* Rafinesque, 1810),

TABLE 5 Biodiversity indices for each sampling station computed using both the trawl surveys and eDNA (12S marker) data.

Sampling method	Station name	Species richness	Pielou evenness	Shannon	Simpson	Inversed Simpson	Unbiased Simpson
eDNA 12S	1S	6	0.309	0.554	0.259	1.350	0.259
eDNA 12S	5S	6	0.236	0.423	0.229	1.297	0.229
eDNA 12S	13S	6	0.214	0.383	0.163	1.195	0.163
eDNA 12S	21S	5	0.134	0.216	0.088	1.096	0.088
eDNA 12S	25S	7	0.632	1.229	0.655	2.900	0.655
eDNA 12S	31S	6	0.627	1.124	0.598	2.486	0.598
eDNA 12S	33S	4	0.792	1.098	0.653	2.884	0.653
eDNA 12S	37S	6	0.540	0.968	0.531	2.131	0.531
eDNA 12S	4A	9	0.162	0.357	0.150	1.177	0.150
eDNA 12S	10A	10	0.246	0.566	0.292	1.413	0.292
eDNA 12S	16A	7	0.564	1.098	0.552	2.234	0.552
eDNA 12S	18A	13	0.228	0.585	0.216	1.275	0.216
eDNA 12S	25A	12	0.150	0.374	0.128	1.146	0.128
eDNA 12S	27A	12	0.119	0.295	0.117	1.133	0.117
eDNA 12S	32A	10	0.171	0.393	0.145	1.170	0.145
eDNA 12S	33A	14	0.473	1.248	0.635	2.737	0.635
Trawl	1S	3	0.337	0.371	0.196	1.243	0.196
Trawl	5S	4	0.234	0.325	0.151	1.178	0.151
Trawl	13S	5	0.046	0.073	0.023	1.024	0.233
Trawl	21S	3	0.351	0.386	0.223	1.287	0.223
Trawl	25S	5	0.107	0.173	0.066	1.071	0.066
Trawl	31S	5	0.153	0.246	0.120	1.136	0.120
Trawl	33S	4	0.371	0.514	0.251	1.334	0.254
Trawl	37S	3	0.339	0.372	0.172	1.208	0.173
Trawl	4A	2	0.323	0.224	0.111	1.125	0.118
Trawl	10A	6	0.492	0.882	0.449	1.815	0.450
Trawl	16A	2	0.006	0.004	0.001	1.001	0.001
Trawl	18A	12	0.746	1.853	0.790	4.751	0.792
Trawl	25A	7	0.262	0.510	0.207	1.261	0.208
Trawl	27A	4	0.121	0.168	0.067	1.072	0.067
Trawl	32A	7	0.051	0.099	0.035	1.036	0.035
Trawl	33A	3	0.732	0.804	0.528	2.118	0.533

Sampling stations exhibiting highest diversity indices values are marked in bold.

black goby (*Gobius niger* Linnaeus, 1758), sand goby (*Pomatoschistus minutus* Pallas, 1770), thornback ray (*Raja clavata* Linnaeus, 1758), chub mackerel (*Scomber japonicus* Houttuyn, 1782), Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758) and Atlantic horse mackerel (*Trachurus trachurus* Linnaeus, 1758) that were not caught by trawling but were detected through eDNA. The last four species were likely

missed by the trawl due to gear selectivity and mesh size. Conversely, four species: stellate sturgeon (*Acipenser stellatus* Pallas, 1771), pontic shad (*Alosa immaculata* Bennett, 1835), tub gurnard (*Chelidonichthys lucerna* Linnaeus, 1758) and Atlantic stargazer (*Uranoscopus scaber* Linnaeus, 1758) were captured by trawling but not detected via eDNA (Table 2). Additionally, the number of species detected in eDNA samples during summer

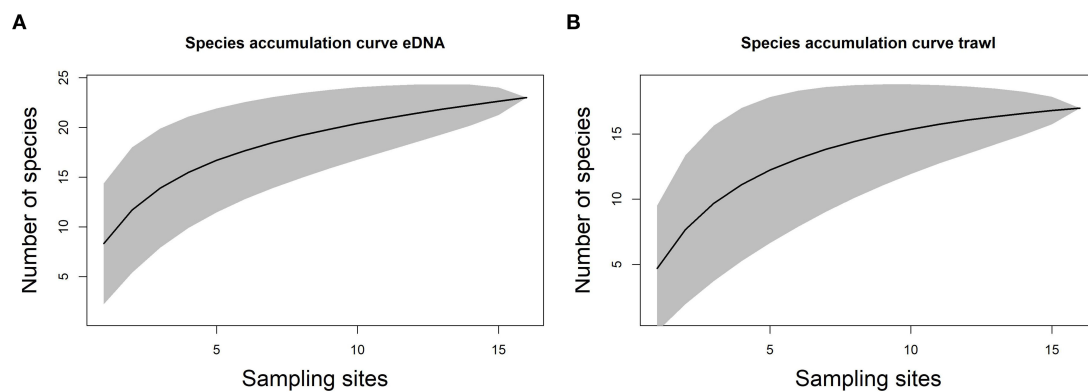


FIGURE 3
Species accumulation curves. (A) computed for eDNA 12S marker and (B) for trawl survey as sampling effort increases.

differed by two species compared to the trawl data (Table 2). Rare species were detected exclusively in eDNA samples during the autumn survey at stations located in the southern region of the Bulgarian Black Sea coast: *E. alletteratus* (33A), *S. japonicus* (27A), *T. thynnus* (27A), and *T. trachurus* (16A, 18A, 25A, 27A, 32A).

Biodiversity Indices

The highest species richness values (Table 5) were predominantly recorded at most stations sampled by eDNA12S during the autumn expedition, with the exception of station 16A, which nonetheless exhibited a species count exceeding the average determined through trawling. The greatest number of species (12), obtained through trawling, was observed at station 18A during the autumn sampling season.

Pielou's evenness ranging from 0 to 1, with values nearing 0 was noted at three stations: trawl survey hauls gathered at stations 13S, 16A, 32A attributed to the significantly large captures of *S. sprattus*

(13S) and *T. mediterraneus* (16A and 32A), which predominated the catch composition.

The Shannon index reached its maximum values during the trawl survey at station 18A and in the eDNA (12S marker) assessment at stations 25S, 31S, 33S, 16A, and 33A. The first three eDNA samples were obtained during the summer expedition, whereas stations 16A and 33A were sampled in the autumn expedition, revealing heightened biodiversity indices in both seasons, likely due to particular developmental stages (in summer) and/or migratory species (in autumn) whose pathways include the coastal and shelf waters of the Bulgarian Black Sea.

Species accumulation curves

Species accumulation curves were used to illustrate the increase in species count relative to sampling effort. The eDNA species accumulation curve (Figure 3A) indicated that roughly 10 more species could be detected using eDNA (12S marker) compared to

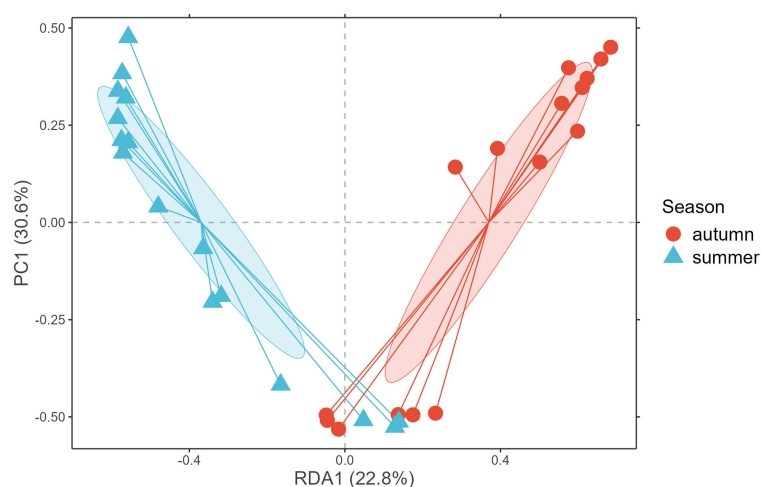


FIGURE 4
Redundancy analysis (RDA) ordination plot showing the variation in species composition constrained by season.

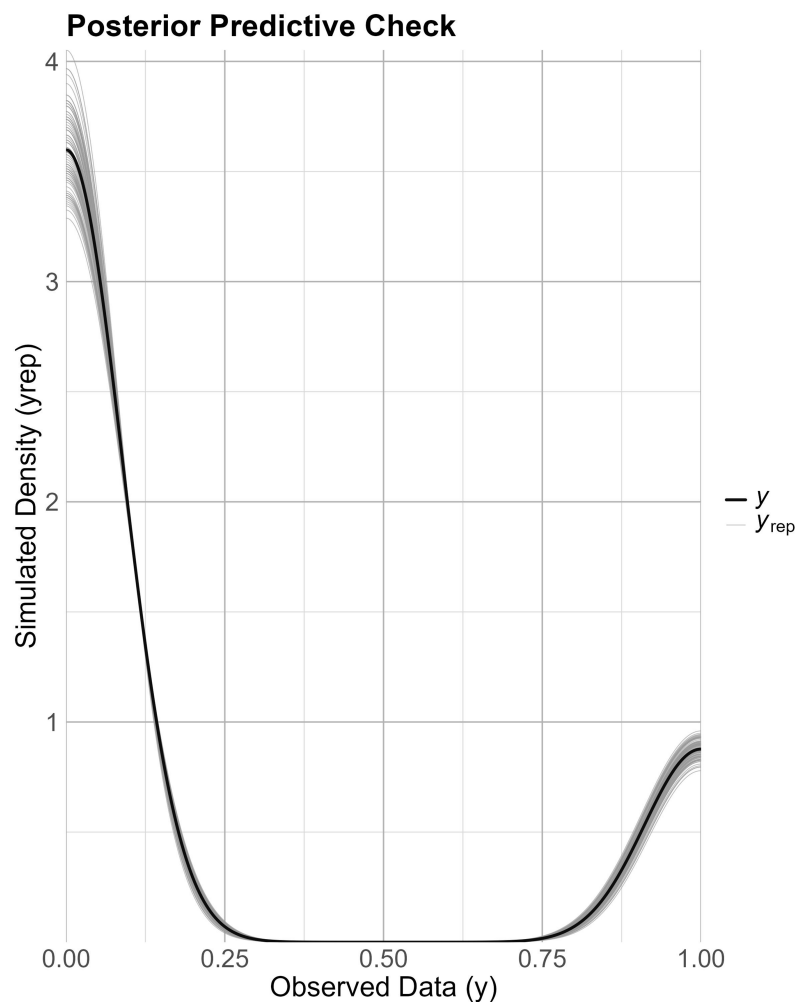


FIGURE 5

Posterior predictive check for the detection model. Comparison of observed data (bold black line) with simulated posterior predictive distributions (grey density curves) to assess model fit.

those identified through trawl surveys considering the sampling effort (Figure 3B). Thus, the extensive reach and non-invasive characteristics of eDNA analysis enable the identification of a greater variety of species within the environment.

Seasonality and species preferences

Seasonal patterns in species communities, detected by both sampling techniques, were analyzed using constrained ordination (Redundancy Analysis - RDA). Seasonal clustering, as indicated by confidence ellipses, was distinctly observable for both seasons (Figure 4), presumably attributable to species habitat suitability and/or particular biotic interactions relevant to each season. Only a limited number of species within the detected community structure during the autumn season showed preferences for an abiotic environment comparable to that of the summer season, possibly due to species interactions or early recruitment developmental stages.

The seasonal pattern explained over 50% of the variance in community structure (based on abundance/eDNA 12S sequence count data). Identical clustering patterns were identified for species associations constrained to environmental covariates (temperature, salinity, and pH), which also show seasonal cycles. The RDA, that includes all continuous and categorical variables, accounted for 65% of the variance in species CPUE (kg)/sequence counts ($R^2 = 0.7$; $R^2_{adj} = 0.51$), demonstrating that the model effectively captured general relationships while accounting for the significant contribution of temperature and pH.

Sampling method detection sensitivity and species-specific detection sensitivity

The posterior predictive check for the Bayesian Hierarchical model, utilized to evaluate method detection sensitivity and species-specific detection sensitivity per method (Figure 5), demonstrated a

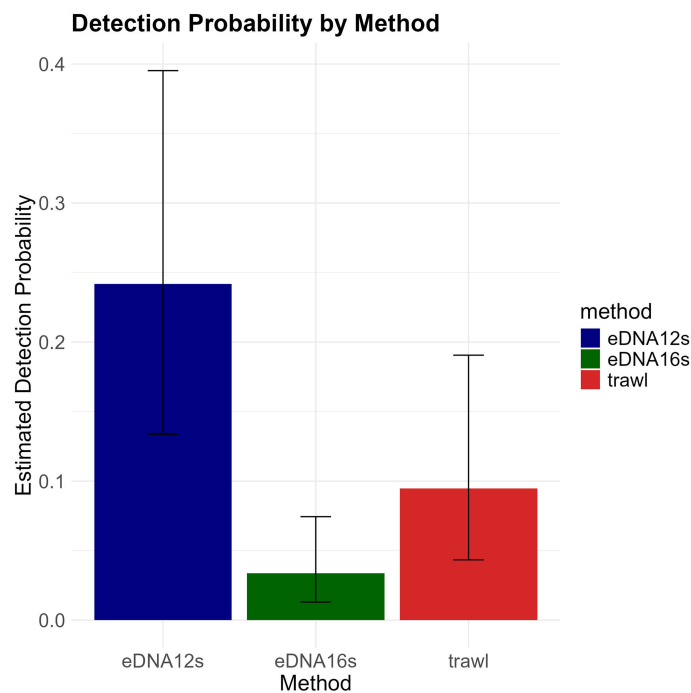


FIGURE 6
Estimated probability of detection by method, comparing eDNA and trawl surveys. Bars represent the mean estimated detection probability, with error bars indicating the 95% credible intervals.

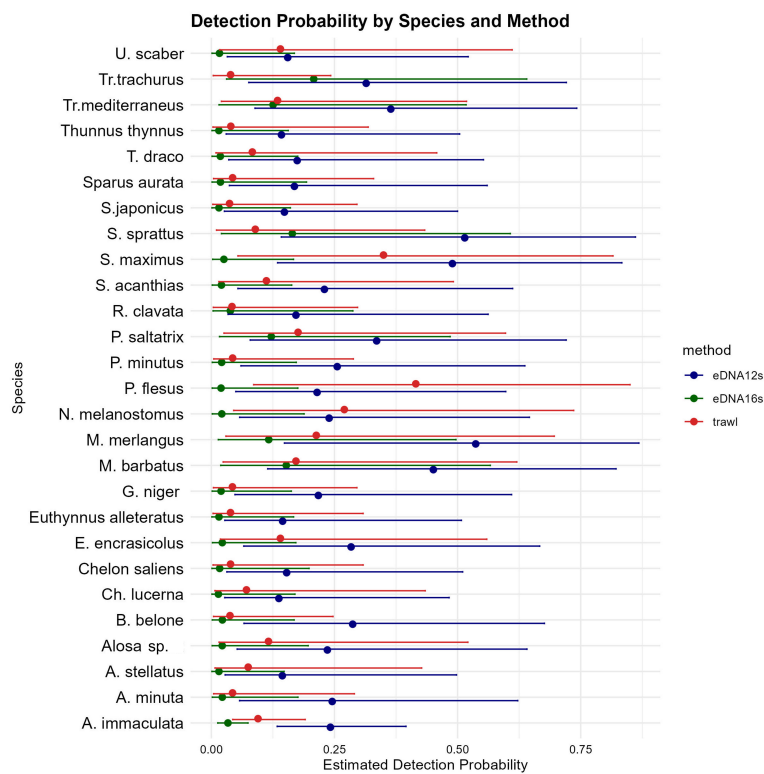


FIGURE 7
Estimated detection probability for each species, comparing eDNA 12S and 16S markers and trawl sampling methods. The points represent mean estimated detection probabilities, with error bars indicating 95% confidence intervals.

TABLE 6 Effects of environmental covariates on sampling method detection sensitivity.

Variable	Estimate	95% CI	Interpretation
Temp	-0.15	[-0.24; -0.05]	Significant negative effect/ higher temperatures reduce detection of species
pH	-0.08	[-1.88, 2.01]	No significant effect
Sal	0.04	[-0.34, 0.42]	No significant effect

strong agreement between the actual data and the simulated posterior predictive distribution. The posterior predictive check was conducted utilizing new observations unknown to the model (train-test split 80–20%), indicating that the model sufficiently captured the data variability.

The model results highlight the influence of sampling methods and environmental covariates on method detection sensitivity (Figure 6) and determine the probability of detection for each species, utilizing the sampling method as a predictor while considering the effects of environmental covariates (Figure 7).

The detection probability for each method was obtained from the posterior distribution, based on the null hypothesis that trawling will identify more species than eDNA. The results indicated the chance of $p_{\text{trawl detection}} > p_{\text{eDNA 12S detection}}$ equals 0, signifying that in all posterior samples the detection probability of eDNA 12S was greater than detection probability of trawl, and $p_{\text{eDNA 16S detection}} > p_{\text{eDNA 12S detection}}$ equals 0, signifying that in all posterior samples the detection probability of eDNA 12S was greater than detection probability of eDNA 16S, hence validating the advantages of 12S primers over the eDNA 16S primers and trawl sampling for species detection.

The estimated species-specific detection probability evidently differs among various species; those that are naturally more abundant and prevalent in the environment (such as *M. merlangus*, *S. maximus*, and *S. sprattus*) and those with seasonal occurrences (*E. encrasicolus*, *P. saltatrix*, and *T. mediterraneus*) have a greater probability of detection. Furthermore, these species exhibit elevated detection probability across all sampling methodologies, despite the natural selectivity of trawling, which targets particular species based on trawl design and fishery type.

TABLE 7 Best model, diagnostics (diagnostic plots – Supplementary Figures S4, S6, S8, S10; Observed vs. predicted – Supplementary Figures S5, S7, S9, S11) and model metrics for small sample size – $n = 16$.

Species	Best model	Deviance explained (%)	R^2_{adj}	Pearson correlation (OOF)	Spearman correlation (OOF)	Comment
<i>M. merlangus</i>	Tweedie (Compound Poisson–Gamma)	76.7	0.938	0.053	0.203	The model accurately fits the observed data but fails to generalize to novel data. The only credible predictor is spatial structure, provided this is not a functional model that accounts for abundance via eDNA. Overfitting to spatial structure (low Pearson correlation coefficient) and a small sample size may be contributing factors
<i>M. barbatus</i>	Reduced Negative Binomial GAM (simplified predictor set)	0	0	-0.218	-0.042	Potential reasons for model failure include insufficient data and low signal indicative for actual rarity in the sample coupled with a small sample size, as well as zero-rich data indicating underdispersion.
<i>S. sprattus</i>	Reduced Negative Binomial GAM (simplified predictor set)	63.4	-0.667	-0.136	0.489	The model's underperformance may be attributed to a low sample size, overparameterized smooth terms, and data with a substantial number of zeros. The Pearson correlation coefficient suggests a likelihood of overfitting. A non-linear relationship between eDNA count data and trawl abundance was discovered.
<i>S. maximus</i>	Gaussian GAM (Identity-link)	28.5	0.253	0.106	0.074	A potential cause of model underperformance is a small sample size and an abundance of zero-rich data. The Pearson correlation coefficient suggests a likelihood of overfitting. The significant within-sample correlations fail to generalize effectively to validation subsets.

TABLE 8 Best model, diagnostics (diagnostic plots – [Supplementary Figures S12, S14, S16, S18](#); Observed vs. predicted – [Supplementary Figures S13, S15, S17, S19](#)) and model metrics for moderate sample size – $n=100$ (synthetic dataset).

Species	Best model	Deviance explained (%)	R_{adj}^2	Pearson correlation (OOF)	Spearman correlation (OOF)
<i>M. merlangus</i>	Tweedie (Compound Poisson–Gamma)	82.2	0.812	0.931	0.795
<i>M. barbatus</i>	Gaussian GAM (Log-link)	39.2	0.43	-0.038	0.01
<i>S. sprattus</i>	Reduced Negative Binomial GAM (simplified predictor set)	63.1	-0.047	0.078	0.675
<i>S. maximus</i>	Gaussian GAM (Identity-link)	52.4	0.362	0.557	0.697

Rare species exhibit low detection probabilities, evident at the detection threshold, and are likely to be identified solely through eDNA (12S), given the constraints of trawling as a sampling technique and the lower sensitivity of 16S primers, which captures only the most abundant species and exhibited inability of detection of rare species and such present in low abundance.

The model findings revealed a statistically significant negative effect of temperature ([Table 6](#)) on method sensitivity, suggesting that high temperatures may adversely affect eDNA detection sensitivity, with no discernible influence from salinity and negligible impact from the pH.

The reliability and accuracy of the model were assessed using the Brier score, AUC, TSS, and accuracy metrics, providing the following results: *Brier score* = 0.105; *AUC – ROC Score* = 0.901; *accuracy* = 0.862 and *TSS* = 0.6375. A *Brier score* of 0.105 signifies that the model's probability estimates are well-calibrated, indicating accurate reflection of species detection likelihood ([Brier, 1950](#); [Trick et al., 2023](#)) *AUC – ROC Score* of 0.8912 suggests that the model has very good discriminatory capacity, meaning that it correctly ranks both detected and undetected instances; An accuracy of 86% is strong, indicating that 86% of the model's predictions are correct.

TABLE 9 Best model, diagnostics (posterior predictive check plots – [Supplementary Figures S20–S27](#)) and model metrics for moderate sample size – $n = 100$ (synthetic dataset).

Species	Best model	Pearson correlation	Spearman correlation	$R_{Bayesian}^2 - mean$	$R_{Bayesian}^2 - sd$	Sample size n
<i>M. merlangus</i>	Negative Binomial with spline	0.973	0.902	0.489	0.306	16
<i>M. barbatus</i>	Bayesian Negative Binomial model	0.585	0.621	0.296	0.240	16
<i>S. maximus</i>	Bayesian Negative Binomial model	0.715	0.662	0.348	0.272	16
<i>S. sprattus</i>	Bayesian Negative Binomial model	0.790	0.483	0.320	0.280	16
<i>M. merlangus</i>	Negative Binomial with spline	0.955	0.792	0.488	0.376	100
<i>M. barbatus</i>	Bayesian Negative Binomial model	0.195	0.222	0.231	0.201	100
<i>S. maximus</i>	Negative Binomial with spline	0.521	0.617	0.277	0.186	100
<i>S. sprattus</i>	Negative Binomial with spline	0.094	0.698	0.261	0.193	100

Modelling species abundance based on eDNA 12S sequence counts

Best models identified for the test case species through the application of the GAMs multi-model framework

Increasing the sample size enabled the GAM smoothers to identify functional relationships instead of being susceptible to noise overfitting (Tables 7, 8). A higher number of samples led to improved model stability, an increased proportion of deviance explained by the models, and demonstrated distinct, biologically credible relationships between eDNA 12S sequence counts and CPUE-derived abundance for multiple species. The results indicated that selecting optimal distribution families is essential for accurately capturing the underlying data dispersion and zero prevalence among species. Cross-validation metrics further verify that predictive generalization, particularly rank-based consistency, may be achieved with moderate sample size, while small-n models were predominantly influenced by noise and sampling variance.

Best models identified for the test case species through the application of the Bayesian regression multi-model framework

The results clearly indicated that the Bayesian regression multi-model framework effectively identified the relationships between eDNA 12S and CPUE-derived abundance for both small ($n = 16$) and intermediate ($n = 100$) sample sizes (Table 9). The Negative Binomial family with spline extensions demonstrated higher performance across all tested species, effectively capturing nonlinear eDNA-abundance trends while addressing overdispersion and zero inflation. *M. merlangus* exhibited robust correlations ($r > 0.9$) and moderate $R^2_{\text{Bayesian}} (\approx 0.5)$, so confirming a reliable eDNA - CPUE-derived abundance relationship. The remaining species demonstrated greater variability in fits, perhaps due to biological and sampling limitations in the detectability of the eDNA 12S marker and/or trawl survey experiment design. The Bayesian regression approach demonstrated effectiveness with small, noisy datasets, yielding consistent parameter estimation and robust uncertainty quantification.

Discussion

DNA metabarcoding is a powerful tool for assessing biodiversity in environments that are challenging to explore with conventional approaches. This research employed simultaneous trawling and eDNA metabarcoding to compare trawl catches with eDNA data from corresponding water samples along the Bulgarian Black Sea coast. Consistent with other studies (Díaz-Ferguson and Moyer, 2014; Gilbey et al., 2021; Rishan et al., 2023; Suren et al., 2024; Çevik and Çevik, 2025; Sahu et al., 2025) our findings demonstrate that eDNA represents an excellent complementary technique, especially for improving detection capacities and providing a more comprehensive understanding of marine fish diversity. Our results demonstrated that eDNA reliably identifies a greater number of species across multiple habitat types. Collectively, our findings underline the value of eDNA as an additional approach that improves detection

capabilities, strengthens biodiversity monitoring, and presents promising prospects for its incorporation into routine stock assessment frameworks.

Biodiversity

Our findings indicate that the eDNA 12S primer pair revealed greater taxonomic diversity than trawling, while requiring less sampling effort. Moreover, the eDNA-based method may detect organisms at various life stages and sizes, in contrast to conventional trawling, which selectively capture mature individuals within specific size ranges (Afzali et al., 2021). Although eDNA metabarcoding cannot provide estimates of absolute biomass, developmental stage, and size distribution, it offers substantial insights into the presence of numerous fish species within the studied ecosystem.

Another advantage of eDNA metabarcoding is its efficiency in a broader functional spectrum compared to bottom trawling and is capable of detecting both demersal and pelagic species present in the water column (Veron et al., 2023). Along the Bulgarian Black Sea coast, eDNA metabarcoding (12S) detected 13 pelagic species, including four rare species (*T. trachurus*, *S. japonicas*, *E. alletterattus*, and *T. thynnus*), eight migratory species, and twelve demersal species (Table 4). Nevertheless, some case studies indicate that pelagic species are captured during demersal surveys in the Black Sea, as summer stratification influences pelagic species such as *S. sprattus* and *T. mediterraneus* which remain beneath the seasonal thermocline, and only ascend during the summer-autumn mixing (Tserkova, 2013; Panayotova and Todorova, 2015; European Commission, Joint Research Centre (JRC), 2025). The latter is not relevant for juvenile fish which typically inhabits shallower waters. Accordingly, it is reasonable for the region to expect co-occurrence of both pelagic and demersal species in the deeper layers (25-100m). The rare species, although not captured by trawling, are known to occur in the Black Sea (Yankova et al., 2014). Overall, eDNA metabarcoding significantly enhanced detection rates of rare and migratory species across diverse habitats without requiring physical capture or direct observation. This capability is especially beneficial for monitoring species that are difficult to detect using traditional methods.

Biodiversity indices revealed substantial seasonal fluctuations in species communities throughout the Bulgarian Black Sea coastline. Both methods revealed seasonal clustering patterns in fish communities (Table 4), with autumn exhibiting greater biodiversity and species richness compared to summer. eDNA detected higher number of species compared to trawling, including rare and those that are less prevalent during specific seasons, such as *E. encrasicolus* and *M. barbatus* in July, and *S. sprattus* in November. Accordingly, eDNA metabarcoding may represent a viable instrument in monitoring of shifts in species distribution and behavior associated with climate change, and assist conservation efforts by identifying locations of the core-density areas for key-species, such as *S. maximus*, *S. sprattus*, and *M. barbatus* for targeted conservation management. These new discoveries improve our understanding of migration timing and mechanisms, aiding the formulation of successful conservation measures.

Only six species were identified to overlap when employing both sampling approaches (trawl and eDNA 12S and 16S). Eleven species were detected only with eDNA, while four species (Table 4) were exclusively identified through trawling. Additionally one species was identified on the genus level (*Alosa* sp.). The efficacy of eDNA-based biodiversity assessments depends on the thoroughness and precision of reference databases relevant to local biological populations, as emphasized in the study of Zhang et al. (2020). The lack of reference data might hinder taxonomic assignment, resulting in ambiguous species identification or, in severe instances, misidentification due to dependence on 'best match' algorithms (Gold et al., 2022). To address this, our study used custom reference databases, that included species known to inhabit the Black Sea, however, sequences for many Black Sea species are absent from NCBI and other public repositories, underscoring the need for greater efforts to incorporate additional species-specific mitochondrial DNA sequences into open access databases (Kasmi et al., 2023; Kumar et al., 2022; Xiong et al., 2022). Another potential explanation for the non-detection of some species via eDNA could be their rare occurrence or low abundance in the survey area, resulting in insufficient detectable eDNA. Species detected exclusively through trawling (*Alosa immaculata*, *Chelidonichthys lucerna*, *Uranoscopus scaber*, and *Acipenser stellatus*), exhibited minimal biomass in the catches (individual catch ≤ 4 kg). Furthermore, low-abundance taxa are often under-detected by traditional survey gear because encounter rates and capture probability decline with density (Williams et al., 2019; Adamo et al., 2020).

Scientific trawling remains the conventional method for assessing marine ecosystems and fish populations along the Bulgarian Black Sea coast, while eDNA-based species identification signifies an emerging scientific field and one of the most rapidly evolving tools in fisheries science and management (Lacoursière-Roussel et al., 2016; Casas Castaño and Saborido-Rey, 2022). The efficacy of fisheries management and conservation strategies depends heavily on the accurate identification of exploited species. This issue is especially challenging when dealing with cryptic species, larval stages, or morphologically similar bycatch (Casas Castaño and Saborido-Rey, 2022; Hansen, 2019). Accurate species-level identification is crucial for ensuring accurate catch records while supporting sustainable fisheries management. An illustrative instance of a challenging scenario involves the Ponto-Caspian species of the genus *Alosa*, which are assigned as vulnerable on the IUCN Red List. Morphological and genetic variations illustrate the taxonomic complexity of *Alosa* species in the Black Sea (Dobrovolev et al., 2012). The inconsistencies may result from misidentification in catches, as shads exhibit significant morphological plasticity, with overlapping morphological traits among species (Orlova et al., 2024). Genetic markers are essential for acquiring accurate information regarding species status and enhancing conservation efforts. Given these identification issues, some *Alosa* specimens gathered during our concurrent trawl research were identified as *Alosa immaculata*, while others could not be identified to the species level and were reported as *Alosa* sp. Similarly, environmental DNA (eDNA) metabarcoding at the same sampling locations (33S, 18A, and 25A), as well as an additional site (27A), using solely molecular approaches, also failed to resolve determination to

species level. The absence of genetic differentiation within the genus *Alosa*, based on various mitochondrial markers (Antognazza et al., 2019; Orlova et al., 2024; Bhendarkar et al., 2025), including those used in the present study, hampered species-level identification. Thus, regardless of the fact the ASVs were affiliated with *A. falax* (the only *Alosa* species represented in the reference library used) in the present study, the results could only be interpreted at the genus level, and intragenus differentiation remains highly challenging.

Species-specific detection sensitivity and methods detection sensitivity

Our findings indicated that species that are naturally more common and prevalent in the ecosystem have a higher likelihood of detection. Moreover, these species demonstrate increased detection probability across all sampling methods, including the trawling, which is selective and targets specific species according to trawl design and fisheries type. Moreover, species with high abundance are anticipated to exhibit a greater probability of correlating eDNA sequence counts with biomass or abundance, as their eDNA signal is expected to be more pronounced due to their naturally higher densities in the environment, thus increasing the likelihood of detection by both conventional and eDNA methodologies. Higher abundance implies higher DNA shedding rates (Tillotson et al., 2018), and effective eDNA sampling with species-specific mitochondrial markers (12S rRNA, COI) can provide quality data to infer actual abundance and spatial distribution.

In contrast, rare species demonstrated low detection rates, apparent at the detection threshold, and are likely to be discovered only via the 12S eDNA marker, due to the limitations of trawling as a sampling method and the reduced sensitivity of the 16S primers.

The concentration of eDNA in marine ecosystems may vary due to numerous abiotic factors influencing its dispersal and degradation rates, including currents, temperature, and pH (Collins et al., 2018; Andruszkiewicz et al., 2019; Fukaya et al., 2021; McCartin et al., 2022). Our data revealed statistically significant adverse impacts of temperature on method sensitivity. Elevated temperatures, particularly in shallow coastal waters, may induce behavioral avoidance in fish, compelling them to seek cooler environments (Pörtner and Peck, 2010; Perry et al., 2005), and may also lead to reduced eDNA persistence due to accelerated degradation (Strickler et al., 2015; Barnes and Turner, 2016). The latter emphasizes the importance of considering environmental fluctuations when evaluating methods sensitivity, and particularly when inferring abundance from eDNA sequence counts (Parsley et al., 2024; Jo, 2025).

Estimation of CPUE-derived abundance from the sequence read counts

There is increasing interest in employing eDNA sequence counts to assess actual fish abundance and biomass (Dukan et al., 2024). Many studies have indicated substantial correlations between

eDNA data and fish abundance/biomass in marine ecosystems (Thomsen et al., 2016; Salter et al., 2019; Afzali et al., 2021; Stoeckle et al., 2021; Rourke et al., 2022; Russo et al., 2021). Several research studies have found that using specific statistical methodologies, including Generalized Linear Models (GLM), Generalized Additive Models (GAM), non-linear models, or machine learning, can enhance the precise modeling of species abundance based on sequence counts (Collins et al., 2022; Fowler et al., 2024; Kasmi et al., 2025; Ip et al., 2025). However, some studies have demonstrated a lack of quantitative association between biomass/abundance and metabarcoding sequences, coupled with significant uncertainty (Lamb et al., 2019; Knudsen et al., 2019; Fraija-Fernández et al., 2020).

This research presents a robust multi-model analytical approach providing two multi-model frameworks implementing Bayesian hierarchical regression and frequentist Generalized Additive Models (GAMs) to establish relationship between eDNA 12S metabarcoding data and CPUE-derived abundance indices. The method addresses the primary challenges of eDNA–abundance modeling including constrained and noisy datasets, excessive zeros, and the potential for nonlinear environmental effects. In all frameworks, the models consistently identified biologically significant correlations between the abundance counts environment, spatial structure and/or eDNA signal strength. For *Merlangius merlangus*, both the Tweedie GAM and Bayesian Negative Binomial-spline models exhibited significant explanatory power. However, the GAM-derived model failed to account for any explanatory power of eDNA counts in relation to the predicted abundance, and the fit was significantly affected by the spatial structure.

Nonetheless, the uniformity of results from diverse statistical frameworks enhances the credibility of eDNA counts as a measure of abundance, assuming that analytical models adequately address dispersion and environmental variability. The Bayesian regression method facilitated comprehensive uncertainty quantification and reliable parameter inference in limited sample sizes, while Generalized Additive Models (GAMs) offered a sophisticated depiction of possibly nonlinear relationships among environmental DNA, environmental variables, and abundance. The latter illustrates that this complex approach enables eDNA data to replicate the spatial and environmental trends identified in trawl surveys, so facilitating the monitoring of fish populations with less environmental impact and increased frequency.

Our findings revealed that, depending on the strength of species-specific abundance and eDNA signals, many taxa (such as *M. barbatus*) had limited or absent relationships between observed and predicted abundance. This underscores the need for integrating additional molecular, ecological, and physical variables in abundance estimation to improve model explanatory power. A significant problem is the necessity for a carefully designed targeted sampling approach in both eDNA and trawl surveys to accurately capture actual species-specific signals, particularly when species coexist, leading to mixed catches. Concerning the latter, it is essential to highlight that the outcomes provided by the Bayesian regression framework can enable the direct translation of model uncertainty into recommendations for future eDNA survey design. The posterior variance and cross-model

agreement identify species for which specific variables strongly affect predictive uncertainty, emphasizing areas where more sampling would improve model accuracy. Species displaying broad credible intervals or unstable correlations may require further spatial replication or more accurate temporal coverage, particularly for species that are seasonally present in the sampling area and have distinct migratory patterns. In contrast, strong relationships (such as those evident in *M. merlangus* model) suggest that eDNA signal strength reliably reflects abundance based on the present effort.

Another observation concerns the integration of environmental variables, including temperature and salinity, which consistently improved fit stability. This highlights the need of jointly predicting eDNA concentration and habitat context. The latter demonstrate that a probabilistic, multi-model Bayesian framework can improve adaptive sampling and concentrate sampling efforts, hence strengthening confidence in eDNA-based monitoring especially through application of quantitative markers as an alternative or supplement to traditional trawl surveys.

However, as with many emerging technologies, eDNA faces several limitations. The accuracy of reference databases, environmental heterogeneity, and DNA degradation rates are critical factors that can compromise the reliability of eDNA-based analyses. Establishing standardized protocols for data acquisition, sampling, and interpretation remains essential (Çevik and Çevik, 2025).

Improvements remain feasible through the application of internal DNA standards in 12S metabarcoding (Stoeckle et al., 2021) and qMiSeq approach (Tsuji et al., 2022), combined with an understanding of species-specific DNA shedding rates, eDNA transport dynamics, and signal degradation. The latter may undoubtedly provide a more robust basis for the precise assessment of biomass in less abundant species, particularly uncommon, newly introduced, and invasive ones. Moreover, several challenges persist, including the need for reliable data on DNA shedding and degradation rates, species mobility in the water column, hydrodynamic conditions influencing DNA transport and decay, and the necessity for precise ecological and abundance data to validate the model outcomes. Furthermore, the integration of eDNA data with ecological models and traditional approaches is essential to improve precision and deepen the understanding of ecological dynamics. By overcoming these constraints, eDNA could emerge as an essential instrument for biodiversity evaluation and conservation efforts (Çevik and Çevik, 2025). In conclusion, current advancements in molecular biomonitoring have expanded opportunities for aquatic ecosystem assessment (Blancher et al., 2022), and its application is becoming increasingly widespread (Ruppert et al., 2019). eDNA metabarcoding is an evolving method that is likely to continue developing as technology advances and standardization progresses. We conclude that it is a promising instrument that can complement and substitute the scientific trawling for biodiversity monitoring and that it can be successfully adapted for abundance estimation, in agreement with Veron et al. (2023). Further advancements are needed before this method can be widely used for fisheries assessments in the Black Sea basin, with a reasonable initial emphasis on the most abundant species. Future biomonitoring programs in the region should integrate eDNA metabarcoding with conventional surveys to yield a more

comprehensive and precise evaluation of ichthyological diversity and fish stock, hence facilitating evidence-based policy formulation.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because Sampling studies were carried out within the scope of the permission given by the Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture and Food, Bulgaria, according to Contract No D-156 of 13.03.2023. This study does not require Local Ethics Committee Approval as experimental animals were not used in this study.

Author contributions

PI: Writing – review & editing, Writing – original draft. IZ: Writing – review & editing, Writing – original draft. ND: Writing – review & editing, Writing – original draft. IP: Writing – review & editing, Visualization. VR: Writing – review & editing, Resources. DD: Writing – review & editing, Resources. SM: Resources, Writing – review & editing. YR: Writing – review & editing, Resources. KS: Writing – review & editing, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2025.1648741/full#supplementary-material>

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