SPECIAL ISSUE ARTICLE

Using eDNA to estimate biomass of bycatch in pelagic fisheries

Paulina Urban[1] | Dorte Bekkevold[1] | Brian Klitgaard Hansen[1] | Magnus Wulff Jacobsen[1] | Anders Nielsen[2] | Einar Eg Nielsen[1]

[1]Section for Marine Living Resources, National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark (DTU), Silkeborg, Denmark

[2]Section for Marine Living Resources, National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark (DTU), Lyngby, Denmark

Correspondence
Paulina Urban, Section for Marine Living Resources, National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark (DTU), Silkeborg, Denmark.
Email: paur@aqua.dtu.dk

Abstract

In recent years, the analysis of environmental DNA (eDNA) has significantly improved, allowing for high-resolution species identification and possible biomass quantification from water samples. Fisheries management typically requires monitoring of catches, including precise information about bycatch quantities to make sound assessments of exploitation rates. Bycatch assessment is particularly challenging in large catches (>500 T), and the current practice of visual assessment of subsampled catches is time-consuming, requires extensive labor, and often has low precision. We explored the feasibility for applying eDNA-based methods for studying catch composition using the pelagic North Sea herring fishery with bycatch of mackerel as a case. First, we experimentally simulate a series of catches using a range of herring and mackerel weight proportions to establish relationships under real fisheries scenarios. The relationship is subsequently used to estimate the biomass of mackerel bycatch from eDNA from three herring catches, by sampling and comparing processing water both onboard ships and at the processing factory. All samples are analyzed using species-specific quantitative PCR (qPCR). The experiments reveled a strong correlation between DNA and weight fractions characterized by a constant overrepresentation of mackerel DNA compared to expected mackerel weight. We found that eDNA-based and visual methods applied to the same landing reflect the within catch variability in species composition alike, however, the methods can show disparity in total estimates of mackerel biomass. Accounting for haul mixing within total landed catches increases the precision of the factory and ship eDNA-based estimates for the same catch. We show that eDNA-based bycatch estimates provide coherent quantitative data, and likely improve quality and reduce costs of collecting fisheries-dependent data and thereby contribute to securing sustainable fisheries.

KEYWORDS
bycatch, eDNA, fisheries, landing obligation, species-specific quantitative PCR

1 | INTRODUCTION

Over the past decade, environmental DNA (eDNA) has evolved into an effective, noninvasive tool for species monitoring in natural environments (Hongo et al., 2021; Salter et al., 2019; Taberlet et al., 2012; Thomsen et al., 2016; Yates et al., 2021). The basis for the eDNA concept is that all organisms constantly release DNA into their surroundings via the skin, mucus, defecation, and other processes (Rodríguez-Ezpeleta et al., 2021; Taberlet et al., 2012). The DNA left in the surrounding environment can be retrieved and analyzed to deduce the species' identity. Environmental DNA has been studied in different environments, including water (Ficetola et al., 2008; Knudsen et al., 2022; Stoeckle et al., 2021; Thomsen et al., 2012; Thomsen et al., 2016), air (Clare et al., 2022; Roger et al., 2022), and soil (Buxton et al., 2018; Ryan et al., 2022). Typically, it is used to assess the presence of either a single target species (Ficetola et al., 2008; Knudsen et al., 2022; Yates et al., 2021) or the composition of species from larger taxonomic groups, that is, biodiversity (Bakker et al., 2019; Boussarie et al., 2018; Hongo et al., 2021; Roger et al., 2022; Russo et al., 2021). Because eDNA allows for easier, cheaper, and faster species monitoring compared to the labor-intensive traditional visual methods (Fediajevaite et al., 2021; Goldberg et al., 2013; Lugg et al., 2018; Thomsen et al., 2012), there is an ongoing effort to explore the potential of eDNA studies beyond species detection toward estimation of biomass and abundance (Russo et al., 2021; Stoeckle et al., 2021; Thomsen et al., 2012; Yates et al., 2021).

The fisheries management sector has a long tradition of high need for extensive monitoring, with high associated costs, in relation to assessment of fisheries resources and food safety (Arnason et al., 2000; Link et al., 2002; Richards et al., 2022; Wallis & Flaaten, 2003). With the development of DNA-based monitoring tools, the sector is looking toward the potential for practical application of eDNA for some monitoring purposes such as in stock assessment, product traceability, and quality assurance (Cusa et al., 2022; Hansen, Farrant, et al., 2020; Helyar et al., 2014; Jacobsen et al., 2019; Roungchun et al., 2022; Salter et al., 2019; Stoeckle et al., 2021). However, for these applications there is so far an insufficient understanding of the quantitative aspects of the DNA signal measured. In natural open environments such as the sea, the reliability of quantitative applications of eDNA is tightly coupled to an understanding of the origin, production rates, degradations rates, and transport of DNA which are to some degree specific to the environment the DNA particle has been sampled from (Hansen et al., 2018). Therefore, establishing very tight relationships between fish abundance/biomass and DNA copy number in ambient water samples is generally considered difficult (Beng & Corlett, 2020; Hansen et al., 2018; Knudsen et al., 2019). Instead, a potentially more tangible application of eDNA in fisheries is for reconstructing catch composition (Russo et al., 2021) including bycatch estimation. Unlike in natural environments, where species biomass and water movements (i.e., flow intensity and direction) can change fast, thereby affecting the retrieved eDNA-signal and making interpretations difficult, the hold of a fisheries vessel represents a confined environment. The composition and biomass of fish in the holdings tanks are unchanged from when the fishing operation ends until the catch is landed to a factory. Onboard modern pelagic trawlers, seawater is used for cooling the catch. For this, the seawater is stored in tanks with the fish and mixed constantly leading to a well-mixed solution (del Valle & Aguilera, 1991), that potentially can provide an integrated signal of the catch composition (Figure 1). Factors associated with fish physiology (i.e., metabolism) and fish movement can also be neglected, as all fish are dead once stored onboard the ship. This controlled setting allows for studying the relationship between eDNA and species abundance/biomass that is far less complex than under ambient conditions at sea.

Bycatch is defined as the accidental intake of nontarget species during fisheries operations. Bycatch is often of low economic value and limited interest for the fisherman, leading not seldom to discarding or slipping and thus poor overall catch records (Tenningen et al., 2021). In pelagic fisheries even small bycatch rates (~1%) can result in high biomass of nontarget species caught due to the generally large size of the catches (>500 t) (Nøttestad et al., 2016). Thus, the information about the quantity of the unwanted part can still be of high value for fisheries management as it serves for predicting catchable amounts. Poorly done bycatch assessment can lead to issues related to spawning stock biomass and associated recruitment and thus lead to instability of the harvested populations and in turn affect the future economic profit (Dickey-Collas et al., 2007; Rudd & Branch, 2016). A poor assessment also potentially prevents appropriate conservation actions in the understanding of the real impact of fisheries on the ecosystem (Gray & Kennelly, 2018) and verifications of eco-labeling (Clegg et al., 2021). Thus, to ensure the long-term economical profitability of fisheries operations without jeopardizing marine biodiversity all catch components need to be reliably monitored (Booth et al., 2021).

The Atlantic herring (*Clupea harengus*) fishery has historically been one of the most economically important fisheries in the North Sea region. Accordingly, the herring fishery is one of the most data-rich and after its collapse in 1970s, a species with a particularly high management focus in the North Sea (Tenningen et al., 2021). Bycatch species in the herring fishery can be diverse, but with the most likely bycatch of Atlantic mackerel (*Scomber scombrus*), whiting (*Merlangius merlangus*), haddock (*Melanogrammus aeglefinus*), and horse mackerel (*Trachurus trachurus*) (ICES, 2017). Out of these, mackerel is a quota-regulated and economically very profitable species (Trenkel et al., 2014). As such, mackerel biomass caught and reported as bycatch is directly subtracted from the species' main quota. Accordingly, there is a need for methods, which can effectively monitor mackerel bycatch, even when at low abundance (>50 kg) in large pelagic catches of herring.

We explored the opportunity to apply eDNA-based methods for studying catch composition and thereby derive quantitative bycatch information for mackerel with high precision and at a low cost. In Denmark, bycatch quantities in the herring fishery are estimated for all landings using a so-called "bucket method". In brief, the method consists of taking a 20–30-kg subsample of the catch for every 25
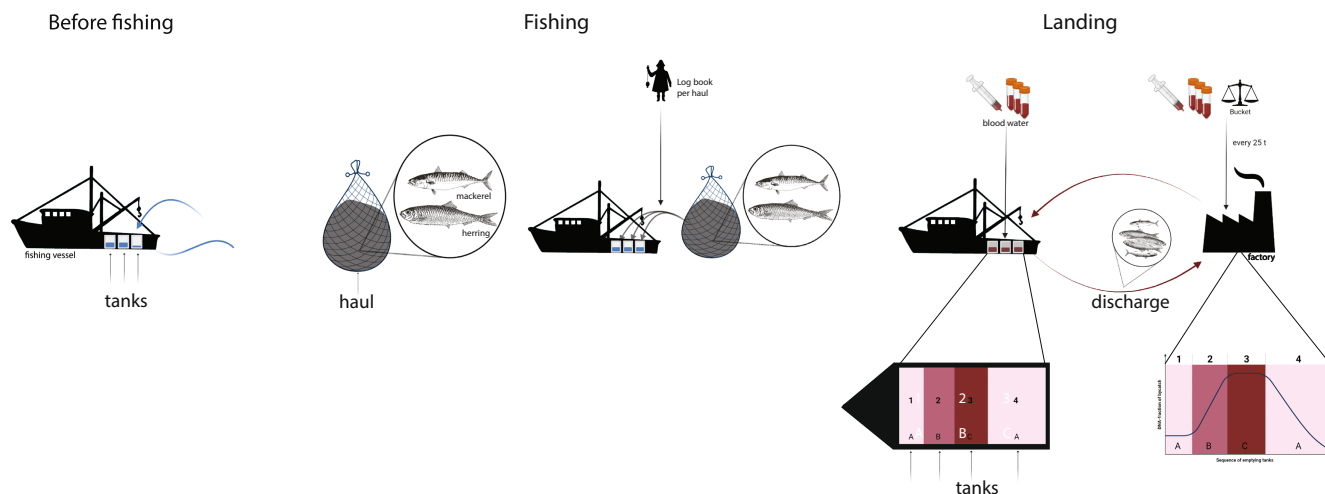
FIGURE 1 A visualization of the three phases in a pelagic industrial fisheries. The first phase, "before fishing" shows the uptake of seawater into tanks on board the ship. The seawater is used during the fishing stage to keep the catch fresh and it is used to transfer the catch from the ship to the factory. During fishing, separate casts of the fishing net are performed ("hauls") to collect the total catch. The letter "A," "B," and "C" represent three different hauls distributed among four ship tanks. The species composition from each haul is determined and noted in the logbook. During landing, fish and the seawater (now called blood water) are transported from the ship to the factory. Each tank is emptied once at the time in a predefined sequence (1–4). During the discharge, the species composition is further determined using the bucket method. The genetic sampling took place on the ship (before the discharge started) and at the factory at the same rate as the bucket method. Importantly, during landing blood water is constantly re-circulated between the factory and the ship

tons as the catch is flushed from the ship to the processing factory via chutes. For each bucket sample, all species are identified and weighed. Subsequently, the species-specific weight composition in the buckets is extrapolated to the total catch and the overall bycatch rate is determined (Fiskeristyrelsen, 2021). The method and its results can be a source of conflict among fishers, fisheries agencies, and industry officials, because of the methods' large and inevitable variation and thus uncertainty (approx. 10%).

The catch onboard a fishing vessel can be seen as a standalone entity from which species composition could be determined through eDNA sampling and analysis. We use the pelagic North Sea herring fishery with bycatch of mackerel as a case study to test the application of DNA analysis from blood water onboard fisheries vessels and at the processing factory for its suitability for reliable eDNA-based bycatch quantification. This is the first study to determine relationships between eDNA copy number fraction and weight fraction for different artificial herring/mackerel mock samples where fractions of mackerel varied. Results were used to establish a model to estimate mackerel biomass from eDNA estimates. Secondly, we applied inference from the model to estimate the weight of mackerel in three landings from the herring fishery. For these landings, eDNA was collected and analyzed from blood water both on board fishing vessels (hereafter eDNA at ship) and in factories (hereafter eDNA at factory). These eDNA-based weight estimates were compared with the estimates derived from the log book (hereafter visual (log book)) and the bucket method (hereafter visual (bucket)). We also investigated if and how eDNA-based estimates of catch composition are affected by the distribution of fish in the individual hauls in different holding tanks onboard the ship, and the mixing of the process water, during the unloading process.

Finally, we evaluated the potential of the method for routine implementation to document bycatch and for control and enforcement.

2 | MATERIAL AND METHODS

2.1 | Experiment: weight to eDNA relationship

The relationship between species biomass and DNA fractions was studied in experimental mixtures of herring and mackerel. Two types of experiments were conducted, DNA-shedding (i.e., DNA production) and DNA-decay (i.e., DNA degradation). Two "DNA-shedding" experiments were performed for this purpose, (1) a laboratory-based DNA-shedding experiment to test the DNA release of species under controlled temperature conditions and (2) a ship-based DNA-shedding experiment that simulated real fisheries conditions. In the laboratory-based DNA-shedding experiment each of 2×7 experimental units was constructed with 5 kg mixed, freshly caught herring–mackerel, and 2.5 L of seawater. Mixtures were prepared based on weight exploring from low (2.5%) to substantial (50%) bycatch contribution (Table 1). The ship-based experiment consisted of four experimental units, each consisting of 5 kg mixed fresh herring and thawed mackerel, and 2.5 L of seawater, with mixtures ranging from 2.5% to 20% of mackerel bycatch (Table 1). For both experiments, only whole specimens were used and thus the actual gram-to-gram proportion in each mixture deviated slightly from the target (Supporting Information 11). All experimental units were prepared in separate containers double wrapped with plastic bags (Cater Line, Freezer bags, 40 L). Before starting the experiments, fish were briefly

TABLE 1 Overview of the experiments performed to study the eDNA to weight relationship of herring and mackerel mock samples

| Experiment | Replicates | Proportions anticipated in mock units (herring/mackerel) | Sampling time points | Catch region | | | Water | Comments |
				Herring	Mackerel			
Shedding experiment in the lab	2	97.5/2.5 95/5 90/10 80/20 70/30 60/40 50/50	24h 48h 72h 96h 120h	2A	3A		Artificial seawater (32 PSU)	Herring 5 days older than mackerel (kept chilled until setup)
Decay experiment in the lab	2	97.5/2.5 95/5 90/10 80/20 70/30 60/40 50/50	2 h 4 h 6 h 12h 24h 24h 48h	2A	3A		Artificial seawater (32 PSU)	
Shedding experiment onboard	1	97.5/2.5 95/5 90/10 80/20	24h 48h	4B	3A		4B	Thawed mackerel used for experiment

Note: In total, three experiments were performed from which two under controlled conditions in the lab and one under "real" conditions onboard a ship, as indicated in "experiment" column. In the laboratory-based experiments, for each anticipated proportion we prepared two mock units (replicates), indicated in the "replicates" column. The end-proportions in the experimental units can deviate from the anticipated because only whole specimens were used. Each experiment was sampled at different time intervals, in total ranging from 2 to 120h. The specimen used in the experiments were commercially caught at different places, as indicated in "catch region." The region of the catch was identified following the ecoregion description from ICES ("2A" is in the Norwegian Sea/North Atlantic, "3A" is the Skagerrak-Kattegat area and "4B" is the southern North Sea).

washed with seawater. The DNA shedding experiments started with placing the predefined herring–mackerel mix (Table 1) and seawater into the container and closing the plastic bags to prevent contamination. The experimental units were then subject to either (1) temperature-controlled laboratory settings simulating real fishery conditions (average temp. −1.25°C ± 1.43, more details see Supporting Information 8 and 9), or (2) real fisheries conditions onboard a ship. During both DNA shedding experiments, up to 6 ml of blood water was collected at regular intervals (Table 1) using a sterile 20-ml syringe (Inject® Solo, B. Braun), into a sterile collection tube (Sarstedt, Screw cap tube, 10 ml). All samples were frozen at −20°C immediately after sampling. In the laboratory, after the DNA-shedding experiment ended, the "DNA-decay experiment" was initiated to study the potential effect of decay of DNA, after removal of the fish from the water, on the estimated fraction of herring and mackerel. The experiment started by separating the fish from the blood water and subsequently maintaining the blood water at same experimental conditions for additional 48 h. Also during this experiment, up to 6 ml of the decaying blood water was sampled at regular intervals (Table 1) using a sterile 20-ml syringe (Inject® Solo, B. Braun), into a sterile tube (Sarstedt, Screw cap tube, 10 ml). All samples were frozen at −20°C immediately after sampling. At all times, all experimental units were thoroughly mixed prior to sampling.

2.2 | Application to fisheries samples: estimating bycatch weight

2.2.1 | General description of the fishery process

We define three phases during herring fisheries: the before fishing, the fishing, and the landing phase (Figure 1). In the first phase, the holding tanks (separate compartments of a ship designed to keep water and fish) onboard the ship are filled with seawater up to 1/3 of their volume. The uptake of seawater happens on the voyage to the fishing grounds. During fishing, each casting and pulling in of the fishing net is called a "haul". Depending on the size of the haul, its content is transferred to one or more tanks. One to several hauls are performed to fill the capacity of the ship. Once all tanks are filled, the total amount of fish stored is referred to as "total catch" or "catch". In the logbook, the fishermen records the

species composition as species (kg)/ haul. The composition is usually determined by the fishermen using the bucket method. Here, each haul is periodically subsampled and species composition is assessed through visual identification and weighing of each species separately. The estimated proportions are used to extrapolate the species composition to the total haul and catch, and reported to the fisheries authorities in a log book. The frequency of this subsampling is not recorded.

While at sea, the seawater inside the tanks keeps the catch fresh until landed. With time, fish remains (i.e., blood, urine, skin cells, gametes, and scales) accumulate and change the appearance of the water, which is subsequently called "blood water". When the ship reaches the processing factory, commonly 72 h, the landing phase begins. In this phase, catch and blood water are transported via chutes from the ship to the factory (discharged). During transport, the blood water is constantly re-used by pumping to lift the next portion of the catch into the factory. During the discharge, the bucket method is applied at the factory to determine the species composition. Here, the method consists of subsampling 20–30 kg of the catch at regular intervals (every 25 tons). The species composition in the subsamples is assessed through visual identification and weighing of each species separately, and these proportions are then used to extrapolate the bycatch fraction in the subsamples to the total catch and reported to both, fishermen and fisheries control.

2.3 | Sampling blood water from landings

We sampled data from a total of three landings ("landing 1–3"). Each sampling started with collecting blood water from the ship. We collected three replicates (one sample = three replicates) of blood water from each holding tank of the ship (individual ships had 8–11 tanks). All replicates were collected at the opening ("top-part") of each tank. Once the landing process started, we collected three replicates of blood water for every 25 t at the factory, synchronized to the visual (bucket) method. Both sampling onboard and at the factory consisted of collecting up to 50 ml of the blood water using a sterile 60-ml syringe (Codan™), into a sterile 50-ml falcon tube (Sarstedt, Screw cap tube, 50 ml). All samples were kept on ice during the sampling and frozen at −20°C immediately after the end of the landing. In each landing bycatch

TABLE 2 Overview of the three landings from which blood water was collected for eDNA-based estimation of mackerel biomass

Landing ID	Time between catch and the landing (h)	Total catch size (t)	Size of the ship (m)	Number of hauls	Number of tanks holding	Number of eDNA at ship samples	Number of eDNA at factory samples
1	48–72	1185.4	75	5	11	9 (out of 11 collected)	6 (out of 36 collected)
2	120	940.2	63	3	8	8	42
3	72–144	902.1	75	3	11	11	37

Note: Only a part of samples from landing 1 was analyzed, that is, only 9 out of 11 samples collected from the ship were analyzed using the eDNA-based approach to explore the possibility of mackerel being present in the catch. Estimates of mackerel biomass for each landing were obtained from the logbook and buckets method.

6 of 16 | **WILEY** Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences Open Access

URBAN ET AL.

was also estimated using visual methods (logbook and bucket). For one landing (no. 3), additional information was recorded about the volume of each of the 11 tanks of the ship, which haul was placed in which tank and the sequence of discharging individual tanks to the factory (Table 2).

2.4 | DNA-extraction

DNA was extracted from 1 ml of each water sample using the Omega Bio-tek E.Z.N.A. Tissue DNA kit (Omega Bio-tek, USA). The standard extraction protocol was adjusted for higher sample volume (with 2.5× the recommended volume of buffers during DNA lysis and adjustment before silica-membrane binding). DNA extraction took place in a molecular laboratory. Blank samples were collected throughout the sampling (using DNA-free water that did not get into contact with either catch or blood water), DNA-extraction and analysis process (nontemplate controls) to monitor possible contamination.

$$\text{logit}(\mu_i) = \alpha + \beta \cdot \text{logit}(\text{true weight fraction}_i) + \delta\left(\text{treatment}_i\right) + \gamma \cdot \text{time}_i + E\left(\text{Replicate}_i\right) + \varepsilon_i, \text{ and } E\left(\text{Replicate}_i\right) \sim N(0, \sigma^2_E) \text{ and } \varepsilon_i \sim N(0, \sigma^2) \text{ independent.}$$

2.5 | Species-specific qPCR

Species-specific assays targeting herring and mackerel cytochrome b sequence of the mitochondrial genome were used for DNA quantification in the samples (Supporting Information 1). Assays used were optimized and tested on a StepOnePlus Real-Time PCR System (Life Technologies, USA). Assay specificity was assessed using genomic DNA from the two target species (herring and mackerel) cross-tested on both assays. Each qPCR reaction was conducted in 10 μl volume with 3 μl of sample and 4 μl TaqMan Universal PCR Master Mix (Thermo Fisher Scientific) and assay-specific volumes of primers and probes to obtain optimal reaction conditions (Supporting Information 1). Thermal cycling conditions for both assays were the same (5°C for 5 min and 95°C for 10 min followed by 50 cycles at 95°C for 30 s and 60°C for 1 min). Samples were analyzed in duplicates with triplicate negative controls and a triplicate standard curve ranging from 3×10^6 to 3×10^0 copies per reaction in each run. The experimental samples were analyzed in a multiplex (duplex) setting, while factory samples were analyzed as singleplex reactions. Primer concentration was the same for both setups with overall higher concentration of mackerel primer, to prevent dominance of the herring assay (see Supporting Information 2). The herring assay was modified for the multiplexing approach by using a NED-dye for the herring probe to separate its fluorescence signal from the FAM-dyed mackerel probe (see Supporting Information 2). To increase delta-fluorescence assays used double-quencher probes (singleplex: 5′FAM/ZEN/3′IB®FQ) (Integrated DNA Technologies, USA; multiplex: 5′TAMRA/ZEN/3′BHQ-2®) (Integrated DNA Technologies, USA), in all reactions. The singleplex and multiplex approaches were compared for 36 out of 162 experimental samples to ascertain estimate consistency. An internal positive control (hereafter IPC) dyed

with VIC was used in each reaction to monitor inhibition. For the multiplexing we used the VetMAX Xeno Internal Positive Control containing the BHQ-3 quencher (Applied Biosystems) and for the singlplex approach we used the TaqMan™ Exogenous Internal Positive Control containing the TAMRA quencher (Applied Biosystems).

2.6 | Modeling of the catch composition

2.6.1 | Model 1

The experimental data served for establishing an eDNA-to-biomass model, which subsequently was used to predict the biomass of mackerel from eDNA measurements from fisheries samples. We used generalized linear mixed models (GLM) built using the package *glmmTMB* 1.0.2.9 (Brooks et al., 2017). The DNA quantities estimated from herring and mackerel were converted into mackerel-to-herring fractions; hence the genetic observations are continuous numbers between 0 and 1, naturally described by a beta distribution:

$O_i \sim \text{Beta}(\mu_i, \phi)$ independent where:

The model parameter ϕ is a precision parameter scaling the variance of O_i as $Var(O_i) = \mu_i(1 - \mu_i)/(1+\phi)$. The logit-scale genetic fractions (O_i) are described as a linear function of time and logit of the true weight-based fractions with separate levels corresponding to each level of *treatment* (DNA-shedding lab, DNA-shedding ship, DNA decay). The uncertainty structure of the model accounts for variations among replicates of mixtures and individual measurement noise.

To describe the mackerel DNA fraction to biomass relationship with resemblance to the conditions of the catch during landing we chose a specific treatment ('DNA-shedding experiment' from laboratory), and the results from the time period of 72 h, as well as a range of true weight fraction from 0.001 to 0.05.

$O_i \sim \text{Beta}(\mu_i, \phi)$ independent where:

$$\text{logit}\left(\mu_i\right) = \alpha + \beta^* \text{logit}\left(\text{true weight fraction}_i\right)$$

2.6.2 | Model 2

For landing 3, additional information was collected about total catch separation into individual hauls and holding tanks. We explored this in an analysis where the mackerel weight fractions estimated from all four methods (logbook, eDNA- from ship, eDNA from factory, and factory bucket method) were modeled to understand how the variation in mackerel fractions in individual hauls and the mixing of the water during the landing process influence the estimations of the total mackerel bycatch.

Again, eDNA-based weight fractions and logbook fractions are continuous numbers between 0 and 1, naturally described by a beta distribution. Fractions from the bucket method have a higher occurrence of zero observations than can be explained by a beta distribution, due to sampling of whole fish, hence the bucket observations are described by a zero inflated beta distribution.

URBAN ET AL.

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences
WILEY | 7 of 16

$$P(O_i = o_i) = \begin{cases} p_i & \text{if } o_i = 0 \\ (1-p_i)f(o_i, \mu_i, \phi_{type_i}) & \text{otherwise} \end{cases} \quad (1)$$

Here, the function f is the density of a beta distribution with mean μ_i and measurement-type specific precision parameter ϕ_{type}, such that variance of the observations O_i scales as $Var(O_i) = \mu_i(1-\mu_i)/(1+\phi_{type})$.

As zero inflation is only relevant for measurements originating from the bucket method it is assumed that $p_i = 0$ unless measurements are from the bucket method. The probability of zero bycatch could further be haul specific, so the model assumed for the zero probability is:

$$\text{logit}(p_i) = \alpha(\text{haul}_i) \quad (2)$$

if i'th observation is from the bucket method.

The conditional expected non-zero fractions μ_i are assumed to be:

$$\text{logit}(\mu i) = \sum_{tank=1}^{n_{tank}} \beta(type_i, tank_i) W_{i,tank} \quad (3)$$

W is matrix of data weights, which is intended to describe how the sample is composed of fish from different tanks. If, for example, the i'th sample is exclusively from the first tank then the first $W_{i,1} = 1$ and the following $W_{i,2} = 0, W_{i,3} = 0,...$ If the j'th sample is taken from an even mixture of tank 1 and tank 2, then $W_{j,1} = 0.5$, $W_{j,2} = 0.5$ and the rest zero.

Using W we tested three different mixing approaches between tanks that contain different fish hauls. We tested the "no mixing," "100 T mixing," and "full mixing" scenario. In "100 T mixing" W describes the changing composition of each sample collected within the first 100 T of the discharge of a tank, after 100 T all subsequent samples collected from the same tank were not considered under W, hence did not mix with the previous tank. In "Full mixing" W describes the changing composition of each sample taken during the discharge at the factory, hence every sample collected is a mixture of previously discharged fractions, and thus subject to W.

Finally, the eDNA-based weight estimates (ship and factory) were analyzed in triplicates and hence those measurements can be expected to be correlated, which was accounted for by introducing sample-level as a random effect.

The generalized linear mixed model 2 was created in C++ and imported into R (4.1.0) using the package *TMB* (Kristensen et al., 2016).

3 | RESULTS

3.1 | qPCR assay performance

The study used previously published and verified qPCR assays to target both species, herring and mackerel (Hansen, Jacobsen, et al., 2020; Knudsen et al., 2019) (Supporting Information 1). The standard curves used for the quantitative estimation of DNA copies from the fisheries samples showed comparable efficiencies (herring average efficiency: 91.08%, mackerel average efficiency: 92.56%, multiplex: 94.91%) and correlation coefficients ($R^2 > .997$ for all) between species (See Supporting Information 2 and 3). Similarly, the standard curves used for the estimation of the DNA quantity in experimental samples also showed comparable efficiencies between species (herring average multiplex: 98.68%, mackerel average efficiency multiplex: 94.91%). None of the assays showed unspecific amplification (See Supporting Information 4). Estimates of contamination were extremely small throughout the analytical process, and did not reach quantifiable amounts (above LOQ, [Merkes et al., 2019]). A subset of the experimental data was analyzed using respectively singleplex and multiplex approach results, and showed highly comparable efficiencies E% (for mackerel, multiplex = 92.89% and singleplex = 95.49%, for herring, multiplex = 99.06% and singleplex = 92.29%) and precision (for all $R^2 \geq .996$) derived from the standard curves (Supporting Information 5–7). Nevertheless, these samples showed significant differences in C_t-value (Wilcoxon signed-rank for herring $N = 36$, $V = 493$, $p < 0.05$ and for mackerel $N = 36$, $V = 42$, $p < 0.001$) and estimated species fraction (Wilcoxon signed-rank for herring $N = 36$, $V = 164$, $p = <0.001$ and for mackerel $N = 36$, $V = 502$, $p = <0.001$) (Supporting Information 12).

3.2 | Mackerel-herring weight to eDNA relationship

The shedding experiment in the laboratory, the shedding experiment onboard, and the decay experiment all showed a strong relationship between estimated DNA fractions in the blood water and input weight fractions for all mock samples (Figure 2). DNA fractions of mackerel were at all times overrepresented compared to the weight-based input fractions. The relationship between DNA fractions and input weight fractions was influenced by the time span of DNA release, and by the different treatments tested (DNA-shedding in the laboratory, DNA-shedding on the ship, and DNA-decay in the laboratory). In general, the mackerel DNA fraction showed a tendency to increase with time within the mock units (Figure 2a,b). The difference in fractions over time was not statistically significant in any treatment, but was marginally nonsignificant for the laboratory-based DNA-shedding experiment ($p = 0.0519$; ship-based DNA-shedding experiment $p = 0.8128$, DNA-decay experiment $p = 0.1557$). Overall, the three different treatments tested show statistically significant differences in estimated mackerel fractions (for all, $p < 0.001$). Because the weight fractions and the DNA fractions are logit transformed, it is difficult to translate the change into a single value per treatment. Hence, the Figure 2C shows how the change in weight fraction translates into changes in DNA fractions. We would like to highlight two examples that visualize the extent of the differences recorded by the different treatments. That is, a mackerel weight fraction of 0.1 (10%) corresponds to 0.456 (45.6%), 0.329 (32.9%), and 0.291 (29.1%) DNA fraction, respectively when estimated from a DNA-decay, laboratory-based DNA-shedding experiment, and
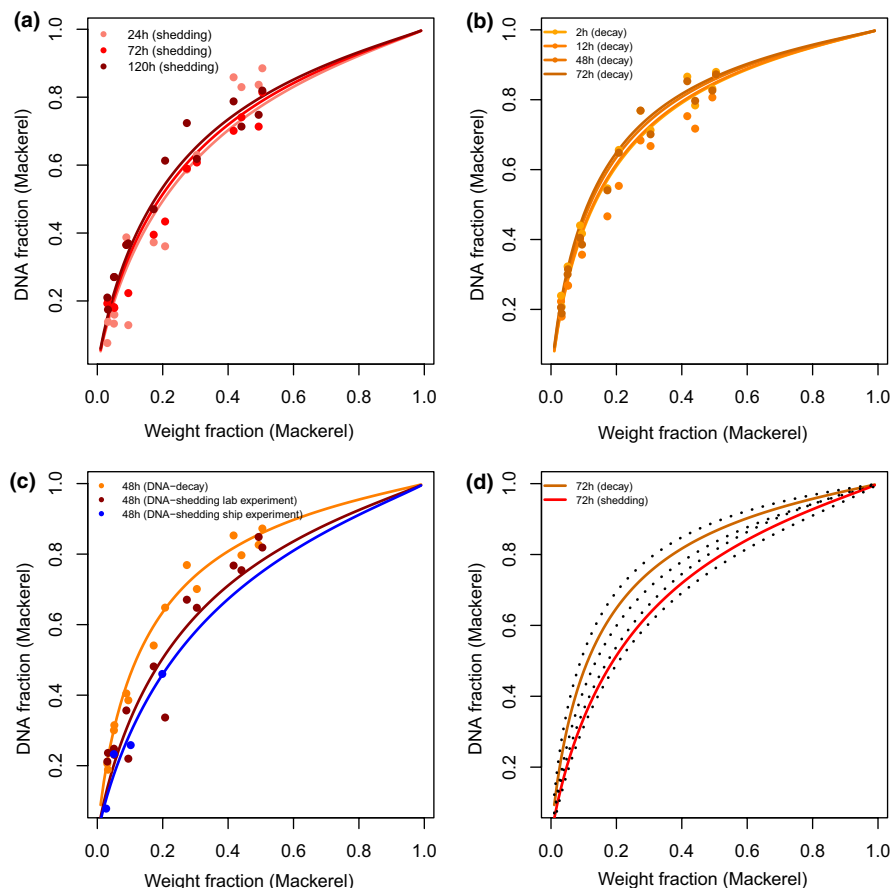
8 of 16 | WILEY— Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences
Open Access

URBAN ET AL.

FIGURE 2 Predictions of the DNA to biomass relationship using model 1 for (a): The effect of time on "DNA-shedding estimated from the lab-based DNA shedding experiment," (b): The effect of time on "DNA-decay." (c): The effect of three treatments ("Shedding experiment in the laboratory," "Shedding experiment on board a ship," "decay experiment in the laboratory"). The two shedding experiments besides of being conducted at different places, used mackerel of different quality, that is, in the lab fresh mackerel was used whereas on the ship defrosted mackerel. (d) shows the assessment of the precision of the model-prediction for "DNA-shedding (lab-based experiment)" and "DNA-decay". Solid lines in each graph show the model predictions of weight-based fractions for DNA-based fractions ranging from 0.01–0.99, dotted lines in (d) show the 95% confidence intervals for the predictions. The dots in (a–c) reflect the measured mackerel eDNA fractions in the different experiments

ship-based DNA-shedding experiment. A mackerel weight fraction of 0.5 (50%) corresponds to 0.858 (85.8%), 0.774 (77.4%), and 0.747 (74.7%) DNA fraction respectively when estimated from a DNA-decay, laboratory-based DNA-shedding experiment, and ship-based DNA-shedding experiment. The DNA-shedding experiments showed a higher mackerel fraction to weight fraction relationship when using fresh (laboratory-based DNA-shedding experiment) compared to thawed fish (ship-based DNA-shedding experiment) (Figure 2C). Overall, the model outcomes are more precise when using the DNA-shedding data compared to using DNA-decay data (Figure 2D).

3.3 | Estimating weight of mackerel bycatch in three fisheries landings

For landing 1, a part of the samples collected was analyzed using the qPCR approach (9 out of 11 samples from the ship and 6 out of 36 samples from the factory). Very low raw DNA copy numbers of mackerel were recorded in some samples. No samples analyzed from landing 1 reached LOQ for mackerel (see Supporting Information 2), thus, all measurements were regarded as un-quantifiable detections. In this landing, the average raw DNA copy number for mackerel in both eDNA at ship and eDNA at factory samples was 5.19 copies/reaction (in the range of 0–41 DNA copies/reaction). The highest raw DNA copy number was recorded in one out of three replicates of a

eDNA at factory sample analyzed (41 copies). In contrast to this, the average herring raw DNA copy number was 50,084 (ranging from 1433–238,734 DNA copies in all samples analyzed). Thus mackerel fraction based on raw DNA in both, eDNA at factory and eDNA at ship samples, was on average 0.0001 (ranging from 0–0.0025). The highest mackerel fraction (based on raw DNA) of 0.0025 (i.e., 0.25%) was recorded in one replicate of an eDNA at ship sample. This replicate had generally one of the lowest outputs of DNA (sum of raw DNA copies of both species = 1692) among all samples, with a mackerel DNA copy number estimated at four (resulting in a fraction of 0.0025 [i.e., 0.25%]). When translating the raw DNA fractions to biomass using model 1, all factory samples ended with negative biomass estimations (hence, no biomass of mackerel detected). For the eDNA at ship samples, five of the analyzed replicates (out of 27 replicates analyzed) yielded positive biomass fraction estimates, with the highest biomass fraction estimated to 0.00029 (i.e., 0.029%, corresponding to 346.0 kg of mackerel). However, the arithmetic mean of all ship observations resulted in −0.000012 (i.e., −0.0012%); hence no biomass. The absence of mackerel biomass estimates in samples from landing 1 assessed using the eDNA-based approach was in agreement with both visual methods (log book mackerel fraction = 0.0, bucket mackerel fraction = 0.0) (Figure 3). Small fractions of mackerel DNA were detected in samples from landing 2 and 3 in both eDNA at ship and eDNA at factory samples, allowing total weight fractions to be estimated using model 1. In both landings, the total mackerel fractions estimated using eDNA-based analysis were
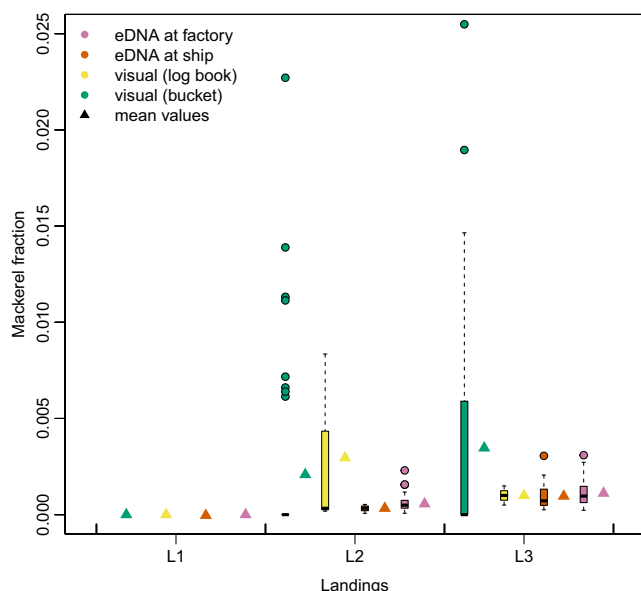
FIGURE 3 Box and whisker plots showing the estimated mackerel biomass per method for landings 1–3. Box and whisker plots show the median, 1st quantile and 3rd quantile as a box and whiskers (1.5 times the interquartile range, i.e., difference between 3rd quantile and 1st quantile, above and below the 3rd quantile and 1st quantile, respectively) display as dashed lines; the open circles show outliers. The results from the eDNA-based method are shown as "eDNA at ship," and "eDNA at factory." The visual methods (visual (log book) and visual (bucket)) are both estimated using the bucket method applied at different time points during the industrial fisheries. The visual (log book) data consists of fractions assessed per haul (each catch within the landing consisted of three to five hauls). The triangles in the graph show the mean fractions recorded per method per landing. The mean fraction is converted to the total mackerel biomass (Table 3), which subsequentially would be recorded to authorities

TABLE 3 Overview of the estimates of the total mackerel biomass in each of the landings analyzed following the methods

Method	Landing 1	Landing 2	Landing 3
eDNA at factory	0 kg	526.5 kg	1001.3 kg
eDNA at ship	0 kg	300.9 kg	866.0 kg
Logbook	0 kg	950 kg	800 kg
Bucket	0 kg	1899.7 kg	3067.1 kg

Note: This estimate is based on the arithmetic mean of all subsamples collected per method per landing. The true mackerel biomass in each landing is not known.

lower from the estimates derived from the visual (bucket) method, however to a large extent aligned with the visual (log book) estimates derived for the same catch (Table 3). The arithmetic mean of fractions estimated from all eDNA at ship and eDNA at factory samples was 0.00032 (i.e., 0.032%) and 0.00056 (i.e., 0.056%) for landing 2 and 0.00096 (i.e., 0.096%) and 0.00111 (i.e., 0.111%) for landing 3 (Figure 3 and Table 3). This corresponded to total mackerel biomass of 300.9 kg and 526.5 kg in landing 2, based on, respectively, eDNA

at ship and eDNA at factory, and to 866.0 kg and 1001.3 kg of mackerel in landing 3 based on, respectively, eDNA at ship and eDNA at factory (Figure 3 and Table 3). The mean mackerel fraction estimated using the visual (bucket) method was larger for both landings with 0.002 (i.e., 0. 2%) for landing 2 and 0.0034 (i.e., 0.034%) in landing 3, which corresponds to 1899.7 kg and 3067.1 kg mackerel, respectively (Figure 3 and Table 3) (see also Supporting Information 10). In comparison, mackerel bycatch weights reported by the visual (log book) method for landing 2 and landing 3 were 950 kg and 800 kg (that would correspond to a fraction of 0.001 (i.e., 0.1%) and 0.0009 (i.e., 0.09%) for landing 2 and 3). The results from the visual (log book) method thus either overestimated the mackerel fraction or aligned with the data from the eDNA-based methods (Figure 3 and Table 3).

3.4 | Assessing variability within catch with the different methods

Additional insights emerged when analyzing results for the different tank, haul, and factory discharge periods of landing 3 (Figure 4). Thus, when discharging haul A, both at the beginning and at the end of the discharge, (0.00045 [i.e., 0.045%] mackerel fraction indicated from visual [logbook] data), all methods resulted in relatively low mackerel estimates (Figure 4). In contrast, bycatch estimates increased in haul B and C (0.00149 (i.e., 0.149%) and 0.00099 (i.e., 0.099%) from the visual (logbook) data respectively) discharged in the middle of the discharging process (Figure 4). Throughout the discharge, process we observe continuous and relatively low mackerel fractions in all eDNA at factory samples, hence we observed smooth transitions between discharging of different tanks consisting of different hauls (Figure 4). On the contrary, the visual (bucket) method yielded highly fluctuating (from 0 to 0.025) pattern in fractions throughout the discharge. Both visual and eDNA methods indicated an increase in the mackerel fraction when 300–500 tons of the total catch (in total 902.1 tons) was discharged to the factory (Figure 4). The two eDNA estimates (ship and factory) indicated the same trend in the mackerel fraction within the catch. However, the integrated estimate (arithmetic mean) for the total catch differed between eDNA at ship versus eDNA at factory, coming out at 0.00096 (i.e., 0.096%) versus 0.00111 (i.e., 0.111%), translating into 866.0 kg or 1001.3 kg of mackerel (Figure 3).

3.5 | The effect of mixing of water on the eDNA-based estimates

The distribution of the individual hauls into different holding tanks on board influenced the eDNA-based estimates. Only eDNA at factory samples were influenced by the sequential mixing of the water from different holding tanks, as a result of continued re-usage of the blood water for the transport of fish from ship to factory. Accounting for mixing of water further increased the similarity of the two eDNA-based estimates. Following the model, the estimated
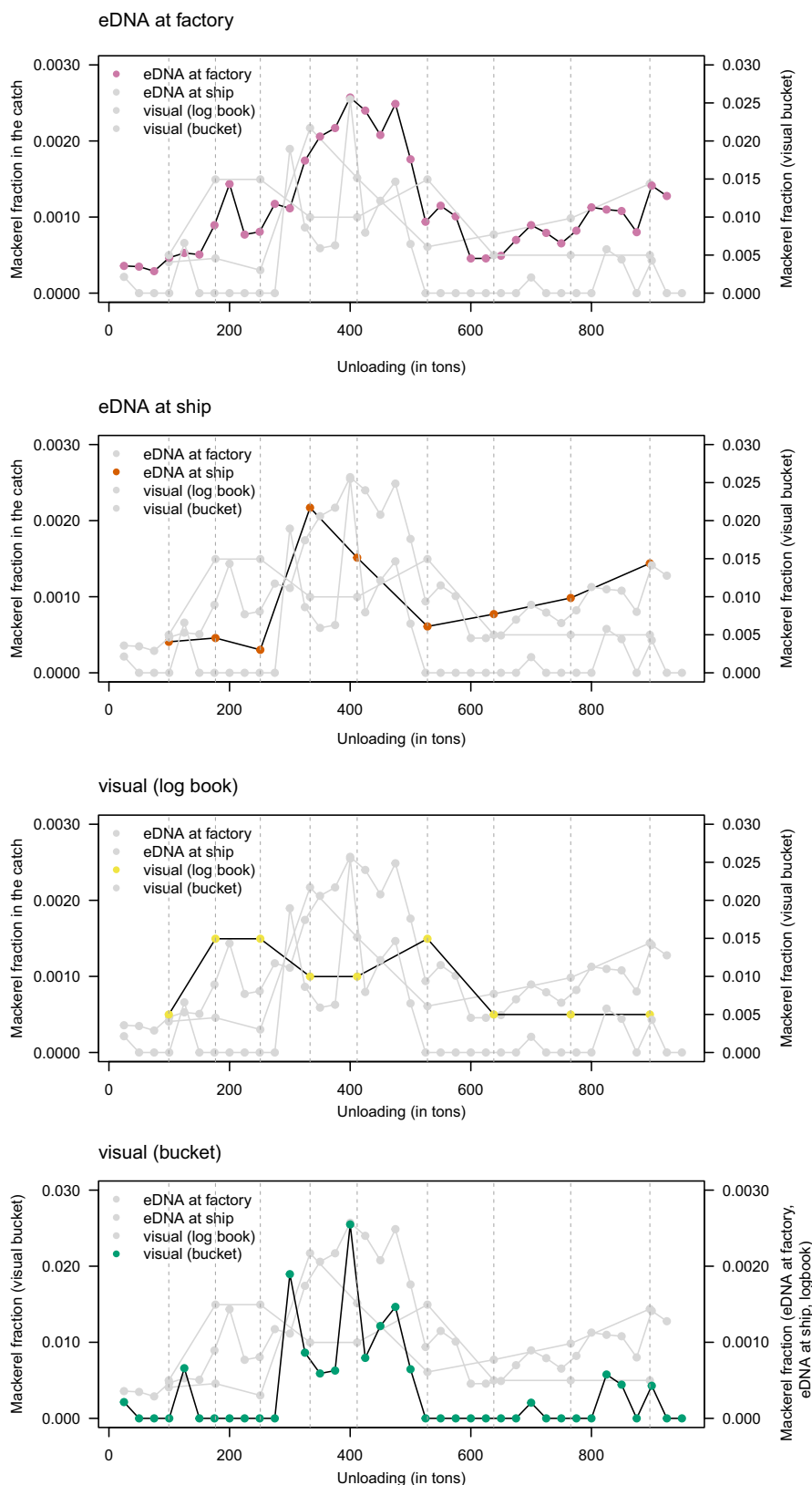
FIGURE 4 Variation in estimated mackerel fractions across landing 3 based on the different methods used: "eDNA at factory," "eDNA at ship," "visual (log book)," "visual (bucket)." The y-axis scale of the eDNA-based results from ship factory is different from the scale used for the visual (log book and bucket) methods. The difference is because the eDNA-based methods provide a continuous, relative low mackerel fraction in all samples. On the contrary, the visual methods yield highly fluctuating (from 0 to 0.025) fractions. The eDNA-based results (eDNA at factory and eDNA at ship) are shown as means of the three replicates collected at each sampling point. For each method, the sampling points are connected using solid black line to ease the visualization. The vertical, dashed lines indicate a change in discharge of fish from different tanks onboard the vessel (1–10), filled with fish from one of 3 hauls. "A," "B," and "C" each haul has potentially different bycatch fraction. According to the visual (logbook) data haul a has a mackerel fraction of 0.00045, haul B 0.00149, and haul C 0.00099

fraction of mackerel from the eDNA at ship samples in the total catch was 0.00085 (i.e., 0.085%) while for the eDNA at factory samples the estimates were, respectively, 0.0099 (i.e., 0.099%), 0.0010 (i.e., 0.10%), and 0.0009 (i.e., 0.09%) in the three different mixing scenarios tested ("no mixing," "100 T-mixing," and "full-mixing")

(Figure 5, Supplementary Information 13). This corresponded to a total of 804.0 kg of mackerel in the total catch estimated using eDNA at ship, and to 934.294 kg, 950.686 kg, and 849.343 kg of mackerel estimated using eDNA at factory (respectively, "no mixing," "100 T-mixing," and "full-mixing"). Under the hypothesis that
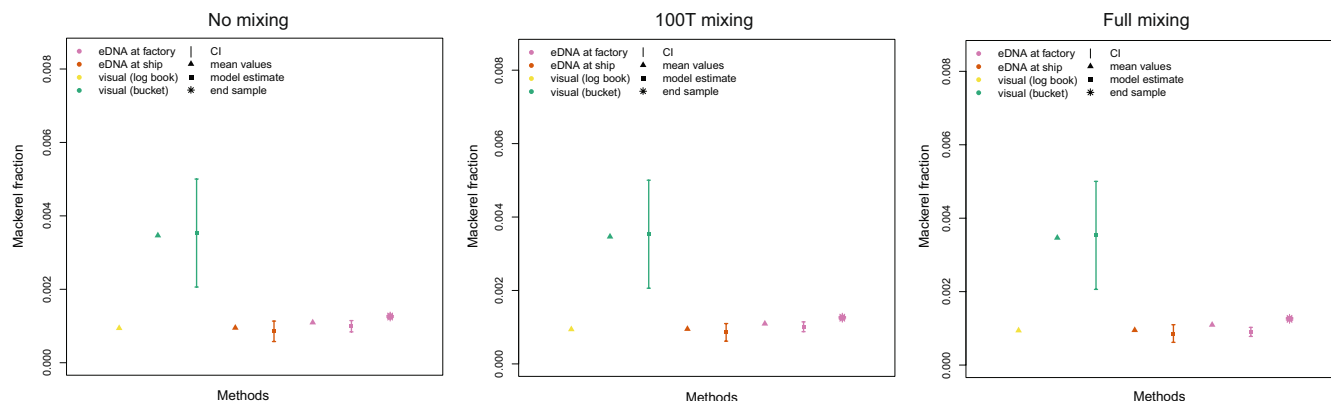
FIGURE 5 Sequential effect of blood water re-usage during discharge of the catch. The effect of re-usage of the blood water largely affects the eDNA at factory derived samples in the total mackerel estimation (the model estimate). In the "no mixing" scenario, no re-usage of the water is taken into account, thus the total mackerel estimate (the model estimate) is an arithmetic mean of eDNA at factory measurements regardless of succession point in discharge. "100 T mixing" assumes that when changing from one haul to the other during discharge, blood water from the first 100 T represents a mixture of mackerel eDNA fractions of both hauls. After 100 T the assumption is that the eDNA results corresponds solely to the currently unloaded haul. In "full mixing" we assume that samples taken at any point in the discharge process (so any eDNA sample collected at the factory) give an integral value of for the previous and current haul being discharged (i.e., all water was re-reused at all times). Following the idea of the "full mixing," the last eDNA at factory sample collected should have the same mackerel fraction as the model estimate derived from all eDNA at factory samples

the entire blood water was mixed thoroughly during the discharge ('full-mixing'), the last sample taken from the blood water at the factory should reflect the integrated signal of the total mackerel weight in the whole catch. However, according to the last eDNA sample collected at the factory 1136.646 ± 27 kg mackerel were in the catch (mean fraction estimate of 0.00126 ± 0.00003 i.e. 0.126% ± 0.003%) (Figure 5). This weight estimate was different from the factory-based estimate of 849.343 kg that takes into account "full mixing."

4 | DISCUSSION

A tangible, large scale application of eDNA for fisheries purposes, like the monitoring of catch composition, was for a long time a scientific vision (Gilbey et al., 2021; Hansen, Farrant, et al., 2020; Rourke et al., 2021; Russo et al., 2021). Turning possibility into reality, we highlight how quantification of bycatch using eDNA can be achieved. In this study, we undertook experimental work to establish an eDNA-to-biomass model, which subsequently was used to estimate the weight of bycatch using actual fisheries process water from three landings. The fractions/weights of mackerel estimated with DNA analysis were comparable to routinely used visual-based estimation metrics, moreover the eDNA-based method stood out in precision of the estimates. Still, there appear to be some systematic differences related to the accuracy of the eDNA-based and the precision of the visual methods, which we discuss below. eDNA-based bycatch estimates conducted for the same catch at the ships and at the factory showed sufficient similarity and robustness to reject potentially confounding factors relating to the distribution of different fishing hauls within the total landing. In contrast, the same confounding factors did apparently affect the visual-based estimates, where subsampling the catch onboard the vessel and at the factories

returned highly divergent estimates. All in all, eDNA-based assessments, like the here presented bycatch estimation, have realistic prospects to be applied for monitoring activities within fisheries science.

4.1 | Mackerel-herring weight to eDNA relationship

We found weight fractions and DNA fractions from mock samples to correlate strongly. However, mackerel DNA fractions were consistently higher than the expected fractions based on weight. Because of the stable abiotic conditions in the experiment, we expect biotic factors to be the main driver of the difference observed. Shedding is known to vary between species based on external features such as body shape (Andruszkiewicz Allan et al., 2021; Wood et al., 2020) and size (Yates et al., 2021). However, since the two species were in general similar in size and shape, other biological factors, such as the mtDNA content in cells and the type and rate of material shed into the environment were considered to be the primary drivers of the differences (Hansen, Farrant, et al., 2020; Sassoubre et al., 2016). Herring and mackerel belong to distinct phylogenetic families (mackerel: Scombridae, herring: Clupeidae). Mackerel are swift, active predators related to tuna-like species and their high needs for energy could be responsible for a higher amount of mitochondria, and thus higher mtDNA content in cells shed from mackerel, identified using the qPCR approach. From studies on Pacific Chub Mackerel and Pacific Clupeid species, it was observed that mackerel tend to release slime in the water, in contrast to Clupeids, which shed scales (Sassoubre et al., 2016). Thus, the difference in the material and cell content shed into the environment could also be responsible for the observed higher mackerel DNA fraction. However, little specific

information is available on these biotic differences between species and their effect on the qPCR results. Thus, for now, we only speculate that a combination of factors could be responsible for discrepancies between weight and eDNA fractions observed.

The DNA-shedding experiment conducted onboard the ship, using thawed mackerel yielded slightly lower mackerel DNA fractions than the laboratory experiment on fresh fish. Because the results were significantly different, one would need to account for this in using the appropriate model, in case freezing of the fish would occur during fisheries. During fisheries operations, blood water is usually cooled down close to the freezing point of the water to maintain a good quality of the fish (−1 to −1.7°C), but the freezing of fish is prevented (Sampels, 2014). The magnitude of difference measured between fresh and thawed fish even though significant, was very small. Thus, if unequal cooling would be observed at all, the overall effect of it on the measured DNA fractions of bycatch would be of little impact to the end estimates.

4.2 | Estimating weight of mackerel bycatch in three fisheries landings

The eDNA tool for bycatch estimation worked equally well for the quantitative detection of species as the visual methods applied. Some of the eDNA at ship and eDNA at factory samples from landing 1 had very low levels of mackerel DNA, with quantities too low to be quantifiable using our species-specific PCR setup. In these samples, the measured copy numbers were too low to result in estimates of mackerel biomass in the modeling approach, hence returning the result that the catch did not contain mackerel bycatch. The low levels of mackerel DNA detected were possibly contamination from previous catches. Fishing vessels pump new seawater into their holding tanks during each sail-out, before fishing. However, for as long as vessels fish for the same species, the holding tanks are only rinsed with fresh seawater after each landing, and are not thoroughly cleaned with detergents. Thus, DNA remains from any previous bycatch can be carried over to the next catch likely in such small quantities that it has little to minimal effect on eDNA-based bycatch biomass estimates for subsequent catches. Overall, this illustrates the robustness of the DNA-based method toward contamination from fishing operations and likely also from natural contamination from the seawater used for holding the fish, as well as from potential contamination from stomach content (Russo et al., 2021). The underlying cause is that the fresh DNA from the catch is so much in excess compared to potential sources of contamination.

For landings 2 and 3, where nonzero mackerel fractions were estimated from the eDNA-based approach, the estimates showed comparable fractions to visual (log book) estimates, and similar but consistently lower fractions compared to estimates from the visual (bucket) method. The estimation of catch fractions from the eDNA-based method is dependent on the model prediction following the DNA shedding experiments. One limitation of our experimental setup was the range of tested catch-bycatch fractions, with the lowest tested mackerel fraction of 0.025 based on weight. In the analyzed landings, mackerel bycatch hardly reached 0.01 of the total weight. Therefore, to predict mackerel weight from the eDNA-based fractions below 0.025 we used the extrapolation of the modeled experimental outcome. As with any predictions made outside the range of empirically gathered data, interpretations need to be made cautiously. Even though it was unrealistic to prepare mock samples in the composition needed (i.e., for a 0.001 fraction we would need to mix 50 g mackerel with 49.95 kg of herring), we expect the model 1 to robustly convert DNA fractions to weight fractions outside the range investigated, as the low range mackerel DNA fractions (0.025–0.05) showed a good fit to the model.

The difference in total estimates of mackerel fraction between the eDNA-based and the visual (bucket) method can also be due to the limitations of the latter. The bucket provides an accurate, however, less precise, estimation of catch composition. The uncertainty of the estimates provided using the bucket method is directly related to the number of subsamples collected (with a higher frequency of sampling, chances of finding bycatch are higher) (Fiskeristyrelsen, 2021). Following the methods description, the bycatch estimates derived from the visual methods should be within ±10%, that is, for a catch with 100 kg of mackerel, the estimates is somewhere between 90 and 110 kg (Fiskeristyrelsen, 2021). For landing 3 this variation corresponds to a difference in estimated bycatch of 3067.1 kg ± 306.7 kg. On top, the difference between the two visual assessments is considerably higher (bucket method 3067 kg, log book 800 kg) then the allowed 10% margin in difference between the two assessments following the Council Regulation (EC) No 1224/2009 (European Union, 2009). The high uncertainty of the visual assessments is often the source of the conflict between the fisherman and control authorities.

4.3 | Assessing variability within catch with the different methods

Fisheries catches typically consist of discrete hauls, which can vary in species composition. For fisheries management, information about variation among individual hauls is unimportant, and thus only estimates of species quantities from total catches are reported in the logbook system. However, to assess the precision of the different methods used for bycatch estimation it is necessary to investigate which method can reliably reflect species distribution within and between hauls, regardless of whether the species distribution within the catch is uniform, random, or clumped. The eDNA-based method provides an integrated signal of the total catch and shows high sensitivity and precision in estimates regardless of the distribution of bycatch. At the same time, it allows studying very subtle differences in the catch composition from different hauls and tanks, as opposed to the bucket method, that only coarsely reflects the variation in bycatch distribution. The reason for the difference is that the eDNA-based method is a continuous measurement of the mackerel fraction, in contrast to the bucket method that measures whole fish, which as

such appear at random in the buckets (Fiskeristyrelsen, 2021). Even though the bucket method could be accurate, the high uncertainty of the measurements (10%) lowers the precision and thus limits the reliability and replicability of the method to the same catch. For instance, changing the sequence of emptying the holding tanks could very likely result in a very different bycatch estimate when using the bucket method, in contrast to the eDNA-based method. The aspect of reliability of estimates is important, as ideally, the estimates of the bycatch fraction should be within a 10% margin, following the legislation on the allowed derivations of visual estimates (log book and bucket) (Article 14(3), European Union, 2009). Following this, we showed that the visual methods, logbook and bucket, are not in accordance to the legislation, because in both landings (landing 2 and landing 3) mackerel biomass estimates differ more than 10%. Alongside this, we show that the eDNA-based approach performed on the ship and at the factory for the same catch result in estimates following the margin of 10% to one another. In the case of landing 3, both eDNA-based estimates are accordance to visual log book, hence align with the 10% margin defined in the legislation (Article 14(3), European Union, 2009). Since the true weight of mackerel is unknown, it is impossible to tell if the violation of the legislation between the two visual methods is because of human error or the error of the methodological approach. However, the precision of the eDNA-based method gives enough support to believe in the methods robustness. It is of central importance that if a new method is implemented, it would provide robust estimates within the scope of the legal regulation of fisheries activities that ensures a sustainable usage of maritime resources.

4.4 | The effect of mixing of water on the eDNA-based estimates

The goal of this study was to evaluate if an eDNA-based tool can be more reliable in bycatch estimation than the visual assessments used currently. We observed deviations between eDNA at ship and eDNA at factory assessed mackerel bycatch estimates. These discrepancies, however, are of a much smaller magnitude than the ones observed between visual assessments, that is, logbook and bucket method. The differences between the eDNA-based approaches can be the result of the restricted potential for true replication of the ship-derived samples compared to the factory-derived samples. Ship-derived eDNA samples were collected only at the surface of each tank as deeper parts of the tanks cannot be sampled, once filled with the catch. The differences observed between the ship and factory eDNA-based estimates can be evaluated when trying to account for different scenarios for mixing of blood water during the discharging. The different mixing scenarios produce comparable results, with more similar ship and factory estimates with increasing mixing. Thus, the most likely scenario is that during landing 3 the total blood water content was mixed during discharge. Following the hypothesis that the full mixing is occurring during the

discharge, the blood water sample collected at the end of the discharge could in principle, provide an integrated signal of the overall catch composition. Reducing the eDNA-based assessment to a single last sample would potentially speed up the bycatch assessment analysis, while simultaneously decreasing the cost and increasing time efficiency. However, this hypothesis could not be directly supported with the note of caution that we do not know the "true" bycatch rate and the results therefore should be interpreted with precaution. The eDNA-based fraction of mackerel in the last sample is higher than the eDNA at ship and the eDNA at factory estimates (eDNA at ship: 804.0 kg, eDNA at factory: 849.343 kg, last sample estimate: 1136.646 kg). Although not big, the differences still lead to somewhat substantial differences in the estimated bycatch biomass. The difference could be caused by a random effect during sampling (i.e., minute variations in the distribution of DNA) or a process of accumulation of mackerel DNA over time. With time, fewer fish remain in the water, hence the fractions in the fisheries process water reflect the DNA-decay scenario more. In the DNA-decay study we observed higher fractions of mackerel DNA compared to the DNA-shedding experiment for the same weight fraction of the species. Thus, the difference between the overall estimate and the estimate from the end-sample might be due to the change in the treatment (DNA-shedding or DNA-decay) to which the samples are subjected. The discrepancy between ship and factory eDNA-based estimates visualizes that there is a need for proper understanding of the whole process from individual hauls, distributions in tanks, and the discharge process that can result in discrepancies between samples collected at different points in the pipeline. Thus, specific Standard Operating Procedures (SOPs) need to be in place to eliminate discrepancies.

4.5 | Implementation scope

Overall, it is likely that the eDNA-based approach is more time and cost-effective, and more precise and consistent in estimating catch fractions than the currently used methods. Building on the experience from this study, the implementation of the method for routine measurements appears to be achievable within a relatively short period. However, some uncertainties need to be addressed and controlled. First, an optimal sampling scheme needs to be designed that takes into account the full process of mixing fish and water from individual fish hauls to factory discharge. It is of paramount importance that sampling design and sample comparisons are operating on a fully transparent foundation. Secondly, the eDNA to biomass translation needs to be addressed further, including exploration of very low weight fractions, which is the reality for some fisheries and for explaining the difference between the approaches in measuring the DNA content in samples. Additional experiments should also ideally encompass the full enumeration (or very extensive subsampling) of large catches allowing evaluation of the accuracy of the method, which was not

14 of 16 | **WILEY**—Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences
Open Access

URBAN ET AL.

feasible for this pilot study. Implementation of the method across pelagic fisheries in Europe would allow a level playing field for pelagic fishermen in Europe and a common framework for control and enforcement. At the same time, the eDNA-based method, if applied for regular bycatch monitoring, would contribute to faster, cheaper, and highly reproducible bycatch estimates ultimately benefitting stock assessment and reducing conflict over bycatch estimation between fisherman and control agencies.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data from the experimental work is submitted as part of the supporting information (Supporting Information 11). The data from the eDNA-based estimation of bycatch from the fisheries is available under https://github.com/12PU/DNAMIX_pelagic_fisheries.git. Raw data from the logbook and the bucket method cannot be made public due to confidentiality agreements with the private enterprises.

ORCID

Paulina Urban https://orcid.org/0000-0002-3823-596X
Dorte Bekkevold https://orcid.org/0000-0002-5297-032X
Magnus Wulff Jacobsen https://orcid.org/0000-0002-7701-7025
Anders Nielsen https://orcid.org/0000-0001-9683-9262
Einar Eg Nielsen https://orcid.org/0000-0002-7009-9814

REFERENCES

Andruszkiewicz Allan, E., Gordon Zhang, W., Lavery, A. C., & Govindarajan, A. F. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA*, 3, 492–514. https://doi.org/10.1002/edn3.141

Arnason, R., Hannesson, R., & Schrank, W. E. (2000). Costs of fisheries management: The cases of Iceland, Norway and Newfoundland. *Marine Policy*, 24(3), 233–243. https://doi.org/10.1016/S0308-597X(99)00029-9

Bakker, J., Wangensteen, O. S., Baillie, C., Buddo, D., Chapman, D. D., Gallagher, A. J., Guttridge, T. L., Hertler, H., & Mariani, S. (2019). Biodiversity assessment of tropical shelf eukaryotic communities via pelagic eDNA metabarcoding. *Ecology and Evolution*, 9(24), 14341–14355.

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, 29(7), 2089–2121. https://doi.org/10.1007/S10531-020-01980-0/FIGURES/2

Booth, H., Arlidge, W. N. S., Squires, D., & Milner-Gulland, E. J. (2021). Bycatch levies could reconcile trade-offs between blue growth and biodiversity conservation. *Nature Ecology & Evolution*, 5(6), 715–725. https://doi.org/10.1038/s41559-021-01444-w

Boussarie, G., Bakker, J., Wangensteen, O. S., Mariani, S., Bonnin, L., Juhel, J.-B., Kiszka, J. J., Kulbicki, M., Manel, S., Robbins, W. D., Vigliola, L., & Mouillot, D. (2018). Environmental DNA illuminates the dark diversity of sharks. *Science Advances*, 4(5), eaap9661.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378–400.

Buxton, A. S., Groombridge, J. J., & Griffiths, R. A. (2018). Seasonal variation in environmental DNA detection in sediment and water samples. *PLoS ONE*, 13(1), e0191737. https://doi.org/10.1371/JOURNAL.PONE.0191737

Clare, E. L., Economou, C. K., Bennett, F. J., Dyer, C. E., Adams, K., McRobie, B., Drinkwater, R., & Littlefair, J. E. (2022). Measuring biodiversity from DNA in the air. *Current Biology*, 32(3), 693–700. e5. https://doi.org/10.1016/J.CUB.2021.11.064

Clegg, T. L., Steven, J. K., Geir, B., & Kjell, N. (2021). Applying global best practices for estimating unreported catches in Norwegian fisheries under a discard ban. *Reviews in Fish Biology and Fisheries*, 31(1), 1–23. https://doi.org/10.1007/s11160-020-09624-w

Cusa, M., St John Glew, K., Trueman, C., Mariani, S., Buckley, L., Neat, F., & Longo, C. (2022). A future for seafood point-of-origin testing using DNA and stable isotope signatures. *Reviews in Fish Biology and Fisheries*, 32, 597–621. https://doi.org/10.1007/s11160-021-09680-w

del Valle, J. M., & Aguilera, J. M. (1991). Physicochemical characterisation of raw fish and stickwater from fish meal production. *Journal of the Science of Food and Agriculture*, 54(3), 429–441. https://doi.org/10.1002/JSFA.2740540314/FORMAT/PDF

Dickey-Collas, M., Pastoors, M. A., Van, O. A., Dickey, K., Dickey-Collas, M., Pastoors, M. A., & Van Keeken, O. A. (2007). Precisely wrong or vaguely right: Simulations of noisy discard data and trends in fishing effort being included in the stock assessment of North Sea plaice. *ICES Journal of Marine Science*, 64(9), 1641–1649. https://doi.org/10.1093/ICESJMS/FSM155

European Union. (2009). Council Regulation (EC) No 1224/2009 of 20 November 2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy, amending Regulations (EC) No 847/96, (EC) No 2371/2002, (EC) No 811/2004, (EC) No 768/2.

Fediajevaite, J., Priestley, V., Arnold, R., & Savolainen, V. (2021). Meta-analysis shows that environmental DNA outperforms traditional

surveys, but warrants better reporting standards. *Ecology and Evolution*, 11(9), 4803–4815. https://doi.org/10.1002/ECE3.7382

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4, 423–425. https://doi.org/10.1098/rsbl.2008.0118

Fiskeristyrelsen. (2021). Fiskeristyrelsens prøvetagningsplan for vejning af fiskevarer, der landes usorteret til industriformål (Case nr.:19–4000-000459).

Gilbey, J., Carvalho, G., Castilho, R., Coscia, I., Coulson, M. W., Dahle, G., Derycke, S., Francisco, S. M., Helyar, S. J., Johansen, T., Junge, C., Layton, K. K. S., Martinsohn, J., Matejusova, I., Robalo, J. I., Rodríguez-Ezpeleta, N., Silva, G., Strammerj, I., Vasemägi, A., … Volckaert, F. A. M. (2021). Life in a drop: Sampling environmental DNA for marine fishery management and ecosystem monitoring. *Marine Policy*, 124, 104331. https://doi.org/10.1016/J.MARPOL.2020.104331

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (Potamopyrgus antipodarum). *Freshwater Science*, 32(3), 792–800. https://doi.org/10.1899/13-046.1/ASSET/IMAGES/LARGE/I2161-9565-32-3-792-F02.JPEG

Gray, C., & Kennelly, S. (2018). Bycatches of endangered, threatened and protected species in marine fisheries. *Reviews in Fish Biology and Fisheries*, 28, 521–541. https://doi.org/10.1007/s11160-018-9520-7

Hansen, B. K., Bekkevold, D., Clausen, L. W., & Nielsen, E. E. (2018). The sceptical optimist: Challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish and Fisheries*, 19(5), 751–768.

Hansen, B. K., Farrant, G. K., Ogden, R., Humble, E., Olafsdottir, G., Bekkevold, D., Knudsen, S. W., Møller, P. R., & Nielsen, E. E. (2020). From DNA to biomass: Opportunities and challenges in species quantification of bulk fisheries products. *ICES Journal of Marine Science*, 77(7–8), 2557–2566. https://doi.org/10.1093/ICESJMS/FSAA115

Hansen, B. K., Jacobsen, M. W., Middelboe, A. L., Preston, C. M., Marin, R., Bekkevold, D., Knudsen, S. W., Møller, P. R., & Nielsen, E. E. (2020). Remote, autonomous real-time monitoring of environmental DNA from commercial fish. *Scientific Reports*, 10(1), 13272. https://doi.org/10.1038/S41598-020-70206-8

Helyar, S. J., Lloyd, H. A. D., De Bruyn, M., Leake, J., Bennett, N., & Carvalho, G. R. (2014). Fish Product Mislabelling: Failings of Traceability in the Production Chain and Implications for Illegal, Unreported and Unregulated (IUU) Fishing. *PLOS ONE*, 9(6), e98691. https://doi.org/10.1371/JOURNAL.PONE.0098691

Hongo, Y., Nishijima, S., Kanamori, Y., Sawayama, S., Yokouchi, K., Kanda, N., Oori, S., Uto, Y., Ishii, M., Mita, H., Akimoto, S., Kusano, A., Okabe, K., Yamazaki, T., Fukuda, N., Onitsuka, T., Minagawa, M., Okamura, H., Niwa, K., … Kurogi, H. (2021). Fish environmental DNA in TokyoBay: A feasibility study on the availability of environmental DNA for fisheries. *Regional Studies in Marine Science*, 47, 101950. https://doi.org/10.1016/J.RSMA.2021.101950

ICES. (2017). Stock Annex: Herring (Clupea harengus) in Subarea 4 and divisions 3.a and 7.d, autumn spawners (North Sea, Skagerrak and Kattegat, eastern English Channel). https://doi.org/10.17895/ices.pub.9427

Jacobsen, M. W., Hansen, B. K., & Nielsen, E. E. (2019). Possible uses of genetic methods in fisheries under the EU Landing Obligation. *In The European Landing Obligation*, 407–427.

Knudsen, S. W., Ebert, R. B., Hesselsøe, M., Kuntke, F., Hassingboe, J., Mortensen, P. B., Thomsen, P. F., Sigsgaard, E. E., Hansen, B. K., & Nielsen, E. E. (2019). Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, 510, 31–45.

Knudsen, S. W., Hesselsøe, M., Thaulow, J., Agersnap, S., Hansen, B. K., Jacobsen, M. W., Bekkevold, D., Jensen, S. K. S., Møller, P. R., &

Andersen, J. H. (2022). Monitoring of environmental DNA from nonindigenous species of algae, dinoflagellates and animals in the North EastAtlantic. *Science of the Total Environment*, 821, 153093. https://doi.org/10.1016/J.SCITOTENV.2022.153093

Kristensen, K., Nielsen, A., Berg, C. W., Skaug, H., & Bell, B. M. (2016). *Journal of Statistical Software TMB*. AutomaticDifferentiation and Laplace Approximation. https://doi.org/10.18637/jss.v070.i05

Link, J. S., Brodziak, J. K. T., Edwards, S. F., Overholtz, W. J., Mountain, D., Jossi, J. W., Smith, T. D., & Fogarty, M. J. (2002). Marine ecosystem assessment in a fisheries management context. *CanadianJournal of Fisheries and Aquatic Sciences*, 59(9), 1429–1440.

Lugg, W. H., Griffiths, J., van Rooyen, A. R., Weeks, A. R., & Tingley, R. (2018). Optimal survey designs for environmental DNAsampling. *Methods in Ecology and Evolution*, 9(4), 1049–1059. https://doi.org/10.1111/2041-210X.12951

Merkes, C., Klymus, K., Allison, M., Goldberg, C., Helbin, C., Hunter, M., Jackson, C., Lance, R., Mangan, A., Monroe, E., Piagio, A., Stokdyk, J., Wilson, C., & Richter, C. (2019). *Generic qPCR Limit ofDetection (LOD)/Limit of Quantification (LOQ) calculator. Data*. U.S.Geological Survey data release. https://doi.org/10.5066/P9AKHU1R

Nøttestad, L., Utne, K. R., Óskarsson, G. J., Jónsson, S. P., Jacobsen, J. A., Tangen, Ø., Anthonypillai, V., Aanes, S., Vølstad, J. H., Bernasconi, M., Debes, H., Smith, L., Sveinbjörnsson, S., Holst, J. C., Jansen, T., & Slotte, A. (2016). Quantifying changes in abundance, biomass, and spatial distribution ofNortheast Atlantic mackerel (Scomber scombrus) in the Nordic seas from 2007 to 2014. *ICES Journal of Marine Science*, 73(2), 359–373. https://doi.org/10.1093/ICESJMS/FSV218

Richards, J. L., Sheng, V., Chung, H. W. Y., Liu, M., Tsang, R. H. H., McIlroy, S. E., & Baker, D. (2022). Development of an eDNA-basedsurvey method for urban fish markets. *Methods in Ecology and Evolution*, 13(7), 1568–1580. https://doi.org/10.1111/2041-210X.13842

Rodríguez-Ezpeleta, N., Morissette, O., Bean, C. W., Manu, S., Banerjee, P., Lacoursiere-Roussel, A., Beng, K. C., Alter, S. E., Roger, F., Holman, L. E., Stewart, K. A., Monaghan, M. T., Mauvisseau, Q., Mirimin, L., Wangensteen, O. S., Antognazza, C. M., Helyar, S. J., de Boer, H., Monchamp, M. E., … Deiner, K. (2021). Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: Comment on "Environmental DNA: What's behind the term?" by Pawlowski et al. The total pool of DNA isolated from the environment. *Molecular Ecology*, 30, 4601–4605. https://doi.org/10.1111/mec.15942

Roger, F., Ghanavi, H. R., Danielsson, N., Wahlberg, N., Löndahl, J., Pettersson, L. B., Andersson, G. K. S., Olén, N. B., & Clough, Y. (2022). Airborne environmental DNA metabarcoding for the monitoring of terrestrial insects—A proof of concept from the field. *Environmental DNA*, 4(4), 790–807. https://doi.org/10.1002/EDN3.290

Roungchun, J. B., Tabb, A. M., & Hellberg, R. S. (2022). Identification of tuna species in raw and processed products using DNA mini-barcoding of the mitochondrial control region. *Food Control*, 134, 108752. https://doi.org/10.1016/J.FOODCONT.2021.108752

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Walburn, J. W., & Furlan, E. M. (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, 4, 9–33. https://doi.org/10.1002/EDN3.185

Rudd, M. B., & Branch, T. A. (2016). Does unreported catch lead to overfishing? *Fish and Fisheries*, 18, 313–323. https://doi.org/10.1111/faf.12181

Russo, T., Maiello, G., Talarico, L., Baillie, C., Colosimo, G., D'Andrea, L., Di Maio, F., Fiorentino, F., Franceschini, S., Garofalo, G., Scannella, D., Cataudella, S., & Mariani, S. (2021). All is fish that comes to the net: Metabarcoding for rapid fisheries catch assessment. *Ecological Applications*, 31(2), e02273. https://doi.org/10.1002/EAP.2273

16 of 16 | **WILEY** Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences
Open Access

URBAN ET AL.

Ryan, E., Bateman, P., Fernandes, K., van der Heyde, M., & Nevill, P. (2022). eDNA metabarcoding of log hollow sediments and soils highlights the importance of substrate type, frequency of sampling and animal size, for vertebrate species detection. *Environmental DNA*, 4(4), 940–953. https://doi.org/10.1002/EDN3.306

Salter, I., Joensen, M., Kristiansen, R., Steingrund, P., & Vestergaard, P. (2019). Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. *Communications Biology*, 2(1), 1–9. https://doi.org/10.1038/s42003-019-0696-8

Sampels, S. (2014). The effects of storage and preservation technologies on the quality of fish products: a review. *Journal of Food Processing and Preservation*, 39, 1206–1215. https://doi.org/10.1111/jfpp.12337

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science and Technology*, 50, 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., & Vanmorter, S. M. (2021). Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, 78(1), 293–304. https://doi.org/10.1093/ICESJMS/FSAA225

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

Tenningen, M., Zimmermann, F., & Enberg, K. (2021). Pre-catch and discard mortality in Northeast Atlantic herring and mackerel fisheries: Consequences for stock estimates and advice. *ICES Journal of Marine Science*, 78(7), 2603–2614. https://doi.org/10.1093/icesjms/fsab135

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., & Rasmussen, M. (2012). Detection of a diverse marine fish Fauna using environmental DNA from seawater samples. *PLoS One*, 7(8), 41732. https://doi.org/10.1371/journal.pone.0041732

Thomsen, P. F., Moller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jorgensen, O. A., & Willerslev, E. (2016). Environmental DNA from seawater samples correlate with trawl catches of subarctic, Deepwater Fishes. *PLoS ONE*, 11(11), e0165252. https://doi.org/10.1371/journal.pone.0165252

Trenkel, V. M., Huse, G., MacKenzie, B. R., Alvarez, P., Arrizabalaga, H., Castonguay, M., Goñi, N., Grégoire, F., Hátún, H., Jansen, T., Jacobsen, J. A., Lehodey, P., Lutcavage, M., Mariani, P., Melvin, G. D., Neilson, J. D., Nøttestad, L., Óskarsson, G. J., Payne, M. R., ... Speirs, D. C. (2014). Comparative ecology of widely distributed pelagic fish species in the North Atlantic: Implications for modelling climate and fisheries impacts. *Progress in Oceanography*, 129, 219–243. https://doi.org/10.1016/J.POCEAN.2014.04.030

Wallis, P., & Flaaten, O. (2003). Fisheries management costs: Concepts and studies. In W. Shrank, R. Arnason, & R. Hannesso (Eds.), *The cost of fisheries management* (pp. 211–223). Ashgate Publishing.

Wood, S. A., Biessy, L., Latchford, J. L., Zaiko, A., von Ammon, U., Audrezet, F., Cristescu, M. E., & Pochon, X. (2020). Release and degradation of environmental DNA and RNA in a marine system. *Science of the Total Environment*, 704, 135314. https://doi.org/10.1016/J.SCITOTENV.2019.135314

Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Molecular Ecology*, 30(13), 3068–3082. https://doi.org/10.1111/MEC.15543

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.