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ORIGINAL ARTICLE

Environmental DNA reveals fine-scale habitat associations for sedentary and resident marine species across a coastal mosaic of soft- and hard-bottom habitats

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Abstract

Accurate knowledge on spatiotemporal distributions of marine species and their association with surrounding habitats is crucial to inform adaptive management actions responding to coastal degradation across the globe. Here, we investigate the potential use of environmental DNA (eDNA) to detect species–habitat associations in a patchy coastal area of the Baltic Sea. We directly compare species-specific qPCR analysis of eDNA with baited remote underwater video systems (BRUVS), two non-invasive methods widely used to monitor marine habitats. Four focal species (cod *Gadus morhua*, flounder *Platichthys flesus*, plaice *Pleuronectes platessa*, and goldsinny wrasse *Ctenolabrus rupestris*) were selected based on contrasting habitat associations (reef- vs. sand-associated species), as well as differential levels of mobility and residency, to investigate whether these factors affected the detection of species–habitat associations from eDNA. To this end, a species-specific qPCR assay for goldsinny wrasse is developed and made available herein. In addition, potential correlations between eDNA signals and abundance counts (MaxN) from videos were assessed. Results from Bayesian multilevel models revealed strong evidence for a sand association for sedentary flounder (98% posterior probability) and a reef association for highly resident wrasse (99% posterior probability) using eDNA, in agreement with BRUVS. However, contrary to BRUVS, eDNA sampling did not detect habitat associations for cod or plaice. We found a positive correlation between eDNA detection and MaxN for wrasse (posterior probability 95%), but not for the remaining species and explanatory power of all relationships was generally limited. Our results indicate that eDNA sampling can detect species–habitat associations on a fine spatial scale, yet this ability likely depends on the mobility and residency of the target organism, with associations for sedentary or resident species most likely to be detected. Combined sampling with conventional non-invasive methods is advised to improve detection of habitat associations for mobile and transient species, or for species with low eDNA concentrations.

KEYWORDS
Baltic Sea, Bayesian multilevel modeling, BRUVS, eDNA, goldsinny wrasse qPCR assay, method comparison, rocky reefs, species mobility

1 | INTRODUCTION

Coastal marine habitats offer numerous ecosystem services that can benefit societal well-being and support critical ecological links with surrounding environments (Barbier et al., 2011; Duarte et al., 2020; Lau et al., 2019). For example, coastal reefs act as important nursery grounds for juvenile fishes and invertebrates, while offering benefits in the form of coastal protection, fisheries, and recreational values (Barbier, 2017; Lefcheck et al., 2019). Safeguarding coastal habitats from cumulative human impacts requires adaptive management actions guided by accurate scientific information. This includes knowledge on spatiotemporal distributions of marine organisms and their association with the surrounding habitat, which provides insights into species-specific responses to environmental change and allows for effective conservation efforts (Espinoza et al., 2020). Traditional catch surveys can be destructive to the environment, costly to perform and highly selective in sampled taxa and size classes (Murphy & Jenkins, 2010). While all marine monitoring techniques are recognized to have their own intrinsic biases and limitations (Danovaro et al., 2016; Murphy & Jenkins, 2010), there is a growing demand for standardized cost-effective methods that enable low-impact assessments of species distributions across a diverse range of marine systems.

A common sampling method is the use of baited remote underwater video systems (BRUVS), offering a non-invasive assessment of aquatic environments from video recordings that can be permanently archived and shared within global repositories (Langlois et al., 2020). BRUVS are used to document a wide variety of marine organisms from the tropics to the polar regions, with well-established protocols on system design, sampling methodology and data processing (Langlois et al., 2020; Whitmarsh et al., 2017). The non-destructive nature of video sampling, with minimal disturbance to marine life and the seabed, is particularly useful for monitoring vulnerable species and habitats, for example, endangered species in protected areas (Espinoza et al., 2020) or biogenic habitats (Orfanidis et al., 2021). BRUVS sampling allows for the recording of marine community metrics, including relative species abundance, diversity indices, community composition, and behavioral aspects (e.g., predator–prey interactions and spawning events). The use of bait to attract individuals to the camera's field of view reduces between-sample variability in species counts and the risk of false negatives, thereby promoting the power of statistical inference from BRUVS data, relative to unbaited cameras (Bernard & Götz, 2012). Several limitations associated with the use of bait attractants remain important to consider, including a possible underrepresentation of herbivores in BRUVS samples and potential difficulties in discriminating between fine-scaled community patterns when attracting species from surrounding habitats. While several studies employing BRUVS have indeed reported higher piscivore abundances relative to unbaited methods (Goetze et al., 2015; Harvey et al., 2007), herbivores generally remain well represented in BRUVS samples (Harvey et al., 2007; Watson et al., 2010). In addition, the use of bait often results in better discrimination of fish assemblages (Harvey et al., 2007) and more robust species–habitat associations relative to unbaited cameras (Rhodes et al., 2020). BRUVS sampling has therefore become an established marine monitoring technique, yet sampling and analysis of the video data remain costly and time-consuming and the data quality highly depends on the taxonomic expertise of video observers.

Monitoring marine communities from environmental DNA (eDNA) has gained significant interest since the pioneering study by Thomsen et al. (2012), which showed that it is possible to detect macro-organisms from eDNA molecules in seawater. Marine organisms continuously release DNA particles into the surrounding water through scales, skin tissue, feces, metabolic waste, gametes, and carcasses (Takahara et al., 2012; Takeuchi et al., 2019; Thomsen & Willerslev, 2015). From the DNA material, species-specific detection can be achieved using real-time quantitative polymerase chain reaction (qPCR), which involves measuring amplification-dependent increases in fluorescence signals if DNA fragments from targeted species are found within the samples (Hansen et al., 2018; Heid et al., 1996). Alternatively, a DNA metabarcoding approach enables broad assessments of community structure and biodiversity in marine systems (Oka et al., 2021; Sigsgaard et al., 2020), providing a taxonomic breadth unparalleled by any single sampling method (Stat et al., 2017). Given the opportunity to rapidly obtain large sample sizes with high sensitivity for species detection, there is strong interest from a conservation and fisheries management perspective in exploring the potential for inferring fish abundance, and eventually population status, from eDNA data. Previous studies have confirmed that the amount of released DNA material correlates to both the size (Maruyama et al., 2014) and number of individuals (Lacoursière-Roussel, Rosabal, & Bernatchez, 2016) in controlled aquatic environments. While a number of studies have provided evidence of a correlation for specific natural systems and target organisms (Doi et al., 2017; Itakura et al., 2019; Lacoursière-Roussel, Côté, et al., 2016; Salter et al., 2019), the explanatory power of the relationship is generally lower relative to controlled environments (Yates et al., 2019). This difference can be attributed to the dynamic conditions inherent to aquatic environments, resulting in transportation and dilution of DNA molecules, as well as a diverse range of biotic and abiotic factors affecting DNA production and degradation (Hansen et al., 2018; Strickler et al., 2015; Yates et al., 2019). Direct comparisons with abundance metrics obtained from traditional methods can shed light on species-specific relationships, in particular if data are concurrently collected at the same sampling location. BRUVS offer an opportunity in this regard, as the video systems can be rapidly deployed at the exact location where eDNA is being sampled, while

the bait may attract individuals in the vicinity that were also captured in the eDNA signal. Comparisons between eDNA and BRUVS have so far focused on biodiversity and community assessments (Boussarie et al., 2018; Jeunen, Lamare, et al., 2020; Marques et al., 2021; Mercaldo-Allen et al., 2021; Stat et al., 2019), yet potential correlations between species-specific qPCR detection and abundances recorded from BRUVS remain, to the best of our knowledge, untested.

Currently, inferences based on BRUVS or eDNA surveys are limited by their undefined spatial resolution. In BRUVS, the soaking bait produces a bait plume that may attract individuals from considerable distances to the stationary camera system, implying that the total area being sampled is difficult to assess. Efforts have been made to estimate bait plume size based on current velocity (Heagney et al., 2007; Taylor et al., 2013), yet accurate description of the sampled area ultimately requires complex three-dimensional hydrodynamic modeling and depends on odor detection thresholds of target species (Taylor et al., 2013; Westerberg & Westerberg, 2011). Similarly, eDNA molecules are transported by ocean currents, leading to uncertainty about the origin of the DNA material and therefore the spatial explicitness of eDNA data (Roussel et al., 2015). Still, there is growing evidence that eDNA surveys can differentiate between fine-scaled patterns in community assemblages (Port et al., 2016; Staehr et al., 2022; Stat et al., 2019; West et al., 2020), even across a wide geographical extent (700 km of coastline; West et al., 2021). While such localized signals may indicate high site fidelity of eDNA to the source community (Harrison et al., 2019), variations in mobility and residency levels across marine species could affect the ability of eDNA sampling to detect species–habitat associations, which has thus far received little attention (but see Monuki et al., 2021).

Here, we investigated the potential of BRUVS and eDNA to record habitat associations for four temperate marine species on a small spatial scale (~20 km^2) in the western Baltic Sea. We selected two sand-associated and two reef-associated fish species, ranging in mobility from sedentary to mobile and in residency from highly resident to transient species, in order to test our three main hypotheses. First, we assessed whether BRUVS and eDNA detected comparable habitat associations for the four focal species, that is, higher abundance of reef fishes recorded on reefs relative to sand and vice versa for sand species (H1). Second, we tested the hypothesis that eDNA sampling yielded higher species detection rates (i.e., presence–absence signals) for all focal species due to higher sensitivity relative to BRUVS (H2). Lastly, we tested whether eDNA signals were positively correlated with relative abundance of the focal species as recorded by BRUVS (H3).

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in Sønderborg Bay within Flensborg Fjord, a Natura 2000 designated protected area shared between Denmark and Germany. The seabed of the bay consists mainly of muddy sand, with patches of fine sand along the coastline occasionally covered by glacial till ranging from gravel to boulders (Figure 1). We sampled four different field sites with depths between 6 and 7 m: Kegnæs Ende (SE) and Viemose (NW) represented soft-bottom habitats (sand sites), while Spar Es (SW) and Vesterhage (N) were characterized by hard-bottom glacial till (i.e., rocky reefs). However, the mosaic nature of the seabed in the bay implied that reef habitat was located in close proximity (i.e., at a few 100 meters distance) to sand sites and vice versa (Figure 1). Sampling was conducted from late April until late June of 2018, during which sea surface temperature (SST) increased from 6.4°C to 20.8°C in the study area. Current velocities in the bay ranged from 0 to 20 cm s^{-1} during the study period and were strongest at the more exposed field sites of Kegnæs Ende and Spar Es (Figure 1; Figure S1).

2.2 | Field sampling

Sampling focused on four relatively abundant marine fish species with documented habitat associations. Atlantic cod (*Gadus morhua*; hereafter "cod") and goldsinny wrasse (*Ctenolabrus rupestris*; hereafter "wrasse") are reef-associated fish species from the area, while European flounder (*Platichthys flesus*; hereafter "flounder") and European plaice (*Pleuronectes platessa*; hereafter "plaice") are associated with soft sediment habitats (Rhodes et al., 2020).

Water samples were collected directly from the surface water and extracted using a disposable 60-ml sterile syringe and injected into an enclosed Sterivex-GP capsule filter (0.22 µm pore size, SVGPL10RC, Millipore, CA, USA). A total of 1000 ml seawater was filtered for each sample; however, on one occasion, due to clogging, only 850 ml could be filtered. After filtration, the sterivex filters were closed using sterile luer lock caps (Cole Palmer, Vernon Hills, IL, USA), put in individual zip-locked bags and stored in a cooling box on ice before subsequently transferring them to a −20°C freezer upon return from the field. A maximum of two water samples were taken on a given sampling day, for which site selection was based on accessibility of the site (depending on weather conditions) or otherwise balanced among sites as much as possible. One field blank was taken on June 18, 2018, at 12:50 h by filtering 900 ml nuclease free water at site to control for potential DNA contamination related to the field sampling procedure. Further details on the sampling scheme are provided in the Supporting Information (Table S1).

Immediately following the eDNA water sampling, we deployed two BRUVS at the same field site. BRUVS consisted of GoPro's Hero 3 or 4 (GoPro, USA), attached to a steel pole (1 m height; 3 cm diameter) at 20 cm above the seabed and positioned firmly on a concrete tile with the camera field of view parallel to the seabed. BRUVS deployments were separated by at least 100 m to minimize the risk of double counting individuals between the two BRUVS while staying within the designated habitat type. We used chopped Atlantic herring (*Clupea harengus*) as bait, packed tightly in a mesh bait bag. The bait bag was positioned 15 cm above the seabed and attached via an 80 cm bait arm in the lower center of the field of view. Soak time

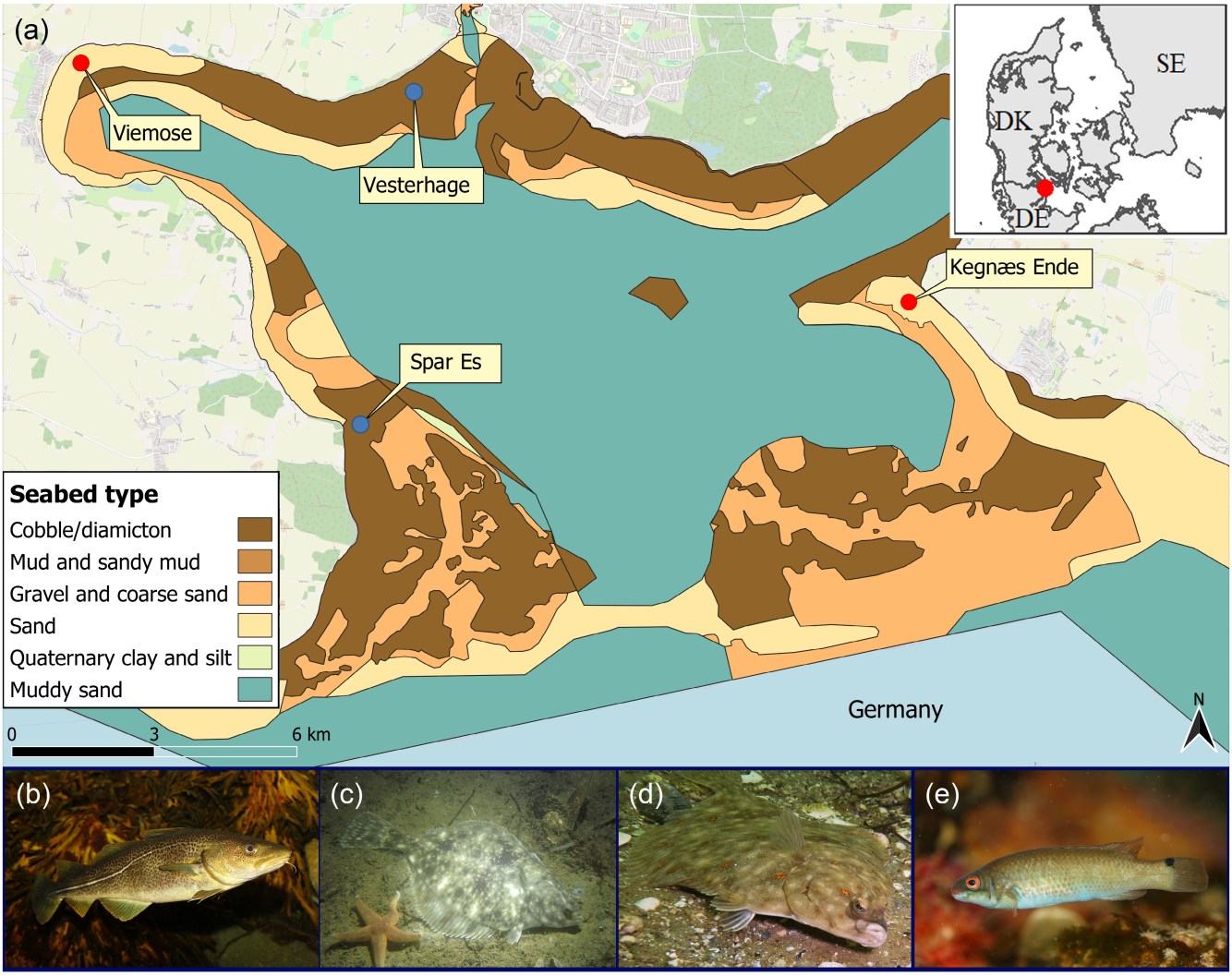

FIGURE 1 Map of the study location in the western Baltic Sea. The seabed structure and composition has gradually been shaped by glacial erosion and deposition, resulting in a mosaic of different seabed types across the bay (a). Glacial till deposits constitute the main type of hard substrate within the bay, also classified as diamicton; an unsorted mixture of sediment with particle sizes ranging from small pebbles to boulders. However, the bay is generally dominated by soft sediment, including extensive areas of fine sand and muddy sand bottom. Paired BRUVS and eDNA samples were collected from sand sites (red circles) and reef sites (blue circles), to document habitat associations for (b) Atlantic cod (*Gadus morhua*), (c) European flounder (*Platichthys flesus*), (d) European plaice (*Pleuronectes platessa*), and (e) goldsinny wrasse (*Ctenolabrus rupestris*). Background data of sediment map obtained from GEUS (Leth, 2021). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article. Photo credits: Nicolai Ulrik Hansen (b,e), Micha Baum (c) and Rita Jansen (d)

of the BRUVS varied between 1 h and 2 h 40 min depending on the camera's battery life, with a small number of BRUVS (<5) marked unsuccessful due to battery failure or loss of the bait bag. Accordingly, a total of 41 seawater samples were taken for eDNA extraction and coupled with 78 BRUVS deployments across the four different field sites (Figure 1; Table S1).

2.3 | Video analysis

We analyzed the video recordings using VLC media player (https://www.videolan.org/vlc/). The four focal species were identified and counted by trained video observers making use of reference images and conspicuous morphological features for identification. Individuals that were challenging to identify to species level (e.g., due to distance from the camera or poor visibility) were instead labeled on a genus or family level and omitted from the analysis in the present study. We expressed the abundances of focal species as the maximum number of individuals per species in a single video frame (MaxN), a metric widely used to avoid duplicate counts of individuals that are visible in multiple frames throughout the video (Cappo et al., 2004). In addition, video observers estimated the functional visibility in each video clip from taped markers on the bait pole and by comparing the field of view to reference images.

2.4 | Quantitative PCR

All DNA extractions and setup of quantitative PCRs (qPCRs) were conducted in a dedicated clean laboratory facility. DNA extraction was based on a modified version of a previously published eDNA extraction protocol (Spens et al., 2017). This protocol uses the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) to extract eDNA directly from the Sterivex filters (Supporting information, Note S1). Each extraction batch included a negative control (extraction blank) to test for exogenous DNA contamination through the used reagents or from the laboratory. Final DNA concentration was measured for every sample using a Qubit™ fluorometer and the Qubit™ dsDNA high sensitivity kit (Thermo Fisher Scientific, Waltham, MA, USA).

Four different qPCR assays targeting the mitochondrial cytochrome b gene (*cytb*) were used to analyze eDNA from the collected filters (Table 1). Assays for cod, flounder and plaice were based on published species-specific primers and probes (Knudsen et al., 2019), while the assay for goldsinny wrasse was designed for the study. This assay was developed based on thorough in silico and in vitro tests to ensure specificity. In order to ensure a broader use of the assay, the in silico analysis included all closely related species of wrasse with an available genetic *cytb* sequence in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). After database establishment, all primers and probes were aligned to the reference sequences using Geneious Prime® 2019.0.4 (https://www.geneious.com/) to verify specificity (Table S2). Specificity was further tested in vitro via qPCR on 1 ng of extracted DNA from the target species, as well as Ballan wrasse (*Labrus bergylta*) and Corkwing wrasse (*Symphodus melops*), which are the only other wrasse species potentially occurring in the sampling area (Muus et al., 1999; Peter Rask Møller, Natural history Museum of Denmark, personal communication). Finally, the assay targeting goldsinny wrasse was optimized for sensitivity by testing all possible combinations of different concentrations of primers (200, 400, 600 and 800 nM) and probes (200, 300 and 400 nM). The combination showing the lowest Ct value (cycle-threshold) was chosen for final qPCR analysis.

Quantitative PCRs were performed in 20 µl reaction volumes containing the optimal concentration of primers and probe (Table 1) with 8 µl of TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific, Waltham, MA, USA) and 4 µl DNA template. All qPCRs were analyzed on a StepOne Plus Real-time PCR instrument (Life Technologies, Carlsbad, CA, USA) using the following thermal cycling profile: 5 min at 50°C and 10 min initial denaturation at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. Initially, all samples were analyzed using a positive control (IPC, Applied Biosystems) to ensure that potential PCR inhibitors did not interfere with the PCR. Subsequently, all samples were run in triplicates. These included one field blank, two extraction blanks, and six NTCs per analyzed species to test for potential exogenous DNA contamination from the field or laboratory. The number of DNA copies per reaction was estimated using a dilution series. DNA amplicons were generated by conventional PCR using the qPCR primers and were purified using the Nucleospin Gel and PCR clean-up kit (Macherey-nagel, Düren, Germany). Amplicon concentrations were measured using a Qubit™ fluorometer and the Qubit™ dsDNA High Sensitivity Kit to enable estimation of copy numbers.

All qPCR results were compared to a standard dilution series and categorized in terms of limit of detection (LOD) and limit of

TABLE 1 Assay information for the four assays used in this study

Assay	Sequence (5′-3′)	Length (bp)	Concentration (nm)[a]	Reference
Atlantic cod (*Gadus morhua*)—80 bp of mtCytB				
Gadmor_F15076	TTCGCACCTAATTTACTCGGAG	22	400	Knudsen et al. (2019)
Gadmor_R15155	TCGGGCTTAACATGAGGTGG	20	800	Knudsen et al. (2019)
Gadmor_P15102	FAM-AGATAATTT/ZEN/CACCCCTGCTAACCCCATC-BKFQ	28	300	Knudsen et al. (2019)
European flounder (*Platichthys flesus*)—88 bp of mtCytB				
Plafle_F15107	TAGGCTTTGCAGTTCTCCTT	20	200	Knudsen et al. (2019)
Plafle_R15194	GCAGGCGTAAAGTTGTCCG	19	800	Knudsen et al. (2019)
Plafle_P15131	FAM-CACTGGCTT/ZEN/CGCTCGCCCTATTTTC-BKFQ	26	300	Knudsen et al. (2019)
European plaice (*Pleuronectes platessa*)—90 bp of mtCytB				
Plepla_F15107	TAGGCTTCGCAGTCCTCCTC	20	1000	Knudsen et al. (2019)
Plepla_R15196	TTGCAGGCGTGAAGTTGTCT	20	200	Knudsen et al. (2019)
Plepla_P15169	FAM-CTAAAAGAT/ZEN/ TTGGGGAAAATAGGGCGAGT-BKFQ	29	300	Knudsen et al. (2019)
Goldsinny wrasse (*Ctenolabrus rupestris*)—138 bp of mtCytB				
CterupR_F705	TCTCATCGCCCTAACTTCCCTA	22	600	This study
Cterup_R842	AGGATGGCATAAGCAAACAGGA	22	800	This study
Cterup_P791	FAM-CCCCACCG/ZEN/CACATTAAACCAGAGTGG-BKFQ	26	400	This study

[a]To ensure high sensitivity, all assays have been optimized for optimal concentration of primer and probes.

quantification (LOQ). We defined the LOD as the lowest copy number detected in at least one of the triplicates in the standard dilution series, and LOQ as the lowest copy number detected in all triplicates in the standard dilution series (Ellison et al., 2006). The sensitivity of all assays was evaluated before analyzing the extracted samples based on analysis of a standard dilution series of 1–1 × 10^5 copies of DNA targets per reaction. Estimation of eDNA copy numbers in the collected samples was based on a slightly higher dilution series of 4–4 × 10^5 copies of DNA targets. Evaluation of LOD/LOQ was based on the lowest values observed from these tests. However, most qPCR estimates of copy numbers were below the LOQ due to low DNA concentrations of the four target species in the collected samples (see Results), which precluded a quantitative data treatment (Klymus et al., 2020). Thus, as a proxy for DNA concentration, we used the proportions of positive amplifications of the individual samples (similar to Biggs et al., 2015), as it is reasonable to expect that amplification rates increase with the relative concentration of target DNA molecules (Furlan et al., 2016).

2.5 | Statistical analysis

All analyses were performed in R statistical software version 3.6.3 (R Core Team, 2020). We used a Bayesian multilevel modeling approach to obtain a probabilistic interpretation of our study hypotheses (Gelman et al., 2013). All models were fitted using the *brms* package (Bürkner, 2017), which facilitates Bayesian model fitting through the R interface of the STAN programming language (Carpenter et al., 2017). We used four Markov Chains for each model, with a total of 40,000 iterations of which 20,000 were discarded as warm-up samples (for exceptions see Table S4). We assessed model convergence by visually inspecting MCMC chains from trace plots and by ensuring that effective sample sizes were > 1000 and the potential scale reduction ($\widehat{R}$) < 1.1 for all model parameters (Table S5; Gelman et al., 2013). Model parameters were assigned uninformative prior distributions (Table S4), and prior predictive checks were performed following the protocol from Gabry et al. (2019) to verify the use of appropriate prior distributions for all model parameters. We defined varying intercepts at the site level, a structure that was retained for all candidate models to incorporate group-level effects and allow for correlations between samples from the same field site. In addition, model residuals were tested for spatial dependencies using the *DHARMa* package after converting the Bayesian models to DHARma objects with the "createDHARMa" function (Hartig, 2018), revealing no significant spatial auto-correlation in residuals across the final models. Posterior predictive checks (e.g., dispersion and zero-inflation tests) were performed to select the probability distributions used in the models by validating underlying distributional assumptions (Gabry et al., 2019). Finally, we identified the optimal model for each hypothesis by comparing model predictive accuracies of candidate models through leave-one-out (LOO) cross-validation (Vehtari et al., 2017).

2.5.1 | Species–habitat associations

Since MaxN counts recorded by BRUVS varied 1–2 orders of magnitude depending on the focal species, we fitted separate Generalized Linear Multilevel Models (GLMLMs) for each species to adjust the exponential distributions accordingly. Habitat (factor with two levels: sand and reef) was the main linear predictor in all BRUVS models. Functional visibility and soak time varied between BRUVS deployments, which we accounted for by adding the logarithm of both exposure terms as covariates in the GLMLMs. This allows the model to estimate the coefficients of the exposure terms instead of defining the terms as an offset (by fixing the coefficient to 1), with the latter approach making the unlikely assumption that recorded species abundance is always twice as high when the exposure doubles (McElreath, 2016; Zuur & Ieno, 2016). Cod and flounder abundance followed a negative binomial distribution (quadratic parameterization), as substantial overdispersion was diagnosed in the initial Poisson models. Plaice abundance followed a regular Poisson distribution, while wrasse abundance was modeled using a zero-inflated Poisson model to address slight zero-inflation in the regular Poisson model (Figure S4).

We examined the proportion of qPCR replicates that amplified (π_{Amp}) per sample as the response variable for the eDNA data, implying that with a total of 3 replicates the response variable was restricted to the values of 0, 0.33, 0.66, and 1. Since a traditional beta regression does not allow inclusion of the boundary values 0 and 1, and we preferred not to transform the y-values, we fitted a zero-one-inflated beta (ZOIB) model to allow for outcome values in the [0,1] interval (Liu & Eugenio, 2018). The ZOIB model includes two distributional parameters in addition to the population mean μ and dispersion parameter ν. The zero-inflation parameter p captures the probability of observing a zero, or $p_{ij} = \Pr(y_{ij} = 0)$ for the jth response on the ith experimental unit, while the conditional-one parameter q refers to the probability of observing a one given the outcome is not zero, or $q_{ij} = \Pr(y_{ij} = 1 | y_{ij} \neq 0)$. Parameters μ, p and q were modeled through a logit-link to ensure values to be constrained between 0 and 1, while dispersion ν was modeled through a log-link to restrict values to the domain of positive real numbers. In contrast to separate species models in the BRUVS analysis, we fitted one pooled model for the eDNA data since the response variable was within the same range for all focal species. The most parsimonious ZOIB model included a Habitat by Species interaction for μ, p and q, varying intercepts per site level for μ and an interaction term between Species and standardized SST on parameters p and q.

To obtain a probabilistic interpretation to the BRUVS and eDNA model outputs, we extracted fitted values (i.e., the linear predictor on the response scale; $\widehat{Y}$) separately for each of the focal species and computed posterior ratios denoting relative habitat associations of the focal species for each model iteration as:

$$\left(R_{\text{Habitat}}\right)_i = \frac{\left(\widehat{Y}_{\text{Reef}}\right)_i}{\left(\widehat{Y}_{\text{Sand}}\right)_i} \quad (1)$$

where $(R_{Habitat})_i \in (0, \infty)$, $i = 1, \ldots N$ with N denoting the total number of post warm-up iterations across all chains for each Bayesian model (Table S4). Hence, a posterior ratio of 1 indicates equal fitted values at reef and sand, whereas ratios of 0.5 and 2 imply fitted values twice as large at sand and reef habitat, respectively. We then computed the posterior density corresponding to study hypothesis H1 (i.e., $R_{Habitat} > 1$ for cod; $R_{Habitat} < 1$ for flounder) to obtain the associated posterior probabilities.

2.5.2 | Comparison of detection rates between methods

We converted all MaxN and π_{Amp} values into presence–absence format to obtain comparable values of species detection for both sampling methods. Here, positive eDNA detection was attained if at least one out of three replicate qPCR reactions amplified (hereafter: "Amp1"). We then used a pooled Bernoulli GLMLM to analyze the detection rates, with Method (factor, 2 levels), Species (factor, 4 levels), and their interaction as the main model predictors. We defined a common exposure term for both methods, by setting 1-h soak time (BRUVS) and 1000 ml water sample (eDNA) to an exposure value of 1 and adjusting deviations from these standard exposure values accordingly, prior to log-transforming the covariate. We retained the varying intercepts per site grouping level to allow for within-site correlations induced by our sampling design, while we simultaneously accounted for inherent method-specific correlations by defining varying slopes per sampling method. Similar to equation (1), we defined posterior ratios separately for each focal species to obtain a probabilistic interpretation to between-method differences:

$$(R_{\text{Method}})_i = \frac{\left(\hat{Y}_{eDNA}\right)_i}{\left(\hat{Y}_{BRUVS}\right)_i} \quad (2)$$

We then computed the posterior density corresponding to hypothesis H2 (i.e., $R_{\text{Method}} > 1$ for all species) to obtain the associated posterior probabilities.

2.5.3 | Correlation between eDNA signals and MaxN

We investigated potential correlations between eDNA signals and MaxN of focal species by performing a direct comparison of eDNA and the paired BRUVS samples. Specifically, we expressed the eDNA response variable as either π_{Amp} or Amp1 and coupled each eDNA sample with MaxN counts of the two subsequently deployed BRUVS samples to assess whether either eDNA metric correlated with MaxN. Similar to the eDNA analyses above, π_{Amp} was modeled with a ZOIB regression while Amp1 followed a Bernoulli distribution. To account for different exposures in MaxN counts (i.e., varying soak times and functional visibility), we standardized all counts per hour and per m visibility (MaxN · mh^{-1}). We defined separate ZOIB and Bernoulli models for each focal species, with a single population effect of MaxN, allowing us to inspect the amount of variance in eDNA signals explained by species abundance recorded from the BRUVS. In addition to varying intercepts per site ID retained throughout all models, we included a varying MaxN effect per site (i.e., varying slopes) in the ZOIB and Bernoulli model. This additional grouping structure allows for potential site-specific effects of MaxN on eDNA signals, for example, due to local differences in vertical mixing at field sites yielding potential variations in eDNA molecule transportation rates.

We made use of the "hypothesis" function within the *brms* package to obtain a probabilistic interpretation to hypothesis H3, that is, $\beta_{MaxN} > 0$ for each of the focal species. This function allows for post hoc testing of contrasts in Bayesian models by computing the posterior probability of a particular hypothesis contrasted against its alternative (Bürkner, 2017). We computed a Bayesian variant of R^2 for the final regression models (Gelman et al., 2019), yielding posterior distributions of the variance explained by the models for all focal species.

3 | RESULTS

3.1 | Summary of BRUVS and eDNA data

3.1.1 | Video analysis

We identified a total of 757 cod, 35 flounder, 20 plaice, and 100 wrasse individuals from video samples. However, the number of flounder and plaice combined (55 individuals) comprised only 55% of the total number of flatfish individuals observed, since 45% of flatfish individuals could not be identified to species level due to distance from the camera, low visibility or lack of visible distinctive features. In comparison, 9.9% and 5.7% of individuals were identified to family level for cod and wrasse, respectively, while all remaining individuals were identified to species level. Individuals identified to family level were subsequently excluded from the analyses. The most frequently observed species was cod (64.1% frequency of occurrence), followed by wrasse (47.4%), plaice (21.8%), and flounder (16.7%). Functional visibility ranged from 0.9 m to 4 m during the study period.

3.1.2 | qPCR analysis

Neither the field blank nor laboratory negative controls showed amplification of the focal species. Calculated efficiencies and goodness-of-fit (R^2) of all analyzed standard dilution series were within the ranges considered appropriate for quantitative qPCR assessment (Bustin et al., 2009) ($R^2 > 0.99$ and efficiency $90\% < x < 110\%$, Table S3). Estimated eDNA copy numbers were low for all four targeted species. Cod showed the highest estimates ranging from 0–752.4

copies/L, followed by Flounder 0–569.8 copies/L, Wrasse 0–370.0 copies/L and Plaice 0–52.4 copies/L. Evaluation of LOQ and LOD showed overall similar thresholds for the different assays with LOQ estimates ranging from 10 copies per reaction (cod and flounder assays) to 4 copies per reaction (plaice and wrasse assays; Table S3). For the individual samples, most copy number estimates were below LOQ and in many cases lower than LOD based on the analyses of the standard dilution series, which represented the lowest standard dilution with positive amplification. In cases where copy number estimates were lower than LOD, estimates still approximated the theoretical minimum detection threshold of one copy per reaction and hence likely represented true positive detections. The measured copy numbers showed positive correlations with the proportion of amplifications (π_{Amp}) for all focal species (Figure S2), with posterior probabilities of 99.9% (cod), 99.8% (flounder), 87.6% (plaice) and 99.9% (wrasse) in favor of a positive correlation, which supported the use of π_{Amp} as a proxy of concentration.

3.2 | Species–habitat associations

3.2.1 | BRUVS

The BRUVS recordings generally revealed strong habitat patterns in accordance with the expected habitat associations for all four focal species (Figure 2). Cod was on average 22 (CI: 1.1–94) times more abundant on reefs relative to sand habitat. The distribution of the posterior ratios for cod indicated a 98% probability of higher cod abundance on reefs compared with sand habitat (Figure 2a; Table 2). By contrast, flounder abundance was on average 17 (CI: 1.2–81) times higher at sand sites relative to reef sites (Figure 2b), with a 98% posterior probability of higher flounder abundance at sand habitat (Table 2). The recorded habitat pattern for plaice was similar yet slightly less pronounced, with on average 9.4 (CI: 0.5–42) times more individuals recorded at sand versus reefs (Figure 2c). The distribution of posterior ratios revealed a 94% probability of observing more plaice at sand habitat relative to reefs (Table 2). Finally, BRUVS recorded a reef association for wrasse (Figure 2d), with on average 12 (CI: 0.4–58) times higher wrasse abundance compared with sand habitat and a 92% probability of observing more wrasses on reefs (Table 2).

3.2.2 | eDNA

The proportion of qPCR replicates that amplified (π_{Amp}) was generally high for cod irrespective of the sampled habitat (Figure 3a). We found only a 37% probability in support of hypothesis H1 for cod (i.e., higher π_{Amp} at reefs; Table 2), with an average posterior ratio of 0.99 (CI: 0.74–1.30) indicating similar amplification rates of cod eDNA across habitats. The eDNA signals for flounder revealed a habitat association in accordance with BRUVS sampling, with a 98% probability of more replicates amplifying at sand habitat (Figure 3b; Table 2). On average, π_{Amp} for flounder was 1.88 (CI: 1.00–3.48) times higher at sand relative to reef habitat. The detection rate for plaice was generally the lowest of the four focal species in our study (Figure 3c) and provided limited support for a sand habitat association. Posterior ratios indicated a 71% probability of a higher π_{Amp} for plaice at sand habitat compared with reefs, with on average 1.32 (CI: 0.54–2.84) times more replicates amplifying at sand habitat. By

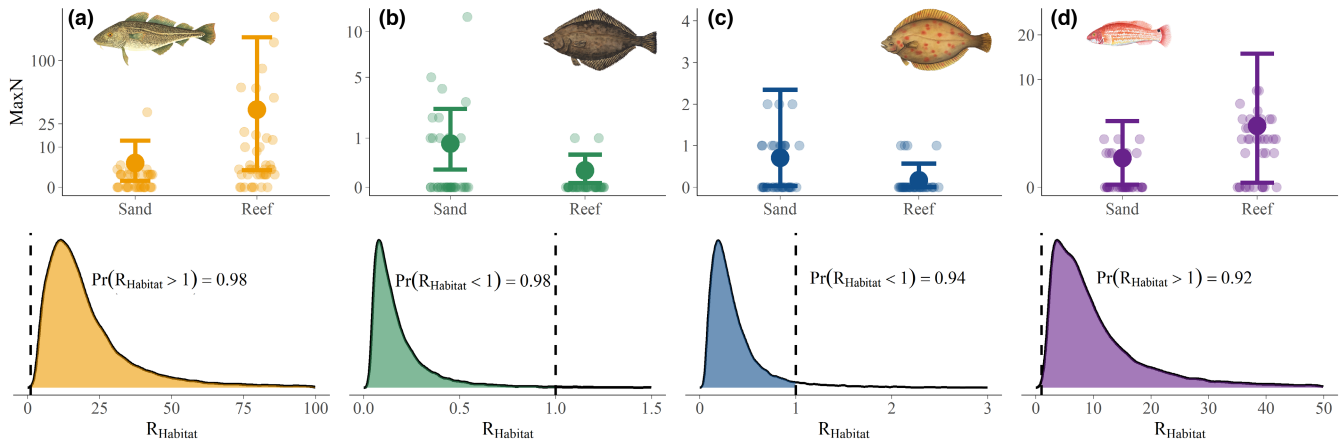

FIGURE 2 Habitat associations for the focal species as recorded by BRUVS. The upper row of plots shows the observed video data, with relative abundance of focal species expressed as MaxN counts (i.e., the maximum number of individuals for that species observed in a single video frame) and represented by small transparent circles. The large solid circles with error bars denote the posterior means and 95% credible interval (CI), respectively, from the separate multilevel models fitted for each focal species. Note that some of the y-axes of the upper plots are root-transformed to improve visualization. The lower row of plots shows the distributions for the posterior ratios, computed according to equation (1). The black vertical dashed line stands at a ratio of 1, implying equal species abundance between reef and sand habitats, while probabilities of observing expected habitat associations (i.e., in favor of hypothesis H1) are included next to the curves for each species. The four focal species include (a) Atlantic cod (*Gadus morhua*); (b) European flounder (*Platichthys flesus*); (c) European plaice (*Pleuronectes platessa*); and (d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells

TABLE 2 Hypothesis table showing the posterior probabilities associated with the study hypotheses for each of the target species. $R_{Habitat}$ and R_{Method} represent the posterior ratios computed from fitted values of the Bayesian models according to equation 1 (H1) and equation 2 (H2), respectively. Detection rates between methods were compared using a binary response variable of species detection (1) and non-detection (0), that is, $\hat{y}_{pres-abs}$. The β_{MaxN} parameter represents the slope of the relationship between MaxN and eDNA presence–absence (Amp1) or the proportion of replicates amplifying (π_{Amp}), for which we computed the probability of taking on positive values in hypothesis H3

	Hypothesis	Cod (*G. morhua*)	Flounder (*P. flesus*)	Plaice (*P. platessa*)	Wrasse (*C. rupestris*)
$\Pr(R_{Habitat} > 1 \mid \hat{y}_{MaxN})$	H1	0.98	0.98[a]	0.94[a]	0.92
$\Pr(R_{Habitat} > 1 \mid \hat{y}_{\pi_{Amp}})$	H1	0.37	0.98[a]	0.71[a]	0.99
$\Pr(R_{Method} > 1 \mid \hat{y}_{pres-abs})$	H2	0.99	1.00	0.99	0.71
$\Pr(\beta_{MaxN} > 0 \mid \hat{y}_{Amp1})$	H3	0.90	0.85	0.53	0.95
$\Pr(\beta_{MaxN} > 0 \mid \hat{y}_{\pi_{Amp}})$	H3	0.52	0.72	0.24	0.53

[a]For sand species in hypothesis H1, probabilities are computed as 1 − Pr(R > 1) as this corresponds to the hypothesis of observing higher abundance at sand habitat (see Equation 1).

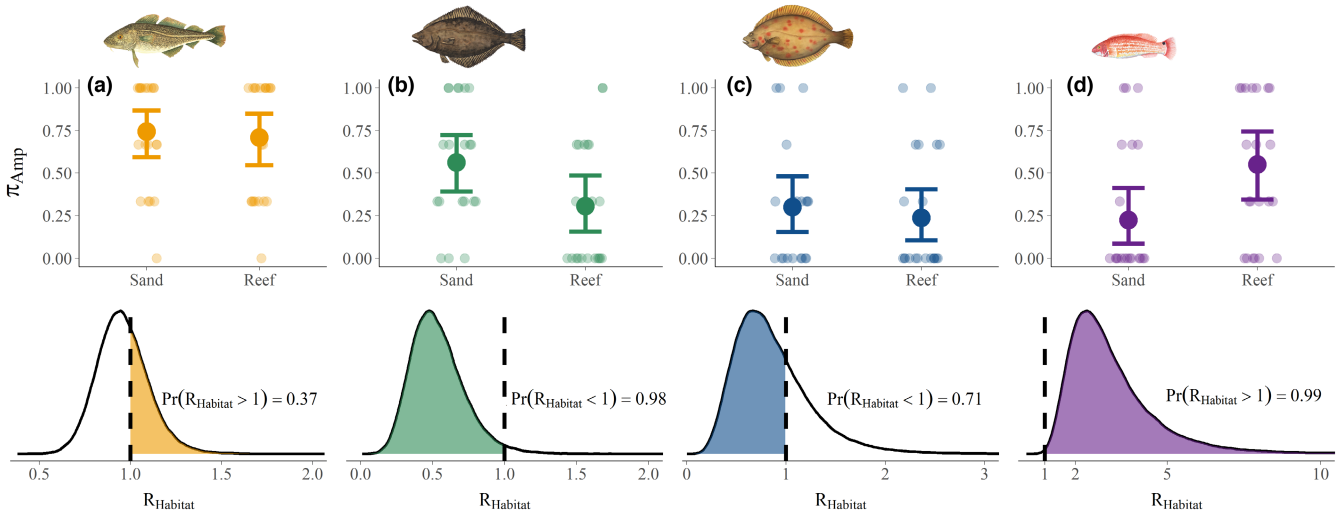

FIGURE 3 Habitat associations for the focal species as recorded by eDNA. The upper row of plots shows the observed amplification signals, expressed as the proportion of qPCR replicates amplifying (π_{Amp}; with 3 qPCR replicates per eDNA sample the possible response values include 0, 0.33, 0.66, and 1). The large circles and error bars denote the posterior mean and 95% credible interval (CI) from the zero-one-inflated beta (ZOIB) model, respectively. The lower row of density curves shows the distributions of the posterior ratios similar to Figure 2, with probabilities of observing expected habitat associations (i.e., in favor of hypothesis H1) included next to the curves. The four focal species include (a) Atlantic cod (*Gadus morhua*); (b) European flounder (*Platichthys flesus*); (c) European plaice (*Pleuronectes platessa*); and (d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells

contrast, eDNA sampling of wrasse confirmed the reef association recorded by BRUVS, with 2.13 (CI: 0.98–4.44) times higher π_{Amp} at reefs and a 99% probability that more qPCR replicates amplified for wrasse at reefs relative to sand sites (Figure 3d). Exploratory analysis of the estimated copy numbers at reef and sand habitat for each focal species showed trends in agreement with our qualitative analysis (Figure S3). However, no further statistical analyses were conducted on the quantitative data as copy numbers were generally below the LOQ for all focal species (Klymus et al., 2020). The ZOIB model additionally revealed species-specific effects of SST on the distributional parameters p and q. Specifically, SST had a positive effect on p for plaice (i.e., higher SST increased the probability of 0/3 amplifications; Figure S9) and a positive effect on q for wrasse (i.e., higher SST increased the probability of 3/3 amplifications; Figure S10).

3.3 | Method comparison

3.3.1 | Species detection

Differences in species detection rates from our two sampling methods (BRUVS and eDNA) varied between the focal species (Figure 4). Across habitat type, cod presence was detected on average 1.5 (CI: 1.2–1.8) times more often in eDNA relative to BRUVS samples, with a 99% probability of a higher cod detection rate using eDNA sampling (Table 2). This pattern remained consistent when accounting for habitat type, with a 99% and 100% probability of detecting cod more frequently using eDNA sampling at reef and sand sites, respectively (Figure S5). A positive detection signal for flounder was on average 4.2 (CI: 2.4–7.3) times more likely using eDNA compared with BRUVS sampling. Posterior ratios indicated a 100% probability of

higher flounder detection rate using eDNA sampling (Table 2), which remained consistent when accounting for habitat type (Figure S5). Similarly, plaice was on average detected 2.3 (CI: 1.3–3.8) times more often with eDNA relative to BRUVS sampling, with a 99% probability of higher plaice detection rates across habitat and >96% within habitat type (Table 2; Figure S5). By contrast, we did not find a strong difference in detection rate of wrasse between the two sampling methods across habitats (Figure 4). On average, eDNA sampling was only 1.2 (CI: 0.8–1.7) times more likely to result in a positive wrasse detection relative to BRUVS, with a moderate 71% probability of a higher detection rate using eDNA sampling (Table 2). Accounting for habitat type revealed that wrasse was more likely to be detected by eDNA compared with BRUVS sampling at sand (85%) than at reef (56%) habitat (Figure S5).

3.3.2 | Relationships between eDNA signals and MaxN

While we found moderate to strong evidence of positive relationships between eDNA signals and MaxN counts for a number of focal species, the explanatory power of the relationships was generally limited. Specifically, there was a 90% probability of a positive relationship between detection (presence-absence) of cod eDNA (Amp1) and MaxN recorded by BRUVS (Figure 5a; Table 2), but this relationship had very low explanatory power (median $R^2 = 0.05$; Figure S11a). Detection of flounder eDNA had a moderate 85% probability of being positively correlated to flounder MaxN (Figure 5b), with the relationship explaining a moderate amount of variation in the eDNA data ($R^2 = 0.20$; Figure S11b). No correlation was found for plaice and explanatory power of the relationship was low (Figure 5c; Figure S11c; $R^2 = 0.04$). Finally, detections of wrasse eDNA provided the strongest evidence for a correlation out of all focal species (Figure 5d), showing a 95% probability of a positive relationship with MaxN, which explained a moderate amount of the variation in eDNA data ($R^2 = 0.16$; Figure S11d). Inspecting the proportion of amplifications (π_{Amp}) as a function of MaxN did not reveal positive correlations for any of the focal species (Figure S6; Figure S12; Table S6).

4 | DISCUSSION

We performed a direct comparison between BRUVS and eDNA monitoring of four coastal marine species in the western Baltic Sea. Despite the patchiness of the focal habitat in the bay combined with very low copy numbers in the eDNA samples, our comparative analysis showed that eDNA was capable of detecting species–habitat associations for two species in agreement with BRUVS. We explored a semi-quantitative approach by expressing eDNA signals as the proportion of qPCR replicates amplifying within a sample, π_{Amp}, which revealed higher proportions at sand sites for sedentary flounder and at reefs for highly resident wrasse, in agreement with BRUVS. However, contrary to BRUVS, no species–habitat associations were detected from eDNA signals of highly mobile cod and sedentary plaice, the latter showing the lowest DNA concentrations of all focal species. Conversely, eDNA sampling yielded higher detection rates (i.e., presence–absence) for cod, flounder, and plaice, with equal detection rates for wrasse, adding to the growing body of evidence indicating superior species detection using eDNA relative to traditional survey methods (Hinlo et al., 2017; Schmelzle & Kinziger, 2016; Valdivia-Carrillo et al., 2021; Weldon et al., 2020). In particular, this result suggests that eDNA can outperform BRUVS in detecting predatory fish like cod, which generally are well represented in BRUVS samples due to their highly conspicuous appearance and strong affinity to bait. Finally, eDNA detection rates showed a moderate to high probability of a positive correlation with MaxN counts for cod, flounder, and wrasse, yet we found no correlations when expressing eDNA signals as π_{Amp}. In the following sections, we discuss our results in relation to the ecology of the focal taxa and reflect on discrepancies between BRUVS and eDNA results to highlight some of the benefits and challenges inherent to both

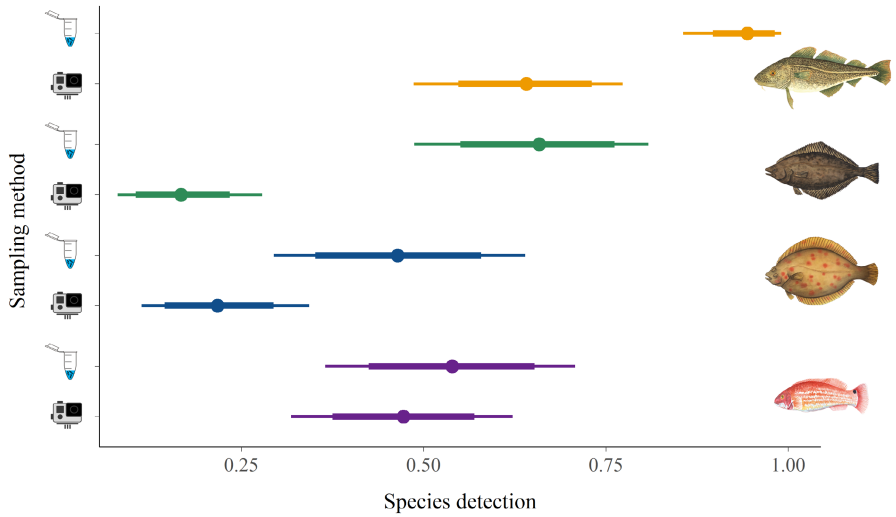

FIGURE 4 Species detection rates for each of the focal species achieved by using either video (BRUVS) or eDNA monitoring. Circles denote the posterior mean, thick bars the 80% confidence interval and thin bars 95% confidence interval. Species drawings by Elisenda Casabona Balcells

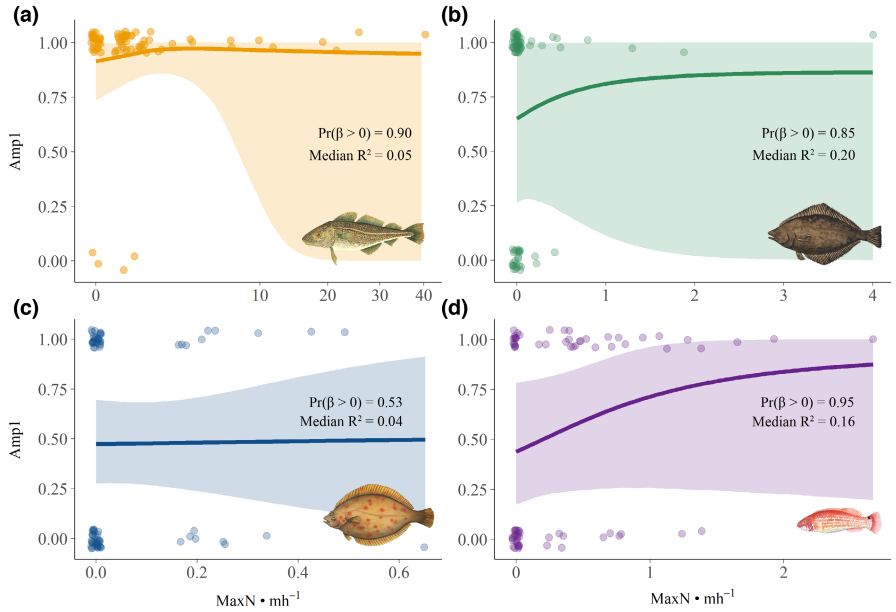

FIGURE 5 Relationships between eDNA detection rates and relative fish abundance recorded by BRUVS for each of the focal species. Positive eDNA detection is attained when at least 1 out of 3 qPCR replicates amplifies (Amp1). Solid lines and shaded areas represent the posterior means and 95% credible intervals from Bayesian models, respectively, while jittered transparent circles show the raw observations. The MaxN metric was standardized (per meter and hour) to account for variations in functional visibility and soak time between deployments. Probabilities of a positive relationship and the median variance explained (Bayesian R^2) are shown within the shaded areas. Note that the x-axis is root-transformed in (a) to improve visualization of smaller MaxN values. Relationships are shown for (a) Atlantic cod (*Gadus morhua*); (b) European flounder (*Platichthys flesus*); (c) European plaice (*Pleuronectes platessa*); and (d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells

methods and to provide suggestions for optimizing future monitoring efforts.

4.1 | Species–habitat associations and ecology

The four focal species in our study were selected based on documented habitat associations (i.e., either reef or sand-associated) and varying mobility and residency levels. Both BRUVS and eDNA monitoring revealed strong habitat associations for flounder and wrasse, while associations for cod and plaice were only apparent from BRUVS samples (Table 2). We suggest that these results at least partly reflect the ecology of our focal species.

4.1.1 | Cod

Atlantic cod is a generalist apex predator in the Baltic Sea and multiple studies have previously highlighted the importance of hard-bottom structures for survival and growth of cod (Gotceitas & Brown, 1993; Lindholm et al., 1999; Schwartzbach et al., 2020). However, habitat use by this mobile demersal fish has been shown to be highly dynamic and to vary with diel and seasonal movement patterns in response to changes in food availability, sea surface temperature and thermal stratification (Freitas et al., 2016; Funk et al., 2020). Therefore, despite a documented preference for hard substrates of high structural complexity in older juveniles and adults, cod individuals exhibit active movement patterns within the larger seascape and still make use of soft-bottom areas to some degree (Cote et al., 2004). For example, cod may use soft-bottom areas to forage on shore crab (*Carcinus maenas*), which is one of the main prey items for adult cod in the SW Baltic Sea (Funk et al., 2021). Still, the use of MaxN as a relative abundance metric derived from BRUVS data enabled us to discern a strong reef association for cod, while eDNA sampling did not detect any habitat association. This apparent discrepancy between the two sampling methods might be partly explained by the relatively low resolution obtained from qualitative eDNA data, combined with the high mobility of cod. In particular, transient visits to soft-bottom habitats by cod may have resulted in substantial shedding of eDNA particles and subsequent high detection rates of cod eDNA at both soft- and hard-bottom habitats. Similarly, vertical use of the water column by cod (Freitas et al., 2021), as opposed to limited movement within the vertical dimension by the remaining focal species, also likely contributed to the ubiquitous detection of cod eDNA across our surface water samples (Figure 3; Figure S5). As we only used 1 L samples and three qPCR replicates in this study, this possibly limited the ability for statistically discerning habitat patterns for cod. Larger sample volumes and a higher number of qPCR replicates (e.g., >1 L volume and 6 to 12 replicates; Furlan et al., 2016) might instead have improved the resolution and statistical power to detect differences in amplification rates between habitats, or alternatively have allowed for a quantitative assessment using eDNA concentrations. In addition, the eDNA traces from cod may represent a substantially wider temporal

coverage compared with the 1–2 h snapshot provided by BRUVS, given documented persistence time of eDNA molecules within marine environments (although strongly dependent on species, eDNA source and environmental conditions; Saito & Doi, 2021b) in the order of hours to days (Thomsen et al., 2012; Weltz et al., 2017). This implies that observed eDNA signals of cod could represent diel variations in movement patterns of cod that we were unable to observe using daytime BRUVS deployments. Future studies employing BRUVS and/or eDNA should thus consider sampling larger water volumes, using a higher number of qPCR replicates and comparing day and nighttime sampling (Jensen et al., 2022), to shed more light on inter-method discrepancies in documenting habitat associations for highly mobile organisms.

4.1.2 | Flatfishes

Flounder and plaice are sedentary benthic predators that mainly feed on tube-building polychaetes, small crustaceans and mollusks (De Groot, 1971; Doornbos & Twisk, 1984). Although both species are known to undertake ontogenetic migrations in response to predation risk and temperature changes (Amorim et al., 2018; Gibson et al., 2002), they generally exhibit a sedentary lifestyle with substantial periods of immobility typical of cryptic benthic predators (Gibson et al., 2005). For flounder, both BRUVS and eDNA monitoring documented a strong association with sand habitat. Since flounders are generally confined to the seafloor, our results indicate that the surface water samples we collected still contained sufficient particles of flounder DNA to detect an association with sand habitat. Despite increasing thermal stratification in the western Baltic Sea during the study period (Funk et al., 2020) and thus a potential for vertical zonation in eDNA signals (Jeunen, Lamare, et al., 2020), mixing appears to have induced sufficient transportation of DNA material from the seabed (~6–7 m depth across sites) to the surface layer to be detected in the eDNA samples. In addition, given the patchy distribution of habitats in our study area with reefs often in close proximity to sand sites and vice versa (few 100 m's away, Figure 1), our results provide evidence of localized flounder eDNA as we did not detect a homogenous eDNA signal across the bay. This implies that flounder DNA was either not transported very far from the source or rapidly became too diluted for detection with increasing distance. Average current velocities across our study sites ranged from 0.9 to 4.8 cm/s, (Figure S1) and combined with an estimated time of 6.7 days until flounder eDNA degrades beyond the detection threshold (Thomsen et al., 2012), we roughly estimate that DNA molecules could have been transported between 5.2 and 27.8 km away from the source on average. As the farthest distance between our field sites (~15 km; Figure 1) falls within this estimated range, we hypothesize that rapid dilution of flounder eDNA in the marine environment was the most important factor contributing to the localized signals found in this study.

By contrast, eDNA traces for plaice were generally low in this study (0–52.4 copies/L) and we did not detect any habitat association for plaice using eDNA monitoring. One possible explanation for the low copy numbers for plaice could be that this species is relatively less common in the western Baltic Sea than flounder, which is widely distributed across most of the Baltic Sea (Nissling et al., 2002). However, BRUVS recorded roughly equal occurrences of flounder and plaice across our study sites, both in terms of relative abundance (Figure 2) and species detection (Figure 4). Both flatfish species were highly attracted to the bait and often observed to be actively feeding for prolonged periods once attracted into the camera's field of view. This bait plume effect in BRUVS may therefore have provided a significant advantage over eDNA monitoring, by attracting individuals in the vicinity to the camera and thereby increasing the statistical power to detect a habitat association for plaice. In case plaice individuals were highly dispersed across the study sites, the eDNA shed by these individuals may have been too diluted to produce detectable signals in seawater samples. An interesting future avenue for studies comparing BRUVS and eDNA monitoring could therefore be to assess whether eDNA detection of target species increases post-BRUV deployment due to the bait plume effect.

4.1.3 | Goldsinny wrasse

Goldsinny wrasses are actively swimming reef fishes, yet they are highly site attached with males defending territories with a mean area of 1.4 m^2 and no larger than 2 m^2 (Hilldén, 1981). Individuals are strongly associated with reef structures such as holes and crevices, and are rarely observed far away from suitable refuges (Sayer et al., 1993). Our results confirm a reef association for goldsinny wrasse, as both BRUVS and eDNA provided strong evidence for higher wrasse detections at reef sites. In addition, eDNA and BRUVS sampling showed comparable detection rates for goldsinny wrasse (Figure 4). Similar to cod, goldsinny wrasse is a conspicuous reef fish and therefore easily identified and counted in BRUVS recordings. While wrasses were generally not responding directly to the bait in our study, they seemed to be attracted to overall increased activity levels of feeding organisms as has also been reported for non-piscivorous fishes in other regions (Harvey et al., 2007; Watson et al., 2010). Combined, the strong site fidelity in wrasse, the equal detection rates between BRUVS and eDNA, and the clear reef association documented by both techniques indicate that wrasse eDNA traces were localized within the reef sites and either not transported far away from the source or too diluted to be detected at the nearby sand sites.

Commercial catches of goldsinny wrasse in Northern Europe have increased significantly over the past decade due to exploitation of wrasse as a cleaner fish in the salmon aquaculture industry (Bjordal, 1991; Blanco Gonzalez & de Boer, 2017; Skiftesvik et al., 2014). Given the ongoing need to monitor the effects of the intensifying wrasse fisheries, our newly developed qPCR assay for goldsinny wrasse could be used to facilitate rapid and low-cost detection of wrasse eDNA in marine environments. We suggest that combined use of eDNA sampling and video surveillance is a promising, non-invasive monitoring strategy to document spatiotemporal distributions of goldsinny wrasse in coastal waters.

4.2 | Environmental effects

The use of ZOIB models in our study revealed some species-specific effects of temperature on the proportion of qPCR replicates amplifying. Specifically, water temperature had a positive effect on the zero-inflation parameter (*p*) for plaice (Figure S9), implying that the probability of observing 0/3 amplifications increased at higher temperatures. One explanation for this effect could be that plaice were less abundant at the study sites in summer (late June) compared with spring (late April), potentially moving to deeper waters to avoid the higher water temperatures and thereby causing a higher proportion of samples to yield zero amplifications. Alternatively, eDNA particles are known to degrade more rapidly when exposed to higher temperatures (Caza-Allard et al., 2021; Saito & Doi, 2021a), which could also explain the observed temperature effect for plaice. However, no similar effects of temperature were found for the remaining three focal species (95% CI's included zero, Figure S8), making it more likely that the effect in plaice was due to a temperature-driven shift in the spatial distribution of plaice within the study area, a trend at least partly confirmed by BRUVS observations (Figure S7c). By contrast, the conditional-one parameter (*q*) was positively affected by temperature for wrasse (Figure S10), implying a higher probability of 3/3 amplifications at higher water temperatures. This result likely reflects the preference of goldsinny wrasse for warmer waters (Olsson et al., 2012), which is also confirmed by the higher MaxN counts for wrasse during the summer part of our study period (early-mid June; Figure S7). In addition, wrasse from the region spawn mainly in June (Skiftesvik et al., 2015), which could produce significant spikes in eDNA signals due to the excretion of gametes (Tsuji & Shibata, 2021), while increased metabolism and activity levels at higher temperatures may also induce an increase in eDNA shedding (Jo et al., 2019; Lacoursière-Roussel, Côté, et al., 2016). It currently remains challenging to disentangle the diverse ways in which temperature may affect eDNA concentrations in marine systems. The contrasting effects of increased shedding and decay rates, with inherent variability across different species and animal forms (Andruszkiewicz Allan et al., 2021), combined with seasonal changes in species distributions and activity levels, likely yield varying effects across study systems and organisms (Rourke et al., 2021). Still, our study exemplifies how the use of ZOIB models can provide further species-specific information on factors influencing the absence and/or complete amplification of qPCR replicates in marine environments with low DNA concentrations.

4.3 | Lack of correlations and limitations of this study

Although we found some evidence in our results of higher eDNA detection rates when the relative abundance (MaxN) of focal species was high (Figure 5), there was a general lack of quantitative relationships between the proportion of eDNA amplifications (π_{Amp}) and MaxN for all focal species (Table 2; Figure S6). There have been a number of studies reporting a similar lack of correlation with abundance metrics from traditional methods (Deutschmann et al., 2019; Hinlo et al., 2018; Knudsen et al., 2019), which can be expected as correlations weaken significantly in dynamic natural systems relative to controlled environments (Yates et al., 2019). Still, a recent literature review revealed that up to 90% of published comparative studies have documented a positive relationship between eDNA signals and fish abundance or biomass recorded by traditional methods (Rourke et al., 2021). While a publication bias against studies reporting no effect is likely affecting this finding (Rourke et al., 2021), the overwhelming trend in positive correlations reported across study systems and organisms offers a promising outlook in terms of using eDNA as a complementary tool to monitor the relative abundance of aquatic organisms. The comparative assessment of our study included two methods with undefined spatial coverage and we suggest that the lack of correlations may at least be partly explained by an expected difference in sampled area between the two methods. The temporal coverage in the signals obtained from both sampling methods is also likely to be different (Jeunen, Urban, et al., 2020), since BRUVS recorded for 1–2 h and continuously attracted individuals that may not have been included in the signal of the eDNA sample taken prior to BRUVS deployments. Furthermore, it is possible that the use of π_{Amp} as the response variable in the eDNA analysis masked potential correlations with MaxN that would otherwise have been apparent when using traditional eDNA concentrations. We suggest that similar future studies conducted within systems of low concentrations should increase their sampling volume (i.e., >1 L of seawater) and use a larger number of qPCR replicates, which could potentially yield additional ecological insights. Here, the use of three qPCR replicates implied that π_{Amp} was limited to only four discrete values. Instead, 6–12 replicates would have increased the resolution of the eDNA data and could thereby benefit statistical inference (Biggs et al., 2015; Furlan et al., 2016), in particular when uncertainties during field and laboratory procedures can subsequently be incorporated through hierarchical model structures (Burian et al., 2021). Further, incorporation of multiple intra-site samples taken simultaneously in the field would be of interest to facilitate a better understanding of the levels of variability within the field and laboratory components of eDNA surveys, ultimately to optimize the sampling design in terms of appropriate replication (Mathieu et al., 2020). Finally, we note that the challenge of identifying organisms with cryptic appearance in BRUVS likely contributed to the lack of correlations for the two flatfish species in our study. Specifically, flatfish individuals were assigned to family level instead of species level in 45% of observations, which mostly represented cases in which individuals were clearly right-eyed (family *Pleuronectidae*) but subtle features distinguishing the different species were not visible on the videos. These observations had to be excluded from the analysis since they could not be confidently assigned to either flounder or plaice, and this exclusion could thus have significantly affected correlations with eDNA signals. However, given the similarly weak relationships for conspicuous cod and wrasse (assigned to family level on 9.9% and 5.7% of BRUVS observations, respectively),

it is safe to argue that the lack of correlations cannot be solely attributed to the challenge of cryptic species identification in BRUVS. This challenge does, however, highlight the benefit of using eDNA monitoring in tandem with other non-invasive methods to improve the taxonomic precision of monitoring efforts.

5 | CONCLUSIONS

Our study has demonstrated that eDNA sampling can detect habitat associations for sedentary and highly resident species within a small coastal area (~20 km^2) characterized by a mosaic of different seabed types. These results add to the growing evidence of localized eDNA signals within coastal marine ecosystems and simultaneously suggest that the ability of eDNA monitoring to document species–habitat associations is to some extent dependent on the mobility and ecology of focal taxa. Notably, this finding is in agreement with a recent study on kelp forest ecosystems, in which the temporal depth variation in eDNA signals from teleost fish and elasmobranchs appeared reflective of species behavioral patterns (Monuki et al., 2021). For highly mobile species and species detected at very low concentrations, our results indicate that monitoring studies might benefit from supplementing eDNA with BRUVS sampling to yield more informative inference through a higher resolution offered by the MaxN metric combined with use of bait attractants to facilitate monitoring of widely dispersed organisms within field sites. Conversely, eDNA sampling outperformed BRUVS in overall detection rates for three out of four species, with equal detection for the remaining species, and therefore offered superior sensitivity in detecting target species in our study, even for piscivorous fishes that are well represented in BRUVS recordings (e.g., cod). While presence–absence of qPCR amplifications was positively correlated with MaxN for wrasse, there was no evidence for relationships between π_{Amp} and MaxN for any of the focal species in this study, suggesting limited scope for directly relating qPCR amplifications to species abundances recorded by paired BRUVS. We recommend that future eDNA studies conducted within systems of low DNA concentrations further explore the use of replicate amplifications (i.e., π_{Amp}) as a response variable and how this metric relates to species abundances derived from conventional methods.

AUTHOR CONTRIBUTIONS

TJGW, MWJ, JCS, EEN, JGS, and HB contributed to the conception and design of the study; JCS, JGS, and EEN contributed to funding acquisition; TJGW, BKH, EEN, and JCS involved in field sampling and methodology; MWJ, BKH, EGAG, and EEN involved in lab analysis and methodology; TJGW, JB, CHSO, and JLB performed video data analysis; TJGW, MWJ, and HB performed statistical analysis; TJGW and MWJ wrote the manuscript with critical contributions from the remaining co-authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with the publication of this article.

DATA AVAILABILITY STATEMENT

Data is available via the Dryad Digital Repository https://doi.org/10.5061/dryad.69p8cz93p (Wilms et al., 2022). An R script with instructions on running the analysis and producing the figures is archived on Zenodo https://doi.org/10.5281/zenodo.6505457.

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