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ORIGINAL ARTICLE OPEN ACCESS

Roaming the Seas—Assessing Marine Invertebrate Biodiversity Along Salinity Gradients With Zooplankton and eDNA Metabarcoding

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Received: 6 March 2024 | **Revised:** 28 October 2024 | **Accepted:** 30 October 2024

Funding: This work was supported by POF IV, Research Program of the HIFMB as part of the Helmholtz Association of German Research Centers, Bundesministerium für Bildung und Forschung, CREATE (03F091A), L.K. further acknowledge funding by the German Science Foundation (DFG HI 848/26-2 as part of Research Unit DynaCom and HI 848/29-1).

Keywords: biodiversity | marine fauna | spatial resolution | zooplankton

ABSTRACT

Marine metazoan biodiversity is accretively being explored through environmental DNA (eDNA) metabarcoding of seawater. However, knowledge gaps in the use of eDNA to study changes in diversity resulting from changing abiotic conditions still do exist. In order to address these gaps, we analyzed patterns of marine invertebrate biodiversity based on eDNA from water and sediment samples along a decreasing salinity gradient from the North Sea toward the Baltic Sea. eDNA was collected from surface (SW) and bottom (BW) water, and from the uppermost sediment layer (SE). To supplement the eDNA approach, we conducted parallel zooplankton (ZP) metabarcoding and morphological identification. DNA was extracted from eDNA and ZP samples, amplified using two universal primers that target of the mitochondrial cytochrome c oxidase subunit 1 (COI) and the nuclear ribosomal 18S rRNA genes, and paired-end sequenced on Illumina Miseq. Metabarcoding detected 279 metazoan species (from 16 phyla) of which > 87% are known from the study area or adjacent regions. Communities identified in SW eDNA were a subset of communities identified in ZP metabarcoding. BW eDNA had additional benthic (mainly bivalve) species. Communities identified in SE eDNA were distinct from those in water eDNA and ZP metabarcoding, and mainly represented by in- and meiofauna. Out of all approaches, only ZP metabarcoding uncovered the expected decrease in species richness toward brackish conditions. Neither salinity nor spatial distance had a significant effect on species composition. All approaches revealed regional differences of which SE eDNA was least informative. The detection of holoplanktonic species from SE eDNA provided evidence for sinking of eDNA particles, dead organisms or the presence of resting eggs. Our study confirms the value of metabarcoding to identify the North Sea and Baltic Sea invertebrates and underscores the importance of combining multiple approaches to understand invertebrate biodiversity and its change in the marine realm.

1 | Introduction

Comprehensive knowledge of local and regional diversity, along with its changes due to anthropogenic activities, is essential for understanding the driving forces of biodiversity and predicting future scenarios (Collins et al. 2018; Hansen et al. 2018). Over the last decades, marine fauna biodiversity assessment experienced fundamental changes from exclusively morphologically based species identification techniques toward molecular multiple-species identification (Beng and Corlett 2020; Sahu et al. 2023). Identification of zooplankton (ZP) including holo-, mero-, and ichthyoplankton using metabarcoding has proven to be a useful tool to detect overall diversity. This is especially true for cryptic and rare species and for life-stages that are difficult to be identified based on morphology (Lindeque et al. 2013; Mohrbeck et al. 2015; Laakmann et al. 2020; Lira et al. 2022; Ohnesorge et al. 2023). However, the success of identifying meroplanktonic and therefore benthic species from net catches is restricted by the seasonality of their reproductive periods and it is virtually impossible for taxa that lack a planktonic phase. Metabarcoding of benthic communities from bulk samples such as sessile fauna from settlement plates (Koziol et al. 2019; Leite et al. 2021), mature biofouling (Zaiko et al. 2021), mobile epi-macrofauna (Cowart et al. 2015), and meiofauna (Rossel et al. 2019; Brandt et al. 2021) provides more comprehensive insights into biodiversity, complementing the detected community with benthic invertebrates.

In recent years, these approaches have been supplemented by eDNA metabarcoding, a noninvasive sampling and species identification technique that offers the advantage of partially circumventing the challenges associated with traditional sampling approaches (Goldberg et al. 2016). Similar to bulk sample metabarcoding, species detections derived from eDNA metabarcoding contain a subset of the community present, as demonstrated by proof-of-concept studies. These studies compare eDNA from seawater and ZP from net catches identified by morphology (Leduc et al. 2019) or both morphological characteristics and organismal metabarcoding (Djurhuus et al. 2018; Ohnesorge et al. 2023), as well as benthic communities (epi- and infauna) (Leduc et al. 2019) and sediment (Holman et al. 2019). In fact, sampling approach (eDNA from SW or bulk samples from sediment, settlement plates, and planktonic tows) was found to be the most important factor driving the taxonomic composition detected, followed by spatial variation (Koziol et al. 2019). As a consequence, to achieve a holistic view of marine invertebrate biodiversity, it is necessary to combine data from several communities (pelagic, benthic) and sources (target organisms, eDNA) to compile complementary and comprehensive information.

Cross-validating eDNA metabarcoding against bulk samples from catches remains important to verify successful resolution across metazoan taxa present in the studied system. Live catches analyzed using established morphological identification methods provide a realistic spatial–temporal resolution and proof of true site occurrences, abundances, and current life histories. Therefore, comparing bulk samples to eDNA is essential to determine the time window represented by eDNA from both the water column and the sediment surface. Understanding vertical eDNA particle turnover (e.g., sinking from the water column to the seafloor or leaching from the sediment into the water column) is necessary to interpret positive species detections by eDNA. Directly comparing various eDNA sources is vital to comprehend the processes and consequences causing differences in the subsets of communities detected by specific sampling strategies. This understanding enhances our ability to draw accurate conclusions when interpreting results in a spatial or temporal context. Despite this importance, only about 4% of eDNA metabarcoding studies targeting eukaryotes employ multiple sampling approaches for cross-validation, with water being the most commonly chosen substrate, followed by sediment (Koziol et al. 2019).

Despite the potential for different sampling approaches to introduce artifacts in detected species compositions, eDNA obtained from various sampling methods may still reflect habitat-specific communities, thereby representing genuine differences. This phenomenon was demonstrated in studies analyzing water samples from different depths (Andruszkiewicz et al. 2017) and comparing water and sediment eDNA (Holman et al. 2019). It is worth noting that sediment eDNA may consistently contain substantial amounts of DNA from meiofauna, thus automatically incorporating them into the analysis.

While (eDNA) metabarcoding is increasingly utilized to characterize regional marine fauna, its ability, compared to other identification methods to reliably detect community changes resulting from shifting abiotic conditions such as salinity, has received limited attention thus far. As only a minority of studies compare different eDNA sampling approaches (Koziol et al. 2019), the evaluation on these approaches regarding their ability to identify spatial differences in marine fauna caused by abiotic conditions, as well as their temporal resolution, remains largely unknown.

In our study, we evaluate the use of eDNA metabarcoding to detect community changes resulting from shifting abiotic conditions. For this, we choose our study area which is characterized by a declining salinity gradient, with marine conditions in the North Sea decreasing toward the Baltic Sea, which is one of the largest brackish regions worldwide (Kautsky and Kautsky 2000; Geburzi et al. 2022). The primary connections between the North Sea and the Baltic Sea are the sea passages Skagerrak, Kattegat, and the Great Belt (Zettler et al. 2014; Geburzi et al. 2022). The Limfjord, a sound cutting through the Danish mainland with its North Sea inflow at the Thyborøn Canal flowing into the Kattegat in the East, emerges as a secondary passage (Riisgård et al. 2012). Unlike other sampling regions, the Limfjord is very shallow, with a mean depth of < 5 m. Its fauna faces massive pressure from eutrophication and stratified water, leading to large-scale oxygen depletion in near-BWs in summer. These conditions create a distinct environment that is favored by predators such as the scyphozoan *Aurelia aurita* and the invasive ctenophore *Mnemiopsis leidyi* (Riisgård et al. 2012). While North Sea surface salinities are approximately 30 PSU, they decrease rapidly to about 20–25 PSU in the central Limfjord, 15–20 PSU in the southern Kattegat, and typically as low as 5 PSU in the Baltic Proper (Kautsky and Kautsky 2000; Riisgård et al. 2012;

Momigliano et al. 2018; Geburzi et al. 2022). Indeed, strong water inflows from the North Sea transport high saline and oxygen-rich waters to the Baltic Sea, resulting in a permanent and pronounced halocline in the deeper basins at depths of about 70–100 m, along with a thermocline in summer (Ojaveer et al. 2010; Neumann et al. 2017). Due to the abrupt changing abiotic and biotic conditions along our study area from the North Sea via the Limfjord to the Baltic Sea, considerable changes in marine biodiversity may be expected. The low salinity environment within the Baltic Sea has direct consequences for marine fauna inhabiting this area, as they must engage in energetically expensive osmoregulation to survive (Plotnikov and Aladin 2011). As a consequence, many marine species in the low saline areas of the Baltic Sea often exhibit dwarfism, and/or their distribution range is restricted to non-brackish conditions (Kautsky and Kautsky 2000; Geburzi et al. 2022). This results in a decline in biodiversity with decreasing salinity toward the Baltic Sea, a phenomenon well documented from morphological species identification studies for invertebrates (Zettler et al. 2014; Geburzi et al. 2022) as well as fish (Koehler et al. 2022). In fact, the richness of marine macrofauna species decreases from about 1500 species at the Swedish North Sea coast to only 70 species in low saline areas of the central Baltic Sea (Kautsky and Kautsky 2000).

While the pronounced salinity gradient from the North Sea toward the Baltic Sea is an important factor shaping their fauna, as determined by traditional sampling campaigns, we aim to test whether this pattern can be identified through indirect and noninvasive metazoan biodiversity assessments based on eDNA metabarcoding. This will be complemented by metabarcoding and traditional morphological identification of ZP from net catches. Specifically, we evaluated (a) whether eDNA mirrors the metazoan community known and typical for the study area and present at the time of sampling, (b) the vertical turnover of eDNA from surface to bottom, (c) the ability of eDNA metabarcoding to identify the same trends known from morphological identification methods, and (d) changes in metazoan assemblages as a function of spatial distance and decreasing salinity from the North Sea to the Baltic Sea. We therefore analyzed spatial differences in the marine metazoan community along a salinity gradient from the North to the Baltic Seas, using a combination of eDNA from water (surface and bottom) and sediment, ZP metabarcoding, and morphological identification.

2 | Methods

We analyzed the metazoan invertebrate biodiversity from seawater, sediment, and ZP samples based on multiple-marker metabarcoding along a salinity gradient from the North Sea via the Limfjord to the Western Baltic Sea to identify spatial changes in marine fauna with decreasing salinities.

2.1 | Water, Sediment, and Zooplankton Sampling and Sample Processing

We sampled water and ZP from 28 stations between August and September 2020 on board of RV Uthörn (UT2020-01) (Table S1). Physical oceanography data were recorded using a CTD at all stations, except Stations 6 and 22, and the raw data are available in the public repository PANGEA (Krock et al. 2023). Sampling started in the North Sea, through the Limfjord and Kattegat and continued in the Western Baltic Sea. For 14 of the 28 stations, marine invertebrate communities were analyzed using all four approaches, that is, (i) eDNA from surface waters (SW), (ii) eDNA from bottom waters (BW), (iii) eDNA from sediments (SE), and (iv) ZP from mainly vertical hauls (150 µm mesh size). For the remaining stations, a subset of the sampling approaches was used (Figure 1 and Table S1).

Water for eDNA analysis was sampled from the bottom (BW; ~5 m above the seafloor) and surface (SW; ~5 m below surface) at all stations, except Station 22, with a CTD rosette equipped with 4 L Niskin bottles. At stations where water depth was shallow, sampling was conducted close below the surface and above ground to maximize the distance between surface and BW eDNA sampling. From each depth, 3 × 1 L seawater (technical replicates) were vacuum-filtered through 0.2 µm nitrocellulose filters (ø 47 mm, Whatman, WHA10401712). Filters were preserved in low-bind tubes containing 720 µL DNeasy Blood & Tissue ATL buffer (Qiagen) as well as 0.5 g Zirconia-Silicate beads (ø 0.5 mm, Biospec) and frozen at −28°C until DNA extraction. In addition, filtration negative controls (UV-treated water) were included and treated as regular samples to control for potential contamination (for details on contamination prevention, see Supplement S2).

Sediment for eDNA analysis was collected at all stations using a Van-Veen grab. About 0.25–1 g of the surface SE was scratched with a sterile spatula in technical replicates, placed in 2 mL low-bind tubes and immediately frozen at −28°C until DNA extraction.

ZP was sampled using vertical WP2 net hauls (150 µm mesh size, 57 cm mouth opening) with maximum depth of 24.7 m. Vertical hauls were taken at 16 stations, while at two shallow stations (8, 15), ZP was sampled by towing the net horizontally at three knots for 5 min (Table S1). Since we did not use a flow meter, the exact water volume cannot be determined. To get an approximate idea, we calculated the analyzed water volume based on reference data. In a previous study, an average of 36,617 L of water was sampled by vertically towing a WP2 net with similar mesh size (200 µm instead of the 150 µm we used) from 150 m depth to the surface (Altukhov et al. 2015). Applying this to our sampling depth between 6 and 24.7 m, the sampled water volume would range from approximately 1500–6000 L across stations. On board, ZP was concentrated on a metal sieve (63 µm mesh size), preserved in absolute ethanol, stored at 7°C and re-fixed after 24 h. In the home laboratory, samples were split into four equal parts with a Motoda splitter (Motoda 1959). In each case, the splitter was rotated 20 times. One quarter of each sample was analyzed for morphological species identification and the second quarter for metabarcoding. The remaining half was kept as a back-up sample. Metabarcoding was conducted for all 18 ZP samples, while morphological identification was conducted on the three most distant samples: the westernmost North Sea station 7, the easternmost Kattegat station 21, and the easternmost Baltic Sea station 27.

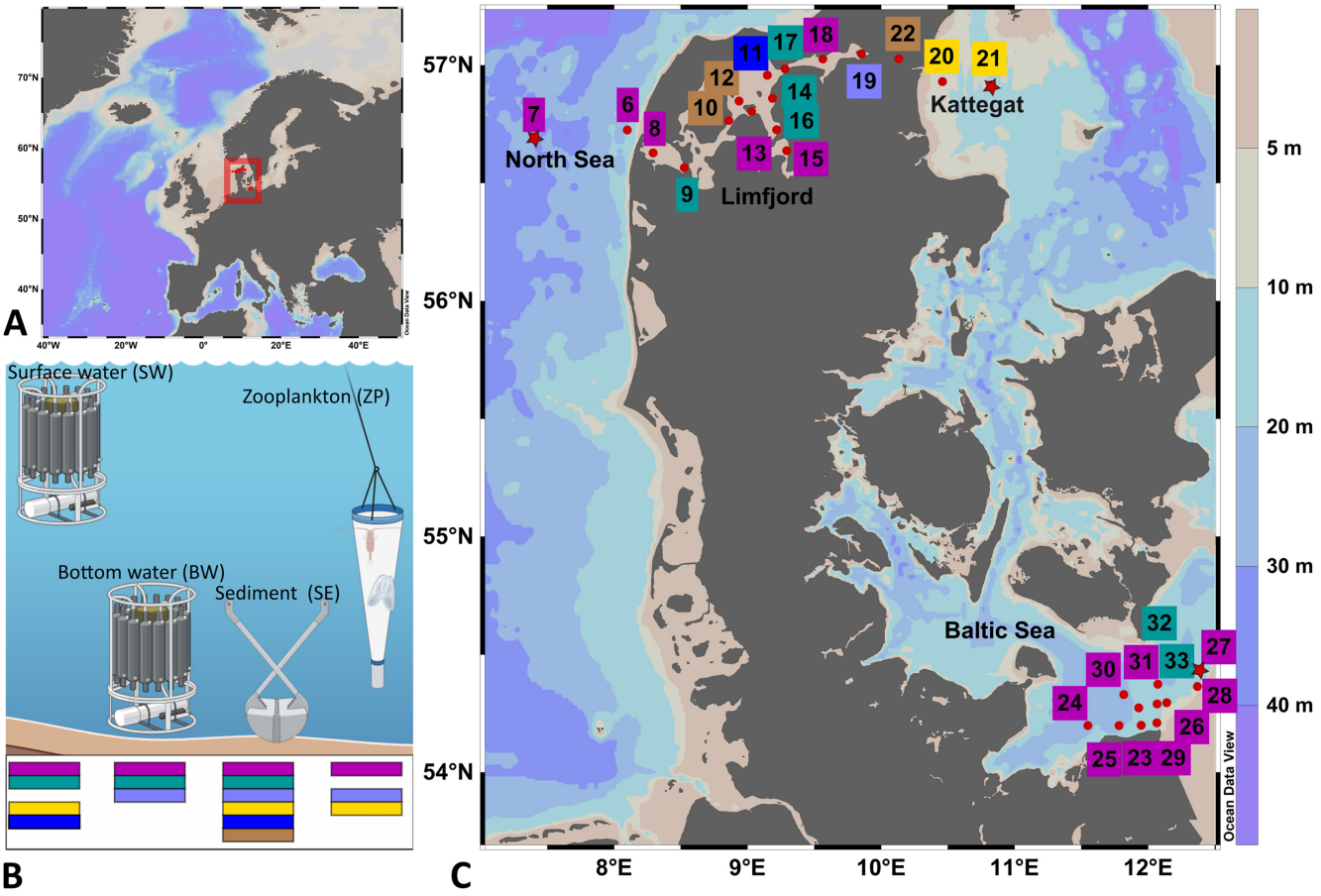

FIGURE 1 | (A) Geographical overview; (B) sampling for eDNA was performed via CTD rosette for surface water (SW), bottom water (BW), and via Van Veen grab for sediment (SE); as well as by vertical WP2 net hauls for zooplankton (ZP). (C) Magnified map of the study area: North Sea, Limfjord, Kattegat, and Western Baltic Sea. Stations for which metabarcoding data of all four sampling approaches are available are marked in pink. Turquoise: SW, BW, and SE; purple: BW, SE, and ZP; yellow: SW, SE, and ZP; blue: SW (18S only) and SE; brown: SE (only). Zooplankton was additionally identified by morphological species-specific characteristics for three stations (7, 21, 27), marked with asterisks. The maps were generated with Ocean Data View (Schlitzer 2018), the sampling illustration was created with BioRender.com.

2.2 | DNA Extraction, Library Preparation, and Sequencing

DNA was isolated from filters and bulk ZP using the DNeasy Blood and Tissue kit whereas sediment samples were extracted using the DNeasy PowerSoil kit (Qiagen). Filters were thawed at room temperature and DNA extracted following bead homogenization according to Ohnesorge et al. (2023) with the modification of using 80 μL proteinase K. The one quarter ZP samples designated for metabarcoding were further split into three pseudoreplicates using the Motoda splitter as described above. DNA extraction was performed according to Ohnesorge et al. (2023) with the exception of first eluting the DNA with 100 μL and thereafter with 50 μL AE buffer (150 μL total elution volume).

Sediment samples were extracted according to the manufacturer's protocol with adjustments as follows: samples were thawed on ice and then vortexed at 10,000*g* for 30 s to remove excess water. For removal of salt, the sample was washed with 1 mL Dulbeccos's Phosphate Buffered Saline (DPBS) without calcium and magnesium. After vortexing, 450 μL of the suspended sediment was transferred to the PowerBead tube (without beads and C1) and centrifuged at 10,000*g* for 2 min and the fluid discarded. After repeating the washing step, beads and C1 were re-added to the tube and the tube was incubated at 70°C for 10 min. The remaining steps followed the manufacturer's protocol with the exception of an elution in 2 × 50 μL Solution C6.

All DNA extracts were checked for purity and concentration on a NanoDrop spectrophometer. Samples were stored at −20°C until amplification. For each station and approach, the DNA extract of one of the technical replicates was used for sequencing. We amplified two molecular markers, the 313 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) using the primer pair mlCOIintF-XT (Wangensteen et al. 2018), jgHCO2198 (Geller et al. 2013) and the approximately 430 bp fragment of the nuclear ribosomal 18S rRNA gene variable region 4 (V4) (will be named "18S" hereafter) using the primer pair Uni18S and Uni18SR (Zhan et al. 2013). For library preparation, we applied a two-step PCR following the 16S Metagenomic Sequencing Library Preparation Guide from Illumina (Illumina 2013), which was adjusted for metazoans (Ohnesorge et al. 2023). All PCR clean-ups were conducted with CleanNGS magnetic beads (GC biotech) and amplicon PCR products were checked on 1.5% agarose gel stained with ethidium bromide for quality control. Five of the filtration negative

controls amplified with 18S primers showed a product on the gel and therefore were sequenced for further investigation. For some stations, PCR failed for all SW/ BW triplicates for either one or both markers (Limfjord: 7/12 stations affected, Kattegat: both BW, see Figure 1 and Table S1), probably due to large amounts of PCR inhibitors. These failed samples were excluded from downstream analysis. Library concentrations were determined on a LabChip (PerkinElmer). The final concentration of the pooled library was validated on a BioAnalyzer (Agilent). For both markers, 15% PhiX were spiked in as an internal control. 96 libraries per run were sequenced on an Illumina Miseq using MiSeq v3 reagent kits generating 2×300 bp paired-end reads aiming for about 120,000 raw reads per sample.

2.3 | Sequence Data Processing

Sequence processing was carried out as in Ohnesorge et al. (2023) with DADA2 (Callahan et al. 2016) and cutadapt (Martin 2011) pipelines. Truncation lengths in this study were adapted to 270 bp (forward) and 240 bp (reverse) for COI and 280 bp (forward) and 250 bp (reverse) for 18S. Taxonomic assignment was conducted with the RDP Naïve Bayesian Classifier (Wang et al. 2007) against the MetaZooGene database (Bucklin et al. 2021) for COI and for 18S V4 against a subset of SILVA and PR2 both curated against WoRMS (WoRMS Editorial Board 2022). In addition, BLAST (Altschul et al. 1990) against NCBI GenBank (Sayers et al. 2020) was performed for both markers and 10 best hits screened in order to eliminate non-metazoan assignments (i.e., top hits based on max score that did not indicate metazoan origin) (five COI and two 18S ASVs). To further examine metabarcoding results for potential incorrect assignments (false-positive detections), species were checked for their distribution ranges as described in OBIS (obis.org), WoRMS (WoRMS Editorial Board 2022) as well as species inventory lists of the North Sea (Armonies et al. 2018) and Baltic Sea (Zettler et al. 2018) and through consultation with taxonomic experts (pers. communication). Based on this, we ranked a species' likeliness of occurrence as "very likely" (i.e., known to be present in the study area), "likely" (i.e., unknown from the study area but recorded distribution in adjacent waters), or "very unlikely" (i.e., species' known distribution outside the study area, the entire North Sea and North East Atlantic). All downstream analyses were performed on species level in R (R Core Team 2021) if not stated otherwise.

2.4 | Zooplankton Identification by Morphology and DNA Barcoding

ZP from three stations (7, 21, and 27) was identified to the lowest taxonomic rank possible based on morphological diagnostic characters using identification guides (Larink and Westheide 2011; Conway 2012b; Conway 2012a; Conway 2015; Castellani and Edwards 2017; Hayward and Ryland 2017). For this, we split the one quarter samples designated for morphological identification further into eight sub-samples as described above and processed them individually. The first subsamples were counted entirely, whereas in the remaining subsamples only taxa with a total abundance of < 50 individuals in the previous subsamples were considered. Total abundance of each taxon was extrapolated correspondingly. The identity of 15 unknown specimens was confirmed by COI barcoding through Sanger sequencing (see Laakmann et al. (2013) for details). Sanger sequencing was performed at LGC Genomics, Berlin, Germany. Raw sequence data were processed using Geneious v 2022.2.1 (Biomatters Ltd.) and taxonomic assignment of species was performed using BLAST (Altschul et al. 1990) against NCBI Genbank.

2.5 | Data Analysis

Maps and oceanographic profiles were created with Ocean Data View (Schlitzer 2018) and BioRender.com. For the analysis of the effects of sampling approach, distance, and salinity, COI and 18S site-by-species matrices were combined. To test for differences in community composition resulting from sampling approach, vertical transport as well as spatial distance and salinity, occurrence-based species turnover values (i.e., true replacement of species, independent of species richness differences between samples) were calculated based on a qualitative species matrix and the function *vegdist* on Jaccard dissimilarity indices (Jaccard 1912). Turnover thus describes the dissimilarity in species composition (e.g., across samples) and ranges from 0 (identical species composition) to 1 (no species in common between the two samples) (Baselga et al. 2021).

Turnover values across all possible combinations of sampling approaches were calculated using site-by-species matrices summarizing all species of the respective approach. For this, only stations at which all eDNA and ZP sampling approaches have been conducted were considered.

In order to assess the effects of spatial distance and salinity on species turnover, we conducted multiple matrix regression (Multi Mantel tests with 999 permutations) with *phytools* (Revell 2012). As sample numbers across sampling approaches were unbalanced and the detected community compositions were expected to be influenced by sampling approach (SW eDNA, BW eDNA, SE eDNA, ZP), the Multi Mantel tests were performed individually per sampling approach. To remove the effect of temporal bias (as samples were taken during two subsequent months), we used the residuals of the relationship between turnover and time (Julian days) as the response variables in the multiple matrix regressions. These residuals were estimated with simple Mantel tests using *daee* (Debastiani 2021). To quantify the dissimilarities between species assemblages introduced by spatial distance and salinity, explanatory variables were transformed into Euclidean distances. Spatial distance was represented by the combined effects of longitude and latitude. Salinity values were obtained at 2.5 m for SW eDNA, at average depth for ZP samples, and at maximal depth for both BW eDNA and SE eDNA.

To determine which species were significantly associated with a certain sampling approach and sampling region, we conducted indicator species analyses using the R package *indicspecies* (with default settings and 999 permutations) (Cáceres and Legendre 2009). The indicator species analysis tests for (a) the probability of a species being exclusively detected with a specific sampling approach or exclusively occur in a specific sampling

region and (b) the sensitivity of that species as an indicator of the sampling approach or sampling region (i.e., in what percentage of samples belonging to a certain target group was the species detected). For a more detailed description, see S6. Firstly, we tested which species were significantly associated with a certain sampling approach with SW eDNA, BW eDNA, SE eDNA, and ZP net catches as target groups. Similarly, we assessed which species were typical representatives of a certain region (North Sea: Stations 6,7, Limfjord: Stations 8–19, 22, Kattegat: Stations 20, 21, and Baltic Sea: Stations 23–33). Indicator species analyses testing the regional differences were performed on all sampling approaches combined as a first step and as a second step on the four sampling approaches separately in order to gain a more detailed resolution on which sampling approach may best resolve the regional differences in the species community.

3 | Results

3.1 | Oceanographic Parameters

Both sea surface temperature (SST) and sea surface salinity (SSS) were higher in the western sampling area (North Sea, Limfjord, and Kattegat) compared to the Baltic Sea stations (Figure 2). SSTs were generally higher in the shallow Limfjord area (20.6°C–22.6°C) compared to the North Sea and Kattegat while SSS decreased from West (North Sea: 32.4 PSU) to East (Kattegat: 20.2 PSU) (Figure 2A). The vertical temperature and salinity profiles indicated a mixed water column in large areas. Exceptions were the deeper North Sea and Kattegat stations as well as two Limfjord stations (15 and 16) located in the southern central part in the Lovns Bredning area with an apparent thermocline (Figure 2A). Baltic Sea stations were characterized by an overall lower SSTs (about 17°C at all stations) and lower overall salinity (max. 22 PSU) compared to the North Sea-Limfjord-Kattegat region with a decreasing trend in SSS from West to East (Figure 2B). All stations (except the shallow station 31) had a distinct halocline with salinity leveling within the upper 5 m at coastal stations and at approximately 10 m at stations further offshore and thereafter increased sharply in parallel to depth. The difference between surface and BW salinity ranged between 2.2 PSU (shallow station 31) and 10.2 PSU at the station furthest from the shore (Station 30). An additional thermocline was apparent at stations with a greater depth (western stations) below 12 m but did not exceed a temperature difference of more than 3.2°C between SW and BW layers (Figure 2B).

3.2 | Invertebrate Community Identification

In total, the metabarcoding approaches identified 279 metazoan species from 16 phyla (S3 and S4). Out of these, 182 species were detected based on COI and 126 based on 18S, with 29 species identified by both markers. Most of these species belong to Arthropoda (65 species), followed by Mollusca (48) and Annelida (37) (Table S4). The majority of these species belong to the zoobenthos (134), followed by meiofauna (82), holoplankton (38), species with alternating asexually and sexually reproducing life cycle such as scyphozoa and hydrozoa (16), parasitic (7), and nekton (2). Of the 279 species, a majority is known from these waters, as 74.6% (89.6% COI, 58.7% 18S) were classified as "very likely" and 12.5% (3.2% COI, 23.0% 18S) as "likely." Among those 36 species without a record in the sampling area

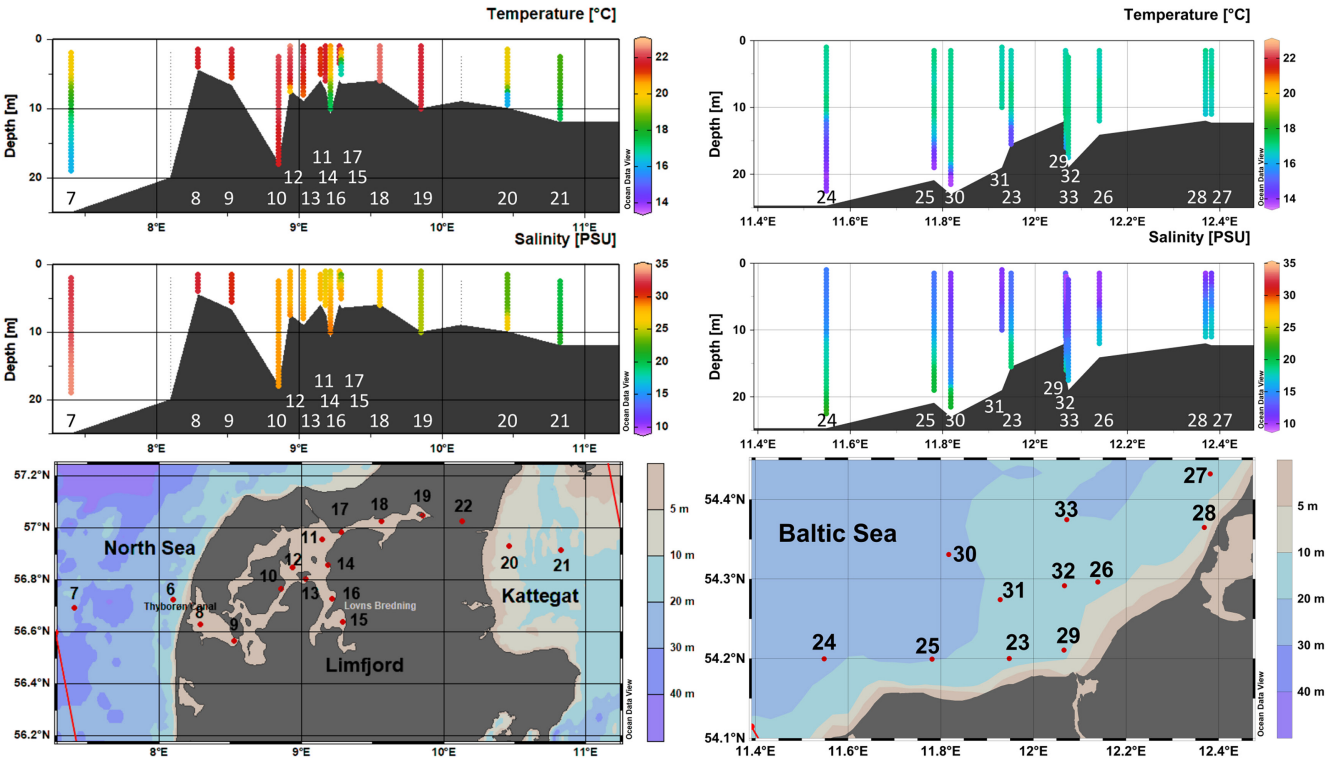

FIGURE 2 | Vertical temperature and salinity profiles of the sampling regions. (A) North Sea–Limfjord–Kattegat–region; (B) Baltic Sea region.

or adjacent regions, many belong to the meio- and infauna (6 Nematoda, 3 Platyhelminthes, 3 Xenacoelomorpha, and 7 Annelida) (S4). Fifteen of the 279 species were solely detected by SW and BW eDNA.

Morphological species identification from ZP net catches was applied as a reinsurance of the metabarcoding success to identify invertebrates across taxa and as a proof of on-site occurrence. In combination with COI barcoding, species identification by morphological diagnostic characters identified all the relevant (i.e., very species rich and dominant) phyla and sub-ranks detected by metabarcoding (S5), demonstrating the successful identification and coverage of metazoan taxa by our metabarcoding methodology. The few obvious false-negative metabarcoding detections on phylum level were Chaetognatha, Hemichordata, and Phoronida, which were represented by only a few species within the sampling area. The North Sea sample revealed the highest biodiversity in terms of number of taxa (30), followed by the Baltic Sea (17) and Kattegat sample (9). Among the young developmental stages, copepodite and nauplii stages of copepods were the most numerous groups. Calanoid copepods were the prevalent group in terms of quantity among the holoplanktonic organisms together with the appendicularian *Oikopleura* (*Vexillaria*) *dioica*. The North Sea sample was dominated by meroplankton (especially polychaete and echinoderm larvae). In the Kattegat sample, many specimens of the Branchiopoda *Penilia avirostris* were found. A typical representative for the Baltic Sea was the copepod *Pseudocalanus acuspes*. ZP metabarcoding of the respective samples reflected the same trend in decreasing species numbers from west to east with North Sea samples showing a biodiversity twice as high (61 species) as Kattegat (26 species), or Baltic Sea (22 species). Compared to morphological identification, metabarcoding of samples from the same stations resulted in > 6 times (North Sea) and 2.4 times (Baltic Sea) more species (S5). Many of the dominant species detected using morphological identification were also detected at most stations by SW and BW eDNA and ZP metabarcoding. For instance, this applied to the ctenophore *Mnemiopsis leidyi* and the copepod *Acartia* (*Acanthacartia*) *tonsa*. Although not specifically counted due to its shrinking behavior in ethanol, we noted many *M. leidyi* specimens in fresh net catches at the coastal North Sea station (station 6), in the Limfjord and in the Baltic Sea. Other dominant species were the Appendicularia *Oikopleura* (*Vexillaria*) *dioica* as well as the polychaete *Polydora cornuta*. In contrast to the most common species, more than half of the identified species from metabarcoding were detected exclusively at one or two stations in each sampling approach (60% from SW eDNA and BW eDNA; 61.3% from SE eDNA; 68.5% from ZP net catches).

3.3 | Effects of Sampling Approach on Diversity Detection

For many taxonomic groups, one of the sampling approaches was considerably more effective in detecting high species numbers (S4). ZP and SE eDNA detected more species than SW and BW eDNA (ZP: 178; SE: 163; SW: 74; BW: 89). ZP metabarcoding worked particularly well for many holo- and meroplanktonic taxa, particularly for Arthropoda (50 species) such as Branchiopoda, Hexanauplia, Malacostraca, and Thecostraca. In addition, all nine Echinodermata species and the majority of Hydrozoa and Gastropoda were identified from ZP metabarcoding. In the SE eDNA, we detected many infaunal taxa such as Nematoda, Platyhelminthes, Xenacoelomorpha, and all Gastrotricha. BW eDNA was the most effective approach in detecting Bivalvia and other benthic taxa, thus likely adults. Biodiversity recovered from SW eDNA was most similar to the biodiversity recovered from ZP net catches. SW eDNA contributed the least, in terms of additional taxa, to the biodiversity assessment.

Turnover values are a measure of dissimilarity (here across sampling approaches) with a value of 1 representing maximum difference in community compositions. They provide information about (a) the usefulness of markers to resolve differences in the identified species assemblages, (b) the similarities and differences of species assemblages across the sampling approaches, and (c) the presence and sinking of (dead) organisms (ZP) and eDNA within the water column (Figure 3, S6 for station-specific turnover). Turnover values for the community identified from ZP net catches increased with eDNA sampling depth, that is, ZP communities were most similar to the communities identified from SW eDNA, less similar to the one from BW and most different to SE eDNA. Alike ZP, communities derived from SW eDNA decreased in similarity inversely to depth, that is, turnover values were lower for BW than for SE. In general, the community from SE eDNA displayed a distinct community compared to water eDNA and ZP, with the highest turnover values across all other sampling approaches. However, among the species detected in the sediment were also holoplanktonic species (mainly calanoid copepods).

3.4 | Changing Communities Along the Salinity Gradient Based on Metabarcoding

We assessed the expected and known differences in marine metazoan species assemblages driven by the confounding factors spatial distance and salinity. In order to estimate to what extent each sampling approach would be suited to display these differences, the four sampling approaches were analyzed separately.

ZP metabarcoding identified > 60 species in the North Sea and the species numbers decreased sharply in the eastern direction (S7). Based on SW eDNA, the highest species number was identified at the westernmost Limfjord station (32 species). BW eDNA results did not resolve a clear trend in species numbers by region, although it was low at most Baltic Sea stations. Sediment samples neither demonstrated an evident pattern regarding species numbers.

PCoA revealed a clustering of area-specific communities for all sampling approaches and emerged to explain between 24% and 35% of variance on the first two axes (SW eDNA: 33.8%; BW eDNA: 32.6%; SE eDNA: 24.2%; ZP: 35.4%) (Figure 4). We ran multi Mantel tests to statistically examine whether spatial distance and salinity had an effect on species assemblages identified by eDNA and ZP metabarcoding. Neither spatial distance nor salinity significantly explained changes in the community composition for any of the four sampling approaches (S8). In

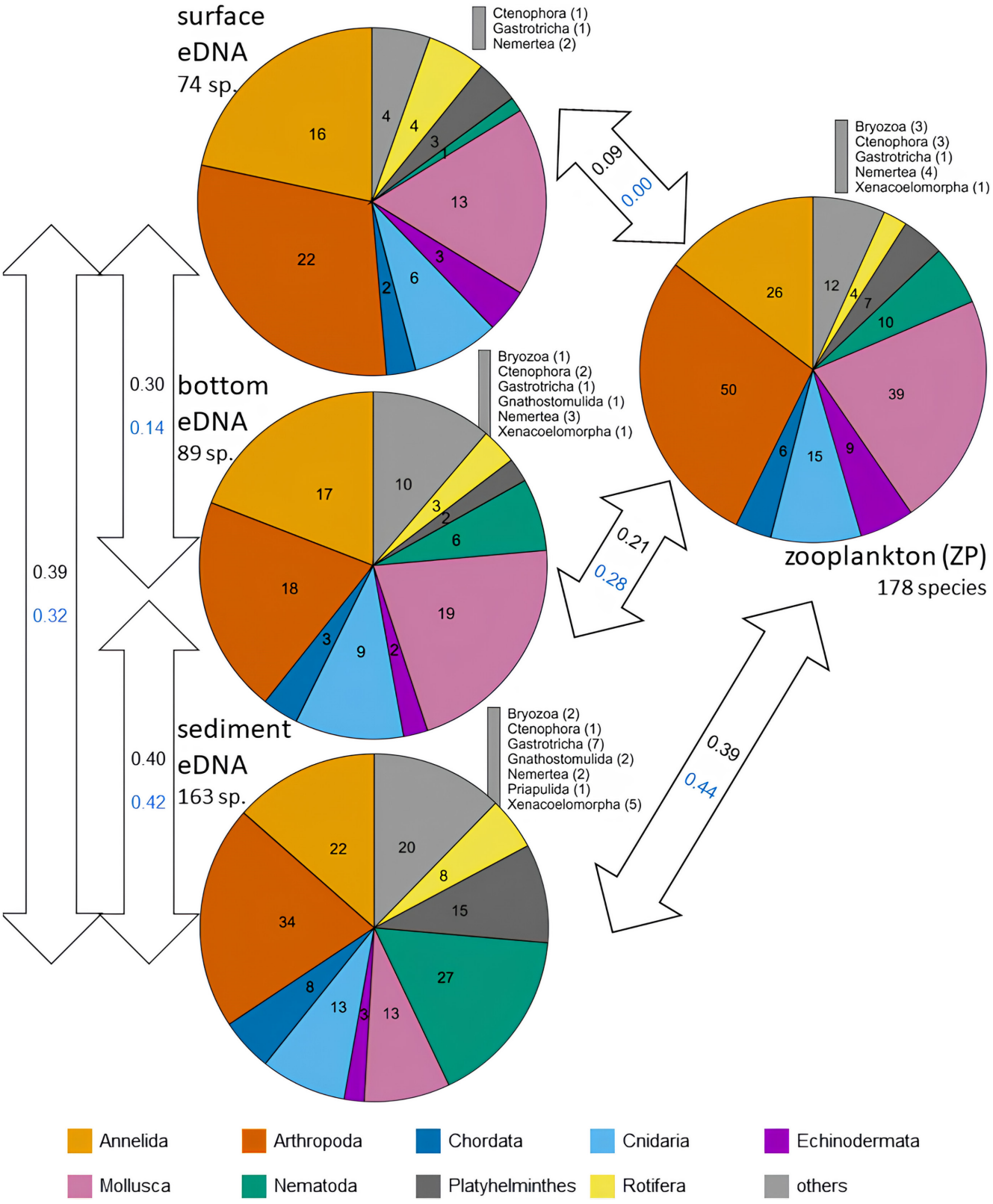

FIGURE 3 | Species identified per sampling approach (left from top to bottom: surface water (SW) eDNA, bottom water (BW) eDNA, sediment (SE) eDNA; right: zooplankton (ZP) net catches grouped by phylum of the entire dataset. Numbers in the pie chart correspond to the number of species per phylum. Turnover values were generated for those stations only at which all four sampling approaches were performed. Numbers in arrows represent turnover values between the sampling approaches. Black: COI; blue: 18S V4.

order to disentangle the confounding effects of spatial distance, salinity, and day at which sampling was conducted, we performed additional variance partitioning (S9).

In accordance with the PCoA, variance partitioning revealed the highest explanatory power for the community identified from SW (16% explained variation), followed by ZP (14%), BW (12%),

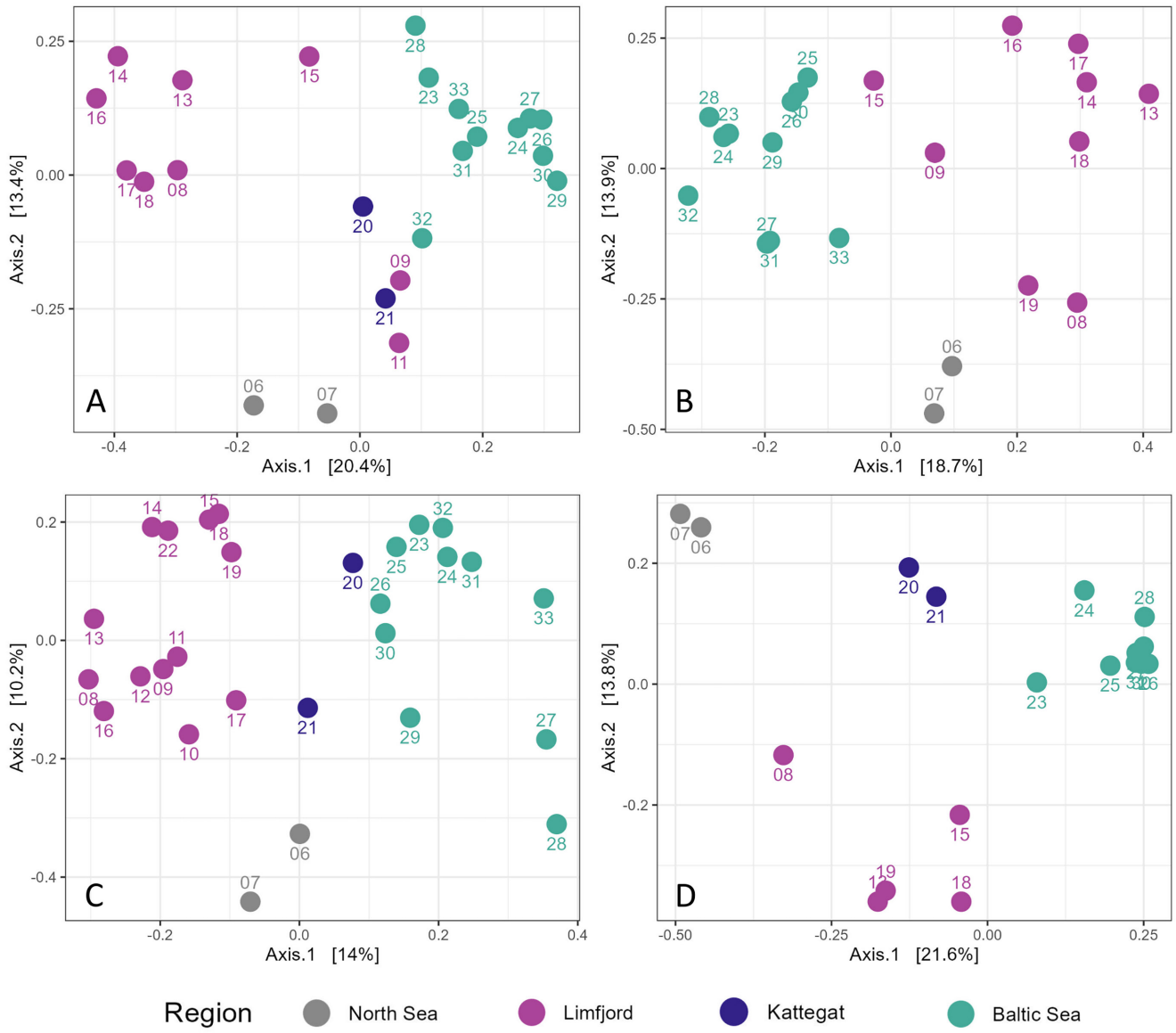

FIGURE 4 | PCoA of species dissimilarities (Jaccard distances) based on communities identified (COI and 18S detections were merged). Colors represent sampling areas; gray: North Sea; magenta: Limfjord; blue: Kattegat; green: Baltic Sea. Subplots were separated by sampling approach; (A) SW eDNA; (B) BW eDNA; (C) SE eDNA; and (D) ZP net catches.

and SE (5%). For both SW and ZP, which showed very congruent results in both PCoA as well as variance partitioning, the largest part of the explained variation belonged to the shared portion of the three variables (10% for SW eDNA and 11% for ZP), that is, the most predictive variable cannot be determined. Across all sampling approaches, spatial distance in BW communities had the highest predictive power (3%) for a single variable.

3.5 | Indicator Species Analysis

The indicator species analysis (S10) examined in detail, which species were significant indicators of any of the applied sampling approaches. The high number of species detections from the ZP net catches and SE eDNA was confirmed by indicator species analysis, which assigned 44 species as significantly associated with the ZP nets (i.e., these species were identified in the majority of ZP net samples and/ or this method was superior in the detection of these species in comparison to other sampling approaches). Similarly, the indicator species analysis identified 19 species significantly associated with SE samples, which overall represented the infauna. It also confirmed that SW eDNA contributed the least number of additional taxa to the biodiversity assessment, since none of the identified species were significantly associated with this sampling approach.

Further, indicator species analysis was used to assess which species are significantly associated with a certain region, with (a) all sampling approaches combined and (b) analyzed individually per sampling approach (S10). Indicator species analysis combining all sampling approaches confirmed that most of the significantly associated species exclusively occurred in the North Sea area and that holo- and meroplanktonic species shaped the regional differences across communities. SE eDNA, which mainly identified meiofauna, least reflected regional differences since out of the 163 species detected with this sampling approach, only

three species significantly explained spatial distribution based on indicator species analysis (S10 2D). From the water eDNA 9 (SW) and 14 (BW) species were significantly associated with a certain region (S10 2B & 2C, S4). There was strong evidence that several species were consistently associated with high saline areas. For instance, the hydrozoan *Lizzia blondina* was significantly associated with the North Sea based on water eDNA and ZP metabarcoding. The two copepod species *Centropages typicus* and *Calanus helgolandicus* were associated with the North Sea (based on water eDNA and ZP) and to the Kattegat (ZP). In total, 22 species were uniquely associated with the North Sea region based on ZP metabarcoding, confirming the distinct and relatively higher biodiversity in this area. For the Limfjord, Kattegat and Baltic Sea regions, significantly associated species largely differed across sampling approaches with the exception of the Rotifer *Synchaeta triophthalma*, which significantly contributed to the Baltic Sea communities in congruence with BW eDNA and ZP net catches.

4 | Discussion

The invertebrate biodiversity we identified with metabarcoding in the North Sea, Kattegat, Limfjord, and Baltic Sea is consistent with species known from these regions, with 87% of the species previously documented in this area. Moreover, our comparison of morphological and molecular identification of ZP highlights the recovery of all relevant phyla and groups and demonstrates identification success for invertebrates across groups based on our multi-marker metabarcoding approach. We only identified 36 species by metabarcoding, which are not yet recorded for these areas. Most of them were detected based on 18S V4, which partly does not resolve congeneric species (revealed by BLAST top 10 hits; S4). This applies especially for meiofauna and benthic species such as Nematoda and Xenacoelomorpha, which has already been observed earlier (Ohnesorge et al. 2023). We therefore encourage careful scrutiny and cross-validation of annotations for invertebrate sequence data based on 18S V4. In addition, we identified some potential errors in sequence references, which resulted in untrustworthy annotations (e.g., data-deficient sequence references) and thus high probability of dubious assignments to species. However, some of the species previously not recorded in this area could be true positive detections such as the annelids *Pisione puzae* and *Fabriciola liguronis*. For these species, we could not find any plausible explanation that would indicate an incorrect annotation.

4.1 | Sampling Approaches Map Different Communities

All sampling approaches appear to differ considerably in terms of the identified species and communities. SW eDNA mainly reflected the holo- and mero-ZP communities from the nets that were present in the water column at the time of sampling. This is confirmed by low turnover among the two sampling approaches. This implies that the eDNA remains in the same water layer in which it was released by the species present. The higher species numbers identified with ZP net catches is achieved by sampling substantially larger volumes of water. In a previous study, we demonstrated that the higher the volume of water sampled for eDNA, the higher the number of species detected (Ohnesorge et al. 2023). Even with very high eDNA sampling efforts that were still far beyond volumes usually sampled with ZP nets, this was demonstrated to be not enough water to capture the entire community present (Ohnesorge et al. 2023). Nevertheless, repetitive sampling can potentially yield a higher overlap up to 42% of species identified in surface water eDNA and ZP (Ohnesorge et al. 2023). A common finding is that higher species numbers of Actinopteri and Arthropoda are identified from ZP net catches compared to eDNA, while from eDNA samples, other taxa are supplemented such as many Ascidians, Anthozoa, and Porifera species (Djurhuus et al. 2018; Leduc et al. 2019; Ohnesorge et al. 2023).

BW eDNA additionally recovered macrozoobenthos, especially bottom-dwelling bivalve species. Our results of high species turnover between SW and BW eDNA indicates the useful application of eDNA metabarcoding to resolve the differences in species communities between different water layers. This was also demonstrated for some holoplanktonic species that were only detected by eDNA from the upper water column, but not from near-BWs (Parry et al. 2020).

SE eDNA (and partly organismal DNA) successfully identified the in- and meiofauna. Our findings are supported by previous studies where recovery of species differed between eDNA sampling approaches (Brandt et al. 2021; Clarke et al. 2021).

Mechanistic models suggest that patterns of waterborne eDNA distribution are shaped, to a limited extent, by vertical displacement such as advection, dispersion and settling (Allan et al. 2021). Hence, eDNA is expected to remain in its water depth of origin/ shedding with merely 10–20 m deviation and vertical movement being of less importance than biological (e.g., shedding and migration) or eDNA degrading parameters (breakdown and decay rate) (Allan et al. 2021). There is an ongoing debate whether stratified water particularly at greater depths does act as a mixing barrier for eDNA (Zhang et al. 2020). The sampling depth of water for eDNA analysis thus may determine which species or groups could be detected (Andruszkiewicz et al. 2019; Larson et al. 2022). Based on our findings of large differences between communities detected using SW and BW eDNA, we argue that even very shallow, mixed water columns (sometimes not exceeding 4 m) resolve differences in the communities detected. Thus, vertical eDNA profiles may have the potential to draw conclusions on a species' depth range profile including vertical migration pattern, as previously modeled (Allan et al. 2021).

Results from SE eDNA in this and other studies revealed high numbers of species from in- and meiofauna-typical phyla, such as Platyhelminthes, Gastrotricha, Nematoda, Nemertea and Xenacoelomorpha (Cordier et al. 2019; Holman et al. 2019; Brandt et al. 2021; Clarke et al. 2021). This is underlined by the distinct species composition with high turnover values across the other sampling approaches in our study, indicating that (a) we did not only identify eDNA but mainly organismal DNA, and (b) vertical mixing or sinking of particles was very limited, even between aboveground water and sediment in shallow waters. Similarly, distinct communities between SE eDNA and BW eDNA have been demonstrated on samples in the English

Channel, with again in- and meiofaunal representatives dominating the sediment samples (Holman et al. 2019).

Next to the meiofauna organismal DNA, SE (e)DNA may originate from genetic traces shed by meiofauna, from dormant forms or material from dead organisms sinking within the water column (Bruce et al. 2021). In our study, the evidence of pelagic taxa such as calanoid copepods likely originated from resting eggs in the sediment or from parts of dead organisms. Therefore, it also reveals the (previous) occurrence of these species in this region at an unknown span of time before sampling. This appears possible because of the preservation properties of marine sediment for (e)DNA, which allows the detection of species-specific (e)DNA several months after shedding and thus the reconstruction of past species occurrences (Ogata et al. 2021). As a consequence, including SE eDNA not only complements the species with typical in- and meiofaunal taxa, but also allows to recover past species occurrences and the existence of resting eggs in sediments. How long the remains of dead organisms, which are partially preserved in the sediment, do trigger positive detection after the absence of the original or source organism in a particular environment, still needs to be investigated in more detail.

Especially in this context, there is the need for a critical consideration of present and past species occurrence based on eDNA identifications. For example in our study, at both North Sea and Western Limfjord stations the jellyfish *Aurelia aurita* was absent in the ZP, SW, and BW eDNA but present in the SE eDNA, indicating their occurrence days or weeks before sampling. In contrast to this, the frequent and simultaneous detection of *Mnemiopsis leidyi* and *Acartia* (*Acanthacartia*) *tonsa* in SE eDNA and ZP nets (the latter based on metabarcoding and morphology) confirm their on-site occurrence at the time of sampling as well as in the past.

For the meroplanktonic species, detections by ZP metabarcoding prove the current presence of their larvae and hence their active reproduction. For instance, the polychaete *Alitta succinea* was significantly associated with SW and BW eDNA, and ZP net. The identification of high amounts of juveniles and trochophora larvae by morphological assessment from the North Sea underlines the high reproductive state of this species at the time of sampling. Thus, the comparison of several sampling approaches allows the reconstruction of species occurrences at different points in time as well as species-specific reproductive seasons.

In conclusion, the strong agreement of ZP with SW eDNA suggests that species detected in SW eDNA reflect the community currently present in the water column. In contrast, BW eDNA rather identified the benthos. Our results demonstrate the different sampling approaches do complement each other for the fauna they identified. Their synergy gives more comprehensive insights in invertebrate biodiversity together with the information on current species-specific reproduction activity. To gain a more complete and realistic picture of the factually present diversity solely based on eDNA, we recommend conducting both SW and BW eDNA for increasing chances to recover the pelagic and benthic communities. eDNA derived from the sediment complements the biodiversity with typical in- and meiofauna phyla and may recover past occurrences of some pelagic species.

4.2 | Spatial Differences Across Abiotic Gradients

As per sampling region, abiotic factors changed from west to east with a decreasing trend in surface water salinity and pronounced haloclines at almost all Baltic Sea stations. Temperature decreased inversely to depth, but pronounced thermoclines were solely recorded at few deeper stations.

Species detected at almost all stations by metabarcoding are mainly representatives of euryhaline species known from this area. For instance, this was the case for *A.*(*A.*)*tonsa* (Ojaveer et al. 2010; Geburzi et al. 2022) and the invasive ctenophore *M. leidyi* (Jaspers et al. 2011; Riisgård et al. 2012). Similarly, we identified the polychaete *Polydora cornuta* at all Baltic Sea stations, a very abundant species in this region (Gogina et al. 2016; OBIS 2023).

In our study, patterns of invertebrate species richness decreased in the transition from marine to brackish conditions as already demonstrated in previous long-term morphological and barcoding species inventories (Zettler et al. 2014; Geburzi et al. 2022). Species richness of the marine macrozoobenthos decreases from > 1150 in the Kattegat (salinity about 15–20 PSU) to 421 species in the Bay of Mecklenburg (Zettler et al. 2014) with surface salinities below 15 PSU where our Western Baltic Sea samples originate.

Our study revealed the trend of decreasing diversity to the low salinity areas was only resolved by ZP metabarcoding but not by any of the eDNA approaches (even though overall communities identified from ZP and SW eDNA were similar). We found the ZP samples of the most saline, westernmost stations to be distinct in the high number of species detected similarly to previous findings on the effect of salinity on ZP communities (Casas et al. 2017; Yebra et al. 2022). Contrary to the declining biodiversity with decreasing salinity described, which was confirmed for ZP based on both the molecular and morphological approach, SW eDNA analysis detected the highest number of species at the coastal station where the North Sea enters the Limfjord. The most likely explanation is a higher concentration of eDNA in the shallow, less dynamic and to some degree isolated Limfjord area in comparison to more dynamic waters at the offshore North Sea station, potentially leading to less dilution. This is supported by the fact that extracted DNA concentrations were twice as high at this Limfjord station compared to the offshore North Sea stations. It may be speculated that the transition from offshore to lotic waters accumulated the eDNA of the species-rich community at this high-saline station and increasingly displays local rather than regional biodiversity (Deiner et al. 2017).

Although species numbers in the Baltic Sea recovered from BW eDNA were generally low, two deep stations, which were characterized by a strong halocline, stand out with high species numbers. Such strong haloclines are commonly found during summer in this area (Narberhaus 2012). Here, SW salinities were lower (14 PSU) than BW salinities (> 20 PSU), which suggests an origin from inflowing North Sea water below the halocline and hence, resulted in the identification of many typical North Sea species. However, this pattern was not visible in the communities identified from the SE eDNA. Although metabarcoding studies on meiofauna show

significant differences between high and low salinity areas (Urban-Malinga et al. 2006; Broman et al. 2019), considerably more sediment was analyzed instead of just the top layer of the sediment as in this study. For the resulting species composition, there seems to be no influence of salinity and spatial distance, which could possibly be explained by the drift of the particles through the water column. A similar result was found by Holman et al. (2019), who concluded that the community they detected from eDNA in the narrow surface layer of sediments was the least suitable to explain differences in association with salinity or spatial distance.

Results from the indicator species analyses supported the ZP shaping identified differences in regional communities in our study and not the fauna identified from the sediment. Therefore, various factors should be considered when identifying current species richness (alpha diversity) and when drawing ecological interpretations solely based on eDNA analyses. For instance, as demonstrated, the eDNA sampling approach can cause substantial bias. When water is sampled across stations with heterogeneous depths and flow velocity, this has to be taken into account as the identified species richness will be influenced by these factors, and not necessarily reflect the actual spatial species richness present in the field.

The results from PCoA, variance partitioning and indicator species analyses indicate regional differences in species compositions. In the following paragraph we discuss these regional differences on the example of planktonic copepods, for which we have most of the information on their occurrences. SW eDNA, BW eDNA, and ZP samples reflect the expected pattern of species community distribution on a regional scale as known from literature. Seven species of the copepod genera *Pseudocalanus*, *Acartia*, and *Temora* have been identified in the study area by morphology and/or metabarcoding and these genera belong to the most important ZP taxa in terms of biomass and production in the open Baltic Sea (Ojaveer et al. 2010). The high number of nauplii compared to adults in the Baltic Sea sample indicated high reproduction rates at the time of sampling for one or some of these species, namely *Pseudocalanus acuspes*, *A.* (*Acanthacartia*) *tonsa*, *A.* (*A.*) *bifilosa*, *A.* (*Acartiura*) *hudsonica*, *A.* (*A.*) *clausi*, *A.* (*Acartiura*) *longiremis, and T. longicornis. A.* (*A.*) *bifilosa* was significant for both the North Sea and the Baltic Sea group, which is in line with its ability to tolerate nearly freshwater conditions and thus thrives even in low saline northern parts of the Baltic Sea (Ojaveer et al. 2010). Most of the abovementioned species have been identified from ZP and SW eDNA with the exception of *A.* (*A.*) *hudsonica* with most detections in the SE eDNA, some in the ZP and, only for the North Sea, in the SW and BW eDNA. This species was recently identified to occur in the North Sea (Ohnesorge et al. 2023) and the Baltic Sea (Hahn and Brennan 2024) based on their sequence data. For the Baltic Sea, this species is potentially replaced by *A.* (*A.*) *tonsa* in late summer (Hahn and Brennan 2024) and this observation matches with our findings. We detected this species predominantly in SE eDNA in September, indicating its former occurrence in the water column and/or the existence of resting/dormant eggs in the sediment, a well-known life-history trait of this species (Holm et al. 2018). These resting eggs are mainly produced toward the thermal boundaries of this species' distribution (Holm et al. 2018), which matches with our observations in September, a time when this species is potentially replaced. Another typical species, *C. typicus*, was found throughout the study area but the indicator species analysis only confirmed its association to the North Sea (SW, BW, and ZP) and the Kattegat (ZP) but not for the Baltic Sea. These findings suggest *C. typicus'* presence in the water column in high saline areas at the time of sampling as proven by ZP net samples. Its identification in the Baltic Sea from SE eDNA may result from sinking and drifting carcasses. In contrast, the congeneric co-occurring species *C. hamatus* has a higher salinity tolerance and is better adapted to the low saline conditions in the Baltic Sea (Ojaveer et al. 2010) and was significantly assigned to both the North Sea (BW eDNA) and the Baltic Sea (ZP) group by indicator species analyses.

In conclusion, our results demonstrated that ZP metabarcoding is more efficient in resolving the patterns of decreasing invertebrate species diversity with decreasing salinities from the North to the Baltic Sea compared to eDNA approaches. Nevertheless, our study shows that eDNA metabarcoding is capable of identifying changes in the invertebrate biodiversity across regions with typical species distribution patterns. This was more pronounced in eDNA analyses from water than from sediment samples. Combining several sampling approaches increases overall invertebrate species detection and further provides insights into past and present occurrences as well as succession patterns.

Author Contributions

Design of the study: A.O. and S.L. Sampling: A.O. and B.K. Sample processing: A.O., U.J., and S.L. Bioinformatics/data processing: A.O., S.N., and S.L. Statistics: A.O., L.K., K.C.B., and S.L. Manuscript: A.O. and S.L. All authors discussed the results and contributed to the final version of the manuscript.

Acknowledgments

We would like to thank a number of people for their kind support: RV Uthörn crew for their support with sampling; Annegret Müller for support in cruise preparation; Rohan Henkel, Daniela Voß, and Kristof Möller for providing CTD data; Christoph Held, Andrea Eschbach, Kerstin Klemm, and Nancy Kühne for advices in the laboratory; Helmut Hillebrand and Gabriele Gerlach for advice during the study; Aino Hosia, Luis Martell, Alexander Kieneke, Sven Rossel, and Reid Brennan for advice on species distribution ranges and succession pattern; Yannick Eller for proof reading. We thank the anonymous reviewers for their constructive advice. HIFMB is a collaboration between the Alfred Wegener Institute, Helmholtz Center for Polar and Marine Science, and the Carl von Ossietzky University Oldenburg. A.O, U.J., S.N., K.B., B.K., and S.L. were financially supported through the POF IV, research program of the HIFMB as part of the Helmholtz Association of German Research Centers. L.K. further acknowledge funding by the German Science Foundation (DFG HI 848/26-2 as part of Research Unit DynaCom and HI 848/29-1). A.O. and K.C.B. acknowledge support by the Federal Ministry of Education and Research (BMBF) through the project CREATE (03F091A), part of the research mission "Protection and Sustainable use of Marine Areas" within the German Marine Research Alliance (DAM). This article is a product of the SCOR Working Group MetaZooGene (WG157) and Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT) of the International Council for the Exploration of the Sea (ICES). We acknowledge support by the Open Access publication fund of Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Sequence data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB67348 https://www.ebi.ac.uk/ena/data/view/PRJEB67348, using the data brokerage service of the German Federation for Biological Data (GFBio) (Diepenbroek et al. 2014), in compliance with the Minimal Information about any (X) Sequence (MIxS) standard (Yilmaz et al. 2011).

References

Allan, E. A., M. H. DiBenedetto, A. C. Lavery, A. F. Govindarajan, and W. G. Zhang. 2021. "Modeling Characterization of the Vertical and Temporal Variability of Environmental DNA in the Mesopelagic Ocean." *Scientific Reports* 11, no. 1: 21273. https://doi.org/10.1038/s41598-021-00288-5.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. "Basic Local Alignment Search Tool." *Journal of Molecular Biology* 215, no. 3: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Altukhov, D., I. Siokou, M. Pantazi, et al. 2015. "Intercomparison of Five Nets Used for Mesozooplankton Sampling." *Mediterranean Marine Science* 16, no. 3: 550. 10.12681/mms.1100.

Andruszkiewicz, E. A., J. R. Koseff, O. B. Fringer, et al. 2019. "Modeling Environmental DNA Transport in the Coastal Ocean Using Lagrangian Particle Tracking." *Frontiers in Marine Science* 6: 1538. https://doi.org/10.3389/fmars.2019.00477.

Andruszkiewicz, E. A., H. A. Starks, F. P. Chavez, L. M. Sassoubre, B. A. Block, and A. B. Boehm. 2017. "Biomonitoring of Marine Vertebrates in Monterey Bay Using eDNA Metabarcoding." *PLoS One* 12, no. 4: e0176343. https://doi.org/10.1371/journal.pone.0176343.

Armonies, W., H. Asmus, C. Buschbaum, D. Lackschewitz, K. Reise, and J. Rick. 2018. "Microscopic Species Make the Diversity: A Checklist of Marine Flora and Fauna Around the Island of Sylt in the North Sea." *Helgoländer Wissenschaftliche Meeresuntersuchungen* 72, no. 1: 11. https://doi.org/10.1186/s10152-018-0512-8.

Baselga, A., D. Orme, S. Villeger, J. de Bortoli, F. Leprieur, and M. Logez. 2021. "Betapart: Partitioning Beta Diversity Into Turnover and Nestedness Components."

Beng, K. C., and R. T. Corlett. 2020. "Applications of Environmental DNA (eDNA) in Ecology and Conservation: Opportunities, Challenges and Prospects." *Biodiversity and Conservation* 29, no. 7: 2089–2121. https://doi.org/10.1007/s10531-020-01980-0.

Brandt, M. I., F. Pradillon, B. Trouche, et al. 2021. "Evaluating Sediment and Water Sampling Methods for the Estimation of Deep-Sea Biodiversity Using Environmental DNA." *Scientific Reports* 11, no. 1: 7856. https://doi.org/10.1038/s41598-021-86396-8.

Broman, E., C. Raymond, C. Sommer, J. S. Gunnarsson, S. Creer, and F. J. A. Nascimento. 2019. "Salinity Drives Meiofaunal Community Structure Dynamics Across the Baltic Ecosystem." *Molecular Ecology* 28, no. 16: 3813–3829. https://doi.org/10.1111/mec.15179.

Bruce, K., R. Blackman, S. J. Bourlat, et al. 2021. *A Practical Guide to DNA-Based Methods for Biodiversity Assessment*.Sofia: Pensoft. https://doi.org/10.3897/ab.e68634.

Bucklin, A., K. T. C. A. Peijnenburg, K. N. Kosobokova, et al. 2021. "Toward a Global Reference Database of COI Barcodes for Marine Zooplankton." *Marine Biology* 168, no. 6: 78. https://doi.org/10.1007/s00227-021-03887-y.

de Cáceres, M., and P. Legendre. 2009. "Associations Between Species and Groups of Sites: Indices and Statistical Inference." *Ecology* 90, no. 12: 3566–3574. https://doi.org/10.1890/08-1823.1.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13, no. 7: 581–583. https://doi.org/10.1038/nmeth.3869.

Casas, L., J. K. Pearman, and X. Irigoien. 2017. "Metabarcoding Reveals Seasonal and Temperature-Dependent Succession of Zooplankton Communities in the Red Sea." *Frontiers in Marine Science* 4: 73. https://doi.org/10.3389/fmars.2017.00241.

Castellani, C., and M. Edwards, eds. 2017. *Marine Plankton: A Practical Guide to Ecology, Methodology, and Taxonomy*. Oxford: Oxford University Press.

Clarke, L. J., L. Suter, B. E. Deagle, et al. 2021. "Environmental DNA Metabarcoding for Monitoring Metazoan Biodiversity in Antarctic Nearshore Ecosystems." *PeerJ* 9: e12458. https://doi.org/10.7717/peerj.12458.

Collins, R. A., O. S. Wangensteen, E. J. O'Gorman, S. Mariani, D. W. Sims, and M. J. Genner. 2018. "Persistence of Environmental DNA in Marine Systems." *Communications Biology* 1, no. 1: 185. https://doi.org/10.1038/s42003-018-0192-6.

Conway, D. V. 2012a. *Marine Zooplankton of Southern Britain. Part 1: Radiolaria, Heliozoa, Foraminifera, Ciliophora, Cnidaria, Ctenophora, Platyhelminthes, Nemertea, Rotifera and Mollusca*, edited by A. W. G. John, 138. Plymouth, UK: Occasional Publications. Marine Biological Association of the United Kingdom, No. 25. https://doi.org/10.13140/2.1.3274.3369.

Conway, D. V. P. 2012b. *Marine Zooplankton of Southern Britain. Part 2: Arachnida, Pycnogonida, Cladocera, Facetotecta, Cirripedia and Copepoda*, edited by A. W. G. John, 163. Plymouth UK: Occasional Publications. Marine Biological Association of the United Kingdom, No. 26. https://doi.org/10.13140/2.1.4704.4800.

Conway, D. V. P. 2015. *Marine Zooplankton of Southern Britain. Part 3: Ostracoda, Stomatopoda, Nebaliacea, Mysida, Amphipoda, Isopoda, Cumacea, Euphausiacea, Decapoda, Annelida, Tardigrada, Nematoda, Phoronida, Bryozoa, Entoprocta, Brachiopoda, Echinodermata, Chaetognatha, Hemichordata and Chordata*, edited by A. W. G. John, 271. Plymouth, UK: Occasional Publications. Marine Biological Association of the United Kingdom, No. 27. https://doi.org/10.13140/RG.2.1.1216.2087.

Cordier, T., F. Frontalini, K. Cermakova, et al. 2019. "Multi-Marker eDNA Metabarcoding Survey to Assess the Environmental Impact of Three Offshore Gas Platforms in the North Adriatic Sea (Italy)." *Marine Environmental Research* 146: 24–34. https://doi.org/10.1016/j.marenvres.2018.12.009.

Cowart, D. A., M. Pinheiro, O. Mouchel, et al. 2015. "Metabarcoding Is Powerful Yet Still Blind: A Comparative Analysis of Morphological and Molecular Surveys of Seagrass Communities." *PLoS One* 10, no. 2: e0117562. https://doi.org/10.1371/journal.pone.0117562.

Debastiani, V. J. 2021. "Daee: Data Analysis for Ecology and Evolution." (Version 0.1.7).

Deiner, K., H. M. Bik, E. Mächler, et al. 2017. "Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities." *Molecular Ecology* 26, no. 21: 5872–5895. https://doi.org/10.1111/mec.14350.

Diepenbroek, M., F. O. Glöckner, P. Grobe, et al. 2014. "Towards an Integrated Biodiversity and Ecological Research Data Management and Archiving Platform: The German Federation for the Curation of Biological Data (GFBio). Informatik 2014."

Djurhuus, A., K. Pitz, N. A. Sawaya, et al. 2018. "Evaluation of Marine Zooplankton Community Structure Through Environmental DNA Metabarcoding." *Limnology and Oceanography: Methods* 16, no. 4: 209–221. https://doi.org/10.1002/lom3.10237.

Geburzi, J. C., N. Heuer, L. Homberger, et al. 2022. "An Environmental Gradient Dominates Ecological and Genetic Differentiation of Marine

Invertebrates Between the North and Baltic Sea." *Ecology and Evolution* 12, no. 5: e8868. https://doi.org/10.1002/ece3.8868.

Geller, J., C. Meyer, M. Parker, and H. Hawk. 2013. "Redesign of PCR Primers for Mitochondrial Cytochrome C Oxidase Subunit I for Marine Invertebrates and Application in All-Taxa Biotic Surveys." *Molecular Ecology Resources* 13, no. 5: 851–861. https://doi.org/10.1111/1755-0998.12138.

Gogina, M., H. Nygård, M. Blomqvist, et al. 2016. "The Baltic Sea Scale Inventory of Benthic Faunal Communities." *ICES Journal of Marine Science* 73, no. 4: 1196–1213. https://doi.org/10.1093/icesjms/fsv265.

Goldberg, C. S., C. R. Turner, K. Deiner, et al. 2016. "Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species." *Methods in Ecology and Evolution* 7, no. 11: 1299–1307. https://doi.org/10.1111/2041-210X.12595.

Hahn, A., and R. S. Brennan. 2024. "Phenotypic Plasticity Drives Seasonal Thermal Tolerance in a Baltic Copepod." *Journal of Experimental Marine Biology and Ecology* 576, no. 152: 014. https://doi.org/10.1016/j.jembe.2024.152014.

Hansen, B. K., D. Bekkevold, L. W. Clausen, and E. E. Nielsen. 2018. "The Sceptical Optimist: Challenges and Perspectives for the Application of Environmental DNA in Marine Fisheries." *Fish and Fisheries* 19, no. 5: 751–768. https://doi.org/10.1111/faf.12286.

Hayward, P. J., and J. S. Ryland, eds. 2017. *Handbook of the Marine Fauna of North-West Europe*. Oxford: Oxford University Press.

Holm, M. W., T. Kiørboe, P. Brun, P. Licandro, R. Almeda, and B. W. Hansen. 2018. "Resting Eggs in Free Living Marine and Estuarine Copepods." *Journal of Plankton Research* 40, no. 1: 2–15. https://doi.org/10.1093/plankt/fbx062.

Holman, L. E., M. de Bruyn, S. Creer, G. Carvalho, J. Robidart, and M. Rius. 2019. "Detection of Introduced and Resident Marine Species Using Environmental DNA Metabarcoding of Sediment and Water." *Scientific Reports* 9, no. 1: 11559. https://doi.org/10.1038/s41598-019-47899-7.

Illumina. 2013. "16S Metagenomic Sequencing Library Preparation: Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System." https://support.illumina.com/documents/documentation/chemistry_documentation/16 s/16 s-metagenomic-library-prep-guide-15044223-b.pdf.

Jaccard, P. 1912. "The Distribution of the Flora in the Alpine Zone. 1." *New Phytologist* 11, no. 2: 37–50. https://doi.org/10.1111/j.1469-8137.1912.tb05611.x.

Jaspers, C., L. F. Møller, and T. Kiørboe. 2011. "Salinity Gradient of the Baltic Sea Limits the Reproduction and Population Expansion of the Newly Invaded Comb Jelly *M. leidyi*." *PLoS One* 6, no. 8: e24065. https://doi.org/10.1371/journal.pone.0024065.

Kautsky, L., and N. Kautsky. 2000. "The Baltic Sea, Including Bothnian Sea and Bothnian Bay."

Koehler, B., M. Erlandsson, M. Karlsson, and L. Bergström. 2022. "Species Richness and Functional Attributes of Fish Assemblages Across a Large-Scale Salinity Gradient in Shallow Coastal Areas." *Biogeosciences* 19, no. 8: 2295–2312. https://doi.org/10.5194/bg-19-2295-2022.

Koziol, A., M. Stat, T. Simpson, et al. 2019. "Environmental DNA Metabarcoding Studies Are Critically Affected by Substrate Selection." *Molecular Ecology Resources* 19, no. 2: 366–376. https://doi.org/10.1111/1755-0998.12971.

Krock, B., K. Möller, U. Tillmann, R. Henkel, and D. Voß. 2023. "Physical Oceanography During UTHÖRN Cruise UT2020-01 (HAB-DK/Limfjord)." https://doi.org/10.1594/PANGAEA.957254.

Laakmann, S., L. Blanco-Bercial, and A. Cornils. 2020. "The Crossover From Microscopy to Genes in Marine Diversity: From Species to Assemblages in Marine Pelagic Copepods." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 375, no. 1814: 0446. https://doi.org/10.1098/rstb.2019.0446.

Laakmann, S., G. Gerdts, R. Erler, T. Knebelsberger, P. Martínez Arbizu, and M. J. Raupach. 2013. "Comparison of Molecular Species Identification for North Sea Calanoid Copepods (Crustacea) Using Proteome Fingerprints and DNA Sequences." *Molecular Ecology Resources* 13, no. 5: 862–876. https://doi.org/10.1111/1755-0998.12139.

Larink, O., and W. Westheide. 2011. *Coastal Plankton: Photo Guide for European Seas*. 2nd ed. Pfeil: München.

Larson, W. A., P. Barry, W. Dokai, J. Maselko, J. Olson, and D. Baetscher. 2022. "Leveraging eDNA Metabarcoding to Characterize Nearshore Fish Communities in Southeast Alaska: Do Habitat and Tide Matter?" *Environmental DNA* 4, no. 4: 868–880. https://doi.org/10.1002/edn3.297.

Leduc, N., A. Lacoursière-Roussel, K. L. Howland, et al. 2019. "Comparing eDNA Metabarcoding and Species Collection for Documenting Arctic Metazoan Biodiversity." *Environmental DNA* 1, no. 4: 342–358. https://doi.org/10.1002/edn3.35.

Leite, B. R., P. E. Vieira, J. S. Troncoso, and F. O. Costa. 2021. "Comparing Species Detection Success Between Molecular Markers in DNA Metabarcoding of Coastal Macroinvertebrates." *Metabarcoding and Metagenomics* 5: 70063. https://doi.org/10.3897/mbmg.5.70063.

Lindeque, P. K., H. E. Parry, R. A. Harmer, P. J. Somerfield, and A. Atkinson. 2013. "Next Generation Sequencing Reveals the Hidden Diversity of Zooplankton Assemblages." *PLoS One* 8, no. 11: e81327. https://doi.org/10.1371/journal.pone.0081327.

Lira, N. L., S. Tonello, R. L. Lui, et al. 2022. "Identifying Fish Eggs and Larvae: From Classic Methodologies to DNA Metabarcoding." *Molecular Biology Reports* 50: 1713–1726. https://doi.org/10.1007/s11033-022-08091-9.

Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet.Journal* 17, no. 1: 200. https://doi.org/10.14806/ej.17.1.200.

Mohrbeck, I., M. J. Raupach, P. Martínez Arbizu, T. Knebelsberger, and S. Laakmann. 2015. "High-Throughput Sequencing-The Key to Rapid Biodiversity Assessment of Marine Metazoa?" *PLoS One* 10, no. 10: e0140342. https://doi.org/10.1371/journal.pone.0140342.

Momigliano, P., G. P. J. Denys, H. Jokinen, and J. Merilä. 2018. "*Platichthys Solemdali* Sp. Nov. (Actinopterygii, Pleuronectiformes): A New Flounder Species From the Baltic Sea." *Frontiers in Marine Science* 5: 00225. https://doi.org/10.3389/fmars.2018.00225.

Motoda, S. 1959. "Devices of Sample Plankton Apparatus." *Memoirs of the Faculty of Fisheries Hokkaido University* 7, no. 1–2: 73–94.

Narberhaus, I. 2012. *Threatened Biodiversity in the German North and Baltic Seas: Sensitivities Towards Human Activities and the Effects of Climate Change*. Bonn-Bad Godesberg, Münster: Bundesamt für Naturschutz; BfN-Schriftenvertrieb im Landwirtschaftsverl.

Neumann, T., H. Radtke, and T. Seifert. 2017. "On the Importance of Major Baltic Inflows for Oxygenation of the Central Baltic Sea." *Journal of Geophysical Research: Oceans* 122, no. 2: 1090–1101. https://doi.org/10.1002/2016JC012525.

OBIS. 2023. "Ocean Biodiversity Information System: Intergovernmental Oceanographic Commission of UNESCO." www.obis.org.

Ogata, M., R. Masuda, H. Harino, et al. 2021. "Environmental DNA Preserved in Marine Sediment for Detecting Jellyfish Blooms After a Tsunami." *Scientific Reports* 11, no. 1: 16830. https://doi.org/10.1038/s41598-021-94286-2.

Ohnesorge, A., U. John, S. Taudien, S. Neuhaus, L. Kuczynski, and S. Laakmann. 2023. "Capturing Drifting Species and Molecules—Lessons Learned From Integrated Approaches to Assess Marine Metazoan Diversity in Highly Dynamic Waters." *Environmental DNA* 5: 1541–1556. https://doi.org/10.1002/edn3.478.

Ojaveer, H., A. Jaanus, B. R. Mackenzie, et al. 2010. "Status of Biodiversity in the Baltic Sea." *PLoS One* 5, no. 9: e12467. https://doi.org/10.1371/journal.pone.0012467.

Parry, H. E., A. Atkinson, P. J. Somerfield, and P. K. Lindeque. 2020. "A Metabarcoding Comparison of Taxonomic Richness and Composition Between the Water Column and the Benthic Boundary Layer." *ICES Journal of Marine Science* 78: 3333–3341. https://doi.org/10.1093/icesjms/fsaa228.

Plotnikov, I. S., and N. V. Aladin. 2011. "An Overview of Hybrid Marine and Lacustrine Seas and Saline Lakes of the World." *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use* 16, no. 2: 97–108. https://doi.org/10.1111/j.1440-1770.2011.00452.x.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/.

Revell, L. J. 2012. "Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things)." *Methods in Ecology and Evolution* 3, no. 2: 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x.

Riisgård, H. U., P. Andersen, and E. Hoffmann. 2012. "From Fish to Jellyfish in the Eutrophicated Limfjorden (Denmark)." *Estuaries and Coasts* 35, no. 3: 701–713. https://doi.org/10.1007/s12237-012-9480-4.

Rossel, S., S. Khodami, and P. Martínez Arbizu. 2019. "Comparison of Rapid Biodiversity Assessment of Meiobenthos Using MALDI-TOF MS and Metabarcoding." *Frontiers in Marine Science* 6: 00659. https://doi.org/10.3389/fmars.2019.00659.

Sahu, A., N. Kumar, C. Pal Singh, and M. Singh. 2023. "Environmental DNA (eDNA): Powerful Technique for Biodiversity Conservation." *Journal for Nature Conservation* 71, no. 126: 325. https://doi.org/10.1016/j.jnc.2022.126325.

Sayers, E. W., M. Cavanaugh, K. Clark, J. Ostell, K. D. Pruitt, and I. Karsch-Mizrachi. 2020. "GenBank." *Nucleic Acids Research* 48, no. D1: D84–D86. https://doi.org/10.1093/nar/gkz956.

Schlitzer, R. 2018. "Ocean Data View." https://odv.awi.de/.

Urban-Malinga, B., S. I. Hedtkamp, J. E. van Beusekom, J. Wiktor, and J. M. Węsławski. 2006. "Comparison of Nematode Communities in Baltic and North Sea Sublittoral, Permeable Sands—Diversity and Environmental Control." *Estuarine, Coastal and Shelf Science* 70, no. 1–2: 224–238. https://doi.org/10.1016/j.ecss.2006.06.020.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. "Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences Into the New Bacterial Taxonomy." *Applied and Environmental Microbiology* 73, no. 16: 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Wangensteen, O. S., C. Palacín, M. Guardiola, and X. Turon. 2018. "DNA Metabarcoding of Littoral Hard-Bottom Communities: High Diversity and Database Gaps Revealed by Two Molecular Markers." *PeerJ* 6: e4705. https://doi.org/10.7717/peerj.4705.

WoRMS Editorial Board. 2022. "World Register of Marine Species." VLIZ https://doi.org/10.14284/170. https://www.marinespecies.org.

Yebra, L., C. García-Gómez, N. Valcárcel-Pérez, et al. 2022. "Assessment of Short-Term Spatio-Temporal Variability in the Structure of Mesozooplankton Communities Integrating Microscopy and Multigene High-Throughput Sequencing." *Estuarine, Coastal and Shelf Science* 276, no. 108: 038. https://doi.org/10.1016/j.ecss.2022.108038.

Yilmaz, P., R. Kottmann, D. Field, et al. 2011. "Minimum Information About a Marker Gene Sequence (MIMARKS) and Minimum Information About any (x) Sequence (MIxS) Specifications." *Nature Biotechnology* 29, no. 5: 415–420. https://doi.org/10.1038/nbt.1823.

Zaiko, A., P. Greenfield, C. Abbott, et al. 2021. "Towards Reproducible Metabarcoding Data—Lessons From an International Cross-Laboratory Experiment." *Molecular Ecology Resources* 22: 519–538. https://doi.org/10.1111/1755-0998.13485.

Zettler, M. L., J. Beermann, J. Dannheim, et al. 2018. "An Annotated Checklist of Macrozoobenthic Species in German Waters of the North and Baltic Seas." *Helgoländer Wissenschaftliche Meeresuntersuchungen* 72, no. 1: 5. https://doi.org/10.1186/s10152-018-0507-5.

Zettler, M. L., A. Karlsson, T. Kontula, et al. 2014. "Biodiversity Gradient in the Baltic Sea: A Comprehensive Inventory of Macrozoobenthos Data." *Helgoland Marine Research* 68, no. 1: 49–57. https://doi.org/10.1007/s10152-013-0368-x.

Zhan, A., M. Hulák, F. Sylvester, et al. 2013. "High Sensitivity of 454 Pyrosequencing for Detection of Rare Species in Aquatic Communities." *Methods in Ecology and Evolution* 4, no. 6: 558–565. https://doi.org/10.1111/2041-210X.12037.

Zhang, Y., M. Pavlovska, E. Stoica, et al. 2020. "Holistic Pelagic Biodiversity Monitoring of the Black Sea via eDNA Metabarcoding Approach: From Bacteria to Marine Mammals." *Environment International* 135, no. 105: 307. https://doi.org/10.1016/j.envint.2019.105307.

Supporting Information

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