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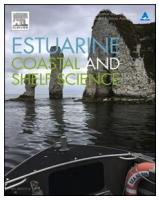

Spatial variability of the coastal Wadden Sea fish community as revealed by environmental DNA

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ABSTRACT

Previous studies have suggested the occurrence of spatial variability in the fish food web structure in the temperate Wadden Sea. However, these studies were carried out in different years and with different fishing devices. To eliminate interannual variability in fish abundance and the impact of sampling design, an environmental DNA (eDNA) study was performed monthly at eight locations over the spatial scale of the Dutch Wadden See year-round in 2018–2019. In total, 40 different individual fish species and 8 fish groups were identified. The number of fish species identified in the samples varied over time and among locations between three and 19 different fish species. Over the year, 20 species were identified at all locations; eight species were found at 6–7 locations and the remaining 30 species were found only incidentally. The spatial variability found in the Wadden Sea fish community is the result of the variability in presence of rare (transient) species, due to location specific differences in hydrographical and geomorphological characteristics.

1. Introduction

Worldwide, coastal zones are known as important nursery areas and feeding grounds for a variety of fish species (e.g. Goodall, 1983; Lefcheck et al., 2019; Whitfield et al., 2002). Species diversity in these transition zones between the marine offshore and the freshwater inner zone is often relatively low (Levin et al., 2001, Selleslagh and Amara, 2008) and a few species account for most of the numbers and biomass, and these species are also widely distributed (Haedrich, 1983; Wolff, 1983; Tulp et al., 2008, 2022; van der Veer et al., 2015; Poiesz et al., 2023).

The international Wadden Sea, along the North Sea, is one of the largest temperate coastal estuarine areas in Europe (for overview see Wolff, 1983). More than 100 fish species have been identified in the area (Witte and Zijlstra, 1983), but most fish species are present during certain parts of the year or during distinct life stages. Moreover, only less than half of the species are common or fairly common (Zijlstra, 1983) and various studies indicate that the fish fauna in the area is dominated by a number of dominant core species especially Clupeidae (herring, sprat), Pleuronectidae (plaice, flounder, dab), Gobiidae (sand goby, common goby) and Ammodytidae (sandeel) (Zijlstra, 1983; Kellnreiter et al., 2012; van der Veer et al., 2015; Tulp et al., 2008, 2022; Meyer et al., 2016; Poiesz et al., 2020, 2023). Also, the benthic in- and epifauna is dominated by a few species of bivalves, polychaetes and crustaceans (see for instance: Meyer et al., 2016; Beukema and Dekker, 2020). This translates in a fish food web structure mainly fueled by a few key prey groups: mainly crustaceans (such as copepods, amphipods, shrimps and crabs) and fish species (especially gobies and juvenile herring) (Kühl and Kuipers, 1983; Kellnreiter et al., 2012; Poiesz et al., 2020, 2023).

The various studies have suggested some local differences in the Wadden Sea fish food web. The fish community showed some variability in the Wadden Sea also with respect to dominant core species. Only four species [herring (*Clupea harengus*), sprat (*Sprattus sprattus*), plaice (*Pleuronectes platessa*) and whiting (*Merlangius merlangus*)] were dominant in all studies and, while other species, such as the Gobiidae species, were only described as highly abundant in one or two areas (Kellnreiter

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et al., 2012; Meyer et al., 2016; Poiesz et al., 2020, 2023). With respect to prey species, copepods and brown shrimp were the most important prey species in all studies, however, mysid shrimp were also important in the Sylt-Rømø bight (Kellnreiter et al., 2012) and Ems basin (Poiesz et al., 2023), while shore crab and juvenile herring were more important in the Marsdiep basin (Poiesz et al., 2020). This variability in fish composition and abundance, and in predator−prey relationships might suggest that it is caused by local differences in predator-prey abundance, as found for two areas by Poiesz et al. (2023). However, most of the studies were carried out in different years and with different sampling gears.

Spatial variability of the Wadden Sea fish community can only be determined when interannual variability in fish community and the impact of sampling design can be excluded. Interannual variability in fish community can be eliminated by simultaneously sampling various locations at multiple timepoints. The selectivity of fishing devices (see for overview Ruth and Berghahn, 1989) can be overcome by sampling environmental DNA (eDNA). eDNA metabarcoding methodology is not selective, can be performed in virtually any marine habitat, and require little expertise or effort in sampling and is less invasive than the traditional fishing devices. Additionally, the molecular identification is more confident and objective than visual identification of species, which is in some cases difficult even for experts (Thomsen et al., 2012). One extraordinary advantage of the eDNA approach is its ability to harness genetic information from an entire waterbody community in a single environmental sample (Yao et al., 2022).

A pilot study with eDNA in the Marsdiep tidal inlet in the western Wadden Sea showed that [1] presence–absence of eDNA corresponded with fyke net catches in the neighborhood; [2] that fish eDNA compositions differed significantly among sample days and months but not between tides; and [3] that patterns in eDNA concentration corresponded to patterns in wet mass for the eight most abundant fish species caught nearby, despite changes in water temperature and changes in fish size (van Bleijswijk et al., 2020).

In this study, the focus is on the scale of spatial variability of the Wadden Sea fish community. Monthly water samples for eDNA were collected in six tidal basins in the Dutch Wadden Sea varying in size and in hydrographical and geomorphological characteristics (for overview see Postma, 1983). Two of these areas [Marsdiep (Texel inlet) and Ems] are incorporated as reference areas since the fish food web has been studied before in these areas (Poiesz et al., 2020, 2023). Both the Marsdiep and Vlie tidal basin are under the direct influence of strong freshwater input from Lake IJssel. In these basins, sampling took place at two different locations. Variability in species composition is analyzed both within locations over the year (sampling every month, and among locations (Dutch Wadden Sea west to east). The hypothesis is tested that spatial variability exists but that common (core) species are found more often and will be more abundant at all locations and that locations differ more with respect to rarer (transient) species not only between tidal basins but also within a tidal basin due to location-specific differences in hydrographical and geomorphological characteristics. To conclude, to what extend the observed differences are due to interannual variability in fish fauna and by differences in sampling design or whether the observed differences in food web structure represent spatial variability in fish community.

2. Materials and methods

2.1. Sampling

Sampling was conducted in the Dutch Wadden Sea at eight stations in six tidal basins (Fig. 1 & Table 1) within 2 h before high water with an interval of one month between February 2018 and February 2019. Scheurrak Omdraai, Inschot and Harlingen were sampled by boat, the other stations were visited by car and sampled from a jetty. During the February sampling campaign of 2018, Harlingen was not sampled due to bad weather conditions.

Water samples were taken and filtered as described in van Bleijswijk et al. (2020). Prior to sampling, a 10 L sampling bucket was rinsed three times with surface seawater (<1 m depth) and dish washer clean glass bottles of 1 L and caps were shaken and rinsed three times with fresh surface seawater (<1 m depth). Next, samples of 10 L were taken just below the surface. After flushing the bottle and cap, 1 L of water was collected from the bucket of 10 L in triplicate into glass bottles. Filled bottles were immediately stored in a dark cool box.

Samples were filtered after collection in a climate room over a 47-

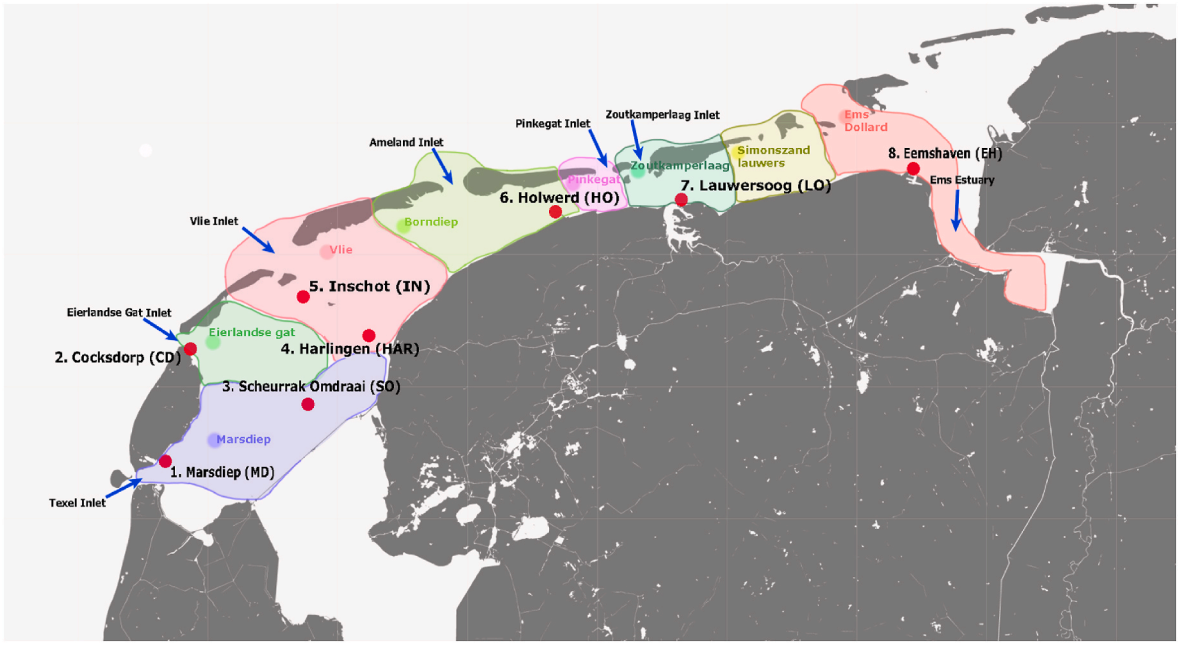

Fig. 1. Monthly eDNA sampling locations (red dots) and the various tidal inlets (blue arrows) in the Dutch Wadden Sea. The colored areas with the corresponding names in the same color indicate the tidal areas. Abbreviations and full names of the locations and their characteristics can be found in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Information of the eDNA sampling locations in the Dutch Wadden Sea. Source: Anon (2018) Getijdetafels voor Nederland 2018. llws is the lowest height of the low waters of spring tides (during a year).

Code	Station	Location	Latitude			Longitude			Water depth at LLWS (m)	Mean tidal range (m)
MD	Marsdiep	NIOZ Jetty	53	0	6.64	4	47	20.66	2.5	1.41
CD	Cocksdorp Jetty	Naast veersteiger Vriendschap	53	10	30.09	4	52	15.55	2	1.50
SO	Scheurrak Omdraai	Next to buoy So21	53	5	53.20	5	10	90.10	8	1.80
IN	Inschot	Next to buoy IN2a	53	14	47.80	5	9	57.80	10	1.80
HAR	Harlingen	Next to buoy P3HAR	53	11	12.30	5	21	7.18	10	1.94
HO	Holwerd	Next to ferryterminal	53	23	42.87	5	52	41.73	2.5	1.36
LO	Lauwersoog	End dike ferry harbour	53	24	41.38	6	12	0.58	3	2.32
EH	Eemshaven	End dike Beatrix harbour	53	27	22.31	6	49	58.16	3	2.62

mm 0.2 μm Nuclepore filter using a high-pressure vacuum pump (Millepore) until clogged within half an hour. The average water volume filtered was 150–200 ml ± 17 (SE). Filters were folded inwards, placed in 2 ml tubes and stored at −80 °C. Blank filters, using milli Q water were taken after all other samples were filtered. After filtration, the Nuclepore filters were rolled up inwards with sterilized tweezers, stored in 2 ml screw cap tubes and put on ice immediately. Support filters were discarded. Sterilization of tweezers was done with 96 % ethanol and burning after storing each filter. The filtration setup and glass bottles for sampling were cleaned with soapy warm water and Milli-Q after finishing filtration (see Supplementary materials Table S1 for all sampling and filtration information) (methods described in van Bleijswijk et al., 2020). For the over 333 samples collected, (including the biological triplicates), the average water volume filtered was 186 ml (±3 StE). Filtration and extraction controls were taken along on 5 days.

2.2. DNA extraction, amplification and sequencing

Filters were cut in small pieces with sterile flamed scissors and DNA was extracted using the DNeasy PowerSoil Pro kit (Qiagen, Inc.) following the manufactures protocol, including a bead-beating step. DNA from all extractions was used as template to amplify a ~106bp fragment from the mitochondrial 12 S rRNA gene in triplicate. The primer pair used (12 S-F1nioz: 5′-AACTGGGATTAGATACCC-3′ and 12 S-R1: 5′-TAGAACAGGCTCCTCTAG-3′) is based on Riaz et al. (2011) with a slight adaptation in the forward primer (van Bleijswijk et al., 2020). Both the forward and reverse primers were extended with 6 nt unique barcodes for sample assignment. PCR reaction volumes were 50 μl and consisted of 10 μl 5x SuperFi I buffer and 0.5 μl SuperFi I polymerase (ThermoFisher Scientific), 2.5 μM of each primer, 200 μM dNTPs, 2.5 μl of EvaGreen (20x), and 5 μl of DNA extract. Negative PCR controls were added for which the DNA extract was replaced by PCR-water. Triplicates were combined after PCR. A total of 120 samples were pooled in three different pools equimolarly based on gel-electrophoresis quantification. The pooled samples were purified using QIAquick PCR purification (Qiagen Inc.) and target products were selected by cutting out the band of interest and using a gel extraction with the QIAquick gel extraction (Qiagen Inc.). Amplicons were submitted for 2 × 150 bp Illumina NovaSeq sequencing at Eurofins Genomics in three different lanes.

The qPCR amplification was carried out at 98 °C for 30 s followed by 37 cycles with 98 °C for 10 s, 57 °C for 15 s and 72 °C for 30 s, followed by a final elongation at 72 °C for 5 min qPCRs were done on all biological triplicates divided over 5 different runs. Each run contained duplicate standard series of fish amplicons in concentrations of 10, 100, 1000, 10 000, and 100 000 copies/μl. The Cq values of these standards ranged between 22 and 37. The Cq values of the 333 field samples ranged between 18.7 and 37 with an average of 32.2 and a standard deviation of 0.8. The Cq values of extraction and filtration controls were always >34.9; Cq values of PCR negatives were 37 or out of range. The efficiency of the amplification was 96.3 %. Differences in the slopes of the exponential phases of the S-curves among the samples used were small (<10 %) indicating that the efficiency of the amplification was constant over the time span used. After bioinformatic processing (see below), fish-specific eDNA concentrations were calculated from the ratios of fish species-specific read counts over the total read count (including assigned and non-assigned reads) of a sample, multiplied by the total eDNA concentration measured with qPCR (according to van Bleijswijk et al., 2020).

2.3. Bioinformatics

Bioinformatics were done according to van Bleijswijk et al., (2020). In short, reads were processed using the Cascabel pipeline (Abdala Asbun et al., 2020) with the OTU workflow. This pipeline is a scalable, flexible, and easy-to-use amplicon sequence data analysis. It takes the raw data as input and delivers a table of operational taxonomic units (OUT's) in BIOM and text format and representative sequences (Abdala Asbun et al., 2020). The quality of the raw reads was verified using FastQC (Andrews, 2010). Subsequently, primers were removed using cutadapt (Martin, 2011) and operational taxonomic units (OTU's) were created using QIIME's trie method with the most abundant sequences in an OTU selected as representative sequences (Caporaso et al., 2010). OTU representatives were blasted against a custom reference database (van Bleijswijk et al., 2020) using blastn (Morgulis et al., 2008) with 98 % sequence identity cut off. For fish, only species assignments with 100 % sequence identity scores were used, except for *Lipophrys folis*, that was identified with 99 % identity cut off. Non-fish species were identified with 98 % sequence identity cut off.

The custom reference database included eight cases in which closely related species have identical sequences:

- Clupeidae complex: *Clupea harengus* and *Sprattus sprattus*;
- Pleuronectidae complex: *Pleuronectes platessa*, *Platichthys flesus* and *Limanda limanda*;
- Ammodytidae complex: *Ammodytes marinus* and *A. tobianus;*
- Gadidae complex: *Merlangius merlangus*, *Pollachius pollachius* and *P. virens*;
- Cottidae complex: *Myoxocephalus scorpius* and *Taurulus bubalis*;
- Mugilidae complex: *Chelon labrosus* and *C. ramada*;
- *Pomatoschitus* spp.: *Pomatoschitus minutus* and *P. lozanoi*;
- *Trisopterus* spp.: *Trisopterus luscus, T. minutus.*

2.4. Data analysis

All fish species found were taxonomically assigned as either core species (species endemic to the area) or transient species (species occurring infrequently and do not maintain viable local populations) (see Table S2 for assigned core/transient or functional groups). Also, functional groups were assigned to fish species in relation to their life habits in the Wadden Sea in line with previous work (van der Veer et al., 2015; Poiesz et al., 2020). These were as follows: pelagic (occurring mainly in the water column, not feeding on benthic organisms), benthopelagic (living and/or feeding on or near the bottom, as well as in midwater) and benthic (living and/or feeding on the bottom), see FishBase (Froese and Pauly, 2019) and based on Witte and Zijlstra (1983), van der Veer et al. (2015) and Poiesz et al. (2020).

Data analysis, statistical analysis and data visualization were all performed in R (R Core Team, 2023). All absolute read abundances [fish eDNA concentrations (12 S copies/L)] were Hellinger transformed in R (Legendre and Gallagher, 2001), executed by the function decostand in R from the vegan package (Oksanen et al., 2022). Statistical analysis was performed with vegan (Oksanen et al., 2022) and stats (R Core Team, 2023). Visualization of the data was done using the ggplot2 package (Wickham, 2016). Fish species accumulation curves for the 8 locations calculated for both the regular accumulation curves plus calculated trough the sample-size- and coverage-based integrations of rarefaction (interpolation) and extrapolation (prediction) of Hill numbers represent a unified standardization method for quantifying and comparing species diversity across multiple assemblages according to Chao et al. (2014, 2016). Presence-absence per species for all locations together and per location separate was calculated and differences between locations were tested by performing an anova. Further analyses were done on both the absolute read abundances as well as the presence-absence data.

Firstly, Nonmetric Multidimensional Scaling (NMDS), using several random starts were performed (Saeed et al., 2018). This so called, 'permutation' is used to arrange a given set of elements in a particular order in a total amount of ways and times. Dissimilarity in distances between samples were calculated using the metaMDS function by means of the Bray-Curtis equation. NMDS was chosen because, it does not depend on linearity and normality (Biswas et al., 2006).

The second analysis done was for partitioning distance matrices among sources of variation and fitting linear models to distance matrices using a permanova by the adonis function. The output of continuous variables (vectors) gives the direction cosines which are the coordinates of the heads of unit length vectors. In the shown NMDS plots these are scaled by their correlation (square root of the column r^2) so that "weak" predictors have shorter arrows than "strong" predictors. With this analysis, vector arrows of only species which have a significant role ($p \leq 0.05$) were shown in the results.

Thirdly, a Jaccard/Sørensen dissimilarity index for community ecologists was calculated (Sørensen, 1948). This index measures the overlap between two populations and divides the number of species shared by both samples by the sum of all species occurring in both samples. The dissimilarity index varies between zero (identical in composition) and one (no common elements). The Jaccard/Sørensen index was calculated on the presence-absence data as done by (Bastow Wilson, 2012).

3. Results

In total 18.2 million reads were found on average 164 914 per samples (minimum: 7545; maximum 127 6536). Supplementary materials Table S3 contains the absolute read abundances data of all found species for each location and time.

3.1. Species composition

In total, 48 taxonomic groups were identified based on their DNA sequences (Table S2). From these, 40 taxonomic groups could be identified at species level and 8 taxonomic groups at genus or family level. The taxonomic groups Clupeidae and Pleuronectidae were present in almost all samples (>94 %), *Pomatoschistus* spp. and also *Pomastoschistus microps* were found frequently, respectively in 86 and 58 % of the samples. Other frequently occurring species or taxa were Ammodytidae (53 %), *Osmerus eperlanus* (50 %), *Dicentrarchus labrax* (31 %), Gadidae (30 %) and *Zoarces viviparus* (30 %).

The fish species accumulation curve of all samples combined levelled off after 7 sampling routines (after about half a year), indicating that from that sampling moment onwards no new fish species were found (Fig. 2).

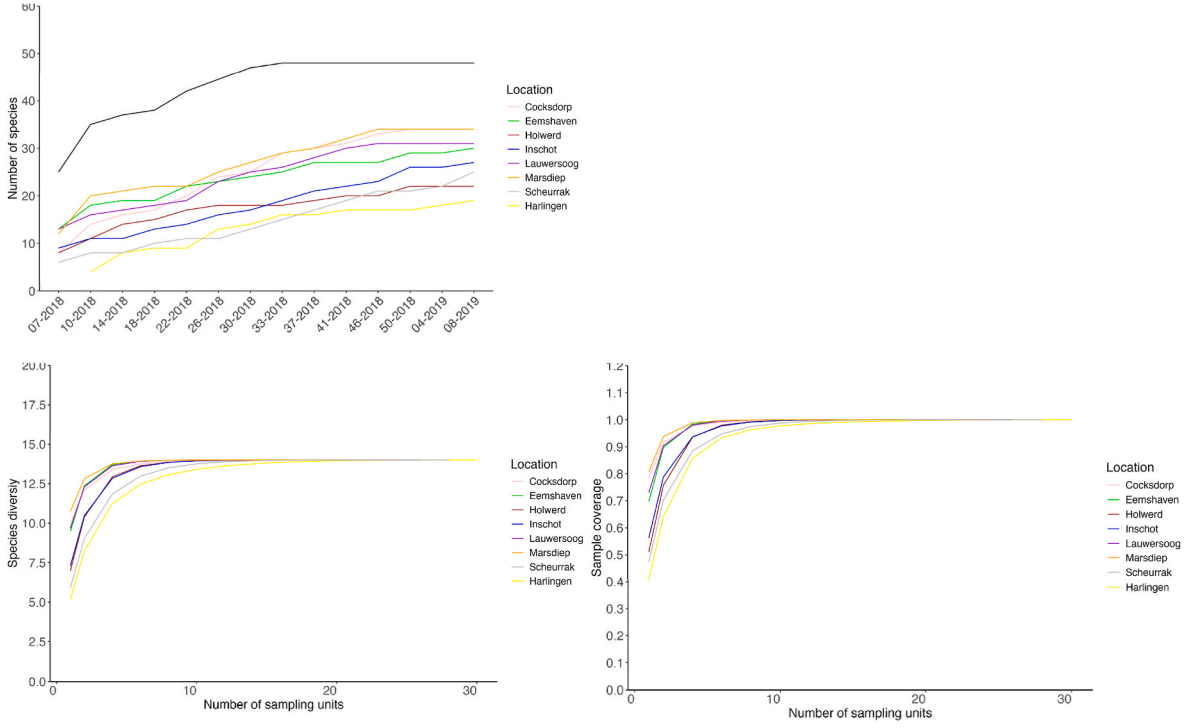

Fig. 2. Upper planel: Fish species accumulation curves for the various locations in the Dutch Wadden Sea based on eDNA sampling in 2018–2019. The black line represents all stations combined. The X-axis represents the sampling times (week-year combination).
Lower panel: Fish species accumulation curves for the various locations in the Dutch Wadden Sea based on eDNA sampling in 2018–2019 calculated trough the sample-size- and coverage-based integrations of rarefaction (interpolation) and extrapolation (prediction) of Hill numbers.
Left: This type of sampling curve plots the diversity estimates with respect to sample size.
Right: This type of sampling curve plots the diversity estimates with respect to sample coverage.

3.2. Spatial variability

3.2.1. Presence/absence of species

The accumulation curves of the various stations were below the fish species accumulation curve of all samples combined (Fig. 2), which means that at none of the sampling locations, all 48 identified taxonomic fish groups were found (Fig. 2). Fish species richness was highest at the three locations near tidal inlets (Marsdiep, Cocksdorp, Eemshaven), respectively 34, 34 and 31 species. At four locations, the fish accumulation curves converge (Holwerd, Lauwersoog, Cocksdorp and Marsdiep). At the other four locations the accumulation curves were still going up, implying that the sampling still might not have identified the total number of species occurring at these stations (Harlingen, Scheurrak, Inschot and Eemshaven). The accumulation curves calculated by sample-size- and coverage-based integrations of rarefaction (interpolation) and extrapolation (prediction) of Hill numbers (Fig. 2), showed that in all locations the levelling of happened when sampling would continue.

Cocksdorp (min 6-max 19) and Marsdiep (min 4-max 17) had the highest species richness per sampling event (Table 2; as minimum and maximum values), followed by Lauwersoog (min 3-max 13), Eemshaven (min 5-max 13), Holwerd (min 5-max 15), Harlingen (min 4-min 9), Inschot (min 4-max 10) and Scheurrak (min 3-max 11). The total number of taxonomic groups found per sampling event and found in total at the various stations differed significantly for Cocksdorp compared to Harlingen and Scheurrak, for Harlingen compared to Eemshaven, for Lauwersoog compared to Marsdiep and for Marsdiep compared to Harllingen, Holwerd and Scheurrak [Anova, F (7, 103) = 9.294, $p \leq 0.05$].

The high species richness at Cocksdorp and Marsdiep, both in total but also per sampling event, was caused by a higher number of core species found compared to the other stations (Table 2). With respect of the number of transient species, no clear pattern between stations was found, because the number of transient species found was overall low (on average 2 species). Most fish species found were benthic species. The average number of benthopelagic (1.6) and pelagic (2.0) species found over all locations and sampling dates was low. Therefore, no clear trend between location or sampling date could be observed in the number of benthic, benthopelagic and pelagic species (Table 2). Clupeidae (*Clupea harengus* and *Sprattus sprattus),* Pleuronectidae (*Pleuronectes platessa*,

Table 2

Heatmap of the number of species found in the samples at the various locations. Colour scheme indicates the categorical difference in number of species found per location over time. Green colours indicate the highest value, yellow the 50 % percentile of the values and red the lowest value. The empty white spots mean that there was no data of the relevant species. (Harlingen, 2018-02 was not sampled due to bad weather conditions). Min and max represent the lowest and the highest value of unique species found per location.

A All species D Benthic species

B Core species E Benthopelagic species

C Transient species F Pelagic species.

A

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	12	8	6		9	8	12	13
10-2018	17	11	6	4	8	8	9	9
14-2018	4	9	4	6	4	8	7	7
18-2018	8	8	6	5	5	9	6	6
22-2018	6	9	5	3	3	6	5	4
26-2018	11	12	3	9	8	7	10	5
30-2018	10	11	9	9	5	5	7	6
33-2018	6	15	5	8	5	6	9	9
37-2018	13	13	5	5	8	6	13	8
41-2018	11	13	9	8	9	10	9	10
46-2018	10	14	6	7	6	6	8	5
50-2018	11	10	10	5	7	6	11	9
04-2019	12	19	8	9	10	8	3	6
08-2019	13	6	9	9	7	9	11	10
min	4	6	3	3	3	5	3	4
max	17	19	10	9	10	10	13	13

B

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	8	7	5		8	7	7	10
10-2018	9	6	5	4	5	6	6	5
14-2018	4	8	3	6	4	5	5	5
18-2018	7	6	5	4	4	6	5	6
22-2018	4	8	5	3	3	6	4	3
26-2018	9	11	3	7	6	6	8	4
30-2018	7	10	7	7	5	4	5	4
33-2018	5	12	5	6	4	4	4	6
37-2018	11	11	4	3	8	5	8	6
41-2018	9	11	7	7	7	8	7	8
46-2018	9	12	5	6	6	5	5	3
50-2018	6	8	7	5	6	5	6	5
04-2019	9	15	7	7	8	6	2	5
08-2019	9	5	8	8	5	8	7	8
min	4	5	3	3	3	4	2	3
max	11	15	8	8	8	8	8	10

C

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	4	1	1		1	1	5	3
10-2018	8	5	1		3	2	3	4
14-2018		1	1			3	2	2
18-2018	1	2	1	1	1	3	1	
22-2018	2	1					1	1
26-2018	2	1		2	2	1	2	1
30-2018	3	1	2	2		1	2	2
33-2018	1	3		2	1	2	5	3
37-2018	2	2	1	2		1	5	2
41-2018	2	2	2	1	2	2	2	2
46-2018	1	2	1	1		1	3	2
50-2018	5	2	3		1	1	5	4
04-2019	3	4	1	2	2	2	1	1
08-2019	4	1	1	1	2	1	4	2
min	1	1	1	1	1	1	1	1
max	8	5	3	2	3	3	5	4

D

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	7	3	3		7	4	6	8
10-2018	11	7	3	2	5	4	4	4
14-2018	3	5	3	3	3	3	3	4
18-2018	5	4	4	3	2	5	3	4
22-2018	3	6	2	1	2	4	2	2
26-2018	7	8	2	6	5	3	5	4
30-2018	8	8	7	5	4	4	4	5
33-2018	3	9	2	5	2	4	2	6
37-2018	7	8	3	4	4	5	5	4
41-2018	6	8	5	5	4	5	5	7
46-2018	8	8	4	3	3	3	6	4
50-2018	7	5	6	3	4	4	6	5
04-2019	8	9	5	5	6	4	2	4
08-2019	9	4	3	6	5	6	6	7
min	3	3	2	1	2	3	2	2
max	11	9	7	6	7	6	6	8

E

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	3	3	1		1	2	4	3
10-2018	2	1	1		2	2	3	4
14-2018		2		2		1	2	1
18-2018	1	3			1	2	1	
22-2018	1		1			1	1	
26-2018	1	1		1	2	1	1	
30-2018	1	1		1				
33-2018	1	1			1		4	1
37-2018	2	1	1		2		4	1
41-2018	2	1	1	1	2	1	2	1
46-2018			1	1	1	1		
50-2018	2	3	2	1	2		4	1
04-2019	2	4	1	2	2	2	1	
08-2019	3	1	2		1	1	3	1
min	1	1	1	1	1	1	1	1
max	3	4	2	2	2	2	4	4

F

week-year	Marsdiep	Cocksdorp	Scheurrak	Harlingen	Inschot	Holwerd	Lauwersoog	Eemshaven
07-2018	2	2	2		1	2	2	2
10-2018	4	3	2	2	1	2	2	1
14-2018	1	2	1	1	1	4	2	2
18-2018	2	1	2	2	2	2	2	2
22-2018	2	3	2	2	1	1	2	2
26-2018	3	3	1	2	1	3	4	1
30-2018	1	2	2	3	1	1	3	1
33-2018	2	5	3	3	2	2	3	2
37-2018	4	4	1	1	2	1	4	3
41-2018	3	4	3	2	3	4	2	2
46-2018	2	6	1	3	2	2	2	1
50-2018	2	2	2	1	1	2	1	3
04-2019	2	6	2	2	2	2		2
08-2019	1	1	4	3	1	2	2	2
min	1	1	1	1	1	1	1	1
max	4	6	4	3	3	4	4	3

Platichthys flesus and *Limanda limanda)*, *Pomatoschistus* spp. (*Pomatoschitus minutus* and *P. lozanoi*), Ammodytidae (*Ammodytes marinus* and *A. tobianus),* Gadidae *(Merlangius merlangus*, *Pollachius pollachius* and *P. virens) and* Cottidae (*Myoxocephalus scorpius* and *Taurulus bubalis*), together with *Pomastoschistus microps*, *Osmerus eperlanus*, *Dicentrarchus labrax, Syngnathus rostellatus*, *Solea solea* and *Zoarces viviparus,* in total potentially 20 species, were identified at all locations (Table S2). Eight other species were found at respectively 7 (*Sardina pilchardus* and *Alosa alosa*) and 6 locations (*Abramis brama, Pholis gunnellus, Lipophrys pholis, Gasterosteus aculeatus, Ciliata mustela* and *Trachurus trachurus*).

3.2.2. Nonmetric Multidimensional Scaling

The NMDS plot of the presence – absence data of all species caught during the year-round sampling showed that the various stations clustered relatively close to each other. Eight taxonomic groups significantly contributed ($p \leq 0.05$): the Clupeidae complex, the Mugilidae complex, the Pleuronectidae complex and *Solea solea*, *Pomatoschitus* spp. and three individual species (*Abramis brama*, *Atherina presbyter*, *Glyptocephalus cynoglossus)* (Fig. 3A). The NMDS plot of the absolute read abundances of all species showed also a clustering relatively close to each other (Fig. 3B). Five taxonomic groups significantly contributed: consisting of

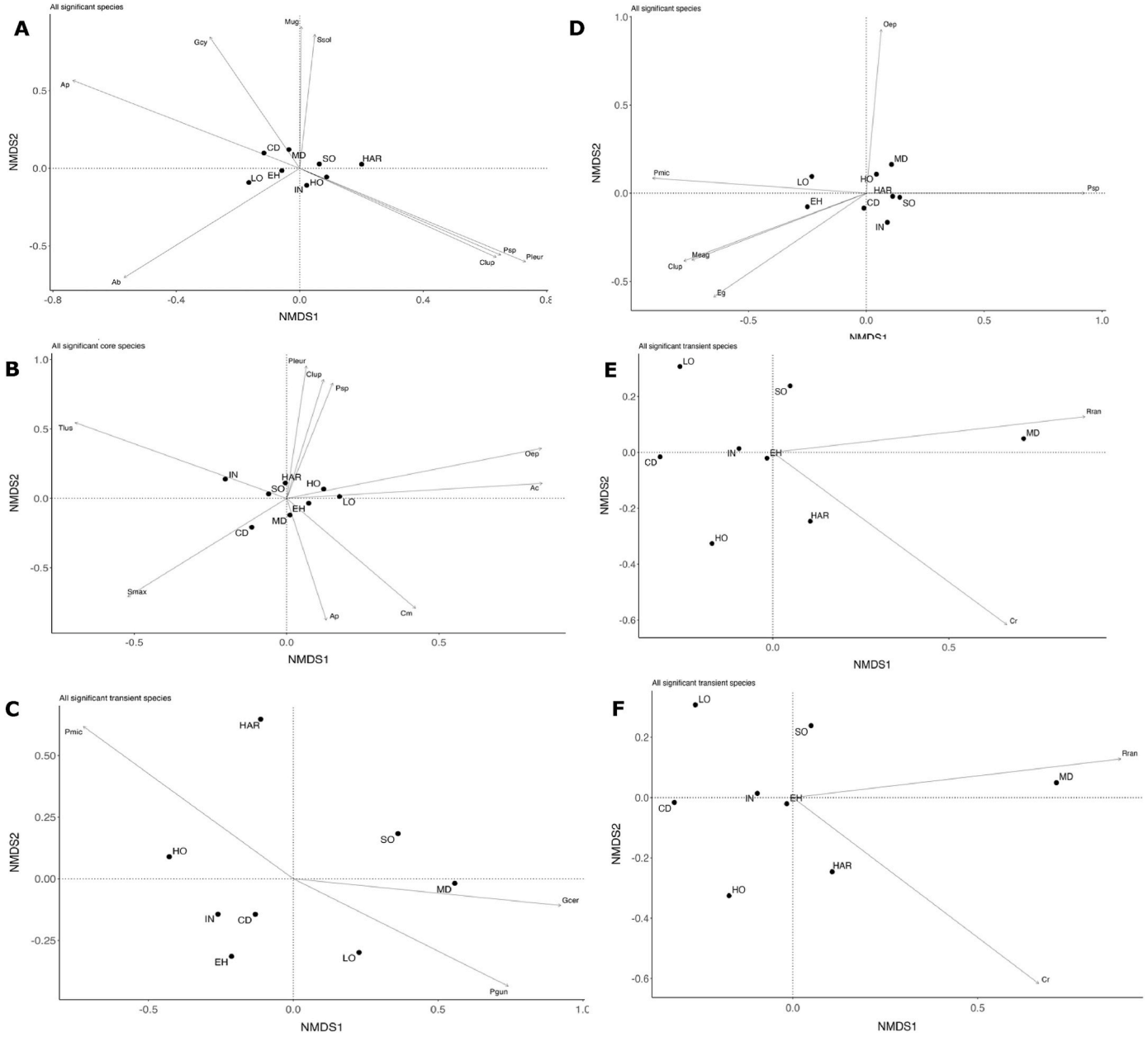

Fig. 3. NMDS of the various stations, all samplings together. Only species with significant vectors are listed ($p \leq 0.05$). Left column: based on presence and absence; right column based on fish eDNA concentrations (12S copies/L). Top row: all species; middle row: core species and bottom row transient species. Abbreviations and their full names of locations and species can be found in Table 1 (locations) and Table S2 (species names).
A: All species from presence-absence data;
B: Core species from presence-absence data;
C: Transient species from presence-absence data;
D: All species from fish eDNA concentrations (12S copies/L);
E: Core species from fish eDNA concentrations (12S copies/L);
F: Transient species from fish eDNA concentrations (12S copies/L).

the Clupeidae complex, three *Pomatoschistus* species, *Eutrigla gurnardus*, *Melanogrammus aeglefinus* and *Osmerus eperlanus*. The NMDS plot of the presence – absence data and the plot of the absolute read abundances differed due to differences in the significantly contributing taxonomic groups. However, in both plots there was a significant contribution of the Clupeidae complex and the three *Pomatoschistus* species (Table S4).

NMDS plots of only core species showed a similar pattern as that for all fish species. The NMDS plot of the presence – absence data, showed the various stations clustered relatively close to each other, whereby nine taxonomic groups significantly contributed ($p \leq 0.05$) (Fig. 3C). Four of these taxonomic groups also significantly contributed to the NMDS plot of the presence – absence data of all species: the Clupeidae complex, the Pleuronectidae complex, *Pomatoschitus* spp. and *Atherina presbyter*. Also, *Agonus cataphractus*, *Ciliata mustela*, *Osmerus eperlanus*, *Scophthalmus maximus* and *Trisopterus* spp. significantly contributed. In the NMDS plot of the absolute read abundances four taxonomic groups significantly contributed to the clustering (Fig. 3D). Three of these taxonomic groups also significantly contributed to the NMDS plot of the presence-absence data: the Clupeidae complex, *Osmerus eperlanus*, and *Pomatoschitus* spp. Furthermore, the Ammodytidae complex (*Ammodytes marinus* and *A. tobianus*) significantly contributed (Table S4).

The NMDS plots of the transient species resulted in more variation between stations and only a few species significantly contributed (Fig. 3E and F): two (*Gymnocephalus cernua* and *Pholis gunnellus*) for the presence-absence plot and two (*Chelon ramada* and *Raniceps raninus*) for the plot of the absolute read abundances (Table S4).

For the complete data set with all species, see Supplementary materials Fig. S2 and Table S5.

3.2.3. Jaccard/Sørensen dissimilarity index

For all fish species caught during the year-round sampling campaign, dissimilarity of a station with the other stations was on average 0.43 with values for most stations between 0.32 and 0.48 and only a higher average value (0.51) for Harlingen (Table S6). For only the core species, dissimilarity between stations was lower, on average 0.37 with some differences between specific stations ranging from 0.43 for Inschot and 0.41 for Harlingen. When only including the core/transient species, dissimilarity between stations was higher than when only considering the core species, on average 0.53 with larger differences between specific stations ranging from 0.69 for Harlingen and 0.61 for Marsdiep.

The monthly sampling showed differences during the year (Table S7). During the cold season (months: 1,2, 10 and 12), dissimilarity was less than during the warmer season (months: 3,5,6,7 and 8). Lauwersoog showed the highest dissimilarity (average 0.79) with the other locations during most of the monthly samplings.

4. Discussion

In this study eDNA-metabarcoding is applied as and assumed to be a reliable sampling tool for spatial analysis of the fish community in the Dutch Wadden Sea. This assumption is based on the fact that in a previous study in the area, presence–absence and concentration of eDNA corresponded with fyke net catches in the neighborhood (van Bleijswijk et al., 2020). Tillotson et al. (2018) also found that concentrations of eDNA did reflect spawning salmon abundance in a small stream at a fine spatial and temporal scales of 10s of meters. Studies in other tidal areas, such as tidal Hood Canal (Washington U.S.A.) revealed that the eDNA signal appeared to be endogenous to the site and water mass sampled (Kelly et al., 2018). Jeunen et al. (2019) showed that in dynamic areas, distinct eDNA signals are found already at a small spatial scale of less than 5 km. Millard-Martin et al. (2024) observed that eDNA can detect differences in nearshore fish distributions even at a small scale of 10s–100s of meters. Also, Harrison et al. (2019) concluded in their review paper that in the marine environment local production and persistence of eDNA appeared to be of more importance than mixing and transport of eDNA from elsewhere. Also, Cornelis et al. (2024) and Dukan et al. (2024) found spatial patterns along a gradient in the Belgian part of the North Sea, where they compared beam trawl data with eDNA samples. This means that despite potential mixing and transport of eDNA in the Dutch Wadden Sea, the risk of false positive species detection in this study seems to be small, especially since the sampling locations in this study, were selected with distinct distances of at least 10s of kilometers between them. However, it is important that despite results found in earlier studies, to what extent abiotic factors, like temperature and salinity, affect results. Similarly, an understanding of the changing metabolism and DNA excretion of species may well have implications for the use of eDNA in monitoring. However, it could also demonstrate that when eDNA degrades rapidly in seawater, detectable DNA is most likely of local origin. For single species detection, the target species DNA must be in detectable concentrations in these open systems which are largely based on the number of target individuals present. For future eDNA studies, it is imperative to take into consideration the importance of species abundance for eDNA yield and species detection (Gonzalez et al., 2023). This has large applications for monitoring of marine biodiversity and in particular fisheries, where data beyond species presence is essential (Thomsen et al., 2012).

4.1. Species composition

A small group of five species from two taxonomical groups (different locations and different samplings), Clupeidae (*Clupea harengus* and *Sprattus sprattus)* and the Pleuronectidae (*Pleuronectes platessa*, *Platichthys flesus* and *Limanda limanda)* were found in nearly all samples (>94 %). In addition, *Pomatoschistus* spp. (*Pomatoschitus minutus* and *P. lozanoi*), Ammodytidae (*Ammodytes marinus* and *A. tobianus),* Gadidae *(Merlangius merlangus*, *Pollachius pollachius* and *P. virens) and* Cottidae (*Myoxocephalus scorpius* and *Taurulus bubalis*), together with *Pomatoschistus microps*, *Osmerus eperlanus*, *Dicentrarchus labrax, Syngnathus rostellatus*, *Solea solea* and *Zoarces viviparus,* were identified at all locations. This group of 20 fish species form the resident and near-resident species community of the Dutch Wadden Sea (Zijlstra, 1983 van der Veer et al., 2015; Poiesz et al., 2020, 2023).

The year round eDNA sampling at eight locations in the Dutch Wadden Sea resulted in a positive identification of in total 40 fish species and 8 fish groups, potentially representing 58 different fish species, which is less than the over 100 fish species that have been identified in the area over the years (Witte and Zijlstra, 1983). However, the fact that the species accumulation curve did level off in the course of the year-round sampling implies that the eDNA sampling did identify most of the species present in the Dutch Wadden Sea during that year. The discrepancy between the low number of fishes identified in this study compared to the total number of fish found in the area illustrates that a large number of fish species can be considered as rare species that are only observed incidentally in the area (van der Veer et al., 2015). Furthermore, various fish species got extinct during the last century (Wolff, 2000), such as most of the skate and shark species (Bom et al., 2020; Poiesz et al., 2021).

4.2. Spatial variability

The aim of this study was to determine spatial variability of the Wadden Sea fish community in the various tidal basins by simultaneous sampling various locations and thereby excluding interannual variability in fish community and the impact of sampling design. In dynamic estuarine areas such as the Wadden Sea, species distribution (presence and abundance) will be dictated by the species' specific abiotic preferences and tolerance ranges (e.g. temperature, salinity, oxygen levels) for the different life stages (Neill et al., 1994; Freitas et al., 2010; Dahlke et al., 2020). Due to local variability hydrodynamic and morphodynamical variation, spatial variability in fish species distribution, composition and abundance within and between tidal basins might be expected as these have also been found in other studies (see for instance

Tulp et al., 2008; Meyer et al., 2016). However, spatial variability of the fish community is constantly being reduced due to ongoing mixing due to tidal currents. The question is to what extend spatial variability still occurs in the Wadden at the scale of tidal basins and if so whether it will translate in differences in the food web structure.

For the individual sampling locations, when considering the species accumulations curves, the picture is different. At four locations (Harlingen, Scheurrak, Inschot and Eemshaven) the accumulation curves still did not level off at the end of the sampling period, implying that at these locations still not all fish species present were identified. For these locations, sampling intensity or duration was too low to identify all species present, which complicates the analysis of spatial variability in species composition. However, when looking at the accumulation curves calculated trough the sample-size- and coverage-based integrations of rarefaction, when sample intensity is higher (20 sampling units), the total number of species caught will be reached. Nevertheless, the fact that the species accumulation curves of Holwerd, Lauwersoog, Cocksdorp and Marsdiep levelled off below that of all samples combined means that [1] that at none of the sampling locations all 48 identified taxonomic fish groups were found, and [2] that spatial variability in fish species composition occurs, at least between these locations.

The total number of fish species identified varied from 19 to 34 among locations with highest numbers near the tidal inlets. Twenty species were identified at all locations [Clupeidae (*Clupea harengus* and *Sprattus sprattus),* Pleuronectidae (*Pleuronectes platessa*, *Platichthys flesus* and *Limanda limanda)*, *Pomatoschistus* spp. (*Pomatoschitus minutus*, *P. lozanoi* and *P. microps*), Ammodytidae (*Ammodytes marinus* and *A. tobianus),* Gadidae *(Merlangius merlangus*, *Pollachius pollachius* and *P. virens) and* Cottidae (*Myoxocephalus scorpius* and *Taurulus bubalis*), *Osmerus eperlanus*, *Dicentrarchus labrax, Syngnathus rostellatus*, *Solea solea* and *Zoarces viviparus*]; eight species were found at 6–7 locations and the remaining 30 species were found only incidentally. Most of these species belonged to the group of core species and also had a high number of reads. Most of these species were found to be abundant not only in the Dutch part of the Wadden Sea (Tulp et al., 2008; van der Veer et al., 2015), but also in other parts (Kellnreiter et al., 2012; Meyer et al., 2016).

Both the presence–absence data and the absolute read abundances showed a clustering of the various locations relatively close to each other. For both, a significant contribution of the Clupeidae complex and the three *Pomatoschitus* species contributed to the clustering. Clupeidae complex and the three *Pomatoschitus* species are among the most abundant fish species both in the Dutch and also German and Danish part of the Wadden Sea (Kellnreiter et al., 2012; Tulp et al., 2008; van der Veer et al., 2015; Meyer et al., 2016; Poiesz et al., 2020, 2023). The number of transient species was overall low and no clear pattern between stations was found. These results illustrated spatial variability in fish community in the Dutch Wadden Sea, but with common (core) species being present at all locations and more differences among locations with respect to rare (transient) species, most probably due to location specific differences in hydrographical and geomorphological characteristics. The various fish surveys in the Wadden Sea also show a large similarity in fish species composition in the different Wadden Sea areas, both with respect to benthic fish (Tulp et al., 2008; van der Veer et al., 2015) and pelagic fish species (van der Veer et al., 2015). Despite differences in sampling methods, strategy and timing, the same species were found in the Marsdiep (Poiesz et al., 2020), the Ems (Poiesz et al., 2023), the Jade (Meyer et al., 2016) and Sylt-Rømø (Kellnreiter et al., 2012).

The observation that common core species are more present and abundant at all locations and that locations differ especially with respect to rarer (transient) species confirms our hypothesis. Although some spatial variability does occur, the main fish components of the Wadden Sea food web will be similar. Also, the most important prey species, such as for instance brown shrimp and small herring can be found all over the Wadden Sea (Kellnreiter et al., 2012; Tulp et al., 2022; Meyer et al., 2016; Poiesz et al., 2020). However, strong temporal changes in both epifauna and infauna prey species have been described (Schrückel and Krőncke, 2013, Beukema and Dekker, 2020) and consequently it cannot be excluded that this might have affected the Wadden Sea fish food web over time.

CRediT authorship contribution statement

Suzanne SH. Poiesz: Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Johannes IJ. Witte:** Data curation. **Judith DL. van Bleijswijk:** Writing – review & editing, Supervision, Methodology, Data curation. **Harry J. Witte:** Methodology, Data curation. **Evaline M. van Weerlee:** Methodology, Data curation. **Maartje Brouwer:** Methodology, Data curation. **Sanne Vreugdenhil:** Methodology, Data curation. **Henk W. van der Veer:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization. **Lise Klunder:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2025.109411.

Data availability

Data will be made available on request.

References

Abdala Asbun, A., Besseling, M.A., Balzano, S., van Bleijswijk, J.D., Witte, H.J., Villanueva, L., Engelmann, J.C., 2020. Cascabel: a scalable and versatile amplicon sequence data analysis pipeline delivering reproducible and documented results. Front. Genet. 11, 489357.

Andrews, S., 2010. FastQC: a Quality Control Tool for High Throughput Sequence Data. ScienceOpen, Inc, Berlin.

Anon. 2018. Getijdetafels voor Nederland.

Bastow Wilson, J., 2012. Species presence/absence sometimes represents a plant community as well as species abundances do, or better. J. Veg. Sci. 23, 1013–1023.

Beukema, J.J., Dekker, R., 2020. Half a century of monitoring macrobenthic animals on tidal flats in the Dutch Wadden Sea. Mar. Ecol. Prog. Ser. 656, 1–18.

Biswas, P., Lian, T.C., Wang, T.C., Ye, Y., 2006. Semidefinite programming-based algorithms for sensor network localization. TOSN 2, 188–220.

Bom, R.A., van de Water, M., Camphuysen, C.J., van der Veer, H.W., van Leeuwen, A., 2020. Historical ecology and demise of the iconic angelshark *Squatina squatina* in the southern North sea. Mar. Biol. 167, 1–10.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84, 45–67.

Chao, A., Ma, K.H., Hsieh, T.C., 2016. iNEXT online: software for interpolation and extrapolation of species diversity Program and User's Guide published at Code 30043, 1-14, http://chao.stat.nthu.edu.tw/wordpress/software_download/inext-online/.

Cornelis, I., De Backer, A., Maes, S., Vanhollebeke, J., Brys, R., Ruttink, T., Derycke, S., 2024. Environmental DNA for monitoring the impact of offshore wind farms on fish and invertebrate community structures. Environ. DNA, 6-e575.
Dahlke, F.T., Wohlrab, S., Butzin, M., Pörtner, H.O., 2020. Thermal bottlenecks in the life cycle define climate vulnerability of fish. Science, 369, 65–70.
Dukan, N., Cornelis, I., Maes, S., Hostens, K., De Backer, A., Derycke, S., 2024. Vertical and horizontal environmental DNA (eDNA) patterns of fish in a shallow and well-mixed North Sea area. Sci. Rep. 14–16748.
Freitas, V., Cardoso, J.F.M.F., Peck, M.A., Kooijman, S.A.L.M., van der Veer, H.W., 2010. Analysis of physiological performance of North Atlantic marine organisms by means of interspecies differences in DEB parameters. Phil. Trans. Royal Soc. B 365, 3553–3565.
Froese, R., Pauly, D., 2019. FishBase. www.fishbase.org.
Gonzalez, F., Barria, P., Ponce, F., Mora, S., 2023. Population genetic structure of marine fishes. In: Behzadi, P. (Ed.), Population Genetics – from DNA to Evolutionary Biology. https://doi.org/10.5772/intechopen.112694 eBook.
Goodall, D.W., 1983. Estuaries and Enclosed Seas. Elsevier, Amsterdam.
Haedrich, R.L., 1983. Estuarine fishes. In: Ketchum, B.H. (Ed.), Ecosystems of the World, Vol. 26, Estuaries and Enclosed Seas. Elsevier, Amsterdam, pp. 183–207.
Harrison, J.B., Sunday, J.M., Rogers, S.M., 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proc. R. Soc. A B 286, 20191409.
Jeunen, G.J., Knapp, M., Spencer, H.G., Lamare, M.D., Taylor, H.R., Stat, M., Bunce, M., Gemmell, N.J., 2019. Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. Mol. Ecol. Res. 19, 426–438.
Kellnreiter, F., Pockberger, M., Asmus, H., 2012. Seasonal variation of assemblage and feeding guild structure of fish species in a boreal tidal basin. Estuar. Coast Shelf Sci. 108, 97–108.
Kelly, R.P., Gallego, R., Jacobs-Palmer, E., 2018. The effect of tides on nearshore environmental DNA. PeerJ 6, e4521.
Kühl, H., Kuipers, B.R., 1983. Qualitative food relationships of Wadden Sea fishes. In: Wolff, W.J. (Ed.), Ecology of the Wadden Sea. Balkema Press, Rotterdam, pp. 5/122–5/123.
Lefcheck, J.S., Hughes, B.B., Johnson, A.J., Pfirrmann, B.W., 2019. Are coastal habitats important nurseries? A meta-analysis. others Conserv. Lett. 12, e12645.
Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.
Levin, L.A., Etter, R.J., Rex, M.A., Gooday, A.J., Smith, C.R., Pineda, J., Stuart, C.T., Hessler, R.R., Pawson, D., 2001. Environmental influences on regional deep-sea species diversity. Annu. Rev. Ecol. Systemat. 32, 51–93.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17, 10–12.
Meyer, J., Kroencke, I., Bartholomae, A., Dippner, J.W., Schückel, U., 2016. Long-term changes in species composition of demersal fish and epibenthic species in the Jade area (German Wadden sea/Southern North Sea) since 1972. Estuar. Coast Shelf Sci. 181, 284–293.
Millard-Martin, B., Sheridan, K., Morien, E., Lemay, M.A., Hessing-Lewis, M., Clemente-Carvalho, R.B.G., Sunday, J.M., 2024. Effect of environmental DNA sampling resolution in detecting nearshore fish biodiversity compared to capture surveys. PeerJ, 17967.
Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.L., Agarwala, R., Schäffer, A.A., 2008. Database indexing for production MegaBLAST searches. Bioinformatics 24, 1757–1764.
Neill, W.H., Miller, J.M., van Der Veer, H.W., Winemiller, K.O., 1994. Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. Neth. J. Sea Res. 32, 135–152.
Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2022. Vegan: Community Ecology Package R Package Version 2.6-4.
Poiesz, S.S.H., Witte, J.I.J., van der Veer, H.W., 2020. Only a few key prey species fuel a temperate coastal fish food web. Mar. Ecol. Prog. Ser. 653, 153–166.
Poiesz, S.S.H., van Elderen, T., Witte, J.I.J., van der Veer, H.W., 2021. Historical trophic ecology of some divergent shark and skate species in the Dutch coastal North Sea zone. Mar. Biol. 168, 165.
Poiesz, S.S.H., Witte, J.I.J., van der Meer, M.T., Jager, Z., Soetaert, K.E., van der Heide, T., van der Veer, H.W., 2023. Stomach content and stable isotopes illustrate large spatial similarity in the Wadden Sea fish food-web structure. Mar. Ecol. Prog. Ser. 707, 57–76.
Postma, H., 1983. Hydrography of the Wadden Sea. Movements and properties of water and particulate matter. In: Wolff, W.J. (Ed.), Ecology of the Wadden Sea. Balkema Press, Rotterdam, pp. 2/1–2/75.
R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. R version 4.3.1.
Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., Coissac, E., 2011. EcoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Res. 39, 1–11.
Ruth, M., Berghahn, R., 1989. Biological monitoring of fish and crustaceans in the Wadden Sea -potential and problems. Helgol. Meeresunters. 43, 479–487.
Saeed, N., Nam, H., Haq, M.I.U., Muhammad Saqib, D.B., 2018. A survey on multidimensional scaling. ACM Comput. Surv. 51, 1–25.
Selleslagh, J., Amara, R., 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). Estuar. Coast Shelf Sci. 79, 507–517.
Schrückel, U., Krőncke, I., 2013. Temporal changes in intertidal macrofauna communities over eight decades: a result of eutrophication and climate change. Estuar. Coast Shelf Sci. 117, 210–218.
Sørensen, T.A., 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content, and its application to analysis of the vegetation on Danish commons. Biologiske Skrifter Kongelige Danske Videnskabernes Selskab 5, 1–34.
Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One 7, e41732.
Tillotson, M.D., Kelly, R.P., Duda, J.J., Hoy, M., Kralj, J., Quinn, T.P., 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biol. Conserv. 220, 1–11.
Tulp, I., Bolle, L.J., Rijnsdorp, A.D., 2008. Signals from the shallows: in search of common patterns in long-term trends in Dutch estuarine and coastal fish. Neth. J. Sea Res. 60, 54–73.
Tulp, I., Bolle, L.J., Chen, C., Dänhardt, A., Haslob, H., Jepsen, N., van Leeuwen, A., Poiesz, S.S.H., Scholle, J., Vrooman, J., Vorberg, R., Walker, P., 2022. Fish. In: Kloepper, S., et al. (Eds.), Wadden Sea Quality Status Report, Common Wadden Sea Secretariat. Wilhelmshaven, Germany. Last updated 06.09.2022. qsr.waddensea-worldheritage.org/reports/fish.
van Bleijswijk, J.D., Engelmann, J.C., Klunder, L., Witte, H.J., Witte, J.I.J., van der Veer, H.W., 2020. Analysis of a coastal North Sea fish community: comparison of aquatic environmental DNA concentrations to fish catches. Environ. DNA 2, 429–445.
van der Veer, H.W., Dapper, R., Henderson, P.A., Jung, A.S., Philippart, C.J.M., Witte, J.I.J., Zuur, A.F., 2015. Changes over 50 years in fish fauna of a temperate coastal sea: degradation of trophic structure and nursery function. Estuar. Coast Shelf Sci. 155, 156–166.
Whitfield, A.K., Able, K.W., Blaber, S.J.M., Elliott, M., 2002. Fish and Fisheries in Estuaries: a Global Perspective. John Wiley & Sons, Chichester UK.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Witte, J.I.J., Zijlstra, J.J., 1983. The species of fish occurring in the Wadden Sea. In: Wolff, W.J. (Ed.), Ecology of the Wadden Sea. Balkema, Rotterdam, pp. 5/10–5/19.
Wolff, W.J., 1983. Ecology of the Wadden Sea. Balkema, Rotterdam, The Netherlands.
Wolff, W.J., 2000. Causes of extirpations in the Wadden Sea, an estuarine area in the Netherlands. Conserv. Biol. 14, 876–885.
Yao, M., Zhang, S., Lu, Q., Chen, X., Zhang, S.Y., Kong, Y., Zhao, J., 2022. Fishing for fish environmental DNA: ecological applications, methodological considerations, surveying designs, and ways forward. Mol. Ecol. 31, 5132–5164.
Zijlstra, J.J., 1983. The function of the Wadden Sea for the members of its fish-fauna. In: Wolff, W.J. (Ed.), Ecology of the Wadden Sea. Balkema, Rotterdam, pp. 5/20–5/26.