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Original Article

Small pelagic fish in the shallow Wadden Sea show opportunistic feeding with a strong benthic link

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Abstract

Small pelagic fish (SPF) are crucial in marine food webs, transferring energy from plankton to higher trophic levels. This study focuses on herring (*Clupea harengus*) and sprat (*Sprattus sprattus*), addressing knowledge gaps in their feeding ecology in a nursery area, the Dutch Wadden Sea. We conducted a year-long, monthly survey, and used DNA metabarcoding to analyse zooplankton samples and stomach contents of two size classes of herring and sprat. Intra-, interspecific, and seasonal variations in fish condition, stomach fullness, and diet composition, along with selective feeding, were studied. Our study showed that condition and diet composition of herring and sprat, along with zooplankton density, exhibited a clear seasonal pattern. Juvenile herring and sprat displayed opportunistic feeding behaviour, rather than showing distinct prey selection. Besides copepods, we regularly observed (larvae of) benthic invertebrates in their diet. This emphasizes the crucial role of SPF as energy transfer agents, not solely between trophic levels, but also from benthic to pelagic habitats. Furthermore, fish post-larvae were part of the diet of larger herring (10–15 cm). Because of its unprecedented temporal and taxonomical detail, this study advances the understanding of seasonal dynamics of dominant components at the base of the Wadden Sea food web.

Keywords: DNA metabarcoding; food web; seasonal dynamics; small pelagic fish; Wadden Sea; zooplankton

Introduction

Small pelagic fish (SPF) play a vital role in marine food webs by transferring energy from plankton to larger predators (Ruzicka et al. 2024). Zooplankton and SPF can occur in vast quantities and are therefore critical components in the functioning of marine ecosystems. Marine ecosystems face significant environmental changes due to human-related stressors, including warming of sea water and intensified extreme weather events (Gissi et al. 2021). Zooplankton communities exhibit strong responses to changes in the marine environment (Martens and Van Beusekom 2008, Richardson 2008). Since SPF primarily feed on zooplankton, they are impacted in turn by changes in zooplankton community compositions (Heneghan et al. 2023). Such changes can potentially trigger regime shifts, also impacting higher trophic levels, as previously observed in regions like the North Sea and Baltic Sea (Beaugrand 2004, Österblom et al. 2007). To explain changes and trends in higher trophic levels, a detailed understanding of the entire marine food web structure is crucial, including the feeding ecology of SPF at the base of the food web.

Studies on SPF are often conducted in open seas, primarily because of technical limitations of observing SPF schools in shallow coastal waters. Meanwhile, in coastal waters, SPF constitutes an important share of fish biomass. This is also true in the Wadden Sea, the world's largest intertidal area and a key area for foraging birds. Furthermore, the Wadden Sea is an important nursery area for juvenile fish in the North Sea ecoregion (van der Veer et al. 2022). Given that SPF are the primary component of the overall fish biomass here (Couperus et al. 2016), they play a crucial role in the marine food web. For example, tern species rely on SPF as their main food source, with the abundance and condition of SPF serving as predictors for successful breeding (Dänhardt and Becker 2011).

The dominant SPF species in the Wadden Sea are Atlantic herring (*Clupea harengus*, hereafter herring) and European sprat (*Sprattus sprattus*, hereafter sprat). Early life stages of both species drift from offshore spawning areas to the Wadden Sea, which is primarily used as juvenile area for growth and development, before moving offshore again. However, detailed studies on their feeding ecology in this area are currently lacking. Diet studies have been conducted in the adjacent North and Baltic Sea, where the copepod *Temora longicornis* is the predominant prey item (e.g. Bernreuther et al. 2018, van Ginderdeuren et al. 2014). Moreover, some studies found *Acartia* as part of their diet (Bernreuther et al. 2018, Ojaveer et al. 2018, Novotny et al. 2022), while in others it was selectively avoided (Casini et al. 2004, van Ginderdeuren et al. 2014). This underscores the seasonal and local variation in prey preference, highlighting the importance of considering seasonality in local studies of SPF diet composition.

In the North Sea, van Ginderdeuren et al. (2014) demonstrated that herring and sprat exhibit selective feeding behaviour, with approximately three-quarters of their diet consisting of only two species. This indicates the vulnerability of the food web to changes in zooplankton dynamics. The most recent study on Wadden Sea seasonal zooplankton abundance was conducted more than four decades ago (Fransz and Arkel 1983). Since then, nutrient inputs have decreased and monthly mean temperatures have increased (van Aken 2008, van Beusekom et al. 2019). Moreover, the incidence of cold winters has become rare, whereas the frequency of hot and dry summers has increased (Beukema and Dekker 2020). In addition, over 100 new species have been introduced in the Wadden Sea (Reise et al. 2023), including potential competitors of SPF, such as the zooplanktivorous ctenophore *Mnemiopsis leidyi* (Kellnreitner et al. 2013, van Walraven et al. 2017). With these recent changes and other stressors related to global warming, there is an increased importance in studying zooplankton dynamics and their impact on the diet of SPF predators. This includes assessing whether predators exhibit prey selection, or simply consume whatever prey is available.

Dietary composition analysis has traditionally been based on the morphological identification of stomach contents (Maes and Ollevier 2002, Casini et al. 2004, van Ginderdeuren et al. 2014, Bernreuther et al. 2018). A principal shortcoming of this approach is that prey organisms are often digested beyond the point of recognition; for instance, Raab et al. (2012) reported 90% of highly digested items in the stomachs of herring and sprat, while van Ginderdeuren et al. (2014) found unidentifiable digested material in 46% of the stomachs of pelagic fish. Furthermore, morphological identification requires taxonomic expertise, is prone to observer bias, and is time-consuming (Leray and Knowlton, 2016). The development of DNA metabarcoding techniques allows dietary composition analysis using prey DNA from stomach contents (Pompanon et al. 2012). This method, used in dietary studies for over a decade, has proven to be valuable for identifying food web structures from fish stomach content samples, especially when prey items are in an advanced stage of digestion (e.g. Albaina et al. 2016, Traugott et al. 2021, Novotny et al. 2022). Meanwhile, the link between abundance and read counts remains a concern due to factors such as difference in DNA quantities among tissues, taxa, and life stages, impacting the relative read abundance (RRA) (Ershova et al. 2023, Lamb et al. 2019). Despite the RRA not directly reflecting abundance, treating the data quantitatively is suggested to be more informative than relying solely on presence-absence (Deagle et al. 2019, Lamb et al. 2019).

This study investigated the diet composition of herring and sprat throughout an entire year in the Dutch Wadden Sea using DNA metabarcoding targeting the Cytochrome Oxidase Subunit I (CO1) gene and 18S ribosomal RNA regions. Monthly fish and mesozooplankton samples were collected, encompassing two size classes of herring and sprat. The aim of this study was to test the following hypotheses: (i) Stomach fullness, condition, and diet composition of herring and sprat follow a seasonal pattern, correlating with zooplankton density and copepod sizes. (ii) No difference exists between the diet composition of herring and sprat, given their similarity in morphology and habitat use. However, ontogenetic differences in diet are expected between small and large size classes. (iii) Herring and sprat exhibit selective feeding behaviour, which will be studied by comparing diet results with the occurrence and community composition of mesozooplankton from the same areas and time periods. This yields a first step in describing and understanding seasonal dynamics in the dominant component at the base of the Wadden Sea food web.

Methods

Fish and zooplankton sampling

Herring, sprat, and mesozooplankton were collected in the Dutch Wadden Sea during a one-year, monthly stow net survey starting March 2021. To cover distinct Wadden Sea basin types (Baptist et al. 2019), samples were obtained from two locations each month: Westgat and either Marsdiep or Vliestroom, depending on site accessibility determined by wind direction (Fig. 1). Stow net fishing is a passive fishing method using water currents driving fish into the net. The stow net was held open by two horizontal bars measuring 8 m during high current speeds at outgoing tide. The net covered the entire water column (average depth 5.2 m, minimum 2.7 m, and maximum 7.4 m), with a stretched mesh size of 20 mm. Temperature ranged from 5°C in February to 21°C in July and salinity fluctuated between 24 and 31 PSU.

Total length and wet weight of collected fish were measured, and herring and sprat were grouped into small (<10 cm) and large (>10 cm) size classes. The small class primarily comprises young-of-the-year and juvenile individuals, representing the main component of the SPF community in the Wadden Sea. The average size of small and large herring was 8.4 and 13.4 cm, respectively, and the average size of small and large sprat was 7.4 and 11.7 cm, respectively. Whenever catch volume allowed, up to 15 fish per species and size class were randomly selected from each haul. The fish were individually stored in 96% ethanol at −20°C, with a ventral incision made from the gill slits towards halfway the abdominal cavity to ensure proper ethanol conservation of the gastrointestinal tract.

The focus of this study was the overall diet of herring and sprat, rather than that of individual fish. To address the challenge of empty stomachs and a large prey variability, within limited sample size, five individual fish of the same species, size class, and haul were pooled into one diet sample. Moreover, Darbyson et al. (2003) demonstrated that schooling fish have a great similarity in stomach content if collected from the same haul because they have been exposed to the same types and concentrations of prey, supporting the rationale for pooling samples. In total, 304 small herring, 186 large herring, 277 small sprat, and 158 large sprat were collected, corresponding to 62, 38, 57, and 32 DNA samples of pooled fish per group, respectively (Supplementary Table S1). All sampling was performed in accordance with Dutch law concerning animal welfare. The protocol was approved by the Animal Ethical Commission of Wageningen UR (experiment code: 2020.D-0026.001; application: 40100202010984).

Additionally, zooplankton samples were collected using a centrifugal pump (pump rate: ±80 l/min) deployed on the ship during fishing operations to capture concurrent prey communities and fish stomach contents. For each zooplankton sample, 100 l of sea water were pumped from a depth of 2–3 m and sieved over a 200-μm plankton net. A total of 66 zooplankton samples were collected: 22 for DNA analysis and 44 for image analysis, corresponding to 1 DNA sample and 2 image

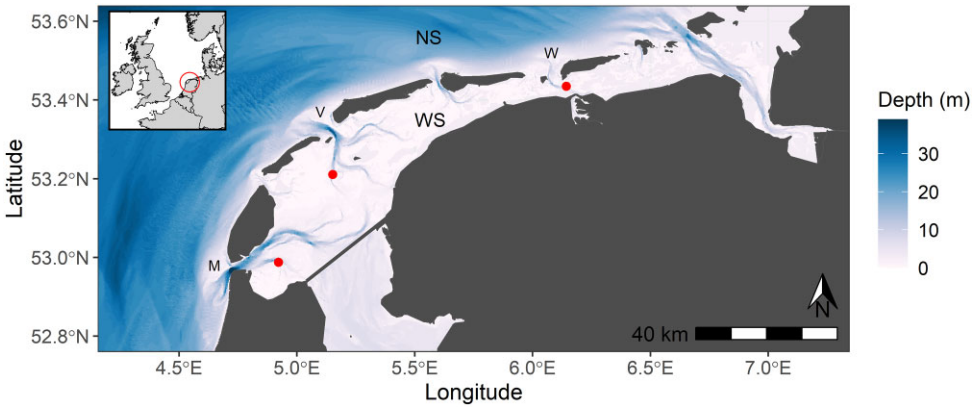

Figure 1. Map of the Dutch Wadden Sea (WS), adjacent to the North Sea (NS), including bathymetry. The inlets are denoted by their initial letters (M = Marsdiep, V = Vliestroom, W = Westgat), and sampling locations are marked with red circles.

analysis samples per fishing location. Zooplankton samples for DNA analysis were preserved in 96% ethanol and stored at −20°C, and samples for image analysis were preserved in 4% formalin and stored at room temperature.

Fish stomach content sampling

For each fish, stomach fullness was visually estimated using categories of total prey content relative to stomach volume: 0% (empty), 5%, 25%, 50%, 75%, and 100% (full). Stomach contents were characterized as either fresh or partially degraded. Contents were collected using a tweezer and scalpel, avoiding inclusion of host stomach tissue as much as possible. To minimize the level of prey degradation, intestinal content was not included, and only the content of the stomach and lower part of oesophagus were used. Additionally, photographs were taken of clearly identifiable prey, and fish larval presence in the stomachs was structurally counted, supporting DNA analyses. Stomach contents of five fish of equal species, size class, and haul were pooled into a 1.5 ml DNA LoBind Microcentrifuge Eppendorf tube. Equipment was sterilized with 0.5% bleach after each sample to prevent cross-contamination. Samples were conserved in 96% ethanol and stored at −20°C.

DNA extraction, amplification, and library preparation

To remove ethanol from samples, Eppendorf tubes were centrifuged at 20.000 *g* for 3 min. Subsequently, ethanol was pipetted off, 1 ml phosphate-buffered saline (PBS) was added to the pellet, and tubes were vortexed and centrifuged again at 20.000 *g* for 3 min. Then, PBS was removed, and pellets were used for DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen). DNA was eluted in a final volume of 100 µl AE buffer. Lab processing was conducted in two separate batches: batch one included samples from March to June, while batch two encompassed samples from July to February.

For metabarcoding, both the CO1 gene and 18S ribosomal RNA gene were used. The CO1 gene is the standard gene region targeted for the identification of animals in metabarcoding studies and is exceptionally well represented in databases (Leray et al. 2013). The 18S rRNA gene is more conserved than CO1, increasing primer versatility at the cost of taxonomic resolution. Studies on zooplankton samples and fish stomach composition have demonstrated that CO1 and 18S rRNA complement each other effectively, resulting in improved species detection (Zhang et al. 2018, Novotny et al. 2022). Leray fragments of the CO1 gene (313 bp) were amplified by polymerase chain reaction (PCR) with primer pair mICOIintF & jgHCO2198 (5'-GGWACWGGWTGAACWGTWTAYCCYCC-3' and 5'-TANACYTCNGGRTGNCCRAARAAYCA-3'; Leray et al. 2013). For the 18S gene, the V4 region (~450 bp) was amplified with primer pairs Uni18S and Uni18SR (5'-AGGGCAAKYCTGGTGCCAGC-3' and 5'-GRCGGTATCTRATCGYCTT-3'; Zhan et al. 2013). The primers were entailed with Oxford Nanopore Technologies (ONT) primer tails according to ONT protocols SQK-LSK110 and SQK-LSK114 for batches one and two, respectively. Both protocols were extended with the EXP-PBC096 kit, according to manufacturer's protocol.

PCR was performed in triplicate for batch one and in duplicate for batch two. PCR reactions were conducted in a total volume of 15 µl, consisting of 7.5 µl of Phire Tissue Direct PCR Master Mix (Thermo Fisher Scientific, MA, USA), 0.3 µl primer mix (10 µM), 6.45 µl nuclease-free water (NFW), and 0.75 µl DNA template. For batch two, the total volume was reduced to 10 µl, comprising of 5 µl of Phire mix, 0.2 µl primer mix, 3.8 µl NFW, and 1 µl DNA template. PCR conditions in the Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories Inc., CA, USA) were optimized as follows: annealing at 98°C for 3 min; followed by 30 cycles of denaturation for 10 seconds at 98°C, annealing for 10 seconds at 55°C, and extension at 72°C for 20 seconds. In batch two, the extension times were adjusted to 15 seconds for 18SV4 and 10 seconds for CO1. The amplification process concluded with an extension step lasting 1–3 min at 72°C. Additionally, for the amplification of the CO1 gene, 5 extra cycles of denaturation, annealing, and extension were applied. Amplification products were visualized using gel electrophoresis and successfully amplified replicates were pooled. Negative controls in DNA extractions and PCR runs detected no significant contamination, except in the first two samples of batch one, which were likely a lab error (Supplementary Fig. S1).

After quantifying DNA concentrations of barcoded amplicons, samples were pooled in equimolar concentrations and cleaned using Agencourt Ampure XP beads (Beckman Coulter Inc., Indianapolis, IN, USA). Pooled products were further processed using SQK-LSK110 and SQK-LSK114 sequencing kits, according to manufacturer's protocol. DNA

concentrations were determined using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). DNA extractions, PCR reaction preparations, and library preparations were performed inside a UV cabinet to prevent contamination.

MinION nanopore sequencing

Barcoded DNA fragments were sequenced using an ONT MinION sequencer, with R9.4.1 flow cells for batch one and R10.4.1 flow cells for batch two. All samples were sequenced across 6 runs, with each sequencing run taking 6–14 hours. The sequencing continued until ∼9 million reads were obtained for runs in batch one and 4.5 million reads for runs in batch two.

Bioinformatic analysis

Fast5 files obtained from the sequencer were processed using the PIMENTA pipeline (van der Vorst et al. 2024, preprint: not peer reviewed). In PIMENTA, basecalling, demultiplexing, and trimming of Nanopore adapters were done using Guppy version 6.4.2 (ONT, Oxford, UK) with default settings. Reads were subsequently filtered for read length (CO1 300–420 bp and 18SV4 400–750 bp) and quality (Q > 12) using PrinseQ 0.20.4 (Schmieder and Edwards 2011). Clustering was performed separately for each sample using CD-HIT-EST (version 4.8.1) with a 90% identity threshold, and a minimum cluster size of 5 (Fu et al. 2012). Multiple sequence alignment (MSA) was performed on sequences within each cluster using MAFFT (version v7.471) (Katoh and Standley 2013). Subsequently, the consensus sequences of samples in single runs were reclustered using CD-HIT-EST with a 99.5% identity threshold, followed by MSA. DNA barcode primers were trimmed using an error rate of 0.15 and a minimum overlap of 20 for CO1 and 14 for 18SV4, using Cutadapt v4.4 (Martin 2011).

Blasting of cluster sequences was done using the nucleotide database from the National Centre for Biotechnology Information (NCBI, accessed April 2023) and R packages rBLAST v0.99.2. (Hahsler and Nagar 2019) and taxonomizr v0.10.2 (Sherrill-Mix 2023). Blast output records were filtered with the following criteria: E-value < 0.001, bit score >50, minimum alignment length >90% of query sequence length, and percentage identity >98%. Records containing "environmental sample" and "uncultured" in species names were removed. Subsequently, the top 10 records per sequence were retained for further filtering. Then, only records within the kingdom Animalia were selected, removing, among others, all bacteria, fungi, and plants. Furthermore, records containing "sp." in the species name were retained only under the conditions that genus-level information was present, and that percentage identity exceeded 99%.

The nomenclature of all identified species was verified and adjusted following the World Register of Marine Species (WoRMS) using R client worrms v0.4.3 (Chamberlain and Vanhoorne, 2024). Subsequently, geographic distribution data for each species was downloaded from Ocean Biodiversity Information System (OBIS, accessed October 2023) using R package robis v2.11.3 (Provoost, 2022). The minimum distance for all records to the Dutch Wadden Sea was calculated, and only those records with distances <1500 km were retained. If multiple records for a single sequence remained, a series of filtering steps followed. If a single record had a 100% identity score, all other records were removed. Otherwise, the record with the highest percentage identity was retained, but only if the query coverage was minimal 97%. In cases with multiple records having equally high percentage identities, all identified species were considered reliable and species names were combined, or the record was downgraded to a genus-level identification.

Image based processing of zooplankton samples

Samples containing high densities of different-sized material were size fractioned using 200, 300, or 500 µm sieves. Fractions with high zooplankton densities were further subsampled using a Motoda box splitter. Clumps of jellyfish or other large material were manually taken out and thoroughly rinsed with seawater to retain attached plankton. Full samples, size fractions, or subsamples were placed in an acrylic tray (dimensions 24.5 × 15.8 × 3.0 cm) to be scanned using an Epson Perfection V850 scanner at 3200 dpi. Captured images were processed using Zooprocess (Gorsky et al. 2010) to isolate individual zooplankton objects and to extract morphological features for each object. Subsequently, the processed images, associated metadata, and morphological features were uploaded to Ecotaxa (Picheral et al. 2023). Annotation of the images was performed in Ecotaxa, aided by its supervised machine learning feature and a learning set consisting of manually classified objects from 30 randomly selected samples of our area. The objects were classified into nine main taxonomic groups: Annelida, Bivalvia, Cirripedia, Copepoda, Foraminifera, Gastropoda, Malacostraca, Ostracoda, and an "other" category encompassing Echinodermata, Daphnia, Dinoflagellates, Bryozoa larvae, and Ctenophora. Additionally, a class was created for artefacts, including bubbles and detritus. Automatically generated annotations were manually verified, corrected, and subsequently exported from Ecotaxa.

Samples from the same months were grouped, and the mean total abundance per taxonomic group per 100 l was calculated to analyse seasonal zooplankton abundance. To examine the size of available food throughout the year, we evaluated copepod size, as copepods are typically the primary food source for SPF. Using Ecotaxa, the average length and width of copepods were calculated, corresponding to the major and minor axes, respectively.

Data analysis

Data analysis was done in R (version 4.3.1) and RStudio (version 2023.06.1). Using wet length (L) and weight (W), Fulton's K was calculated as an indicator of fish condition (Froese 2006). This was done exclusively for the small size class, given that the large size class includes individuals of varying maturity, influencing fish condition.

$$K = 100 * \frac{W}{L^3} \quad (1)$$

Data filtering and statistical analysis of metabarcoding results were facilitated by R package Phyloseq v1.44.0 (McMurdie and Holmes 2013). Rarefaction curves were generated and read count per sample was plotted against observed number of clustered amplicon sequence variants (ASVs, Supplementary Figs S2–S4). Correcting for rarefaction is a topic of significant debate (McMurdie and Holmes 2014, Cameron et al. 2021). Subsampling for rarefaction was applied, given the positive trend observed in sample read count

against ASV diversity. Notably, runs from the first batch were sequenced twice as long, resulting in twice the ASV diversity. Read counts and sample sizes at each stage are detailed in Supplementary Tables S2 and S3 for CO1 and 18SV4, respectively. To homogenize prey diversity across all samples, read counts were standardized to 24 000 reads for all runs using Phyloseqs "rarefy_even_depth" function. Consequently, 7 CO1 and 11 18SV4 samples were excluded owing to insufficient read counts.

Subsequently, queries that could not be identified in BLAST or only identified at a taxonomic level higher than family were excluded. To address barcode hopping (also called index switching), wherein barcode sequences initially assigned to one sample are erroneously assigned to another, 0.2% of the total read count per taxon per run was subtracted from the read count per taxon per sample. This value was selected experimentally using the control samples, ensuring clean results with minimal data loss. Subsequently, host sequences were excluded from CO1 samples (i.e. herring reads from herring stomachs and likewise for sprat), along with all sequences classified as Actinopteri (ray-finned fish) in 18SV4 samples, due to this marker's unsuitability for detecting fish. In the zooplankton samples, removal of Actinopteri reads was necessary to address potential fish contamination, as zooplankton was collected while fishing. Furthermore, several parasite sequences were removed from both CO1 and 18SV4 datasets, assuming these were not part of the diet. Read counts were then transformed to RRA per sample. Subsequently, alpha diversity of stomach contents was calculated using the Shannon index (Shannon 1948), and differences between predator groups (small herring, large herring, small sprat, and large sprat) were tested using ANOVA. Lastly, the minimum leftover read count per sample was set at 100, which led to the exclusion of 12 CO1 samples and 15 18SV4 samples. The low read counts observed in certain samples can be expected due to the (almost) empty stomachs and are considered an inherent aspect of this dataset.

Presence or absence of prey per sample was used to calculate the frequency of occurrence at species and genus levels to classify the 10 most observed prey per sample group (small herring, large herring, small sprat, large sprat, and zooplankton). In cases of equal frequency of occurrence, the read abundance determined the order. Furthermore, barplots were generated at the class level to assess diet and zooplankton composition. For these plots, samples were merged by haul and sample group, eliminating field replicates. To examine the type of food consumed by the fish, diet items were categorized into five functional habitat groups: copepods, pelagic invertebrate (larvae), pelagic fish (larvae), demersal fish (larvae), and benthic invertebrate (larvae). Supplementary Table S4 provides an overview of the prey species belonging to each functional group.

Diet overlap was evaluated following the procedure by Novotny et al. (2022), the Bray-Curtis Index, being (1-Bray–Curtis distance) × 100, was computed across species and size classes. Diet-overlap calculations focused exclusively on dominant diet components, excluding taxa present in less than 15% of the samples. Differences in diet composition were statistically evaluated using permANOVA via the "adonis" function in R package vegan. Pairwise comparisons between fish species and size classes were conducted using the "pairwise adonis" function in R package pairwiseAdonis. Nonmetric multidimensional scaling (NMDS) plots were generated using Bray–Curtis distances with R package vegan. To identify the prey taxa contributing most to the dissimilarity between the groups, the 'simper' function in R package vegan was used.

The selectivity for zooplankton prey was determined using Ivlev's electivity index E (Ivlev 1961). This index compares the abundance of a prey species (i) found in a stomach (r_i) to the abundance of prey in the environment (p_i) using:

$$E_i = (r_i - p_i) / (r_i + p_i) \quad (2)$$

For this analysis, RRA was used as proxy for abundance and samples were aggregated based on predator group and haul, eliminating field replicas. Ivlev electivity index was selected for its application in similar studies on SPF (Bachiller and Irigoien 2015, Ojaveer et al. 2018) and its use with DNA data (Ribeiro et al. 2019). Selectivity was computed for most important prey species, which was based on highest frequency of occurrence and highest average RRA. Calculations were confined to March until August, as outside this period, zooplankton concentrations were insufficient for a sensible comparison. Due to inadequate sample sizes for large fish, significance testing for feeding selectivity was exclusively conducted on the small size class, using a one-sample t-test with a significance level of $P < 0.01$.

Results

Stomach fullness and condition

Herring exhibited slightly higher stomach fullness compared to sprat, particularly when comparing the large size classes (Fig. 2a). There was considerable within-haul variation, with percentage fullness ranging from 0% to 100% within species. Fullness tended to be lower towards the end of the year, with sudden higher levels in November. The highest degree of fullness was observed in large herring in April and May. The percentage of empty stomachs was quite similar for small herring, large herring, and small sprat, with average values of 28.2%, 30.1%, and 30.0%, respectively. Large sprat recorded an average percentage of empty stomachs of 70.9%. The condition of small herring and sprat displayed a distinct seasonal pattern, with the highest Fulton's K observed from April to July, followed by a decline until November and then a subsequent increase (Fig. 2b). Fulton's K values for small sprat were significantly higher than for small herring ($F(1) = 25.27$, $P < 0.001$).

Diet composition and seasonal variation

Metabarcoding of stomach content generated 13.9 and 15.0 million reads for CO1 and 18SV4, respectively. Approximately 37% of the CO1 reads were identified as host, while ∼7% could not be identified by BLAST. Similarly, for 18SV4, ∼33% of the reads were identified as fish, and ∼7% remained unidentified by BLAST. Following quality control and filtering, ∼2.7 million reads were retained for analysis for both CO1 and 18SV4. CO1 identified 170 taxa and 18SV4 identified 86 taxa. After filtering on RRA >0.5% in at least one sample, the number of taxa for CO1 reduced to 80, compared to 61 for 18SV4. By further selecting taxa observed in at least 15% of samples, CO1 yielded 23 taxa, while 18SV4 yielded 20. Both markers detected the main prey items, with differences at species level. For example, CO1 identified fish species that 18SV4 did not, while Appendicularia were exclusively found by 18SV4.

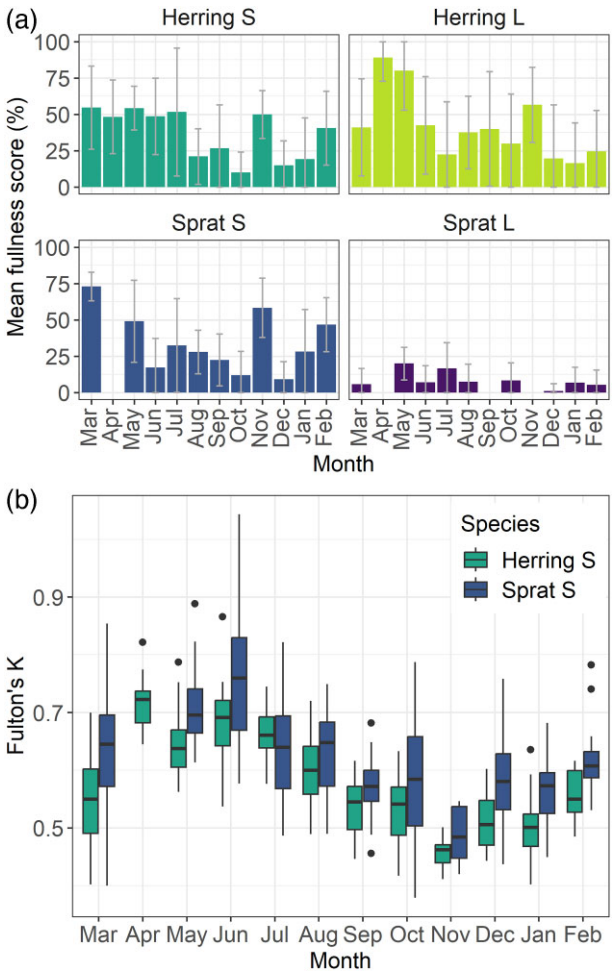

Figure 2. (a) Average stomach fullness of the four predator groups per month, with error bars indicating the standard deviation. Note that there are no observations for sprat in April and for large sprat in November. (b) Boxplots of Fulton's K for small herring and sprat per month. Boxes show minimum, 1st quartile, median, 3rd quartile, and maximum values.

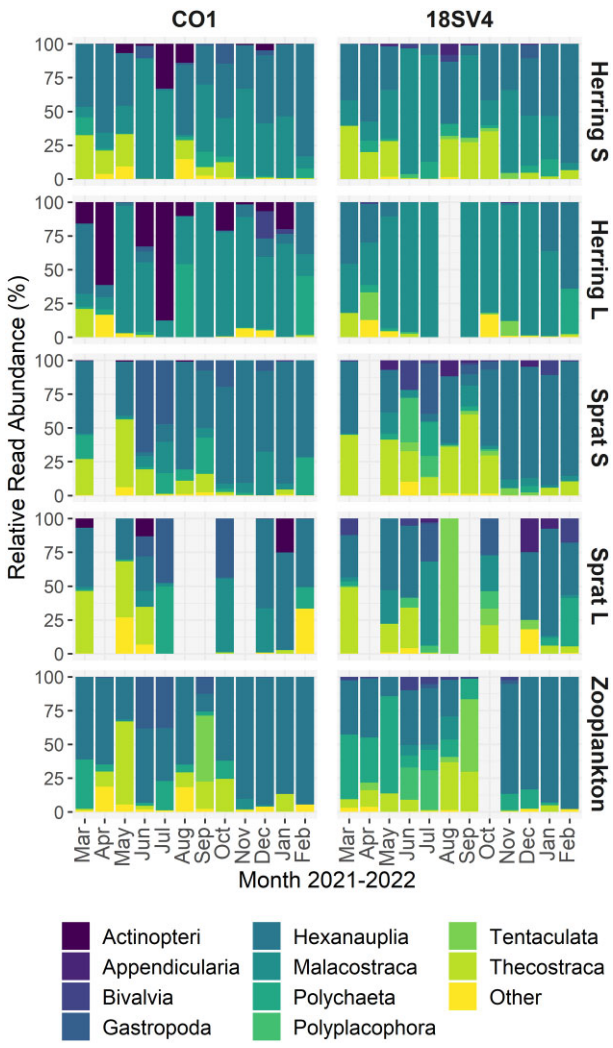

Figure 3. Relative Read Abundances (RRA) indicating diet composition of the four predator groups and the community composition of the zooplankton samples at class level of CO1 (left) and 18SV4 (right). The bars represent different months, with empty bars indicating either no samples could be collected or that samples did not pass quality control. The category "other" includes classes with an average RRA per sample of <1.5%.

The diet of herring and sprat primarily comprised the classes Hexanauplia (copepods), Malacostraca (diverse group of crustaceans), and Thecocostraca (barnacles; Fig. 3). Substantial variation was noted between months, for instance, low RRA of Thecocostraca during winter months. Furthermore, June and July exhibited high RRA of Gastropoda in sprat stomachs, while for herring, these months showed high RRA of Malacostraca. Ordination of the samples in a NMDS plot, showed clustering by month rather than by predator, indicating a pronounced seasonal impact on the diet of herring and sprat (Fig. 4). For instance, *Acartia bifilosa* was positioned between winter months, and *A. tonsa* was positioned between late summer months, corresponding with their seasonal occurrence.

Acartia emerged as most often consumed genus across all samples, based on frequency of occurrence (Table 1). Additionally, in the stomachs of small herring and small and large sprat, *Temora* and *Balanus* were frequently observed, while large herring often consumed *Crangon*, *Sprattus*, and *Mnemiopsis*. The dominant prey species for small herring and sprat, as determined by CO1, were *A. tonsa* and *T. longicornis*. Additionally, 18SV4 revealed *A. bifilosa* and *Paracalanus parvus parvus* as most frequently consumed by small herring, and *Balanus crenatus* and *Pseudocalanus elongatus* for small sprat. Both markers indicated consistent results for large fish: *C. crangon* and *A. bifilosa* for large herring, and *A. bifilosa* and *B. crenatus* for large sprat. Details on the 10 most occurring species per sample group and marker can be found in Supplementary Table S5.

On average, copepods made up about 35% of the reads in the diet of the studied herring and sprat, yet this varies widely per size class (Fig. 5). The copepod fraction was largest in small sprat (CO1: 57%, 18SV4: 49%) and small herring (CO1: 41%, 18SV4: 38%), followed by large sprat (CO1: 36%, 18SV4: 34%), and large herring (CO1: 12%, 18SV4: 17%). Stomachs of larger fish, especially large herring, contained higher percentages of pelagic invertebrates, like mysids and fish. Both herring and sprat exhibited regular consumption of (larvae of) benthic invertebrates. This group encompassed various species of Amphipoda, Decapoda, and Gastropoda, such as *Corophium volutator*, *Carcinas meanas*, and *Peringia ulvae* (Supplementary Table S6). Most benthic

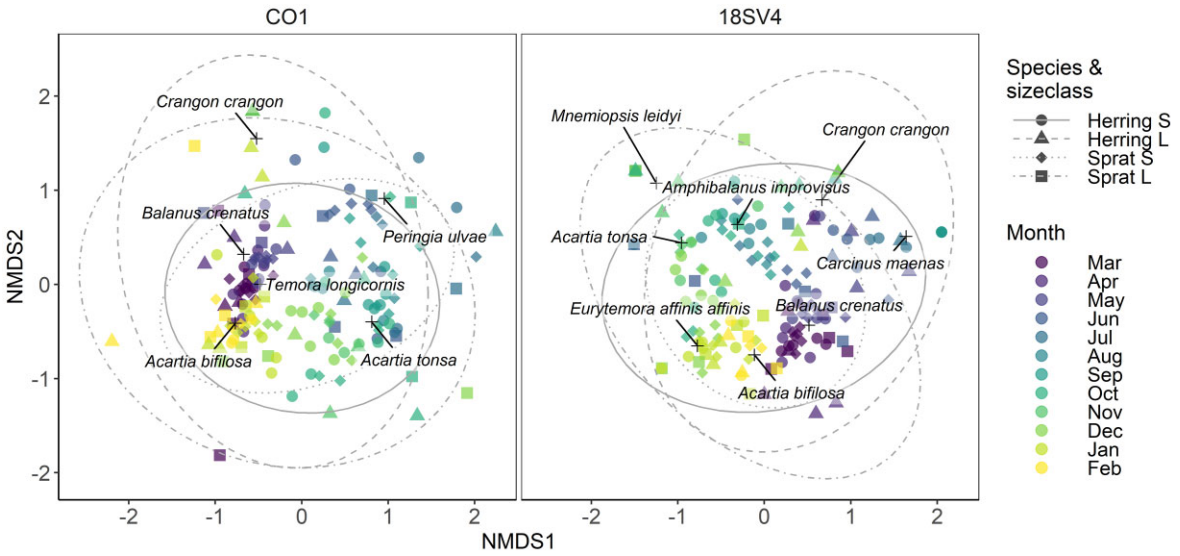

Figure 4. Nonparametric multidimensional scaling (NMDS) plots illustrating fish diet composition based on Bray–Curtis distances of stomach content using CO1 (left) and 18SV4 (right). Each sample is represented as a single symbol, with colour indicating month and shape indicating predator group. Prey contributing most to differences are denoted by a plus sign. Ellipses follow the t-distribution of NMDS scores for each predator group.

Table 1. Frequency of occurrence (FO, %) of top 10 preyed genera in the four predator groups for CO1 and 18SV4.

		CO1		18SV4	
Species	#	Genus	FO %	Genus	FO %
Herring S	1	*Acartia*	86.9	*Acartia*	74.1
	2	*Temora*	65.6	*Paracalanus*	41.4
	3	*Balanus*	52.5	*Amphibalanus*	39.7
	4	*Paracalanus*	42.6	*Oikopleura*	36.2
	5	*Harpacticus*	39.3	*Eurytemora*	34.5
	6	*Euterpina*	34.4	*Balanus*	34.5
	7	*Eurytemora*	32.8	*Pseudocalanus*	34.5
	8	*Bathyporeia*	29.5	*Centropages*	31.0
	9	*Amphibalanus*	29.5	*Calanus*	29.3
	10	*Pseudocalanus*	29.5	*Mnemiopsis*	29.3
Herring L	1	*Acartia*	52.9	*Acartia*	53.3
	2	*Crangon*	38.2	*Crangon*	46.7
	3	*Sprattus*	35.3	*Mnemiopsis*	33.3
	4	*Schistomysis*	32.4	*Schistomysis*	30.0
	5	*Praunus*	32.4	*Praunus*	23.3
	6	*Osmerus*	29.4	*Carcinus*	23.3
	7	*Gammarus*	26.5	*Gastrosaccus*	20.0
	8	*Temora*	26.5	*Calanus*	20.0
	9	*Bathyporeia*	26.5	*Pseudocalanus*	20.0
	10	*Merlangius*	23.5	*Atylus*	16.7
Sprat S	1	*Acartia*	96.2	*Acartia*	79.2
	2	*Temora*	60.4	*Balanus*	56.6
	3	*Paracalanus*	58.5	*Pseudocalanus*	56.6
	4	*Amphibalanus*	47.2	*Harpacticus*	52.8
	5	*Balanus*	45.3	*Amphibalanus*	49.1
	6	*Harpacticus*	45.3	*Paracalanus*	47.2
	7	*Polydora*	45.3	*Oikopleura*	45.3
	8	*Amphiascopsis*	37.7	*Mytilus*	41.5
	9	*Pseudocalanus*	37.7	*Temora*	41.5
	10	*Austrominius*	34.0	*Mnemiopsis*	37.7
Sprat L	1	*Acartia*	66.7	*Acartia*	58.3
	2	*Balanus*	41.7	*Balanus*	45.8
	3	*Temora*	33.3	*Pygospio*	33.3
	4	*Eurytemora*	29.2	*Eurytemora*	25.0
	5	*Pseudocalanus*	25.0	*Mytilus*	25.0
	6	*Osmerus*	25.0	*Temora*	25.0
	7	*Marenzelleria*	20.8	*Pseudocalanus*	25.0
	8	*Rathkea*	20.8	*Ensis*	25.0
	9	*Harpacticus*	20.8	*Harpacticus*	25.0
	10	*Microprotopus*	16.7	*Mnemiopsis*	20.8

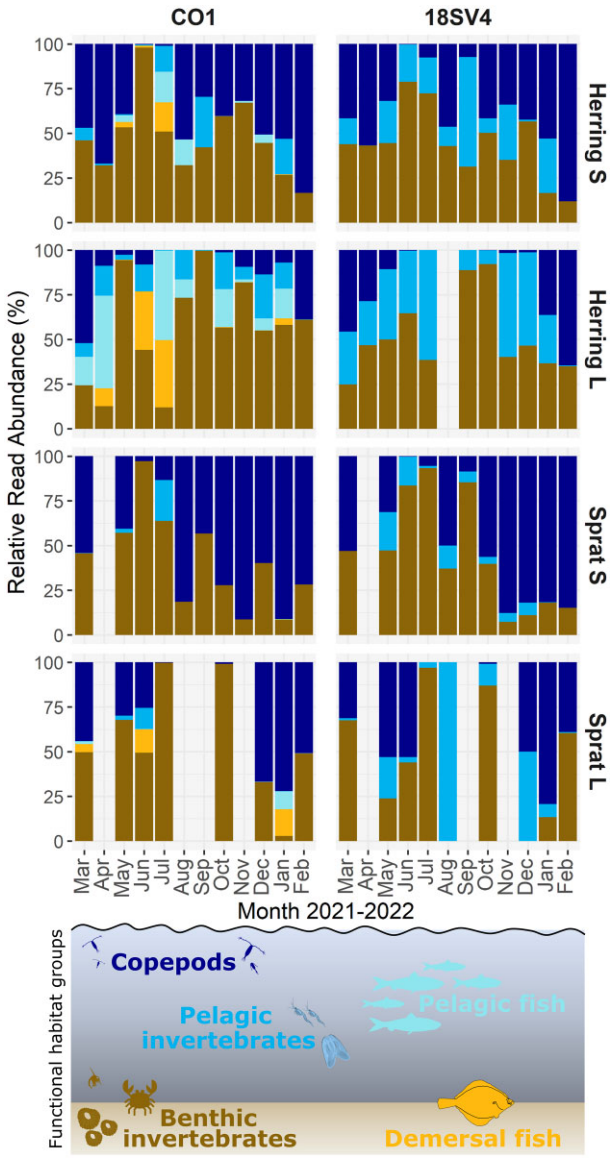

Figure 5. Diet composition of the four predator groups, categorized in functional habitat groups. Coloured bars illustrate RRA of functional habitat groups of CO1 (left) and 18SV4 (right). Empty bars indicate that no samples were collected or that samples did not pass quality control. Species are categorized based on adult appearance but include larval (pelagic) stages. A comprehensive list detailing species composition of functional groups is provided in Supplementary Table S4.

invertebrates observed in the diet were likely (planktonic) larval stages of species that are considered benthic as adults. Metabarcoding lacks resolution to specify life stages, but larvae were regularly observed macroscopically, as well as some benthic adult individuals.

The CO1 marker revealed piscivory in both herring and sprat (Table 2). Large fish ate fish more frequently than the smaller size classes. In herring, sprat was the most consumed species, while the reverse only occurred occasionally. Additionally, herring and sprat regularly consumed *Pomatoschistus* gobies, whiting (*Merlangius merlangus*), and smelt (*Osmerus eperlanus*). Sprat and smelt were eaten throughout the year, while other species were part of their diet in certain seasons. Macroscopically, fish larvae were observed in the stomachs of 10 small herring and 32 large herring, but were not observed in sprat. These observations were mainly clupeid (post-)larvae, alongside sandeel and goby larvae. Most instances of macroscopical observations of fish larvae occurred in April, and some in May, June, July, and October.

Seasonal patterns in zooplankton

Monthly zooplankton sampling in the Wadden Sea identified 61 taxa with CO1 and 52 with 18SV4. After selecting only taxa observed in at least 15% of the samples, CO1 yielded 21 taxa, while 18SV4 yielded 27. Hexanauplia were the most abundant class, especially in winter (Fig. 3). In spring and summer, Thecostraca, Polychaeta, and Gastropoda showed relative high RRA. Additionally, a bloom of Tentaculata was observed in September. The prominent zooplankton genera were *Acartia, Electra, Balanus, Pygospio*, and *Temora* (Table 3). *A. bifilosa*, *A. tonsa*, and *B. crenatus* were the most frequently observed species in zooplankton samples (Supplementary Table S5).

Zooplankton abundance varied widely, ranging from 16 individuals per 100 l in February to 3057 in June (Fig. 6a). From March to August, abundance exceeded 300 individuals per 100 l, with a clear peak of Gastropods in June. Copepoda, the most abundant group, were present year-round, with peak concentrations in April and June and a dip in May. On average, copepods measured 0.9 ± 0.3 mm in length and 0.3 ± 0.1 mm in width, although no clear seasonal pattern was evident (Fig. 6b).

Intra- and interspecific variation

An average stomach comprised 11.3 ± 6.4, 11.1 ± 6.8, 7.5 ± 5.1, and 5.0 ± 4.7 different species for the predator groups small herring, small sprat, large herring, and large sprat, respectively. Stomach content diversity varied throughout the year, with May consistently exhibiting the highest number of different prey species, across all predator groups. Significant differences in prey alpha diversity were found among all predator groups, except for small herring and small sprat (Supplementary Fig. S5). Small fish exhibited significantly higher alpha diversity in their diet compared to large herring and sprat, with large sprat displaying the least diverse diet.

In the NMDS plot of Fig. 4, the ellipses of small herring and sprat almost completely overlap, indicating strong diet overlap. Indeed, based on Bray–Curtis distance for both markers, the diets of small herring and sprat did not differ significantly (Fig. 7). Small herring and sprat had a Bray–Curtis similarity of 18.8% and 15.5% for CO1 and 18SV4, respectively. Least overlap was observed for the combinations of large herring with both sprat size classes. For herring, size influenced diet, with a significant dissimilarity between the diets of small and large herring in both markers. In contrast, in sprat, there was no significant difference between the diets of small and large fish. There was notable variation in diet, characterized by low diet overlap values when comparing diets within predator groups—ranging from 12.5% to 19.3% for CO1 and 11.6% to 18.8% for 18SV4. Large sprat displayed the lowest similarity values. Within field replicas, the average overlap was higher (>55%), especially among small fish (Supplementary Fig. S6). Nonetheless, diet exhibited considerable diversity, with certain field replicate samples showing an overlap of only 25%.

Table 2. Frequency of occurrence (%) of preyed fish species in the four predator groups (Her = herring, Spr = sprat), using marker CO1.

Prey species	Frequency of occurence (%)				Months											
Prey species	Her S	Her L	Spr S	Spr L	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Ammodytes tobianus	1.6	8.8	1.9	4.2												
Atherina presbyter	0.0	14.7	0.0	8.3												
Clupea harengus	*	*	3.8	8.3												
Liparis liparis	1.6	11.8	0.0	4.2												
Merlangius merlangus	1.6	23.5	1.9	8.3												
Osmerus eperlanus	4.9	29.4	0.0	25.0												
Pleuronectes platessa	3.3	2.9	0.0	8.3												
Pomatoschistus spp.	9.8	8.8	5.7	12.5												
Sardina pilchardus	0.0	8.8	0.0	0.0												
Solea solea	3.3	8.8	3.8	8.3												
Sprattus sprattus	19.7	35.3	*	*												

Species are listed if observed at least three times, and asterisks indicate that no results can be provided because host sequences were excluded. Months of occurrence in predator stomachs are indicated on the right side (grey = presence and white = absence)

Table 3. Frequency of occurrence (FO, %) of top 10 occurring genera in the zooplankton samples for CO1 and 18SV4.

	CO1		18SV4	
Rank	Genus	FO %	Genus	FO %
1	*Acartia*	95.5	*Acartia*	95.0
2	*Electra*	59.1	*Balanus*	55.0
3	*Balanus*	45.5	*Pygospio*	50.0
4	*Obelia*	45.5	*Temora*	45.0
5	*Temora*	40.9	*Ensis*	45.0
6	*Paracalanus*	40.9	*Electra*	45.0
7	*Austrominius*	40.9	*Scoloplos*	35.0
8	*Amphibalanus*	36.4	*Amphibalanus*	35.0
9	*Polydora*	36.4	*Paracalanus*	35.0
10	*Peringia*	27.3	*Scrippsiella*	35.0

Selective feeding behaviour

Few selectivity values were significantly different from zero, indicating minimal prey selectively (Fig. 8). However, for CO1, *A. bifilosa* was avoided ($P < 0.01$) by both small herring and sprat, while *Amphibalanus improvises* was selected ($P < 0.01$) by small sprat. Concerning 18SV4, *Scoloplos armiger* ($P < 0.001$) was avoided by small herring, while *A. tonsa* ($P < 0.01$) was avoided by small sprat. For the large fish, mostly negative values were observed, yet these were not tested due to small sample sizes.

Discussion

Gaining insight into the feeding ecology of herring and sprat is crucial for understanding the ecological processes that drive their occurrence in coastal areas like the Wadden Sea. Through our monthly sampling over an entire year, our study provides a unique addition to the understanding of food relations between SPF and zooplankton, contributing to general food web studies in the Dutch Wadden Sea (Christianen et al. 2017, Poiesz et al. 2020, Poiesz et al. 2021). Addressing our initial hypotheses, we found: (i) the condition and diet composition of herring and sprat, along with zooplankton density, exhibited a distinct seasonal pattern, whereas stomach fullness and copepod sizes displayed variability throughout the year; (ii) small herring and sprat displayed a similar diet, and ontogenetic differences were only evident for herring; and (iii) our findings suggest no strong selective feeding behaviour in herring and sprat. Below, we discuss the limitations of our study, put our findings in an ecological context, and discuss their implications.

No single diet method is perfect

Diet studies are all limited in one way or the other, and for several decades, there has been ongoing debate on the most effective methodological approaches to study stomach contents (Amundsen and Sánchez-Hernández 2019). There is a long tradition of morphologically based methods that have resulted in very insightful studies. However, this method has inherent difficulties and biases, such as the time-consuming nature requiring a trained taxonomist (introducing observer bias), as well as challenges posed by cryptic species, organisms disintegrating in fixatives (e.g. gelatinous plankton), and organisms lacking sufficient morphological features for visual identification (Ershova et al. 2021, Leray and Knowlton, 2016).

DNA metabarcoding is a well-established tool for describing the diversity of (pelagic) communities and has been utilized in diet studies for over a decade. One of the major advantages of DNA metabarcoding is the high resolution in identification of degraded prey items and soft-bodied taxa (Ershova et al. 2021, Novotny et al. 2022). This was especially relevant for sprat, which showed more degraded prey in their stomach than herring. The application of two markers enhanced the overall completeness of prey species identification. The degree of detail achieved in this study, both taxonomically and temporally, is necessary to understand processes operating at the base of the food web. However, identifying trophic interactions through DNA metabarcoding has its limitations. With the rapid expansion of reference databases and numerous studies on the impact of technical choices to minimize bias, such as marker selection and bioinformatic approaches (e.g. Alberdi et al. 2018, van der Loos and Nijland 2020), the primary remaining issue is establishing the quantitative value of the proportion of reads. The RRA is impacted by factors such as PCR bias and variable gene copy numbers among tissues and taxa, and is therefore not directly quantitatively equal to prey abundance (Lamb et al. 2019). Furthermore, the developmental stages of prey may impact RRA, particularly in the CO1 marker, as mitochondrial activity is generally higher during growth (Duke and Burton 2020).

Yet, recent years have seen many studies comparing macroscopic methods with metabarcoding. Generally, there is good consistency between methods (e.g. Coguiec et al. 2021,

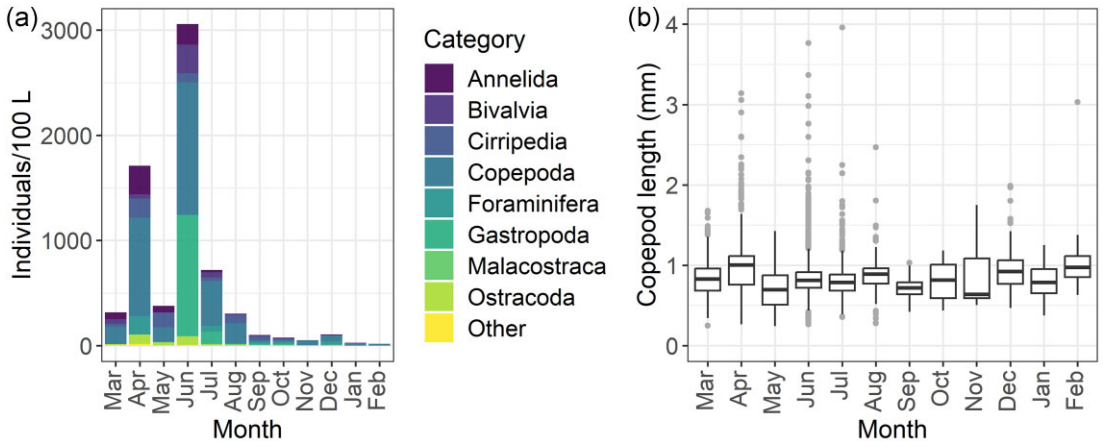

Figure 6. Zooplankton image analysis results per month: (a) abundance and (b) copepod length. Colours indicate taxonomic categories. Boxes show minimum, 1st quartile, median, 3rd quartile, and maximum values.

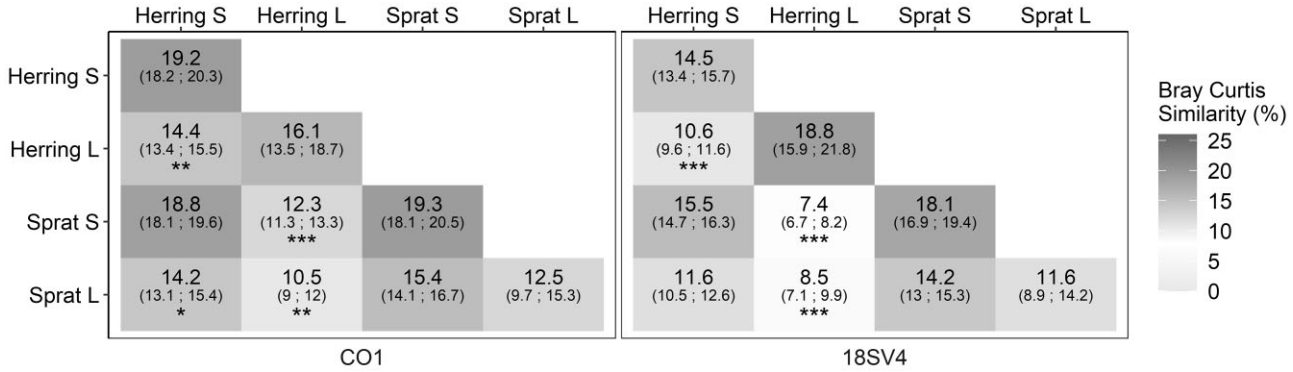

Figure 7. The Bray–Curtis similarity index (BCI) presented in percentage, between and across each predator group, with 95% confidence intervals between brackets. A low BCI indicates low niche overlap. Asterisks indicate statistical significance (∗ = $P < 0.05$, ∗∗ = $P < 0.01$, ∗∗∗ = $P < 0.001$).

Novotny et al. 2022, Vasiliadis et al. 2024), with studies reporting correlations ranging from weak links (Lamb et al. 2019) to robust correlations (Ershova et al. 2021, Ershova et al. 2023, Piñol et al. 2019). It is commonly stated that DNA metabarcoding is able to detect more species and at better taxonomic resolution than traditional methods, and although the RRA is not a direct abundance value, it is suggested that it is more informative to treat the data quantitatively rather than relying solely on presence-absence (Deagle et al. 2019, Lamb et al. 2019). Indeed, several recent papers have successfully utilized DNA metabarcoding of diet samples as a standalone method (e.g. Siegenthaler et al. 2019, Penning et al. 2022, Ingvaldsen et al. 2024).

Unfortunately, despite visual observation herring consuming clupeid larvae, molecular confirmation of cannibalism was not possible through DNA analysis, neither was determining the size and life history stages of prey items. Therefore, to assess cannibalism or feeding selectivity, it is recommended to complement molecular methods with traditional morphological approaches. In conclusion, since no single diet method is perfect, combining different methods is advised to address their limitations. For example, Cordone et al. (2022) studied diet by integrating DNA metabarcoding, direct stomach observations, and stable isotopes.

Seasonal variation in stomach fullness and fish condition

Herring and sprat stomach fullness varied monthly, showing no clear seasonal pattern. Large sprat exhibited exceptionally low stomach fullness throughout the year. Falkenhaug and Dalpadado (2014) reported decreasing fullness with increasing sprat size, and reduced feeding activity of large sprat during winter. Surprisingly, we also observed low values in spring and summer: maturing sprat typically intensify feeding during spring to meet the energy demands of reproduction. The notable prevalence of empty stomachs in our study may indicate a lack of feeding flexibility or the absence of suitable prey for adult sprat in the Wadden Sea. Yet, sprat often displays relatively low stomach fullness in the field as compared to laboratory conditions (Peck et al. 2012). An alternative explanation is that adult sprat primarily feed at night (Bernreuther et al. 2013). With fast prey degradation and fast gastric evacuation rates, e.g. estimated for herring at 1.75 h (Darbyson et al. 2003), nocturnal-feeding fish will have empty stomachs during the day. However, our study setup, limited to one sample per day during daylight, did not allow to study the effect of sampling time on stomach fullness.

Given the priority of growth over energy storage in juvenile fish, Fulton's K values remained low for small herring and sprat. Condition varied seasonally, with higher Fulton's

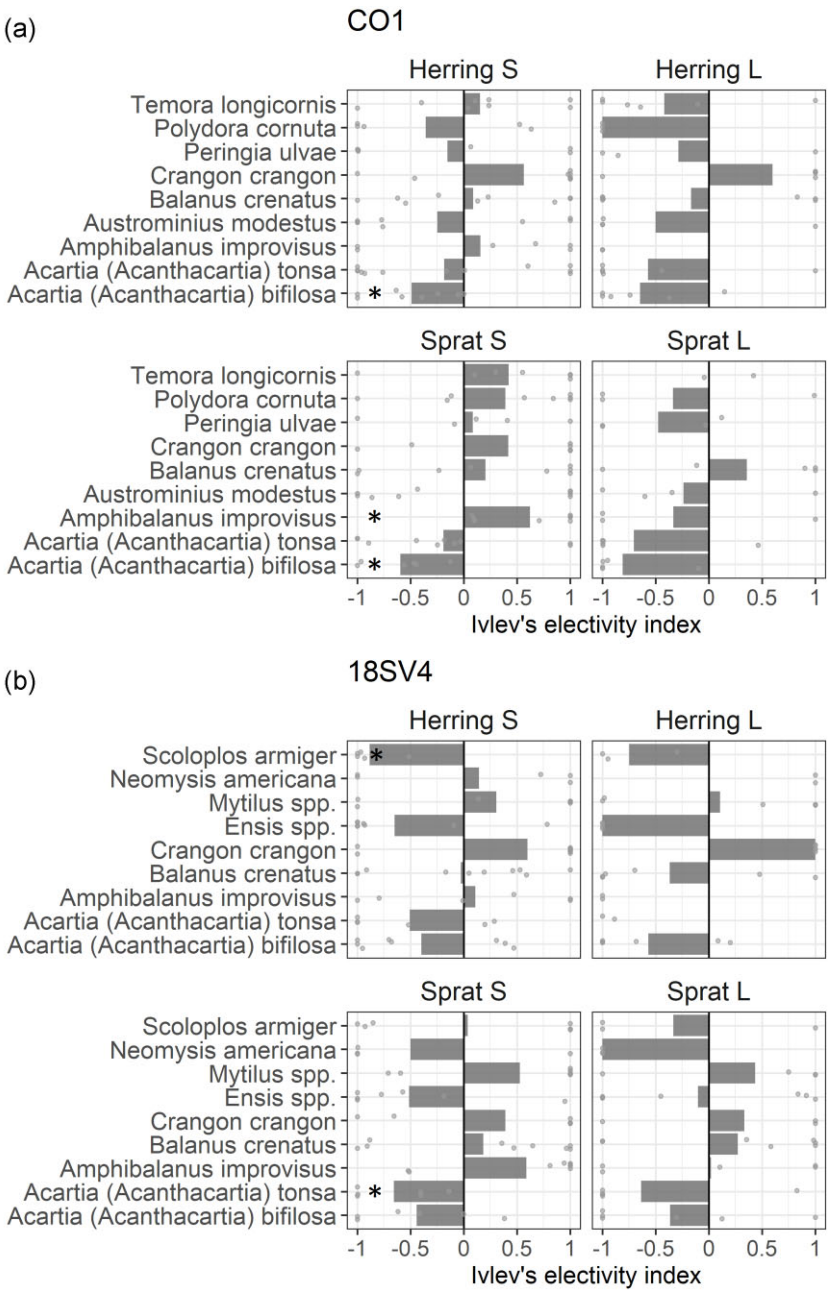

Figure 8. Ivlev's electivity index for the four predator groups for (a) CO1 and (b) 18SV4. Negative values indicate avoidance, positive values indicate selection. Bars indicate means and grey circles indicate individual samples. Means are only given if $n \geq 3$. Asterisks indicate $P < 0.01$, obtained by one sample *t*-tests.

K values observed from April to July, the months of elevated zooplankton abundance. A study in the Baltic Sea on herring and sprat similarly identified a positive correlation between condition and total zooplankton biomass (Casini et al. 2006).

Local and temporal variation in diet composition

Prominent genera identified in the diet included *Acartia, Amphibalanus, Balanus, Crangon, Eurytemora,* and *Temora*, aligning with findings of studies in related areas, where *T. longicornis* is consistently highlighted as main prey item alongside other copepod species (Bernreuther et al. 2018, van Ginderdeuren et al. 2014, Raab et al. 2012, Ojaveer et al. 2018, Novotny et al. 2022). In the North Sea, the copepod *Centropages hamatus* was the second-most encountered prey item in stomachs of herring and sprat (van Ginderdeuren et al. 2014). Intriguingly, however, this species did not rank among the top 10 in the diet of our fish. Despite being a widespread species in coastal waters of the North Atlantic Ocean, its presence in our zooplankton samples was limited. This underscores important local differences in prey availability, highlighting that a species crucial in the diet of herring and sprat in the North Sea may not play a similar role in the Wadden Sea.

The diets of herring and sprat showed a pronounced seasonal effect, which corresponded with the variation observed in zooplankton composition. A striking finding of our study was the significant contribution of (early life stages of)

benthic species to the diet, exceeding what has been reported in literature. In the Baltic Sea, smaller herring and all size classes of sprat were described as strictly zooplanktivorous, excluding early life stages of benthos and fish (Casini et al. 2004). van Ginderdeuren et al. (2014) previously reported mysids, amphipods, and larvae of decapods, and barnacles in diet of herring. Our study confirms similar prey items and extends this list to include species of gastropods, shrimps, and bivalves. Notably, not only larvae (i.e. meroplankton) were observed in the stomachs, adult stages of benthic species were also consumed, such as the mudsnail *P. ulvae*, as confirmed by macroscopic observations. Additionally, brown shrimp (*C. crangon*), a key prey species in the Wadden Sea (e.g. Heindler et al. 2019, Penning et al. 2022), was also eaten by herring and sprat.

The Wadden Sea is a shallow, dynamic ecosystem, with depths typically below 15 m and strong tidal currents. Herring and sprat primarily feed in the water column through particulate feeding, with herring also capable of switching to filter feeding depending on prey concentrations (Möllmann et al. 2004). Consequently, benthic prey can become part of their diet through two main mechanisms: (1) Reproductive activity of benthic species, where eggs or larvae are included in the mesozooplankton. This is often constrained to specific periods, contributing to the observed seasonal variation, e.g. the presence of barnacles in the diet during spring and summer. And (2) species may be lifted from the bottom by currents and the tide. In the shallow and dynamic Wadden Sea, there is no clear distinction between the benthic and pelagic habitats, so herring and sprat can benefit from available benthic prey in the water column. By preying on benthic species, they contribute to the benthic-pelagic coupling, the process of energy and nutrient exchange between benthic and pelagic habitats (Giraldo et al. 2017). This study highlights that, in addition to their established function of transferring energy from plankton to larger predators, herring and sprat also play a crucial role in transferring energy from the benthic to the pelagic habitat in the Wadden Sea.

Intra- and interspecific interactions

Diets of small herring and sprat did not differ significantly, implying potential interspecific competition during periods of limited food availability. This observation might partly explain their generalist diet, as high abundances of clupeids in the Wadden Sea necessitate the use of all available resources. In the Dutch Wadden Sea, juvenile herring and sprat exhibit highest densities from mid-spring to early autumn (Maathuis et al. 2023). This peak coincides with high zooplankton densities, providing favourable conditions for juvenile fish growth and development. Despite winter's reduced zooplankton availability, lower fish densities may alleviate resource scarcity.

In contrast, while no significant difference in diet was observed between the two size classes of sprat, as also noted by Falkenhaug and Dalpadado (2014), herring displayed an ontogenetic shift consistent with observations in the Baltic Sea (Casini et al. 2004). The primary difference in diet between small and large herring was the increased consumption of mysids and fish in large herring. Cannibalism in herring is well-documented (Corten, 2013), and previous studies also noted herring feeding on sprat eggs and larvae (Karaseva et al. 2013), indicating intra-guild predation, an important factor in the dynamics of SPF (Irigoien and de Roos 2011). Particularly in April, large herring were observed consuming clupeid (post-)larvae. While this could potentially impact local early life stage mortality, the sheer abundance of early life stage clupeids largely outnumbers large herring, mitigating its overall influence. Most macroscopic diet studies on herring and sprat do not mention fish larvae as a significant part of their diet, which may be attributed to the rapid digestion rates of early life stages, losing morphological characters required for visual identification (Legler et al. 2010). Furthermore, many prey fish species were only seasonally part of their diet, probably in their early pelagic stages (eggs or larvae), for instance, sole (*Solea solea*), plaice (*Pleuronectes platessa*), and pilchard (*Sardina pilchardus*).

Herring and sprat show opportunistic feeding behaviour

Small herring and sprat showed selectivity values not significantly different from zero, indicating a lack of selective feeding on most prey items. Their higher Shannon diversity index values compared to larger size classes, along with substantial diet overlap, individual and seasonal variation, suggest that juvenile herring and sprat in the Wadden Sea are opportunistic feeders—consuming what is available. The combination of their high densities and the size-limited range of prey options makes an opportunistic feeding strategy advantageous for small herring and sprat. Average copepod sizes ranged between 0.6 and 1 mm throughout the year. Notably, for sprat, these sizes align closely with their preferred size spectrum (250–1000 μm) (Bernreuther et al. 2009). Therefore, herring and sprat are, based on their gape sizes, capable of feeding on copepods year-round, suggesting that copepod size is not a limiting factor in the Wadden Sea.

Contrasting results emerge from different studies on prey selectivity of small pelagics. While van Ginderdeuren et al. (2014) observed strong selective feeding in herring and sprat, contrasting our results, Falkenhaug and Dalpadado (2014) suggested non-selective feeding behaviour in sprat, which was consistent with our findings. When selective feeding was observed, *Acartia* was identified as a preferred prey in some studies (Bernreuther et al. 2018, Ojaveer et al. 2018, Novotny et al. 2022), whereas others observed a selective avoidance of this species (Casini et al. 2004, van Ginderdeuren et al. 2014). In this study, we found avoidance of *A. bifilosa* and, to some extent, *A. tonsa*. *Acartia* species, abundant in the area, are small-sized and suggested to be highly alert to hydrodynamic signals, making them challenging to capture (Viitasalo et al. 2001). Furthermore, Bernreuther et al. (2013) and Viitasalo et al. (2001) describe that life history stages of prey influence selectivity for copepods, with older copepodite stages and reproducing individuals being selected, an aspect worth exploring in future research.

Wider implications

This study revealed that herring and sprat exhibited a generalist diet, consuming the diverse prey community available in the Wadden Sea without strong prey selection. This suggests resilience to future changes; if one prey species declines, they can likely adapt to shifts in community composition. This adaptability is already evident in their seasonally diverse diet, with different species consumed each season. Being an opportunistic, generalist mesopredator provides an advantage in adapting to ecosystem changes, allowing for rapid adjustment to

novel conditions (Olin et al. 2022). Ecosystem models used for predicting future scenarios or carbon fluxes, often rely on broad assumptions regarding diet composition due to the lack of accurate and representative field data. Understanding interactions at the base of the food web is crucial for various management implications, including developing or refining ecosystem models and studying fisheries impact. By elucidating predator-prey relationships and providing detailed data on trophic dynamics for these key species, this study significantly enhances our understanding of ecosystem functioning.

Conclusions

This study showed that juvenile herring and sprat exhibit opportunistic feeding behaviour in the Wadden Sea, with strong seasonal variation in diet composition. In addition to copepods, we discovered a significant share of (early life stages of) benthic prey as part of their diet. This emphasizes the crucial role of herring and sprat in the ecosystem as agents of energy transfer, not solely from plankton to larger predators but also from benthic to pelagic habitats. The larger size classes showed a reduced consumption of copepods, especially large herring, which instead fed on fish (post-)larvae and mysids. In contrast, no significant size-dependent shift in diet was observed for sprat. The dietary overlap between small herring and sprat suggests potential competition during periods of limited food resources. However, the peak densities of SPF align with the months when zooplankton abundance and fish conditions reach their highest levels. Moreover, our study demonstrates the utility of DNA metabarcoding in obtaining detailed results for studying SPF diet, including soft-bodied prey. However, further research on cannibalism and detailed prey selectivity is recommended based on additional morphological approaches. As the world's largest temperate intertidal ecosystem, the Wadden Sea plays a crucial role in the life cycle of many species. This study, characterized by unprecedented temporal and taxonomical detail, represents a significant step in describing and understanding the seasonal dynamics of the dominant components at the base of the Wadden Sea food web.

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Author contributions

Conceptualization and Methodology: M.M., I.T., S.V., B.C., R.N., S.S., and J.P.; Investigation: M.M., S.V., X.B., and M.K.; Formal analysis: M.M., J.P., and V.V. ; Funding acquisition: I.T.; Resources: R.N; Writing - Original Draft: M.M; Writing - Review & Editing: all co-authors contributed critically to the drafts and gave final approval for publication.

Supplementary data

Supplementary data is available at *ICES Journal of Marine Science* online.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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