



# Stomach content and stable isotopes illustrate large spatial similarity in the Wadden Sea fish food-web structure

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**ABSTRACT:** Spatial variability in the Wadden Sea fish food-web structure was studied by comparing stomach content and bulk stable isotopes of fish species caught simultaneously in the Ems and Marsdiep basins during 2012–2014. Almost all 31 fish species caught were generalist feeders. In both basins, similar predator–prey relationships were found in which a few key prey species fuelled the fish food web. Copepods and brown shrimp were the most important prey species in both basins, mysid shrimp were more important as prey in the Ems basin, while shore crab and herring were more important prey species in the Marsdiep basin. The observed spatial variability in prey preferences was most likely the result of local differences in predator and prey abundances. Published absolute trophic positions based on compound-specific stable isotopes were available for some fish species and indicated low variability between the basins. Estimated absolute trophic positions based on stomach content and on bulk stable isotopes could not be used for the analysis of spatial variability due to sensitivity to sampling procedure (stomach content) and sampling size and baseline (bulk stable isotopes). Although estimates based on bulk stable isotopes underestimated absolute trophic levels in both basins, they can be used for the analysis of relative trophic positions of fish species. Relative trophic positions showed a significant correlation for most fish between the Ems and Marsdiep basins, also indicating a large spatial similarity in trophic structure.

**KEY WORDS:** Coastal fish community · Wadden Sea · Stomach content · Stable isotopes · Trophic position · Trophic structure · Marsdiep basin · Ems basin · Predator–prey relationships

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## 1. INTRODUCTION

Fish species require a sequence of suitable habitats for development during their different life stages (see Heesen et al. 2015). These habitat requirements can

differ among species or life stages but can also overlap. Therefore, each marine fish habitat will often contain a fish community consisting of a variety of different species and life stages. Worldwide, coastal areas have been recognized as important foraging

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grounds and habitats for fish species (e.g. Goodall 1983, Lefcheck et al. 2019). This may be due to their often relatively high productivity, driven by their transitional position between the marine and freshwater environment, which allows for nutrient and organic matter inputs from both oceanic and land-based sources (Houde & Rutherford 1993, Nixon 1995, Cloern et al. 2014, Carstensen et al. 2015).

Anthropogenic pressure has negatively impacted coastal areas for centuries, causing major disturbances and structural and functional changes, and leading to system losses (e.g. see Jackson et al. 2001, Lotze 2005, 2007). Anthropogenic stressors such as overfishing, climatic changes (e.g. warming, acidification, deoxygenation), habitat destruction and pollution are expected to increase in the future (Bijma et al. 2013, European Marine Board 2013), which will likely put the functioning of these coastal systems under even more pressure.

The Wadden Sea is an estuarine area of nearly 8000 km<sup>2</sup> bordering the Dutch, German and Danish coast. It is one of the largest European temperate coastal estuarine areas (Wolff 1983) and is important for a variety of fish species (Zijlstra 1972). The Wadden Sea forms an essential habitat for all life stages of resident and near-resident fish species and acts as a nursery area for several marine migrant species during their juvenile stage. The area is also used as a feeding and spawning ground by marine seasonal (summer or winter) visitors. It is visited by both marine and freshwater species, while diadromous migrant species pass through (Zijlstra 1983).

During the last century, the Wadden Sea has suffered mostly from habitat alterations and reductions (embankments), loss of top predators (marine mammals, sharks, rays), the introduction of invasive species (such as the razor clam *Ensis directus* and Pacific or Japanese oyster *Magallana gigas*), pollution and eutrophication events (Wolff 1983, 2000, van der Veer et al. 1989, van Raaphorst & de Jonge 2004, van Beusekom et al. 2019, Jung et al. 2020). These changes have also affected the fish community in the area: in the Dutch Wadden Sea, 2 long-term monitoring programmes of the fish fauna have shown a similar general pattern over the last 50–60 yr (Tulp et al. 2008, 2017a, van der Veer et al. 2015). Total fish biomass increased from 1970 to 1980, with a peak in the mid-1980s and a strong decline from 1980 to 2000, followed by a stabilization at a low level. Marine migrant species, such as the flatfish species plaice *Pleuronectes platessa*, dab *Limanda limanda* and sole *Solea solea*, have, in particular, decreased in biomass since the 1980s (Tulp et al. 2008, 2017a, van der Veer et al.

2015, 2022). Furthermore, mean individual biomass has been decreasing since 1980, with the strongest declines generally in the largest size classes (Tulp et al. 2008, 2017a, van der Veer et al. 2015). Fish species composition in the Wadden Sea appears to be similar at a large scale (Zijlstra 1983), changes in fish community vary considerably for individual species, regions and time periods (Tulp et al. 2017b). In the German and Danish parts of the Wadden Sea, marine migrant species have also been declining in almost all areas since the early or mid-1980s (Tulp et al. 2022). Trends in biomass for demersal migratory and resident species varied from overall stable (flounder *Platichthys flesus*), to ongoing declines (viviparous blenny [eelpout] *Zoarces viviparus* and hooknose *Agonus cataphractus*), overall increase (five-bearded rockling *Ciliata mustela*) or even variable trends (bull-rout *Myoxocephalus scorpius* and pipefishes *Syngnathus* spp.) (Tulp et al. 2022).

Some differences in fish food-web structure have been found between various parts of the Wadden Sea, such as the Ems basin (BOEDE 1985), the Sylt-Rømø basin (Kellnreitner et al. 2012) and the Marsdiep basin (Poiesz et al. 2020, 2021). However, these studies were carried out in different time periods. The observed spatial variability in food-web structure is likely due to a variety of factors, including the hydrography and geomorphology of the area. The Wadden Sea consists of a number of semi-enclosed tidal basins that are separated by shallow watersheds. These tidal basins vary both in size and in the balance and amount of organic matter input from marine and freshwater sources, and therefore exhibit varying local productivity (Postma 1983). In this study, we investigated the spatial variability in the fish food web by sampling simultaneously in 2 tidal basins that are similar in size and freshwater supply, and hence in their 'estuarine character': the Ems basin in the eastern Dutch Wadden Sea and the Marsdiep tidal basin in the western Dutch Wadden Sea (Postma 1983). Spatial variability in the food-web structure was analysed by combining stomach content and bulk stable isotope analysis to compare (1) predator–prey relationships and (2) the trophic structure of the various fish species caught in both basins during 2012–2014.

Predator–prey relationships and prey overlap were based on stomach content information following Hynes (1950), Baker et al. (2014) and Froese & Pauly (2022). These trophic relationships were based on a comparison of the trophic position of the various fish species in both basins. These relative trophic positions of the various species in relation to

each system were analysed by comparing  $\delta^{15}\text{N}$  bulk stable isotope values. Absolute trophic positions were estimated from stomach content composition (Poiesz et al. 2020, Froese & Pauly 2022) and from bulk stable isotope ( $\delta^{15}\text{N}$ ) composition (Post 2002, Boecklen et al. 2011). However, both estimates may have some limitations. Estimates of absolute trophic position based on stomach content are based on only those prey items that can be identified; this offers only a small snapshot in time and can be sensitive to digestion during sampling. Estimates of absolute trophic position based on stable isotopes are especially sensitive to sampling design and the selection of the baselines and their spatial variability (Phillips et al. 2014), a situation that also has been found in the Wadden Sea (Christianen et al. 2017). Recently, Riekenberg et al. (2022) solved the problems caused by baseline variations by applying compound-specific stable isotope analysis to construct a preliminary Wadden Sea food web, including estimates of some fish species. This information was used as a reference in the present study for determining the absolute estimates based on stomach content and bulk stable isotopes.

## 2. MATERIALS AND METHODS

### 2.1. Sampling

The Marsdiep and Ems basins are the 2 largest tidal basins in the Dutch part of the Wadden Sea and are situated, respectively, in the western and eastern parts (Fig. 1). Both tidal basins are characterized by a large freshwater influx: in the Marsdiep basin through the discharge of fresh water from Lake IJssel (the former enclosed inner part of the Marsdiep basin) and in the Ems estuary via freshwater discharge from the River Ems. Tidal range is 1.5–2.0 m in the Marsdiep basin and 3.0–3.5 m in the Ems basin, but mean tidal volume is almost similar:  $1050 \times 10^6 \text{ m}^3$  (Marsdiep basin) versus  $1000 \times 10^6 \text{ m}^3$  (Ems basin) (Postma 1983). In both tidal basins, sampling occurred in the outer part at salinities in the range of 20–25 PSU (Postma 1983, de Jonge 1988).

Samples in the Ems basin were obtained from the cooling water screens of the Ems Centrale power plant (53°2' N, 6°52' E) (Fig. 1). The cooling water inlet is located 300 m from the shoreline in the Doekegat at a water depth of 13 m at high tide, and

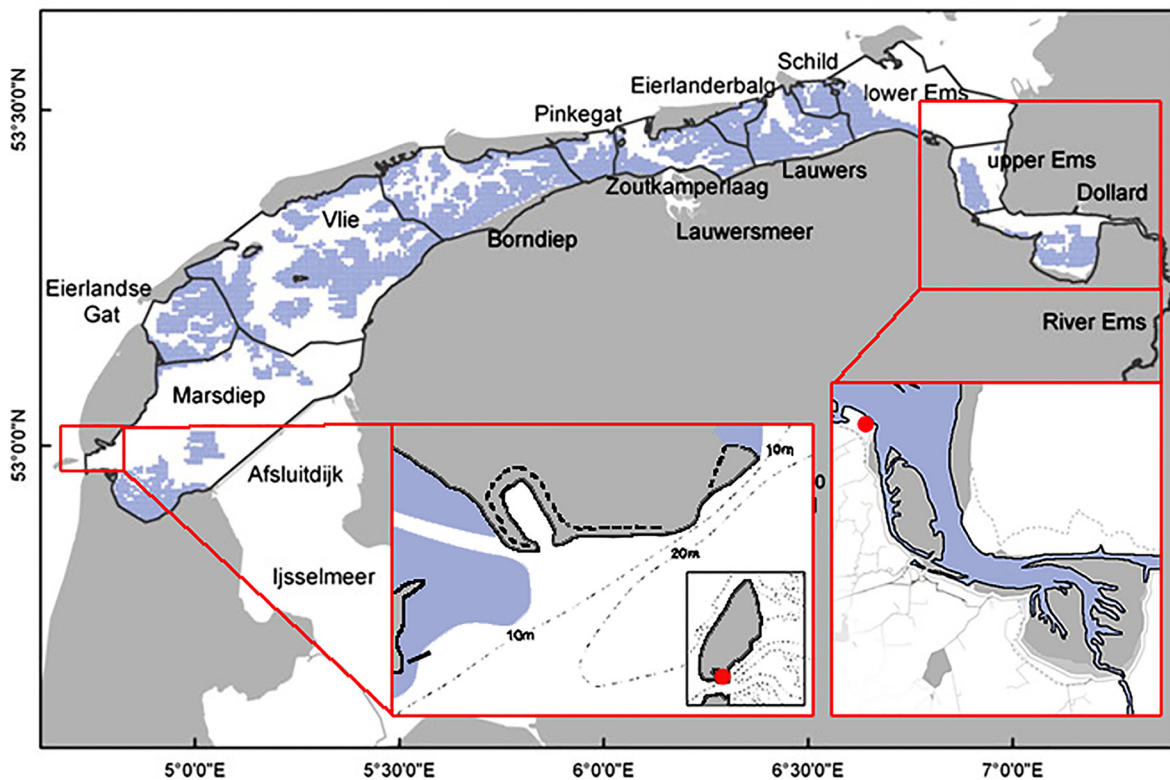


Fig. 1. Sampling stations in the Marsdiep and Ems tidal basins in the Dutch Wadden Sea. Left inset: Sampling location of the NIOZ kom-fyke near the island of Texel (red dot) in the Marsdiep basin. Right inset: Sampling location of the Ems Centrale (red dot) inside the Ems basin. The intertidal areas are indicated in blue (after Compton et al. 2013 and Poiesz et al. 2020)

water intake occurs over the whole water column with a maximum capacity of  $55 \text{ m s}^{-1}$  (Hadderingh & Jager 2002). Seawater is passed through  $6 \times 6 \text{ cm}$  screens, and all impinged biota is flushed from the screens and discharged back into the estuary just below the low water mark via an open return drain. In the present study, all impinged fish and other biota were collected from the return drain with a custom-made frame (with a mesh bag: length 1.5 m, mesh size  $5 \times 5 \text{ mm}$ ) inserted into the drain. Sampling took place monthly from March to November/December in 2012–2014. During sampling, total sampling time was at least 10 min, based on 5 samples of 2 min interrupted by an interval of 15 min. When large amounts of organic matter flooded the gutter, sampling time was shortened to 1 min or even 30 s, and sampling frequency was increased. Despite the short sampling time, a substantial volume of water was sampled during each measurement. For a more detailed description of this sampling method, see Hadderingh & Jager (2002).

The Marsdiep sampling was part of a long-term fish monitoring programme by means of a passive fish trap (Fig. 1). This so-called kom-fyke, with a stretched mesh size of 20 mm, consists of a leader of 200 m running from the beach towards deeper waters. The fish trap is emptied every day (weather permitting) in spring (April, May, June) and in autumn (September, October). For more information, see van der Veer et al. (2015) and Poiesz et al. (2020).

## 2.2. Processing

All samples were processed as described by Poiesz et al. (2020). Samples were sorted immediately, and all fish and other biota caught were identified up to species level, counted, and measured for length within 1 h and stored at  $-20^\circ\text{C}$  until further processing. Each fish species was classified according to guild as juvenile marine migrant (JMM), marine seasonal visitor (MSV) or (near)-resident (resident and near-resident species) and according to functional group as benthic (living and/or feeding on the bottom), benthopelagic (living and/or feeding on or near the bottom as well as in midwater) or pelagic (occurring mainly in the water column, not feeding on benthic organisms). Guilds were assigned based on Witte & Zijlstra (1983), and functional groups were based on FishBase (Froese & Pauly 2022). Classification was in line with previous studies in the area (van der Veer et al. 2015, Poiesz et al. 2020).

### 2.2.1. Stomach content analysis

Within a few weeks of capture, fish were transported to the lab, defrosted and analysed to determine total length, fork length, total weight, gonad weight, sex and ripeness. For each fish, the stomach content was weighted (wet mass; g) and analysed in a petri dish under a binocular microscope (20 $\times$ ). Prey items were identified up to species level or if not possible, up to a higher classification (class, order, genus). Total prey length was measured (mm) if feasible, and incomplete specimens were counted. Taxonomic identification was done by experts from the Royal Netherlands Institute for Sea Research (NIOZ) and was based on an internal reference collection and Hayward & Ryland (2017) for polychaetes, bivalves and crabs and Wheeler (1978) for fish species. For more information, see Poiesz et al. (2020).

### 2.2.2. Stable isotope analyses

Defrosted muscle samples (fish) or (part of) individuals (other species) were taken for stable isotope analyses following Svensson et al. (2014). The samples were placed in a 1.5 ml centrifuge vial and stored at  $-80^\circ\text{C}$  until further processing. Next, samples were freeze-dried for 48 h and ground, and the remaining powder was homogenized. Duplicates were taken of each sample between 0.4 and 0.8 mg, and were weighed and folded into small tin containers for analysis. Nitrogen and carbon isotopes were measured at NIOZ with a Thermo Scientific Delta V Advantage Isotope Mass Spectrometer which was linked with a Flash 2000 Organic Element Analyzer. During each sample run, monitoring gas ( $\text{N}_2$  and  $\text{CO}_2$ ) with a predetermined isotopic composition was used to determine the  $\delta$  values of both the samples as well as the standards. Standards ( $n = 22$ ) with certified isotopic composition were weighted and included on each plate of 94 spots (acetanilide, urea and casein). One standard, acetanilide, was used to correct the measured values, and the 2 other standards, urea and casein, were used to check the correction. Analytical reproducibility was 0.3‰ for  $\delta^{15}\text{N}$  and 0.1‰ for  $\delta^{13}\text{C}$  throughout every sequence.

Isotope value of a sample ( $\delta X$ ) was expressed as a ratio in the  $\delta$  notation in ‰ relative to an internationally defined reference:

$$X = (R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{reference}}$  are the ratio between the ‘heavy’ and ‘light’ isotopes ( $^{15}\text{N}$ : $^{14}\text{N}$  or  $^{13}\text{C}$ : $^{12}\text{C}$ ) of the sample and the reference, respectively.  $\delta^{15}\text{N}$  values were expressed against atmospheric nitrogen and  $\delta^{13}\text{C}$  against Vienna Pee Dee belemnite (VPDB). Lastly,  $\delta^{13}\text{C}$  values were corrected for lipid content according to Svensson et al. (2014). These lipid-content-corrected  $\delta^{13}\text{C}$  values were used in all further analyses. For a detailed description, see Poiesz et al. (2021).

### 2.3. Data analysis

#### 2.3.1. Stomach contents

For each fish species, the percentage of occurrence of each prey item (number of stomachs containing the prey species divided by total number of stomachs examined) was determined as a measure of diet composition following Baker et al. (2014). From the diet composition found in the stomachs, absolute trophic position of each individual fish was calculated according to:

$$\text{TP}_j = 1 + \frac{\sum \text{TP}_i}{i} \quad (2)$$

where  $\text{TP}_j$  is the calculated trophic position of the individual fish  $j$ , and  $\text{TP}_i$  is the trophic position of prey species  $i$  in the stomach of fish  $j$ .

Trophic positions of the various prey species in the stomachs were taken from Froese & Pauly (2022). Finally, for each fish species, the mean absolute trophic position of all individuals was calculated.

#### 2.3.2. Stable isotope analyses

Relative trophic positions were analysed by comparing mean stable isotope  $\delta^{15}\text{N}$  values of the various fish species in both basins. Absolute trophic position of each fish species was estimated according to a dual baseline Bayesian approach which includes a mixing model to discriminate between 2 distinct sources of C and N, i.e. pelagic vs. benthic baselines (van der Zanden et al. 1997, Post 2002). In order to perform the Bayesian analysis, the first equation used was based on 1 baseline with the trophic discrimination factors for nitrogen only:

$$\delta^{15}\text{N}_c = \delta^{15}\text{N}_b + \Delta\text{N}(\text{TP} - \lambda) \quad (3)$$

where  $\delta^{15}\text{N}_c$  is the  $\delta^{15}\text{N}$  value of the consumer,  $\delta^{15}\text{N}_b$  is the  $\delta^{15}\text{N}$  value of the single baseline,  $\Delta\text{N}$  is the trophic discrimination factor for nitrogen,  $\text{TP}$  is the trophic position of the consumer, and  $\lambda$  is the trophic position of the baseline.

Next, the analysis was extended to 2 baselines (pelagic and benthic) with 2 distinct sources (N and C):

$$\delta^{15}\text{N}_c = \Delta\text{N}(\text{TP} + \lambda) + \alpha(\delta^{15}\text{N}_{b1} + \delta^{15}\text{N}_{b2}) - \delta^{15}\text{N}_{b2} \quad (4)$$

where  $\delta^{15}\text{N}_{b1}$ ,  $\delta^{15}\text{N}_{b2}$  is the  $\delta^{15}\text{N}$  of baseline 1 and 2, respectively, and  $\alpha$  is the proportion of N derived from baseline 1 (van der Zanden et al. 1997, Post 2002).

The full model of 2 baselines for C is rewritten to derive  $\alpha$ :

$$\alpha = \frac{\left[ \frac{\delta^{13}\text{C}_{b2} - (\delta^{13}\text{C}_c + \Delta\text{C})}{\text{TP} - \lambda} \right]}{(\delta^{13}\text{C}_{b2} + \delta^{13}\text{C}_{b1})} \quad (5)$$

where  $\delta^{13}\text{C}_{b1}$ ,  $\delta^{13}\text{C}_{b2}$  are the  $\delta^{13}\text{C}$  of baselines 1 and 2, respectively,  $\delta^{13}\text{C}_c$  is the  $\delta^{13}\text{C}$  of the consumer, and  $\Delta\text{C}$  is the trophic fractionation factor for carbon.

Poiesz et al. (2021) showed that stable isotope values between immigrating (spring) and emigrating (autumn) fish in the Wadden Sea were similar, suggesting a similar trophic niche of the various fish species in the coastal zone and inside the Wadden Sea. Therefore, only baseline samples from inside the Wadden Sea were collected, in line with Christianen et al. (2017) and Poiesz et al. (2021). All baseline samples in both basins were collected between 2008 and 2012 by Christianen et al. (2017). In line with Poiesz et al. (2021), the blue mussel *Mytilus edulis* was taken as proxy for the pelagic baseline. The common periwinkle *Littorina littorea* was used as proxy for the benthic baseline (Table 1). *M. edulis*, an obligatory suspension feeder, was collected just below the water surface from buoys in deep channels. *L. littorea* was collected at various locations in the intertidal.

The trophic fractionation factors for nitrogen ( $\delta^{15}\text{N}$ :  $3.4 \pm 0.98\text{‰}$ ) and carbon ( $\delta^{13}\text{C}$ :  $0.39 \pm 1.3\text{‰}$ ) were taken from Post (2002), in line with a previous study in the Marsdiep by Poiesz et al. (2021).

Table 1. Isotopic baselines (mean  $\pm$  SE) for the Ems and Marsdiep basins. Data after Christianen et al. (2017)

Location	Benthic baseline (‰)		Pelagic baseline (‰)	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Ems basin	$-14.00 \pm 0.46$	$12.82 \pm 0.22$	$-18.77 \pm 0.13$	$10.67 \pm 0.15$
Marsdiep basin	$-14.3 \pm 0.26$	$11.96 \pm 0.18$	$-17.8 \pm 1.74$	$12.5 \pm 0.17$



## 2.4. Data exploration and visualization

Only fish data of similar species and similar size range from the Marsdiep data set for the years 2012–2014 were used (see Poiesz et al. 2020) for a comparison with the Ems data. All analyses were based on at least 3 observations of stomach contents and stable isotopes of a fish species in both basins.

All computations and analyses were done in R (R Core Team 2019). Firstly, the data were explored using the protocol described by Zuur et al. (2010), with graphics rendition via the 'ggplot' package (Wickham 2009). Secondly, the 'tRophicPosition' package (Quezada-Romegialli et al. 2018) was used to determine the Bayesian TP model.

## 3. RESULTS

### 3.1. Ems basin

In the Ems basin, 24 surveys were performed over the period 2012–2014, and 45 different species of fish, crustaceans, jellyfish, sea stars and squid were caught (1661 individuals in total). The non-fish species mainly consisted of brown shrimp *Crangon crangon*, mysid shrimp (Mysidae), grass prawn *Palaemon elegans*, common prawn *P. serratus*, common swimming crab *Macropipus holsatus*, shore crab *Carcinus maenas* and common sea star *Asterias rubens*. Thirty-one fish species from 3 functional groups were found (Table 2). Most individuals caught were smaller than 19 cm in size.

#### 3.1.1. Stomach contents

In the Ems basin, most fish species consumed multiple prey, except bass *Dicentrarchus labrax* (Fig. 2), which was a single-prey consumer of brown shrimp. However, the number of observations was low ( $n = 3$ ). Brown shrimp, mysid shrimp, mud shrimp and copepods were the most common prey species. Benthic species preyed especially on mysid shrimp, mud shrimp and brown shrimp. For pelagic species, copepods and mysid shrimp were important prey. Since most benthic fish species belong to the near-resident and resident species, this functional group also preyed mostly on the mysid shrimp and brown shrimp. For guilds, the trend was more diverse and variable (Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/m707p057\\_supp.pdf](http://www.int-res.com/articles/suppl/m707p057_supp.pdf)).

Based on stomach frequency occurrence of at least 50%, various predator–prey relationships could be identified. Bass and tub gurnard *Trigla lucerna* focussed on brown shrimp; common goby *Pomatoschistus microps* on mud shrimp; herring *Clupea harengus* and sprat *Sprattus sprattus* on copepods; sandeel *Ammodytes tobianus* on bristle worms; flounder *Platichthys flesus* on Laver spire shell; bull-rout *Myoxocephalus scorpius* on smelt; and smelt *Osmerus eperlanus*, twait shad *Alosa fallax*, Nillson's pipefish *Syngnathus rostellatus*, Lozano's goby *P. lozanoi*, five-bearded rockling *Ciliata mustela*, hook-nose *Agonus cataphractus* and plaice *Pleuronectes platessa* focussed on mysid shrimp. For the other fish species, prey items had frequency occurrences of  $\leq 50\%$ , with the most common prey item being mysid shrimp for three-spined stickleback *Gasterosteus aculeatus*, whiting *Merlangius merlangus* and sand goby *P. minutus*; mud shrimp for sea-snail *Liparis liparis* and brown shrimp for viviparous blenny *Zoarces viviparus*.

Mean trophic position based on diet ( $\overline{TP}_{\text{diet}}$ ) did not show significant relationships with fish size for the various species (linear regressions:  $p > 0.05$ ; Fig. S2, Table S1). Mean trophic position of the various fish species ranged between 1.7 and 3.6, with the lowest value for viviparous blenny and the highest value for bass and butterflyfish *Pholis gunnellus*. Most values were between 2.5 and 3.0 (Table 3). No trends were found for the functional groups (Table 3), or with functional guild (Fig. S3).

#### 3.1.2. Stable isotopes

Bulk stable isotope values were determined for 31 fish species (Table 3). For isotopic values of the non-fish species caught in the Ems basin, see Table S2.

$\delta^{13}\text{C}$  values ranged from  $-15$  to  $-21\text{‰}$ , although 1 species (the benthic nine-spined stickleback *Pungitius pungitius*) had a more depleted  $\delta^{13}\text{C}$  value ( $-25.5\text{‰}$ , single measurement) (Figs. 3A & 4A). Most species showed stable carbon isotope values between the pelagic and benthic baseline, except for nine-spined stickleback, three-spined stickleback, river lamprey *Lampetra fluviatilis* and twaite shad. Bass had the highest  $\delta^{13}\text{C}$  value of  $-14.9\text{‰}$  (Fig. 3A, Table 3). The average  $\delta^{15}\text{N}$  values for most of the fish species varied between 14 and 18‰ (Figs. 3A & 4B), except for greater pipefish and nine-spined stickleback, with  $\delta^{15}\text{N}$  values of respectively 12.9‰ and 10.8‰ (both single observations). Benthic, benthopelagic and pelagic species showed on average the

Table 2. Total number of individual fish species caught in 2012–2014, together with the number of individuals containing stomach contents and the number of isotope samples taken: (a) Ems basin; (b) Marsdiep basin. MSV: marine seasonal visitor; JMM: juvenile marine migrant

(a) Ems basin Scientific name	Common name	Abbreviation	Functional group	Guild	No. of ind. caught				No. with stomach contents				No. isotope samples	
					2012	2013	2014	Total	2012	2013	2014	Total	Total	Total
<i>Agonus cataphractus</i>	Hooknose	Ac	Benthic	(Near)-resident	3	6	1	10	0	5	0	5	5	10
<i>Alosa fallax</i>	Twaite shad	Af	Pelagic	(Near)-resident	9	8	5	22	7	5	3	15	22	22
<i>Ammodytes tobianus</i>	Sandeel	At	Benthic	MSV	2	9	7	18	0	2	1	3	18	18
<i>Aphia minuta</i>	Transparent goby	Am	Benthic	(Near)-resident	0	0	1	1	0	0	0	0	1	1
<i>Ciliata mustela</i>	Five-bearded rockling	Cm	Benthic	(Near)-resident	12	2	4	18	11	0	4	15	18	18
<i>Clupea harengus</i>	Herring	Ch	Pelagic	JMM	44	51	46	141	19	19	17	55	141	141
<i>Dicentrarchus labrax</i>	Bass	Dl	Benthopelagic	(Near)-resident	7	0	2	9	2	0	1	3	9	9
<i>Gadus morhua</i>	Cod	Gm	Benthopelagic	MSV	1	0	0	1	1	0	0	1	1	1
<i>Gasterosteus aculeatus</i>	Three-spined stickleback	Ga	Benthopelagic	(Near)-resident	36	34	23	93	17	21	15	53	93	93
<i>Lampetra fluviatilis</i>	River lamprey	Lf	Benthic	(Near)-resident	1	0	0	1	0	0	0	0	1	1
<i>Limanda limanda</i>	Dab	Ll	Benthic	MSV	1	1	1	3	0	0	1	1	3	3
<i>Liparis liparis</i>	Sea-snail	Llip	Benthic	(Near)-resident	29	13	8	50	22	10	7	39	50	50
<i>Merlangius merlangus</i>	Whiting	Mm	Benthopelagic	MSV	15	22	8	45	8	18	6	32	45	45
<i>Myoxocephalus scorpius</i>	Bull-rout	Ms	Benthic	(Near)-resident	2	5	1	8	0	4	1	5	8	8
<i>Osmerus eperlanus</i>	Smelt	Oe	Pelagic	MSV	49	55	39	143	30	27	19	76	143	143
<i>Pholis gunnellus</i>	Butterfish	Pg	Benthic	(Near)-resident	4	2	0	6	1	1	0	2	6	6
<i>Platichthys flesus</i>	Flounder	Pf	Benthic	(Near)-resident	8	21	7	36	4	11	2	17	36	36
<i>Pleuronectes platessa</i>	Plaice	Pp	Benthic	JMM	22	38	18	78	6	10	5	21	78	78
<i>Pomatoschistus lozanoi</i>	Lozano's goby	Pl	Benthic	MSV	24	11	19	54	9	5	12	26	54	54
<i>Pomatoschistus microps</i>	Common goby	Pmic	Benthic	(Near)-resident	4	9	4	17	3	0	3	6	17	17
<i>Pomatoschistus minutus</i>	Sand goby	Pmin	Benthic	(Near)-resident	41	54	35	130	13	19	14	46	130	130
<i>Pungitius pungitius</i>	Nine-spined stickleback	Ppun	Benthic	MSV	0	0	1	1	0	0	0	0	1	1
<i>Scophthalmus maximus</i>	Turbot	Sm	Benthic	MSV	0	0	1	1	0	0	0	0	1	1
<i>Solea solea</i>	Sole	Ssol	Benthic	JMM	1	8	0	9	0	0	0	0	9	9
<i>Sprattus sprattus</i>	Sprat	Ss	Pelagic	JMM	17	12	27	56	3	7	3	13	56	56
<i>Syngnathus rostellatus</i>	Nilsson's pipefish	Sr	Benthic	(Near)-resident	45	52	42	139	17	27	18	62	139	139
<i>Syngnathus acus</i>	Greater pipefish	Sa	Benthic	MSV	1	0	0	1	0	0	0	0	1	1
<i>Trachurus trachurus</i>	Scad	Tt	Pelagic	MSV	0	4	0	4	0	1	0	1	4	4
<i>Trigla lucerna</i>	Tub gurnard	Tl	Benthopelagic	MSV	0	5	6	11	0	3	6	9	11	11
<i>Trisopterus luscus</i>	Bib	Tlus	Benthopelagic	MSV	1	0	0	1	1	0	0	1	1	1
<i>Zoarces viviparus</i>	Viviparous blenny	Zv	Benthic	(Near)-resident	9	10	0	19	4	5	0	9	19	19

Table 2 (continued)

(b) Marsdiep basin Scientific name	Common name	Abbreviation	Functional group	Guild	No. of ind. caught				No. with stomach contents				No. isotope samples	
					2012	2013	2014	Total	2012	2013	2014	Total	Total	Total
<i>Agonus cataphractus</i>	Hooknose	Ac	Benthic	(Near)-resident	3	5	2	10	1	1	1	3	3	3
<i>Alosa fallax</i>	Twaite shad	Af	Pelagic	(Near)-resident	13	158	24	195	17	33	79	129	59	59
<i>Ammodytes tobianus</i>	Sandeel	At	Benthic	MSV	0	0	1	1	0	0	1	1	1	1
<i>Aphia minuta</i>	Transparent goby	Am	Benthic	(Near)-resident	0	0	0	0	0	0	0	0	0	0
<i>Ciliata mustela</i>	Five-bearded rockling	Cm	Benthic	(Near)-resident	137	23	48	208	32	34	89	155	36	36
<i>Dicentrarchus labrax</i>	Herring	Ch	Pelagic	JMM	6730	25103	14187	46020	25	79	81	185	134	134
<i>Gadus morhua</i>	Bass	DI	Benthopelagic	(Near)-resident	316	175	381	872	55	23	111	189	189	189
<i>Gasterosteus aculeatus</i>	Cod	Gm	Benthopelagic	MSV	175	247	17	439	10	18	7	35	35	35
<i>Lampetra fluviatilis</i>	Three-spined stickleback	Ga	Benthopelagic	(Near)-resident	381	116	32	529	7	8	14	29	28	28
<i>Limanda limanda</i>	River lamprey	Lf	Benthic	(Near)-resident	0	0	0	0	0	0	0	0	0	0
<i>Liparis liparis</i>	Dab	Ll	Benthic	MSV	50	57	68	175	11	12	16	39	39	39
<i>Merlangius merlangus</i>	Sea-snail	Llip	Benthic	(Near)-resident	2	6	0	8	5	3	0	8	2	2
<i>Myoxocephalus scorpius</i>	Whiting	Mm	Benthopelagic	MSV	107	56	71	234	7	26	81	114	52	52
<i>Osmerus eperlanus</i>	Bull-rout	Ms	Benthic	(Near)-resident	19	18	16	53	12	2	33	47	20	20
<i>Pholis gunnellus</i>	Smelt	Oe	Pelagic	MSV	2	31	31	64	20	14	38	72	38	38
<i>Platichthys flesus</i>	Butterfish	Pg	Benthic	(Near)-resident	3	5	0	8	1	1	0	2	2	2
<i>Pleuronectes platessa</i>	Flounder	Pf	Benthic	(Near)-resident	417	570	401	1388	46	74	102	222	148	148
<i>Pomatoschistus lozanoi</i>	Plaice	Pp	Benthic	JMM	486	873	469	1828	18	27	57	102	86	86
<i>Pomatoschistus microps</i>	Lozano's goby	Pl	Benthic	MSV	0	0	0	0	0	0	0	0	0	0
<i>Pomatoschistus minutus</i>	Common goby	Pmic	Benthic	(Near)-resident	0	0	1	1	0	0	0	0	0	0
<i>Pungitius pungitius</i>	Sand goby	Pmin	Benthic	(Near)-resident	13	52	35	100	0	5	11	16	11	11
<i>Scophthalmus maximus</i>	Nine-spined stickleback	Ppun	Benthic	MSV	0	0	0	0	0	0	0	0	0	0
<i>Solea solea</i>	Turbot	Sm	Benthic	MSV	8	24	45	77	2	1	15	18	18	18
<i>Sprattus sprattus</i>	Sole	Ssol	Benthic	JMM	13	23	9	45	2	5	4	11	11	11
<i>Syngnathus rostellatus</i>	Sprat	Ss	Pelagic	JMM	734	1839	779	3352	2	13	9	24	19	19
<i>Syngnathus acus</i>	Nilsson's pipefish	Sr	Benthic	(Near)-resident	0	0	5	5	0	0	0	0	0	0
<i>Trachurus trachurus</i>	Greater pipefish	Sa	Benthic	MSV	34	31	35	100	6	1	9	16	16	16
<i>Trigla lucerna</i>	Scad	Tt	Pelagic	MSV	13	98	70	181	4	15	17	36	36	36
<i>Trisopterus luscus</i>	Tub gurnard	Tl	Benthopelagic	MSV	13	2	1	16	2	0	2	4	4	4
<i>Zoarces viviparus</i>	Bib	Tlus	Benthopelagic	MSV	1	44	6	51	0	5	2	7	7	7
	Viviparous blenny	Zv	Benthic	(Near)-resident	3	1	1	5	0	4	1	5	5	5



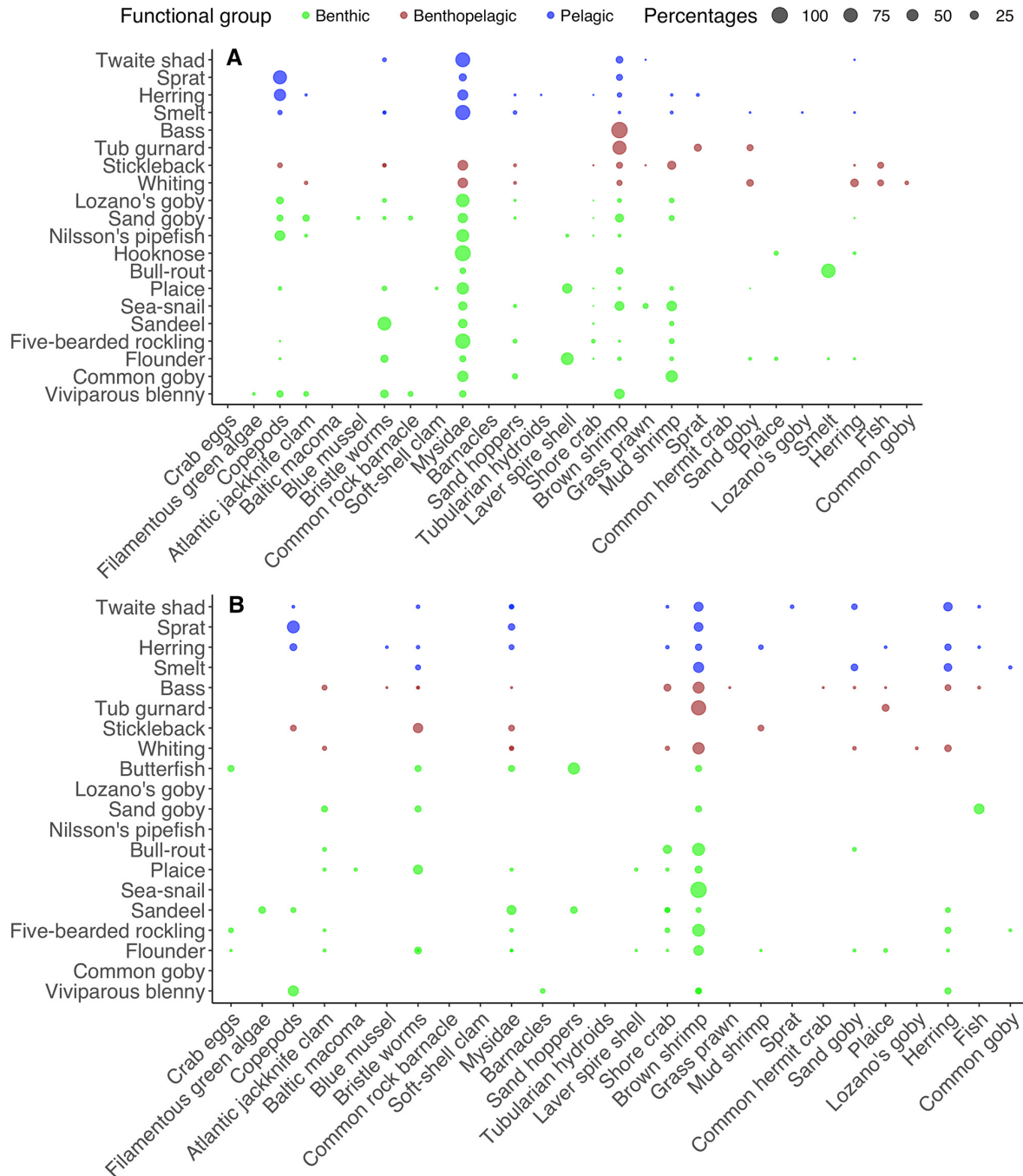


Fig. 2. Prey species occurrence (%; continuous scale of 0–100%) in the stomachs of fish species in the various functional groups (benthic; benthopelagic; pelagic) for the years 2012 – 2014. (A) Ems basin; (B) Marsdiep basin

same values (14–18‰). Highest  $\delta^{15}\text{N}$  values were found for viviparous blenny, smelt and bass.

Mean trophic position (TP) based on  $\delta^{15}\text{N}$  isotope values ranged between 3.0 and 3.9, with most values around 3.5, and with the lowest value for Nilsson's pipefish and the highest value for smelt (Table 3). No differences were found between pelagic, benthopelagic and benthic species or guild (Figs. S4 & S5).

### 3.2. Marsdiep basin

In the Marsdiep basin, 457 fyke catches were done between 2012 and 2014, and in total, 54 fish species were caught. All fish species found in the Ems basin were also caught in the Marsdiep basin, except for transparent goby *Aphia minuta*, river lamprey, nine-spined stickleback and Lozano's goby (Table 2).

Table 3. Trophic position (TP) based on stomach content. Mean isotope values and TP are based on isotope values of fish species caught in the Ems basin with corresponding values for fish caught in the Marsdiep basin in 2012–2014. Data are mean  $\pm$  SE. MSV: marine seasonal visitor; JMM: juvenile marine migrant

Scientific name	Common name	Abbreviation	Functional group	Guild	Stomach content analysis		Stable isotope analysis				TP based on isotopes	
					Ems	Marsdiep	Ems		Marsdiep		Ems baseline	Marsdiep baseline
							AC	AN	AC	AN		
<i>Agonus cataphractus</i>	Hooknose	Ac	Benthic	(Near)-resident	2.84 $\pm$ 0.63	3.60 $\pm$ 0.05	-16.45 $\pm$ 0.11	16.73 $\pm$ 0.18	-16.71 $\pm$ 0.29	16.63 $\pm$ 0.14	3.36 $\pm$ 0.15	3.96 $\pm$ 2.71
<i>Alosa fallax</i>	Twaite shad	Af	Pelagic	(Near)-resident	2.97 $\pm$ 0.24	3.47 $\pm$ 0.05	-19.47 $\pm$ 0.37	16.92 $\pm$ 0.16	-16.67 $\pm$ 0.37	17.14 $\pm$ 0.13	3.79 $\pm$ 0.15	3.05 $\pm$ 0.24
<i>Ammodytes tobianus</i>	Sandeel	At	Benthic	MSV	2.70 $\pm$ 0.45	3.16 v0.14	-17.68 $\pm$ 0.16	16.08 $\pm$ 0.30				3.47 $\pm$ 0.23
<i>Aphia minuta</i>	Transparent goby	Am	Benthic	(Near)-resident			-17.95	14.24				
<i>Ciliata mustela</i>	Five-bearded rockling	Cm	Benthic	(Near)-resident	2.66 $\pm$ 0.24	3.72 $\pm$ 0.02	-16.41 $\pm$ 0.20	17.12 $\pm$ 0.33	-16.61 $\pm$ 0.18	16.75 $\pm$ 0.27	3.48 $\pm$ 0.34	3.11 $\pm$ 0.19
<i>Clupea harengus</i>	Herring	Ch	Pelagic	JMM	2.68 $\pm$ 0.14	3.42 $\pm$ 0.03	-18.58 $\pm$ 0.15	16.06 $\pm$ 0.12	-17.89 $\pm$ 0.09	14.19 $\pm$ 0.12	3.52 $\pm$ 0.14	2.69 $\pm$ 0.14
<i>Dicentrarchus labrax</i>	Bass	Dl	Benthopelagic	(Near)-resident	3.60	3.55 $\pm$ 0.04	-14.92 $\pm$ 0.45	17.66 $\pm$ 0.37	-17.73 $\pm$ 0.64	15.59 $\pm$ 0.63	3.38 $\pm$ 0.33	3.18 $\pm$ 0.2
<i>Gadus morhua</i>	Cod	Gm	Benthopelagic	MSV				14.80	-16.69 $\pm$ 0.13	16.12 $\pm$ 0.15		3.17 $\pm$ 0.2
<i>Gasterosteus aculeatus</i>	Three-spined stickleback	Ga	Benthopelagic	(Near)-resident	2.71 $\pm$ 0.16	3.20 $\pm$ 0.06	-20.18 $\pm$ 0.25	16.61 $\pm$ 0.12	-18.89 $\pm$ 0.20	15.26 $\pm$ 0.26	3.81 $\pm$ 0.15	3.04 $\pm$ 0.19
<i>Lampetra fluviatilis</i>	River lamprey	Lf	Benthic	MSV			-20.27	17.35				
<i>Limanda limanda</i>	Dab	Ll	Benthic	MSV			-17.28 $\pm$ 0.30	15.37 $\pm$ 0.31	-16.48 $\pm$ 0.13	17.28 $\pm$ 0.11	3.12 $\pm$ 0.50	2.49 $\pm$ 0.15
<i>Liparis liparis</i>	Sea-snail	Llip	Benthic	(Near)-resident	2.71 $\pm$ 0.18	3.60 $\pm$ 0.01	-16.40 $\pm$ 0.12	16.82 $\pm$ 0.15	-16.40 $\pm$ 0.40	16.07 $\pm$ 0.16	3.44 $\pm$ 0.13	5.13 $\pm$ 2.27
<i>Merlangius merlangus</i>	Whiting	Mm	Benthopelagic	MSV	2.55 $\pm$ 0.29	3.59 $\pm$ 0.04	-17.30 $\pm$ 0.14	17.11 $\pm$ 0.13	-17.35 $\pm$ 0.42	15.81 $\pm$ 0.21	3.63 $\pm$ 0.14	3.05 $\pm$ 0.17
<i>Myoxocephalus scorpius</i>	Bull-rout	Ms	Benthic	(Near)-resident	2.78 $\pm$ 0.55	3.55 $\pm$ 0.03	-16.37 $\pm$ 0.22	17.27 $\pm$ 0.23	-16.24 $\pm$ 0.15	17.45 $\pm$ 0.13	3.55 $\pm$ 0.18	3.53 $\pm$ 0.19
<i>Osmerus eperlanus</i>	Smelt	Oe	Pelagic	MSV	2.64 $\pm$ 0.14	3.92 $\pm$ 0.05	-18.04 $\pm$ 0.14	17.81 $\pm$ 0.08	-16.72 $\pm$ 0.41	16.61 $\pm$ 0.17	3.97 $\pm$ 0.14	3.28 $\pm$ 0.13
<i>Pholis gunnellus</i>	Butterfish	Pg	Benthic	(Near)-resident	3.60	3.44 $\pm$ 0.08	-17.02 $\pm$ 0.15	17.36 $\pm$ 0.69	-17.70 $\pm$ 0.41	16.52 $\pm$ 0.17	2.69 $\pm$ 0.43	3.31 $\pm$ 1.3
<i>Platichthys flesus</i>	Flounder	Pf	Benthic	(Near)-resident	2.30 $\pm$ 0.30	3.42 $\pm$ 0.03	-17.57 $\pm$ 0.47	17.08 $\pm$ 0.27	-19.14 $\pm$ 0.14	16.17 $\pm$ 0.14	3.51 $\pm$ 0.19	3.09 $\pm$ 0.15
<i>Pleuronectes platessa</i>	Plaice	Pp	Benthic	JMM	2.73 $\pm$ 0.26	3.22 $\pm$ 0.03	-16.44 $\pm$ 0.16	15.49 $\pm$ 0.14	-15.45 $\pm$ 0.15	14.85 $\pm$ 0.15	2.99 $\pm$ 0.17	2.81 $\pm$ 0.11
<i>Pomatoschistus lozanoi</i>	Lozano's goby	Pj	Benthic	MSV	3.00 $\pm$ 0.13		-17.42 $\pm$ 0.10	17.25 $\pm$ 0.10	-16.84 $\pm$ 0.12	16.01 $\pm$ 0.19	2.62 $\pm$ 0.10	
<i>Pomatoschistus microps</i>	Common goby	Pmic	Benthic	(Near)-resident	2.20 $\pm$ 0.47		-16.43 $\pm$ 0.35	16.54 $\pm$ 0.24	-18.94 $\pm$ 0.14	14.28 $\pm$ 0.10	2.41 $\pm$ 0.19	
<i>Pomatoschistus minutus</i>	Sand goby	Pmin	Benthic	(Near)-resident	2.92 $\pm$ 0.15		-17.46 $\pm$ 0.11	17.02 $\pm$ 0.08	-15.58 $\pm$ 0.15	14.88 $\pm$ 0.13	3.6 $\pm$ 0.19	3.11 $\pm$ 0.17
<i>Pungitius pungitius</i>	Nine-spined stickleback	Ppun	Benthic	MSV			-25.50	10.78				
<i>Scophthalmus maximus</i>	Turbot	Sm	Benthic	MSV			-15.97	17.65	-18.76 $\pm$ 0.29	15.28 $\pm$ 0.25		3.11 $\pm$ 0.13
<i>Solea solea</i>	Sole	Ssol	Benthic	JMM			-18.89 $\pm$ 0.17	16.69 $\pm$ 0.30	-15.46 $\pm$ 0.26	15.99 $\pm$ 0.15	3.40 $\pm$ 0.20	2.89 $\pm$ 0.22
<i>Sprattus sprattus</i>	Sprat	Ss	Pelagic	JMM	2.80 $\pm$ 0.17		-18.54 $\pm$ 0.15	15.06 $\pm$ 0.17	-16.95 $\pm$ 0.36	15.30 $\pm$ 0.13	3.38 $\pm$ 0.17	2.83 $\pm$ 0.25
<i>Syngnathus rostellatus</i>	Nilsson's pipefish	Sr	Benthic	(Near)-resident	2.86 $\pm$ 0.08		-18.06 $\pm$ 0.08	16.16 $\pm$ 0.09	-16.29 $\pm$ 0.12	17.36 $\pm$ 0.13	2.31 $\pm$ 0.18	
<i>Syngnathus acus</i>	Greater pipefish	Sa	Benthic	(Near)-resident			-17.74	12.94				
<i>Trachurus trachurus</i>	Scad	Tt	Pelagic	MSV			-18.73 $\pm$ 0.79	16.51 $\pm$ 0.28	-18.63 $\pm$ 0.34	15.46 $\pm$ 0.29	3.61 $\pm$ 0.18	2.87 $\pm$ 0.2
<i>Trigla lucerna</i>	Tub gurnard	Tl	Benthopelagic	MSV	3.26 $\pm$ 0.28		-16.20 $\pm$ 0.54	16.89 $\pm$ 0.30	-17.38 $\pm$ 0.15	13.74 $\pm$ 0.30	3.35 $\pm$ 0.29	3.4 $\pm$ 1.07
<i>Trisopterus luscus</i>	Bib	Tlus	Benthopelagic	MSV			-18.79	17.49	-18.53 $\pm$ 0.21	16.58 $\pm$ 0.12		2.73 $\pm$ 0.18
<i>Zoarces viviparus</i>	Viviparous blenny	Zv	Benthic	(Near)-resident	1.74 $\pm$ 0.30		-16.78 $\pm$ 0.23	18.05 $\pm$ 0.29	-16.67 $\pm$ 0.20	14.77 $\pm$ 0.20	3.8 $\pm$ 0.20	2.98 $\pm$ 0.23

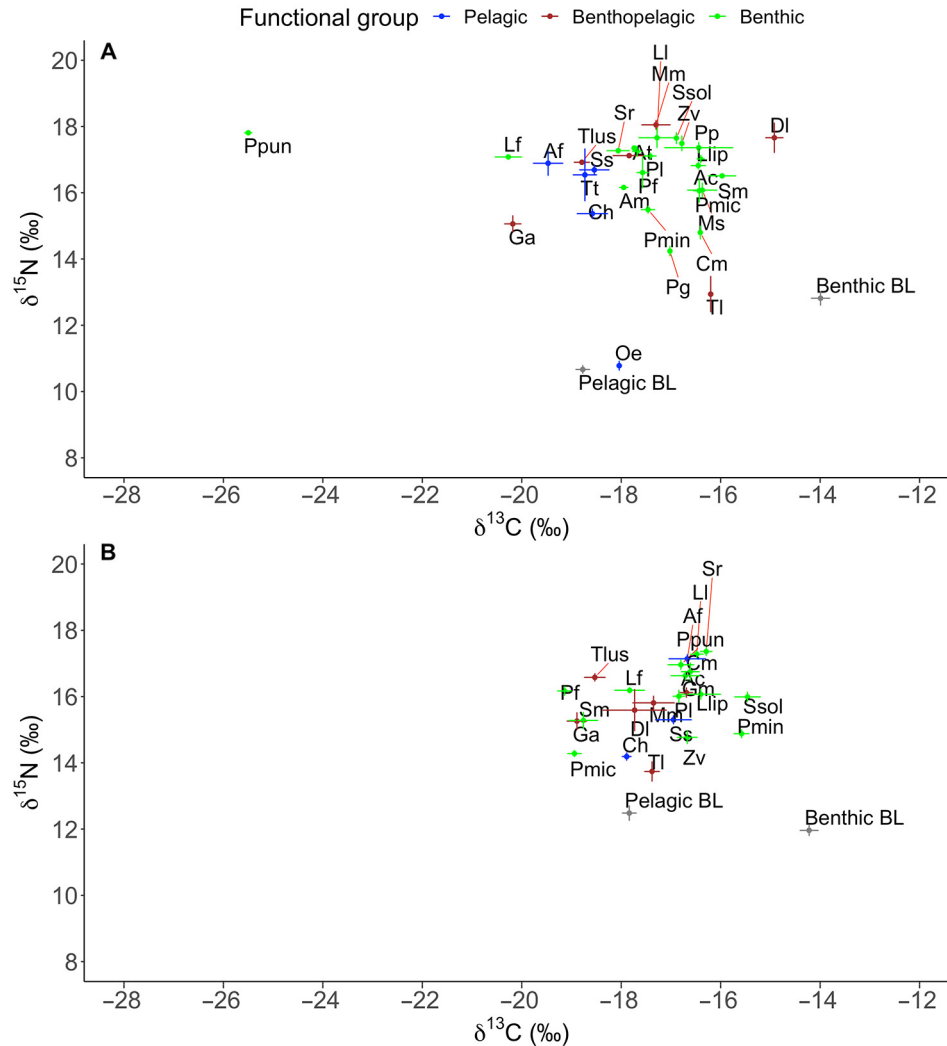


Fig. 3. Average  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  stable isotope values with standard error bars for fish species in 2012–2014 split into the various functional groups. For full species names, values and corresponding abbreviations, see Table 2. BL: baseline. (A) Ems basin; (B) Marsdiep basin

### 3.2.1. Stomach contents

Most fish species consumed multiple prey (Fig. 2B), except for the common sea-snail *Liparis liparis*, which focussed on brown shrimp only. Copepods, mysid shrimp, brown shrimp, bristle worm and herring were the most common prey species. Benthic species preyed especially on brown shrimp. For pelagic species, copepods, mysid shrimp, brown shrimp and herring were important prey. Since most benthic species belong to the (near)-resident species, this functional group also preyed mostly on brown shrimp. For the other guilds, the trend was more diverse and variable, but for marine seasonal visitors, brown shrimp was also a main food item (Fig. S1).

Prey items with a frequency of occurrence of >50% in stomach contents were brown shrimp for

tub gurnard, whiting, bull-rout, five-bearded rockling, hooknose, sea-snail and copepods for sprat. For the other fish species, prey items had frequencies of occurrence of  $\leq 50\%$ , and the most common prey items were copepods for herring and viviparous blenny; mysid shrimp for sandeel; brown shrimp for smelt, twaite shad, bass and flounder; bristle worms for stickleback and plaice; and fish for sand goby.

Mean trophic positions based on diet ( $\overline{\text{TP}}_{\text{diet}}$ ) did not show significant relationships with fish size for the various species (linear regressions:  $p > 0.05$ ; Fig. S2, Table S1). Mean trophic position of the various fish species ranged between 3.1 and 3.9 (Table 3). The lowest values were found for sandeel and sprat, and the highest values were found for smelt and sand goby.

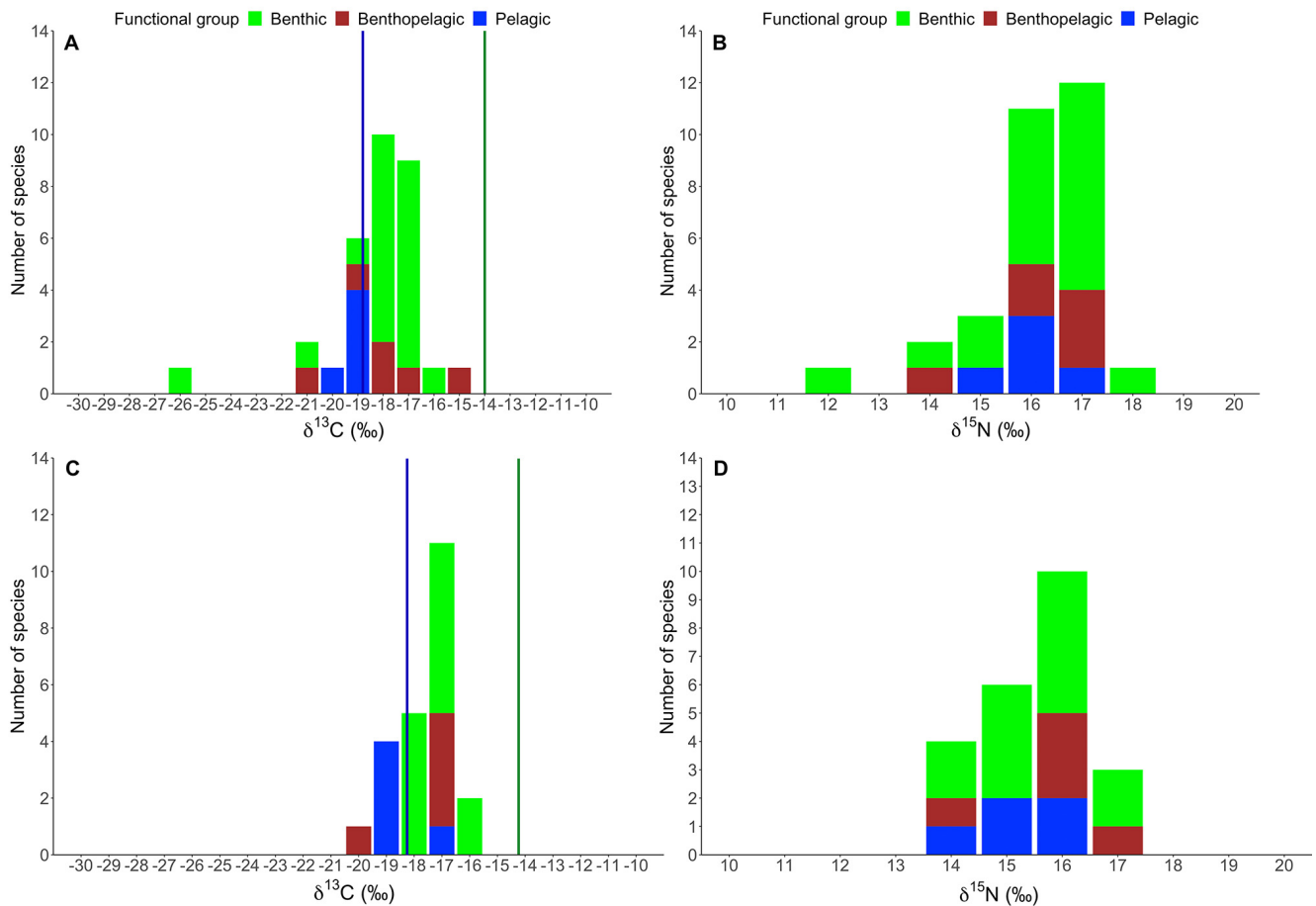


Fig. 4. Frequency distribution of average stable isotope values of the various fish species for the various functional groups in the Ems and Marsdiep basins in 2012–2014. The dark blue line in (A) and (C) represents the pelagic baseline (*Mytilus edulis*), while the dark green line represents the benthic baseline (*Littorina littorea*). (A)  $\delta^{13}\text{C}$  values of Ems species; (B)  $\delta^{15}\text{N}$  values of Ems species; (C)  $\delta^{13}\text{C}$  values of Marsdiep species; (D)  $\delta^{15}\text{N}$  values of Marsdiep species

### 3.2.2. Stable isotopes

In the Marsdiep basin, stable isotope values could be determined for 26 fish species (Table 3).  $\delta^{13}\text{C}$  values of the various fish species ranged between  $-15.5$  and  $-19.1$ ‰ (Figs. 3B & 4C). Five species had  $\delta^{13}\text{C}$  values lower than the pelagic baseline of  $-17.8$ ‰: three-spined stickleback, flounder, common goby, turbot *Scophthalmus maximus* and bib *Trisopterus luscus*; however, values of turbot and bib were based on single observations.

The average  $\delta^{15}\text{N}$  values for the fish species varied between  $13.7$  and  $17.5$ ‰ (Figs. 3B & 4D), with the lowest values for tub gurnard and the highest  $\delta^{15}\text{N}$  values for bull-rout. No differences were found between benthic, benthopelagic and pelagic species (Fig. 4D).

Mean estimated trophic position ( $\overline{\text{TP}}$ ) based on  $\delta^{15}\text{N}$  isotope values varied from  $2.5$  to  $5.1$ , with most

values between  $2.5$  and  $3.5$  (Table 3). Dab had the lowest value, and sea-snail had the highest value. No differences were found between pelagic, benthopelagic and benthic species or between guilds (Figs. S4 & S5).

## 3.3. Comparison between Ems and Marsdiep

### 3.3.1. Predator–prey relationships

In both the Ems and Marsdiep basins, almost all fish species were multiple prey consumers; however, the frequency of occurrence of the prey items in the stomachs varied between the 2 basins (Fig. 5). In the Ems basin, mysid shrimp, brown shrimp, copepods, bristle worms and laver spire shell were the most frequently occurring prey species. In the Marsdiep basin, brown shrimp, copepods, herring, bristle

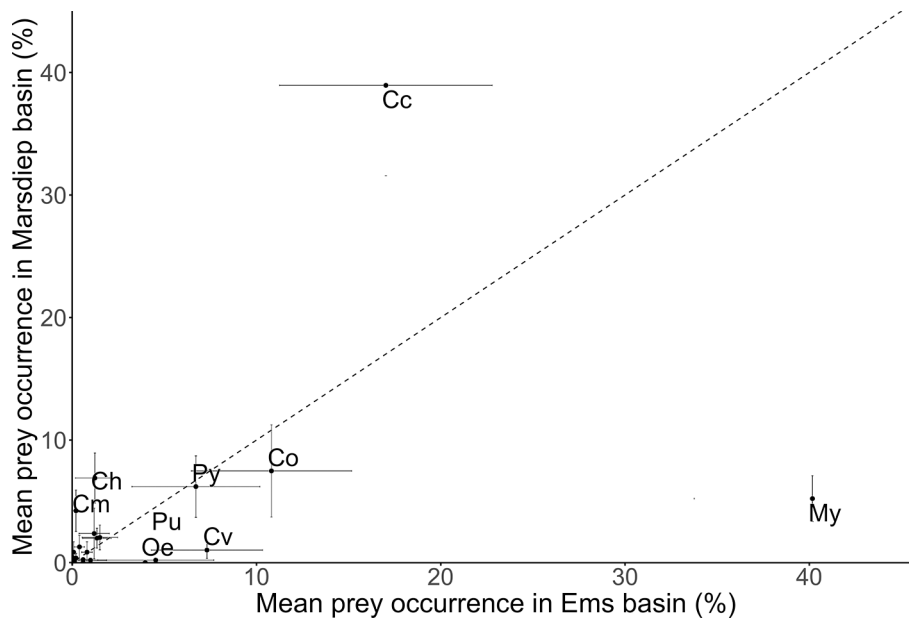


Fig. 5. Comparison of mean occurrence (%) together with SE of the various prey items in the stomachs of all fish species caught in the Ems and in the Marsdiep basin. Data from Fig. 2. The dashed black line represents the 1:1 relationship. For species names and corresponding abbreviations, see Table 2

worms and mysid shrimp were the most common prey species. Overall, in the Ems and in the Marsdiep basins, mysid shrimp and brown shrimp, respectively, were the most important prey.

For pelagic species, copepods and brown shrimp were important prey in both basins in addition to mysid shrimp in the Ems basin and herring in the Marsdiep basin. Since most benthic species belong to the resident and near-residents, this functional group also preyed more on brown shrimp in the Marsdiep basin compared to mysid shrimp in the Ems basin. For the other 2 functional groups, the trend was more diverse and variable.

were also significantly correlated with absolute trophic positions based on compound-specific stable isotopes (Pearson  $r = 0.76$ ;  $n = 7$ ;  $p < 0.02$ ); however, such a correlation was absent in the Marsdiep basin (Fig. 6B). Trophic positions based on stomach contents (Fig. 6C) were correlated in both the Ems and Marsdiep basins with absolute trophic positions based on compound-specific stable isotopes (Ems:  $r = 0.69$ ;  $n = 7$ ;  $p < 0.05$ ; Marsdiep:  $r = 0.61$ ,  $n = 7$ ;  $p < 0.10$ ), although the correlation for the Marsdiep basin was less strong. Trophic positions based on bulk isotopes (Fig. 6D) were correlated in both basins with absolute trophic positions based on compound-

### 3.3.2. Trophic position

As a reference, estimates of trophic positions in the Wadden Sea based on compound-specific stable isotopes are available for 7 fish species: herring, bass, smelt, flounder, plaice, sole and viviparous blenny (Table 4). For these fish, trophic positions from FishBase were related to the absolute trophic positions based on compound-specific stable isotopes, albeit at a lower level (Fig. 6A). Mean  $\delta^{15}\text{N}$  isotope values of the 7 fish species in the Ems basin

Table 4. Estimates of absolute trophic positions (mean, SE) of some fish and epibenthic species in the Dutch Wadden Sea based on compound-specific stable isotopes, together with number of observations (n). After Riekenberg et al. (2022)

Species name	Common name	Abbreviation	n	Trophic position	
				Mean	SE
<i>Clupea harengus</i>	Herring	Ch	10	3.4	0.0
<i>Dicentrarchus labrax</i>	Bass	Dl	10	4.0	0.2
<i>Osmerus eperlanus</i>	Smelt	Oe	8	3.8	0.1
<i>Platichthys flesus</i>	Flounder	Pf	8	3.4	0.1
<i>Pleuronectes platessa</i>	Plaice	Pp	10	3.2	0.1
<i>Solea solea</i>	Sole	Ss	11	3.0	0.1
<i>Zoarces viviparus</i>	Viviparous blenny	Zv	8	3.8	0.1
<i>Crangon crangon</i>	Brown shrimp	Cc	17	3.5	0.1
<i>Carcinus maenas</i>	Shore crab	Cm	18	3.2	0.1



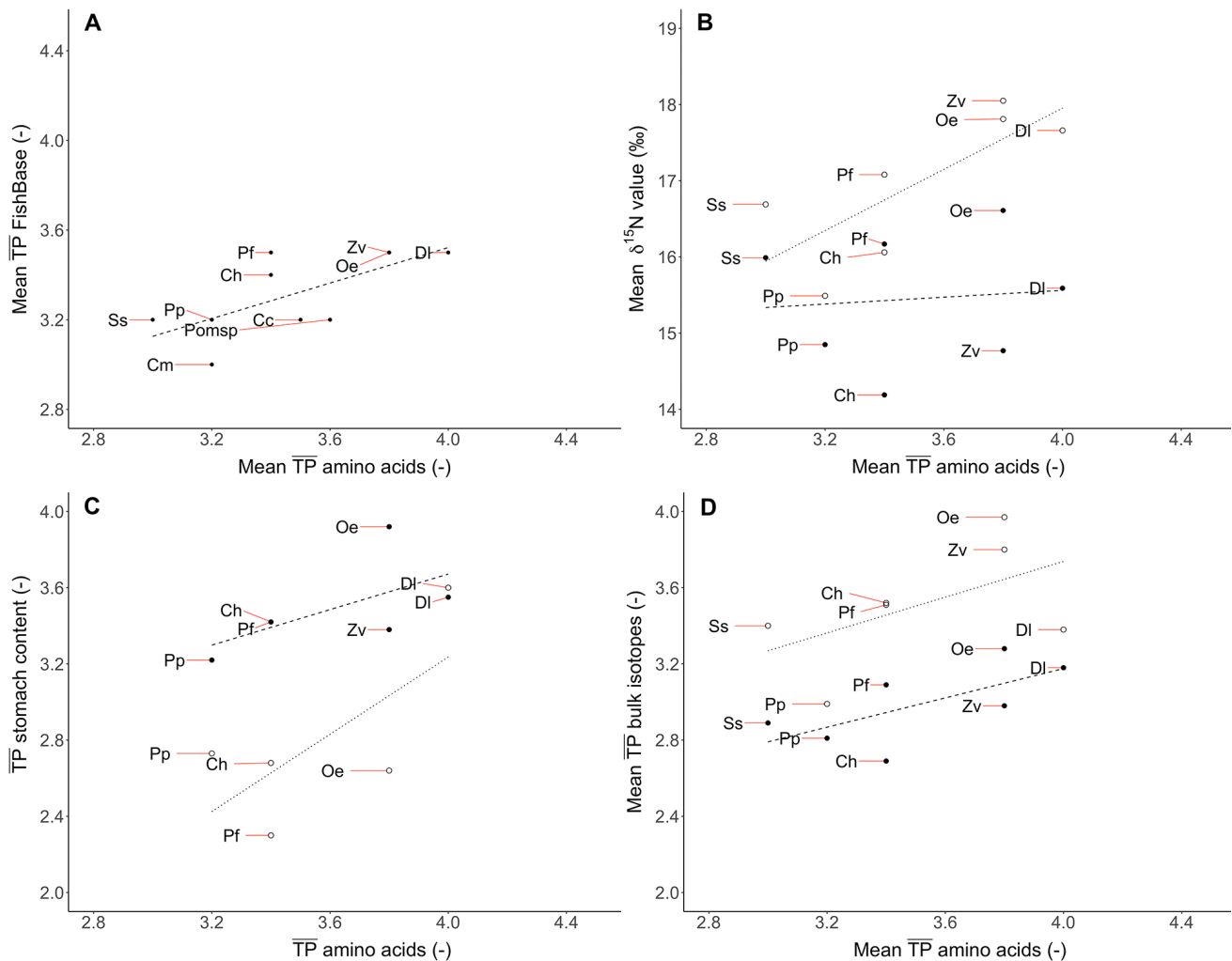


Fig. 6. Relationship between estimated absolute trophic position ( $\overline{TP}$ ) in the Wadden Sea of some fish species by means of compound-specific isotopes (Riekenberg et al. 2022) with (A) trophic position according to FishBase; (B) mean  $\delta^{15}N$  stable isotope values in the Ems (open symbols) and Marsdiep (closed symbols) basins; (C) trophic positions in the Ems (open symbols) and Marsdiep (closed symbols) basins based on stomach content; and (D) trophic positions in the Ems (open symbols) and Marsdiep (closed symbols) basins based on bulk stable isotopes. For species names and corresponding abbreviations, see Table 2

specific stable isotopes (Ems:  $r = 0.67$ ;  $n = 7$ ;  $p < 0.05$ ; Marsdiep:  $r = 0.54$ ,  $n = 7$ ;  $p < 0.10$ ), but the correlation in the Marsdiep basin was less strong.

For 13 fish species, relative trophic positions, as indicated by the mean stable  $\delta^{15}N$  isotope values, were significantly correlated ( $r = 0.66$ ,  $n = 13$ ;  $p < 0.01$ ). For the other species, mean stable  $\delta^{15}N$  isotope values were either higher (dab, twaite shad and bull-rout) or lower (herring, tub gurnard, sand goby, viviparous blenny) in the Marsdiep (Fig. 7A).

Mean absolute trophic position based on stomach content ( $\overline{TP}_{\text{diet}}$ ) of the various fish species in the Ems ranged between 1.7 and 3.6, with most ( $\overline{TP}_{\text{diet}}$ ) values in the Marsdiep varying between 2.5 and 3.5 (Fig. 7B). For most of the fish species, mean trophic

level values were higher in the Marsdiep compared with the Ems. Trophic positions were similar in both basins only for sprat, tub gurnard, bass and butterfish. There was no correlation between the absolute trophic position in the Ems with that in the Marsdiep.

Mean estimated absolute trophic position ( $\overline{TP}$ ) based on  $\delta^{15}N$  isotope values ranged between 2.9 and 3.9 in the Ems basin, with most values around 3.5, and between 2.6 and 3.5 in the Marsdiep basin, with large standard errors for species with low numbers of measurements such as butterfish ('Pg' in Fig. 7C). For most of the species, values were higher in the Ems basin. There was no correlation between the absolute trophic position in the Ems with that in the Marsdiep basin.

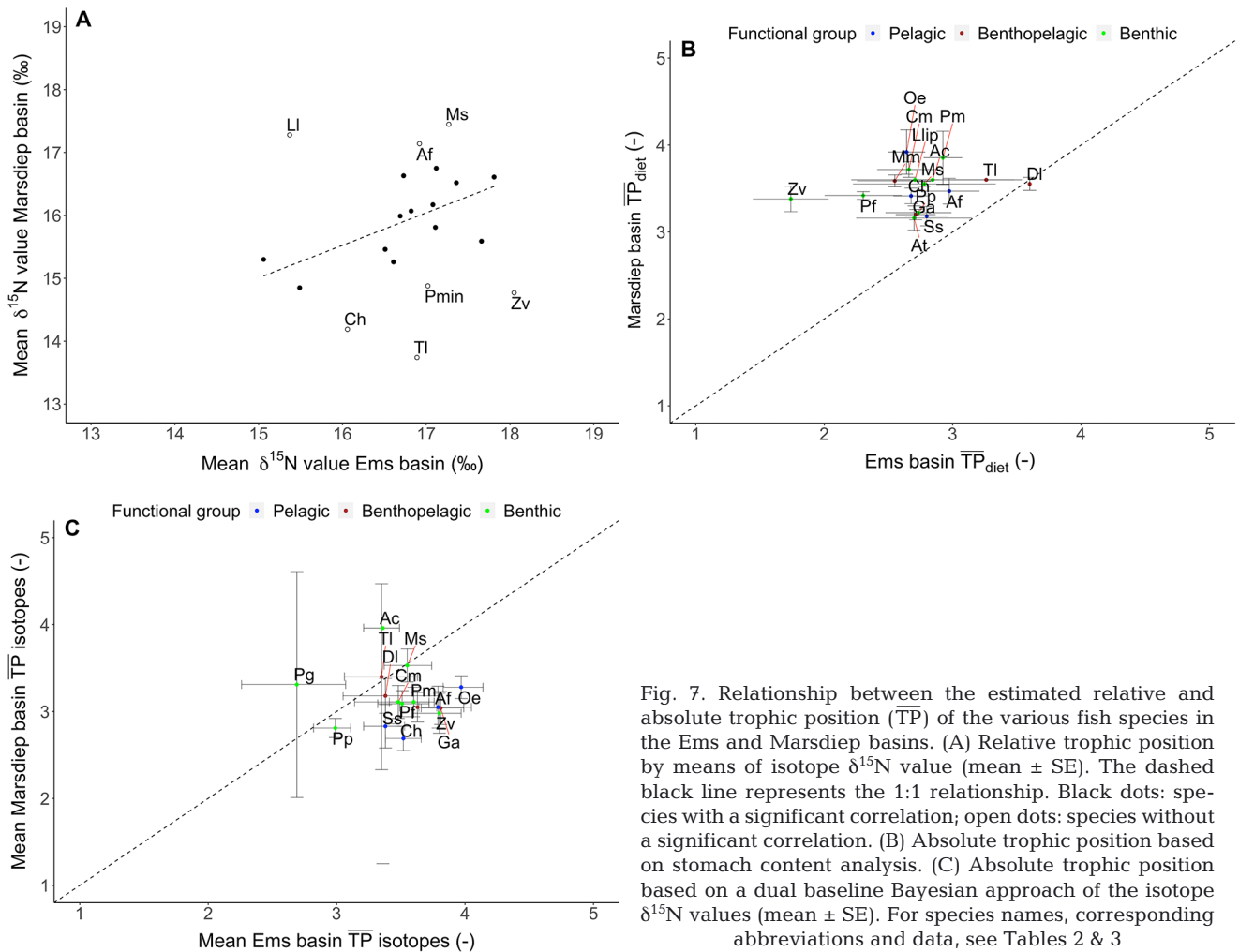


Fig. 7. Relationship between the estimated relative and absolute trophic position (TP) of the various fish species in the Ems and Marsdiep basins. (A) Relative trophic position by means of isotope  $\delta^{15}\text{N}$  value (mean  $\pm$  SE). The dashed black line represents the 1:1 relationship. Black dots: species with a significant correlation; open dots: species without a significant correlation. (B) Absolute trophic position based on stomach content analysis. (C) Absolute trophic position based on a dual baseline Bayesian approach of the isotope  $\delta^{15}\text{N}$  values (mean  $\pm$  SE). For species names, corresponding abbreviations and data, see Tables 2 & 3

#### 4. DISCUSSION

The Wadden Sea is an important dynamic area for around 100 fish species (Witte & Zijlstra 1983). Sampling of such a diverse community comprising a mixture of different life stages (larvae, juveniles, adults) distributed over various habitats (intertidal, subtidal and gullies) is a challenge and always a compromise dictated mainly by study goal, geomorphology of the area, area accessibility, equipment and manpower availability.

Sampling of the complete Wadden Sea fish community is an illusion: fish species collected in the Sylt-Rømø basin totalled 43 different species over 2 yr (Kellnreitner et al. 2012), while in the Marsdiep basin, 54 different species were found over 9 yr (Poiesz et al. 2020). The Ems power plant sampling covered a 3 yr sampling period and resulted in 31 fish species. Even sampling programmes extending over decades have not been able to identify all fish spe-

cies recorded in the area: over a 55 yr time period (1960–2015), 82 different species were found in the western Wadden Sea (van der Veer et al. 2015) out of around 100 fish species that had previously been listed (Witte & Zijlstra 1983).

A sampling strategy should at least be based on collecting a representative part of the different functional groups (pelagic, benthopelagic and demersal) in relation to the total number of species of the fish community present in the area. A minimum might be around 30 fish species, as collected in the Ems basin, since between 30 and 40 fish species are abundant and can be considered as core species (present almost each year) in the area (van der Veer et al. 2015).

##### 4.1. Methodological constraints

The analysis of the spatial variability in the Wadden Sea fish food-web structure in this study is based

on a combination of stomach content and stable isotope analyses of individuals collected simultaneously in the Ems and Marsdiep basins but, for logistic reasons, by means of different sampling designs.

In the Ems basin, sampling was restricted to day-time only, samples were sorted immediately, and all fishes caught were still alive. They were preserved and stored at  $-20^{\circ}\text{C}$  within 1 h, reducing potential digestion of prey items as much as possible. In the Marsdiep basin, fishes were collected once a day from a kom-fyke, and the samples covered a complete day and night cycle. These fishes could have been in the kom-fyke from anything between 0 and 24 h, which means stomach content would have been partly digested in many fishes. Stomach content analysis is sensitive to fish sampling by kom-fykes due to potentially missing small digested prey items with a relatively low trophic position. This might explain the observation that the trophic positions based on stomach contents were higher for most fish species in the Marsdiep compared to the Ems basin. Stable isotope analysis was not affected by the difference in sampling designs in the Ems and Marsdiep basins.

The few estimates of absolute trophic position of some fish species in the Wadden Sea by means of compound-specific stable isotope analysis (Riekenberg et al. 2022) underpins the sensitivity of stomach content analysis to sampling design and bulk isotope analysis to the selection of the baselines and their spatial variability (Phillips et al. 2014). Stable isotope values derived from bulk tissues have other limitations, the diet-to-tissue discrimination factors can be variable in  $\delta^{15}\text{N}$  among different types of tissues within the same organisms, and the differences are sometimes very large (Bowes & Thorp 2015). Compound-specific isotope analysis would have overcome these problems but was outside the scope of the present study. Compound-specific isotope analysis eliminates the need for separate signatures from a primary producer (Bowes & Thorp 2015), and the combination of compound-specific and bulk analytical stable isotopes allows for a better identification of trophic food-web relationships.

Both estimates of stomach content analysis and bulk stable isotope analysis were differently correlated with estimates based on compound-specific stable isotope analysis, but both methods underestimated the absolute trophic levels of the various fish species substantially (Fig. 6C,D). This means that estimates of absolute trophic position based on stomach content analysis and on bulk stable isotopes analysis are not correct and can only be used for rel-

ative comparison of species within a basin. The same holds true for the information provided by FishBase (Fig. 6A).

Despite the shortcomings of stomach content and bulk stable isotope analysis, both methods remain a valuable and complementary source of information about the fish community (see also Sturbois et al. 2022):

- stomach contents provide information about predator–prey relationships in the area and allow a comparison of the main relationships between areas and time periods;
- long-term patterns in stomach composition are the main source of information about trends in predator–prey relationships and food-web dynamics and can reveal significant shifts in diet over time (Holt et al. 2019), especially before the introduction of stable isotope analysis in the 1980s;
- both stomach content and bulk stable isotope analysis provide information about the food sources and relative trophic position of fish species within an area.

#### 4.2. Spatial variability in the Wadden Sea fish food web

Analysing spatial variability in the Wadden Sea fish food web is complicated because the Wadden Sea is a highly dynamic area with fluctuating abiotic conditions such as water temperature and salinity (van Aken 2008a,b), and the area hosts multiple migratory species with large-scale movements. Some species such as seabass can even be a partially migratory species, with some individuals exhibiting long-distance migrations and other individuals showing residency behaviour (de Pontual et al. 2019). In the estuarine Wadden Sea system, tidal basins are the basic units from a geomorphological point of view. Each of the more than 30 tidal inlet systems along the Wadden Sea coastline fuels a tidal basin between the barrier islands and the mainland. These tidal basins are separated from each other by tidal watersheds with relatively low water exchange, and freshwater input varies from high to almost zero. Tidal basins show high variation in size and tidal amplitude (Postma 1983). This variation in hydrography and geomorphology is also reflected in differences in system productivity: mean intertidal macrozoobenthic biomass in 2008–2010 varied between tidal basins in the Dutch Wadden Sea (Compton et al. 2013). For the Marsdiep and outer Ems, intertidal macrozoobenthic biomass is comparable, at around

20 g ash-free dry mass (Compton et al. 2013, 2017), with Sylt-Rømø biomass values of the same order or higher (Baird et al. 2004).

An extensive study in the whole Dutch Wadden Sea based on bulk stable carbon isotope analysis found, despite a large spatial heterogeneity in  $\delta^{13}\text{C}$  values, that microphytobenthos production was the most important energy source supporting the food web in the area (Christianen et al. 2017). Focussing on the Marsdiep basin, Jung et al. (2019) obtained similar results but also pointed to the importance of freshwater suspended particulate organic matter, especially in autumn. Using a combination of bulk and compound-specific stable isotope analysis confirmed the dominant role of microphytobenthos fuelling productivity in the Wadden Sea, including the Marsdiep basin (Riekenberg et al. 2022), albeit to a lesser extent than reported by Christianen et al. (2017). In addition to marine particulate organic matter, microphytobenthos has also been found to contribute most to the food web in the area of the Chanche estuary (Bouaziz et al. 2021).

A detailed analysis of the energy sources supporting the fish food web is outside the scope of this study; however, carbon isotope values lower than the pelagic baseline in both the Ems and Marsdiep basins suggested for some species a signature of organic matter produced in freshwater (Middelburg & Herman 2007). For some species, such as nine-spined stickleback, three-spined stickleback, river lamprey and twaite shad in the Ems basin, this might be in line with their diadromous behaviour (Zijlstra 1983).

Kühl & Kuipers (1983) described the general food-web relationships for the Wadden Sea fishes in 4 overlapping categories: zooplankton feeders, fish feeders, zoobenthos feeders and feeders on minute bottom particles, implicitly suggesting there is no significant spatial variability in predator–prey relationships in the area. The demersal fish survey monitoring programme in the Dutch part of the Wadden Sea also shows similar fish species composition in the different Wadden Sea basins for demersal fish (Tulp et al. 2008). The same holds true for pelagic fish species: despite differences in sampling methods, strategy and timing, the same species were found in the Marsdiep (Poiesz et al. 2020), the Ems (this study), the Jade (Meyer et al. 2016) and Sylt-Rømø (Kellnreitner et al. 2012) basins. Most prey species can also be found throughout the Wadden Sea, such as brown shrimp, shore crab and herring (Kellnreitner et al. 2012, Tulp et al. 2012, Meyer et al. 2016, Poiesz et al. 2020). This might explain the absence of differences

between groups or guilds between the Marsdiep and Ems basins. However, at a smaller scale, within a tidal basin, species distribution (presence and abundance) will vary and be dictated by the species' specific abiotic preferences and acceptable ranges (e.g. temperature, salinity, oxygen levels) in the highly dynamic Wadden Sea (Neill et al. 1994, Freitas et al. 2010, Dahlke et al. 2020) during the different life stages.

Although based on only 30 fish species, the predator–prey relationships found in the Ems basin are in line with the general food relationships described for Wadden Sea fishes in the past (Kühl & Kuipers 1983, BOEDE 1985) and more recently (Kellnreitner et al. 2012, Poiesz et al. 2020). Most fish species appear to be generalists and opportunistic feeders, consuming multiple prey species. The various fish species in the Ems basin also showed overlap in prey consumption, especially for copepods, mysid shrimps and brown shrimps. The same species were also the most important prey species for fish in the Marsdiep basin in the western Wadden Sea (Poiesz et al. 2020) and in the Sylt-Rømø basin (Kellnreitner et al. 2012).

Copepods, brown shrimps, mysid shrimps, shore crabs and herring are the most important prey species in the Ems and Marsdiep basins. This means that predator–prey relationships are to a large extent similar in both basins, but fish species also showed differences in stomach content between the 2 basins. These differences are most likely caused by a combination of differences in both predator and prey species abundance. Large spatial fluctuations have been found for prey species in the Dutch Wadden Sea, such as macrozoobenthos (Compton et al. 2013) and the epibenthic brown shrimp (Tulp et al. 2012).

Riekenberg et al. (2022) published estimates of absolute trophic position of some fish species in the Dutch Wadden Sea by means of compound-specific stable isotope analysis were published, including pelagic (herring, smelt), benthopelagic (bass) and benthic (plaice, flounder, sole and viviparous blenny) species. For all species, standard error of the estimates was very low, suggesting a similarity in food-web structure for these species at least at the scale of the Dutch Wadden Sea.  $\delta^{15}\text{N}$  isotope values and trophic position estimated from both stomach content and bulk isotope analysis also showed a large similarity in relative trophic position of the various fish species and hence in food-web structure in both the Ems and Marsdiep basins. Estimates based on stomach contents and on bulk isotope analysis both underestimated the absolute trophic position based on compound-specific stable isotopes reported by

Riekenberg et al. (2022) in a different way and with different results for the 2 basins. Therefore, future studies on spatial variability in food-web structure should be based on compound-specific stable isotope analysis in line with Riekenberg et al. (2022).

In conclusion, (1) fish fauna composition shows large spatial similarity, and (2) the fish food-web structure is fuelled by a few key prey species such as copepods, mysid shrimp, brown shrimp, shore crab and herring. The observed spatial variability in predator–prey relationships in the Wadden Sea is most likely the result of local differences in predator and prey abundance, mainly driven by local productivity and species- and stage-specific preferences and tolerance ranges to abiotic factors. Fish food-web structure appears to be similar between spatially separated areas, at least at the scale of the Dutch Wadden Sea.

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