Poiesz, S.S.H.; Witte, J.IJ.; van der Meer, M.T.J.; van der Veer, H.W. & Soetaert, K. (2021). Trophic structure and resource utilization of the coastal fish community in the western Wadden Sea: evidence from stable isotope data analysis. *Marine Ecology Progress Series,* 677: 115-128

Published version: https://dx.doi.org/10.3354/meps13855

NIOZ Repository: http://imis.nioz.nl/imis.php?module=ref&refid=347196

Trophic structure and resource utilization of the coastal fish community in the western Wadden Sea: evidence from stable isotope data analysis.

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Keywords: Coastal fish community, Wadden Sea, stable isotopes, trophic position, trophic structure

Abstract

We studied the trophic structure of the western Wadden Sea fish community through stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of 1658 samples from 57 fish species collected between 2012 and 2016. Stable isotope values differed between species but did not vary between years or between seasons, and only for some species with fish size. Stable isotope values were not different between immigrating (spring) and emigrating (autumn) fish suggesting a similar trophic niche of the various fish species in the coastal zone and inside the Wadden Sea. For the majority of the species, average $\delta^{13}C$ values were within the range of -12‰ to -20.5‰, showing that both (marine) pelagic and benthic primary producers were at the base of the food web. Average $\delta^{15}N$ values varied among species from 13‰ to 18‰, resulting in estimated trophic positions (TP) between 2.1 to 5.5 with the majority between 2.2 to 3.5. Thick-lipped grey mullet (*Chelon* *labrosus*), golden grey mullet (*Chelon aurata*), greater pipefish (*Syngnathus acus*) and pilchard (*Sardina pilchardus*) had the lowest trophic position (2.2 – 2.4). Among the common species (> 10 observations), highest TP values (3.4 – 3.5) were found for the twaite shad (*Alosa fallax*), smelt (*Osmerus eperlanus*), bull-rout (*Myoxocephalus scorpius*), bass (*Dicentrarchus labrax*) and cod (*Gadus morhua*). For all species, estimated trophic positions based on isotope values were lower than those based on stomach content composition (2.0 – 4.7), which could be explained by species-specific differences in trophic fractionation or by underestimation of the contribution of smaller prey species in the stomach content analysis. The trophic niche space of benthopelagic species was the smallest and overlapped with that of the pelagic and benthic species. In terms of use of the area, trophic niche space was smaller for juvenile marine migrant species (nursery-type species) and overlapped with that of the (near)-resident species and marine seasonal visitors. Potentially, trophic competition is highest for the functional group of benthopelagic species and the guild of juvenile marine migrant species (nursery-type species).

1. Introduction

Shallow coastal systems are often highly productive areas due to import of nutrients and organic matter from river runoff and from the open sea (Nixon 1995, Cloern et al. 2014). As a consequence, these areas are important foraging grounds for a variety of fish, bird and marine mammal species (e.g. Goodall 1983). Worldwide, these coastal areas are under anthropogenic threat already for centuries which has caused major disturbance and structural and functional changes in these systems (see for instance Jackson et al. 2001, Lotze 2005, Lotze et al. 2006). Also for the future, threats such as overfishing, climate change (e.g. warming, acidification, deoxygenation), habitat destruction and pollution are expected to increase (Bijma et al. 2013, European Marine Board 2013). Any prediction of the consequences of these threats for the future productivity of these coastal areas requires -among other factors- insight in the food web structure of these systems.

Historically, food web studies have been, and still are, based on taxonomic identification of prey items via stomach content analysis (Hynes 1950). The strength of stomach content analysis is that it provides detailed information about predator-prey relationships. However, its limitations are that only visible larger prey items can be identified; that it offers only a small snapshot in time of recent prey items, and that it requires extensive taxonomic knowledge. Stable isotope measurements (Minagawa & Wada 1984) overcame the snapshot problem by providing a more integrated signal of assimilated prey over a longer time period. Stable nitrogen isotope values (δ^{15}N) increase with trophic position (Minagawa & Wada 1984). Carbon isotope (δ^{13}C) values are an indication of different carbon sources (Hecky & Hesslein 1995), provided that these have significantly different values. Therefore, carbon and nitrogen stable isotopes have been increasingly used as indicators of both habitat use and trophic position (Post 2002, McCutchan et al. 2003, Boecklen et al. 2011, Abrantes et al. 2014, Christianen et al. 2017), while insight in predator-prey relationships still relies on taxonomic identification of prey items via stomach content analysis. Food web structure analysis benefits most from a combination of both stomach content and stable isotope analysis. By combining these 2 types of analyses, complementary results of the food web structure and food web functioning and dynamics can be obtained (Preciado et al. 2017, Park et al. 2018, Bissattini et al. 2021).

One of the most important European temperate coastal areas is the international Wadden Sea, an estuarine area bordering the Dutch, German and Danish coast, with recognized importance as a nursery area for a variety of fish species (Zijlstra 1972) and as resting and feeding area for wading birds (Wolff 1983). For the Wadden Sea, food web studies started with static carbon flow models of the intertidal (Kuipers et al. 1981) and the subtidal (de Wilde & Beukema 1984). Later, spatial and temporal fluctuations were investigated by means of ecological network analysis (ENA) (Baird et al. 2011, 2012, Schückel et al. 2015, de Jonge et al. 2019a, 2019b, Jung et al. 2020) and dynamic energy flow budget models (Baretta & Ruardij 1988, Lindeboom et al. 1989). Recently, some aspects of the Wadden Sea food web have been studied by means of stable isotopes. Christianen et al. (2017) concluded from an extensive sampling campaign in the Dutch Wadden Sea that the benthic primary producers (micro-phytobenthos) were the most important energy source for the majority of consumers at higher trophic positions in late summer; but, in line with Deegan & Garritt (1997), large spatial heterogeneity was observed. Jung et al. (2019) pointed out that the Wadden Sea food web also showed seasonal variability, highlighting the important role of freshwater energy inputs. Both studies mainly focussed on the macrobenthic community and although these studies included some information about fish, a detailed stable isotope analysis of the trophic position of the Wadden Sea fish community is still lacking.

So far, trophic food web structure of the Wadden Sea fish community, including predator-prey relationships, was analysed only in detail based on stomach content information in the Sylt-Rømø Bight basin (Kellnreitner et al. 2012) and the Marsdiep basin (Poiesz et al. 2020). In this study, the food web structure of the fish community of the Marsdiep basin in the western Dutch Wadden Sea is analysed based on stable isotopes and combined with information about primary producers in the area (Christianen et al. 2017). Calculated trophic positions are compared with estimates based on dietary information from stomach content data (Poiesz et al. 2020). Furthermore, for all species the size of the trophic niche is determined. These trophic niches comprise all the trophic interactions that connect a species to others in the ecosystem (Elton 1927) and is a representation of a species' overall trophic role (Leibold 1995). In addition, niche overlap within fish communities indicate potential trophic competition among different groups (Dubois & Colombo 2014). The previous analysis of the trophic structure based on stomach content information (Poiesz et al. 2020) showed a pivotal position of a few key prey species, namely amphipods, brown shrimps, juvenile herring and gobies. To allow a link of the present study with Poiesz et al. (2020), the stable isotope value of these key prey species is also determined. Furthermore, the trophic niches of the individual fish species were determined in relation to their use of the area as (near)-resident species, juvenile marine migrant and marine seasonal visitors and in relation to their feeding type (benthic, benthopelagic, pelagic), following Zijlstra (1983) and Elliott & Dewailly (1995).

2. Material and methods

2.1. Sampling

From 2012 to 2016, fish was collected from the catches of a long-term monitoring programme of the fish fauna by means of a passive fish trap near the entrance of the Wadden Sea (Fig 1). This kom-fyke with a stretched mesh-size of 20 mm consisted of a leader of 200m running from the beach towards deeper waters. Fish swimming against the leader are guided towards two chambers (the so-called 'kom') and from there collected into the kom-fyke. Fishing took place in spring (April, May, June) and autumn (September, October) and during this period the kom-fyke was emptied every day whenever weather conditioned permitted. During the winter and summer months the kom-fyke was removed due to the risk of potential damage by ice in winter and extreme algal blooms and high numbers of jellyfish during summer. For more information see van der Veer et al. (2015). Key prey species according to Poiesz et al (2020) were collected nearby the kom-fyke by means of fine-meshed pelagic and demersal trawls.

All fish and prey species caught were taken back to the laboratory, sorted immediately, identified to species level, counted, measured and weighed. Sometimes, fish were damaged by shore crabs and the exact weight could not be determined. A maximum of three individuals per fish species per week, preferably of different size, were selected and stored at -20°C for dissection. Within a few weeks of storage, fish were defrosted and thawed and isotope samples (dorsal muscle tissue directly posterior to the head) were taken in line with Svensson et al. (2014), put in a 1.5-ml centrifuge vial and stored at -80°C. After freeze-drying for 48 h, the isotope samples were ground and homogenized. Next, two samples of between 0.4 – 0.8 mg were weighed and folded into small tin cups for analysis. $\delta^{15}N$ and $\delta^{13}C$, % total organic carbon (%TOC) and % total nitrogen (%TN) contents were measured at the Royal Netherlands Institute for Sea Research (NIOZ)with a Thermo Scientific Delta V Advantage Isotope Mass Spectrometer linked with a Flash 2000 Organic Element Analyzer. During each sample run, monitoring gas (N_2 and CO_2) with a predetermined isotopic composition was used to determine the δ values of both the samples as well as the standards.

Standards with known isotopic composition were weighed and included on each plate of 94 spots (Acetanilide, Urea and Casein) at the beginning of the analysis, after every twelve samples and at the end of each sequence in order to monitor the process of measuring and in order to correct for the offset between the measured and actual isotope ratio. One standard, Acetanilide, was used to correct the measured values and the other two standards, Urea and Casein, to check the correction. Analytical reproducibility was 0.3‰ for $\delta^{15}N$ and 0.1‰ for $\delta^{13}C$ throughout every sequence. Before the standards, each sequence starts with multiple blanks, empty tin cups, to remove air if present and to determine a potential blank contribution to the analysis. Blanks were typically too low to be of any importance.

Isotope value of the sample (δX) was expressed as ratio, delta (δ) notation in per mil (‰), relative to an internationally defined reference:

$$\delta X = (R_{sample}/R_{reference} - 1) * 1000 \qquad [1]$$

where R_{sample} and $R_{reference}$ are the ratio between the 'heavy' and the 'light' isotopes (^{15}N:^{14}N or ^{13}C:^{12}C) of the sample and the reference, respectively. $\delta^{15}N$ values are reported against atmospheric nitrogen and $\delta^{13}C$ against Vienna Peedee-Belemnite (VPDB). All information was added to a database.

2.2. Stable isotopes

$\delta^{13}C$ values were corrected for lipid content according to Svensson et al. (2014):

$$\delta^{13}C_{corr} = \delta^{13}C_{bulk} - 2.21 + 0.82 * C:N \qquad [2]$$

where:

$\delta^{13}C_{corr}$ the calculated $\delta^{13}C$ values corrected for lipid content;

$\delta^{13}C_{bulk}$ the $\delta^{13}C$ values of the bulk tissue ($\delta^{13}C$ values including lipid content);

$C:N$ the ratio of total nitrogen (%TN) / total organic carbon (%TOC).

These lipid content corrected $\delta^{13}C$ values were used in all the further analyses.

Isotopic values of $\delta^{15}N$ and $\delta^{13}C$ were analysed in relation to fish length and season for species with 57 or more isotopic measurements. Linear relationships were calculated by fitting a model according to:

$$\delta^{13}C = \beta_1 * \text{fish species} + \text{factor (season)} + \text{fish length (cm)} \qquad [3]$$

$$\delta^{15}N = \beta_1 * \text{fish species} + \text{factor (season)} + \text{fish length (cm)} \qquad [4]$$

where season refers to spring or autumn sampling.

2.3. Trophic positions

Feeding niches of the fish species distinguishing between their guilds and functional groups were analysed. The guild represents how a species uses the area (Wadden Sea) as a (near)-resident species (NR), juvenile marine migrant (JMM) or marine seasonal visitors (MSV) following Zijlstra (1983). Species were also classified into 3 functional groups (benthic, benthopelagic and pelagic) based on habitat position (e.g. bottom-dwelling, near the bottom or swimming in the water column) and method of food acquisition (Dumay et al. 2004). Trophic positions for each fish species, were estimated according to a dual baseline Bayesian approach which includes a mixing model to discriminate among two distinct sources of C and N, e.g., pelagic vs. benthic baselines (van der Zanden et al. 1997, Post 2002), in line with Christianen et al. (2017). In order to perform the Bayesian analysis, the first step was based on one baseline with the trophic fractionation factor for nitrogen only.

$$\delta^{15}N_c = \delta^{15}N_b + \Delta N(TP - \lambda) \qquad [5]$$

where:

$\delta^{15}N_c$	the δ^{15}N value of the consumer
$\delta^{15}N_b$	the δ^{15}N value of the single baseline
ΔN	the trophic fractionation factor for nitrogen (N)
TP	the trophic position of the consumer
λ	the trophic position of the baseline

In order to extend this analysis to two baselines (pelagic and benthic) with two distinct sources (N and C) the formula for N becomes:

$$\delta^{15}N_c = \Delta N(TP + \lambda) + \alpha(\delta^{15}N_{b1} + \delta^{15}N_{b2}) - \delta^{15}N_{b2} \qquad [6]$$

with additional:

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

The full model of two baselines for C is rewritten to derive α:

$$\alpha = ((\delta^{13}C_{b2} - (\delta^{13}C_c + \Delta C))/(TP - \lambda)/(\delta^{13}C_{b2} + \delta^{13}C_{b1}) \qquad [7]$$

with additional:

$\delta^{13}C_{b1}$, $\delta^{13}C_{b2}$	the δ^{13}C of respectively baseline 1 and 2
$\delta^{13}C_c$	the δ^{13}C of the consumer
ΔC	the trophic fractionation factor for carbon (C)

Freshwater and estuarine suspended particulate organic matter values for the Marsdiep area were taken from Jung et al. (2019). Data on pelagic and benthic baselines were taken from Christianen et al. (2017). In line with Christianen et al. (2017), the blue mussel (*Mytilus edulis*) from deep channel buoys was taken as proxy for the pelagic baseline. In contrast to Christianen et al. (2017), the common periwinkle (*Littorina littorea*) was used as it was considered to be the best suitable proxy for the benthic baseline in the Marsdiep area. These relatively large and long-lived primary consumers integrate temporal variability thereby representing average δ^{15}N baseline values. *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. Isotopic values of *M. edulis and L.littorea* that were used had been collected between 2011 and 2014 from several locations (87 and 60, respectively) in the western part of the Wadden Sea.*L. littorea* feeds primarily on ephemeral filamentous bladed algae, other macrophytic sporelings/germlings and scraping surficial diatoms (Tyrrell et al. 2008). In order to validate this species as proxy for the benthic baseline, δ^{13}C values were compared with those of benthic diatoms and of *Ulva lactuca* and *U. ulva*. The diatoms and *Ulva* samples had a similar temporal (2011-2013) and spatial (western Wadden Sea) coverage as the *L. littorea* data (see Christianen et al. 2017). The δ^{13}C values of *L. littorea* had a range of -17.1‰ to -10.6‰ (average -14.22‰; s.e. 0.18‰), the *Ulva* species a range of -18.47‰ to -9.15‰ (average -13.91‰; s.e. 0.29‰) and the diatoms a range of -19.8‰ to -10.42‰ (average -14.12‰; s.e. 0.17‰), justifying the use of *L. littorea* as a proxy for benthic production.

The trophic fractionation factor of 3.4‰ for nitrogen δ^{15}N (s.d. 0.98‰) and of 0.39‰ for carbon δ^{13}C (s.d. 1.3‰), was taken from Post (2002). The two different baselines were incorporated into the calculation together with the variable trophic fractionation, using the tRophicPosition R package (R Core Team 2019) with a Bayesian TP model following Quezada-Romegialli et al. (2018). Trophic fractionation for nitrogen in the Marsdiep basin was estimated for the various functional groups by determining the relationship between the estimated average trophic position ($\overline{TP}\mathrm{diet}$) of a fish species based on stomach content (taken from Poiesz et al. 2020) and the mean δ^{15}N value.

2.4. Trophic niche

Based on the δ^{15}N and δ^{13}C isotope values, trophic niches were quantified for fish species using niche/community metrics following Layman et al. (2007): (1) δ^{13}C range (CR), which represents the niche diversification with respect to the basal food sources, whereby higher CR reflected the utilization of a broader spectrum of food sources; (2) $\delta^{15}N$ range (NR), which represents the vertical food web structure and therefore the diversity of trophic positions, providing information on the trophic length of the community; (3) total area (TA), which is the convex hull area encompassed by all species in $\delta^{13}C$– $\delta^{15}N$ bi-plot space, reflecting the size of the total niche space occupied and (4) mean distance to centroid (CD), which is the mean distance of the isotopic value of each specimen from the $\delta^{15}N$-$\delta^{13}C$ centroid and is a proxy for the trophic diversity. For the different species, the estimated isotopic niche width, measured as the convex hull total area (TA) and the standard ellipse areas (SEA ‰) and the standard ellipse area corrected for small sample sizes (SEAc; ‰) were calculated using the corresponding trophic values ($\delta^{15}N$ and $\delta^{13}C$). Differences between guilds and between functional groups were determined based on differences in TA and SEAc.

Trophic redundancy (which species fill the same trophic niche), was characterized by (1) the mean nearest neighbor distance (MNND), which is the mean distance in the isotopic space of each predator to its nearest neighbor, and as such reflects the average trophic (dis)similarity of predators, and (2) the standard deviation of nearest neighbor distance (SDNND), which is calculated as the standard deviation of these distances and is a measure of the evenness of the spatial density and packing of the predators in the isotopic space. All metrics were calculated using the Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al. 2011) package in the R statistical computing programme (R Core Team 2019).

3. Results

3.1. Stable isotopes

The pelagic baseline was -17.8‰ ± 0.1‰ and for the benthic $\delta^{13}C$ baseline -14.2‰ ± 0.1‰ (Table 1). Freshwater and estuarine suspended organic matter values were respectively in the range of -22‰ to -25‰ and -18‰ to -16‰. $\delta^{13}C$ values of the key prey items of the fish fauna in the western Wadden Sea varied from -15.9‰ for *Gammarus sp.* to -19.9‰ for *Gastrosaccus spinifer* (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m000p000supp.pdf).

In total 1658 samples from 57 fish species were analysed (see Supplementary material Table S2). The average $\delta^{13}C$ values of the Wadden Sea fishes varied from -11.3‰ to -27.0‰ with most species within the range of -15‰ to -19‰ (Fig 2). The golden grey mullet *(Chelon aurata)* had the highest average $\delta^{13}C$ value of -11.3‰, suggesting macroalgae and/or seagrass as carbon source. Three species had $\delta^{13}C$ average values lower than -20‰: round goby (*Neogobius melanostomus*), vendace (*Coregonus albula*), and the eel (*Anguilla anguilla*), suggesting a freshwater carbon source. Pelagic species were showed carbon isotope values concentrated around the pelagic baseline value (Fig 3A). The benthic species covered the whole $\delta^{13}C$ range, but most species were also clustered around the pelagic baseline value (Fig 3A). No differences were found between the three guilds (Fig 3C).

Average $\delta^{15}N$ values varied from 13‰ to 18.3‰ among species (Fig 2). The thick-lipped grey mullet (*Chelon labrosus*), golden grey mullet, greater pipefish (*Syngnathus acus*) and two clupeoid species pilchard (*Sardine pilchardus*) and anchovy (*Engraulis encrasicolus*) had the lowest values around 13‰ and highest values around 17‰ were found for the twaite shad (*Alosa fallax*), smelt (*Osmerus eperlanus*), cod (*Gadus morhua*), bass (*Dicentrarchus labrax*), bull-rout (*Myoxocephalus scorpius*), tompot blenny (*Parablennius gattorugine*), round goby and vendace. No clear patterns were found in relation to functional group (Fig 3B) or guild (Fig 3D).

$\delta^{15}N$ was significantly ($p<0.001$) related to fish size for some species; positively for bass, bib (*Trisopterus luscus*), bull-rout, cod, plaice *(Pleuronectes platessa*), sand-smelt *(Atherina presbyter*) and sea trout (*Salmo trutta*) and negatively for herring (*Clupea harengus*) (see Supplementary material; Table S3, Fig S1, Fig S2). For all data of all fish species together, the relationship was not statistically significant [$F(1, 1447) = 0.54$, $p = 0.46$]. No significant differences between years and season were found for $\delta^{15}N$ [$t(1470)=0.316$, $P=0.752$; Supplementary material Figure S3]. Also, no significant relationship was found for average fish length (cm) versus average $\delta^{15}N$ [$F(1, 49) = 4.02$, $p = 0.051$] and average $\delta^{13}C$ [$F(1, 49) = 0.76$, $p = 0.387$] (Supplementary material Fig S4).

3.2. Trophic position

The mean trophic positions (TP) based on stable isotopes were estimated for all fish species and ranged from 2.1 to 5.5, with the majority between 2.2 to 3.5 (Supplementary material Fig S5).

In line with $\delta^{15}N$, the two mullet species (thick-lipped grey mullet, golden grey mullet), greater pipefish, pilchard and anchovy had the lowest trophic positions. The less common species (<10 observations) showed overall the highest average trophic positions [vendace, forkbeard (*Phycis blennoides),* recticulated dragonet (*Callionymus reticulatus*), houting (*Coregonus oxyrinchus*), tompot blenny and shanny (*Lipophrys pholis*). Among the common species (> 10 observations), highest TP values were found for twaite shad, smelt, bull-rout and cod (Supplementary material Fig S5).

With respect to the different functional groups, the few benthopelagic species had the smallest range and the benthic and pelagic group included the consumers with the lowest TP values (mullet and clupeid species). The highest trophic positions were almost the same in all three functional groups (Supplementary material Fig S5). MSV had the widest range of trophic positions. JMM, a small but abundant group of juvenile flatfishes and clupeids had the smallest range (Supplementary material Fig S5).

Mean trophic positions calculated based on stable isotope value were significantly lower than based on stomach content data (Table 2; $F(1,26)=10.1$, $P < 0.05$). Only the benthic species showed a significant relationship between the calculated dietary based TP and the $\delta^{15}N$ values ($P >0.05$) (Supplementary material Fig S6). For all species combined a trophic fractionation factor of 3.2‰ per trophic level was found; for the groups separately: benthic species 3.7‰, benthopelagic species 3.0‰ and pelagic species 1.0‰. The pelagic garfish (*Belone belone*) and pilchard were outliers as their stomach content data indicated a mean trophic position value nearly 0.4 units higher than the $\delta^{15}N$ trophic position estimates did (Supplementary material Fig S6; lowest two blue dots).

3.3. Trophic niche

Density plots of standard ellipses areas indicated a larger SEAc for flounder (*Platichthys flesus*), sea trout and thick-lipped grey mullet compared to all other species (Fig 4, Table 3), which was due to a large variability in respectively $\delta^{15}N$ (sea trout) and $\delta^{13}C$ (flounder) or both (thick-lipped grey mullet).

With respect to functional groups, trophic niche space was smallest for benthopelagic species and overlapped with niches of both pelagic and benthic species. The trophic niche space of benthic species also overlapped with that of the pelagic species. In benthic species the largest range of $\delta^{13}C$ values were found compared to the benthopelagic and pelagic species (Fig 5).

In terms of guilds, trophic niche space was smallest for JMM species (0.91). The trophic niche of both NR species and MSV overlapped with the niche of juvenile migrant species. The size of the trophic niche of both NR species and MSV was about the same but overlapped partly with highest TP values in NR species. Highest $\delta^{13}C$ values of -6.5‰ were found among the MSV and highest $\delta^{15}N$ values (25‰) occurred in the NR species (Supplementary materials Fig S1, Fig S2).

Trophic niche sizes were compared based on their SEAc (Table 3). The Layman metrics for the trophic diversity and redundancy confirmed the differences in the trophic structure of the difference groups and guilds (Table 4). The benthopelagic group and the JMM had the smallest mean $\delta^{13}C$ range (CR – 2.02 and 2.55), while the MSV and the benthic species had the highest (CR – 7.90 and 6.94). The JMM had the smallest range in $\delta^{15}N$ (NR – 0.92) and the benthic group had the highest (NR – 4.10). The distance to centroid was smallest for the benthopelagic group (CD – 0.82) (trophic diversity), whereby the other groups were found to be around 1. The smallest mean nearest neighbours' distance (MNND – 0.60 (trophic redundancy) was found for the NR species and the highest (MNND – 1.20) for the MSV species. The highest convex hull areas (TA – 15.16 and 15.95) were observed for the benthic and MSV species, while the smallest was found for the JMM (Fig 5).

4. Discussion

Three different estimates of the trophic structure of the Wadden Sea fish fauna are now available: estimates based on [1] FishBase (www.fishbase.com); [2] "snapshot" dietary information from stomach content data (Poiesz et al. 2020) and [3] stable isotope fractionation (this study). Focussing on the 28 most abundant Wadden Sea fish species (species with 10 or more observations), the estimates of trophic position based on stomach content and on FishBase were in general similar, but also showing differences in both directions. The estimate of trophic position based on stable isotope data was on average about 20% (varying from 4% to 33%) lower than the two other estimates.

4.1. What is fuelling the Wadden Sea fish food web?

Ecological network analysis (ENA) for various time periods in different parts of the Wadden Sea (Balgzand NL; Jade Germany; Sylt-Rømø Germany/DK) illustrated large spatial and temporal variability in the contribution of various local producers versus imported organic matter as energy source of the local food web (Baird et al. 2012, Schückel et al. 2015, Jung et al. 2020). Despite a small enrichment relative to the diet, carbon isotopic values can be used to identify the main energy sources of a species as they reflect their diet within about 1‰ (for overview see Michener & Kaufman 2007). For the Dutch part of the Wadden Sea, Christianen et al. (2017) concluded from an extensive stable carbon isotope analysis that local benthic primary producers were the most important energy source for the majority of the intertidal macrozoobenthic food web. Due to the almost complete absence of macroalgae in this area (Folmer et al. 2016), microphytobenthos appears to be the most important energy source for the majority of the intertidal benthic food web (Christianen et al. 2017). Recently, Jung et al. (2020) confirmed the dominant role of microphytobenthos as primary producers in the Balgzand intertidal area in the western Wadden Sea.

In our study, most of the Wadden Sea fish species had δ^{13}C carbon isotope values in the range of -15‰ to -20‰, whereby pelagic species could be distinguished by their lower stable carbon signals compared to benthic and benthopelagic species, in line with the proxy for pelagic primary producers (Currin et al. 1995, Stribling & Cornwell 1997, Riera et al. 1999). The diet of the western Wadden Sea fish fauna shows a large prey overlap, with a focus on a few key species: amphipod crustaceans, brown shrimps, juvenile herring and gobies (Poiesz et al. 2020). For most of the benthic and benthopelagic species, macrozoobenthic prey is (part of) their diet (Poiesz et al. 2020) and therefore microphytobenthos will also be an important energy source (Christianen et al. 2017) for these functional groups. In addition, most benthic and benthopelagic species also prey partly upon the epibenthic key items with a more pelagic signal such as for instance the copepod consuming juvenile herring. Therefore, in the shallow Wadden Sea micro phytoplankton will not only be an important energy source for the pelagic fish fauna but also for some benthic and epibenthic fish species, as reflected in their relatively low δ^{13}C isotope values. The absence of a clear pattern between the various guilds, NR species, JMM and MSV indicates that their main energy source constitutes prey items from 'local production'.Some fish species had very high or very low δ^{13}C values. Golden grey mullet had the highest stable δ^{13}C value of around -11.3‰ which points to seagrasses and/or marine macroalgae as their main energy source. On the other hand, eel had a very low stable carbon value of about -27‰. These eels were large migrating females caught in autumn, so their stable δ^{13}C values probably indicate a freshwater origin (Harrod et al. 2005, Middelburg & Herman 2007).

Our results for the western Wadden Sea are consistent with data of the fish fauna in the Sylt-Rømø basin in the eastern part of the Wadden Sea (de la Vega et al. 2016). In the Sylt-Rømø basin, δ^{13}C values ranged from on average from -16 to -19‰, and differences in pelagic, benthopelagic and benthic species were also found. Some other studies point to large differences between habitats. For instance, in the Gironde estuary along the French west coast most fish species had different stable carbon isotope values in different habitats along a salinity gradient (Selleslagh et al. 2015). Also, in saltmarsh areas, fish species will assimilate material derived from macrophytes and filamentous algae (see for instance Winemiller et al. 2007). In general, local morphological and hydrographical characteristics will (indirectly) affect the stable carbon isotope values of the fish fauna.

4.2. Wadden Sea fish food web

The calculation of trophic positions of the various Wadden Sea fish species in this study is based on a mean fractionation of 3.4‰ for δ^{15}N, which was derived for a wide range of consumers by van der Zanden & Rasmussen (2001) and Post (2002). However, this calculation of trophic position can only be considered as a rough estimate given the large variability in fractionation in the order of 1.8‰ (van der Zanden & Rasmussen 2001).

The majority of calculated trophic positions based on stable isotopes of the western Wadden Sea fish species ranged from 2.2 to 3.5, with most trophic positions above 2.5. Except for the low trophic positions of mullets and clupeids (herring, sprat (*Sprattus sprattus*) and pilchard) that consume algae (Poiesz et al. 2020), the range in trophic positions was almost similar for the different functional groups (pelagic, benthopelagic, benthic). With respect to guild, MSV had the largest range of trophic positions and JMM the smallest. Maximum trophic positions of the JMM using the area as a nursery (Zijlstra 1983) were between 3.0 and 3.5, a medium trophic position.

The trophic positions estimated from stomach content data resulted in higher values with a range from 2.0 to 4.7 and with most trophic positions above 3.0 (Poiesz et al. 2020). A possible reason for this mismatch between TP based on stable isotopes and dietary-based TP might be that sedimentary organic matter, microbial biomass and smaller benthic marine microphytobenthos were not identified in the stomach content of (benthic) predators. The exclusion of these 'lower' trophic food sources, would therefore result in an overall overestimation of the TP from diet. The low isotope-based trophic positions found for both some benthopelagic and pelagic species might be explained by their diet, such as the benthopelagic bib, feeding on a wide variety of different smaller prey items such as mysidacea and small crustaceans (among others; Heessen et al. 2015; Poiesz et al. 2020) and the pelagic herring, pilchard and sprat, which feed mainly on copepods, bristle worms, mysidacea and small shrimps (Poiesz et al. 2020). An alternative explanation might be that our baseline species are not 100% herbivorous in the area.

Part of the discrepancy will be caused by the fact that the trophic fractionation differs from the average value of 3.4‰ from van der Zanden & Rasmussen (2001) and Post (2002), and that this trophic fractionation is species-specific. According to Minagawa & Wada (1984), van der Zanden & Rasmussen (2001) and Goedkoop et al. (2006), trophic fractionation values could range between 1.0‰ and 9‰, depending on types of diet and environmental factors. This study showed indeed that trophic fractionation differed at the functional group level, with a slightly higher value of 3.7‰ for the benthic species and a somewhat lower value 3.0‰ for the benthopelagic species. For the pelagic species a relatively low value in the order of 1.0‰ was found. Diet quality and food processing mechanisms may affect fractionation (Mill et al. 2007). Therefore, calculating the different trophic fractionation values is a useful tool for distinguishing different fish species. Estimates of trophic position are more sensitive to assumptions and different life history traits about the trophic fractionation of δ^{15}N, than to the isotopic baseline (Post 2002).

Trophic structure of the western Wadden Sea fish community still includes predatory fishes with a trophic position above 3.0 and maximum trophic positions are comparable to the trophic positions observed in other coastal European areas such as the Tagus estuary (Vinagre et al. 2012), where larger more pelagic species showed higher values than smaller benthic species. However, these values are lower than documented for coastal zones (see for instance Rodríguez-Graña et al. 2008). The absence of the highest trophic positions might be due to the loss of predatory species in the Wadden Sea. Whereas skates and sharks used to be common in the North Sea and surrounding coastal areas, nowadays they are either absent or occurring in low densities (Wolff 2005). Predatory shark and skate species had trophic positions (based on historical archive dietary data) in a range of 3.2 to 4.6 (Poiesz et al. 2021). Another explanation might be due to trophic downgrading, where food webs are losing complexity and trophic biodiversity due to changing environmental conditions (changing temperatures, eutrophication) and competition (Saleem 2015, Edwards & Konar 2020, Yan et al. 2020).

4.3. Trophic niche

For the Wadden Sea fish species, stable isotope values, both $\delta^{13}C$ and $\delta^{15}N$, did not vary significantly between spring and autumn. Some species showed a significant ($p<0.001$) increase (for $\delta^{13}C$: herring, sea trout and for $\delta^{15}N$: bass, bib, cod, plaice, sea trout, twaite shad) and some others showed a significant decrease with size [for $\delta^{13}C$: bass, whiting (*Merlangius merlangus*), sole (*Solea solea*) and for $\delta^{15}N$: herring, thick-lipped grey mullet]. For bass, these findings are in line with the significant relationship found by Cardoso et al. (2015).

Spring catches contain fish migrating from the North Sea into the Wadden Sea whilst autumn catches include the locally produced young-of-the-year (Fonds 1983). The absence of a difference in stable isotope values between spring and autumn suggests that the trophic niche of the various fish species in the coastal zone and inside the Wadden Sea is similar. Stomach content composition also did not differ with fish size or between spring and autumn (Poiesz et al. 2020).

The average stable isotope values for the Wadden Sea fish species cover a rather large range for $\delta^{13}C$ from -13‰ to -27‰ and for $\delta^{15}N$ from 13.5‰ to 18.5‰ and clearly differs among species, illustrating high trophic diversity in the area whereby various species occupy different niches. Trophic niche size (SEA; SEAc) was more or less similar for most of the Wadden Sea fish species, except for a few ones with a large variability. These species, flounder, thick-lipped grey mullet and golden grey mullet (diadromous) and sea trout (anadromous) are species which are tolerant to both sea water as well as fresh water during their life cycle) and hence have a large trophic niche size. Both the functional groups, benthic, benthopelagic, pelagic, as well as guilds NR, JMM and MSV showed to a large extent trophic niche overlap illustrating trophic competition (Dubois & Colombo 2014).

Trophic competition appears to be most visible for JMM (nursery-type species), mainly consisting of pelagic juvenile clupeid species and benthic juvenile flatfish species (van der Veer et al. 2015). This reflects the prey overlap in the diet, as also found in the stomach content analysis, whereby a few key prey species (amphipods, brown shrimps, juvenile herring and gobies) could be identified (Poiesz et al. 2020). Present information indicates that for juvenile flatfish, resource limitation does not seem to be an issue: growth during most of the summer is maximum and determined by water temperature conditions only (van der Veer et al. 2016). The same holds true for the abundant group of gobies (Freitas et al. 2011). Present growth conditions and competition in juvenile clupeid species in the Wadden Sea are unclear.

Data archive. Original data and R script for calculations can be found under https://dx.doi.org/10.25850/nioz/7b.b.bb.

Acknowledgements

Thanks to all of our colleagues, especially Rob Dapper, Ewout Adriaans, Willem Jongejan, Sieme Gieles and Marco Kortenhoeven, for assisting in the collection of the samples, and to Thomas Leerink, David Zaat and Vincent van Ernich for grinding, homogenizing and weighting of the stable isotope samples. All fish sampling and handling was done under CCD project number AVD8020020174165. Key prey species were collected within the framework of ZKO project 839.08.242 of the National Ocean and Coastal Research Programme (ZKO), supported by The Netherlands Organization for Scientific Research (NWO).

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