Deep dive into the Wadden Sea

S.S.H. de Fockert - Poiesz

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A diverse approach to better understand spatial and temporal variability in the Wadden Sea fish food web

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Uitnodiging

Voor het bijwonen van
de openbare verdediging
van mijn proefschrift

Deep dive into the Wadden Sea

Op dinsdag
23 september 2025 om
14:15 uur in de Aula in
het Academiegebouw,
Broerstraat 5 te Groningen

Na afloop van de promotie
bent u van harte welkom
op de receptie

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STELLINGEN

Behorende bij het proefschrift

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1. Uiteindelijk blijken er maar enkele prooisoorten te zijn die door (bijna) elke vissoort in de Waddenzee worden gegeten *(dit proefschrift)*.
2. Vergelijkbare isotoopsignalen van vissen die de Waddenzee ingaan in de voorjaarstrek in vergelijking met die de Waddenzee uit gaan in de najaarstrek, suggereren eenzelfde voedselweb in de kustzones van de Noordzee en in de Waddenzee *(dit proefschrift)*.
3. De Waddenzee kent vissoorten (haring en schol/bot en gobies) die spatieel gezien overal voorkomen en zeldzamere soorten (horsmakreel en botervis) die meer gebonden zijn aan specifieke hydrografische (vb. diepte en stromingsverschillen) en geomorfologische omstandigheden (vb.vorm van de kusten en type en vorming van de bodem) *(dit proefschrift)*.
4. Onderzoek wijst uit dat er een grote spatiele trofische homogeniteit heerst in de Waddenzee. De kleine verschillen die toch worden gevonden, komen door de af- of aanwezig zijn van zeldzame soorten als gevolg van lokale geografische of biologische verschillen *(dit proefschrift)*.
5. Het vroegere visvoedselweb in de Waddenzee was complexer doordat veel soorten nu zeldzamer zijn of ontbreken (haaien en roggen) *(dit proefschrift)*.
6. De trofische positie van individuele soorten in de westelijke Waddenzee is vrijwel onveranderd gebleven in de afgelopen 80 jaar *(dit proefschrift)*.
7. Stellingen zijn altijd onderhevig aan de tijd, ze kunnen keer op keer bevestigd worden of ze kunnen worden ontkracht.
8. Promoveren en uiteindelijk werken en het stichten van een gezin zorgt ervoor dat een introverte kluizenaar omgevormd wordt tot een 'alles onder controle hebbende' socialisator.

Suzanne S.H. de Fockert – Poiesz

23 september 2025

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NIOZ
Royal Netherlands
Institute for
Sea Research

Colofon

The research presented in this thesis was carried out at the Department of Coastal Systems (COS) at NIOZ Royal Netherlands Institute for Sea Research, Den Burg (Texel), The Netherlands.

The research was funded by the University of Groningen (RUG), Graduate School of Science and Engineering and facilitated by the NIOZ. The printing was supported by the University of Groningen (RUG).

The preferred citation for this thesis is:
Poiesz, S.S.H. (2025). Deep dive into the Wadden Sea: A diverse approach to better understand spatial and temporal variability in the Wadden Sea fish food web. PhD thesis, University of Groningen, Groningen, The Netherlands

Layout and design by Ryanne Keja | persoonlijkproefschrift.nl
Printed by Total Copy Service | Aalsmeer
Cover design by Suzanne de Fockert – Poiesz and Ryanne Keja

rijksuniversiteit
groningen

Deep dive into the Wadden Sea

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Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. ir. J.M.A. Scherpen
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 23 september 2025 om 14.30 uur

door

Suzanne Sophia Hendrike Poiesz
geboren op 8 december 1985

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General introduction

General Introduction

The Wadden Sea is a large estuarine tidal flat system along the mainland of the Netherlands, Germany and Denmark. A chain of barrier islands intersected by deep tidal channels separate the area from the adjacent North Sea. The tidal amplitude varies between 1,5 meters in the western part to about 4 meters in the eastern part. During low water, vast areas of tidal flats emerge leaving more than two third of the entire area exposed. Because of the various rivers that discharge into the Wadden Sea directly (Ijssel, Ems, Weser, Elbe) or indirectly, the salinity varies and fluctuates yielding brackish conditions (Wolff 1983).

The Wadden Sea has been and is under long-term anthropogenic influence, including large scale habitat transformations, exploitation pollution and eutrophication (Lotze 2005, Lotze et al. 2005, Lotze & Worm 2009). In the future, global warming, acidification and deoxygenation might become dominant factors (Bijma et al. 2013). Over time, the extinction or severe depletion of more than hundred plant and animal species have been documented (Lotze et al. 2005) and this will have affected the functioning of the area. Lotze et al. (2005) state that human impact has caused a simplification and homogenisation of the fish food web structure (Fig. 1) and ecosystem functioning of the Wadden Sea.

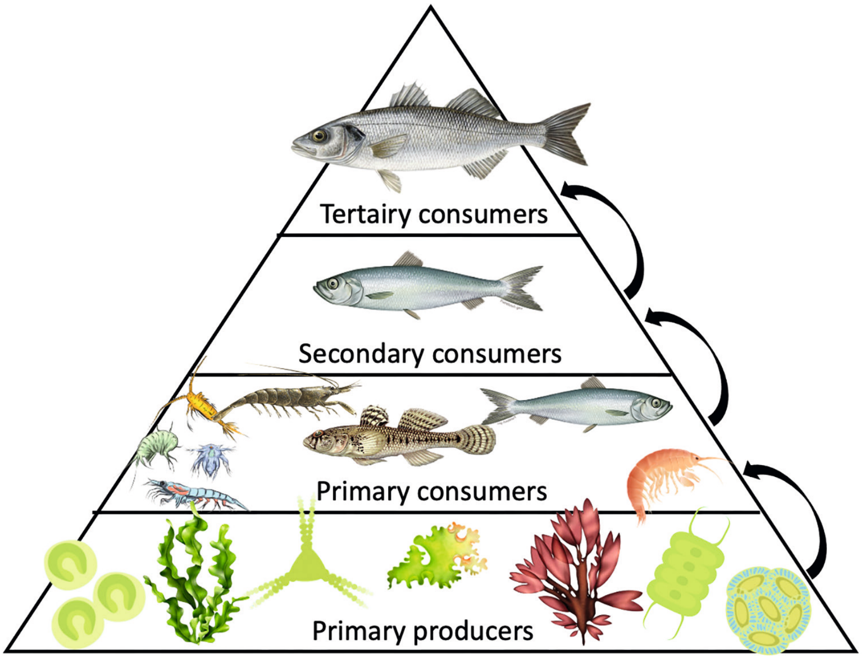

Figure 1 Simplified food web pyramid. Shown food web species represent predator-prey interactions found in the Wadden Sea.

One of the important components of the Wadden Sea ecosystem is its fish community as is illustrated by the long fishing history in the area, already dating back to passive fyke fishing in Roman times (Prummel 1999, Knottnerus 2001, Prummel & Heindrich 2005). During centuries the Wadden Sea was used extensively and fished with different passive and active gears targeting a variety of fish species such as eel *(Anguilla anguilla*), various flatfishes (*Pleuronectes platessa*, *Platichthys flesus*, *Solea solea, Limanda limanda*), herring (*Clupea harengus*) and anchovy (*Engraulis encrasicolus*) (see for instance de Jonge et al. 1993). Fishing and hunting has driven large animals such as the grey whale, sturgeon and salmon to extinction (Wolff 2000). After the cut off of the brackish Zuiderzee from the Wadden Sea in 1932, commercial fisheries in the Wadden Sea strongly decreased especially due to the disappearance of the Zuiderzee herring. Commercial fishing with passive gears ended after the second world war, mainly caused by a decreasing fish community in the area (Tulp et al. 2008, van der Veer et al. 2015). Nowadays, commercial fishery in the Wadden Sea is focussed mainly on invertebrates, in particular shrimp (*Crangon crangon*), mussels and the harvest for oysters (Lotze 2005).

There are indications that the Wadden Sea fish fauna has even changed and decreased over the last decades (Tulp et al. 2008, 2017, van der Veer et al. 2011, 2015). Despite this decrease, the Wadden Sea is still an important area for numerous fish species and many fish species rely on the Wadden Sea in at least one of their life stages (Zijlstra 1983, Tulp et al. 2017). Many species are near-resident and resident species and prefer or depend on the Wadden Sea during most of their life cycle or even during their whole life cycle). The Wadden Sea is also an important nursery area for a group of commercial and non-commercial fish species. Furthermore, various marine fish species visit the area seasonally either as juveniles or also as adults and various anadromous catadromous species still can be found.

For many fish species, the basic information concerning migration, feeding habits, physiological preference and tolerance and their role in the food web is lacking and largely unknown (Tulp et al. 2017). Only for some Wadden Sea individual fish species, detailed information about their food relationships is available (see for instance Kühl 1961, 1973, de Vlas 1979, Kühl & Kuipers 1983, del Norte-Campos & Temming 1994, Nijssen 2001). The Wadden Sea fish food web complex has only been described in a quantitative way by Kühl & Kuipers (1983), whereby they distinguished four different groups: the zooplankton feeders, the fish feeders, the zoobenthos feeders and the feeders on minute particles from the bottom. Since their description only one detailed analysis of the fish food web was published for the Sylt-Romo tidal basin in the German-Danish part of the Wadden Sea by Kellnreitner et al. (2012). Therefore, despite the fact that some data is available about the occurrence of certain fish species and their numbers in time, no detailed description of the current fish food web (at least not in the Dutch Wadden Sea) is available and little to no information is known about the past fish food web.

Historically, food web studies have been 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 it is labour intense and requires extensive taxonomic knowledge. Furthermore, only visible larger prey items can be identified and it offers only a small snapshot in time of recent prey items. Nowadays, food web studies are based on measurements of especially stable nitrogen isotope ($\delta^{15}N$) values and stable carbon isotope ($\delta^{13}C$) values (Minagawa & Wada 1984). Stable isotopes provide a more integrated signal of assimilated prey over a longer time period; whereby stable nitrogen isotope values increase with trophic position (Minagawa & Wada 1984) and carbon isotope ($\delta^{13}C$) values are an indication of different carbon sources (Hecky & Hesslein 1995). 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).

This study focusses on spatial and temporal variability in the Dutch Wadden Sea fish food web, whereby the aim is threefold:

[1] a detailed analysis of the present fish food web to fill in the gap of knowledge for the western Dutch Wadden;

[2] an analysis of the spatial variability in the Wadden Sea fish food web;

[3] an analysis of the temporal variability in the Wadden Sea fish food web, focussing on the last century.

General introduction

1

2

Thesis outline

Thesis outline

This thesis is structured into three parts. The first part (chapters 3 and 4) focuses on the **present western Wadden Sea fish food web structure** for the Marsdiep basin both by stomach content analyses and stable isotopes.

The second part (chapters 5 and 6) investigates **the spatial variability in the occurrence of Wadden Sea fish species** and in **Wadden Sea fish food web structure** based on environmental DNA and the comparison of both stomach content and stable isotope analysis of the western Wadden Sea (Marsdiep basin) and the eastern Wadden Sea (Ems basin).

The third part (chapter 7 and 8) deals with **temporal variability in Wadden Sea fish food web** focussing on information of predator-prey interactions of some fish species over the last century, including the historical trophic ecology of species that are presently extinct. This part will be based on the NIOZ archive of historical information of stomach content data of Wadden Sea fish species dating back to 1930 (de Vooys et al. 1991).

Part I: Western Wadden Sea fish food web structure

In **chapter 3** the food web structure of a coastal fish community (western Dutch Wadden Sea) was studied based on stomach content data from samples collected between 2010 and 2018. In total, 54 fish species were caught of different guilds (pelagic, benthopelagic or demersal/benthic) and functional groups (marine seasonal visitors, (near)residents or juvenile marine migrants). In total, 72 different prey items were identified. Results showed the pivotal position of a few key prey species (amphipod crustaceans, brown shrimps, juvenile herring and gobies) for the coastal Wadden Sea fishes and that the substantial prey overlap in the diet of some predators cannot exclude intra- and inter-specific competition among these predators.

Chapter 4 investigates the trophic structure of the western Wadden Sea fish community by means of 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 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. Results showed that the estimated trophic positions based on isotope values were lower than those based on stomach content composition, 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).

Part II: Spatial variability in Wadden Sea fish food web structure

Chapter 5 investigates the spatial variability in the fish community composition by means of monthly sampling of environmental DNA concentrations at eight stations spread out over the Dutch Wadden Sea from west to east. The number of fish species identified in the samples varied over time and among locations between three and 19 fish species. Species accumulation curves indicated that the number of samples were sufficient to identify all fish species at four locations. Total number of fish species identified varied from 19 to 34 between locations with highest numbers near the tidal inlets. Over the year, twenty species were identified at all locations; eight species were found at 6-7 locations and the remaining 30 species were found only incidentally. These results illustrated spatial variability in fish community in the Dutch Wadden Sea with common (core) species being present at all locations and differences between locations with respect to rare (transient) species, most probably due to location specific differences in hydrography and geomorphology.

In **chapter 6** the spatial variability in food web structure for the Wadden Sea fish community was investigated by simultaneously analysing stomach content and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in the Marsdiep and Ems basin in the Dutch Wadden Sea. 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.

Part III: Past Wadden Sea fish food web structure

Chapter 7 focusses on the historical trophic ecology of some divergent shark and skate species in the Dutch coastal North Sea zone. In this study historical dietary data of four species of sharks and skates being in the past (near)-residents, juvenile marine migrants and marine seasonal visitors of the Dutch coastal North Sea zone, were analysed for the period 1946 - 1954. Based on stomach content composition, the trophic position of four of the various shark and skate species could be reconstructed. The (near)-resident species, the lesser spotted dogfish, the marine juvenile migrant, the starry smooth hound, and the benthopelagic marine seasonal visitor, the thornback ray, had a benthic/demersal diet (polychaetes, molluscs and crustaceans), while the pelagic marine seasonal visitor, the tope shark, fed dominantly on cephalopods and fishes. Diet overlap occurred for fish (tope shark and lesser spotted dogfish), for hermit crabs (lesser spotted dogfish and starry smooth hound) and for shrimps (thornback ray and starry smooth hound). Trophic position ranged from 3.2 for thornback ray preying exclusively on crustaceans to 4.6 for the tope shark consuming higher trophic prey (crustaceans and fish). The analysis indicates that most of the shark and skate species were generalist predators. The calculated trophic positions of shark and skate species indicate that those species were not necessarily at the top of the marine ecosystem food web, but they might have been the top predators of their particular ecological assemblage.

Chapter 8 investigates the temporal variability in fish food web structure of the western Wadden Sea over almost the last century. Information about stomach content composition over the period 1930 – 2019 was analysed to reconstruct long-term trends in trophic position of individual species. For 18 fish species, all being omnivorous and belonging to different functional groups (pelagic, benthopelagic, demersal) and guilds [(near)-resident, juvenile marine migrants, marine seasonal visitors], prey consumption and trophic position over time could be analysed. Prey occurrence in the stomachs of different fish species showed variability over time, most likely due to fluctuations in prey abundance, but without a trend. For all species, individual fish showed variability in trophic position in the order of 1 unit or even more both within and between years. However, in all 18 species, no significant trend in mean trophic position over time could be found, despite the serious anthropogenic stress (pollution, eutrophication events, climate change) and the decrease in fish abundance in the area during the last 50 years. The present study does not indicate any changes in trophic position of individual species in the western Dutch Wadden Sea over the last 80 years. At the community level, trophic structure varies due to interannual fluctuations in species composition and year-to year fluctuations in the relative abundance of the various fish species. At the ecosystem level the trophic role of the fish community has been degraded due to the decrease in total fish biomass in the area.

PART I

Western Wadden Sea fish food web structure

3

Only a few key prey species fuel a temperate coastal fish food web

Poiesz SSH, Witte, JIJ, van der Veer HW (2020). Only a few key prey species fuel a temperate coastal fish food web. Marine Ecology Progress Series, 653, 153-166.

Keywords:
Food web structure, Wadden Sea, Stomach content analysis,
Fish community, Trophic position, Predator-prey interactions

Running title:
Coastal Wadden Sea food web structure

Abstract

The food web structure of a coastal fish community (western Dutch Wadden Sea) was studied based on stomach content data from samples collected from 2010 to 2018. In total 54 fish species were caught and 72 different prey items were identified. Fish species had consumed from a few up to more than 30 different prey species, suggesting the presence of both opportunistic and more specialized feeders. No significant differences between years nor switches in food source with fish size were found. The trophic positions of the Wadden Sea fish community ranged between 2.0 and 4.7, with most trophic positions above 3.0. In the past, (near)-resident species were the most abundant functional group in spring and juvenile marine migrants in autumn. Nowadays, all functional groups are present in almost similar but low abundances. The (near)-resident community consisted of about 20 species which especially were feeding on amphipod crustaceans, brown shrimps and juvenile herring. There was only a slight overlap in diet with the group of marine juvenile migrants (5 species of juvenile flatfishes and clupeids), whose preferred preys were copepods, polychaetes and brown shrimps. About 15 species of marine seasonal visitors showed an overlap in diet with both the (near)-resident and the marine juvenile migrant especially for brown shrimps and to a lesser extent herring and gobies. Our results illustrate (1) the pivotal position of a few key prey species for the coastal Wadden Sea fishes: amphipod crustaceans, brown shrimps, juvenile herring and gobies, and that (2) the substantial prey overlap in the diet of some predators cannot exclude some intra- and inter-specific competition for some predators.

1. Introduction

Temperate coastal zones are dynamic areas, experiencing fluctuations in temperature and salinity on short (tidal) to medium (seasonal) time scales. Since only few species can cope with these rapidly changing conditions of especially temperature and salinity, species diversity in these transition zones between the marine offshore and the freshwater inner zone is relatively low (Levin et al. 2001). Temperate coastal zones are also highly productive ecosystems because of their inputs of nutrients and organic matter from river runoff and the neighbouring open sea (Nixon 1995, Cloern et al. 2014). Consequently, those species present can occur in high numbers [see for the European coast for instance Gibson (1994), Freitas et al. (2007, 2010), Jung et al. (2017)] and thereby also attracting large numbers of predators. As such, coastal zones are important foraging areas/grounds for a variety of fish, bird and marine mammal species [see for instance Goodall (1983)].

One such coastal area is the temperate Wadden Sea, an estuarine area bordering the Dutch, German and Danish North Sea coast, an important nursery area for a variety of fish species (Zijlstra 1972) and also a resting and feeding area for wading birds (Wolff 1983). Over the last decades, the trophic structure of the coastal zone fish fauna has changed and the nursery function of the Wadden Sea for flatfish juveniles has decreased (Tulp et al. 2008, 2017, van der Veer et al. 2011, 2015). A detailed analysis of the present functioning of the Wadden Sea for the various fish species would require detailed information about the various predator-prey relationships. Although some information is available (see for instance Kühl 1961, 1973, de Vlas 1979, Kühl & Kuipers 1983, Norte-Campos & Temming 1994, Nijssen 2001, Kellnreitner et al. 2012), a comprehensive, detailed analysis of the trophic structure of the coastal Dutch Wadden Sea fish community is still lacking.

Taxonomic identification of prey items using stomach content analysis has been (Hynes 1950) and is still an important tool for the analysis of predator-prey interactions, however it only offers a small temporal snapshot of recent prey items only. Nevertheless, all historical data is based on stomach content identification, therefore any comparison with previous work requires the same methodology. For these reasons, stomach content analysis is still a tool to provide an overview of the most important food web components and predator-prey relationships.

This study elaborates on previous stomach content studies of Wadden Sea fish and analyses the complete fish community of the western Dutch Wadden Sea collected in 2010-2018 with a focus on the competitive interactions between the most important functional groups. In addition to (near)-resident species, present year-round and spending (almost) their entire live-cycle in the area, the study also encompasses marine juvenile migrants using the area as a nursery and marine seasonal (summer of winter) visitors or users visiting the area as adults. Furthermore, marine adventitious visitors, which appearing irregularly, diadromous (catadromous or anadromous) migrant species and freshwater adventitious species, which occasionally enter brackish waters are also found (Zijlstra 1983, Elliott & Dewailly 1995).

In this study, the role and impact of (near)-resident fish species is compared with that of marine juvenile migrants and marine seasonal (summer of winter) visitors. Firstly, the trophic structure of the fish community will be described based on stomach content information in relation to fish size (or age) following FishBase (Froese & Pauly 2019). Subsequently, the food web structure (trophic position, predator-prey relationships, prey overlap) of the (near)-resident species is determined. Next, the food web structure for the marine juvenile migrants and marine seasonal visitors is constructed and the extent of overlap and interaction with that of the (near)-resident species analysed.

Our analysis is based on a long-term monitoring programme of the fish fauna in the western part of the Dutch Wadden Sea by means of fyke nets. The programme started in 1960 and has continued without methodological change until now (van der Veer et al. 2015). Previous papers dealt with long-term patterns in fish abundance and phenology (van der Veer et al. 2015, Cardoso et al. 2015, van Walraven et al. 2017). This study focusses on present food web structure.

2. Materials and methods

2.1. Field sampling

Fish were collected from the catches of a long-term monitoring programme 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 fyke. The kom-fyke was emptied every day, weather permitting. During the winter (November-March) and summer (July-August) months the kom-fyke was removed due to the risk of potential damage by storm and ice in winter and extreme algal blooms and high numbers of jellyfish during summer. For more information see van der Veer et al. (2015).

All fish caught were taken to the laboratory and sorted within an hour, identified up to species level, counted and their length measured. During 2010 to 2018, a maximum of three individuals per species per week (Monday – Sunday) were selected and stored at -20°C for further stomach content analysis.

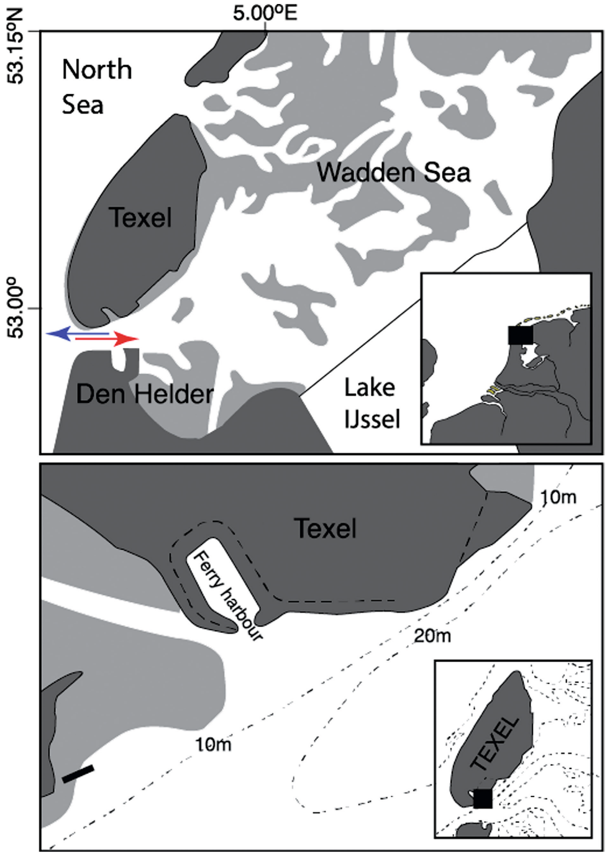

Figure 1 Sampling location near the island of Texel. Top panel: western Dutch Wadden Sea black box); red arrow indicates inwards migration in spring and blue arrow outward migration in autumn. Lower panel: fyke net position (black bar). Grey: intertidal areas.

2.2. Fish abundance

All daily fyke catches for the period 1980 - 2018 were included for the months April-June and September-October, except those with a fishing duration less than 12 h (exclusion of 0.1% of the records), or more than 48 h (6.6% of the records), or when the gear was damaged or seriously clogged with debris (0.3% of the records).

For each catch, numbers per species were determined. Next, weekly (Monday – Sunday) and monthly average numbers caught per fishing day were determined. Finally, mean average catch in spring (April – June) and autumn (September – October) was estimated.

2.3. Stomach content analysis

Within a few weeks of capture, fish selected for dissection were defrosted and total length, fork length, standard length, frozen weight, gonad weight, sex and ripeness were determined. In addition, the sagittal otoliths were removed for age determination. Stomach content was analysed in a petri dish under a binocular (20x). For each individual fish, the stomach content was weighted (wet mass; g) and the prey items were identified up to species level or if not possible, up to a higher classification (class, order, genus). Also, if possible, total length of the prey was measured (mm). Incomplete specimens, often from species that were eaten in pieces such as *Alitta virens* or *Ensis leei*, were counted. For each prey item percentage of occurrence was calculated (= number of stomachs containing a prey species divided by total number of stomachs examined) as measure of diet composition following Baker et al. (2014). Taxonomic identification was based on an internal reference collection and Hayward & Ryland (2017) for polychaetes, bivalves and crabs and Wheeler (1978) for fish species.

2.4. Data analysis

Functional groups were assigned to all predatory fish species in relation to their use of the Wadden Sea in line with previous work (van der Veer et al.2015). These were as follows: pelagic (occurring mainly in the water column, not feeding on benthic organisms); benthopelagic (living and/or feeding on or near the bottom, as well as in midwater) and benthic (living and/or feeding on the bottom), see also FishBase Froese & Pauly 2019). Furthermore, species were classified according to their use of the Wadden Sea area [(near)-resident species, marine juvenile migrants, marine seasonal visitors] based on Witte & Zijlstra (1983), also in line with van der Veer et al. (2015). *Dicentrarchus labrax* (bass) was considered to have become a resident species in the Wadden Sea in recent time, due to the presence of small juveniles and adults almost year-round (Cardoso et al. 2015).

For each individual fish and therefore each unique stomach *j*, the trophic position (TP_j) was not taken directly from FishBase, but calculated from their diet compositions based on the fixed trophic positions of prey items and the procedure from FishBase (Froese & Pauly 2019) with a slight modification to compensate for digestion, including the following steps:

Step 1: If all food items were plants or detritus (TP=1): then trophic position of the predator $TP_j = 1 + 1 = 2$ and standard error (s.e.) = 0;

Step 2: In case there was only one food item in the stomach, which was neither a plant nor detritus then: $TP_j = 1 +$ the trophic position TP of food item according to FishBase and s.e. = s.e. of the food according to FishBase;

Step 3: If there were several food items, and at least one was not a plant or detritus, then trophic position was determined based on the relative contributions of each prey to the total diet. To eliminate the effect of the state of digestion on the calculation, the relative contribution of the various prey item to the

> total diet was determined on the basis of back-calculated consumed fresh biomass, reconstructed by means of length-weight relationships. When no length measurement was available (often small prey items), a mean wet mass was taken. The weighted average of the trophic positions of the various food items was considered to represent the trophic position of the prey. The trophic position of the predator was estimated according to:
>
> TP_j = 1 + mean weighted trophic position of all food items inside the stomach.

For all species with at least 2 stomach contents analyses, mean trophic position was calculated, and for all species with at least 10 stomach contents analyses, prey occurrence was estimated.

All computations and analyses were done in R (R Core Team 2019). The graphics were made using the ggplot package (Wickham 2009).

3. Results

3.1. Fish abundance

Mean fish abundance of the different functional groups is shown in Fig 2. All three groups showed a decrease in time both in spring and in autumn. The decrease was clearer in spring and for the (near)-resident and the marine juvenile migrants.

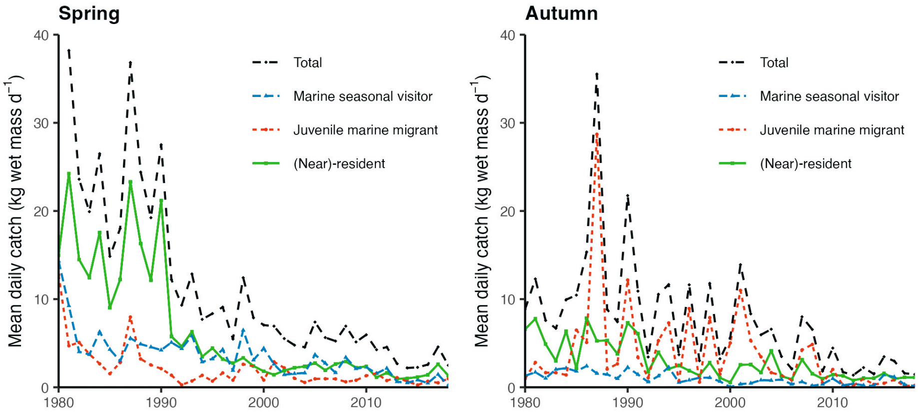

Figure 2 Mean daily fyke catch (kg wet mass d^{-1}), total and for the different groups in spring (left panel) and autumn (right panel).

In the 1980s, (near)-resident species were the most abundant functional group in spring. From the mid 1990s onwards, abundance of the three groups was low and more or less similar. In autumn, marine juvenile migrants were the most dominant group until about 2010. Hereafter, all functional groups were present in similar, but low, numbers.

3.2. Fish community

Over the period 2010 – 2018, 54 different fish species were caught and 74 different prey items were identified (Supplementary materials Table S1). Mean trophic position could be calculated for 51 species and prey composition and occurrence for 41 species.

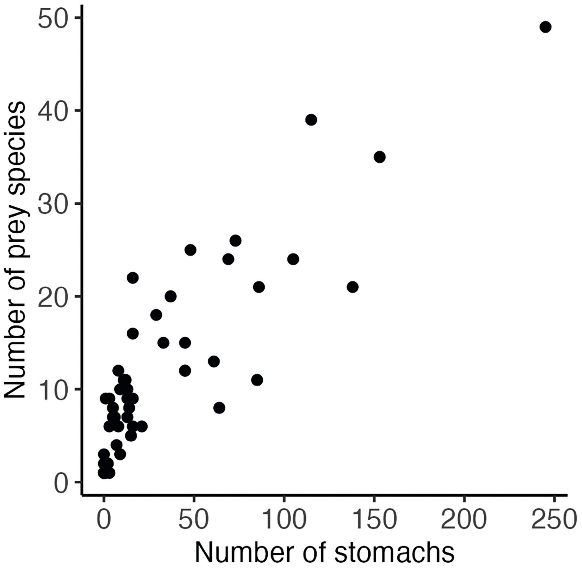

Figure 3 Number of stomachs analyzed for each fish species compared in relation to the total number of different prey species found inside those stomachs. All years combined (2010 – 2018).

Number of prey species found in the stomachs showed an increase with number of stomachs analysed, at least for up to 50 stomachs. Hereafter, the pattern was more variable (Fig 3). All fishes were preying on multiple species, mostly varying between 3 and 10 to up to more than 30 species, indicating that most fishes were opportunistic feeders.

For most species, number of observations and/or size range was too low to analyse any relationship of trophic position (TP) with fish size (Fig 4). In some species a slight positive [*Dicentrarchus labrax* (bass), *Clupea harengus* (herring), *Scophthalmus rhombus* (brill)] or negative [*Belone belone* (garfish), *Microstomus kitt* (lemon sole), *Chelon ramada* (thin-lipped grey mullet)] trend between TP and fish size could be observed, however these relationships were statistically not significant (linear regressions: $p > 0.05$).

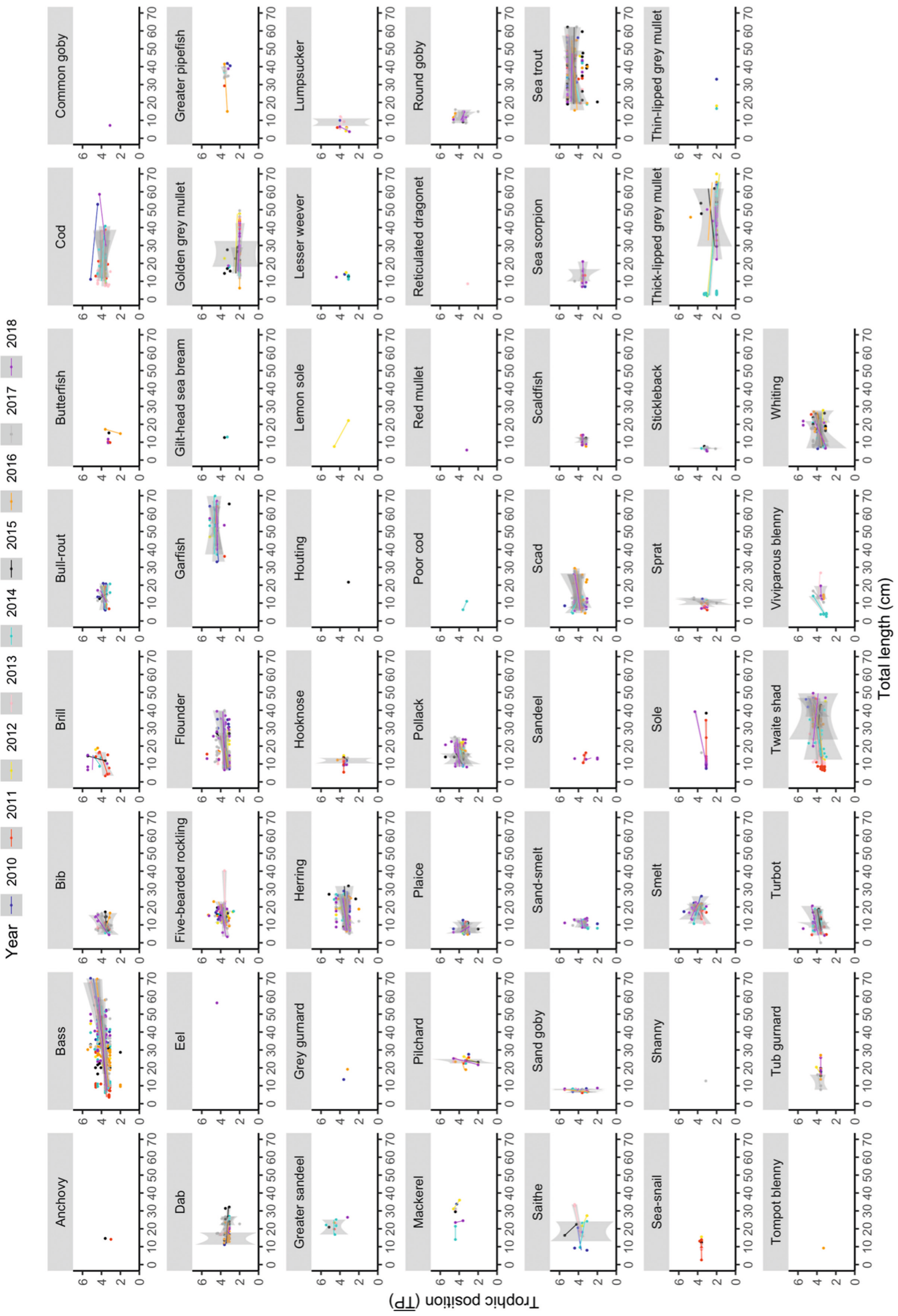

Figure 4 Calculated trophic positions ($\overline{TP}$) based on the stomach content as a function of the total length for each predatory species. A linear regression with a 95% confidence interval for each year (2010 –2018) is added to visualize trends.

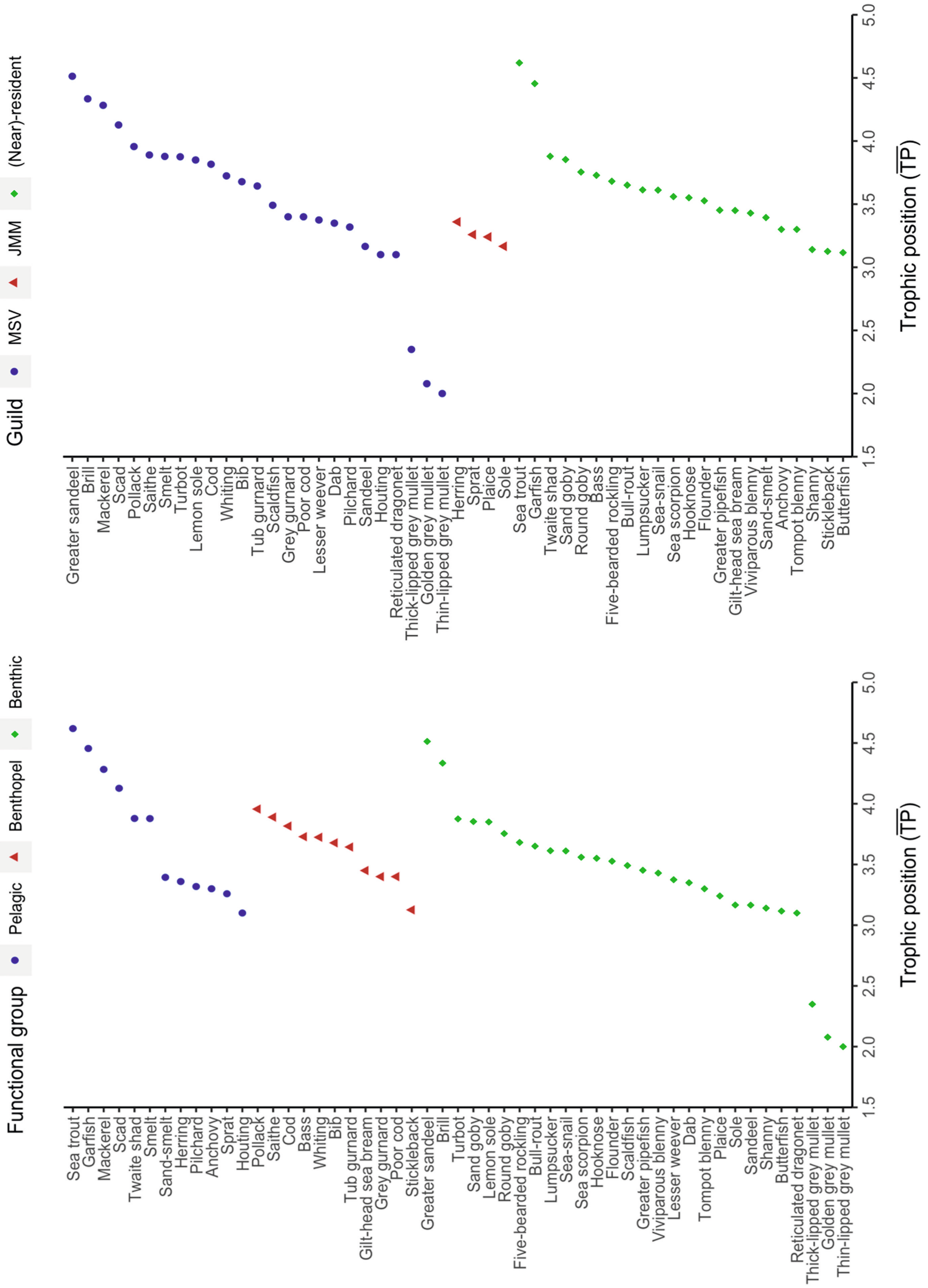

Figure 5 Trophic position ($\overline{TP}$) of the fish species based on stomach content analysis. divided into the functional groups (left panel. with blue: pelagic. green: benthopelagic and brown: demersal/benthopelagic species) and into species guild (right panel. blue: marine seasonal visitors (MSV). Green: (near)-residents and brown: juvenile marine migrants (JMM). Species are listed from lowest TP to highest TP.

The mean TP of the fish community ranged between 2.0 and 4.7 with most trophic positions above 3.0 (Fig 5). Low values (<3.0) were found for the mullet species [*Chelon ramada* (thin-lipped grey mullet), *Chelon aurata* (golden grey mullet) and *Chelon labrosus* (thick-lipped grey mullet)]. The TP of *Trachurus trachurus* (scad), *Scomber scombrus* (mackerel), *Belone belone* (garfish), *Scophthalmus rhombus* (brill), *Salmo trutta* (sea trout) and *Hyperoplus lanceolatus* (greater sandeel) were above 4.0. The marine seasonal visitors showed the largest range of trophic positions and the marine juvenile migrants the smallest (Fig 5).

There was no 1:1 relationship between the mean TP of the fish species in FishBase and the calculated TP based on stomach contents (Fig 6).

3.3. Differences between functional groups

(Near)-resident species varied in trophic position from 3.2 to 4.7. Their food ranged from copepods to fish species, with a dominance of prey species with a higher TP (Fig 7). Whereas the diet of some species consisted of a variety of prey items, for a number of species (multiple) preferred prey items -defined as items with an occurrence in the stomachs- of > 25%- could be identified and a number of prey it ems occurred in the stomachs with a presence of 50% or more.

Gasterosteus aculeatus (stickleback) preferred copepods; *Zoarces viviparus* (viviparous blenny): copepods and sand hoppers; *Pholis gunnellus* (butterfish): sand hoppers; *Agonus cataphractus* (hooknose) and *Liparis liparis* (sea snail): shrimps (> 50%); *Dicentrarchus labrax* (bass): shrimps and *Clupea harengus* (herring); sea scorpion: shore crabs (>50%) and shrimps; bull-rout: shrimps (>50%); flounder: shrimps; greater pipefish: shrimps (>50%); five bearded rockling: shrimps (>50%); gobies: shrimps and fish (>50%); twaite shad: shrimps and *Clupea harengus* (herring),; garfish: herring (>50%) and fish and sea trout: *Clupea harengus* (herring), and sandeel (>50%).

There was a large overlap in prey species consumed by the various (near)-resident species, with a few prey items having a high occurrence (sand hoppers, brown shrimps and juvenile herring) in the stomachs of different fish species , however for a large number of prey items their occurrence in the stomachs was low (Fig 7).

Marine juvenile migrants consisted mainly of flatfish species and clupeids and they had a trophic position between 3.2 and 3.4 (Fig 7). Marine juvenile migrants also preyed upon a variety of prey items, most of them in low occurrence in the stomachs. Herring were cannibalistic. For herring and sprat, copepods were a preferred prey, for plaice and sole it was polychaetes. All species consumed brown shrimps. For these three prey species, overlap in diet occurred between marine juvenile migrants. With (near)-resident species, overlap in diet occurred for copepods, sand hoppers, brown shrimps and herring.

Marine seasonal visitors consisted of a variety of species with a trophic position between 2.4 and 4.7 (Fig 7). Most marine seasonal visitors also preyed on multiple prey items, mostly with a low frequency of occurrence. For most marine seasonal visitors, brown shrimps and herring were preferred prey items. Furthermore, sandeel preferred Mysidae; dab: Atlantic jackknife clam and shore crabs; lesser weever: Atlantic jackknife clam; scaldfish: Mysidae; turbot: sand goby; brill: sand- and common goby and greater sandeel preferred fishes. Overlap in diet with (near)-resident and marine juvenile migrant species occurred mainly for brown shrimps and to a lesser extent for herring and fish.

A Principal Component Analysis (PCA) was used to visualize the differences and similarities between the various fish species with respect to the main prey items. The relation between the predatory fish species and prey was based on the average prey biomass found inside the stomachs of the predators all years combined. The PCA illustrated the clustering around algae, copepods, polychaetes, brown shrimps and herring as main prey items (Fig 8).

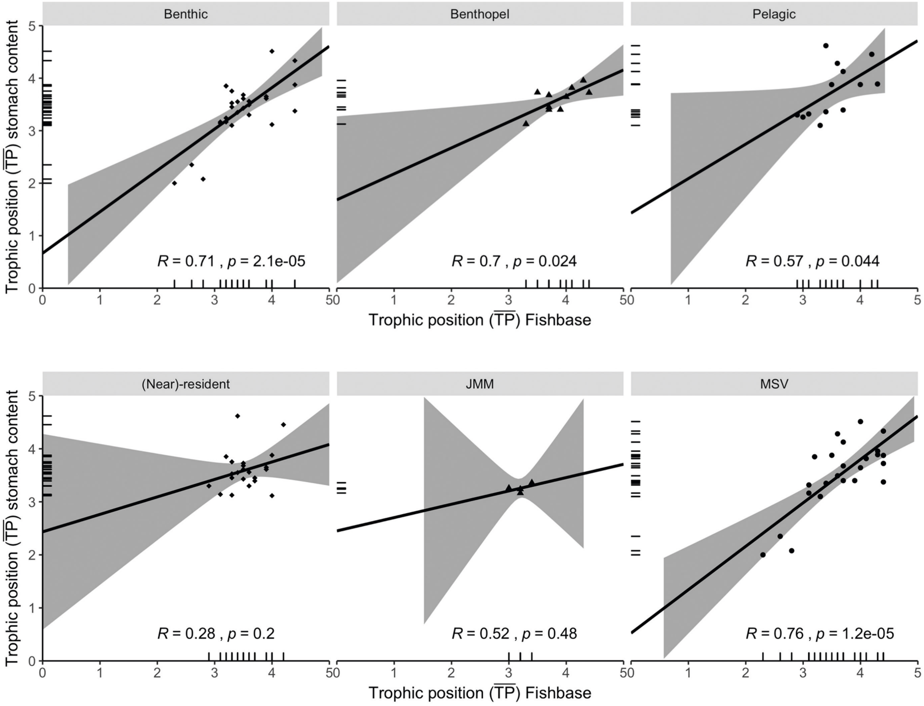

Figure 6 Linear relationship with 95% confidence interval between trophic position ($\overline{TP}$) from FishBase and the trophic position ($\overline{TP}$) based on the stomach content analysis. Upper panel for the various functional group. Lower panel for the various guild.

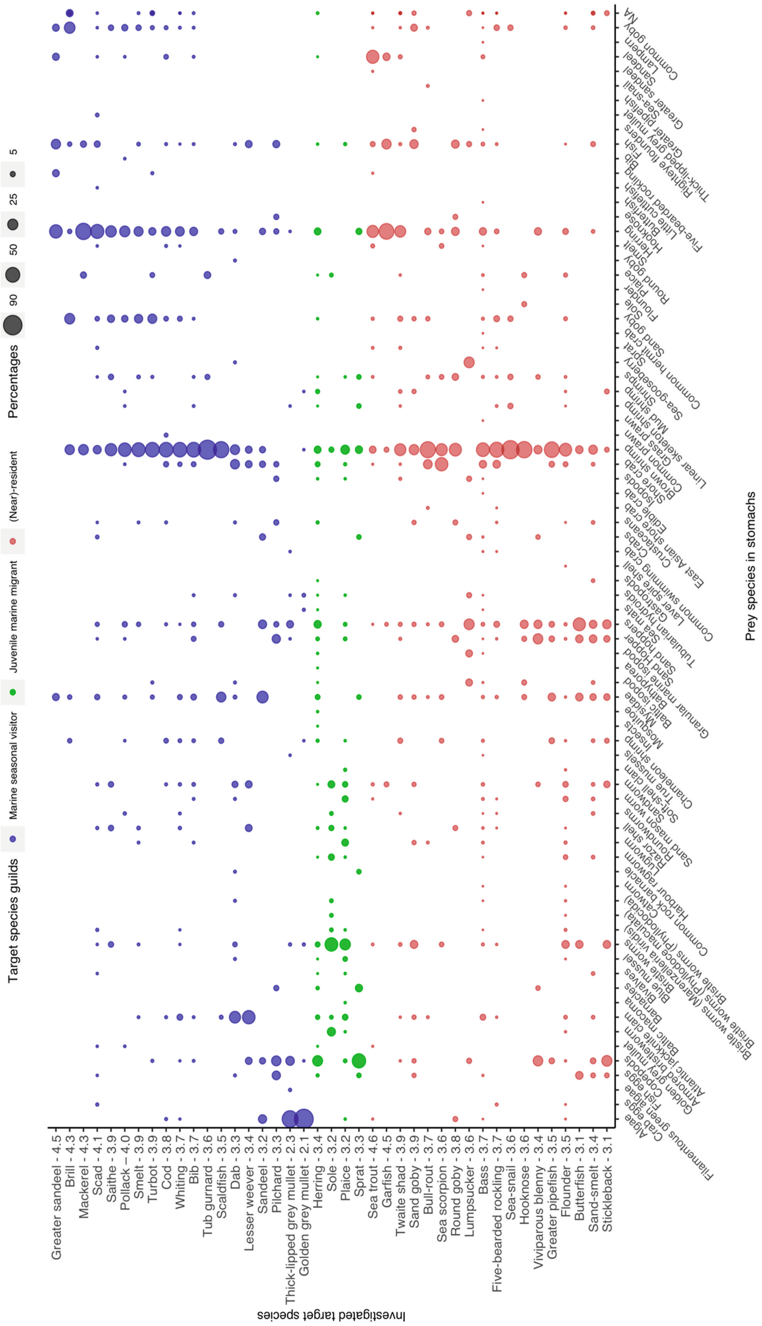

Figure 7 Occurrence of prey species (%) in the stomach of fish species in the years 2010 – 2018 together with corresponding calculated trophic position (TP) and guild. Predatory fish species are listed from guild type and lowest TP to highest TP and prey species are listen from lowest TP to highest TP.

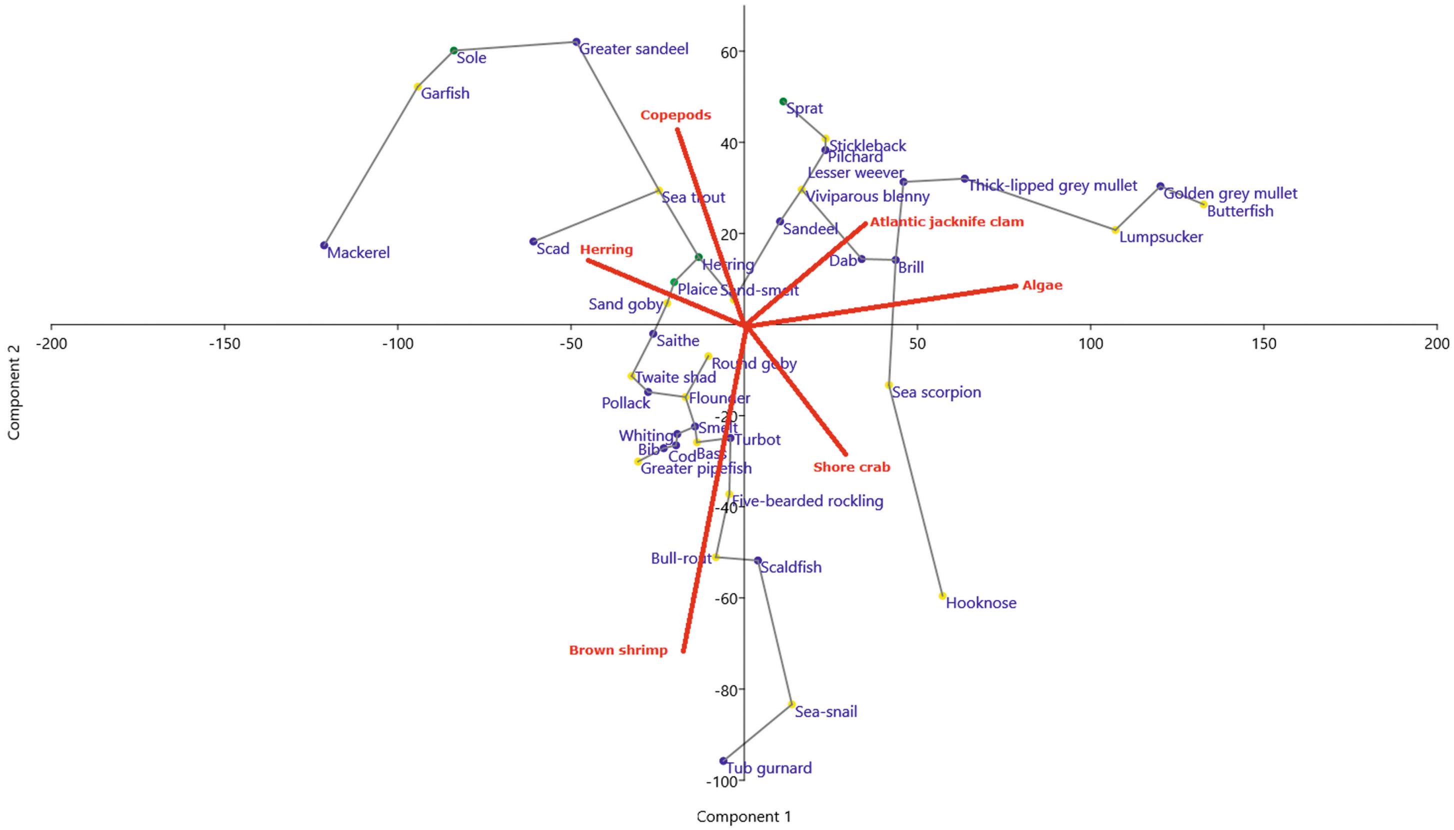

Figure 8 Principal Component Analysis (PCA) per species for each guild based on the stomach content. Yellow: (near)-residents. blue: marine seasonal visitor and green: juvenile marine migrant. The PCA illustrates the clustering around algae, copepods, brown shrimps and herring.

4. Discussion

Food web analysis requires a spatial and temporal sampling of the important food at the appropriate spatial and temporal scales. Ideally it would combine different sampling gears in various habitats and locations over a number of years. The sampling design in this study is limited to a single gear at a single spot. Nevertheless, the large number of species caught by the kom-fyke (54) is comparable to

Kellnreitner et al. (2012) in the Sylt-Rømø bight, Germany (43). Over the time period, 1960 – 2015, 82 fish species were caught by the kom-fyke (van der Veer et al. 2015), indicating that in this study some species will be missed and some others are caught in low numbers.

Sampling was performed during the period of fish immigration in spring and emigration in autumn only. Although no differences between spring and autumn were found, it cannot be guaranteed that this does not hold true for the summer and winter period as has been found by Kellnreitner et al. (2012) in the German Wadden Sea. The large number of (near)-resident, marine juvenile migrants and marine seasonal visitors caught belong to different functional groups (pelagic, benthopelagic, demersal) indicating that they represent different habitats. Furthermore, the predator-prey relationships found in this study corresponded with the general food relationships found for Wadden Sea fishes in the past as summarized by Kühl & Kuipers (1983) and recently in the Sylt-Rømø bight, Germany by Kellnreitner et al. (2012) suggesting that the results of this study might be applicable for a larger area than the western Dutch Wadden Sea only.

All species analysed consumed a variety of prey items. However, taxonomic identification of prey items via stomach content analysis only offers a small snapshot in time as it details only recently ingested prey items, while regurgitation and digestion are factors that result in missing or overlooking prey items. While our extended period of sampling may have partly overcome these limitations, the relationship between number of stomachs analysed and number of prey species found in the stomachs does not seem to level off below 50 stomachs, indicating that for the rare species or for species having a very wide diet, insufficient stomachs may have been sampled to cover all possible prey species (Mulas et al. 2015).

4.1. Trophic structure

This study shows that the trophic information provided by FishBase allows an impression of the trophic structure of a fish community: information from FishBase correlates significantly with estimates based on stomach content composition. However, for the Dutch Wadden Sea, except for the marine seasonal visitors, the information from FishBase gave an underestimation of the trophic positions calculated from the stomach contents. This might be caused by differences in size or age between this study and the reference values of FishBase given that there is a positive relationship between fish size and trophic position (see for instance Ursin 1973). These relationships have also been observed in other studies such as growing juvenile cod and plaice (Daan 1973, Kuipers 1977). No significant relationships between fish size and trophic position were found in this study, but the size and age of the (near)-resident species and marine juvenile migrants is relatively small and only marine seasonal visitors include larger more adult fish (van der Veer et al. 2015, van Walraven et al. 2017). Another factor might be area-specific differences in feeding pattern or in contribution of different items to the diet for both (near)-resident species and marine juvenile migrants. For instance, *Pomatischistus minutus*, a (near)-resident species, mainly consumes *Corophium volutator* in Swedish bays (Pihl 1985) and small *Crangon crangon* in the Dutch Wadden Sea (Kühl & Kuipers 1983). Also, for marine juvenile migrants differences occur. For 0-group plaice an important contribution of *Corophium volutator* to the diet was found in Swedish bays (Pihl 1985), while at the Balgzand intertidal in the Dutch Wadden Sea tail-tips and bivalve siphons were the most important components of the diet (de Vlas 1979).

In terms of species and abundance, the main components of the coastal Wadden Sea fish fauna are (near)-resident species, marine juvenile migrants and marine seasonal visitors. The trophic position of the (near)-resident species covers a range from 3.2 to 4.7, meaning that these (near)-resident species are more secondary consumers (carnivores) and tertiary consumers (carnivores consuming other carnivores). The marine juvenile migrants cover a narrow range in trophic position from 3.2 to 3.4, reflecting that these juvenile fish are not tertiary consumers. Marine seasonal visitors cover the largest range from 2.4 to 4.7. They include herbivore species up to tertiary consumers.

The trophic positions of the present fish community illustrate that the trophic structure still covers the various levels up to tertiary consumers, despite the disappearance of species such as some skates and sharks (Wolff 2000, Lotze 2007). The observed degradation of trophic structure and nursery function over the last decades (van der Veer et al. 2015) might not be through loss of trophic positions but rather strong reductions in abundance of a various trophic positions due to the great decrease in abundance (Tulp et al. 2008, van der Veer et al. 2015). The importance of the Wadden Sea as a nursery area (Zijlstra 1972) is still observed and reflected in the increased catches of marine juvenile migrants in autumn compared to spring.

4.2. Food web structure

Stomach content analysis shows that all species are consuming multiple preys indicating opportunistic feeding. On the other hand, for most species preferred prey items could be identified suggesting at least some kind of specialization. Cluster analysis of the stomach content support this, with clustering around algae, copepods, polychaetes, brown shrimps and herring. This clustering was also partly found in the German

Wadden Sea (Kellnreitner et al. 2012) and corresponds with the classification of Wadden Sea fish by Kühl & Kuipers (1983) into [1] feeders on minute particles from the bottom; [2] zooplankton feeders; [3] zoobenthos feeders and [4] fish feeders.

Notably, (near)-resident species and marine seasonal visitors show dietary overlap in prey items, indicating that for some predators intra- and inter-specific competition cannot be excluded. The decrease in fish abundance over the last decades (Tulp et al. 2008, van der Veer et al. 2015, this study) suggests that competition will be less likely nowadays than in the past.

Marine juvenile migrants appear to have their own niche: the clupeids prey mainly on copepods while juvenile flatfishes prefer polychaetes. In terms of energy, the nursery function of the areas is mainly a conversion of energy: the energy influx of the massive amounts of marine juvenile migrant larvae is in the same order of magnitude as the energy export of larger juveniles at the end of the growing season (Wolff 1980).

In the Dutch Wadden Sea, juvenile herring and brown shrimps are abundant and are the most preferred prey items by many fish species and thereby form important links in the fish food web. Juvenile herring form a link of the plankton to the secondary consumers by their consumption of copepods (Last 1989); while brown shrimps by their consumption of (epi)benthic prey items (Wolff & Zijlstra 1983, Pihl & Rosenberg 1984) link the benthos to the secondary consumers. Copepods, brown shrimps and mysid shrimps were also the most abundant prey items of the fish in the Sylt-Rømø bight, Germany (Kellnreitner et al. 2012), indicating that at a large geographic scale, key prey items for the fish community are the same, however their contribution might vary due to differences in absolute and relative prey and predator abundance.

4.3. Top down or bottom-up control

Similar to other estuarine food webs, the Wadden Sea food web is supported by local pelagic and benthic primary production, as well as import of dead organic matter from the open sea and freshwater discharges [see de Jonge & Postma (1974), Kuipers et al. (1981), de Wilde & Beukema (1984), de Jonge (1990)]. In the Dutch part, benthic primary producers (micro-phytobenthos) are the most important energy source for the majority of consumers of the food web, but in line with Deegan & Garritt (1997), large spatial heterogeneity was observed (Christianen et al. 2017). Recently, Jung et al. (2019) highlighted the important role of the influx of freshwater carbon as energy source, indicating that the importance of the various energy sources might vary spatially as well as temporally.

There has long been discussion as to whether trophic control in these continental shelf ecosystems is bottom-up (resource-driven) or also top-down (consumer driven). Jones (1989) has argued that in the past before exploitation started in general fish populations might have been determined by resource limitation. Anecdotic information indicates that fish biomass in the Wadden Sea has been substantially higher in the past even allowing a community of fisherman a living with passive fyke nets until it came to an end in about the 1960-ties due to decreasing catches. This did not stop a further decrease in fish abundance in the area especially from the 1980ties onwards (Tulp et al. 2008, 2017, van der Veer et al. 2015). It is therefore questionable that at present the trophic control of the fish community in the temperate coastal Wadden Sea would be bottom-up (resource-driven).

Frank et al. (2007) provided evidence that the type of trophic forcing might be strongly correlated with species richness and temperature, whereby very-cold and species-poor areas might succumb to top-down control. Although only a few fish species are abundant, species richness in the Wadden Sea is still substantial with about 100 different species being recorded (Witte & Zijlstra 1983). On the other hand, however, species abundance has seriously declined over the last decades (Tulp et al. 2008, 2017, van der Veer et al. 2015). Furthermore, the area is situated in the temperate zone and temperatures are not notably low. Also, the fact that most Wadden Sea fish species are not highly specialized predators but rather opportunistic feeders make resource limitation less likely. On the other hand, resource limitation might be an issue since the Wadden Sea fish food web structure relies on a few abundant species only, especially juvenile herring and brown shrimps that are the most preferred prey items by many fish species.

Various methods have been suggested to analyse bottom-up control, such as the proportion of prey production that is consumed by their predators (Evans 1984), per capita population growth rate in relation to the population density of a habitat in line with MacCall's theoretical basin model (McCall 1990) in the form of metabolic biomass (van der Veer et al. 2000), applying self-thinning (Nash et al. 2007), and the analysis of the growth potential (van der Veer & Witte 1993, Freitas et al. 2007). Most studies have been conducted on demersal fish, with partially contradictory results. Recently, Chevillot et al. (2019) concluded based on an Ecopath modelling exercise that the Gironde estuary reached its trophic carrying capacity with resource limitation for demersal fish. Also, Day et al. (2020) and Saulnier et al. (2020) suggested the occurrence of trophic limitation for marine juvenile migrants based on estimates of benthic production. On the other hand, a detailed seasonal growth analysis for a (near)-resident species in the Wadden Sea, the sand goby *Pomatoschistus minutus*, indicated that growth was not food-limited (Freitas et al. 2011), suggesting the absence of such a bottom-up control. For some marine juvenile migrants, juvenile flatfishes, both van der Veer et al. (2000) and Nash et al. (2007) concluded that these populations rarely approached the carrying capacity of the nursery grounds. To what extent this holds also true for pelagic marine juvenile species (herring, sprat) and (near)-resident is unclear. It cannot be excluded that control is not linked to latitude (temperature) as suggested by Frank et al. (2007) but also on feeding guild, whereby especially zooplankton feeders such as herring (marine juvenile migrants) and fish feeders (such as much of the marine seasonal visitors) are more sensitive for bottom-up control.

A food web that depends on a on a few abundant species might be a characteristic of temperate coastal areas in general: these are highly productive systems due to nutrient and organic matter input (Nixon 1995, Cloern et al. 2014) and only a few species can cope with their rapidly changing abiotic conditions (Levin et al. 2001). This is in line with the observation by Rice (1995) that in many marine food webs, particularly in boreal and subboreal areas, a single taxon in a middle trophic position passes most of the energy to higher predators.

Acknowledgements

Thanks are due to all our colleagues who assisted in the collection and analyses of the samples, especially Rob Dapper, Ewout Adriaans, Willem Jongejan, Sieme Gieles and Marco Kortenhoeven. Nelleke Barten-Krijgsman assisted in the final preparation of the figures. All fish sampling and handling was done under CCD project number: AVD8020020174165.

Supplementary materials

Table S1 General information of species analysed.

Species	SpeciesLatin	TP calculated by stomach content	TP according to FishBase	Functional group	Guild
Anchovy	*Engraulis encrasicolus*	3,3	2,9	Pelagic	(Near)-resident
Bass	*Dicentrarchus labrax*	3,7	3,5	Benthic	(Near)-resident
Bib	*Trisopterus luscus*	3,7	3,7	Benthopelagicpelagic	MSV
Brill	*Scophthalmus rhombus*	4,3	4,4	Benthic	MSV
Bull-rout	*Myoxocephalus scorpius*	3,7	3,9	Benthic	(Near)-resident
Butterfish	*Pholis gunellus*	3,1	4	Benthic	(Near)-resident
Cod	*Gadus morhua*	3,8	4,1	Benthopelagic	MSV
Dab	*Limanda limanda*	3,3	3,4	Benthic	MSV
Five-bearded rockling	*Ciliata mustela*	3,7	3,5	Benthic	(Near)-resident
Flounder	*Platichthys flesus*	3,5	3,3	Benthic	(Near)-resident
Garfish	*Belone belone*	4,5	4,2	Pelagic	(Near)-resident
Gilt-head sea bream	*Sparus aurata*	3,5	3,7	Benthopelagic	(Near)-resident
Golden grey mullet	*Chelon aurata*	2,1	2,8	Benthic	MSV
Greater pipefish	*Syngnathus acus*	3,5	3,3	Benthic	(Near)-resident
Greater sandeel	*Hyperoplus lanceolatus*	4,5	4	Benthic	MSV
Grey gurnard	*Eutrigla gurnardus*	3,4	3,9	Benthopelagic	MSV
Herring	*Clupea harengus*	3,4	3,4	Pelagic	JMM
Hooknose	*Agonus cataphractus*	3,6	3,4	Benthic	(Near)-resident
Houting	*Coregonus oxyrinchus*	3,1	3,3	Pelagic	MSV
Lemon sole	*Microstomus kitt*	3,9	3,2	Benthic	MSV
Lesser weever	*Echeiichthys vipera*	3,4	4,4	Benthic	MSV
Lumpsucker	*Cyclopterus lumpus*	3,6	3,9	Benthic	(Near)-resident
Mackerel	*Scomber scombrus*	4,3	3,6	Pelagic	MSV
Pilchard	*Sardine pilchardus*	3,3	3,1	Pelagic	MSV
Plaice	*Pleuronectes platessa*	3,2	3,2	Benthic	JMM
Pollack	*Pollachius pollachius*	4,0	4,3	Benthopelagic	MSV
Poor cod	*Trisopterus minutus*	3,4	3,7	Benthopelagic	MSV

Table S1 General information of species analysed. *(continued)*

Species	SpeciesLatin	TP calculated by stomach content	TP according to FishBase	Functional group	Guild
Reticulated dragonet	*Callionymus reticulatus*	3,1	3,3	Benthic	MSV
Round goby	*Neogobius melanostomus*	3,8	3,3	Benthic	(Near)-resident
Saithe	*Pollachius virens*	3,9	4,3	Pelagic	MSV
Sand goby	*Pomatoschistus minutus*	3,9	3,2	Benthic	(Near)-resident
Sand-smelt	*Atherina presbyter*	3,4	3,7	Pelagic	(Near)-resident
Sandeel	*Ammodytes tobianus*	3,2	3,1	Benthic	MSV
Scad	*Alosa alosa*	4,1	3,7	Pelagic	MSV
Scaldfish	*Arnoglossus laterna*	3,5	3,6	Benthic	MSV
Sea scorpion	*Taurulus bubalis*	3,6	3,6	Benthic	(Near)-resident
Sea trout	*Salmo trutta*	4,6	3,4	Pelagic	(Near)-resident
Sea-snail	*Liparis liparis*	3,6	3,5	Benthic	(Near)-resident
Shanny	*Lipophrys pholis*	3,1	3,1	Benthic	(Near)-resident
Smelt	*Osmerus eperlanus*	3,9	3,5	Pelagic	MSV
Sole	*Solea solea*	3,2	3,2	Benthic	JMM
Sprat	*Sprattus sprattus*	3,3	3	Pelagic	JMM
Stickleback	*Gasterosteus aculeatus*	3,1	3,3	Benthopelagic	(Near)-resident
Thick-lipped grey mullet	*Chelon labrosus*	2,3	2,6	Benthic	MSV
Thin-lipped grey mullet	*Chelon ramada*	2,0	2,3	Benthic	MSV
Tompot blenny	*Parablennius gattorugine*	3,3	3,6	Benthic	(Near)-resident
Tub gurnard	*Chelidonichthys lucerna*	3,6	4	Benthopelagic	MSV
Turbot	*Scophthalmus maxima*	3,9	4,4	Benthic	MSV
Twaite shad	*Alosa fallax*	3,9	4	Pelagic	(Near)-resident
Viviparous blenny	*Zoarces viviparus*	3,4	3,5	Benthic	(Near)-resident
Whiting	*Merlangus merlangus*	3,7	4,4	Benthopelagic	MSV

4

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

Poiesz SSH, Witte JIJ, van der Meer MTJ, van der Veer HW, Soetaert KER (2021)
Trophic structure and resources utilization of the western Wadden sea fish community:
evidence from stable isotope data analysis. Marine Ecology Progress Series 677: 115–128.

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

Running title:
Coastal Wadden Sea food web structure by stable isotopes

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 ($\delta^{15}N$) increase with trophic position (Minagawa & Wada 1984). Carbon isotope ($\delta^{13}C$) 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.

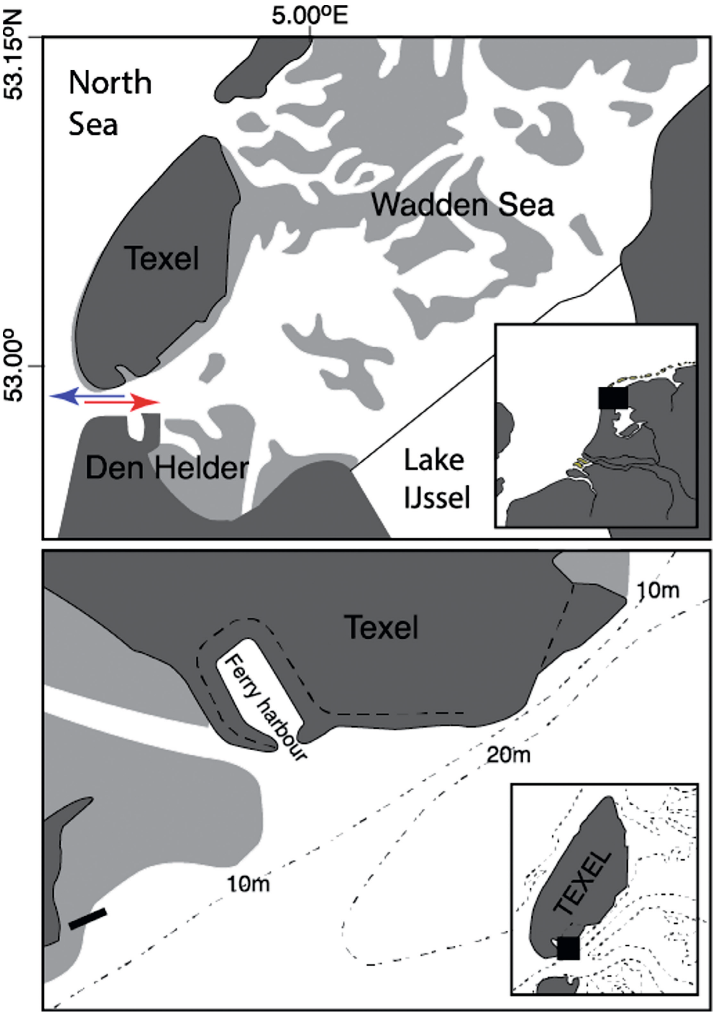

Figure 1 Sampling location of the NIOZ kom-fyke near the island of Texel. Top panel: western Dutch Wadden Sea (black box); red arrow indicates inwards migration in spring and blue arrow outward migration in autumn. Lower panel: kom-fyke position (black bar). Grey: intertidal areas (After Poiesz et al. 2020).

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:

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

where $\boldsymbol{R}_{sample}$ and $\boldsymbol{R}_{reference}$ 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

δ^{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 δ^{13}C values corrected for lipid content;
$\delta^{13}C_{bulk}$	the δ^{13}C values of the bulk tissue (δ^{13}C values including lipid content);
$C:N$	the ratio of total nitrogen (%TN) / total organic carbon (%TOC).

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

Isotopic values of δ^{15}N and δ^{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:

δ^{13}C = β_1 * fish species + factor (season) + fish length (cm) [3]

δ^{15}N = β_1 * fish species + factor (season) + fish length (cm) [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 extent this analysis to two baselines (pelagic and benthic) with two distinct sources (N and C) the formula for N becomes:

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

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:

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

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 $\delta^{15}N$ (s.d. 0.98‰) and of 0.39‰ for carbon $\delta^{13}C$ (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 ($\overline{TP}diet$) of a fish species based on stomach content (taken from Poiesz et al. 2020) and the mean $\delta^{15}N$ value.

2.4. Trophic niche

Based on the $\delta^{15}N$ and $\delta^{13}C$ isotope values, trophic niches were quantified for fish species using niche/community metrics following Layman et al. (2007): (1) $\delta^{13}C$ 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 Supplementary material Table S1).

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).

Table 1 Overview of the $\delta^{13}C$ baselines for the western Dutch Wadden Sea. SPOM refers to suspended particulate organic matter.

	Source	Range	Mean ± SE	Source
Pelagic	freshwater SPOM	-22‰ to -25‰		Jung et al. (2019)
	estuarine SPOM	-18‰ to -16‰		Jung et al. (2019)
	Mytilus edulis baseline		-17.8‰ ± 0.1‰	Christianen et al. (2017)
Benthic	*Littorina littorea*		-14.2‰ ± 0.1‰	Christianen et al. (2017)

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).

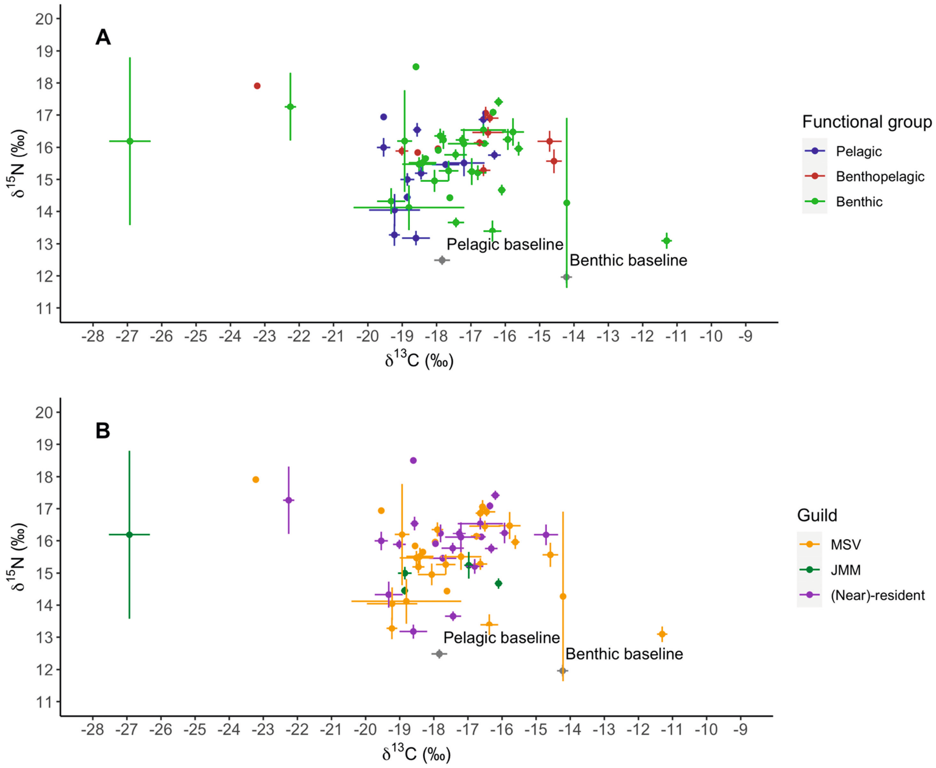

Figure 2 Average $\delta^{15}N$ and $\delta^{13}C$ stable isotope values with standard error bars for $\delta^{15}N$ (vertical) and $\delta^{13}C$ (horizontal) for all Wadden Sea fish species A: Functional groups; B: Guilds. The benthic baseline and the pelagic baseline are added for comparison. For values and species names see Supplementary materials Table S2.

$\delta^{15}N$ 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 materials; 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 materials Fig 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 materials Fig S4).

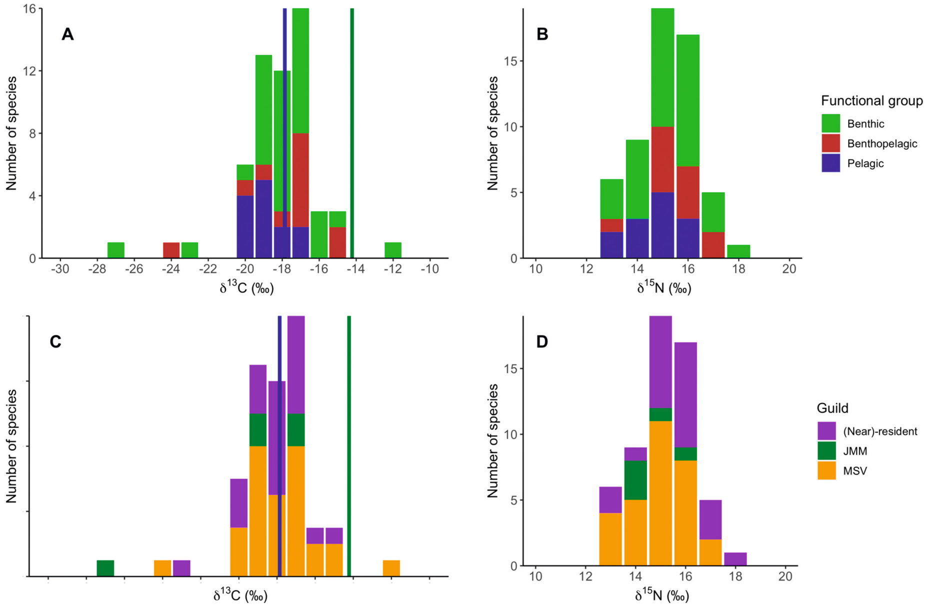

Figure 3 Frequency distribution of average $\delta^{13}C$ values (left panel) and average $\delta^{15}N$ values (right panel) for the Wadden Sea fish species, split up in functional groups (top panel) and guilds (bottom panel). The dark blue lines represent the pelagic baseline of Mytulis edulis [$\delta^{13}C$ -17.8‰ ± 0.1‰ (mean ± SE)] and dark green benthic baseline of Littorina littorea [$\delta^{13}C$ -14.2‰ ± 0.1‰ (mean ± SE).

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 materials 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 materials 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 materials 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 materials 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 δ^{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 δ^{15}N trophic position estimates did (Supplementary materials Fig S6; lowest two blue dots).

Table 2 Functional group, guild and mean trophic positions for Wadden Sea fish species with more than 10 observations based on stomach content analysis (TP stomach content) after Poiesz et al. 2020, mean trophic position according to FishBase (http://www.fishbase.org) and derived from stable isotope analysis (mean TP isotope based) after this study.

Common name	Scientific name	Functional group	Guild	TP stomach content	TP Fishbase	TP isotope based
Bass	*Dicentrarchus labrax*	Benthic	NR	3.70	3.50	3.42
Bib	*Trisopterus luscus*	Benthopelagic	MSV	3.56	3.70	2.88
Bull-rout	*Myoxocephalus scorpius*	Benthic	NR	3.57	3.90	3.52
Cod	*Gadus morhua*	Benthppelagic	MSV	3.75	4.10	3.36
Dab	*Limanda limanda*	Benthic	MSV	3.32	3.40	2.59
Five-bearded rockling	*Ciliata mustela*	Benthic	NR	3.65	3.50	3.13
Flounder	*Platichthys flesus*	Benthic	NR	3.47	3.30	3.12
Garfish	*Belone belone*	Pelagic	NR	4.65	4.20	2.88
Golden grey mullet	*Chelon auratus*	Benthic	MSV	2.13	2.80	2.32
Greater pipefish	*Syngnathus acus*	Benthic	NR	3.60	3.30	2.37
Herring	*Clupea harengus*	Pelagic	JMM	3.44	3.40	2.57
Pilchard	*Sardina pilchardus*	Pelagic	MSV	3.52	3.10	2.24
Plaice	*Pleuronectes platessa*	Benthic	JMM	3.23	3.20	2.73
Pollack	*Pollachius pollachius*	Benthopelagic	MSV	3.70	4.30	3.25
Saithe	*Pollachius virens*	Pelagic	MSV	4.13	4.30	2.84
Sand goby	*Pomatoschistus minutus*	Benthic	NR	3.84	3.20	3.24
Sand-smelt	*Atherina presbyter*	Pelagic	NR	3.26	3.70	3.06
Scad	*Trachurus trachurus*	Pelagic	MSV	4.13	3.70	2.80
Sea trout	*Salmo trutta*	Peagic	NR	4.58	3.40	3.05
Smelt	*Osmerus eperlanus*	Pelagic	MSV	3.93	3.50	3.36
Sole	*Solea solea*	Benthic	JMM	3.10	3.20	2.85
Sprat	*Sprattus sprattus*	Pelagic	JMM	3.13	3.00	2.73
Stickleback	*Gasterosteus aculeatus*	Benthopelagic	NR	3.13	3.30	3.00
Thick-lipped grey mullet	*Chelon labrosus*	Benthic	MSV	2.36	2.60	2.33
Turbot	*Scophthalmus maximus*	Benthic	MSV	3.85	4.40	3.14
Twaite shad	*Alosa fallax*	Pelagic	NR	3.86	4.00	3.20
Viviparous blenny	*Zoarces viviparus*	Benthic	NR	3.46	3.50	3.13
Whiting	*Merlangius merlangus*	Benthopelagic	MSV	3.64	4.40	3.13

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 δ^{15}N (sea trout) and δ^{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 δ^{13}C values were found compared to the benthopelagic and pelagic species (Fig 5).

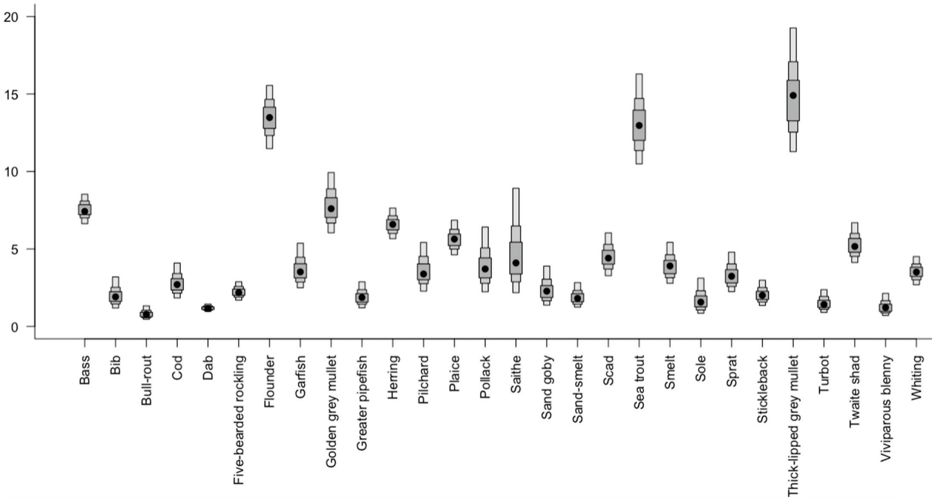

Figure 4 Density plots of corrected standard ellipses areas (SEAc) (black dots) for all Wadden Sea species with more than 10 observations with credible intervals (50% inside dark grey boxes, 75% middle grey boxes, 95 % outer light grey boxes).

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 δ^{13}C values of -6.5‰ were found among the MSV and highest δ^{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 δ^{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).

Table 3 Convex hull area (TA) and Standard ellipse areas (SEA) for Wadden Sea fish species with more than 10 observations. TA is the convex hull area (total area); SEA(c) = standard ellipse area corrected for a small sample size, representing the isotopic niche metrics calculated for both all species in sympatry based on the $\delta^{15}N$ and $\delta^{13}C$ values.

Common name	TA	SEA	SEAc
Bass	87.61	7.53	7.56
Bib	5.51	1.99	2.11
Bull rout	2.14	0.80	0.86
Cod	6.66	2.77	2.89
Dab	8.56	1.19	1.20
Five bearded ockling	11.86	2.23	2.27
Flounder	119.18	13.47	13.54
Garfish	14.43	3.70	3.85
Golden grey mullet	48.21	7.78	7.90
Greater pipefish	5.68	1.85	1.95
Herring	42.62	6.57	6.61
Pilchard	10.65	3.60	3.78
Plaice	28.53	5.62	5.68
Pollack	9.14	3.89	4.17
Saithe	5.93	3.83	4.37
Sand goby	6.38	2.35	2.52
Sand smelt	5.20	1.89	1.97
Scad	18.10	4.49	4.60
Sea trout	78.82	13.05	13.22
Smelt	23.77	3.92	4.04
Sole	3.65	1.70	1.91
Sprat	11.25	3.29	3.42
Stickleback	6.33	2.04	2.12
Thick lipped grey mullet	106.43	14.80	15.07
Turbot	4.14	1.50	1.59
Twaite shad	32.53	5.27	5.35
Viviparous blenny	3.18	1.27	1.37
Whiting	23.03	3.52	3.58

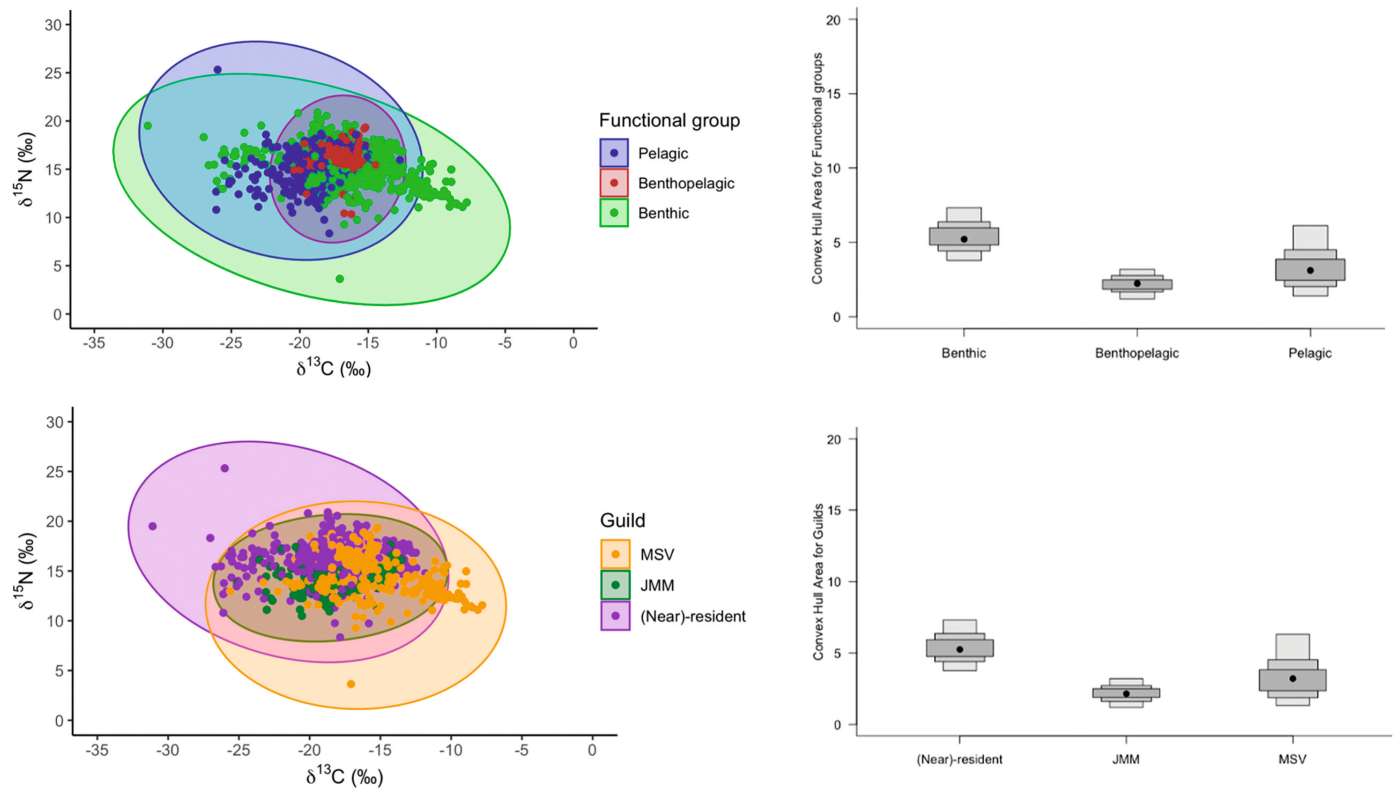

Figure 5 Total convex hulls area for the various functional groups (left top panel) and guilds (left bottom panel) based on mean isotope values of the individual Wadden Sea fish species with more than 10 observations. Right column: Density plots of standard ellipses areas (black dots) for the three functional groups (top) and guilds (bottom) with credible intervals (50% inside dark grey boxes, 75% middle grey boxes, 95% outer light grey boxes).

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.

Table 4 Layman metrics for the functional groups and guilds of Wadden Sea fish species (JMM for juvenile marine migrants and MSV for marine seasonal visitors). With CR is the $\delta^{13}C$ range, NR is the $\delta^{15}N$ range, TA is the convex hull area, CD is the mean distance to centroid, MNND is the mean nearest neighbour distance and the SDNND is the standard deviation of nearest neighbour distance.

	Benthic	Benthopelagic	Pelagic	(Near)resident	JMM	MSV
NR	4.104	1.513	3.728	3.59	0.915	3.854
CR	6.949	2.026	3.075	3.669	2.557	7.908
TA	15.164	1.518	6.063	6.414	1.199	15.951
CD	1.748	0.822	1.223	1.236	1.046	1.852
MNND	1.000	0.725	0.805	0.608	0.879	1.208
SDNND	1.286	0.646	0.409	0.459	0.237	1.349

In our study, most of the Wadden Sea fish species had $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values. Golden grey mullet had the highest stable $\delta^{13}C$ 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 $\delta^{13}C$ 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, $\delta^{13}C$ 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 $\delta^{15}N$, 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 $\delta^{15}N$, 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:

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).

Supplementary materials

Table S1 δ^{13}C signatures of the key prey items of the fish fauna in the western Wadden Sea. Data collected in 2012. Number of observations (N), together with mean δ^{13}C and standard deviation (s.d.). Data after van der Veer & Witte (unpublished.).

Common name	Scientific name	N	δ^{13}C	s.d.
Gammarus	*Gammarus sp*	4	-15.9	0.49
Amphipod	*Hyperia galba*	11	-19.1	0.79
Isopod	*Idotea linearis*	17	-18.6	0.85
Chameleon shrimp	*Praunus flexuosus*	15	-17.9	0.93
Gastrosaccus spinifer	*Gastrosaccus spinifer*	15	-19.9	0.87
Brown shrimp	*Crangon crangon*	15	-16.4	1.22
Shore crab	*Carcinus meanas*	11	-16.8	0.54
Transparent goby	*Aphia minuta*	8	-18.8	1.00
Lozano's goby	*Pomatoschistus lozanoi*	14	-18.5	1.09
Common goby	*Pomatoschistus microps*	16	-17.1	1.19
Sand goby	*Pomatoschistus minutus*	17	-17.5	1.05
Sprat	*Sprattus sprattus*	20	-19.4	0.94
Herring	*Clupea harengus*	28	-19.4	1.75

Table S2 Total number of samples analysed per year together with mean δ^{15}N and δ^{13}C values with corresponding standard error bars.

Common name	Scientific name	2012	2013	2014	2015	2016	Total	δ^{15}N Mean	δ^{15}N s.e.	δ^{13}C Mean	δ^{13}C s.e.
Bass	*Dicentrarchus labrax*	50	22	90	22	56	240	17,09	0,09	-16,36	0,11
Flounder	*Platichthys flesus*	24	54	46	14	39	177	16,23	0,09	-17,80	0,28
Herring	*Clupea harengus*	18	58	37	19	42	174	14,45	0,10	-18,86	0,12
Plaice	*Pleuronectes platessa*	8	20	38	14	22	102	14,67	0,11	-16,10	0,16
Dab	*Limanda limanda*	10	12	14	39	23	98	14,43	0,06	-17,61	0,06
Sea trout	*Salmo trutta*	5	21	9	26	19	80	16,00	0,19	-19,54	0,29
Golden grey mullet	*Chelon auratus*	12	0	18	13	25	68	16,54	0,12	-18,57	0,21
Twaite shad	*Alosa fallax*	7	19	26	4	12	68	13,10	0,15	-11,30	0,25
Whiting	*Merlangius merlangus*	6	16	25	3	11	61	16,12	0,13	-16,61	0,10
Five-bearded rockling	*Ciliata mustela*	9	8	16	9	15	57	16,14	0,17	-16,75	0,11
Thick-lipped grey mullet	*Chelon labrosus*	34	2	13	5	2	56	15,19	0,18	-18,45	0,19
Scad	*Trachurus trachurus*	4	12	9	0	18	43	13,39	0,26	-16,37	0,32
Smelt	*Osmerus eperlanus*	4	9	19	1	3	36	16,86	0,14	-16,64	0,26
Garfish	*Belone belone*	11	4	9	1	3	28	16,90	0,25	-16,45	0,14
Sprat	*Sprattus sprattus*	2	10	5	3	7	27	15,89	0,19	-19,01	0,14
Cod	*Gadus morhua*	10	12	4	0	0	26	15,00	0,20	-18,85	0,19
Stickleback	*Gasterosteus aculeatus*	2	8	10	1	5	26	15,46	0,40	-17,74	0,11
Sand-smelt	*Atherina presbyter*	1	1	16	0	6	24	15,76	0,18	-16,32	0,15
Greater pipefish	*Syngnathus acus*	6	1	9	3	3	22	17,41	0,12	-16,20	0,14
Pilchard	*Sardina pilchardus*	6	6	3	0	7	22	16,24	0,14	-15,93	0,32
Turbot	*Scophthalmus maximus*	1	1	15	2	0	19	13,66	0,24	-17,44	0,15
Bib	*Trisopterus luscus*	0	5	2	8	3	18	13,28	0,16	-19,23	0,34
Pollack	*Pollachius pollachius*	11	0	4	0	1	16	16,46	0,46	-16,50	0,17
Sand goby	*Pomatoschistus minutus*	0	4	6	0	6	16	15,96	0,13	-15,61	0,21
Bull-rout	*Myoxocephalus scorpius*	4	2	7	1	1	15	15,28	0,20	-16,63	0,18
Viviparous blenny	*Zoarces viviparus*	0	4	1	0	9	14	15,51	0,60	-17,20	0,41
Sole	*Solea solea*	0	5	4	0	1	10	16,23	0,19	-17,25	0,15
Saithe	*Pollachius virens*	3	3	1	1	1	10	15,24	0,13	-16,98	0,41
Brill	*Scophthalmus rhombus*	2	2	2	1	1	8	16,47	0,32	-15,78	0,42

Table S2 Total number of samples analysed per year together with mean $\delta^{15}N$ and $\delta^{13}C$ values with corresponding standard error bars. *(continued)*

Common name	Scientific name	2012	2013	2014	2015	2016	Total	$\delta^{15}N$ Mean	$\delta^{15}N$ s.e.	$\delta^{13}C$ Mean	$\delta^{13}C$ s.e.
Scaldfish	*Arnoglossus laterna*	1	5	0	0	2	8	15,52	0,40	-18,41	0,27
Eel	*Anguilla anguilla*	0	1	0	2	4	7	16,19	0,60	-26,93	2,61
Mackerel	*Scomber scombrus*	2	3	1	0	1	7	14,04	0,74	-19,22	0,50
Tub gurnard	*Trigla lucerna*	2	0	2	0	3	7	17,06	0,11	-16,57	0,21
Hooknose	*Agonus cataphractus*	1	1	1	1	2	6	16,11	0,46	-17,21	0,46
Lesser weever	*Echiichthys vipera*	3	0	0	1	2	6	16,35	0,17	-17,89	0,22
Lumpsucker	*Cyclopterus lumpus*	2	3	1	0	0	6	14,33	0,41	-19,32	0,40
Round goby	*Neogobius melanostomus*	0	0	0	0	6	6	17,26	0,16	-22,26	1,05
Butterfish	*Pholis gunnellus*	1	1	0	0	3	5	15,77	0,33	-17,45	0,17
Gilt-head sea bream	*Sparus aurata*	0	0	2	1	1	4	16,19	0,35	-14,71	0,32
Red sea-bream	*Pagellus bogaraveo*	0	0	4	0	0	4	15,57	0,23	-14,58	0,37
Anchovy	*Engraulis encrasicolus*	0	0	0	0	3	3	13,18	0,41	-18,60	0,22
Dragonet	*Callionymus lyra*	1	0	1	0	1	3	15,47	0,49	-18,51	0,21
Sea scorpion	*Taurulus bubalis*	0	0	2	0	1	3	15,21	0,25	-16,80	0,23
Sea-snail	*Liparis liparis*	1	1	0	0	1	3	16,54	0,66	-16,64	0,18
Greater sandeel	*Hyperoplus lanceolatus*	0	1	0	0	1	2	14,96	0,40	-18,06	0,34
Lemon sole	*Microstomus kitt*	2	0	0	0	0	2	15,27	0,29	-17,65	0,31
Poor cod	*Trisopterus minutus*	0	0	2	0	0	2				
Red mullet	*Mullus surmuletus*	1	0	0	0	1	2	16,20	0,22	-18,93	1,58
Sea lamprey	*Petromyzon marinus*	0	1	1	0	0	2	14,12	1,61	-18,81	0,70
Thin-lipped grey mullet	*Chelon ramada*	1	0	1	0	0	2	14,27	0,08	-14,21	2,64
Forkbeard	*Phycis blennoides*	0	1	0	0	0	1	15,96		-17,96	
Grey gurnard	*Eutrigla gurnardus*	0	0	0	0	1	1	15,84		-18,55	
Houting	*Coregonus oxyrinchus*	0	1	0	0	0	1	16,94		-19,54	
Reticulated dragonet	*Callionymus reticulatus*	0	1	0	0	0	1	15,65		-18,32	
Shanny	*Lipophrys pholis*	0	0	1	0	0	1	15,91		-17,95	
Tompot blenny	*Parablennius gattorugine*	0	0	0	0	1	1	18,50		-18,60	
Vendace	*Coregonus albula*	0	0	0	0	1	1	17,91		-23,22	

Table S3 Regression values for each species for δ^{15}N ~ Length (cm) and δ^{13}C ~ Length (cm). ***: $p<0.001$; **: $p<0.01$- $p<0.001$).

Common name	δ^{15}N ~Length (cm)	δ^{13}C ~ Length (cm)
Anchovy	$y=14.6-0.0833x$, $R^2=0.003$, $P=0.97$	$y=-23.4+0.286x$, $R^2=0.12$, $P=0.78$
Bass	$y=16.4+0.0264x$, $R^2=0.038$, $P<0.001$***	$y=-14.7-0.0596x$, $R^2=0.32$, $P<0.001$***
Bib	$y=13.2+0.221x$, $R^2=0.66$, $P<0.001$***	$y=-17.6+0.103x$, $R^2=0.18$, $P=0.078$
Brill	$y=14.3+0.6x$, $R^2=0.42$, $P<0.083$	$y=-15.4-0.025x$, $R^2=0.0059$, $P=0.86$
Bull-rout	$y=15.4+0.139x$, $R^2=0.44$, $P<0.001$***	$y=-15.6-0.0465x$, $R^2=0.082$, $P=0.17$
Butterfish	$y=14.2+0.102x$, $R^2=0.23$, $P=0.42$	$y=-17.1-0.0223x$, $R^2=0.041$, $P=0.74$
Cod	$y=15.2+0.102x$, $R^2=0.54$, $P<0.001$***	$y=-16.4-0.0127x$, $R^2=0.012$, $P=0.54$
Dab	$y=14.3+0.00859x$, $R^2=0.0032$, $P=0.56$	$y=-17.7+0.0026$, $R^2=0.0004$, $P=0.84$
Dragonet	$y=6.09+0.62x$, $R^2=0.98$, $P=0.083$	$y=-14.6-0.257x$, $R^2=0.89$, $P=0.22$
Eel	$y=14.1+0.0338x$, $R^2=0.034$, $P=0.69$	$y=-17.8-0.739x$, $R^2=0.87$, $P=0.002$**
Five-bearded rockling	$y=14.9+0.0769x$, $R^2=0.12$, $P=0.004$**	$y=-17.2+0.038x$, $R^2=0.045$, $P=0.082$
Flounder	$y=16.4-0.0116x$, $R^2=0.0023$, $P=0.48$	$y=-21.2+0.235x$, $R^2=0.079$, $P<0.001$***
Forkbeard		
Garfish	$y=10+0.0982x$, $R^2=0.13$, $P=0.058$	$y=-17.6-0.00348x$, $R^2=0.0022$, $P=0.81$
Gilt-head sea bream	$y=21.1-0.357x$, $R^2=0.40$, $P=0.37$	$y=-21.7+0.509x$, $R^2=0.96$, $P=0.018$
Golden grey mullet	$y=12.7+0.0214x$, $R^2=0.012$, $P=0.36$	$y=-14-0.0192x$, $R^2=0.0035$, $P=0.62$
Greater pipefish	$y=5.89+0.21x$, $R^2=0.36$, $P=0.003$**	$y=-20.1+0.0724x$, $R^2=0.11$, $P=0.13$
Greater sandeel		
Grey gurnard		
Herring	$y=16.6-0.15x$, $R^2=0.46$, $P<0.001$***	$y=-17.7-0.0849x$, $R^2=0.11$, $P<0.001$***
Hooknose	$y=19.9-0.236x$, $R^2=0.096$, $P=0.55$	$y=-13.2-0.311x$, $R^2=0.17$, $P=0.42$
Houting		
Lemon sole		
Lesser weever	$y=12.8+0.262x$, $R^2=0.31$, $P=0.25$	$y=-18.1+0.0159x$, $R^2=0.0066$, $P=0.96$
Lumpsucker	$y=15.2+0.077x$, $R^2=0.53$, $P=0.1$	$y=-19.3-0.0027x$, $R^2=0.00068$, $P=0.96$
Mackerel	$y=14.5-0.0189x$, $R^2=0.0074$, $P=0.85$	$y=-21.4+0.0824x$, $R^2=0.3$, $P=0.2$
Pilchard	$y=13.4-0.0054x$, $R^2=0.00047$, $P=0.92$	$y=-21.9+0.107x$, $R^2=0.041$, $P=0.37$
Plaice	$y=13.+0.213x$, $R^2=0.15$, $P<0.001$***	$y=-18+0.232x$, $R^2=0.085$, $P<0.001$***
Pollack	$y=17.2-0.0375x$, $R^2=0.006$, $P=0.74$	$y=-18.1+0.0945x$, $R^2=0.22$, $P=0.031$**
Poor cod		
Red mullet		
Red sea-bream	$y=22.2-0.484x$, $R^2=0.98$, $P=0.012$**	$y=-24.4+0.713x$, $R^2=0.79$, $P=0.11$
Reticulated dragonet		
Round goby	$y=17.6-0.0259x$, $R^2=0.024$, $P=0.77$	$y=-27.5+0.454x$, $R^2=0.18$, $P=0.41$
Saithe	$y=16.1-0.0079$, $R^2=0.0007$, $P=0.92$	$y=-16.5-0.0387x$, $R^2=0.043$, $P=0.46$
Sand goby	$y=16.5-0.020x$, $R^2=0.00008$, $P=0.89$	$y=-18.2+0.336x$, $R^2=0.05$, $P=0.28$
Sand-smelt	$y=12.9+0.27x$, $R^2=0.45$, $P<0.001$***	$y=-17.8+0.134x$, $R^2=0.19$, $P=0.023$
Scad	$y=15.4-0.017x$, $R^2=0.0$, $P=0.4$	$y=-19.8+0.0842x$, $R^2=0.21$, $P<0.001$***
Scaldfish	$y=12.5+0.279x$, $R^2=0.16$, $P=0.33$	$y=-15.9-0.231x$, $R^2=0.24$, $P=0.21$
Sea lamprey		
Sea scorpion	$y=13.6+0.182x$, $R^2=0.98$, $P=0.093$	$y=-15.3-0.164x$, $R^2=0.99$, $P=0.057$
Sea trout	$y=12.5+0.095x$, $R^2=0.49$, $P<0.001$***	$y=-25.9+0.179x$, $R^2=0.52$, $P<0.001$***

Table S3 Regression values for each species for $\delta^{15}N$ ~ Length (cm) and $\delta^{13}C$ ~ Length (cm). ***: p<0.001; **: p<0.01- p<0.001). *(continued)*

Common name	$\delta^{15}N$ ~Length (cm)	$\delta^{13}C$ ~ Length (cm)
Sea-snail	y=14.4+0.209x, R^2=0.68, P=0.38	y=-16-0.0644x, R^2=0.89, P=0.22
Shanny		
Smelt	y=14.7+0.121x, R^2=0.20, P=0.003**	y=-20+0.185x, R^2=0.13, P=0.017
Sole	y=15.3-0.003x, R^2=0.002, P=0.89	y=-14.8-0.144x, R^2=0.72, P<0.001***
Sprat	y=15.6-0.057x, R^2=0.005, P=0.71	y=-18.8-0.00715x, R^2=0.000083, P=0.96
Stickleback	y=22-0.92x, R^2=0.15, P=0.021**	y=-19-0.0293x, R^2=0.00021, P=0.93
Thick-lipped grey mullet	y=14.1-0.017x, R^2=0.037, P=0.14	y=-18.4+0.043x, R^2=0.21, P<0.001***
Thin-lipped grey mullet		
Tompot blenny		
Tub gurnard	y=16.6+0.0268x, R^2=0.18, P=0.35	y=-17.5+0.048x, R^2=0.15, P=0.39
Turbot	y=15.2+0.0931x, R^2=0.14, P=0.092	y=-15.1-0.0473x, R^2=0.017, P=0.57
Twaite shad	y=16.2+0.012x, R^2=0.021, P=0.2	y=-18.3-0.0106x, R^2=0.049, P=0.54
Vendace		
Viviparous blenny	y=16+0.013x, R^2=0.005, P 0.81	y=-15.7-0.111x, R^2=0.56, P=0.002**
Whiting	y=15.8+0.09x, R^2=0.12, P=0.0036**	y=-15.4-0.0908x, R^2=0.27, P<0.001***

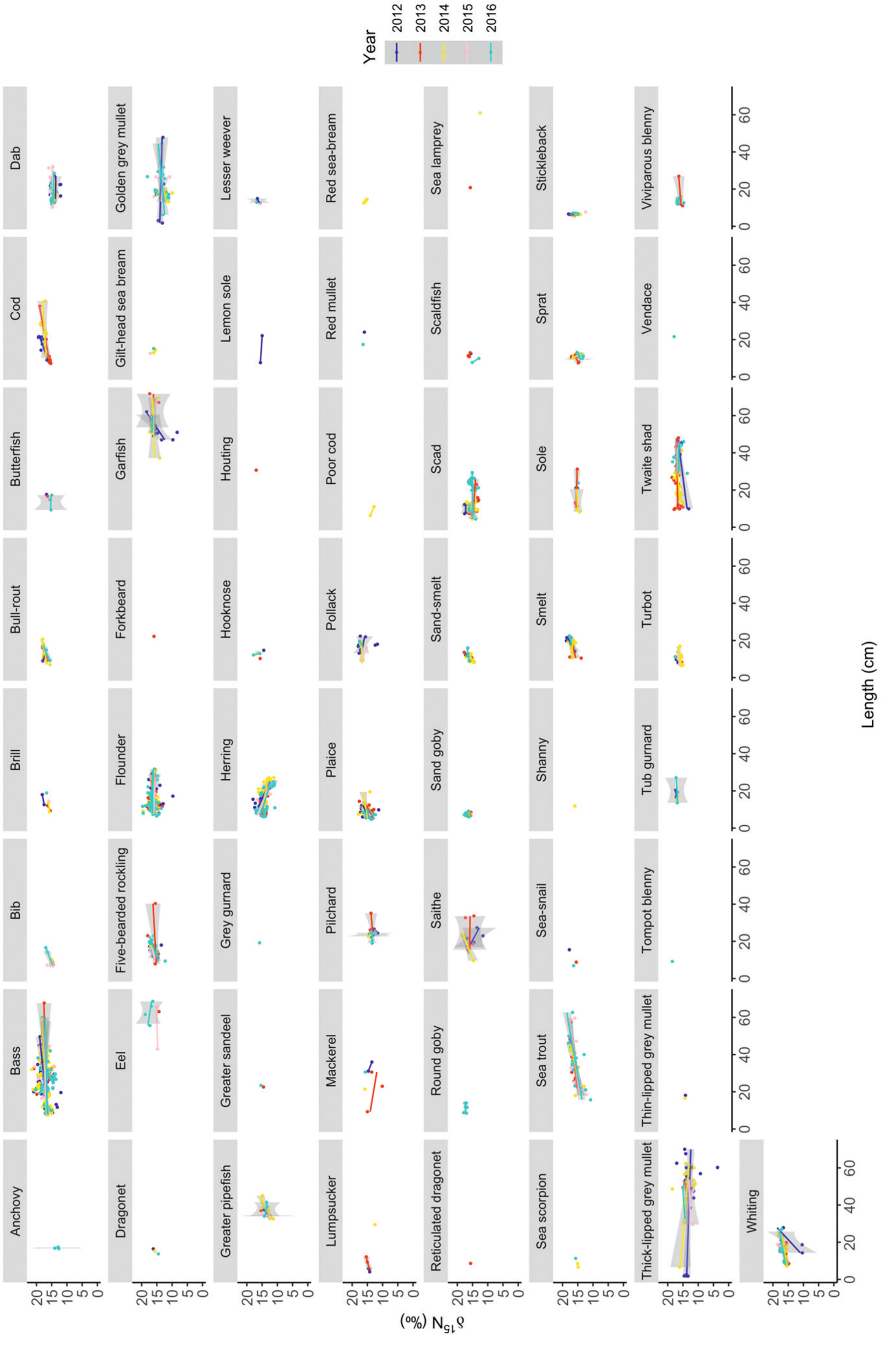

Figure S1 δ^{15}N stable isotope value in relation to total fish length for all species caught over the years 2012 -2016 together with linear regression and 95%-confidence interval.

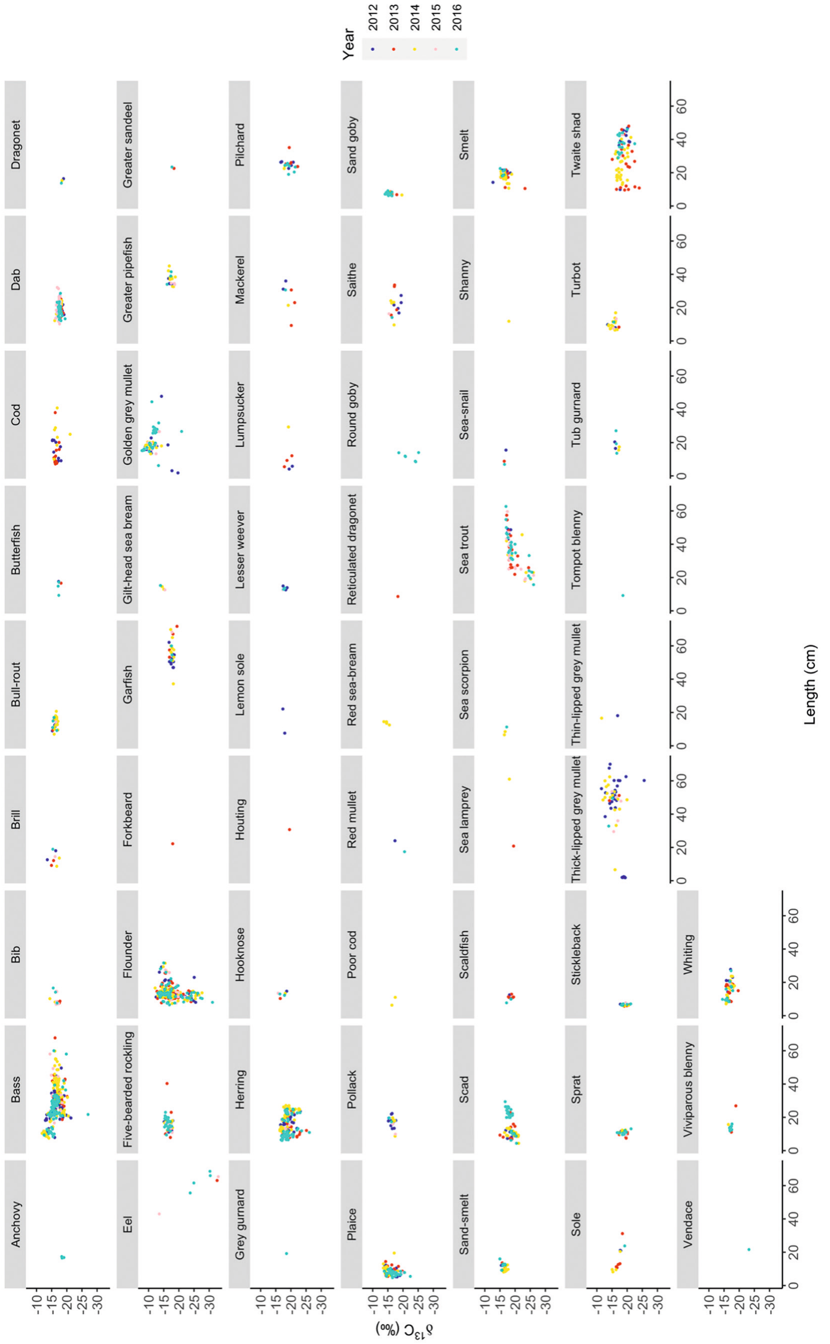

Figure S2 δ^{13}C stable isotope value in relation to total fish length for all species caught over the years 2012 - 2016 together with linear regression and 95%-confidence interval.

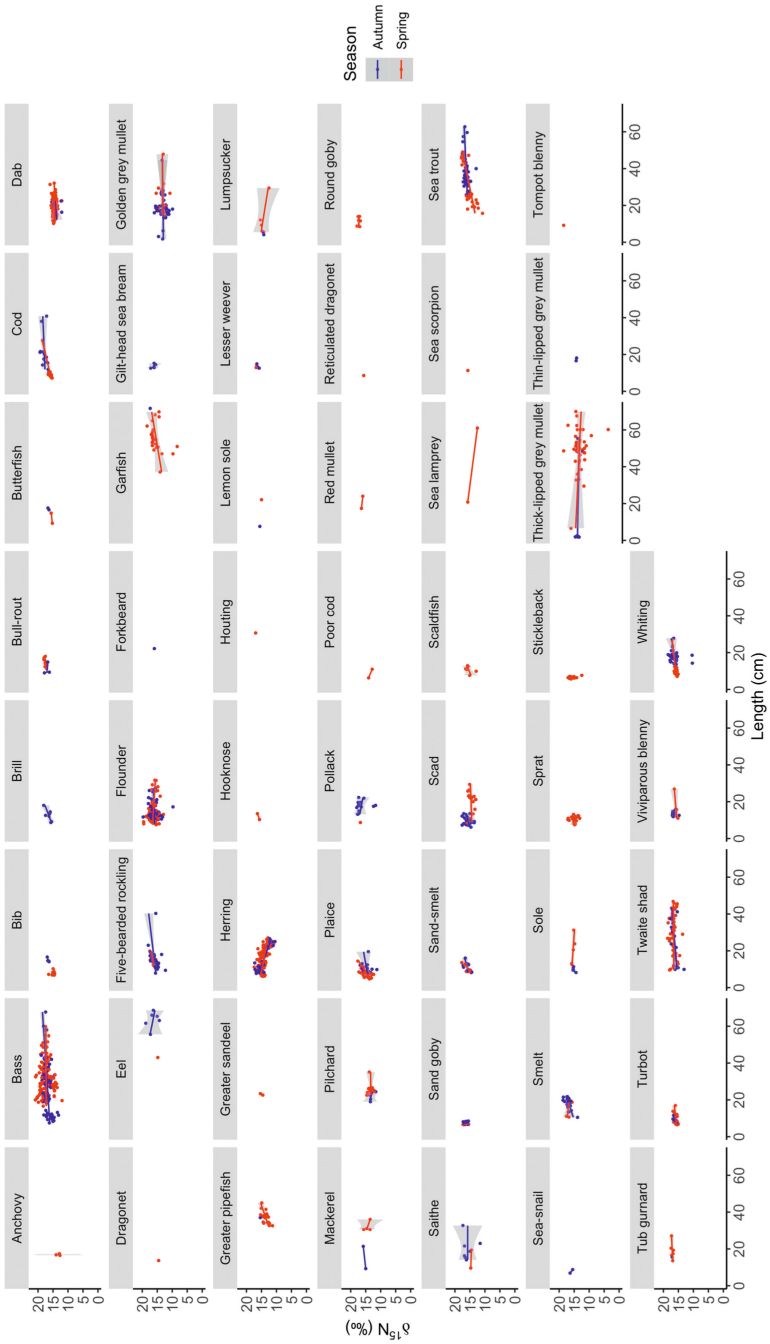

Figure S3 δ¹⁵N stable isotope value in relation to total fish length for all species in spring (April, May and June) and autumn (September and October) for the years 2012 – 2016 together with linear regression and 95%-confidence interval.

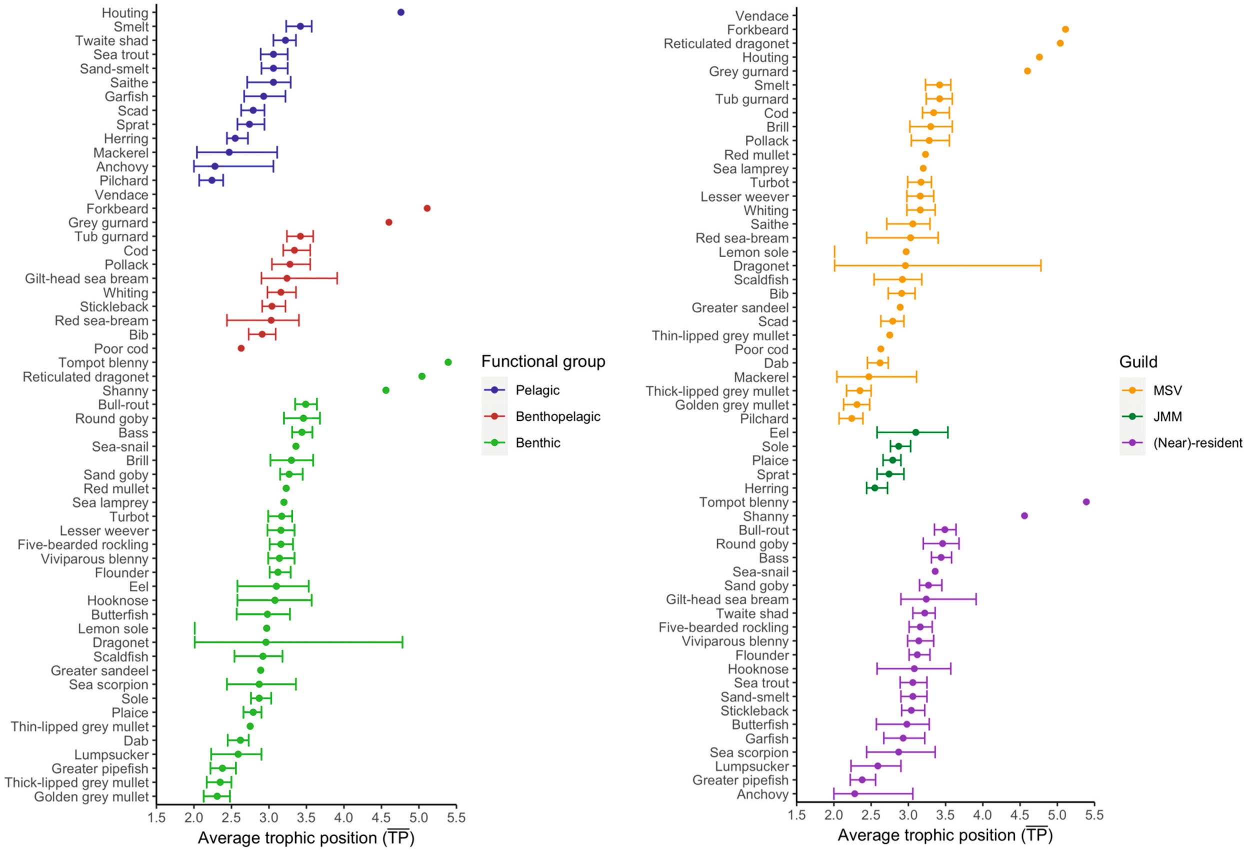

Figure S4 Trophic position ($\overline{TP}$) based on stable isotope analysis with standard error bars for functional groups (left panel) and guilds (right panel) of western Wadden Sea fish species. Species are listed from lowest to highest average ($\overline{TP}$). Errors bars greater than (+/-) 5.5 are omitted.

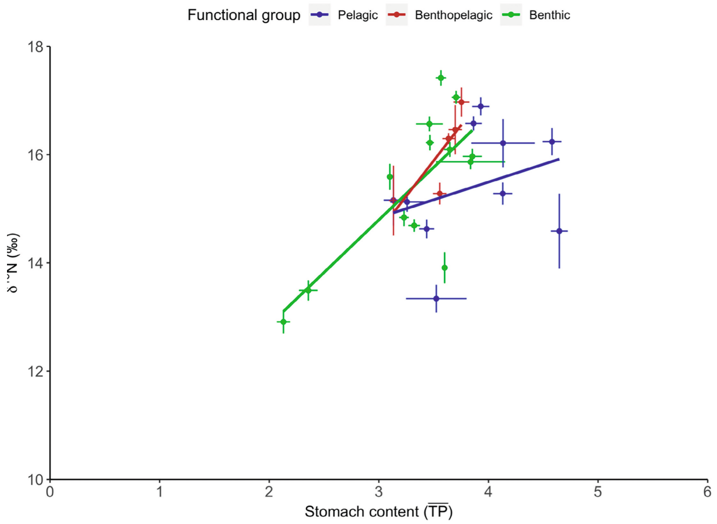

Figure S5 Relationship between dietary-based calculated mean trophic position ($\overline{TP}$) and mean δ^{15}N stable isotope values for western Wadden Sea fish species, split up into benthic, benthopelagic and pelagic species, mean values together with standard error bars.

benthic:	δ^{15}N = 8.9 + 1.94* $\overline{TP}diet$;	N=11; R=0.75; p<0.01
benthopelagic:	δ^{15}N = 6.7 + 2.62* $\overline{TP}diet$;	N= 5; R=0.82; p=0.09
pelagic:	δ^{15}N = 12.9 + 0.65* $\overline{TP}diet$;	N= 9; R=0.32; p=0.37

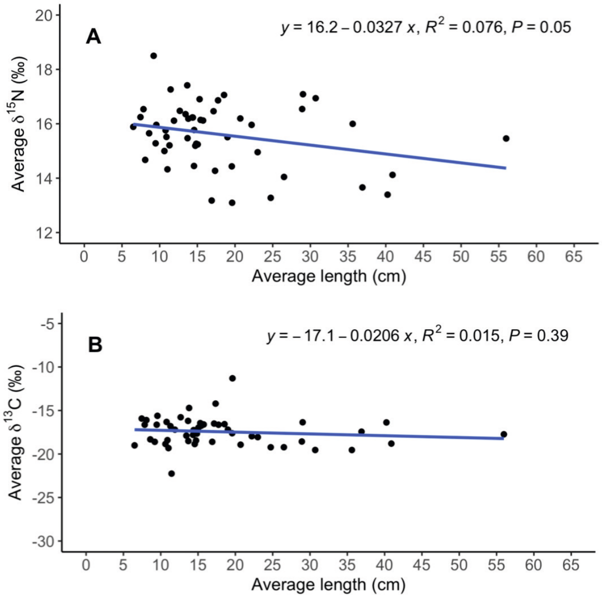

Figure S6 Linear regressions between average δ^{15}N (A) and δ^{13}C (B) and average total length (cm) of the various fish species.

PART II

Spatial variability in Wadden Sea fish food web structure

5

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

Poiesz SSH, Witte Johannes JIJ, Bleijswijk JDL, Witte HJ, van Weerlee EM, Brouwer M, Vreugdenhil S, van der Veer HW, Klunder L (2025) Spatial variability of the coastal Wadden Sea fish community as revealed by environmental DNA. Estuarine, Coastal and Shelf Sciences, 323, 109411.

Keywords:
Core fish species, transient species, Dutch temperate coastal estuarine, environmental DNA-metabarcoding, biodiversity monitoring, spatial similarity and differences, DNA-sequencing, 12S barcode, riaz primers

Running title:
Spatial variability in the Wadden Sea fish community by eDNA

Abstract

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

1. Introduction

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

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

The various studies have suggested some local differences in the Wadden Sea fish food web. The fish community showed some variability in the Wadden Sea also with respect to dominant core species. Only four species [herring (Clupea harengus), sprat (*Sprattus sprattus*), plaice (*Pleuronectes platessa*) and whiting (*Merlangius merlangus*)] were dominant in all studies and, while other species, such as the Gobiidae species, were only described as highly abundant in one or two areas (Kellnreitner et al. 2012, Meyer et al. 2016, Poiesz et al. 2020, 2023). With respect to prey species, copepods and brown shrimp were the most important prey species in all studies, however, mysid shrimp were also important in the Sylt-Rømø bight (Kellnreitner et al. 2012) and Ems basin (Poiesz et al. 2023), while shore crab and juvenile herring were more important in the Marsdiep basin (Poiesz et al. 2020). This variability in fish composition and abundance, and in predator–prey relationships might suggest that it is caused by local differences in predator-prey abundance, as found for two areas by Poiesz et al. (2023). However, most of the studies were carried out in different years and with different sampling gears.

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

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

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

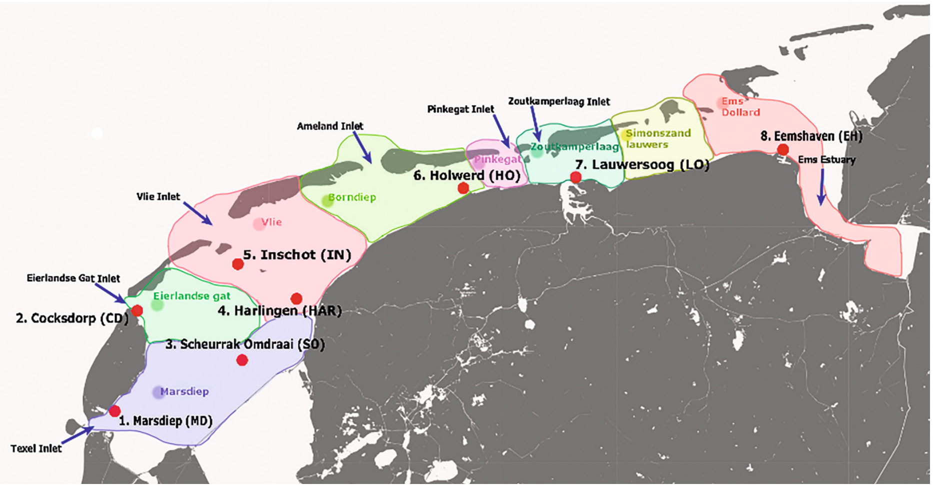

Figure 1 Monthly eDNA sampling locations (red dots) and the various tidal inlets (blue arrows) in the Dutch Wadden Sea. The colored areas with the corresponding names in the same color indicate the tidal areas. Abbreviations and full names of the locations and their characteristics can be found in Table 1.

2. Materials and methods

2.1. Sampling

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

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

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

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

Samples were filtered after collection in a climate room over a 47-mm 0.2 µm Nuclepore filter using a high-pressure vacuum pump (Millepore) until clogged within half an hour. The average water volume filtered was 150-200 ml ±17 (SE). Filters were folded inwards, placed in 2ml tubes and stored at -80 °C. Blank filters, using milli Q water were taken after all other samples were filtered. After filtration, the Nuclepore filters were rolled up inwards with sterilized tweezers, stored in 2 ml screw cap tubes and put on ice immediately. Support filters were discarded. Sterilization of tweezers was done with 96% ethanol and burning after storing each filter. The filtration setup and glass bottles for sampling were cleaned with soapy warm water and Milli-Q after finishing filtration (see Supplementary materials Table S1 for all sampling and filtration information) (methods described in van Bleijswijk et al. 2020). For the over 333 samples collected, (including the biological triplicates), the average water volume filtered was 186 ml (± 3 StE). Filtration and extraction controls were taken along on 5 days.

2.2. DNA extraction, amplification and sequencing

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

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

2.3. Bioinformatics

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

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

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

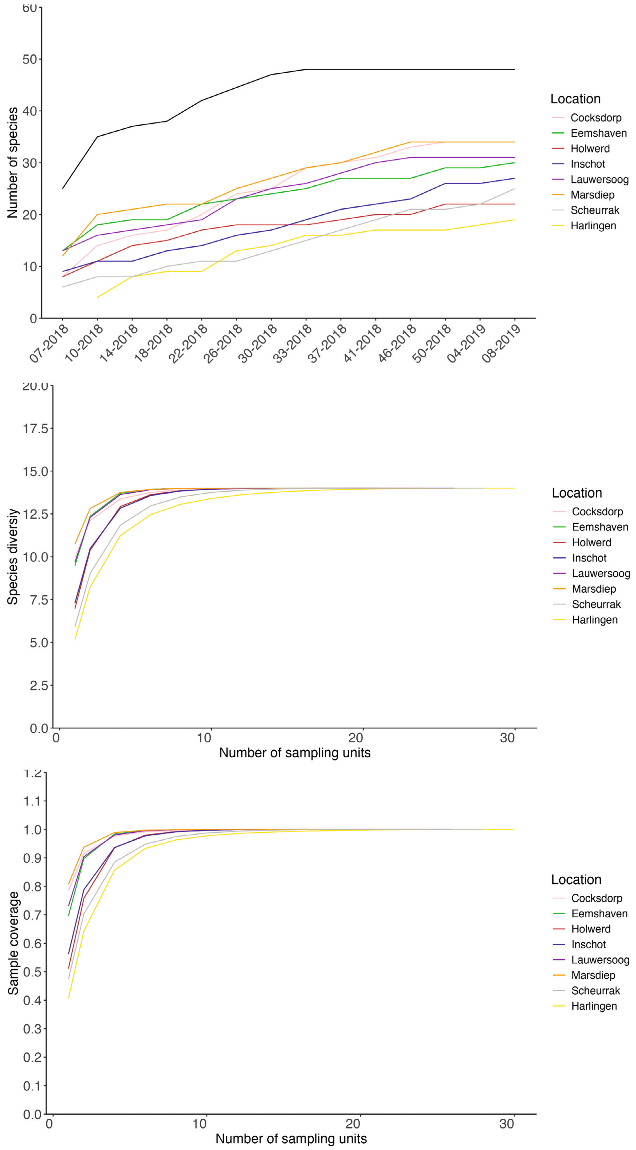

Figure 2 Top figure: Fish species accumulation curves for the various locations in the Dutch Wadden Sea based on eDNA sampling in 2018-2019. The black line represents all stations combined. The X-axis represents the sampling times (week-year combination).
Lower figures: Fish species accumulation curves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

Table 2 Heatmap of the number of species found in the samples at the various locations. Colour scheme indicates the categorical difference in number of species found per location over time. Green colours indicate the highest value, yellow the 50% percentile of the values and red the lowest value. The empty white spots mean that there was no data of the relevant species. (Harlingen 2018-02 was not sampled due to bad weather conditions). Min and max represent the lowest and the highest value of unique species found per location.

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

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

C: Transient species

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

D: Benthic species

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

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

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

2.4 Data analysis

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

Data analysis, statistical analysis and data visualisation were all performed in R (R Core Team 2023). All absolute read abundances [fish eDNA concentrations (12S copies/L)] were Hellinger transformed in R (Legendre and Gallagher 2001), executed by the function decostand in R from the vegan package (Oksanen et al. 2022). Statistical analysis was performed with vegan (Oksanen et al. 2024) and stats (R Core team 2023). Visualization of the data was done using the ggplot2 package (Wickham 2016). Fish species accumulation curves for the 8 locations calculated for both the regular accumulation curves plus calculated trough the sample-size- and coverage-based integrations of rarefaction (interpolation) and extrapolation (prediction) of Hill numbers represent a unified standardization method for quantifying and comparing species diversity across multiple assemblages according to Chao et al. (2014, 2016). Presence-absence per species for all locations together and per location separate was calculated and differences between locations were tested by performing an anova. Further analyses were done on both the absolute read abundances as well as the presence-absence data.

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

The second analysis done was for partitioning distance matrices among sources of variation and fitting linear models to distance matrices using a permanova by the adonis function. The output of continuous variables (vectors) gives the direction cosines which are the coordinates of the heads of unit length vectors. In the shown NMDS plots these are scaled by their correlation (square root of the column r2) so that "weak" predictors have shorter arrows than "strong" predictors. With this analysis, vector arrows of only species which have a significant role ($p \leq 0.05$) were shown in the results.

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

3. Results

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

3.1. Species composition

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

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

3.2. Spatial variability

3.2.1. Presence/absence of species

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

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

The high species richness at Cocksdorp and Marsdiep, both in total but also per sampling event, was caused by a higher number of core species found compared to the other stations (Table 2). With respect of the number of transient species, no clear pattern between stations was found, because the number of transient species found was overall low (on average 2 species). Most fish species found were benthic species. The average number of benthopelagic (1.6) and pelagic (2.0) species found over all locations and sampling dates was low. Therefore, no clear trend between location or sampling date could be observed in the number of benthic, benthopelagic and pelagic species (Table 2). Clupeidae (*Clupea harengus* and *Sprattus sprattus),* Pleuronectidae (*Pleuronectes platessa*, *Platichthys flesus* and *Limanda limanda*), *Pomatoschistus* spp. (*Pomatoschitus minutus* and *P. lozanoi*), Ammodytidae (*Ammodytes marinus* and *A. tobianus),* Gadidae *(Merlangius merlangus*, *Pollachius pollachius* and *P. virens) and* Cottidae (*Myoxocephalus scorpius* and *Taurulus bubalis*), together with *Pomastoschistus microps*, *Osmerus eperlanus*, *Dicentrarchus labrax*, *Syngnathus rostellatus*, *Solea solea* and *Zoarces viviparus,* in total potentially 20 species, were identified at all locations (Table S2). Eight other species were found at respectively 7 (*Sardina pilchardus* and *Alosa alosa*) and 6 locations (*Abramis brama, Pholis gunnellus, Lipophrys pholis, Gasterosteus aculeatus, Ciliata mustela* and *Trachurus trachurus*).

3.2.2. Nonmetric Multidimensional Scaling

The NMDS plot of the presence – absence data of all species caught during the year-round sampling showed that the various stations clustered relatively close to each other. Eight taxonomic groups significantly contributed (p≤0.05): the Clupeidae complex, the Mugilidae complex, the Pleuronectidae complex and *Solea solea*, *Pomatoschitus* spp. and three individual species (*Abramis brama*, *Atherina presbyter*, *Glyptocephalus cynoglossus)* (Fig 3A). The NMDS plot of the absolute read abundances of all species showed also a clustering relatively close to each other (Fig 3B). Five taxonomic groups significantly contributed: consisting of the Clupeidae complex, three *Pomatoschistus* species, *Eutrigla gurnardus*, *Melanogrammus aeglefinus* and *Osmerus eperlanus*. The NMDS plot of the presence – absence data and the plot of the absolute read abundances differed due to differences in the significantly contributing taxonomic groups. However, in both plots there was a significant contribution of the Clupeidae complex and the three *Pomatoschistus* species (Table S4).

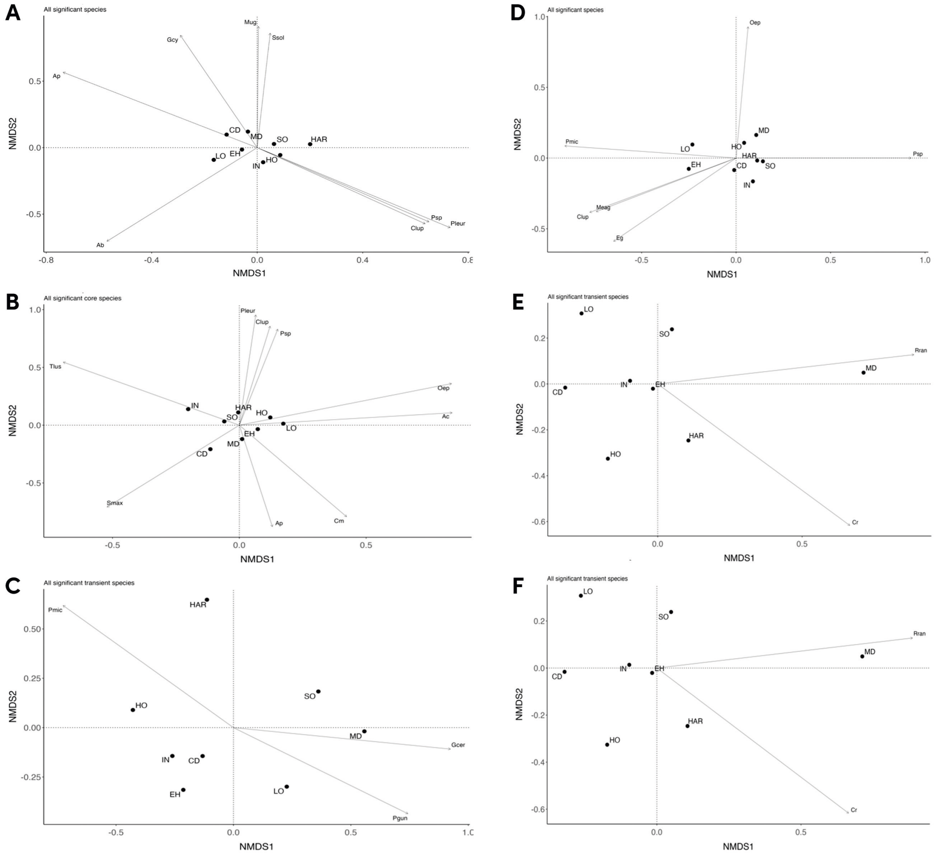

Figure 3 NMDS of the various stations, all samplings together. Only species with significant vectors are listed ($p \leq 0.05$). Left column: based on presence and absence; right column based on fish eDNA concentrations (12S copies/L). Top row: all species; middle row: core species and bottom row transient species. Abbreviations and their full names of locations and species can be found in Table 1 (locations) and Table 4 (species names).
A: All species from presence-absence data;
B: Core species from presence-absence data;
C: Transient species from presence-absence data;
D: All species from fish eDNA concentrations (12S copies/L);
E: Core species from fish eDNA concentrations (12S copies/L);
F: Transient species from fish eDNA concentrations (12S copies/L).

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

The NMDS plots of the transient species resulted in more variation between stations and only a few species significantly contributed (Fig 3E and 3F): two (*Gymnocephalus cernua* and *Pholis gunnellus*) for the presence-absence plot and two (*Chelon ramada* and *Raniceps raninus*) for the plot of the absolute read abundances (Table S4). For the complete data set with all species, see Supplementary materials Fig S2 and Table S5.

3.2.3. Jaccard/Sørensen dissimilarity index

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

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

4. Discussion

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

4.1. Species composition

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

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

4.2. Spatial variability

The aim of this study was to determine spatial variability of the Wadden Sea fish community in the various tidal basins by simultaneous sampling various locations and thereby excluding interannual variability in fish community and the impact of sampling design. In dynamic estuarine areas such as the Wadden Sea, species distribution (presence and abundance) will be dictated by the species' specific abiotic preferences and tolerance ranges (e.g. temperature, salinity, oxygen levels) for the different life stages (Neill et al. 1994, Freitas et al. 2010, Dahlke et al. 2020). Due to local variability hydrodynamic and morphodynamical variation, spatial variability in fish species distribution, composition and abundance within and between tidal basins might be expected as these have also been found in other studies (see for instance Tulp et al. 2008, Meyer et al. 2016). However, spatial variability of the fish community is constantly being reduced due to ongoing mixing due to tidal currents. The question is to what extend spatial variability still occurs in the Wadden at the scale of tidal basins and if so whether it will translate in differences in the food web structure.

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

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

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

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

Acknowledgements

Thanks to all of our colleagues, especially Sieme Gieles and Marco Kortenhoeven, for assisting in the collection of the fish samples of the fyke. And to our colleagues and students, Andre Dijkstra and Ewout Adriaans, Amber Keurhorst, Iris Grashuis, Kevin Sarelse, Floortje Heres, Rowan Stavast and Vincent van Ernich who helped us to collect all eDNA samples trough the good and the bad weather and even by snow, blizzards and temperatures below 0°C.

Supplementary materials

Table S1 Overview of all raw information about sampling and water to DNA filtration.

Table S2 Species and species groups identified by means of eDNA in water samples at eight stations in the Dutch Wadden Sea, together with presence of species in all samples (-).

Family group	Species	Species groups	Abbreviation	Core/ Transient	Functional group	
Clupeidae		*Clupea harengus - Sprattus sprattus*	Clup	Core	Pelagic	
Pleuronectidae		*Pleuronectes platessa - Platichthys flesus - Limanda limanda*	Pleur	Core	Benthic	
	Pomatoschistus spp.	*Pomatoschitus minutus - Pomatoschitus lozanoi*	Psp	Core	Benthic	
	Pomatoschistus microps		Pmic	Transient	Benthic	
Ammodytidae		*Ammodytes marinus - Ammodytes tobianus*	Amm	Core	Benthic	
	Osmerus eperlanus		Oep	Core	Pelagic	
	Abramis brama		Ab	Transient	Benthopelagic	
	Sardina pilchardus		Sp	Core	Pelagic	
	Dicentrarchus labrax		Dl	Core	Benthopelagic	
	Syngnathus rostellatus		Sros	Transient	Benthic	
Gadidae		*Merlangius merlangus - Pollachius pollachius - Pollachius virens*	Gad	Core	Benthopelagic	
	Solea solea		Ssol	Core	Benthic	
	Pholis gunnellus		Pgun	Transient	Benthic	
	Gymnocephalus cernua		Gcer	Transient	Benthopelagic	
	Chelon ramada		Cr	Core	Benthic	
	Melanogrammus aeglefinus		Meag	Transient	Benthopelagic	
	Rutilus rutilus		Rrur	Transient	Benthopelagic	
	Lipophrys pholis		Lp	Transient	Benthic	
	Gasterosteus aculeatus		Gac	Transient	Benthopelagic	
	Zoarces viviparus		Zv	Core	Benthic	
Cottidae		*Myoxocephalus scorpius - Taurulus bubalis*	Cot	Core	Benthic	
	Salmo trutta		St	Core	Pelagic	
	Anguilla anguilla		Aan	Core	Benthic	
	Ciliata mustela		Cm	Core	Benthic	
	Atherina presbyter		Ap	Core	Pelagic	
	Alosa alosa		Aal	Transient	Pelagic	
	Arnoglossus laterna		Al	Core	Benthic	
	Trachurus trachurus		Ttrac	Core	Pelagic	
	Raniceps raninus		Rran	Transient	Benthic	
	Coregonus oxyrinchus		Cox	Transient	Pelagic	
Mugilidae		*Chelon labrosus - Chelon auratus*	Mug	Core	Pelagic	

				CD Cocksdorp	EH Eemshaven	HAR Harlingen	HO Holwerd	IN Inschot	LO Lauwersoog	MD Marsdiep	SO Scheurrak Omdraai
	Total reads	% present	Counts >0	% present	% present	% present	% present	% present	% present	% present	% present
	6279563	0,97	108	1,00	0,93	0,92	0,93	1,00	0,93	1,00	1,00
	2820889	0,94	104	1,00	0,86	1,00	1,00	1,00	0,86	0,86	0,93
	2361369	0,86	95	1,00	0,79	0,77	0,93	0,86	0,79	0,86	0,86
	1164287	0,58	64	0,71	0,71	0,54	0,71	0,43	0,57	0,64	0,29
	1103791	0,53	59	0,93	0,29	0,38	0,36	0,64	0,43	0,71	0,50
	1093185	0,50	56	0,36	0,43	0,69	0,86	0,14	0,79	0,36	0,43
	839525	0,14	15	0,14	0,14	0,00	0,07	0,07	0,57	0,00	0,07
	486296	0,15	17	0,43	0,14	0,00	0,14	0,07	0,14	0,21	0,07
	355855	0,31	34	0,71	0,21	0,31	0,21	0,21	0,14	0,36	0,29
	161997	0,18	20	0,43	0,36	0,15	0,14	0,07	0,07	0,07	0,14
	160469	0,30	33	0,36	0,21	0,23	0,36	0,43	0,21	0,43	0,14
	157910	0,28	31	0,64	0,21	0,31	0,14	0,14	0,14	0,50	0,14
	152898	0,24	27	0,07	0,36	0,00	0,00	0,07	0,36	0,71	0,36
	135730	0,10	11	0,00	0,00	0,00	0,00	0,00	0,36	0,29	0,14
	122402	0,12	13	0,29	0,07	0,15	0,29	0,00	0,00	0,14	0,00
	102480	0,08	9	0,14	0,21	0,00	0,00	0,07	0,21	0,00	0,00
	88526	0,10	11	0,07	0,00	0,08	0,00	0,00	0,43	0,14	0,07
	71266	0,05	6	0,07	0,07	0,00	0,07	0,07	0,07	0,07	0,00
	63833	0,19	21	0,21	0,14	0,00	0,43	0,14	0,36	0,21	0,00
	63783	0,30	33	0,21	0,43	0,46	0,14	0,14	0,36	0,36	0,29
	62687	0,20	22	0,21	0,14	0,08	0,14	0,14	0,07	0,64	0,14
	39815	0,05	6	0,21	0,00	0,00	0,07	0,00	0,00	0,07	0,07
	33614	0,06	7	0,00	0,21	0,00	0,00	0,07	0,14	0,07	0,00
	28406	0,11	12	0,21	0,07	0,00	0,07	0,00	0,14	0,29	0,07
	28180	0,06	7	0,29	0,07	0,00	0,00	0,00	0,07	0,07	0,00
	26775	0,11	12	0,07	0,00	0,23	0,14	0,14	0,07	0,14	0,07
	26577	0,03	3	0,07	0,00	0,00	0,00	0,14	0,00	0,00	0,00
	24444	0,10	11	0,29	0,07	0,15	0,00	0,07	0,14	0,00	0,07
	18584	0,02	2	0,00	0,07	0,00	0,00	0,00	0,00	0,07	0,00
	18377	0,01	1	0,00	0,00	0,00	0,00	0,00	0,00	0,07	0,00
	17644	0,08	9	0,29	0,07	0,08	0,00	0,00	0,00	0,14	0,07

Table S2 Species and species groups identified by means of eDNA in water samples at eight stations in the Dutch Wadden Sea, together with presence of species in all samples (-). *(continued)*

Family group	Species	Species groups	Abbreviation	Core/ Transient	Functional group	
	Cyclopterus lumpus		Clum	Core	Benthic	
	Trisopterus spp.	*Trisoperus luscus – Trisopterus minutus*	Tlus	Core	Benthopelagic	
	Belone belone		Bbe	Core	Pelagic	
	Leuciscus idus		Lid	Transient	Benthopelagic	
	Scophthalmus maximus		Smax	Core	Benthic	
	Engraulis encrasicolus		Een	Core	Pelagic	
	Eutrigla gurnardus		Eg	Transient	Benthopelagic	
	Ballerus ballerus		Bba	Transient	Benthopelagic	
	Parablennius gattorugine		Pgat	Transient	Benthic	
	Gobiusculus flavescens		Pflav	Transient	Benthic	
	Hyperoplus lanceolatus		Hl	Core	Benthic	
	Agonus cataphractus		Ac	Core	Benthic	
	Syngnathus acus		Sac	Core	Benthic	
	Liparis liparis		Lli	Transient	Benthic	
	Scomber scombrus		Ssc	Core	Pelagic	
	Microstomus kitt		Mk	Transient	Benthic	
	Glyptocephalus cynoglossus		Gcy	Transient	Benthic	

Table S3 Overview of the absolute read abundances data of all found species for each location and time.

				CD Cocksdorp	EH Eemshaven	HAR Harlingen	HO Holwerd	IN Inschot	LO Lauwersoog	MD Marsdiep	SO Scheurrak Omdraai
	Total reads	% present	Counts >0	% present	% present	% present	% present	% present	% present	% present	% present
	17084	0,05	6	0,00	0,07	0,00	0,07	0,00	0,07	0,21	0,00
	14716	0,07	8	0,00	0,00	0,08	0,00	0,29	0,00	0,07	0,14
	12729	0,05	5	0,00	0,00	0,00	0,00	0,07	0,00	0,14	0,14
	12699	0,03	3	0,00	0,00	0,00	0,00	0,00	0,21	0,00	0,00
	10943	0,09	10	0,50	0,07	0,00	0,00	0,07	0,00	0,07	0,00
	8537	0,04	4	0,14	0,07	0,00	0,00	0,00	0,00	0,00	0,07
	6896	0,05	5	0,07	0,14	0,00	0,00	0,07	0,07	0,00	0,00
	6533	0,02	2	0,00	0,00	0,00	0,00	0,00	0,14	0,00	0,00
	5510	0,02	2	0,00	0,00	0,00	0,00	0,00	0,00	0,14	0,00
	3814	0,01	1	0,00	0,00	0,00	0,00	0,00	0,00	0,07	0,00
	3056	0,02	2	0,00	0,00	0,00	0,00	0,14	0,00	0,00	0,00
	1767	0,04	4	0,00	0,07	0,00	0,07	0,00	0,14	0,00	0,00
	1581	0,01	1	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	575	0,01	1	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	432	0,02	2	0,07	0,00	0,00	0,00	0,00	0,07	0,00	0,00
	394	0,01	1	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00
	292	0,02	2	0,07	0,00	0,00	0,00	0,00	0,00	0,07	0,00

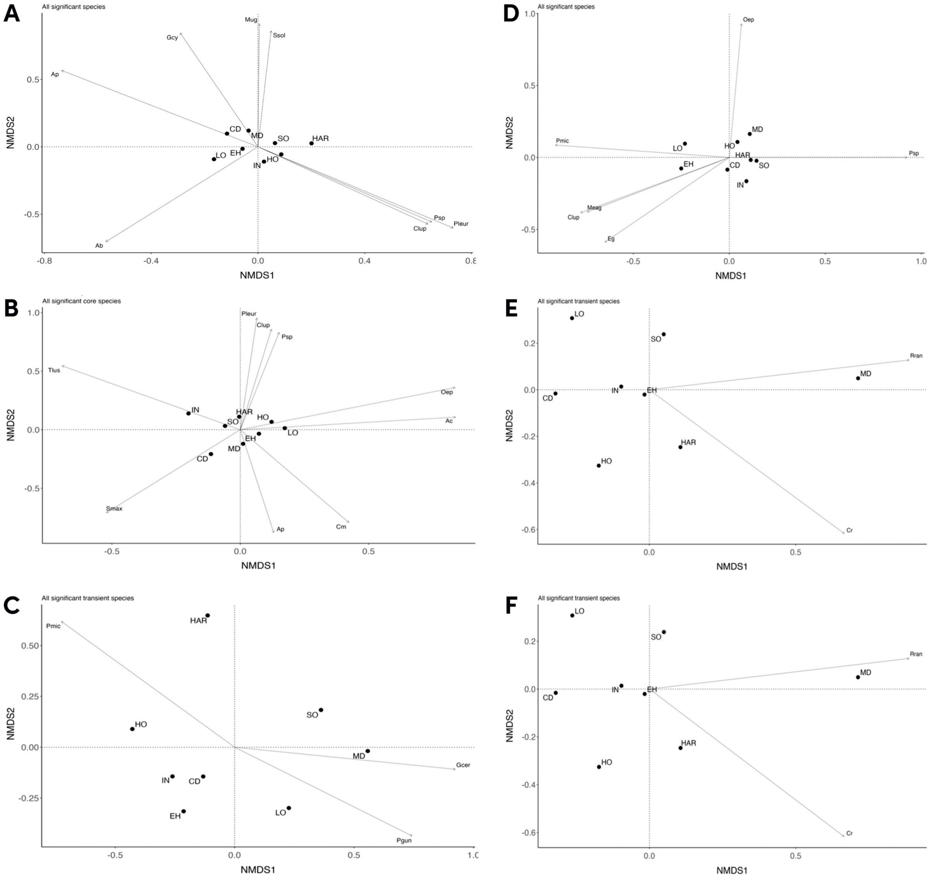

Figure S1 NMDS of the various stations, all samplings together. Left column: based on presence and absence; right column based on fish eDNA concentrations per L water sampled. Top row: all species; middle row: core species and bottom row transient species. Abbreviations and their full names of locations and species can be found in Table 1 (locations) and Table S2 (species names).
A: All species from presence-absence data;
B: Core species from presence-absence data;
C: Transient species from presence-absence data;
D: All species from fish eDNA concentrations (12S copies/L);
E: Core species from fish eDNA concentrations (12S copies/L);
F: Transient species from fish eDNA concentrations (12S copies/L).

Table S4 Detailed data of the different NMDS graphs of Fig 3. Table S4a represents the data from presence-absence of the different species. Table S4b represents the data from the eDNA concentrations (12S copies/L) of the different species.
I: Axis NMDS data for each location considering: all species, core species and transient species.
II: Axis NMDS data and p-values for all species.
III: Axis NMDS data and p-values for core species.
IV: Axis NMDS data and p-values for transient species.

A		All species		Core		Transient	
I	**Locations**	**NMDS1**	**NMDS2**	**NMDS1**	**NMDS2**	**NMDS1**	**NMDS2**
	CD	-0,12	0,10	-0,11	-0,21	-0,13	-0,14
	EH	-0,06	-0,01	0,07	-0,03	-0,21	-0,32
	HAR	0,20	0,03	0,00	0,11	-0,11	0,65
	HO	0,09	-0,06	0,12	0,07	-0,43	0,09
	IN	0,02	-0,11	-0,20	0,14	-0,26	-0,14
	LO	-0,16	-0,09	0,17	0,01	0,23	-0,30
	MD	-0,04	0,12	0,01	-0,12	0,56	-0,02
	SO	0,06	0,03	-0,06	0,03	0,36	0,18

II	**All species**		**NMDS1**	**NMDS2**	**p-value**
		Ab	-0,57	-0,70	0,01
		Ap	-0,74	0,57	0,01
		Clup	0,64	-0,57	0,05
		Gcy	-0,29	0,85	0,04
		Mug	0,00	0,91	0,01
		Pleur	0,73	-0,60	0,01
		Psp	0,65	-0,56	0,04
		Ssol	0,05	0,86	0,04

III	**Core species**		**NMDS1**	**NMDS2**	**p-value**
		Ac	0,84	0,11	0,04
		Ap	0,13	-0,88	0,02
		Cm	0,42	-0,79	0,01
		Clup	0,12	0,86	0,04
		Oep	0,84	0,36	0,01
		Pleur	0,06	0,95	0,00
		Psp	0,15	0,83	0,04
		Smax	-0,52	-0,71	0,02
		Tlus	-0,69	0,55	0,03

IV	**Transient species**		**NMDS1**	**NMDS2**	**p-value**
		Gcer	0,92	-0,11	0,03
		Pgun	0,74	-0,44	0,05
		Pmic	-0,73	0,62	0,01

Table S4 Detailed data of the different NMDS graphs of Fig 3. Table S4a represents the data from presence-absence of the different species. Table S4b represents the data from the eDNA concentrations (12S copies/L) of the different species. *(continued)*
I: Axis NMDS data for each location considering: all species, core species and transient species.
II: Axis NMDS data and p-values for all species.
III: Axis NMDS data and p-values for core species.
IV: Axis NMDS data and p-values for transient species.

B		All species		Core		Transient	
I	**Locations**	**NMDS1**	**NMDS2**	**NMDS1**	**NMDS2**	**NMDS1**	**NMDS2**
	CD	-0,01	-0,08	-0,15	-0,20	-0,32	-0,02
	EH	-0,25	-0,08	0,12	-0,20	-0,02	-0,02
	HAR	0,11	-0,02	-0,05	0,07	0,11	-0,25
	HO	0,04	0,11	0,14	0,21	-0,17	-0,33
	IN	0,09	-0,16	-0,25	0,05	-0,10	0,01
	LO	-0,23	0,10	0,23	-0,06	-0,26	0,31
	MD	0,11	0,16	0,08	0,05	0,71	0,05
	SO	0,14	-0,02	-0,12	0,08	0,05	0,24

II	All species		NMDS1	NMDS2	p-value
		Clup	-0,77	-0,38	0,03
		Eg	-0,65	-0,59	0,04
		Meag	-0,74	-0,38	0,00
		Oep	0,06	0,93	0,01
		Pmic	-0,91	0,09	0,01
		Psp	0,93	0,00	0,00

III	Core species		NMDS1	NMDS2	p-value
		Amm	-0,86	-0,17	0,03
		Clup	0,35	-0,84	0,01
		Oep	0,68	0,63	0,01
		Psp	-0,46	0,86	0,00

IV	Transient species		NMDS1	NMDS2	p-value
		Cr	0,67	-0,62	0,02
		Rran	0,89	0,13	0,05

Table S5 Detailed data of the different NMDS graphs of Fig 3. Table S5a represents the data from presence-absence of the different species. Table S5b represents the data from the fish eDNA concentrations per L water sampled of the different species.
I: Axis NMDS data and p-values for all species;
II: Axis NMDS data and p-values for core species;
III: Axis NMDS data and p-values for transient species.

A			
I: All species			
	NMDS1	NMDS2	p-value
Ab	-0,57	-0,70	0,01
Ac	-0,30	-0,61	0,21
Aal	0,69	-0,09	0,18
Amm	0,05	0,20	0,90
Aan	-0,48	-0,46	0,26
Al	-0,10	-0,27	0,81
Ap	-0,74	0,57	0,01
Bba	-0,48	-0,53	0,13
Bbe	0,11	0,20	0,85
Cr	0,22	0,48	0,43
Cm	-0,55	0,61	0,07
Clup	0,64	-0,57	0,04
Cox	-0,09	0,59	0,51
Cot	-0,01	0,60	0,36
Clum	-0,33	0,09	0,71
Dl	0,23	0,74	0,12
Een	-0,21	0,43	0,50
Eg	-0,55	-0,51	0,12
Gad	0,25	-0,30	0,64
Gac	-0,45	-0,46	0,26
Gcy	-0,29	0,85	0,03
Pflav	-0,09	0,59	0,51
Gcer	-0,38	0,16	0,61
Hl	0,06	-0,53	0,61
Lid	-0,48	-0,53	0,13
Lli	-0,32	0,53	0,38
Lp	-0,54	-0,43	0,21
Meag	-0,67	-0,49	0,06
Mk	0,68	0,17	0,24
Mug	0,00	0,91	0,01
Oep	0,37	-0,30	0,50
Pgat	-0,09	0,59	0,51
Pgun	-0,50	0,28	0,38
Pleur	0,73	-0,60	0,00
Pmic	0,20	-0,28	0,77
Psp	0,65	-0,56	0,04
Rran	-0,19	0,38	0,61
Rrur	-0,30	0,21	0,70

B			
I: All species			
	NMDS1	NMDS2	p-value
Ab	-0,44	-0,23	0,47
Ac	-0,64	0,54	0,06
Aal	0,50	-0,33	0,35
Amm	0,47	-0,67	0,07
Aan	-0,76	-0,17	0,10
Al	0,17	-0,65	0,22
Ap	-0,30	0,31	0,59
Bba	-0,54	0,43	0,22
Bbe	0,33	-0,53	0,29
Cr	0,07	0,44	0,56
Cm	-0,11	0,65	0,24
Clup	-0,77	-0,38	0,03
Cox	0,21	0,61	0,37
Cot	-0,26	0,17	0,74
Clum	0,07	0,70	0,16
Dl	0,41	0,00	0,63
Een	-0,28	-0,47	0,41
Eg	-0,65	-0,59	0,03
Gad	0,07	-0,26	0,82
Gac	-0,22	0,72	0,14
Gcy	0,16	0,27	0,86
Gcer	-0,35	0,48	0,28
Hl	0,17	-0,61	0,51
Lid	-0,54	0,43	0,22
Lli	-0,02	-0,31	0,89
Lp	0,18	0,59	0,43
Meag	-0,74	-0,38	0,01
Mk	0,28	-0,08	1,00
Mug	0,22	0,43	0,53
Oep	0,06	0,93	0,01
Pgat	0,21	0,61	0,37
Pgun	-0,22	0,18	0,78
Pleur	0,75	-0,30	0,08
Pflav	0,21	0,61	0,37
Pmic	-0,91	0,09	0,01
Psp	0,93	0,00	0,01
Rran	0,05	0,50	0,53
Rrur	-0,24	0,36	0,55

A

I: All species

	NMDS1	NMDS2	p-value
Sp	-0,74	0,29	0,06
Ssc	-0,74	-0,02	0,14
Smax	-0,41	0,48	0,30
Ssol	0,05	0,86	0,04
Sac	-0,32	0,53	0,38
Sros	0,02	0,24	0,84
Ttrac	-0,10	-0,06	0,96
Tlus	0,48	-0,17	0,46
Zv	0,30	0,16	0,74

II: Core species

	NMDS1	NMDS2	p-value
Ac	0,84	0,11	0,05
Amm	-0,77	-0,16	0,09
Aan	0,29	-0,01	0,84
Al	-0,80	0,08	0,07
Ap	0,13	-0,88	0,02
Bbe	-0,49	0,08	0,48
Cr	0,20	-0,33	0,68
Cm	0,42	-0,79	0,01
Clup	0,12	0,86	0,04
Cot	-0,12	-0,43	0,52
Clum	0,71	-0,28	0,12
Dl	-0,56	-0,53	0,11
Een	-0,23	-0,52	0,35
Gad	-0,23	0,30	0,66
Hl	-0,64	0,50	0,13
Mug	-0,27	-0,69	0,14
Oep	0,84	0,36	0,01
Pleur	0,06	0,95	0,00
Psp	0,15	0,83	0,03
St	-0,16	-0,60	0,30
Sp	0,10	-0,74	0,14
Ssc	0,22	-0,44	0,50
Smax	-0,52	-0,71	0,02
Ssol	-0,21	-0,69	0,17
Sac	-0,36	-0,72	0,25
Ttrac	-0,24	-0,02	0,86
Tlus	-0,69	0,55	0,02
Zv	0,44	0,18	0,53

B

I: All species

	NMDS1	NMDS2	p-value
Sp	0,01	-0,20	0,98
Ssc	-0,52	0,12	0,41
Smax	0,01	-0,49	0,51
Ssol	0,12	0,80	0,07
Sac	-0,02	-0,31	0,89
Sros	-0,29	-0,41	0,50
Ttrac	-0,19	-0,53	0,38
Tlus	0,50	-0,59	0,12
Zv	0,12	-0,18	0,90

II: Core species

	NMDS1	NMDS2	p-value
Ac	0,83	-0,05	0,06
Amm	-0,86	-0,17	0,04
Aan	0,40	-0,57	0,19
Al	-0,65	0,09	0,27
Ap	0,19	-0,63	0,25
Bbe	-0,62	0,27	0,22
Cm	0,64	0,35	0,13
Clup	0,35	-0,84	0,01
Cot	0,31	-0,22	0,66
Clum	0,42	0,16	0,66
Dl	-0,23	0,10	0,86
Een	-0,03	-0,62	0,32
Gad	-0,23	-0,46	0,46
Gac	0,71	0,22	0,12
Hl	-0,60	0,16	0,52
Mug	0,03	-0,14	0,95
Oep	0,68	0,63	0,01
Pleur	-0,49	0,56	0,12
Psp	-0,46	0,86	0,00
St	-0,34	0,14	0,71
Sp	-0,27	-0,49	0,53
Ssc	0,22	-0,53	0,37
Smax	-0,47	-0,63	0,06
Ssol	0,51	0,20	0,40
Sac	-0,33	-0,58	0,23
Ttrac	-0,11	-0,44	0,56
Tlus	-0,70	0,40	0,07
Zv	-0,05	-0,01	0,99

A			
III: Transient species			
	NMDS1	NMDS2	p-value
Ab	-0,17	-0,69	0,17
Aal	-0,13	0,81	0,06
Bba	0,25	-0,39	0,75
Cox	0,66	-0,03	0,25
Eg	-0,37	-0,74	0,06
Gac	-0,41	-0,60	0,16
Gcy	0,34	-0,18	0,75
Pflav	0,66	-0,03	0,25
Gcer	0,92	-0,11	0,03
Lid	0,25	-0,39	0,75
Lli	-0,15	-0,19	1,00
Lp	-0,40	-0,73	0,07
Meag	-0,30	-0,78	0,04
Mk	-0,12	0,83	0,12
Pgat	0,66	-0,03	0,25
Pgun	0,74	-0,44	0,04
Pmic	-0,73	0,62	0,00
Rran	0,26	-0,35	0,68
Rrur	0,71	0,30	0,12
Sros	-0,48	0,26	0,43

B			
III: Transient species			
	NMDS1	NMDS2	p-value
Ab	-0,61	0,50	0,09
Aal	-0,13	-0,39	0,65
Bba	-0,23	0,62	0,24
Cr	0,66	-0,62	0,01
Cox	0,88	0,14	0,13
Eg	-0,21	0,22	0,73
Gcy	0,48	0,09	0,47
Gcer	-0,04	0,83	0,06
Lid	-0,23	0,62	0,24
Lli	-0,40	-0,05	0,74
Lp	0,84	0,02	0,10
Meag	-0,15	0,15	0,87
Mk	0,09	-0,47	0,50
Pgat	0,88	0,14	0,13
Pgun	0,37	0,70	0,08
Pflav	0,88	0,14	0,13
Pmic	-0,27	-0,65	0,18
Rran	0,89	0,13	0,06
Rrur	-0,03	0,58	0,37
Sros	0,00	-0,35	0,73

Table S6 Jaccard/Sorensen dissimilarity index measures the overlap between two populations and divides the number of species shared by both samples by the sum of all species occurring in both samples (dissimilarity = 1 – similarity). The index varies between zero (identical in composition) and one (no common elements). Analyses according to the presence absence data of each species for each location. Green colors indicate the highest value, yellow the 50% percentile of the values and red the lowest value.

A: All species

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,32	0,53	0,44	0,39	0,38	0,42	0,41
Eemshaven	0,32		0,56	0,38	0,37	0,31	0,40	0,47
Harlingen	0,53	0,56		0,48	0,52	0,57	0,53	0,37
Holwerd	0,44	0,38	0,48		0,47	0,39	0,44	0,43
Inschot	0,39	0,37	0,52	0,47		0,39	0,48	0,42
Lauwersoog	0,38	0,31	0,57	0,39	0,39		0,45	0,44
Marsdiep	0,42	0,40	0,53	0,44	0,48	0,45		0,41
Scheurrak	0,41	0,47	0,37	0,43	0,42	0,44	0,41	
Dissimilarity	0,41	0,40	0,51	0,43	0,43	0,42	0,45	0,42
Similarity	0,59	0,59	0,50	0,57	0,56	0,57	0,55	0,57

B: Core species

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,28	0,43	0,42	0,46	0,40	0,35	0,33
Eemshaven	0,28		0,41	0,32	0,44	0,23	0,25	0,38
Harlingen	0,43	0,41		0,42	0,40	0,48	0,41	0,32
Holwerd	0,42	0,32	0,42		0,52	0,30	0,32	0,38
Inschot	0,46	0,44	0,40	0,52		0,43	0,38	0,36
Lauwersoog	0,40	0,23	0,48	0,30	0,43		0,38	0,43
Marsdiep	0,35	0,25	0,41	0,32	0,38	0,38		0,30
Scheurrak	0,33	0,38	0,32	0,38	0,36	0,43	0,30	
Dissimilarity	0,38	0,33	0,41	0,38	0,43	0,38	0,34	0,36
Similarity	0,62	0,67	0,59	0,62	0,57	0,62	0,66	0,64

C: Transient species

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,38	0,69	0,50	0,25	0,33	0,53	0,54
Eemshaven	0,38		0,83	0,50	0,20	0,43	0,63	0,67
Harlingen	0,69	0,83		0,63	0,73	0,71	0,71	0,50
Holwerd	0,50	0,50	0,63		0,33	0,54	0,64	0,56
Inschot	0,25	0,20	0,73	0,33		0,31	0,63	0,55
Lauwersoog	0,33	0,43	0,71	0,54	0,31		0,56	0,46
Marsdiep	0,53	0,63	0,71	0,64	0,63	0,56		0,57
Scheurrak	0,54	0,67	0,50	0,56	0,55	0,46	0,57	
Dissimilarity	0,46	0,52	0,69	0,53	0,43	0,48	0,61	0,55
Similarity	0,54	0,48	0,31	0,47	0,57	0,52	0,39	0,45

Table S7 Jaccard/Sorensen dissimilarity index measures the overlap between two populations and divides the number of species shared by both samples by the sum of all species occurring in both samples (dissimilarity = 1 – similarity). The index varies between zero (identical in composition) and one (no common elements). Analyses according to the presence absence data of each species for each sampling campaign between locations. Sampling campaigns are indicated as year-month and (when necessary) per week (May 2018). Green colors indicate the highest value, yellow the 50% percentile of the values and red the lowest value.

2018-02

Location	Cocksdorp	Eemshaven	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,24	0,25	0,41	0,52	0,40	0,29
Eemshaven	0,24		0,43	0,55	0,46	0,36	0,47
Holwerd	0,25	0,43		0,41	0,43	0,30	0,29
Inschot	0,41	0,55	0,41		0,64	0,52	0,47
Lauwersoog	0,52	0,46	0,43	0,64		0,28	0,37
Marsdiep	0,40	0,36	0,30	0,52	0,28		0,33
Scheurrak	0,29	0,47	0,29	0,47	0,37	0,33	
Dissimilarity	0,35	0,42	0,35	0,50	0,45	0,37	0,37
Similarity	0,65	0,58	0,65	0,50	0,55	0,63	0,63

2018-03

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,70	0,47	0,47	0,37	0,50	0,43	0,53
Eemshaven	0,70		0,69	0,53	0,53	0,56	0,62	0,73
Harlingen	0,47	0,69		0,50	0,50	0,54	0,62	0,40
Holwerd	0,47	0,53	0,50		0,50	0,53	0,52	0,57
Inschot	0,37	0,53	0,50	0,50		0,41	0,36	0,43
Lauwersoog	0,50	0,56	0,54	0,53	0,41		0,54	0,20
Marsdiep	0,43	0,62	0,62	0,52	0,36	0,54		0,57
Scheurrak	0,53	0,73	0,40	0,57	0,43	0,20	0,57	
Dissimilarity	0,50	0,62	0,53	0,52	0,44	0,47	0,52	0,49
Similarity	0,50	0,39	0,46	0,47	0,55	0,54	0,46	0,52

2018-04

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,50	0,33	0,53	0,38	0,63	0,38	0,54
Eemshaven	0,50		0,54	0,60	0,45	0,57	0,45	0,27
Harlingen	0,33	0,54		0,71	0,40	0,69	0,40	0,40
Holwerd	0,53	0,60	0,71		0,50	0,47	0,67	0,67
Inschot	0,38	0,45	0,40	0,50		0,64	0,25	0,25
Lauwersoog	0,63	0,57	0,69	0,47	0,64		0,64	0,64
Marsdiep	0,38	0,45	0,40	0,67	0,25	0,64		0,25
Scheurrak	0,54	0,27	0,40	0,67	0,25	0,64	0,25	
Dissimilarity	0,47	0,48	0,50	0,59	0,41	0,61	0,43	0,43
Similarity	0,53	0,52	0,48	0,40	0,58	0,39	0,56	0,59

2018-05,19

Location	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Cocksdorp		0,57	0,38	0,41		0,33	0,38	0,29
Eemshaven	0,57		0,45	0,47	0,38	0,38	0,57	0,50
Harlingen	0,38	0,45		0,43	0,64	0,50	0,38	0,09
Holwerd	0,41	0,47	0,43		0,20	0,38	0,41	0,47
Inschot	0,38	0,64	0,20	0,57	0,57	0,67	0,54	0,27
Lauwersoog	0,33	0,38	0,50	0,38	0,67		0,47	0,38
Marsdiep	0,38	0,57	0,38	0,41	0,54	0,47		0,29
Scheurrak	0,29	0,50	0,09	0,47	0,27	0,38	0,29	
Dissimilarity	0,39	0,51	0,35	0,45	0,47	0,44	0,43	0,33
Similarity	0,61	0,50	0,66	0,55	0,53	0,54	0,56	0,67

2018-05,22

Location	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Cocksdorp		0,69	0,67	0,33	0,50	0,71	0,60	0,57
Eemshaven	0,69		0,71	0,80	0,71	0,78	0,60	0,78
Harlingen	0,67	0,71		0,56	0,33	0,25	0,33	0,50
Holwerd	0,33	0,80	0,56		0,56	0,64	0,50	0,27
Inschot	0,50	0,71	0,33	0,56		0,50	0,56	0,50
Lauwersoog	0,71	0,78	0,25	0,64	0,50		0,45	0,60
Marsdiep	0,60	0,60	0,33	0,50	0,56	0,45		0,64
Scheurrak	0,57	0,78	0,50	0,27	0,50	0,60	0,64	
Dissimilarity	0,58	0,73	0,48	0,52	0,52	0,56	0,53	0,55
Similarity	0,42	0,27	0,55	0,45	0,47	0,46	0,49	0,45

2018-06

Location	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Cocksdorp		0,53	0,43	0,47	0,60	0,48	0,39	0,60
Eemshaven	0,53		0,43	0,33	0,38	0,50	0,63	0,25
Harlingen	0,43	0,43		0,38	0,41	0,40	0,60	0,50
Holwerd	0,47	0,33	0,38		0,47	0,33	0,44	0,40
Inschot	0,60	0,38	0,41	0,47		0,47	0,68	0,45
Lauwersoog	0,48	0,50	0,40	0,33	0,47		0,55	0,57
Marsdiep	0,39	0,63	0,60	0,44	0,68	0,55		0,57
Scheurrak	0,60	0,25	0,50	0,40	0,45	0,57	0,57	
Dissimilarity	0,50	0,44	0,45	0,40	0,50	0,47	0,55	0,48
Similarity	0,50	0,58	0,55	0,61	0,52	0,53	0,42	0,54

2018-07								
Location	**Cocks-dorp**	**Eems-haven**	**Harlingen**	**Holwerd**	**Inschot**	**Lauwers-oog**	**Mars-diep**	**Scheurrak**
Cocksdorp		0,53	0,43	0,47	0,60	0,48	0,39	0,60
Eemshaven	0,53		0,43	0,33	0,38	0,50	0,63	0,25
Harlingen	0,43	0,43		0,38	0,41	0,40	0,60	0,50
Holwerd	0,47	0,33	0,38		0,47	0,33	0,44	0,40
Inschot	0,60	0,38	0,41	0,47		0,47	0,68	0,45
Lauwersoog	0,48	0,50	0,40	0,33	0,47		0,55	0,57
Marsdiep	0,39	0,63	0,60	0,44	0,68	0,55		0,57
Scheurrak	0,60	0,25	0,50	0,40	0,45	0,57	0,57	
Dissimilarity	0,50	0,44	0,45	0,40	0,50	0,47	0,55	0,48
Similarity	0,50	0,58	0,55	0,61	0,52	0,53	0,42	0,54

2018-08								
Location	**Cocks-dorp**	**Eems-haven**	**Harlingen**	**Holwerd**	**Inschot**	**Lauwers-oog**	**Mars-diep**	**Scheurrak**
Cocksdorp		0,42	0,39	0,62	0,60	0,60	0,43	0,60
Eemshaven	0,42		0,53	0,33	0,57	0,68	0,47	0,57
Harlingen	0,39	0,53		0,43	0,38	0,67	0,57	0,38
Holwerd	0,62	0,33	0,43		0,45	0,63	0,50	0,45
Inschot	0,60	0,57	0,38	0,45		0,60	0,64	0,20
Lauwersoog	0,60	0,68	0,67	0,63	0,60		0,50	0,60
Marsdiep	0,43	0,47	0,57	0,50	0,64	0,50		0,64
Scheurrak	0,60	0,57	0,38	0,45	0,20	0,60	0,64	
Dissimilarity	0,52	0,51	0,48	0,49	0,49	0,61	0,53	0,49
Similarity	0,48	0,47	0,51	0,53	0,53	0,39	0,45	0,53

2018-09								
Location	**Cocks-dorp**	**Eems-haven**	**Harlingen**	**Holwerd**	**Inschot**	**Lauwers-oog**	**Mars-diep**	**Scheurrak**
Cocksdorp		0,24	0,44	0,47	0,43	0,46	0,31	0,56
Eemshaven	0,24		0,38	0,43	0,50	0,43	0,33	0,38
Harlingen	0,44	0,38		0,45	0,54	0,56	0,56	0,40
Holwerd	0,47	0,43	0,45		0,57	0,58	0,47	0,45
Inschot	0,43	0,50	0,54	0,57		0,52	0,52	0,38
Lauwersoog	0,46	0,43	0,56	0,58	0,52		0,46	0,67
Marsdiep	0,31	0,33	0,56	0,47	0,52	0,46		0,67
Scheurrak	0,56	0,38	0,40	0,45	0,38	0,67	0,67	
Dissimilarity	0,42	0,39	0,48	0,49	0,50	0,53	0,47	0,50
Similarity	0,58	0,59	0,52	0,51	0,49	0,46	0,50	0,51

2018-10

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,39	0,52	0,39	0,45	0,57	0,33	0,45
Eemshaven	0,39		0,33	0,50	0,37	0,50	0,43	0,37
Harlingen	0,52	0,33		0,44	0,41	0,33	0,37	0,53
Holwerd	0,39	0,50	0,44		0,37	0,50	0,33	0,37
Inschot	0,45	0,37	0,41	0,37		0,37	0,20	0,33
Lauwersoog	0,57	0,50	0,33	0,50	0,37		0,52	0,58
Marsdiep	0,33	0,43	0,37	0,33	0,20	0,52		0,40
Scheurrak	0,45	0,37	0,53	0,37	0,33	0,58	0,40	
Dissimilarity	0,44	0,41	0,42	0,42	0,36	0,48	0,37	0,43
Similarity	0,56	0,58	0,60	0,58	0,66	0,53	0,62	0,57

2018-11

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,60	0,45	0,52	0,62	0,57	0,44	0,62
Eemshaven	0,60		0,33	0,27	0,45	0,23	0,47	0,27
Harlingen	0,45	0,33		0,23	0,38	0,33	0,65	0,54
Holwerd	0,52	0,27	0,23		0,33	0,29	0,63	0,33
Inschot	0,62	0,45	0,38	0,33		0,43	0,50	0,50
Lauwersoog	0,57	0,23	0,33	0,29	0,43		0,44	0,43
Marsdiep	0,44	0,47	0,65	0,63	0,50	0,44		0,38
Scheurrak	0,62	0,27	0,54	0,33	0,50	0,43	0,38	
Dissimilarity	0,55	0,38	0,42	0,37	0,46	0,39	0,50	0,44
Similarity	0,45	0,66	0,59	0,65	0,57	0,64	0,49	0,59

2018-12

Location	Cocksdorp	Eemshaven	Harlingen	Holwerd	Inschot	Lauwersoog	Marsdiep	Scheurrak
Cocksdorp		0,52	0,63	0,44	0,37	0,30	0,55	0,52
Eemshaven	0,52		0,60	0,41	0,56	0,55	0,43	0,40
Harlingen	0,63	0,60		0,50	0,38	0,65	0,75	0,33
Holwerd	0,44	0,41	0,50		0,47	0,47	0,56	0,29
Inschot	0,37	0,56	0,38	0,47		0,30	0,58	0,44
Lauwersoog	0,30	0,55	0,65	0,47	0,30		0,39	0,45
Marsdiep	0,55	0,43	0,75	0,56	0,58	0,39		0,33
Scheurrak	0,52	0,40	0,33	0,29	0,44	0,45	0,33	
Dissimilarity	0,48	0,50	0,55	0,45	0,44	0,45	0,51	0,40
Similarity	0,52	0,51	0,46	0,55	0,54	0,53	0,49	0,62

2019-01								
Location	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Cocksdorp		0,54	0,50	0,41	0,45	0,91	0,48	0,50
Eemshaven	0,54		0,25	0,20	0,29	0,64	0,37	0,13
Harlingen	0,50	0,25		0,18	0,16	0,85	0,24	0,11
Holwerd	0,41	0,20	0,18		0,11	0,83	0,30	0,18
Inschot	0,45	0,29	0,16	0,11		0,86	0,27	0,16
Lauwersoog	0,91	0,64	0,85	0,83	0,86		0,75	0,69
Marsdiep	0,48	0,37	0,24	0,30	0,27	0,75		0,24
Scheurrak	0,50	0,13	0,11	0,18	0,16	0,69	0,24	
Dissimilarity	0,54	0,34	0,33	0,31	0,33	0,79	0,38	0,29
Similarity	0,46	0,69	0,70	0,70	0,69	0,23	0,64	0,75

2019-02								
Location	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Cocksdorp		0,38	0,33	0,47	0,08	0,29	0,37	0,60
Eemshaven	0,38		0,26	0,37	0,41	0,33	0,39	0,47
Harlingen	0,33	0,26		0,33	0,38	0,30	0,45	0,33
Holwerd	0,47	0,37	0,33		0,50	0,30	0,45	0,44
Inschot	0,08	0,41	0,38	0,50		0,33	0,40	0,63
Lauwersoog	0,29	0,33	0,30	0,30	0,33		0,33	0,50
Marsdiep	0,37	0,39	0,45	0,45	0,40	0,33		0,55
Scheurrak	0,60	0,47	0,33	0,44	0,63	0,50	0,55	
Dissimilarity	0,36	0,37	0,34	0,41	0,39	0,34	0,42	0,50
Similarity	0,64	0,63	0,66	0,60	0,56	0,65	0,57	0,51

Average dissimilarity	0,46	0,40	0,42	0,41	0,41	0,50	0,44	0,43
Average similarity	0,53	0,60	0,57	0,58	0,57	0,52	0,55	0,58

	Cocks-dorp	Eems-haven	Harlingen	Holwerd	Inschot	Lauwers-oog	Mars-diep	Scheurrak
Overall average dissimilarity	0,47	0,46	0,43	0,45	0,44	0,49	0,46	0,44
Overall average similarity	0,53	0,54	0,56	0,55	0,55	0,52	0,53	0,56

6

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

Poiesz SSH, Witte JIJ, van der Meer MTJ, Jager Z, Soetaert KER, van der Heide T, van der Veer HW (2023) Stomach content and stable isotopes illustrate large spatial similarity in the Wadden Sea fish food web structure. Marine Ecology Progress series. 707, 57-76.

Keywords:
Coastal fish community, Wadden Sea, stomach content, stable isotopes, trophic position, trophic structure, Marsdiep basin, Ems basin, predator-prey relationships

Running title:
Similarity in Wadden Sea fish food-web structure

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 basin 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 the Ems and Marsdiep. Mysid shrimp were more important as prey in the Ems, while shore crab and herring were more important prey species in the Marsdiep. The observed spatial variability in prey preferences was most likely the result of local differences in predator and prey abundance. Published absolute trophic position based on compound specific stable isotopes were available for some fish species and indicated a low variability between Ems and Marsdiep. 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 Ems and Marsdiep, also indicating a large spatial similarity in trophic structure.

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 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 already for centuries, causing major disturbances and structural and functional changes, and leading to system losses (see for instance Jackson et al. 2001, Lotze 2005, 2007). Anthropogenic stressors like 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 would put the functioning of these coastal systems under even more pressure.

The Wadden Sea is an estuarine area of nearly 8000 km^2 bordering the Dutch, German and Danish coast. It is one of the largest European temperate coastal estuarine areas (Wolff 1983) and important for a variety of fish species (Zijlstra 1972). The Wadden Sea forms an essential habitat for fish species of all life stages of resident and near-resident species and acts as a nursery area for a group of marine migrant species during their juvenile stage. The area is also used as 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, shark, 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 impacts have also affected the fish community in the area: in the Dutch Wadden Sea two long-term monitoring programmes of the fish fauna show a similar general pattern over the last 50 – 60 years (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 flatfish species plaice *Pleuronectes platessa*, dab *Limanda limanda* and sole *Solea solea* have particularly decreased since the 1980s (Tulp et al. 2008, 2017a, van der Veer et al. 2015, 2022). Furthermore, mean individual biomass decreased between 1980 and the present, with the strongest declines generally in the largest size classes (Tulp et al. 2008, 2017a, van der Veer et al. 2015). Although 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 part of the Wadden Sea, marine migrant species also declined in almost all areas since the early or mid-1980s (Tulp et al. 2022). Trends for other demersal migratory and resident species varied from overall stable trends (flounder *Platichthys flesus*), to ongoing declines (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* sp.) (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 (Anon 1985), the Sylt-Rømø basin (Kellnreitner et al. 2012) and the Marsdiep basin (Poiesz et al. 2020, 2021). However, the 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 investigate the spatial variability in the fish food web by sampling simultaneously in two tidal basins that are similar in size and fresh water supply, and hence in their "estuarine character": the Ems 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 the Ems and the Marsdiep basin during 2012 – 2014. Predator-prey relationships and prey overlap are based on stomach content information following Hynes (1950), Baker et al. (2014), Froese & Pauly (2022). Trophic structure is based on a comparison of the trophic position of the various fish species in both basins. Relative trophic position of the various species to each other within each system was analyzed by comparing $\delta^{15}N$ bulk stable isotope values. Absolute trophic position was estimated from stomach content composition (Froese & Pauly 2022, Poiesz et al. 2020) and from bulk stable isotopes ($\delta^{15}N$) composition (Post 2002, Boecklen et al. 2011). However, both estimates may potentially suffer from limitations. Estimates of absolute trophic position based on stomach content are based on only those prey items that could be identified; it offers only a small snapshot in time and it can be sensitive to digestion during sampling. Estimates of absolute trophic position based on stable isotope 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. 2017a). Recently, Riekenberg et al. (2022) overcame the effects from baseline variations by applying compound-specific stable isotopes analysis to construct a preliminary Wadden Sea food web, including estimates of some fish species. This information will be used as a reference for the absolute estimates based on stomach content and bulk stable isotopes.

2. Materials and methods

2.1. Sampling

The Marsdiep and the Ems are the two largest tidal basins in the Dutch part of the Wadden Sea and are situated respectively in the western and eastern part (Fig 1). Both tidal basins are characterized by a large freshwater influx: in the Marsdiep basin through the discharge of freshwater from Lake IJssel (the former enclosed inner part of the Marsdiep basin) and in the Ems estuary via fresh water discharge by 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: 1050x10^6 m^3 (Marsdiep basin) versus 1000x10^6 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).

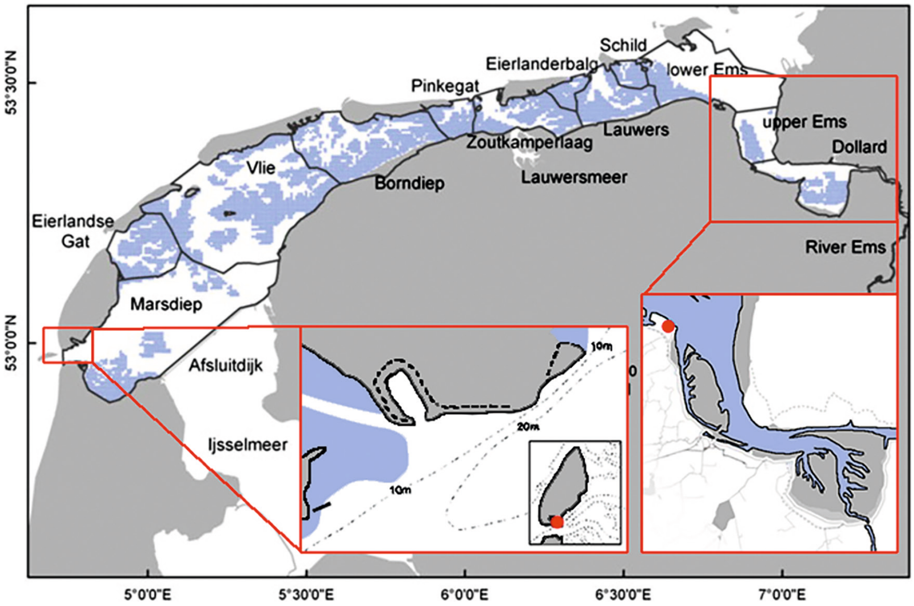

Figure 1 Sampling stations in the Marsdiep and Ems tidal basins in the Dutch Wadden Sea. Inner left panel: Sampling location of the NIOZ kom-fyke near the island of Texel (red dot and black line) in the Marsdiep basin. Inner right panel: Sampling location of the Ems Centrale (red dot) inside the Ems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

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 inlet is located 300 m from the shoreline in the Doekegat at a water depth of 13 m at high tide, and water intake occurred over the whole water column with a capacity of maximum 55 m s^{-1} (Hadderingh & Jager 2002). Seawater passed through a screen of 6x6 cm and all impinged biota was flushed from the screens and discharged back into the estuary just below the low water mark via an open return drain. All impinged fish and other biota were collected from the return drain with a custom-made frame (with a bag-shaped network: length 1.5 m, mesh size 5x5 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 amounts of organic matter flooded the gutter sampling time was shortened to 1 min. or even 30 sec., 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 see Hadderingh & Jager (2002).

Table 1 The isotopic baselines for Ems and Marsdiep. Data after Christianen et al. (2017). For more information see text.

	Benthic baseline				Pelagic baseline			
Location	δ^{13}C +/- s.e		δ^{15}N +/- s.e		δ^{13}C +/- s.e		δ^{15}N +/- s.e	
Ems basin	-14,00	0,46	12,82	0,22	-18,77	0,13	10,67	0,15
Marsdiep basin	-14,3	0,26	11,96	0,18	-17,8	1,74	12,5	0,17

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 in Poiesz et al. (2020). Samples were sorted immediately and all fish and other biota caught were identified up to species level, counted, measured for length within an hour and stored at -20˚C until further processing. Each fish species was classified according to guild as JMM (juvenile marine migrant), MSV (marine seasonal visitor) or (Near)-resident (resident and near-resident species) and according to functional group in 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

Within a few weeks of capture, fish were transported to the lab, defrosted and analysed, which determined 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 (20x). 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 NIOZ experts and 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, 2023).

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˚C until further processing. Next, samples were freeze-dried for 48 hours and grinded, and the remaining powder was homogenized. Duplicates were taken of each sample between 0.4 – 0.8 mg and were weighted and folded into small tin containers for analysis. Nitrogen and carbon isotopes were measured at the 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 (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. 22 standards 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 two other standards, Urea and Casein, were used to check the correction. Analytical reproducibility was 0.3‰ for $\delta^{15}N$ and 0.1‰ for $\delta^{13}C$ throughout every sequence.

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

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

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

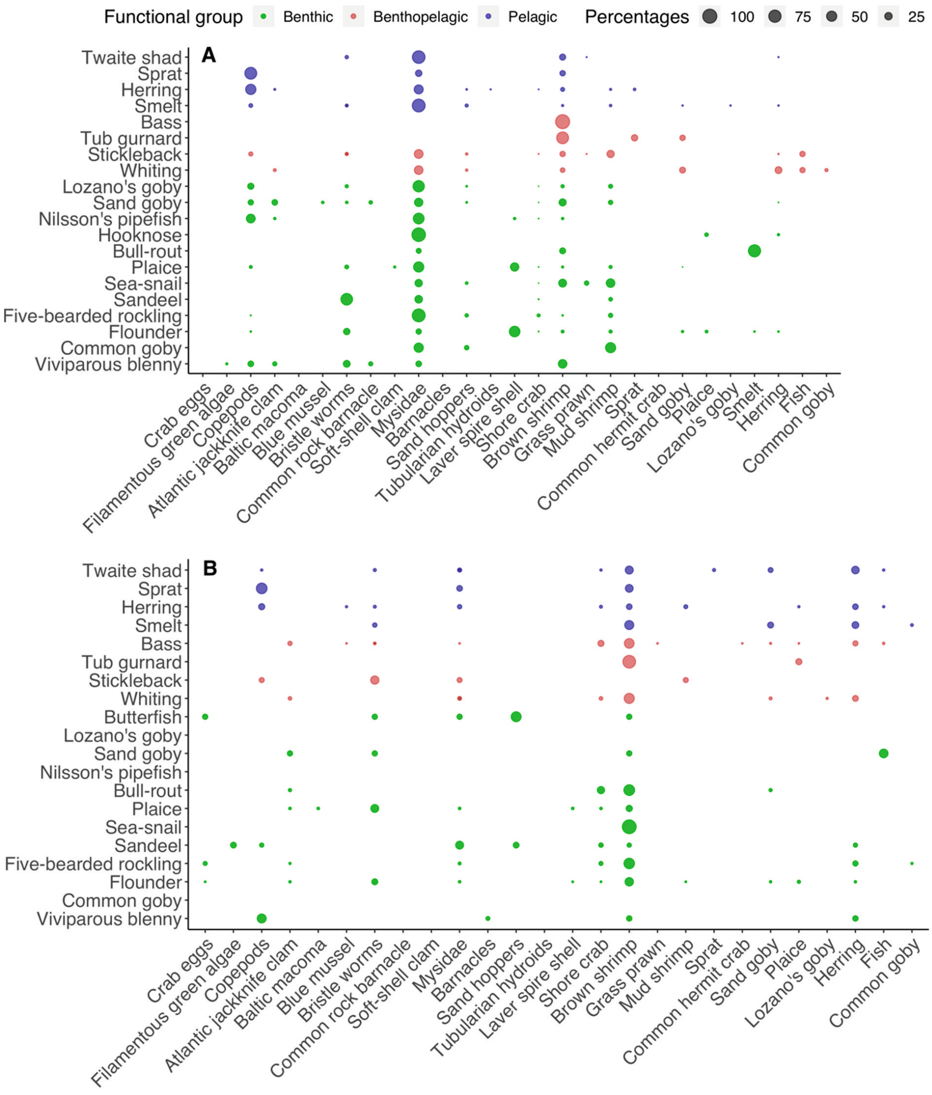

Figure 2 Prey species occurrence (%; continues scale of 0-100%) in the stomachs of fish species in the various functional groups (benthic; benthopelagic; pelagic) for the years 2012 – 2014. A: Ems basin B: Marsdiep basin

2.3. Data analysis

2.3.1. Stomach content

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:

$$TP_j = 1 + \frac{\sum TP_i}{i} \qquad [2]$$

with:

TP_j as the calculated trophic position of the individual fish j;

TP_i as 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 $\delta^{15}N$ 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 among two distinct sources of C and N, e.g. 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 one baseline with the trophic discrimination factors for nitrogen only:

$$\delta^{15}N_c = \delta^{15}N_b + \Delta N(TP - \lambda) \qquad [3]$$

with:

$\delta^{15}N_c$ the $\delta^{15}N$ values of the consumers

$\delta^{15}N_b$ the $\delta^{15}N$ values of the single baseline

ΔN the trophic discrimination factor for nitrogen (N)

TP the trophic position of the consumers

λ the trophic position of the baseline

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

$$\delta^{15}N_c = \Delta N(TP + \lambda) + \alpha\left(\delta^{15}N_{b1} + \delta^{15}N_{b2}\right) - \delta^{15}N_{b2} \qquad [4]$$

where

$\delta^{15}N_{b1}$, $\delta^{15}N_{b2}$	is the δ^{15}N of respectively baseline 1 and 2
α	is 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:

$$\alpha = ((\delta^{13}C_{b2} - \left(\delta^{13}C_c + \Delta C\right))/(TP - \lambda)/(\delta^{13}C_{b2} + \delta^{13}C_{b1}) \qquad [5]$$

with

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

Poiesz et al. (2021) has shown 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 factor for nitrogen δ^{15}N 3.4‰ (s.d. 0.98‰) and carbon δ^{13}C 0.39‰ (s.d. 1.3‰), was taken from Post (2002) in line with a previous study in the Marsdiep by Poiesz et al. (2021).

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 three observations of stomach content 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 was explored using the protocol described in Zuur et al. (2010), with graphics rendition via the ggplot package (Wickham 2009). Secondly, the tRophicPosition R package (R Core Team 2019) was used to determine the Bayesian TP model following Quezada-Romegialli et al. (2018).

Table 2 Total number of individual fish species caught in 2012-2014, together with number of individuals containing stomach content and number of isotope samples taken.
Upper table: Ems basin.
Lower table: Marsdiep basin.

Ems Dollard				
Scientific name	**Common name**	**Abbreviation**	**Functional group**	**Guild**
Agonus cataphractus	Hooknose	*Ac*	Benthic	(Near)-resident
Alosa fallax	Twaite shad	*Af*	Pelagic	(Near)-resident
Ammodytes tobianus	Sandeel	*At*	Benthic	MSV
Aphia minuta	Transparent goby	*Am*	Benthic	(Near)-resident
Ciliata mustela	Five-bearded rockling	*Cm*	Benthic	(Near)-resident
Clupea harengus	Herring	*Ch*	Pelagic	JMM
Dicentrarchus labrax	Bass	*Dl*	Benthopelagic	(Near)-resident
Gadus morhua	Cod	*Gm*	Benthopelagic	MSV
Gasterosteus aculeatus	Stickleback	*Ga*	Benthopelagic	(Near)-resident
Lampetra fluviatilis	River lamprey	*Lf*	Benthic	(Near)resident
Limanda limanda	Dab	*Ll*	Benthic	MSV
Liparis liparis	Sea-snail	*Llip*	Benthic	(Near)-resident
Merlangius merlangus	Whiting	*Mm*	Benthopelagic	MSV
Myoxocephalus scorpius	Bull-rout	*Ms*	Benthic	(Near)-resident
Osmerus eperlanus	Smelt	*Oe*	Pelagic	MSV
Pholis gunnellus	Butterfish	*Pg*	Benthic	(Near)-resident
Platichthys flesus	Flounder	*Pf*	Benthic	(Near)-resident
Pleuronectes platessa	Plaice	*Pp*	Benthic	JMM
Pomatoschistus lozanoi	Lozano's goby	*Pl*	Benthic	MSV
Pomatoschistus microps	Common goby	*Pmic*	Benthic	(Near)-resident
Pomatoschistus minutus	Sand goby	*Pmin*	Benthic	(Near)-resident
Pungitius pungitius	Nine-spined stickleback	*Ppun*	Benthic	MSV
Scophthalmus maximus	Turbot	*Sm*	Benthic	MSV
Solea solea	Sole	*Ssol*	Benthic	JMM
Sprattus sprattus	Sprat	*Ss*	Pelagic	JMM
Syngnathus rostellatus	Nilsson's pipefish	*Sr*	Benthic	(Near)-resident
Syngnatus acus	Greater pipefish	*Sa*	Benthic	MSV
Trachurus trachurus	Scad	*Tt*	Pelagic	MSV
Trigla lucerna	Tub gurnard	*Tl*	Benthopelagic	MSV
Trisopterus luscus	Bib	*Tlus*	Benthopelagic	MSV
Zoarces viviparus	Viviparous blenny	*Zv*	Benthic	(Near)-resident

	Numbers of individuals caught				Number with stomach content				No isotope samples
	2012	2013	2014	Total	2012	2013	2014	Total	Total
	3	6	1	10	0	5	0	5	10
	9	8	5	22	7	5	3	15	22
	2	9	7	18	0	2	1	3	18
	0	0	1	1	0	0	0	0	1
	12	2	4	18	11	0	4	15	18
	44	51	46	141	19	19	17	55	141
	7	0	2	9	2	0	1	3	9
	1	0	0	1	1	0	0	1	1
	36	34	23	93	17	21	15	53	93
	1	0	0	1	0	0	0	0	1
	1	1	1	3	0	0	1	1	3
	29	13	8	50	22	10	7	39	50
	15	22	8	45	8	18	6	32	45
	2	5	1	8	0	4	1	5	8
	49	55	39	143	30	27	19	76	143
	4	2	0	6	1	1	0	2	6
	8	21	7	36	4	11	2	17	36
	22	38	18	78	6	10	5	21	78
	24	11	19	54	9	5	12	26	54
	4	9	4	17	3	0	3	6	17
	41	54	35	130	13	19	14	46	130
	0	0	1	1	0	0	0	0	1
	0	0	1	1	0	0	0	0	1
	1	8	0	9	0	0	0	0	9
	17	12	27	56	3	7	3	13	56
	45	52	42	139	17	27	18	62	139
	1	0	0	1	0	0	0	0	1
	0	4	0	4	0	1	0	1	4
	0	5	6	11	0	3	6	9	11
	1	0	0	1	1	0	0	1	1
	9	10	0	19	4	5	0	9	19

Marsdiep				
Scientific name	**Common name**	**Abbreviation**	**Functional group**	**Guild**
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Zoarces viviparus	Viviparous blenny	*Zv*	Benthic	(Near)-resident

	Numbers of individuals caught				Number with stomach content				No isotope samples
	2012	2013	2014	Total	2012	2013	2014	Total	Total
	3	5	2	10	1	1	1	3	3
	13	158	24	195	17	33	79	129	59
	0	0	1	1	0	0	1	1	1
	0	0	0	0	0	0	0	0	0
	137	23	48	208	32	34	89	155	36
	6730	25103	14187	46020	25	79	81	185	134
	316	175	381	872	55	23	111	189	189
	175	247	17	439	10	18	7	35	35
	381	116	32	529	7	8	14	29	28
	0	0	0	0	0	0	0	0	0
	50	57	68	175	11	12	16	39	39
	2	6	0	8	5	3	0	8	2
	107	56	71	234	7	26	81	114	52
	19	18	16	53	12	2	33	47	20
	2	31	31	64	20	14	38	72	38
	3	5	0	8	1	1	0	2	2
	417	570	401	1388	46	74	102	222	148
	486	873	469	1828	18	27	57	102	86
	0	0	0	0	0	0	0	0	0
	0	0	1	1	0	0	0	0	0
	13	52	35	100	0	5	11	16	11
	0	0	0	0	0	0	0	0	0
	8	24	45	77	2	1	15	18	18
	13	23	9	45	2	5	4	11	11
	734	1839	779	3352	2	13	9	24	19
	0	0	5	5	0	0	0	0	0
	34	31	35	100	6	1	9	16	16
	13	98	70	181	4	15	17	36	36
	13	2	1	16	2	0	2	4	4
	1	44	6	51	0	5	2	7	7
	3	1	1	5	0	4	1	5	5

Table 3 Trophic position based on stomach content, mean isotopic values and trophic position based on isotope values of fish species caught in the Ems with corresponding values for fish caught in the Marsdiep in 2012-2014. Mean and standard error.

					Stomach content analysis			
					Ems		Marsdiep	
Scientific name	**Common name**	**Abbreviation**	**Functional group**	**Guild**	**Mean**	**se**	**Mean**	**se**
Agonus cataphractus	Hooknose	*Ac*	Benthic	(Near)-resident	2,84	0,63	3,60	0,05
Alosa fallax	Twaite shad	*Af*	Pelagic	(Near)-resident	2,97	0,24	3,47	0,05
Ammodytes tobianus	Sandeel	*At*	Benthic	MSV	2,70	0,45	3,16	0,14
Aphia minuta	Transparent goby	*Am*	Benthic	(Near)-resident				
Ciliata mustela	Five-bearded rockling	*Cm*	Benthic	(Near)-resident	2,66	0,24	3,72	0,02
Clupea harengus	Herring	*Ch*	Pelagic	JMM	2,68	0,14	3,42	0,03
Dicentrarchus labrax	Bass	*Dl*	Benthopelagic	(Near)-resident	3,60		3,55	0,04
Gadus morhua	Cod	*Gm*	Benthopelagic	MSV				
Gasterosteus aculeatus	Stickleback	*Ga*	Benthopelagic	(Near)-resident	2,71	0,16	3,20	0,06
Lampetra fluviatilis	River lamprey	*Lf*	Benthic	MSV				
Limanda limanda	Dab	*Ll*	Benthic	MSV				
Liparis liparis	Sea-snail	*Llip*	Benthic	(Near)-resident	2,71	0,18	3,60	0,01
Merlangius merlangus	Whiting	*Mm*	Benthopelagic	MSV	2,55	0,29	3,59	0,04
Myoxocephalus scorpius	Bull-rout	*Ms*	Benthic	(Near)-resident	2,78	0,55	3,55	0,03
Osmerus eperlanus	Smelt	*Oe*	Pelagic	MSV	2,64	0,14	3,92	0,05
Pholis gunnellus	Butterfish	*Pg*	Benthic	(Near)-resident	3,60		3,44	0,08
Platichthys flesus	Flounder	*Pf*	Benthic	(Near)-resident	2,30	0,30	3,42	0,03
Pleuronectes platessa	Plaice	*Pp*	Benthic	JMM	2,73	0,26	3,22	0,03
Pomatoschistus lozanoi	Lozano's goby	*Pl*	Benthic	MSV	3,00	0,13		
Pomatoschistus microps	Common goby	*Pmic*	Benthic	(Near)-resident	2,20	0,47		
Pomatoschistus minutus	Sand goby	*Pmin*	Benthic	(Near)-resident	2,92	0,15	3,85	0,17
Pungitius pungitius	Nine-spined stickleback	*Ppun*	Benthic	MSV				
Scophthalmus maximus	Turbot	*Sm*	Benthic	MSV				
Solea solea	Sole	*Ssol*	Benthic	JMM				
Sprattus sprattus	Sprat	*Ss*	Pelagic	JMM	2,80	0,17	3,18	0,10
Syngnathus rostellatus	Nilsson's pipefish	*Sr*	Benthic	(Near)-resident	2,86	0,08		
Syngnatus acus	Greater pipefish	*Sa*	Benthic	(Near)-resident				
Trachurus trachurus	Scad	*Tt*	Pelagic	MSV				
Trigla lucerna	Tub gurnard	*Tl*	Benthopelagic	MSV	3,26	0,28	3,60	0,03
Trisopterus luscus	Bib	*Tlus*	Benthopelagic	MSV				
Zoarces viviparus	Viviparous blenny	*Zv*	Benthic	(Near)-resident	1,74	0,30	3,38	0,08

	Stable isotope analysis								Trophic position based on isotopes			
	Ems				Marsdiep				Ems		Marsdiep	
	ΔC		ΔN		ΔC		ΔN		Ems baseline TP		Marsdiep baseline TP	
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
	–16,45	0,11	16,73	0,18	–16,71	0,29	16,63	0,14	3,36	0,15	3,96	2,71
	–19,47	0,37	16,92	0,16	–16,67	0,37	17,14	0,13	3,79	0,15	3,05	0,24
	–17,68	0,16	16,08	0,30					3,47	0,23		
	–17,95		14,24									
	–16,41	0,20	17,12	0,33	–16,61	0,18	16,75	0,27	3,48	0,34	3,11	0,19
	–18,58	0,15	16,06	0,12	–17,89	0,09	14,19	0,12	3,52	0,14	2,69	0,14
	–14,92	0,45	17,66	0,37	–17,73	0,64	15,59	0,63	3,38	0,33	3,18	0,2
			14,80		–16,69	0,13	16,12	0,15			3,17	0,2
	–20,18	0,25	16,61	0,12	–18,89	0,20	15,26	0,26	3,81	0,15	3,04	0,19
	–20,27		17,35									
	–17,28	0,30	15,37	0,31	–16,48	0,13	17,28	0,11	3,12	0,50	2,49	0,15
	–16,40	0,12	16,82	0,15	–16,40	0,40	16,07	0,16	3,44	0,13	5,13	2,27
	–17,30	0,14	17,11	0,13	–17,35	0,42	15,81	0,21	3,63	0,14	3,05	0,17
	–16,37	0,22	17,27	0,23	–16,24	0,15	17,45	0,13	3,55	0,18	3,53	0,19
	–18,04	0,14	17,81	0,08	–16,72	0,41	16,61	0,17	3,97	0,14	3,28	0,13
	–17,02	0,15	17,36	0,69	–17,70	0,41	16,52	0,17	2,69	0,43	3,31	1,3
	–17,57	0,47	17,08	0,27	–19,14	0,14	16,17	0,14	3,51	0,19	3,09	0,15
	–16,44	0,16	15,49	0,14	–15,45	0,15	14,85	0,15	2,99	0,17	2,81	0,11
	–17,42	0,10	17,25	0,10	–16,84	0,12	16,01	0,19	2,62	0,10		
	–16,43	0,35	16,54	0,24	–18,94	0,14	14,28	0,10	2,41	0,19		
	–17,46	0,11	17,02	0,08	–15,58	0,15	14,88	0,13	3,6	0,19	3,11	0,17
	–25,50		10,78									
	–15,97		17,65		–18,76	0,29	15,28	0,25			3,11	0,13
	–16,89	0,17	16,69	0,30	–15,46	0,26	15,99	0,15	3,40	0,20	2,89	0,22
	–18,54	0,15	15,06	0,17	–16,95	0,36	15,30	0,13	3,38	0,17	2,83	0,25
	–18,06	0,08	16,16	0,09	–16,29	0,12	17,36	0,13	2,31	0,18		
	–17,74		12,94									
	–18,73	0,79	16,51	0,28	–18,63	0,34	15,46	0,29	3,61	0,18	2,87	0,2
	–16,20	0,54	16,89	0,30	–17,38	0,15	13,74	0,30	3,35	0,29	3,4	1,07
	–18,79		17,49		–18,53	0,21	16,58	0,12			2,73	0,18
	–16,78	0,23	18,05	0,29	–16,67	0,20	14,77	0,20	3,8	0,20	2,98	0,23

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; in total 1661 individuals. The non-fish species mainly consisted of brown shrimp (*Crangon crangon*), mysid shrimp (*Mysida*), grass prawn (*Palaemon elegans*), common prawn (*Palaemon serratus*), common swimming crab (*Macropipus holsatus*), shore crab (*Carcinus maenas*) and common sea star (*Asterias rubens*). Thirty-one fish species of different functional groups were found (Table 2). Most individuals caught were smaller than 19 cm in size.

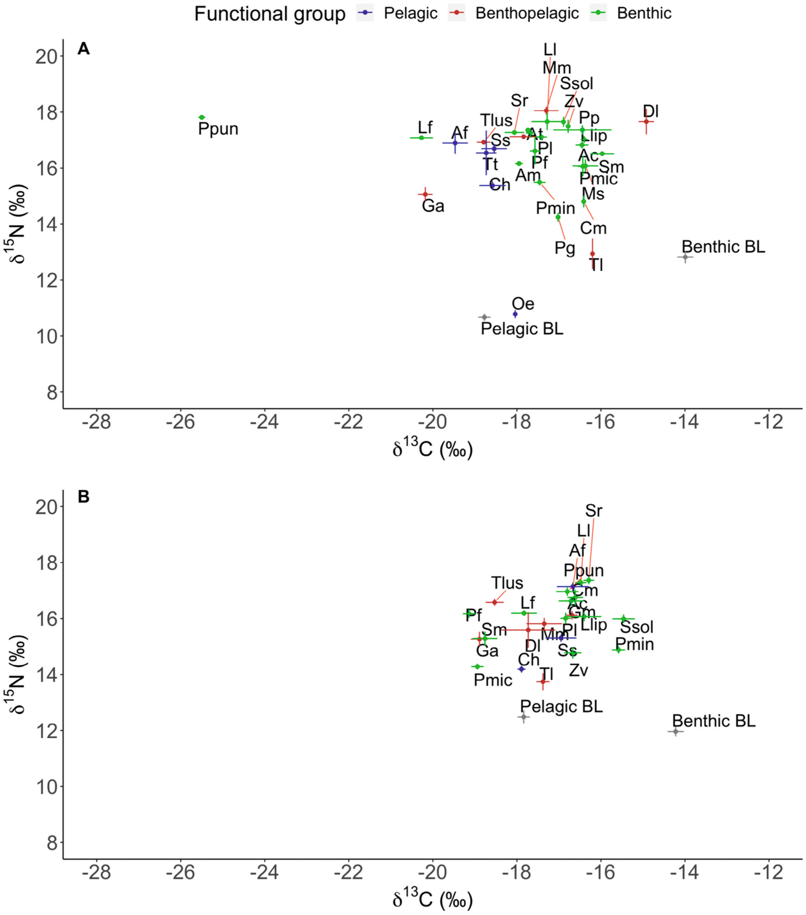


Figure 3 Average δ^{15}N and δ^{13}C stable isotope values with standard error bars for fish species caught in 2012 – 2014, split up into the various functional groups. For species names, values and corresponding abbreviations see Table 2.
A: Ems basin B: Marsdiep basin

3.1.1. Stomach content

In the Ems, most fish species consumed multiple prey, except bass (*Dicentrarchus labrax*) (Fig 2), which was a single prey consumer of the brown shrimp. However, the number of observations was low (n=3). The brown shrimp, mysid shrimp, mud shrimp and copepods were the most common prey species. Benthic species were preying 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 was also preying mostly on the mysid and brown shrimp. For guilds the picture was more diverse and variable (Supplementary materials Fig S1).

Based on stomach frequency occurrence of at least 50%, various predator-prey relationships could be identified. Bass and tub gurnard (*Trigla lucerne)* were focussing 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*), hooknose (*Agonus cataphractus*) and plaice (*Pleuronectes platessa)* focussed on mysid shrimp. For the other fish species prey items had frequency occurrences of ≤ 50%, with the most common prey item being mysid shrimp for 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*).

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

3.1.2. Stable isotopes

For 31 fish species bulk stable isotope values were determined (Table 3). For isotopic values for the non-fish species caught in the Ems: see Supplementary materials Table S2.

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

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

3.2. Marsdiep basin

In the Marsdiep, 457 fyke catches were done between 2012 – 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 the Lozano's goby (Table 2).

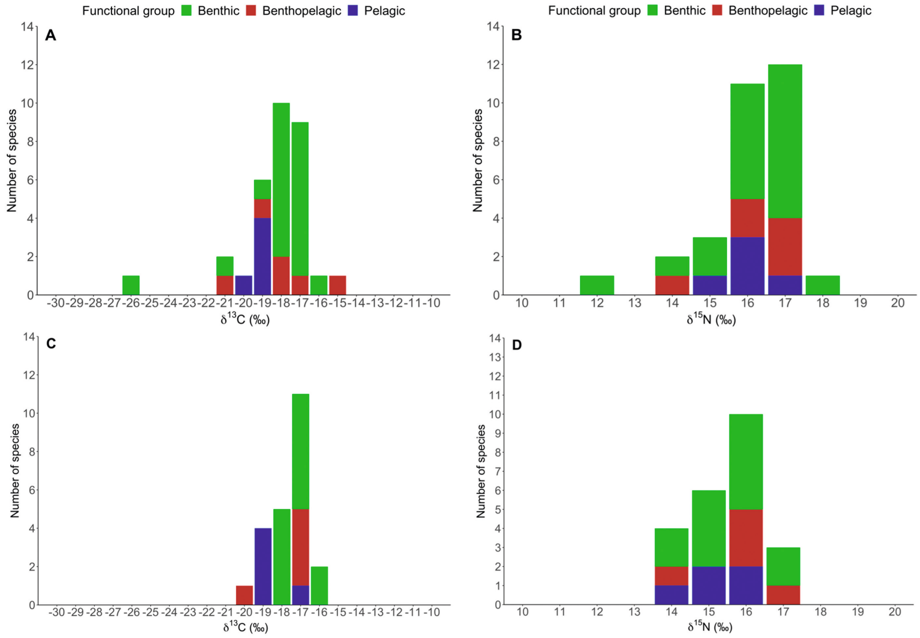


Figure 4 Frequency distribution of average stable isotope values of the various fish species for the various functional groups in the Ems and Marsdiep basin in 2012-2014. The dark blue lines in panels A and C represent the pelagic baseline (*Mytilus edulis*), while the dark green line represents the benthic baseline (*Littorina littorea*).
A: δ^{13}C values Ems species
B: δ^{15}N values Ems species
C: δ^{13}C values Marsdiep species
D: δ^{15}N values Marsdiep species

3.2.1. Stomach content

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 were preying 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 was also preying mostly on brown shrimp. For the other guilds the picture was more diverse and variable but for marine seasonal visitors brown shrimp was also a main food item (Supplementary materials Fig S1).

The prey items with a stomach frequency occurrence of > 50% 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 frequency occurrences of ≤ 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.

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

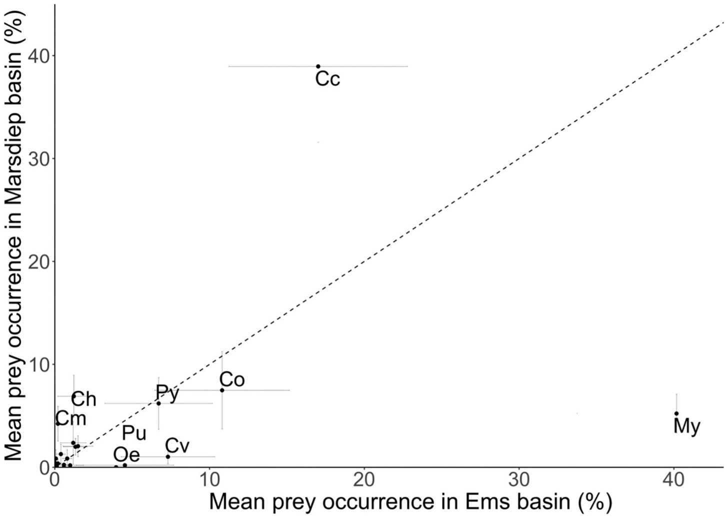


Figure 5 Comparison of mean occurrence (%) together with SE of the various prey items in the stomachs of all the fish species caught in the Ems and in the Marsdiep basin. Data from Fig 2. The dashed black line represents the 1:1 relationship.

3.2.2. Stable isotopes

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

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

Mean estimated trophic position ($\overline{TP}$) based on δ^{15}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 guild (Supplementary materials Fig S4 & S5).

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

Species name	Common name	Abbreviation	Numbers	TP AA	se
Clupea harengus	Herring	Ch	10	3,4	0,0
Dicentrarchus labrax	Bass	Dl	10	4,0	0,2
Osmerus eperlanus	Smelt	Oe	8	3,8	0,1
Platichthys flesus	Flounder	Pf	8	3,4	0,1
Pleuronectes platessa	Plaice	Pp	10	3,2	0,1
Solea soles	Sole	Ss	11	3,0	0,1
Zoarces viviparus	Eelpout	Zv	8	3,8	0,1
Crangon crangon	Brown shrimp	Cc	17	3,5	0,1
Carcinus maenas	Shore crab	Cm	18	3,2	0,1

3.3. Comparison between Ems and Marsdiep

3.3.1. Predator-prey relationships

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

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

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 seven fish species: herring, bass, smelt, flounder, plaice, sole and viviparous blenny (Table 4). For these fish species, trophic positions from FishBase were related to the absolute trophic positions based on compound-specific stable isotopes, however at a lower level (Fig 6A). Mean δ^{15}N isotope values of the seven fish species in the Ems 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 (Fig 6B). Trophic positions based on stomach content (Fig 6C) were correlated in both Ems and Marsdiep with absolute trophic positions based on compound-specific stable isotopes (Ems: $r=0.69$, $n=7$; $p<0.05$; Marsdiep: $r=0.61$, $n=7$; $p<0.10$), however the correlation for the Marsdiep was less strong. Trophic positions based on bulk isotopes (Fig 6D) were correlated in both Ems and Marsdiep with absolute trophic positions based on compound-specific stable isotopes (Ems: $r=0.67$; $n=7$; $p<0.05$; Marsdiep: $r=0.54$, $n=7$; $p<0.10$), however the correlation in the Marsdiep was less strong.

For thirteen fish species, relative trophic positions, as indicated by the mean stable δ^{15}N isotope values were significantly correlated ($r=0.66$, $n=13$; $p<0.01$). For the other species, mean stable δ^{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 ($\overline{TP}diet$)'s of the various fish species in the Ems ranged between 1.7 and 3.6, with most ($\overline{TP}diet$)'s in the Marsdiep varied 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. Only for sprat, tub gurnard, bass and butterfish trophic positions were similar in both basins. 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 δ^{15}N isotope values ranged between 2.9 and 3.9 in the Ems, with most values around 3.5 and between 2.6 to 3.5 in the Marsdiep and with large standard errors for species with low number of measurements such as butterfish (Pg) (Fig 7C). For most of the species values were higher in the Ems. There was no correlation between the absolute trophic position in the Ems with that in the Marsdiep.

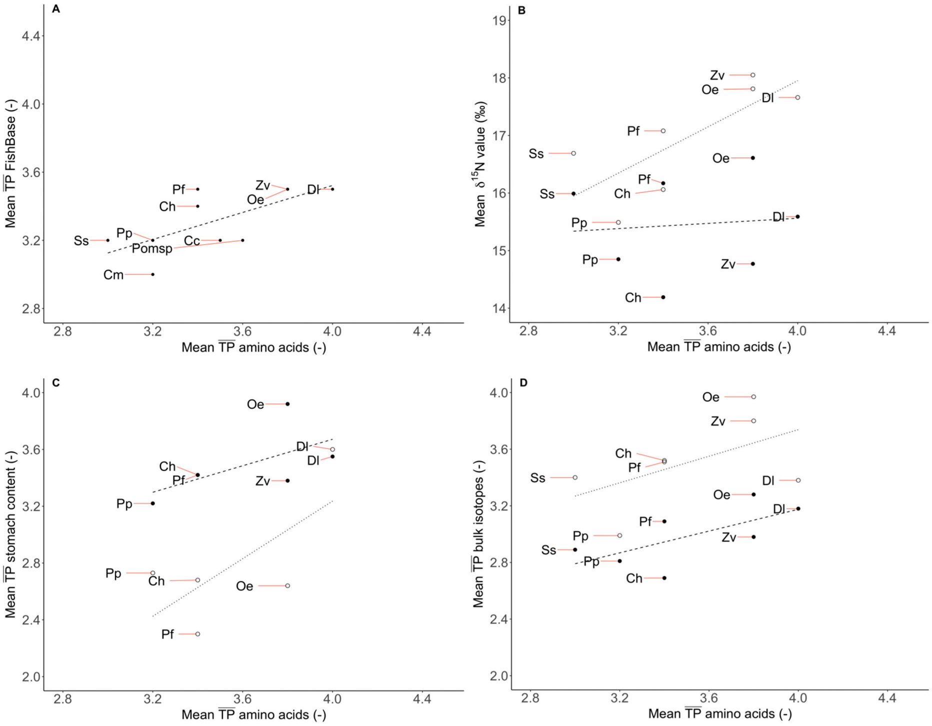


Figure 6 Relationship between estimated absolute trophic position in the Wadden Sea of some fish species by means of compound specific isotopes (Riekenberg et al. 2022) with:

A: trophic position according to FishBase;
B: mean $\delta^{15}N$ stable isotope values in Ems (open symbols) and Marsdiep (closed symbols);
C: trophic positions in Ems (open symbols) and Marsdiep (closed symbols) based on stomach content;
D: trophic positions in Ems (open symbols) and Marsdiep (closed symbols) based on bulk stable isotopes.

For species names and corresponding abbreviations see Table 2.

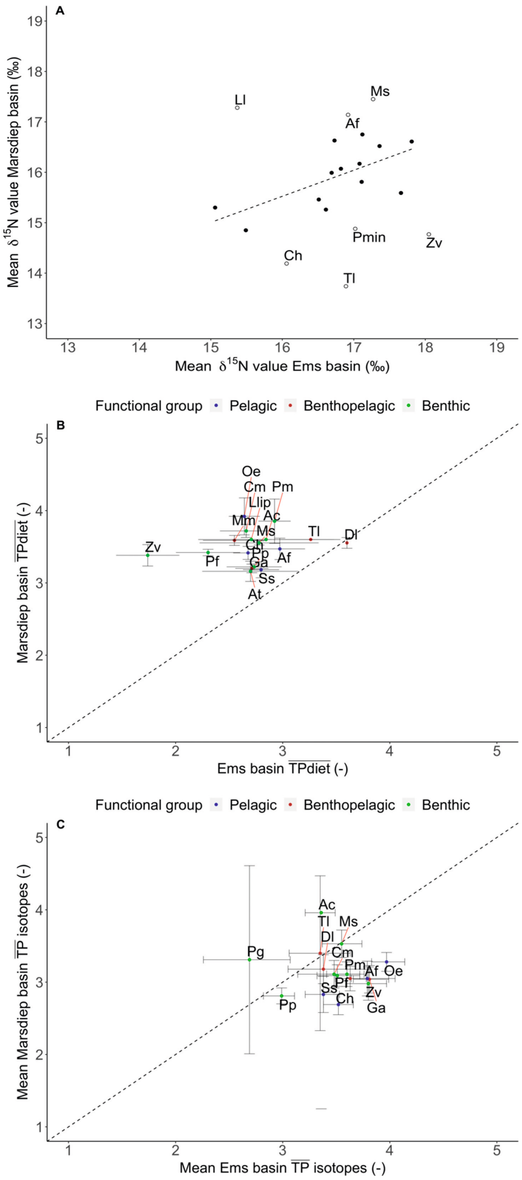


Figure 7 Relationship between the estimated relative and absolute trophic position of the various fish species in Ems and Marsdiep.
A: Relative trophic position by means of mean isotope δ^{15}N value (mean ± s.e.). The dashed black line represents the 1:1 relationship. Black dots: species with a significant 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 δ^{15}N values (mean ± s.e.).
For species names, corresponding abbreviations and data see Table 2 & 3.

4. Discussion

The Wadden Sea is an important dynamic area for a variety of around 100 fish species (Witte & Zijlstra 1983). Sampling of such a diverse community comprising a mixture of different life stage (larvae, juveniles, adults) distributed over various habitats (intertidal, subtidal and gullies) is a challenge and always a compromise dictated mainly by 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 two years (Kellnreitner et al. 2012), while in the Marsdiep basin 54 different species were found over 9 years (Poiesz et al. 2020). The Ems power plant sampling covered a 3-year sampling period and resulted in 31 fish species. Even sampling programmes extending over decades were not able to identify all fish species recorded in the area: over a 55-year time period (1960 – 2015), 82 different species were found in the western Wadden Sea (van der Veer et al. 2015) of around 100 fish species being previously 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 thirty fish species as collected in the Ems, 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 a combination of stomach content and stable isotope analyses of individuals collected simultaneously in the Ems and Marsdiep basin but, for logistic reasons, by means of different sampling designs.

In the Ems, 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°C within an hour, reducing potential digestion of prey items as much as possible. In the Marsdiep, 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 hours, 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. Stable isotope analyses is 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 $\delta^{15}N$ 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 isotope allows for a better identification of trophic food web relationships.

Both estimates of stomach content analysis and bulk stable isotopes 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 relative comparison of species within a basin. The same holds true for the information provided by FishBase (Fig 6A).

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

- stomach content provides information about predator-prey relationships in the area and allows 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 isotopes analysis provide information about the food sources and relative trophic position of fish species within an area.

4.2. Spatial variability in 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 exhibited 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 fuel 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 on 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 δ^{13}C values that microphytobenthos production was the most important energy source supporting the food web in the area (Christianen et al. 2017). Jung et al. (2019), focussing on the Marsdiep basin, 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), however to a lesser extent than Christianen et al. (2017). Also, for the Chanche estuary, microphytobenthos besides marine particulate organic matter has been found to contribute most to the food web in the area (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, suggested for some species a signature of organic matter produced in freshwater (Middelburg & Herman 2007). For some species, such as nine-spined stickleback, stickleback, river lamprey and twaite shad in the Ems, 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 four 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). Most prey species can also be found all over the Wadden Sea, such as brown shrimp, shore crab and herring (Poiesz et al. 2020, Tulp et al. 2012, Kellnreitner et al. 2012, Meyer et al.2016). This might explain the absence of differences between groups or guilds between both areas. 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 are in line with the general food relationships described for Wadden Sea fishes in the past (Kühl & Kuipers 1983, Anon 1985) and 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 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 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 basin. This means that predator–prey relationships are to a large extent similar in both the Ems and Marsdiep basin but fish species also showed differences in stomach content between the Ems and Marsdiep. 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).

Recently, a few 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 (Riekenberg et al. 2022). 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. Also δ^{15}N isotope values and trophic position estimated from both stomach content and bulk isotope analysis shows a large similarity in relative trophic position of the various fish species and hence in food-web structure in both Ems and Marsdiep. Estimates based on stomach content and on bulk isotope analysis, both underestimate the absolute trophic position based on compound specific stable isotopes by Riekenberg et al. (2022) in a different way and differently for Ems and Marsdiep. 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] the fish fauna composition is rather similar over a large range 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, driven mainly by local productivity and species' and stage specific preferences and tolerance ranges to abiotic factors. Fish food web structure appears to be similar at least at the scale of the Dutch Wadden Sea.

Acknowledgements

Thanks to all of our colleagues for assisting in the collection and analyses of the samples, especially Rob Dapper, Ewout Adriaans, Willem Jongejan, Sander Holthuijsen, Sieme Gieles, Marco Kortenhoeven and Thomas Leerink. All fish sampling and handling was done under CCD project number AVD 8020020174165. The comments of three anonymous reviewers improved the manuscript considerably.

Supplementary materials

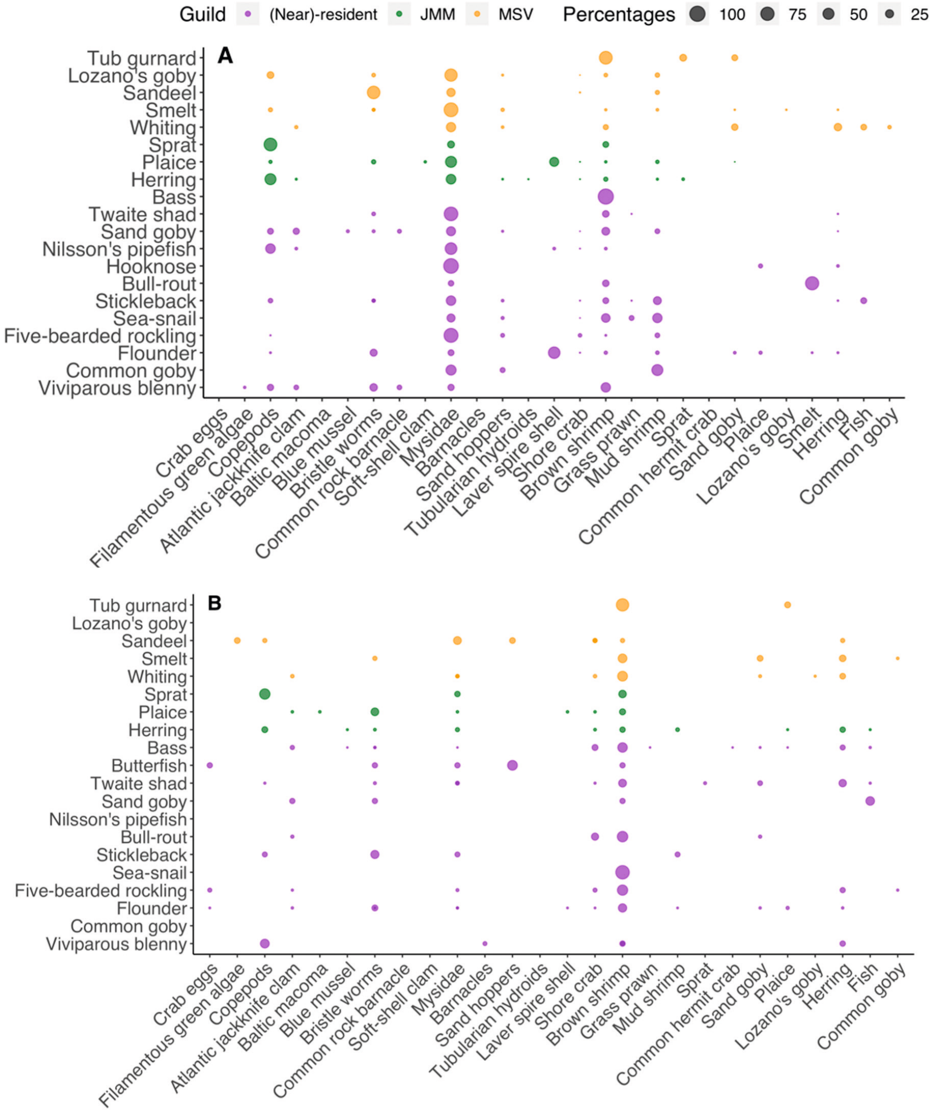


Figure S1 Prey species occurrence (%; continues scale of 0-100%) in the stomachs of fish species for the various guilds [(near)-resident species, JMM; juvenile marine migrant species and MSV; marine seasonal visitors] for the years 2012 – 2014.
A: Ems basin
B: Marsdiep basin

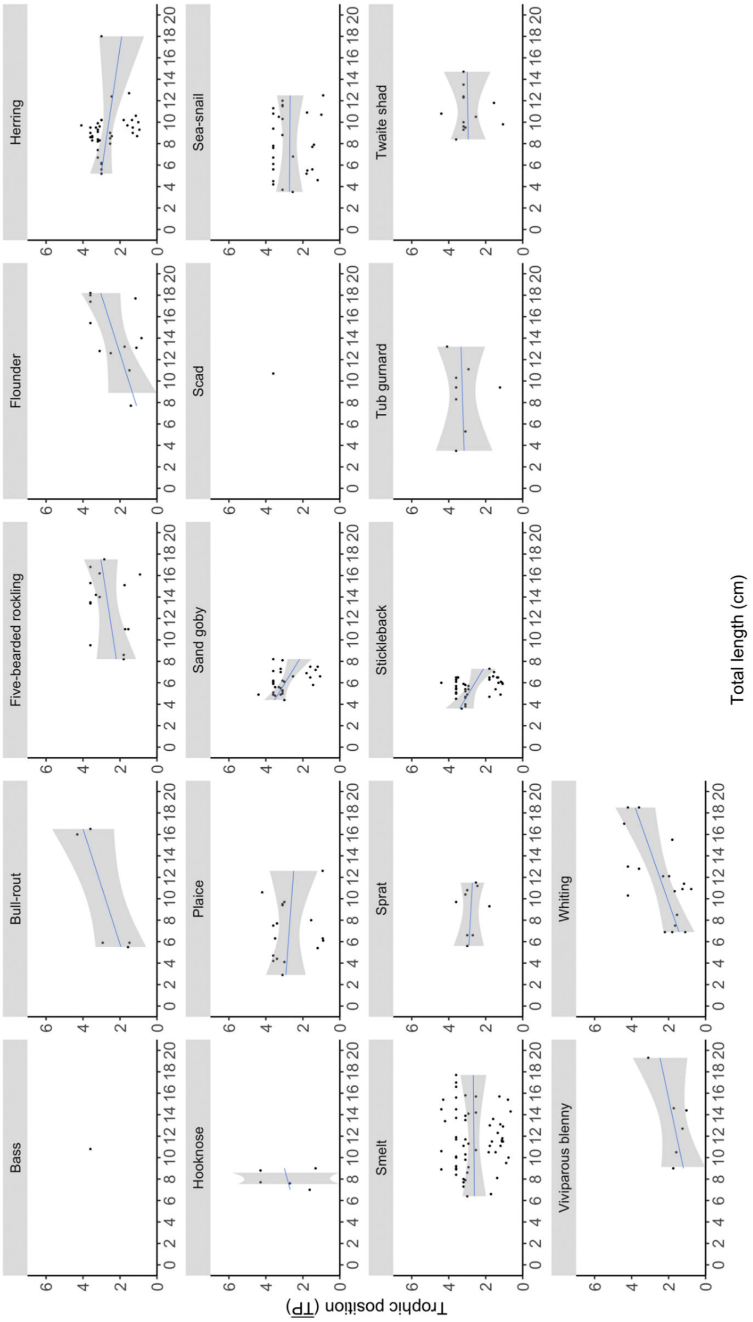


Figure S2 Calculated trophic positions ($\overline{TP}$) based on the stomach content in relation to the total length for the various fish species caught in the Ems basin for all years (2012 -2014) combined. A linear regression with a 95% confidence interval (in grey) is added to visualize trends. For relationships see Supplementary material S1.

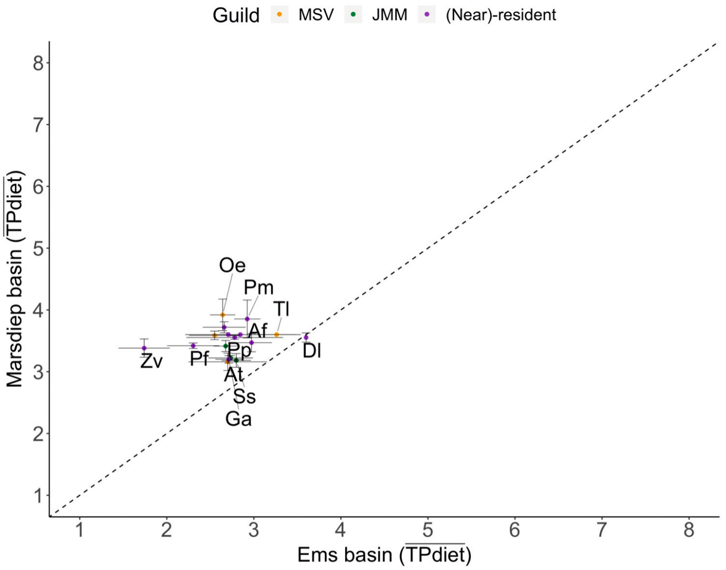


Figure S3 Relationship between the trophic position of various fish species (mean ± s.e.) in the Ems and Marsdiep basins based on stomach content analysis for the various guilds [(near)-resident species, JMM; juvenile marine migrant species and MSV; marine seasonal visitors]. For species names and corresponding abbreviations see Table 2. The black line indicates the 1:1 relationship.

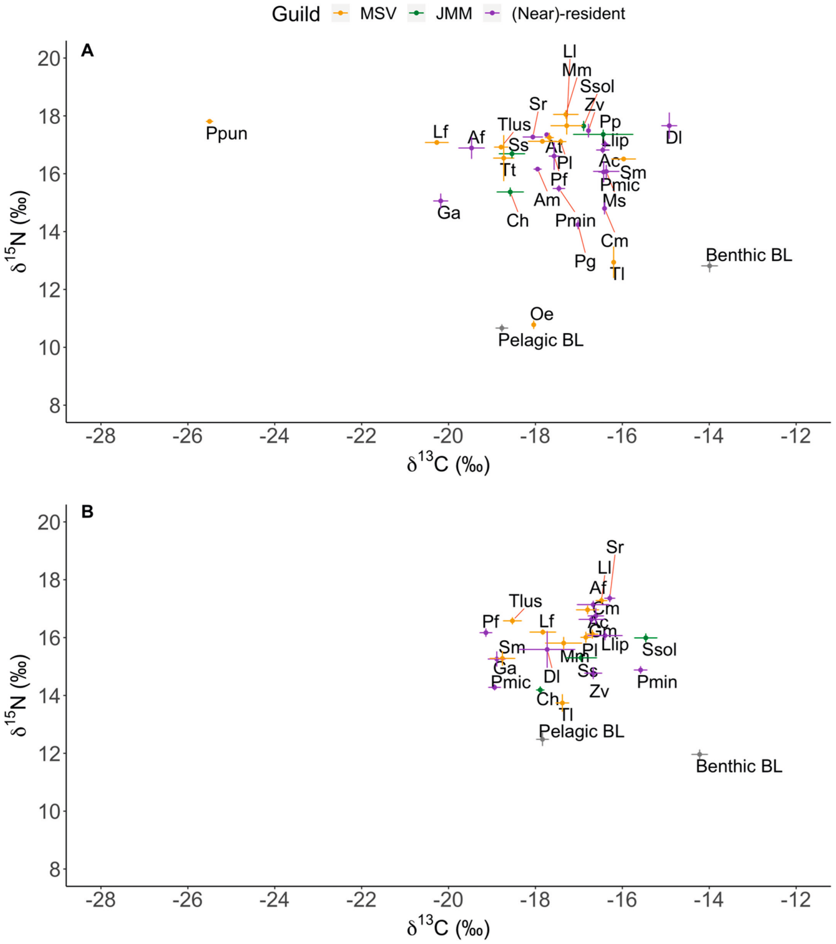


Figure S4 Average δ^{15}N and δ^{13}C stable isotope values with standard error bars for fish species in 2012-2014, split up into the various guilds [(near)-resident species, JMM; juvenile marine migrant species and MSV; marine seasonal visitors]. For species names, values and corresponding abbreviations see Table 2.
A: Ems basin
B: Marsdiep basin

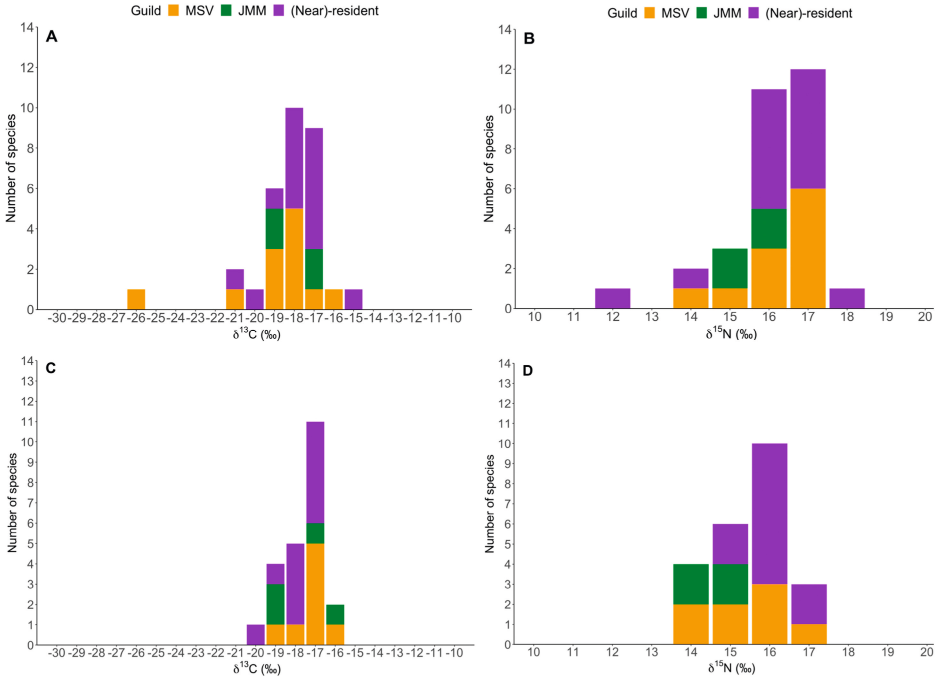


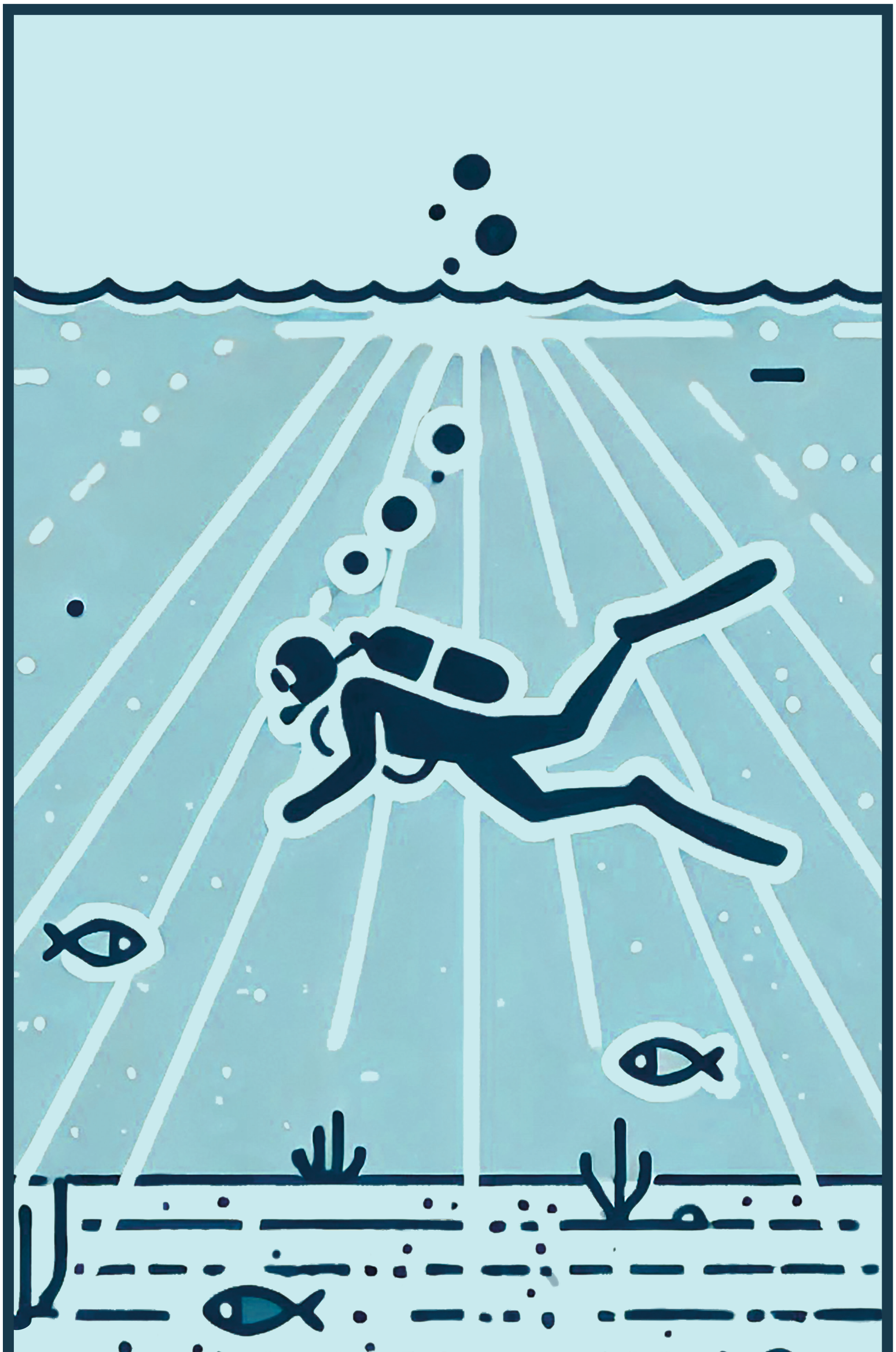
Figure S5 Frequency distribution of average stable isotope values of the various species (guilds [(near)-resident species, JMM; juvenile marine migrant species and MSV; marine seasonal visitors] for the Ems basin in the years 2012-2014. The dark blue lines in panels A and C represent the pelagic baseline (*Mytilus edulis*), the dark green line indicates the benthic baseline (*Littorina littorea*).
Panel A: Average δ^{13}C values for Ems species;
Panel B: Average δ^{15}N values for Ems species;
Panel C: Average δ^{13}C values for Marsdiep species;
Panel D: Average δ^{15}N values for Marsdiep species.

Table S1 Linear regression values for the relationship between calculated trophic positions ($\overline{TP}$) based on the stomach content and total length of the various fish species caught in the Ems basin for all years (2012 -2014) combined. NA: not applicable (due to lack of data).

Common name	Estimate	Std. error	t-statistic	p-value
Bass	NA	NA	NA	NA
Bull-rout	0.046	0.096	0.482	0.6304
Five-bearded rockling	-0.050	0.098	-0.515	0.6067
Flounder	-0.029	0.086	-0.341	0.7337
Herring	-0.227	0.087	-2.610	0.0095
Hooknose	0.019	0.584	0.033	0.9739
Plaice	-0.184	0.100	-1.837	0.0672
Sand goby	-0.495	0.177	-2.800	0.0055
Scad	NA	NA	NA	NA
Sea-snail	-0.145	0.080	-1.822	0.0696
Smelt	-0.135	0.064	-2.132	0.0339
Sprat	-0.173	0.163	-1.064	0.2883
Stickleback	-0.488	0.185	-2.634	0.0089
Tub gurnard	-0.123	0.127	-0.965	0.3355
Twaite shad	-0.131	0.161	-0.816	0.4153
Viviparous blenny	-0.017	0.130	-0.132	0.8948
Whiting	0.140	0.053	2.642	0.0156

Table S2 Isotopic values for the non-fish species caught in the Ems during 2012-2014, with mean and standard error.

						Ems basin			
						$\delta^{13}C$		$\delta^{15}N$	
Abbreviation	Scientific name	Common name	Phylum	Class	No of observations	Mean	s.e.	Mean	s.e.
Am	*Arenicola marina*	Lugworm	Annelida	Polychaeta	2	-17.4	0.11	14.9	0.68
Cp	*Cancer pagurus*	Edible crab	Arthropoda	Malacostraca	8	-17.02	0.37	16.38	0.66
Cm	*Carcinus maenas*	Shore crab	Arthropoda	Malacostraca	125	-16.80	0.12	16.33	0.11
Cc	*Crangon crangon*	Brown shrimp	Arthropoda	Malacostraca	146	-16.25	0.09	16.48	0.09
Es	*Eriocheir sinensis*	Chinese mitten crab	Arthropoda	Malacostraca	2	-24.49	3.86	17.88	0.07
Hp	*Hemigrapsus penicillatus*	Brush crab	Arthropoda	Malacostraca	6	-17.12	0.63	15.17	0.48
Hs	*Hemigrapsus sanguineus*	(East) Asian shore crab	Arthropoda	Malacostraca	4	-17.86	0.18	16.12	0.35
Hg	*Hyperia galba*	Amphipoda	Arthropoda	Malacostraca	1	-18.97		14.15	
Lh	*Liocarcinus holsatus*	Common swimming crab	Arthropoda	Malacostraca	34	-17.60	0.22	15.30	0.21
My	*Mysidae*	Mysidae	Arthropoda	Malacostraca	44	-17.15	0.18	15.24	0.27
Pb	*Pagurus bernhardus*	Common hermit crab	Arthropoda	Malacostraca	2	-16.09	1.99	14.76	0.02
Ps	*Palaemon serratus*	Common prawn	Arthropoda	Malacostraca	20	-17.00	0.27	16.43	0.21
Pf	*Praunus flexuosus*	Chameleon shrimp	Arthropoda	Malacostraca	26	-17.14	0.32	15.55	0.48
Ms	*Metridium senile*	Sea anemone	Cnidaria	Anthozoa	57	-18.77	0.14	16.2	0.17
Cl	*Cyanea lamarckii*	Blue jellyfish	Cnidaria	Scyphozoa	2	-18.41		15.05	
Ppil	*Pleurobrachia pileus*	Comb jellies	Ctenophora	Tentaculata	2	-17.53	0.78	14.80	0.37
Ar	*Asterias rubens*	Common sea star	Echinodermata	Asteroidae	60	-16.54	0.20	14.64	0.16
Lv	*Loligo vulgaris*	European common squid	Mollusca	Cephalopoda	2	-18.16	0.42	15.65	0.29
Pe	*Palaemon elegans*	Grass prawn	Arthropoda	Malacostraca	263	-18.35	0.20	17.50	0.12



PART III

Past Wadden Sea fish food web structure

7

Historical trophic ecology of some divergent shark and skate species in the Dutch coastal North Sea zone.

Poiesz SSH, Witte JIJ, van der Veer HW (2021) Historical trophic ecology of some divergent shark and skate species in the Dutch coastal North Sea zone. Marine Biology 168:165.

Running title:
Historical Dutch coastal trophic ecology of divergent fish species

Abstract

Over the last century the fish community of the Dutch coastal North Sea zone has lost most its shark and skate species. Whether their disappearance has changed the trophic structure of these shallow waters has not been properly investigated. In this study historical dietary data of sharks and skates, being in the past (near)-residents, juvenile marine migrants and marine seasonal visitors of the Dutch coastal North Sea zone were analyzed for the period 1946 - 1954. Near-resident and juvenile marine migrant species were demersal while all marine seasonal visitors species were pelagic. Based on stomach content composition, the trophic position of four of the various shark and skate species could be reconstructed. The (near)-resident species, the lesser spotted dogfish, the marine juvenile migrant, the starry smooth hound, and the benthopelagic marine seasonal visitor, the thornback ray, had a benthic/demersal diet (polychaetes, molluscs and crustaceans), while the pelagic marine seasonal visitor, the tope shark, fed dominantly on cephalopods and fishes. Diet overlap occurred for fish (tope shark and lesser spotted dogfish), for hermit crabs (lesser spotted dogfish and starry smooth hound) and for shrimps (thornback ray and starry smooth hound). Trophic position ranged from 3.2 for thornback ray preying exclusively on crustaceans to 4.6 for the tope shark consuming higher trophic prey (crustaceans and fish). The analysis indicates that most of the shark and skate species were generalist predators. The calculated trophic positions of shark and skate species indicate that those species were not necessarily at the top of the marine ecosystem food web, but they might have been the top predators of their particular ecological assemblage.

1. Introduction

Worldwide, major structural and functional changes have occurred in coastal ecosystems due to overfishing (Pauly et al. 1998, Jackson et al. 2001, Lotze 2005). Pauly et al. (1998) state that this so-called "fishing down the marine food web" reflects the removal of long-lived, high trophic level, piscivorous fish, including sharks and skates. It is unclear what effect the removal of top predators can have on the stability of a community (Shurin et al. 2002), because for instance the relationship between food chain stability and food chain length is unclear (Sterner et al. 1997). While it is easy to predict that carnivores have a high trophic position and exert a degree of top-down effects, these effects are still very poorly understood (Cortés 1999). Consequences of the removal of top predators could have a cascading effect down the food web, through to lower trophic positions such as bivalves and polychaetes (Hussey et al. 2015). These cascades potentially could extent to the level of the primary producers (Myers et al. 2007). Considering these possible consequences, eliminating larger predators carries more risks of broader ecosystem degradation than previously thought. Top-down effects must be widely expected whenever entire groups of predators are eliminated or removed.

The loss of top predators will cause a reduction of the mean trophic level in fish communities (Estes et al.1998, Worms & Myers 2003, Dulvy et al. 2004, Frank et al. 2005, Myers et al. 2007), but the impact can vary across niches and communities (Borer et al. 2005, Frank et al. 2007). Predator reductions can also cause a shift in niche availability, which can subsequently alter the niche of other predatory species (Frid et al. 2007). As top predatory species, sharks and skates might play pivotal roles in the regulation of lower trophic level organisms and, therefore, of marine ecosystems (Myers et al. 2007). For instance, model studies on the impact of shark depletion in different ecosystems showed differences in the response of prey species and a larger increase in the abundance of minor prey species compared to major prey species (Stevens et al. 2000).

Whereas sharks and skates used to be more common in the North Sea and surrounding coastal areas, nowadays these species are one of the most vulnerable groups of marine fishes (Dulvy et al. 2004, Stevens et al. 2000) and are under pressure and either absent or occurring in low densities (de Vooys et al. 1991, Walker & Heessen 1996, Walker & Hislop 1998, Heessen et al. 2015, Bom et al. 2020). For instance, the thornback ray *Raya clavata* was a common species in Dutch coastal waters but has disappeared from the late 1950s onwards (Walker & Heessen 1996). The trophic position of the species that are still present in the North Sea indicate also a relatively high trophic position (Jennings et al. 2002).

Sharks and skates were common in Dutch coastal waters and estuaries in the past (Egmond 2005). These waters also have been subject to pervasive human disturbance for centuries (Lotze 2005, 2007). A compilation of available information by Witte & Zijlstra (1983) listed ten shark and skate species that were components of the coastal fish community in the past but were already considered extremely rare by the 1970s. For one of the species, the common smooth-hound *Mustelus mustelus* doubt occurred about the taxonomic identification. Heessen et al. (2015) state that species identification between the common smooth-hound and the starry smooth-hound (*Mustulus asterias)* has been quite problematic and that the common smooth-hound is not as common as suggested or may not even occur in the North Sea, implying that all identification in the past of *M. mustulus* would have been *M. asterias.* In an update, Wolff (2005) and Bom et al. (2020) concluded that nowadays, additional rare species had become extinct in or near Dutch coastal waters. Conversely, recently commercial catches have recorded the re-occurrence of some shark species in the Dutch coastal zone (source: Sportvisserij (Dutch fishing society), The Netherlands).

FishBase (Froese & Pauly 2019) provide average trophic positions of individual sharks and skates, but these might not correspond with local observations as a recent analysis of the Wadden Sea coastal fish food web by Poiesz et al. (2020) has shown. Therefore, the past role and trophic position of these lost sharks and skates in the Dutch coastal zone fish food web is unknown. Recent isotope studies showed that the trophic ecology of shark and skate species is potentially very complex (Hussey et al. 2015, Bird et al. 2018, Flowers et al. 2020).

The aim of this study is to reconstruct the trophic position for shark and skate species listed by Witte & Zijlstra (1983) in the past food web of the Dutch coastal North Sea zone. This information on the trophic position of these lost ecosystem components is important with respect to our perspective regarding past food web structure and functioning. The reconstruction is based on information on stomach content composition of fish species in the Dutch coastal zone and estuarine Wadden Sea, dating back to the 1930's (de Vooys et al. 1991). The data were extracted from the archive of the Royal Netherlands Institute for Sea Research (NIOZ). These reconstructions of the trophic position of different shark and skate species was done in line with a recent analysis of the present fish food web structure in the western Wadden Sea by Poiesz et al. (2020).

First, the various shark and skate species were listed according to their mode of life (pelagic, benthopelagic or demersal) following FishBase (Froese & Pauly 2019) and their functional group [marine seasonal visitors, (near)-residents or juvenile marine migrants] after Zijlstra (1983) and Elliott & Dewailly (1995). Next, stomach content and ontogenetic shifts in diet were analyzed and described for the various species. Subsequently, the trophic positions of shark and skate species were reconstructed based on the stomach composition. Since the historical data set only contained information on prey species found, trophic position was estimated based on the average trophic position of the various prey species, ignoring differences in mass between the various prey species. The potential bias of ignoring differences in prey mass was investigated with a recent data set of stomach content data of fish community of the Wadden Sea collected between 2010 and 2018 (Poiesz et al. 2020). Lastly, the relative degree of specialization in the diet of the various shark and skate species was reconstructed and dietary overlap among species was quantified and the niche type for each species was determined.

2. Materials and methods

2.1. The NIOZ archive

In the period from 1930 until 1969, trained staff of the Royal NIOZ registered landings of rare fish and invertebrate species. Specimens were delivered at the port of Den Helder, mainly by local fishermen from Wieringen, Texel and Den Helder and were paid for with a price slightly above the auction value at the local market. Most of the landings originated from Dutch coastal North Sea waters and the rest from inside the Dutch Wadden Sea.

All elasmobranchs were identified to the species level. All information about species, date of catch, catch location and depth, size of the fish and stomach content was stored. A detailed description of the NIOZ archive can be found in de Vooys et al. (1991, 1993).

Table 1 List of shark and skate species extracted from the NIOZ archive. The average trophic positions ($\overline{TP}$) and the mode of life were extracted from FishBase (Froese & Pauly 2019). Functional group according to Witte & Zijlstra (1983). MSV: marine seasonal visitor; NR: (near)-resident species; JMM: juvenile marine migrants.

Group	Scientific name	Common name	Trophic position FishBase ($\overline{TP}$)	Mode of life	Functional group
Sharks	*Alopias vulpinus*	Common thresher	4.6 (±0.0 SE)	Pelagic	MSV
Sharks	*Lamna nasus*	Porbeagle	4.6 (±0.0 SE)	Pelagic	MSV
Sharks	*Cetorhinus maximus*	Basking shark	3.4 (±0.3 SE)	Pelagic	MSV
Sharks	*Scyliorhinus caniculus*	Lesser spotted dogfish	4.01 (±0.3 SE)	Demersal	NR
Sharks	*Mustelus asterias*	Starry smooth-hound	3.88 (±0.3 SE)	Demersal	JMM
Sharks	*Galeorhinus galeus*	Tope shark	4.37 (±0.1 SE)	Benthopelagic	MSV
Sharks	*Squatina squatina*	Angelshark	4.33 (±0.5 SE)	Demersal	JMM
Skates	*Raja clavata*	Thornback ray	3.59 (±0.2 SE)	Demersal	JMM
Skates	*Dasyatis pastinaca*	Common stingray	3.48 (±0.63 SE)	Demersal	MSV

2.2. Stomach content analysis

Prey items were in most cases identified and registered to species level. When prey items were (partly) digested, identification was made to a higher taxonomic level. Almost all prey items (total of 364) could be identified at least at the class level. In only a few cases unidentified prey items were registered. These were removed from further analysis. For prey species belonging to fishes, shrimps and crabs, total length was also registered. Incomplete specimens, often from species that were eaten in pieces such as *Alitta virens* or *Ensis leei* or when species were in part such as the *Crangon crangon*, were counted only by the number of 'heads'. Nine classes were identified (worms, sea stars, bivalves, gastropods, crabs, shrimps, lobsters, cephalopods and fishes).

For each prey item percentage of occurrence was calculated (= number of stomachs containing a prey species divided by total number of stomachs examined) as measure of diet composition following Baker et al. (2014).

2.2.1. Trophic position (TP)

For each prey species, the mean trophic position was taken for their class, order and family as taxonomic group (see Supplementary materials Table A1). FishBase (Froese & Pauly 2019) was used as it provided dietary information of over 800 mostly peer-reviewed references.

Trophic position (TP_j) for each individual skate or shark *j*, was calculated in line with a previous study of the trophic structure of the Wadden Sea fish fauna (Poiesz et al. 2020). as 1 + the mean value of the trophic positions of the different prey species found in a stomach:

$$TP_j = 1 + \sum(\overline{TP}_{i1} + \overline{TP}_{i2} + \overline{TP}_{i3}\ldots) \qquad [1]$$

where

TP_j	being the calculated trophic position of the individual predator j;
TP_{i1}	the mean trophic position of the first prey species $i1$;
$TP_{i2}, TP_{i3}\ldots$	the mean trophic position of the second and third etc. prey species.

2.2.2. Potential bias in trophic position

From the data set of stomach content data of fish community of the Wadden Sea collected between 2010 and 2018 (Poiesz et al. 2020), all individual fishes with at least two different prey species were selected. For these individuals (n = 5300), trophic position was estimated in two different ways:

Estimate 1: Based on mean trophic position of the prey items in line with this study. Trophic position was calculated as 1 + the mean trophic position of the various prey species according to FishBase.

Estimate 2: Based on weighted mean trophic position of the different prey masses. First, the contribution of the various prey item to the total diet was determined on the basis of back-calculated consumed fresh biomass, reconstructed by means of length-weight relationships, whereby for small prey items a mean wet mass was taken. Next the trophic position of the predator was estimated as 1 + the weighted average of the trophic positions of the various food items.

The potential bias associated with not taking prey mass into account was assessed by constructing a linear regression between both estimates.

2.2.3. Niche overlap

To determine the relative degree of specialization in diet and to compare the diets between the different shark and skate species, the Levins' index of niche breath was used (Levins 1968). For this analysis the contribution of each different prey species within a stomach was used and calculated according to:

$$P_{ij} = \frac{N_{ij}}{N_{jtot}} \qquad [2]$$

where

p_{ij} being the proportion of each prey species i in the diet of each individual predator j;

N_{ij} the number of individuals of the species i in the stomach of the predator j;

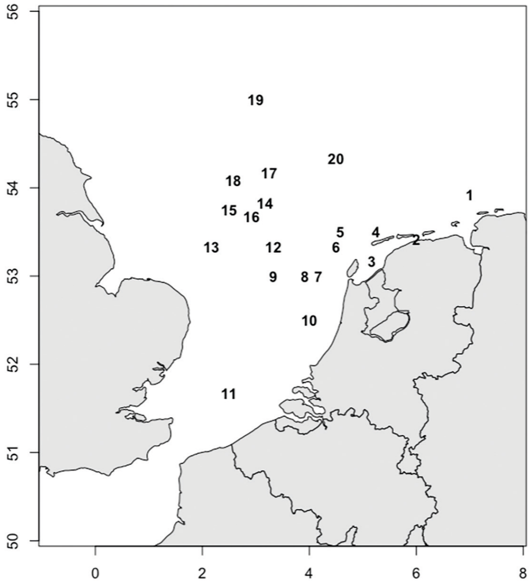
N_{jtot} the total number of preys counted of each individual predator j.

Next, the average proportion (p_{ij}) of each prey species was taken for each predatory species.

In order to compare the different diets, the average proportion of the different prey items ($\boldsymbol{p}_{ij}$) were classified up to the highest taxonomic level (order) level. Next, Levins' standardized measure of niche breadth ($\boldsymbol{B}_j$) was calculated according to:

$$B_j = \frac{1}{\sum Pij^2} \qquad [3]$$

Levins' measure of niche breadth can range from 1 (indicating a highly specific diet with only one prey species) to <1 (indicating a less specific diet with more prey species).



Location	Name	*Alopias vulpinus*	*Lamna nasus*	*Cetorhinus maximus*	*Scyliorhinus caniculus*	*Mustelus asterias*	*Galeorhinus galeus*	*Squatina squatina*	*Raja clavata*	*Dasyatis pastinaca*
1	Borkummer Stones				▒	▒	▒		▒	
2	Eastern Wadden Sea					█	█			
3	Western Wadden Sea	█	█		█	█	█	█	█	█
4	Terschelling Bank		▒	▒	▒	▒	▒	▒	▒	▒
5	Frisian Front		▒		▒	▒	▒		▒	▒
6	Vlieland Ground		▒	▒	▒	▒	▒		▒	▒
7	Texel Hole				▒	▒	▒	▒	▒	▒
8	Texel Ground	▒			▒	▒	▒		▒	▒
9	Brown Bank				▒	▒	▒		▒	▒
10	The Broad Fourteen	▒	▒	▒	▒	▒	▒	▒	▒	▒
11	Southern North Sea	▒		▒	▒	▒	▒	▒		
12	Tea Kettle Hole				▒	▒	▒		▒	
13	Leman Ground			▒	▒	▒			▒	
14	Botney Cut				▒	▒	▒		▒	▒
15	Markham’s Hole				▒	▒			▒	
16	Botney Grounds	▒			▒		▒			
17	Cleaver Bank				▒	▒	▒		▒	
18	Silver Pit			▒	▒			▒	▒	
19	Doggers Bank			▒	▒	▒			▒	
20	Oyster Ground				▒	▒	▒			

Figure 1 Catch location in the North Sea and Wadden Sea of shark and skate species extracted from the NIOZ archive. The names correspond with the locations are given below. The numbers of the locations in the table correspond with the numbers in the map. Grey: North Sea locations; black: Wadden Sea locations; white no records.

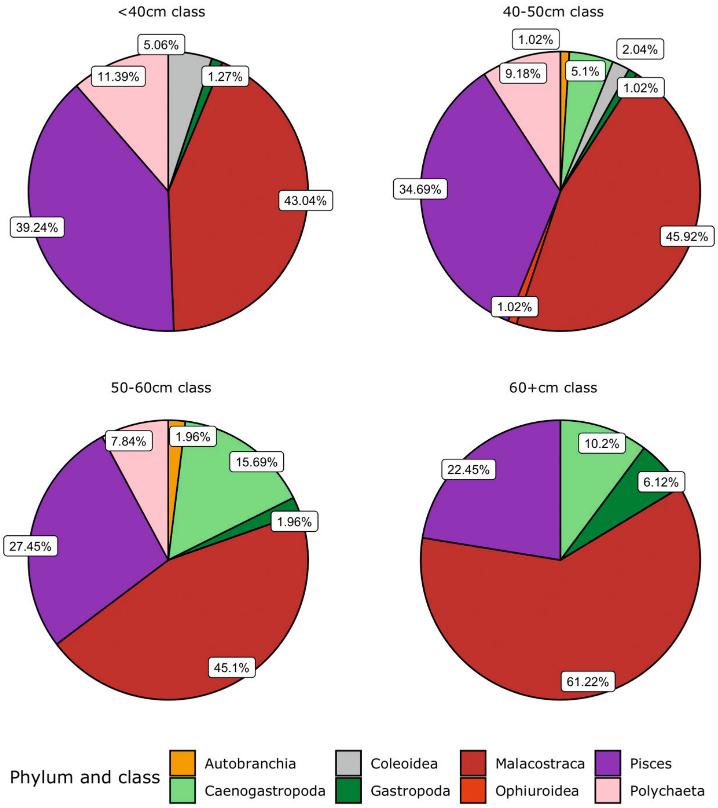


Figure 2 Occurrence of each prey phylum and class (%) in the stomach of the lesser spotted dogfish (*Scyliorhinus canicula*) for different size classes (cm) between 1946 and 1954.

To quantify dietary overlap, the MacArthur-Levins' method was used (MacArthur & Levins 1967, Feinsinger et al. 1981). It estimates the extent to which the prey of consumer species ***k*** overlaps with that of species ***j***. For instance, if species ***j*** specializes on a certain type of food source which is also eaten by a more generalist species ***k***, then from species ***j***'s viewpoint its niche overlaps completely with the other species, but from species ***k's*** viewpoint the niche only partially overlaps with the niche of species ***j***. Therefore, the calculated values can differ between ***j*** and ***k*** and vice versa. The MacArthur-Levins' method is calculated using the following equation:

$$M_{jk} = s\,\frac{\sum p_{ij}{}^{*}p_{ik}}{\sum p_{ij}^{2}} \quad \text{and} \quad M_{kj} = s\,\frac{\sum p_{ij}{}^{*}p_{ik}}{\sum p_{ik}^{2}} \qquad [4]$$

where M_{jk} and M_{kj} are the degree of overlap on species j by species k and vice versa, and p_{ij} and p_{ik} are the proportions that food resource $\boldsymbol{i}$ contributes to the diets of species j and k, respectively (Ellis 1996, Sa-Oliveira et al. 2014).

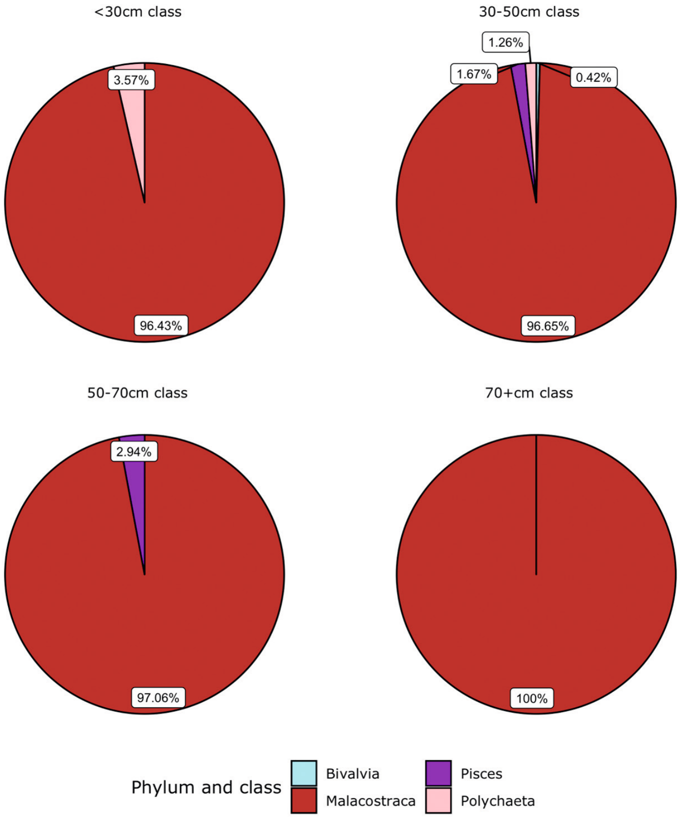


Figure 3 Occurrence of each prey phylum and class (%) in the stomach of the thornback ray (*Raja clavata*) for different size classes (cm) between 1946 and 1954.

2.4. Data exploration and visualization

The data was explored using the protocol described in Zuur et al. (2010). Ontogenetic shifts in diet and trophic position were explored with length as categorical variable. Local Polynomial Regression (LPR) were used by means of LOESS (span = 0.75) with the ggplot package (Wickham 2016).

Visualizations of the network data was made by the bipartite package in Dormann et al. (2009) and Levins' index of niche breath calculation was made using the MicroNiche package (Finn 2020). All further data analysis and data manipulations were done in R (R Core Team 2019).

3. Results

3.1. Species composition

The NIOZ-archive contained information from nine shark and skate species (Table 1). No records were found from the common smooth-hound. Therefore, all records have been considered to have been starry smooth-hound *Mustelus asterias*. One species, the lesser spotted dogfish, belonged to the (near)-resident species and three species were juvenile marine migrants: the thornback ray, the starry smooth-hound and the angelshark. All other species were marine seasonal visitors. (Near)-resident and juvenile marine migrants were demersal species, and all marine seasonal visitors were pelagic species.

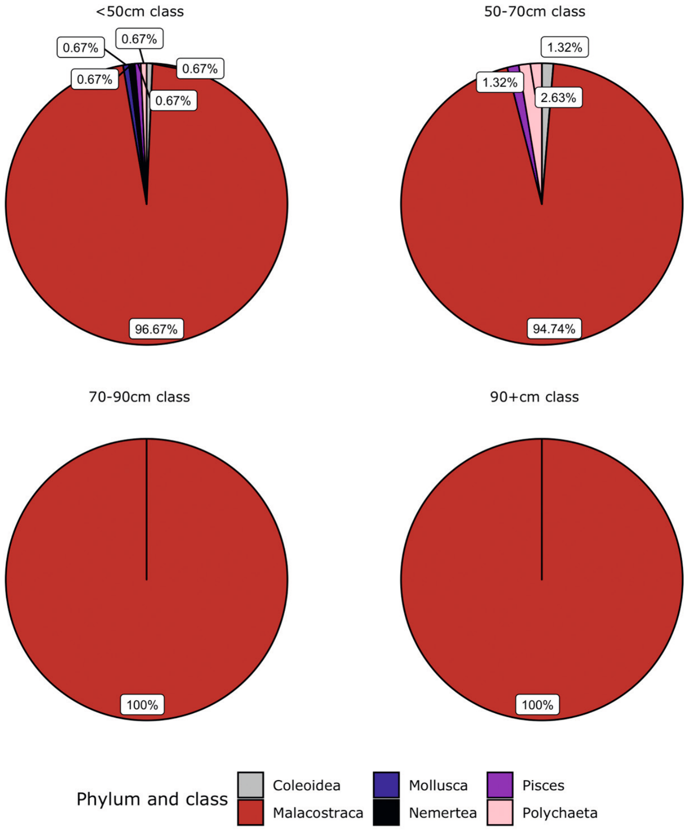


Figure 4 Occurrence of each prey phylum and class (%) in the stomach of the starry smooth-hound (*Mustelus asterias*) for different size classes (cm) between 1946 and 1954.

Capture locations of individual sharks and skates could be grouped into wider geographical areas (e.g., banks, grounds and holes) in the North Sea and Wadden Sea (Fig 1). The lesser spotted dogfish, starry smooth-hound, tope shark and thornback ray were captured at almost all locations. All other species were caught throughout the North Sea and Wadden Sea (Fig 1). The only species that did not occur in the Terschelling Bank was the common thresher shark, while only the starry smooth-hound and tope shark occurred in the eastern Wadden Sea.

Most stomach content data were collected between 1946 and 1954, therefore the analysis was restricted to this period. For five species (common thresher shark, basking shark, common sting ray, porbeagle and angelshark), the NIOZ archive contained very few data, i.e., information from less than ten stomachs (see Supplementary materials Table B1). Therefore these species were not included in subsequent analysis.

3.2. Stomach content and trophic position

3.2.1. Lesser spotted dogfish (Scyliorhinus canicula)

The size of the lesser spotted dogfish ranged from 10 to 83 cm total length and they were caught in depths between 2 and 77 meters. Supplementary materials Fig B2 shows the records at the various locations. Stomach data from 180 individuals were available: of these 19 stomachs were empty (10.6%) and for 12 stomachs the information about total fish length was lacking. In total 29 different prey items were found (Supplementary materials Table B2), with only a few groups occurring frequently in the stomachs: Pisces and Malacostraca and to a lesser extent Caenogastropoda and Polychaeta. Pisces consisted mainly of Pleuronectiformes (flatfishes, flounder, sole), Ammoditidae (greater sandeel), and Trigiidae (gurnard). Malacostraca included mainly Crangonidae (shrimps), Anomura (hermit crabs) and Brachyura (crabs). Caenogastropoda comprised Buccinidae (whelks) and Polychaeta Annelidae (Nereididae) (Fig 2). About half (48.4%) of the prey items in the stomachs were crustaceans. With increasing fish size, the occurrence of Brachyura decreased. The occurrence of Annelids (Nereididae) also decreased with size and they were absent above 60 cm in size. Larger lesser spotted dogfish did also consume flatfishes (Fig 2). There was variability in the estimates of trophic position, but there was no significant relationship with fish size [ANOVA; F(3, 149) = 1.492, p = 0.22] (Fig 6). Mean trophic position was 4.01 (±0.43 SE).

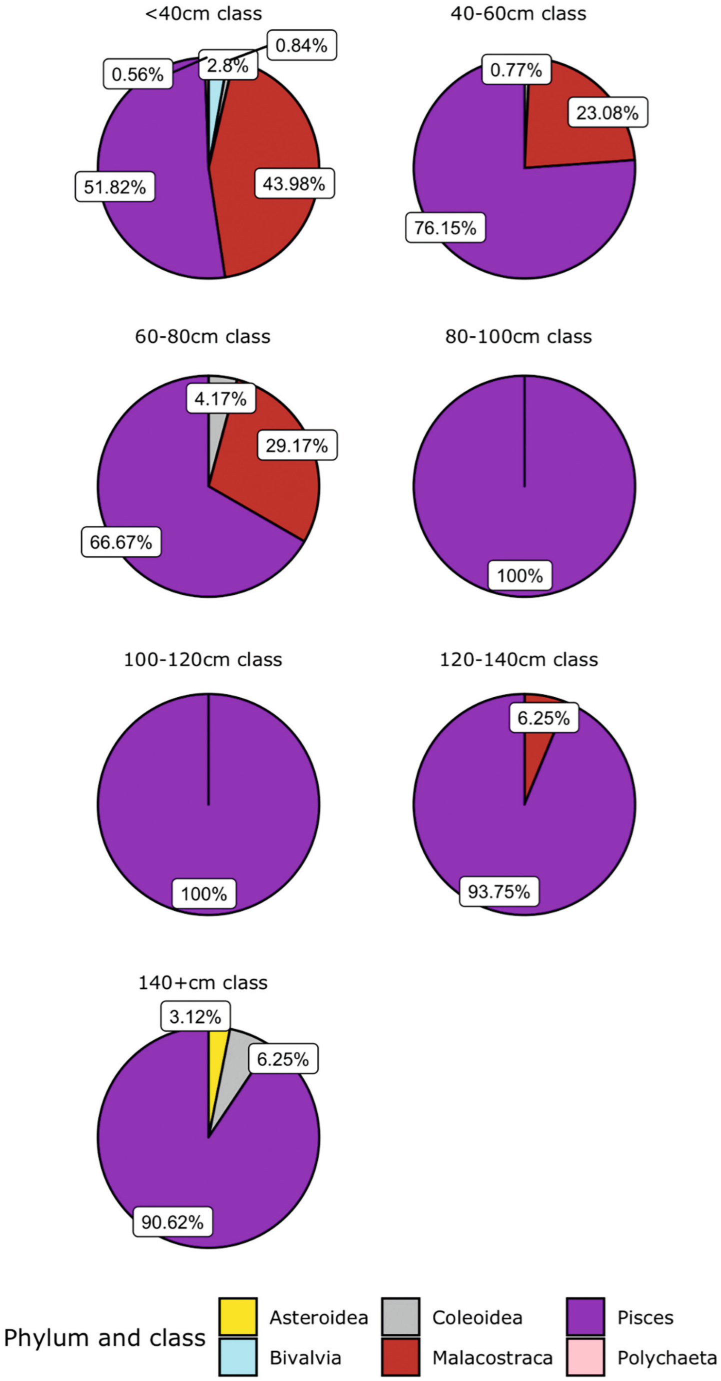


Figure 5 Occurrence of each prey phylum and class (%) in the stomach of the tope shark (*Galeorhinus galeus*) for the different size classes (cm) between 1946 and 1954.

3.2.2. Thornback ray (Raja clavata)

All thornback rays were caught from water depths ranging from 3 to 49 meters and measured from 10 to 85 cm in total length. Supplementary materials Fig B3 shows the number of individuals caught at the various locations. From all thornback rays captured, 174 individuals contained information about stomach content. Ten individuals (5.5%) had empty stomachs, and for 9 individuals no fish size was documented (Supplementary materials Table B3). The diet of thornback rays mainly consisted of Malacostraca and low percentages of Pisces and some Bivalvia and Polychaeta (Fig 3). The Malacostraca included mainly Crangonidae (shrimp species) and Brachyura (crab species) and the Pisces were Ammoditidae (greater) sandeel). Nereididae were the dominant Polychaeta family. No trends between trophic position and size were found [ANOVA; F(3, 152) = 0.892, p = 0.447], which might partly be due to the low number of observations of small (<30 cm) and large (>70 cm) fishes (Fig 6). Mean trophic position was 3.59 (±0.14 SE).

3.2.3. Starry smooth-hound (Mustelus asterias)

Stomach content data of 276 starry smooth-hounds were available. Of these, five individuals (1.8%) had empty stomachs. The total length of the starry smooth-hounds ranged between 27 and 117 centimeters and they were caught at depths ranging between 5 and 56 meters. Supplementary materials Fig B4 shows the number of individuals caught at each location. Almost all prey species were Malacostraca with in addition some Pisces, Polychaeta and Molluscs (Fig 4) (See Supplementary materials Table B4 for more detailed information). The Malacostraca were Crangonidae (various shrimp species), Anomura (hermit crabs) and Brachyura (crabs). The few Pisces belonged to the Callionimidae (dragonets) and the Polychaeta were Nereididae. The prey species remained the same with increasing fish size, however frequency of occurrence did show some variability over the different size classes. Estimated trophic position showed some variability but was not related to the different size classes [ANOVA; F(3, 276) = 0.428, p = 0.73] (Fig 6). Mean trophic position was 3.88 (±0.27 SE).

3.2.4. Tope sharks (Galeorhinus galeus)

The NIOZ archive contained stomach data of 508 tope sharks, but 29 individuals (5.7%) had an empty stomach and for 2 individuals, total length data was not recorded from prey (See Supplementary materials Table B5 for more detailed information). All tope sharks were caught at depths between 3 and 67 meters and the total length ranged from 25 to 166 cm. Supplementary materials Fig B5 shows the number of individuals caught at each location. Pisces were the most important prey item for all size classes (Fig 5). Smaller individuals (<40 cm) also had Malacostraca in their stomachs. Other groups were found infrequently: Polychaeta only occurred in the stomachs below 40 cm total length whereas Asteroidea and Ophiuridae were only present in fish with a total length above 120 cm. Cephalopods occurred in almost all size classes. Several families of Pisces were eaten: both demersal (Pleuronectidae, Solidae, Gobiidae) and pelagic (Clupeidae, Ammodytidae, Scrombidae, Trigiidae, Tadidae, Carangidae) species. Malacostraca were preyed on mainly by the smallest size groups, especially Anomura (hermit crabs) and Brachyura (crabs) and to a lesser extent Crangonidae (shrimp species). Trophic position showed a significant positive relationship with size [ANOVA; F(6, 470) = 15.36, $p < 0.05$], however with some variability (Fig 6). Overall, mean trophic position was 4.37 (± 0.39 SE).

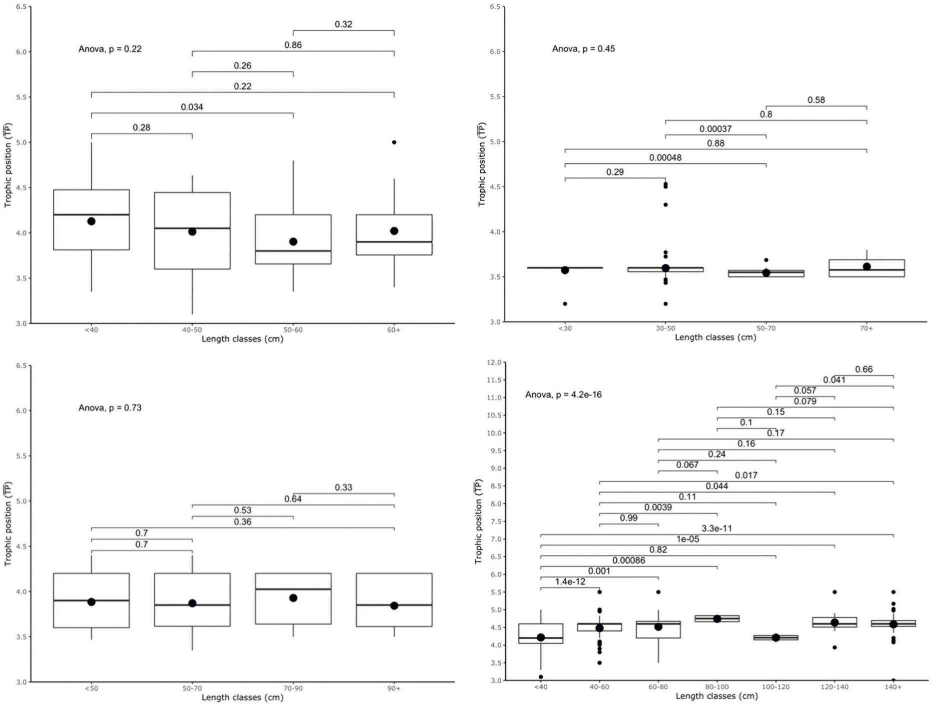


Figure 6 The average trophic positions ($\overline{TP}$;--) based on stomach content composition related to boxplot length classes (cm) with whiskers; black dot as mean for each length class and P value for comparison between each length class. Top left: the lesser spotted dogfish (*Scyliorhinus canicula*); Top right: thornback ray (*Raja clavata*); Bottom left: starry smooth-hound (*Mustelus asterias);* Bottom right: tope shark (*Galeorhinus galeus*).

3.2.5. Other species

Five species (common thresher shark, basking shark, common stingray, porbeagle and angelshark) contained very few data (see Supplementary materials Table C1). The diet of the thresher shark consisted mostly of Pisces, while the diet of the basking shark only contained Crustacea. The porbeagle preyed upon Pisces and to a small extent on Cephalopoda. The diet of the common stingray was more variable and consisted of a mixture of Annelida and Crustacea and some Pisces. The angelshark preyed mainly on Pisces, and in addition on Crustacea and Cephalopoda (Supplementary materials Table C2). Supplementary materials Fig C1-C5 shows the number of individuals caught at the various locations for these five species.

3.3. Potential bias in TP estimate

The data set of stomach content data of fish community of the Wadden Sea collected between 2010 and 2018 contained 2876 stomach content records about 54 prey fish species and 72 different prey taxa. For all individual stomachs, the relationship between the two methods (Estimate 1: based on mean trophic position of the prey items; Estimate 2: based on the weighted mean trophic position of the different prey masses) was highly significant ($R^2 = 0.98$, $p < 0.05$) over a range in trophic positions from 2.0 to 4.7 (Fig 7). A selection of stomachs containing only fish and crustaceans, corresponding with the main prey items of the sharks and skates in this study, resulted in a similar significant relationship ($R^2 = 0.91$, $p < 0.05$; Fig 7), with an estimated intercept of 0 and slope 95 confidence limits that overlapped with 1. As such, we concluded that no mass-based correction of TP estimates used in this study was required.

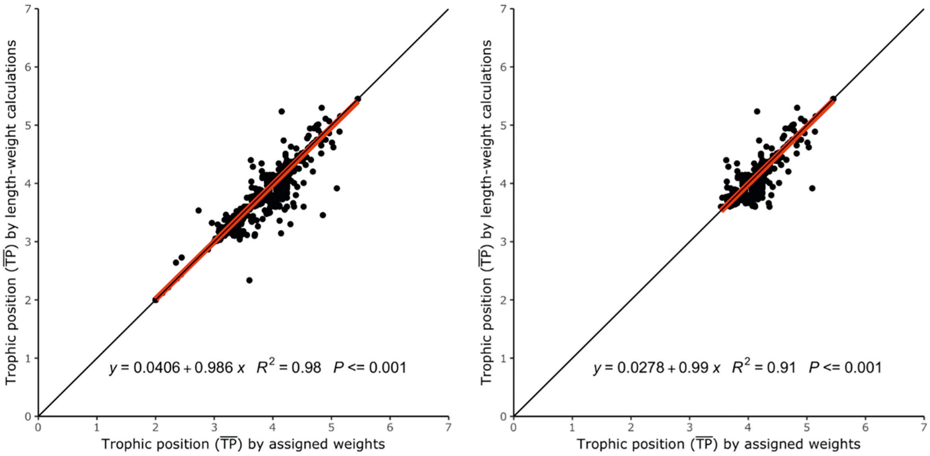


Figure 7 Relationship between two estimates of trophic position (TP) based on stomach content data. X axes: TP calculated based on the mean of the trophic positions of the different prey items. Y axes: TP calculated based on the weighted mean of the trophic positions of the prey items after reconstruction of their mass. The black line represents the y=x. Left panel: all individual data of 27 species. Right panel: all individual data with TP > 4. Data after Poiesz et al. (2020).

3.4. Niche overlap

The four shark and skate species appeared to be generalists, feeding on a variety of different prey items (Fig 8) with differences between the species. The lesser spotted dogfish, the starry smooth hound and thornback ray showed a benthic/demersal diet (polychaetes, molluscs and crustaceans), while the tope shark fed dominantly on cephalopods and fishes. Most diet overlap occurred for three groups of prey: Pisces, Crustacea and Cephalopoda (Fig 8).

Niche overlap occurred for fish (tope shark and lesser spotted dogfish), for hermit crabs (lesser spotted dogfish and starry smooth hound) and for shrimps (thornback ray and starry smooth hound). A niche overlap analysis (Table 2) showed that the lesser spotted dogfish had a significant diet overlap with the other species. The tope shark showed no significant diet overlap with other predatory species (Table 2).

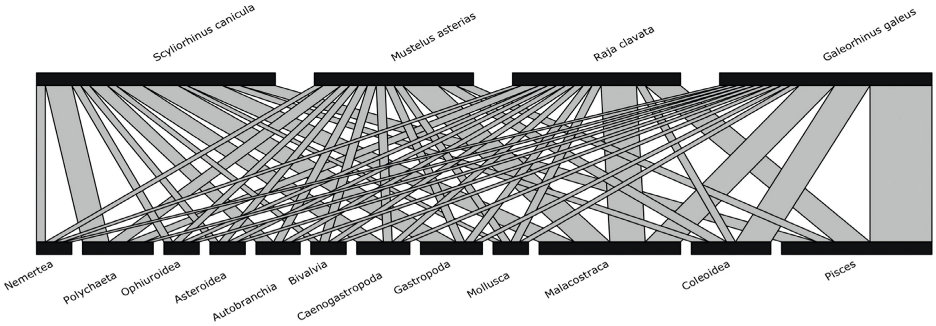


Figure 8 Network analysis based on the overlap in occurrence of prey species in the stomach of the various shark and skate species. Prey classes are listed from lowest average $\overline{TP}$ to highest average $\overline{TP}$.

4. Discussion

We used historical dietary data to examine the trophic ecology of four species of sharks and skates which have disappeared from Dutch coastal and North Sea waters. Our results show that the four species were generalist feeders and had trophic positions between 3.2 and 4.6. Three species, the (near)-resident lesser spotted dogfish, the marine juvenile migrant starry smooth hound, and the benthopelagic marine seasonal visitor thornback ray had a benthic/demersal diet feeding especially on polychaetes, molluscs and crustaceans. The pelagic marine seasonal visitor tope shark fed dominantly on cephalopods and fishes. The diet of the different species overlapped: for fish (tope shark and lesser spotted dogfish), for hermit crabs (lesser spotted dogfish and starry smooth hound) and for shrimps (thornback ray and starry smooth hound).

The catch data of the various species illustrate that the records of most sharks and skates from the NIOZ archive originated from Dutch coastal waters. These shark and skate species can be considered part of the wider Dutch coastal and Wadden Sea food web as they have also been registered inside the Wadden Sea (Witte & Zijlstra 1983). Tagging experiments also indicate that the data may be representative for the Dutch Wadden Sea. Tagging of the tope shark and the starry smooth-hound has shown migration of these species from the south coast of England and the north coast of Wales to the Bay of Biscay in Portugal (Holden & Horrod 1979, Farrell et al. 2010). Also, thornback rays move over several hundred kilometers (Walker et al. 1997) and the lesser spotted dogfish have shown movement up to 30 kilometres (Rodríguez-Cabello et al. 1998, 2004, Sims et al. 2001). The movement of the common stingray has not yet been studied, but the dispersion of this species as shown in de Vooys et al. (1991), indicates a population that lived close to or in the Dutch Wadden Sea.

Table 2 MacArthur and Levins' measure of niche overlap for the shark and skate species from the NIOZ archive between 1946 and 1954. Values are indicating the extent to which the diet of species (X) is overlapped by the diet of species (Y). Significance: ** p≤0.05; *: 0.05<p<0.10. Values from 0.6 are considered to indicate significant niche overlap.

Species	*Scyliorhinus caniculus*	*Raja clavata*	*Galeorhinus galeus*	*Mustelus asterias*
Scyliorhinus caniculus		0.666**	0.914**	0.671**
Raja clavata	0.495		0.261	0.998**
Galeorhinus galeus	0.562*	0.486		0.469
Mustelus asterias	0.494	0.998**	0.253	

4.1. Diet composition

The taxonomic identification of the stomach contents in this study was not always made to species level but to higher taxonomic groups (class and order). More detailed information may have resulted in more detailed information about segregation and overlap in prey items between species. Also, elasmobranchs quickly digest and break down their prey (Córtes et al. 2008, Wieczorek et al. 2018), which might have meant that soft-bodied prey were under-represented.

Due to the limited data available for five species (the common thresher shark, porbeagle, basking shark, the angelshark and the common stingray), it is questionable whether these species are really generalist or specialist feeders. At least for the angelshark, the focus on crustaceans and (flat)fishes in the Irish Sea (Ellis et al. 1996) suggest that this species is a specialist feeder. The other four shark and skate species in the Dutch coastal zone appeared to be generalist predators consuming a variety of polychaetes, mollusks, crustaceans and teleosts, as also found for the North-eastern Atlantic by Ellis et al. (1996). In generalist predators, spatial differences in diet composition might be expected reflecting local variability in prey availability.

In the Dutch coastal zone, the diet of the lesser spotted dogfish contained a wide variety of other taxonomic groups but about half of the prey items were crustaceans, similar to reports from other parts of the North Sea (Pinnegar 2014) and elsewhere (Lyle 1983, Ellis et al. 1996, Wieczorek et al. 2018). The diet of thornback rays in the Dutch coastal zone mainly consisted of crustaceans and low percentages of Pisces and some Bivalvia and Polychaeta. The diet composition of the starry smooth-hound contained Pisces and Crustacea and to a lesser extent Caenogastropoda and Polychaeta. The focus of both species on mainly crustacean species is comparable with feeding patterns in other parts of the North Sea (Daan 1993, Pinnegar 2014). Other studies reported similar diet composition for the common stingray but also included additional benthic invertebrate species (Yeldan et al. 2009, Saglam et al. 2010, Šantić et al. 2011). The diet composition of the starry smooth-hound also corresponded with the findings of Ellis et al. (1996) for the Irish Sea, but the NIOZ archive data showed an additional consumption of shrimps in the early life stage. The results for the tope shark found in this study are in line with the those described for sharks by Cortés (1999).

In this study, ontogenetic shifts in diet were only found the lesser spotted dogfish and the tope shark whereby the prey size and diet composition differed with total length and life stage, in line with other studies (Lyle 1983, Ellis et al. 1996, Henderson & Dunne 2002, Lucifora et al. 2006, Martinho et al. 2012). In the lesser spotted dogfish, the occurrence of crab and polychaeta worms decreased with size while in the tope shark smaller individuals (< 40 cm) had crustaceans in their stomachs and larger individuals had a wider variety of fish species (Pleuronectiformes, Gadidea). Ontogenetic shifts in diet have also been described for the thornback ray in contrast to this study. In other areas, young individuals prey on small crustaceans such as shrimps, while larger individuals consume larger crustaceans, such as swimming crabs (Holden & Tucker 1974, Ellis et al. 1996, Farias et al. 2006). In this study, only large thornback rays > 70 cm showed a higher preference for Pisces.

4.2. Trophic position

Estimates of trophic position might to some extent depend on the methodology used. In a previous analysis of stomach contents of the lesser spotted dogfish from the west coast of Ireland, Wieczorek et al. (2018) found mainly hard-bodied prey or vertebrates, such as decapods or fish with a relatively higher trophic level. However, a simultaneous stable isotope analysis resulted in a lower estimate of the trophic position of the lesser spotted dogfish (Wieczorek et al. 2018). Apparently, elasmobranchs digest soft-bodied prey items very quickly and therefore their contribution is underestimated by stomach content analysis. Conversely, comparable trophic positions estimated from isotope composition were found by Jennings and van der Molen (2015) for the two species in this study (the thornback ray and the starry smooth-hound). This suggests that estimates of trophic position based on historical stomach content data may be biased due to missing digested soft prey items. In this study stomachs with a record of only a single prey item resulted in some cases in an estimate of a very low or high trophic position of the predator. Most likely other less important prey items were not registered or identified in these stomachs and as such, estimates of trophic position based on these data are invalid. However, stomach content analyses remain a simple and valuable tool to reconstruct the historic trophic position of predators, including predator-prey relationships and niche overlap. For future studies, it is highly desirable that stomach content analysis and stable isotope analysis should be combined to unravel the complex trophic ecology of these elusive predators (Cortés 1999, Flowers et al. 2020).

The shark and skate species from the Dutch coastal zone also occurred in large parts of the North Sea (Heessen et al. 2015) and included species from different functional groups (Zijlstra 1983, Elliott & Dewailly 1995). One species, the lesser spotted dogfish, belonged to the (near)-resident species and three species were juvenile marine migrants: the thornback ray, the starry smooth-hound and the angelshark. All other species were marine seasonal visitors. (Near)-resident and juvenile marine migrants were demersal species, and all marine seasonal visitors were pelagic species. Most niche overlap occurred among the benthic/demersal species for hermit crabs (lesser spotted dogfish and starry smooth hound) and for shrimps (thornback ray and starry smooth hound). There was no evidence for overlap between the lesser spotted dogfish, starry smooth-hound and thornback ray. The pelagic tope shark had some overlap for fish with the benthic/demersal lesser spotted dogfish.

Estimates of trophic position ranged from 3.2 for thornback ray preying exclusively on crustaceans to 4.6 for the tope shark consuming higher trophic prey (crustaceans and fish) over the period 1946 - 1954. The calculated trophic position for the shark and skate species in the Dutch coastal zone in this study corresponds closely with that found in other parts of the North Sea by Jiming (1982). The calculated trophic positions of shark and skate species indicate that some species fed at the top of the marine food web, but others fed at a lower trophic level but may be top predators of their ecological assemblage.

4.3. Past role in the food web

Common thresher, porbeagles, basking and angel sharks have been extremely rare for a long period in the Dutch Wadden Sea and coastal area (Witte & Zijlstra 1983, Heessen et al. 2015). As such, predation pressure and potential food competition from these species will have been low for decades. Commercial landings of skates and rays in the past (Heessen et al. 2015, Bom et al. 2020) indicate substantial densities in the Dutch coastal zone at that time and imply that they were an important component of the past food web and that their disappearance might have caused changes in the community through competitive release as stated by Stevens et al. (2000).

The effects of removing large numbers of these top predators on the marine ecosystem is still largely unknown (Stevens et al. 2000) but might be substantial for Dutch coastal waters. For instance, Lynam et al. (2017) suggest that top-down exploitation of predators has an important effect on the dynamics of other fish populations and can initiate complex cascading effects. In the North Sea especially planktivorous (top) predators should play such a central role (Lynam et al. 2017).

A recent analysis of contemporary food web structure of the Dutch coastal fish community based on stomach content analysis resulted in trophic positions between 2.0 and 4.7, with most trophic positions above 3.0. Until a few decades ago, (near)-

resident species were the most abundant functional group in spring and juvenile marine migrants in autumn (Poiesz et al. 2020). Part of the shark and skate species analyzed in the study also belonged to (near)-residents and marine juvenile migrants. Therefore, coastal zone habitats were likely more important for (near)-residents and marine juvenile migrants in the past than nowadays.

The various shark and skate species differ with respect to their prey location, prey depth, prey size and feeding times (Young et al. 2010). Mouth dimensions of skates correlate with their diet and prey specialization (Walker 1998, Scharf et al. 2000). This trophic or behavioral separation also causes niche segregation. Our work has shown that these now rare species likely played important trophic roles in Dutch coastal waters. However, to what extent these species can be considered as top predators within their own ecological assemblage (Pusineri et al. 2008, Young et al. 2010), is still unknown. For instance, some reportedly "top-predator" species such as skates (thornback ray), feed at a relatively low trophic position and prey to other species with a higher trophic position (Flowers et al. 2020).

Acknowledgements

Thanks are due to all former NIOZ colleagues who were responsible for the NIOZ archive. The data set of stomach content data of the shark and skates from the NIOZ archive can be found under https://dx.doi.org/10.25850/nioz/XXXX and the data set of the stomach content of the fish community of the Wadden Sea 2011 – 2018 and the R script for calculation under https://dx.doi.org/10.25850/nioz/7b.b.bb. The comments of the two reviewers are much appreciated and they improved the manuscript substantially.

Supplementary materials A

Table A1 Characterization of prey species found in the stomachs of sharks and skates for the NIOZ archive.

Supplementary materials B

Table B1 The number of records with stomach contents information found in the NIOZ archive for each species in each year.

	Scyliorhinus canicula	*Raja clavata*	*Mustelus asterias*	*Galeorhinus galeus*
1931				2
1932	5			
1945				2
1946	3	1	82	70
1947	12	5	40	339
1948	16	5	28	51
1949	1	47	6	5
1950	45	17	38	3
1951	21	24	56	5
1952	29	47	12	1
1953	11	1	3	
1954	10		2	
1955			1	
1956			1	1
1966			2	
1967		4		
1968		1		2
1969		4		
Total	**153**	**156**	**271**	**481**

Table B2 The occurrence of prey species in the stomach of the lesser spotted dogfish (*Scyliorhinus canicula*) for different size classes, the total number of individuals per size class and the total number of empty stomachs per size class. The values represent mean percentages of occurrence.

	Diet composition of *Scyliorhinus canicula*				
Taxa	<40 cm	40-50 cm	50-60 cm	60+ cm	Total
Annelida	11.4	9.1	6.9		7.1
Nereididae	8.9	8.1	6.9		6.2
Annelida (unidentified)	2.5	1.0			1.0
Crustacea	43.0	46.5	48.3	58.5	48.4
Anomura	21.5	18.2	24.1	26.4	20.8
Paguroidea (unidentified)	21.5	18.2	22.4	26.4	20.5
Pagurus bernhardus			1.7		0.3
Brachyura	13.9	11.1	1.7	3.8	8.8
Portunidae (unidentified)	8.9	3.0	1.7	1.9	4.2
Corystes sp.		1.0			0.3
Hyas sp.		1.0			0.3
Brachyura (unidentified)	5.1	6.1		1.9	3.9
Caridea	7.6	17.2	22.4	28.3	17.9
Processas sp.		1.0	1.7		0.6
Crangon allmanni		2.0	1.7	5.7	2.3
Crangon crangon	3.8	3.0	3.4	11.3	5.5
Pontophilus trispinosus			1.7		0.3
Caridea (unidentified)	3.8	11.1	13.8	11.3	9.1
Crustacea (unidentified)					1.0
Mollusca	6.3	12.1	20.7	20.8	14.6
Gastropoda	1.3	6.1	17.2	17.0	10.1
Buccinum undatum		5.1	15.5	11.3	8.1
Gastropoda (unidentified)	1.3	1.0	1.7	5.7	1.9
Cephalopoda	5.1	5.1	1.7	3.8	3.9
Alloteuthis sp.		1.0			0.3
Loligo sp.		3.0	1.7	3.8	1.9
Sepia sp.	1.3	1.0			0.6
Sepiola sp.	3.8				1.0
Bivalvia		1.0	1.7		0.6
Mya truncata		1.0	1.7		0.6
Echinodermata		1.0			0.3
Ophiuroidea (unidentified)		1.0			0.3
Pisces	39.2	31.3	24.1	20.8	29.5
Pleuronectiformes (unidentified)	6.3	2.0	8.6		3.9
Clupeidae (unidentified)		1.0			0.3
Gadidae (unidentified)	1.3				0.3
Pleuronectidae (unidentified)				3.8	0.6
Buglossidium luteum	1.3	1.0			0.6
Hyperoplus lanceolatus	2.5	1.0	3.4	5.7	2.6
Solea solea	2.5	1.0	1.7		1.3
Pisces (unidentified)	25.3	25.3	10.3	11.3	19.8
Number of empty stomachs	**5**	**5**	**1**	**5**	**19**
Total number of stomachs	**49**	**64**	**25**	**31**	**181**

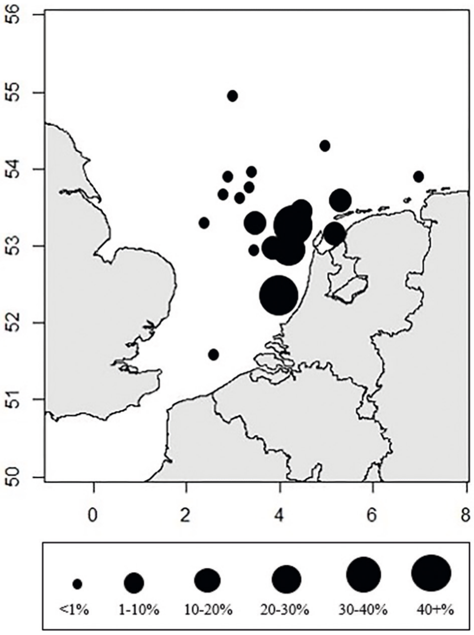


Figure B2 The relative density (%) of the lesser spotted dogfish (*Scyliorhinus canicula*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

Table B3 The occurrence of prey species in the stomach of the thornback ray (*Raja clavata*) for different size classes, the total number of individuals per size class and the total number of empty stomachs per size class. The values represent mean percentages of occurrence.

	Diet composition of *Raja clavata*				
Taxa	<30 cm	30-50 cm	50-70 cm	70+ cm	Total
Annelida	3.2	1.2			1.2
Nereididae (unidentified)	3.2	1.2			1.2
Crustucea	96.8	96.8	95.2	66.7	95.0
Anomura		0.8	2.4		0.9
Paguroidea (unidentified)		0.4			0.3
Pagurus bernhardus		0.4	2.4		0.6
Brachyura	3.2	14.5	42.9	33.3	19.0
Portunidae (unidentified)	3.2	8.4	28.6	33.3	11.1
Corystes sp.		0.4	2.4		0.6
Macropipus sp.		0.4			0.3
Cancer pagurus					0.3
Corystes cassivelaunus		0.4			0.3
Liocarcinus holsatus		3.2	11.9		5.2
Thia scutellata		0.4			0.3
Brachyura (unidentified)		1.2			0.9
Caridea	90.3	77.5	42.9	33.3	71.1
Processas sp.	25.8	11.2	2.4		10.8
Crangon allmanni	12.9	10.4	7.1		9.9
Crangon crangon	19.4	20.1	9.5	16.7	18.4
Philocheras bispinosus bispinosus	3.2	2.8			2.3
Philocheras trispinosus	12.9	16.5	7.1		14.0
Processa canaliculata		6.8	4.8		5.5
Processa parva	3.2	1.6			1.5
Caridea (unidentified)	12.9	8.0	11.9	16.7	8.7
Other crustaceans	3.2	4.0	7.1		4.1
Amphipoda (unidentified)		0.4			0.3
Cumacea (unidentified)		0.8			0.6
Mysida (unidentified)	3.2		2.4		0.6
Gastrosaccus sp.		0.8	2.4		0.9
Gastrosaccus spinifer		2.0			1.5
Pestarella tyrrhena			2.4		0.3
Mollusca		0.4			0.3
Bivalvia (unidentified)		0.4			0.3
Pisces		1.6	4.8	33.3	3.5
Ammodytidae (unidentified)			2.4		0.3
Callionymus sp.			2.4		0.6
Ammodytes tobianus		0.4			0.3
Hyperoplus lanceolatus		0.8			1.2
Pisces (unidentified)		0.4		33.3	1.2
Number of empty stomachs	**0**	**9**	**0**	**0**	**10**
Total number of stomachs	**18**	**124**	**19**	**4**	**174**

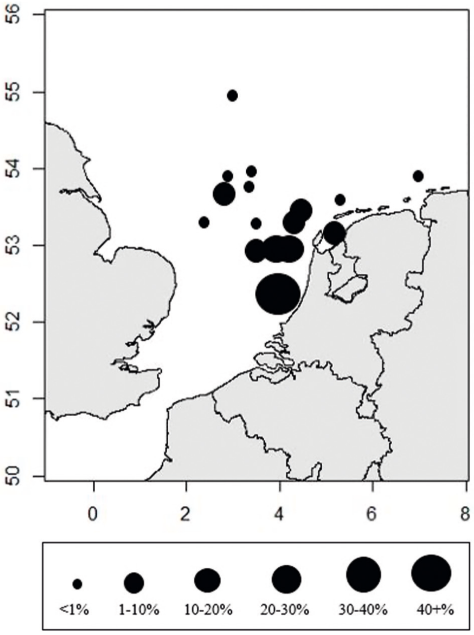


Figure B3 The relative density (%) of the thornback ray (*Raja clavata*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

Table B4 The occurrence of prey species in the stomach of the smooth-hound (*Mustelus asterias*) for different size classes, the total number of individuals per size class and the total number of empty stomachs per size class. The values represent mean percentages of occurrence.

	Diet composition of *Mustelus asterias*				
Taxa	<50 cm	50-70 cm	70-90 cm	90+ cm	Total
Nemertea	0.4				0.2
Annelida	1.5	2.3			1.3
Nereididae	1.5	1.1			1.1
Annelida (unidentified)		1.1			0.2
Crustacea	97.0	95.4	100.0	100.0	97.3
Anomura	46.3	42.5	46.4	47.5	45.7
Pagurus bernhardus				1.6	0.2
Paguroidea (unidentified)	46.3	42.5	46.4	45.9	45.5
Brachyura	20.7	32.2	50.0	49.2	28.7
Portunidea (unidentified)	13.3	24.1	10.7	27.9	17.3
Corystes sp.	0.4	1.1	3.6	1.6	0.9
Corystes cassivelaunus	1.1	2.3	3.6	1.6	1.6
Macropipus sp.		1.1			0.2
Liocarcinus holsatus	3.0		21.4	13.1	4.9
Brachyura (unidentified)	3.0	3.4	10.7	4.9	3.8
Caridea	28.5	17.2	3.6	3.3	21.3
Crangon allmanni	4.1	3.4			3.1
Crangon crangon	5.9	4.6			4.5
Philocheras trispinosus	0.4				0.2
Caridea (unidentified)	18.1	9.2	3.6	3.3	13.5
Other crustuceans	0.4	3.4			0.9
Mysida (unidentified)		1.1			0.2
Upogebia deltaura	0.4	1.1			0.4
Pestarella tyrrhena		1.1			0.2
Crustacea (unidentified)	1.1				0.7
Mollusca	0.7	1.1			0.7
Cephalopoda	0.4	1.1			0.4
Sepiola sp.	0.4				0.2
Alloteuthis sp.		1.1			0.2
Mollusca (unidentified)	0.4				0.2
Pisces	0.4	1.1			0.4
Callionymus sp.		1.1			0.2
Pisces (unidentified)	0.4				0.2
Number of empty stomachs	**4**	**1**	**0**	**0**	**5**
Total number of stomachs	**172**	**52**	**16**	**36**	**276**

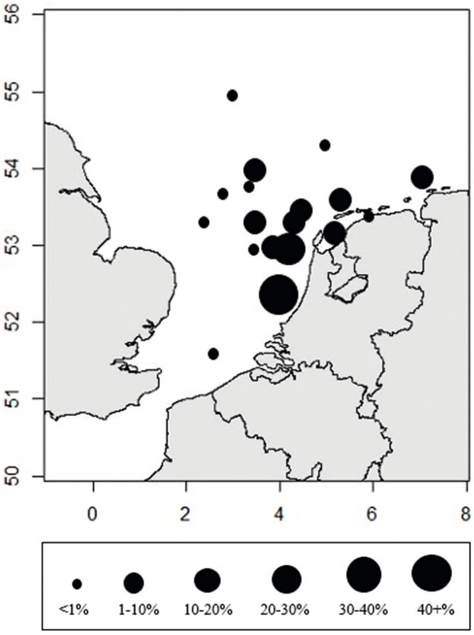


Figure B4 The relative density (%) of the smooth-hound (*Mustelus asterias*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

Table B5 The occurrence of prey species in the stomach of the tope shark (*Galeorhinus galeus*) for different size classes, the total number of individuals per size class and the total number of empty stomachs per size class. The values represent mean percentages of occurrence.

Taxa	Diet composition of *Galeorhinus galeus*							
	<40 cm	40-60 cm	60-80 cm	80-100 cm	100-120 cm	120-140 cm	140+ cm	Total
Annelida	0.6							0.3
Nereididae	0.6							0.3
Crustacea	43.9	21.8	28.9			5.0		27.5
Anomura	20.4	11.2	13.2			2.5		13.0
Paguroidea (unidentified)	20.4	11.2	13.2			2.5		13.0
Brachyura	22.9	8.9	13.2					13.6
Portunidae (unidentified)	2.5	0.6						1.3
Thia scutellata		0.6						0.1
Corystes cassivelaunus			2.6					0.1
Brachyura (unidentified)	20.4	7.8	10.5					12.0
Caridea	0.3	1.7	2.6					0.7
Other crustaceans						2.5		0.1
Upogebia deltaura						2.5		0.1
Crustacea (unidentified)	0.3							0.1
Mollusca	7.3	3.9	2.6		9.1	2.5	6.4	5.8
Cephalopoda	4.5	3.9	2.6		9.1	2.5	6.4	4.5
Teuthida (unidentified)	0.3							0.1
Sepia sp.		0.6					3.2	0.7
Loligo sp.	3.6	2.2			9.1	2.5	0.8	2.6
Loligo forbesii	0.3	0.6	2.6					0.4
Loligo vulgaris							0.8	0.1
Cephalopoda (unidentified)	0.3	0.6					1.6	0.5
Bivalvia	2.8							1.3
Echinodermata						2.5	2.4	0.5
Asteroidea							2.4	0.4
Asteriidae (unidentified)							1.6	0.3
Astropectinidae (unidentified)							0.8	0.1
Ophiuroidea						2.5		0.1
Pisces	48.3	74.3	68.4	100.0	90.9	90.0	91.2	65.9
Clupeiformes	0.6	5.6	7.9		72.7	2.5	10.4	4.9
Clupea harengus	0.3	5.0	7.9		18.2	2.5	7.2	3.3
Sardina pilchardus							0.8	0.1
Engraulis encrasicolus		0.6			45.5			0.8
Sprattus sprattus					9.1		2.4	0.5
Clupeiformes (unidentified)	0.3							0.1
Gadiformes		2.8	5.3	40.0		20.0	10.4	4.1
Gadidae (unidentified)						5.0	0.8	0.4
Merlangius merlangus		2.8	5.3	40.0		15.0	9.6	3.7
Pleuronectiformes	0.8	6.1	5.3	40.0	9.1	32.5	35.2	10.3
Pleuronectidae (unidentified)						5.0	6.4	1.3

Limanda limanda		0.6				12.5	5.6	1.7
Pleuronectes platessa		0.6				2.5	3.2	0.8
Solea solea					9.1	2.5	4.8	1.1
Pleuronectiformes (unidentified)	0.8	5.0	5.3	40.0		10.0	15.2	5.4
Perciformes	6.1	13.4	15.8		9.1	15.0	13.6	10.0
Ammodytidae (unidentified)	0.3							0.1
Gobiidae (unidentified)	3.6	5.6						3.0
Mullus surmuletus							0.8	0.1
Scomber scombrus		1.1	2.6			5.0	1.6	0.9
Hyperoplus lanceolatus	2.0	3.4	5.3				0.8	2.1
Trachurus trachurus	0.3	2.2	7.9		9.1	7.5	10.4	3.3
Perciformes (unidentified)		1.1				2.5		0.4
Anguilliformes		0.6				5.0	0.8	0.5
Anguilla anguilla		0.6				2.5	0.8	0.4
Conger conger						2.5		0.1
Scorpaeniformes							2.4	0.4
Triglidae (unidentified)							0.8	0.1
Eutrigla gurnardus							1.6	0.3
Pisces (unidentified)	40.8	45.8	34.2	20.0		15.0	18.4	35.7
Number of empty stomachs	**12**	**8**	**3**	**2**	**0**	**1**	**3**	**29**
Total number of stomachs	**254**	**146**	**32**	**6**	**2**	**17**	**49**	**508**

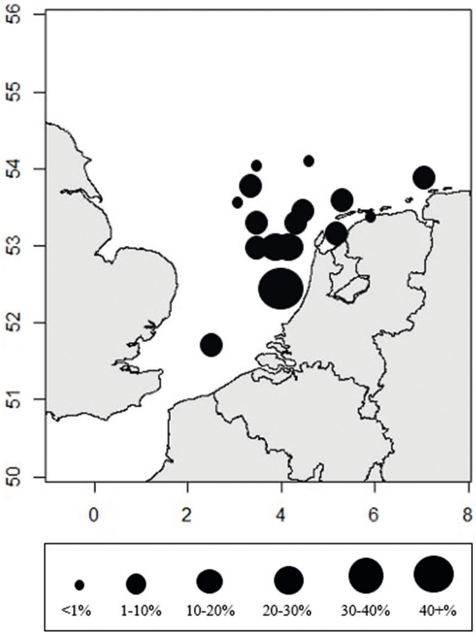


Figure B5 The relative density (%) of the tope shark (*Galeorhinus galeus*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

Supplementary information C

Table C1 Shark and skate species with less than 10 registrations on stomach content composition in the NIOZ archive, together with number of registrations, number of individuals that contain stomach content, the range of the total length, the range of the depth and the mean trophic level with SE.

Species	Number of individuals	Number of stomach data	Total length (cm)	Depth (m)	Trophic level
Common thresher	16	1	127 - 435	7 - 18	4.60
Basking shark	48	3	163 - 1200	6 - 29	3.40 (±0.17 SE)
Common stingray	547	9	30 - 120	2 – 38	3.48 (±0.38 SE)
Porbeagle	15	6	137 – 252	10 – 38	4.60 (±0.28 SE)
Angelshark	29	4	28 – 144	8 - 41	4.33 (±0.26 SE)

Table C2 The frequency of occurrence of prey species in the stomach of the common thresher (*Alopiasvulpinus*), basking shark (*Cetorhinus maximus*), common stingray (*Dasyatis pastinaca*), porbeagle (*Lamna* nasus) and angelshark (*Squatina squatina*), the total number of individuals per species and the total number of empty stomachs per species. The given values are percentages based on the frequency of occurrence of a prey in all stomachs.

	Diet compostitions				
Taxa	*Alopias vulpinus*	*Cetorhinus maximus*	*Dasyatis pastinaca*	*Lamna nasus*	*Squatina squatina*
Annelida			42.9		
Polychaeta (unidentified)			14.3		
Nereididae (unidentified)			14.3		
Alitta virens			14.3		
Crustacea		100.0	42.9		33.3
Caridea (unidentified)			42.9		33.3
Mysida (unidentified)		33.3			
Crustacea (unidentified)		66.7			
Cephalopoda				10.0	16.7
Loligo sp.				10.0	16.7
Pisces	100.0		14.3	90.0	50.0
Triglidae (unidentified)				10.0	
Ammodytes tobianus				10.0	
Gadus morhua				10.0	
Limanda limanda				30.0	
Solea solea				10.0	
Sprattus sprattus				10.0	
Pisces (unidentified)	100.0		14.3	10.0	50.0
Number of empty stomachs	**0**	**0**	**4**	**0**	**0**
Total number of stomachs	**1**	**3**	**9**	**6**	**4**

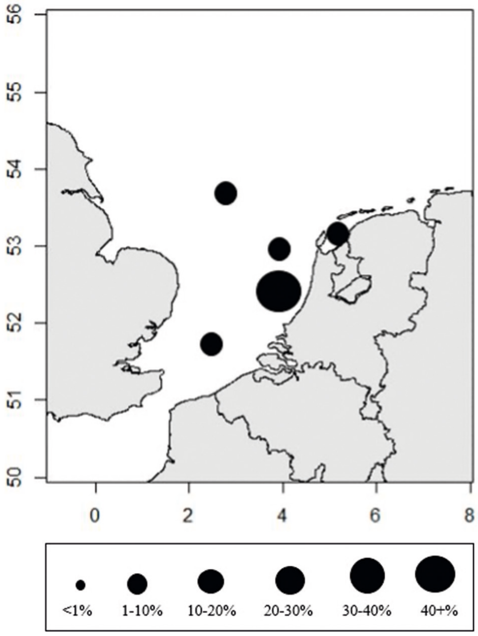


Figure C1 The relative density (%) of the common thresher (*Alopias vulpinus*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

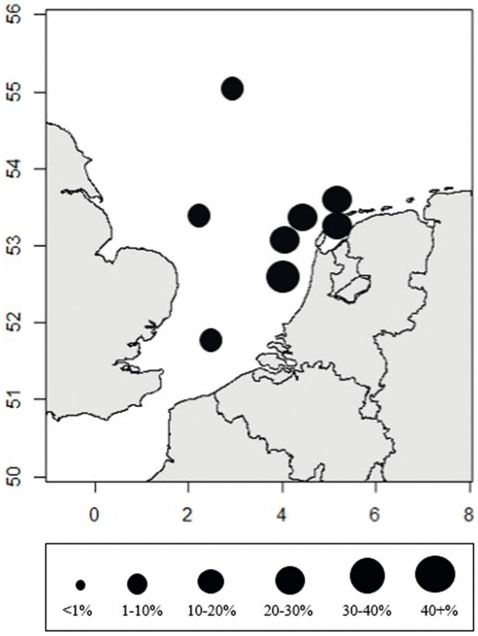


Figure C2 The relative density (%) of the basking shark (*Cetorhinus maximus*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

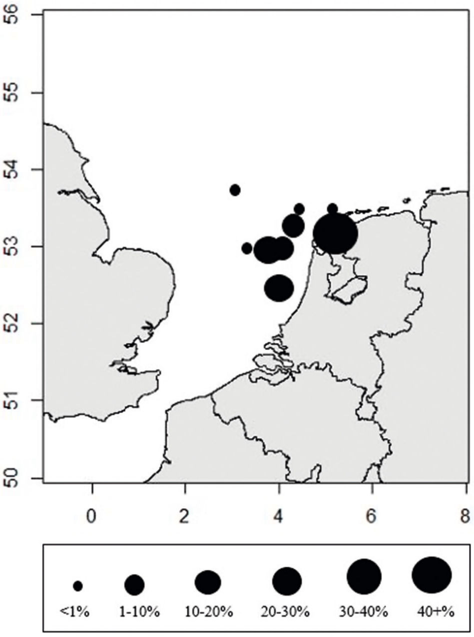


Figure C3 The relative density (%) of the common stingray (*Dasyatis pastinaca*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

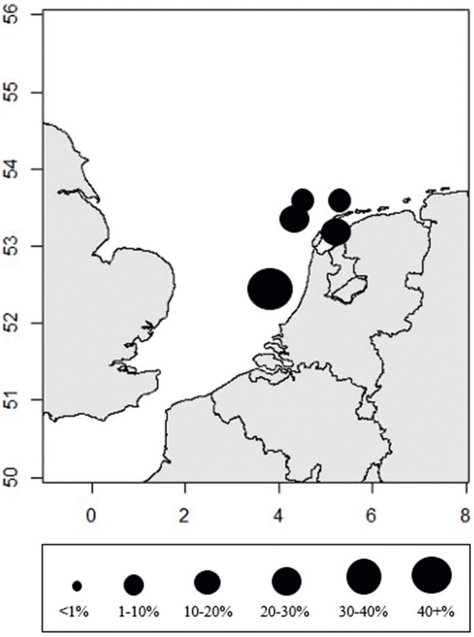


Figure C4 The relative density (%) of the porbeagle (*Lamna nasus*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

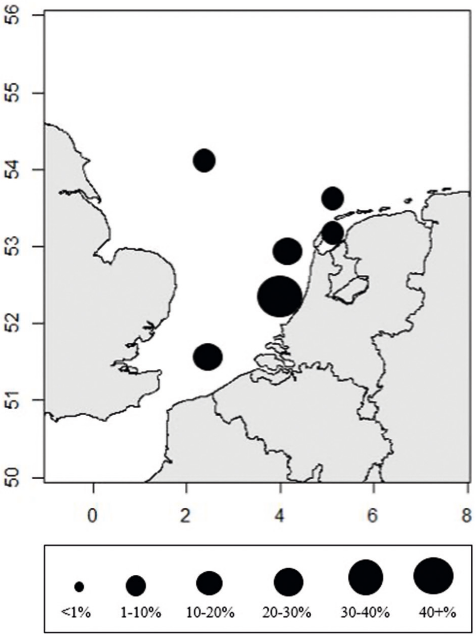


Figure C5 The relative density (%) of the angelshark (*Squatina squatina*) in the North Sea and Wadden Sea based on total number of records in the NIOZ archive.

8

Stomach content analysis indicates multi decadal trophic stability in a temperate coastal fish food web, western Dutch Wadden Sea

Poiesz SSH, Witte JIJ, van der Veer HW (2024) Stomach content analysis indicates multi decadal trophic stability in a temperate coastal fish food web, western Dutch Wadden Sea. Estuarine, Coastal and Shelf Science, 308, 108912.

Keywords:
Coastal fish community, Wadden Sea, Food web structure,
Stomach content analysis, Long-term trends, Stability

Running title:
Long-term stability in fish food web structure

Abstract

Information about stomach content composition of fish species of a temperate coastal fish community (western Dutch Wadden Sea) over the period 1930 – 2019 was analysed to reconstruct long-term trends in trophic position of individual species. Stomach data were not evenly distributed but clustered both with respect to years as well as fish species. For 18 fish species, all being omnivorous and belonging to different functional groups (pelagic, benthopelagic, demersal) and guilds [(near)-resident, juvenile marine migrants, marine seasonal visitiors], prey consumption and trophic position over time could be analysed. Prey occurrence in the stomachs of different fish species showed variability over time, most likely due to fluctuations in prey abundance, but without a trend. For all species, individual fish showed variability in trophic position in the order of 1 unit or even more both within and between years. However, in all 18 species, no significant trend in mean trophic position over time could be found, despite the serious anthropogenic stress (pollution, eutrophication events, climate change) and the decrease in fish abundance in the area during the last 50 years. The present study does not indicate any changes in trophic position of individual species in the western Dutch Wadden Sea over the last 80 years. At the community level, trophic structure varies due to interannual fluctuations in species composition and year-to year fluctuations in the relative abundance of the various fish species. At the ecosystem level the trophic role of the fish community has been degraded due to the decrease in total fish biomass in the area.

1. Introduction

Coastal systems provide a large variety of ecosystem goods and services (see Barbier 2017, Liu et al. 2021) and consequently, their ecosystem value is high (Liu et al. 2021). Coastal systems are known as important foraging grounds for a variety of fish, bird and marine mammal species (e.g. Goodall 1983, Beck et al. 2001), and in these areas fish harvesting has been an important marine ecosystem good for centuries. However, due to human fishing and hunting, coastal ecosystems have also been under pervasive human disturbance for centuries (Jackson et al. 2001, Lotze 2007). For the future, anthropogenic pressure in these areas is expected to continue especially due to the combined pressure of overfishing and habitat destruction, pollution and climate change (Bijma et al. 2013, European Marine Board, 2013).

Predicting the consequences of the still ongoing threats on the future productivity of coastal areas requires (among other factors) insight into the food web structure of these systems. The fact that coastal ecosystems have been under pervasive human disturbance already for centuries makes it difficult to get insight in their 'original pristine state' and to assess the impact of human disturbance over time. First of all, going back in time, information about ecosystem status becomes more and more qualitative and anecdotic. Furthermore, our perspective about the past also suffers from "the shifting baseline phenomenon": ecosystem changes are considered relative to the situation the evaluator can remember and therefore the baseline shifts with each generation (Pauly 1995, Zeller et al. 2005). This stresses the need for long time series of reliable information on ecosystem structure, preferably covering multiple observer generations. In this study, we focus on the fish food web in the international Wadden Sea, one of the largest estuarine areas in the world, bordering the Dutch, German and Danish North Sea coast. The area is an important resting and fuelling area for birds and nursery area for various (non)commercial fish species (Zijlstra 1972, Wolff 1983). From archaeological, historical, fisheries, and ecological records, it is clear that the Wadden Sea have been under pervasive disturbance for centuries already (Lotze 2005, 2007).

Quality status reports about the ecology of the Wadden Sea has been produced periodically since 1999 (https://qsr.waddensea-worldheritage.org), with various ecological monitoring series in the western part of the area on phytoplankton (Philippart et al. 2007, Jacobs et al. 2020), macrozoobenthos (Beukema and Dekker 2020) and fish (Tulp et al. 2008, van der Veer et al. 2015) providing reconstructions over the last 60 years. From the 1970's, no changes in fish biodiversity were found. However, fish abundance of both pelagic and demersal species showed a 10-fold decrease in catches from 1980s onwards (Tulp et al. 2008, van der Veer et al. 2015). At present, various stomach content studies show that most Wadden Sea fish species are omnivorous, feeding on multiple prey items with a pivotal position of a few key prey species (Kellnreitner et al. 2012, Whitehouse et al. 2017, Poiesz et al. 2020, 2023). Stable isotope analyses indicates that the fish food web in this area consists of a spatially stable structure with various trophic levels (Poiesz et al. 2021a, 2023). To what extent the decrease in fish abundance in the 1980s has caused a shift in prey selection and therefore a temporal change in their trophic positions by the omnivorous predatory fish species, is unclear.

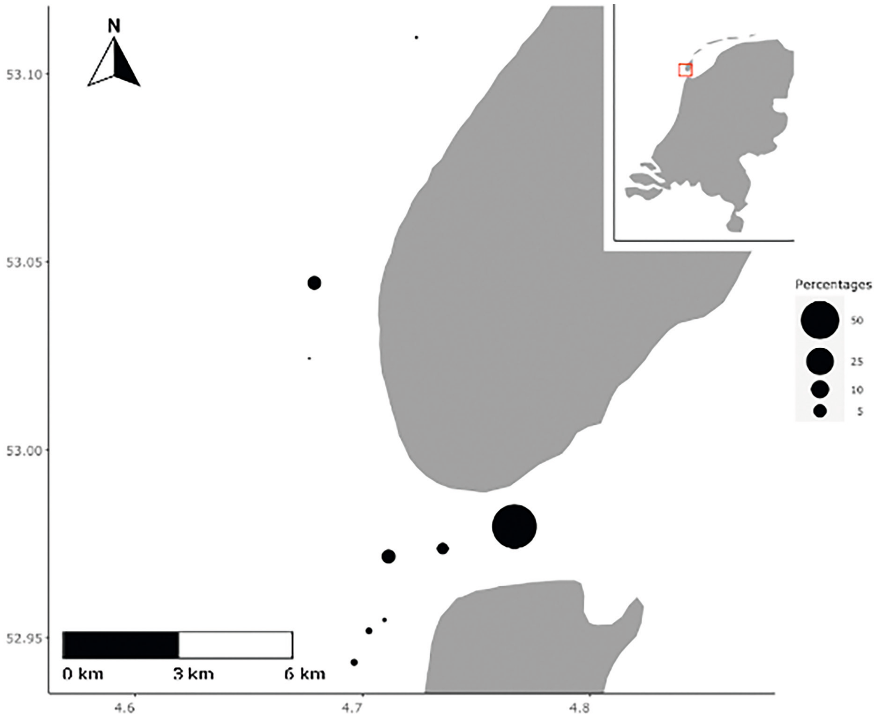


Figure 1 Sampling locations from the North Sea coast and Wadden Sea. Black dot size indicates the contribution (%) for each sampling location to the total number of individuals caught.

Ecological information about the fish food web before the 1970's is mostly qualitative and anecdotic, except for stomach content information of the fish fauna as a by-product of the long-lasting human fishing in the area. Stomach content data is an important source of information (Kellnreitner et al. 2012, Whitehouse et al. 2017, Poiesz et al. 2020), despite the fact that it is labour intensive, requires taxonomic expertise and only offers a small temporal snapshot of recently consumed prey items and might thus be sensitive to sampling design (Poiesz et al. 2023). In the absence of stable isotope information, stomach content information can be used to derive trophic structure of the fish fauna and its predator–prey interactions (Hynes 1950, Baker et al. 2014, Poiesz et al. 2020, 2021). A recent comparison of stomach content information and stable isotopes of fish in the western Wadden Sea, illustrated that both resulted in a similar picture of the trophic structure of the fish fauna (Poiesz et al. 2023).

For long-term stomach content time series, standardised methods of sampling and analysis are important (see for overview Hyslop 1980, Buckland et al. 2017, Amundsen & Sánchez-Hernández 2019). However, time series often suffer from limitations due to differences over time in sampling strategy, sampling intensity and/or in detail and methods of the analyses. In case enough data are present General Additive Models (GAMs) can be applied to visualise and analyse trends in stomach content over time (Hastie and Tibshirani 1995, Kvaarik et al. 2019, Kordubel et al. 2024). In this study we focus on unpublished records of fish stomach content data, mainly from the western part of Wadden Sea form the NIOZ archive, dating back to the 1930's (de Vooys et al. 1991, 1993). For all species, missing observations and/or gaps in the time series occurred. Furthermore, not all records contained information about number of prey found, prey condition, and prey weight. Therefore, Buckland et al. (2017) was followed and the simple presence/absence and frequency of occurrence approach was taken, since it is not affected by prey condition and hence provides a rapid, unambiguous and reliable account of diet composition and prey trophic position.

This NIOZ archive stomach content information is used to analyse fluctuations in predator-prey relationships and in the trophic position of individual fish species over the last century with the aim to get insight in the temporal variability of the Wadden Sea fish food web. The present trophic position of the various fish species (Poiesz et al. 2020, 2021a) will be used as reference to test whether shifts in trophic position of individual fish species has occurred over time. The stomach content data are available for the time span 1930 – present and thus the time series period covers more than a single scientific career. As such, the results of this study can also be used to correct for the "shifting baseline phenomenon".

2. Material and methods

2.1. Data collection

From 1930 onwards the Royal Netherlands Institute for Sea Research (NIOZ) registered observations and landings of fish and invertebrate species from the western Wadden Sea and nearby Dutch coastal waters (Fig 1). Most information originated from NIOZ cruises and fish collected during NIOZ courses. In addition, landings of rare fish species from fishermen were recorded. All individual fish were identified and information about species and stomach content was recorded. From the beginning, data collection, section and stomach content analysis were done by specialised NIOZ personnel only. A more detailed description of the NIOZ archive can be found in de Vooys et al. (1991, 1993).

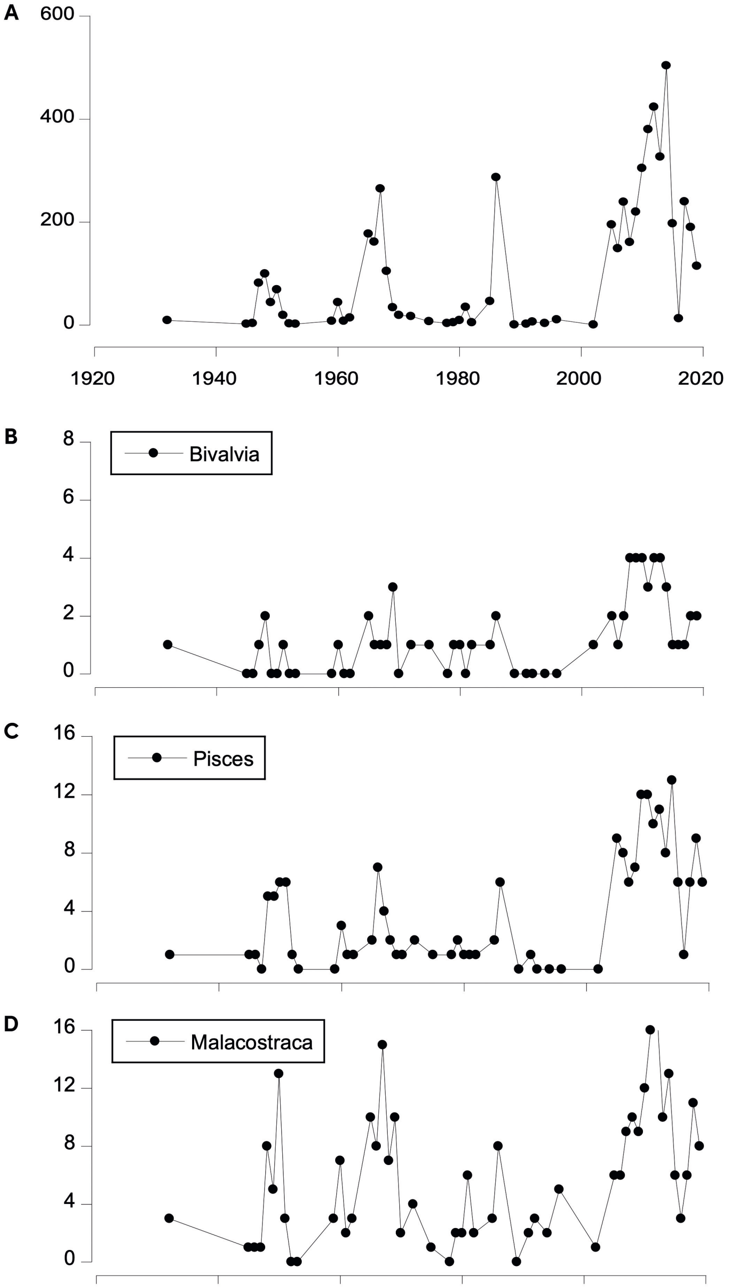


Figure 2 NIOZ archive.
A: Number of stomachs contents analyzed over the years 1932 – 2019.
B: Number of Bivalve species identified in the Wadden Sea fish stomachs.
C: Number of Malacostraca species identified in the Wadden Sea fish stomachs.
D: Number of Pisces species identified in the Wadden Sea fish stomachs.

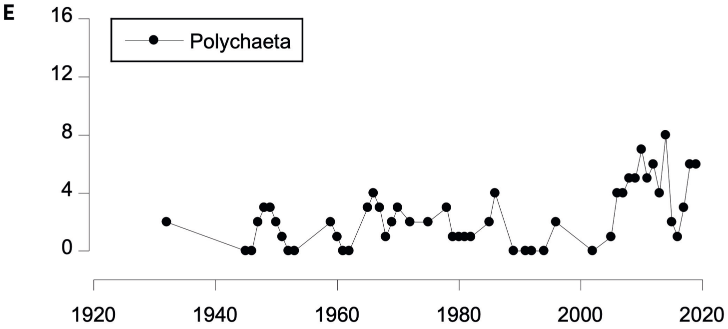


Figure 2 NIOZ archive. *(continued)*
E: Number of Polychaete species identified in the Wadden Sea fish stomachs.

From the 1980's onwards, stomach content data were collected from a long-term monitoring programme of the fish fauna with a passive fish trap near the entrance of the Wadden Sea in spring and autumn (Poiesz et al. 2020). Until 2010, all fish caught on Fridays were taken to the laboratory and sorted within an hour, identified up to species level, counted and length measured. From 2017 onwards, a maximum of three individuals per species per week were selected and stored at -20°C for further analysis. Within a month, individuals were defrosted, and stomach content was taken out and analysed in a petri dish under a binocular (20x). Of each individual fish, total stomach content was determined (wet mass; g) and subsequently, prey items were identified up to species level or sometimes, up to a higher classification (class, order, genus). If possible, total length of the prey was measured (mm). Incomplete specimens, often from species that were eaten in pieces, such as *Alitta virens* or *Ensis leei*, or from species that were in parts, such as the *Crangon crangon*, were counted only by the number of 'heads'. Taxonomic identification was based on an internal reference collection and Hayward and Ryland (2017) for polychaetes, bivalves and crabs and Wheeler (1978) for fish species. For more details see van der Veer et al. (2015) and Poiesz et al. (2020).

2.2. Data processing

All fish records were checked for species name and, if necessary, updated according to WoRMS (http://www.marinespecies.org). Next, fish species were assigned in line with previous work (van der Veer et al. 2015, Poiesz et al. 2020) into: pelagic (occurring mainly in the water column between 0 and 200 m, not feeding on benthic organisms); benthopelagic (living and/or feeding on or near the bottom, as well as in midwater, between 0 and 200 m) and benthic (living and/or feeding on the bottom) according to FishBase (Froese and Pauly 2021).

Table 1 Overview of prey items found in the stomachs of the various fish species of the NIOZ archive between 1931-2019, together with trophic position according to FishBase (www.fishbase.com).

Class	Order	Family	Scientific name	Common name	Trophic position (-)
				Eggs (Crab, shrimp, fish)	1,00
Asteroidea	Forcipulatida	Asteriidae	*Asteriidae*	Sea stars	2,00
Asteroidea	Spatangoida	Loveniidae	*Echinocardium*	Sea urchins	2,00
Bivalvia	Adapedonta			Bivalves	2,10
Bivalvia	Adapedonta	Myridae	*Mya spec*	Solf shell clams	
Bivalvia	Adapedonta	Pharidae	*Ensis*	Razor clams	2,10
Bivalvia	Adapedonta	Pharidae	*Ensis leei*	Atlantic jackknife clam	2,10
Bivalvia	Mytilida	Mytilidae	*Mytilus edulis*	Blue mussel	2,10
Bivalvia	Cardiida	Tellinidae	*Limecola balthica*	Baltic macoma	2,10
Caenogastropoda	Littorinimorpha	Hydrobiidae	*Peringia ulvae*	Laver spire shell	2,40
Chlorophyta	Algae	Algae	*Algae*	Algae	1,00
Chlorophyta	Cladophorales	Cladophoraceae	*Chaetomorpha melagonium*	Chaetomorpha melagonium	1,00
Chlorophyta	Ulvales	Ulvaceae	*Ulva lactuca*	Sea lettuce	1,00
Coleoidea	Cephalopoda	Loliginidae	*Loligo vulgaris*	European squid	3,50
Coleoidea	Cephalopoda	Loliginidae	*Sepia officinalis*	Common cuttlefish	3,50
Coleoidea	Teuthida	Teuthida	*Teuthida*	Squid	3,50
Cydippida	Ctenophora	Ctenophora	*Ctenophora*	Ctenophora	3,00
Cydippida	Cydippida	Pleurobrachiidae	*Pleurobrachia pileus*	Sea-gooseberry	3,00
Discomedusae	Rhizostomeae	Rhizostomatidae	*Rhizostoma pulmo*	Giant jellyfish	3,00
Gastropoda	Littorinimorpha	Littorinimorpha	*Littorinimorpha*	Littorinimorpha	2,40
Heterobranchia	Nudibranchia	Nudibranchia	*Nudibranchia*	Nudibranchs	2,40
Hydrozoa					2,30
Hydrozoa	Anthoathecata	Tubularia	*Tubularia*	Tubularia	2,30
Hydrozoa	Anthoathecata	Corynidae	*Sarsia tubulosa*	Clapper medusa	2,50
Insecta	Insecta	Insecta	*Insecta*	Insects	1,00
Malacostraca	Amphipoda	Isopoda	*Hyperia galba*	Big-eye amphipod	2,30
Malacostraca	Amphipoda	Hyperiidae	*Talitrus saltator*	Sand hopper	2,30

Table 1 Overview of prey items found in the stomachs of the various fish species of the NIOZ archive between 1931-2019, together with trophic position according to FishBase (www.fishbase.com). *(continued)*

Class	Order	Family	Scientific name	Common name	Trophic position (-)
Malacostraca	Amphipoda	Gammaridae	*Gammarus spec*	Gammarus	2,30
Malacostraca	Balanomorpha	Thoracica	*Semibalanus balanoides*	Barnacle	2,10
Malacostraca	Balanomorpha	Thoracica	*Thoracica*	Barnacles	2,30
Malacostraca	Copepoda	Copepoda	*Copepoda*	Copepods	2,00
Malacostraca	Decapoda	Anomura	*Paguroidea*	Hermit crabs	3,20
Malacostraca	Decapoda	Brachyura	*Corystes*	Helmet crabs	2,50
Malacostraca	Decapoda	Brachyura	*Macropipus*	Macropipus	2,50
Malacostraca	Decapoda	Brachyura	*Macropodia rostrata*	Long-legged spider crab	2,50
Malacostraca	Decapoda	Brachyura	*Portunidae*	Swimming crabs	2,50
Malacostraca	Decapoda	Carcinidae	*Cancer pagurus*	Edible crab	2,50
Malacostraca	Decapoda	Carcinidae	*Carcinus maenas*	Shore crab	2,50
Malacostraca	Decapoda	Corophiidae	*Corophium sp.*	Corophium sp.	2,60
Malacostraca	Decapoda	Corophiidae	*Corophium volutator*	Mud shrimp	2,60
Malacostraca	Decapoda	Crangonidae	*Caprella linearis*	Skeleton shrimp	2,60
Malacostraca	Decapoda	Crangonidae	*Crangon allmanni*	Crangon allmanni	2,60
Malacostraca	Decapoda	Crangonidae	*Crangon crangon*	Brown shrimp	2,60
Malacostraca	Decapoda	Crangonidae	*Gastrosaccus spinifer*	Gastrosaccus spinifer	2,20
Malacostraca	Decapoda	Crangonidae	*Mysidae*	Mysidae	2,20
Malacostraca	Decapoda	Crangonidae	*Palaemon serratus*	Aesop prawn	2,60
Malacostraca	Decapoda	Crangonidae	*Pontophilus bispinosus*	Philocheras bispinosus bispinosus	2,60
Malacostraca	Decapoda	Crangonidae	*Pontophilus trispinosus*	Philocheras trispinosus	2,60
Malacostraca	Decapoda	Crangonidae	*Praunus flexuosus*	Chameleon shrimp	2,20
Malacostraca	Decapoda	Crangonidae	*Processa*	Processa	2,60
Malacostraca	Decapoda	Crangonidae	*Processa canaliculata*	Processa canaliculata	2,60
Malacostraca	Decapoda	Cumacea	*Cumacea*	Hooded shrimp	2,60
Malacostraca	Decapoda	Nephropidae	*Homarus gammarus*	European lobster	3,20
Malacostraca	Decapoda	Palaemonidae	*Palaemon elegans*	Grass prawn	2,60

Table 1 Overview of prey items found in the stomachs of the various fish species of the NIOZ archive between 1931-2019, together with trophic position according to FishBase (www.fishbase.com). *(continued)*

Class	Order	Family	Scientific name	Common name	Trophic position (-)
Malacostraca	Decapoda	Polybiidae	*Macropipus holsatus*	Swimming crab	2,50
Malacostraca	Isopoda	Isopoda	*Idotea sp.*	Idotea sp.	2,30
Mollusca	Mollusca	Mollusca	*Mollusca*	Mollusca	2,60
Nematoda	Nematoda	Nematoda	*Nematoda*	Nematodes	2,10
Ophiuroidea	Ophiurida	Ophiuroidea	*Ophiura ophiura*	Serpent star	2,00
Ophiuroidea	Spatangoida	Loveniidae	*Echinocardium cordatum*	Sea-potato	2,00
Pisces	Pisces	Pisces	*Pisces*		3,60
Pisces	Atheriniformes	Aterinidae	*Atherina presbyter*	Sand-smelt	3,70
Pisces	Clupeiformes	Clupeidae	*Alosa fallax*	Twaite shad	2,92
Pisces	Clupeiformes	Clupeidae	*Clupea harengus*	Herring	3,40
Pisces	Clupeiformes	Clupeidae	*Sprattus sprattus*	Sprat	3,09
Pisces	Cyprinodontiformes	Belonidae	*Belone belone*	Garfish	3,68
Pisces	Gadiformes	Gadidae	*Ciliata mustela*	Five-bearded rockling	3,53
Pisces	Gadiformes	Gadidae	*Merlangius merlangus*	Whiting	3,83
Pisces	Gasterosteiformes	Gasterosteidae	*Gasterosteus aculeatus*	Stickleback	3,30
Pisces	Mugiliformes	Mugilidae	*Liza aurata*	Golden grey mullet	2,05
Pisces	Perciformes	Moronidae	*Dicentrarchus labrax*	Bass	3,60
Pisces	Perciformes	Ammodytidae	*Ammodytes tobianus*	Sandeel	4,15
Pisces	Perciformes	Ammodytidae	*Hyperoplus lanceolatus*	Greater sandeel	4,00
Pisces	Perciformes	Trachinidae	*Echiichthys vipera*	Lesser weever	4,40
Pisces	Perciformes	Callionymidae	*Callionymus lyra*	Dragonet	4,41
Pisces	Perciformes	Gobiidae	*Gobius niger*	Black goby	3,30
Pisces	Perciformes	Gobiidae	*Pomatoschistus minutus*	Sand goby	3,20
Pisces	Petromyzontiformes	Petromyzontidae	*Petromyzon marinus*	Lamprey	3,11
Pisces	Pleuronectiformes	Pleuronectidae	*Limanda limanda*	Dab	3,40
Pisces	Pleuronectiformes	Pleuronectidae	*Platichthys flesus*	Flounder	3,26
Pisces	Pleuronectiformes	Pleuronectidae	*Pleuronectes platessa*	Plaice	3,29

Table 1 Overview of prey items found in the stomachs of the various fish species of the NIOZ archive between 1931-2019, together with trophic position according to FishBase (www.fishbase.com). *(continued)*

Class	Order	Family	Scientific name	Common name	Trophic position (-)
Pisces	Pleuronectiformes	Pleuronectidae	*Reinhardtius hippoglossoides*	Greenland halibut	4,60
Pisces	Pleuronectiformes	Solidae	*Buglossidium luteum*	Solenette	3,25
Pisces	Pleuronectiformes	Solidae	*Solea solea*	Sole	3,20
Pisces	Salmoniformes	Osmeridae	*Osmerus eperlanus*	Smelt	3,31
Pisces	Scorpaeniformes	Cottidae	*Myoxocephalus scorpius*	Bull-rout	3,90
Pisces	Scorpaeniformes	Cottidae	*Myoxocephalus quadricornis*	Four-horn sculpin	3,60
Pisces	Scorpaeniformes	Liparidae	*Liparis liparis*	Sea-snail	3,89
Pisces	Scorpaeniformes	Gobiidae	*Pomatoschistus lozanoi*	Lozano's goby	3,30
Pisces	Scorpaeniformes	Gobiidae	*Pomatoschistus microps*	Common goby	4,45
Pisces	Scorpaeniformes	Gobiidae	*Pomatoschistus sp.*	Pomatoschistus sp.	4,45
Pisces	Zeiformes	Zeidae	*Zeus faber*	Dory	4,50
Polychaeta	Annelida	Annelida	*Annelida*	Annelida	2,10
Polychaeta	Arenicolidae	Arenicolidae	*Arenicolidae*	Arenicolidae	2,10
Polychaeta	Phyllodocida	Aphrodita	*Aphrodita*	Sea mouse	2,10
Polychaeta	Phyllodocida	Nereididae	*Alitta virens*	Sandworm	2,10
Polychaeta	Phyllodocida	Nereididae	*Nereididae*	Nereididae	2,10
Polychaeta	Phyllodocida	Nereididae	*Nereis*	Nereis	2,10
Polychaeta	Phyllodocida	Opheliidae	*Ophelia limacina*	Ophelia limacina	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Arenicola marina*	Lugworm	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Lanice conchilega*	Sand mason worm	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Marenzelleria viridis*	Marenzelleria viridis	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Nephtys hombergii*	Catworm	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Phyllodoce maculata*	Phyllodoce maculata	2,10
Polychaeta	Phyllodocida	Phyllodocidae	*Scoloplos armiger*	Scoloplos armiger	2,10
Polychaeta	Polychaeta	Polychaeta	*Polychaeta*	Bristle worms	2,10
Polychaeta	Terebellida	Pectinariidae	*Lagis koreni*	Trumpet worm	2,10

Species were also classified according to their use of the area into near-resident and resident species, marine juvenile migrants and seasonal visitors based on Zijlstra (1983). *Dicentrarchus labrax* (bass) was considered to have become a resident species in the Wadden Sea in recent time, due to the presence of small juveniles and adults almost year-round (Cardoso et al. 2015).

All prey items found in the stomachs of the fish were checked and scientific name, family, order and class were updated according to WoRMS (http:/www.marinespecies.org). Level of taxonomic identification of prey items was variable over the years, often Class level from 1930-1980 versus species level from 1980 onwards. For all prey Classes, Families and species found, trophic position was taken from FishBase (Froese and Pauly, 2021).

Per year, for each fish species, the mean percentage of occurrence (= number of stomachs containing a prey species divided by total number of stomachs with content examined) of each class of prey items was determined as a measure of diet composition following Baker et al. (2014). Furthermore, the trophic position of each individual fish *j* (TP_j) was calculated from the stomach content as the mean trophic position of the different prey species k found in a stomach, according to:

$$TP_j = 1 + \frac{\sum TP_k}{k} \qquad [1]$$

where:

TP_j being the calculated trophic position of the individual fish j;
TP_k the trophic position of prey species k in the stomach of fish j;
k the number of different prey species in the stomach of fish j.

The bias introduced by not correcting for differences in mass of the various prey items in the stomachs is small (Poiesz et al. 2021a). Next, for each fish species, the mean trophic position per year was calculated.

2.3. Data analysis

The impact of level of detail of prey identification on estimated trophic level of stomach content was analysed for the 2010-2019 data (Poiesz et al. 2010). Estimated trophic levels of the stomach contents based on trophic values of identified prey species were compared with estimates after a rerun with Class values instead of species values.

In all species, missing observations and gaps in the time series occurred. For fish species, with minimum 15 years of observation with at least 5 stomach contents analysed were present to apply General Additive Models (GAMs) to visualise and analyse trends over time (Hastie and Tibshirani 1995). For these fish species *i*, trends over time in the most common prey items (PO_i) and in mean trophic position (TP_i) were analysed by fitting

GAMs using locally weighted least squares regression (LOESS), an identity link function and the Gaussian error distribution according to:

$$PO_i \text{ or } TP_i = \alpha + f(Year) + \varepsilon_i \qquad \varepsilon_i \sim N(0,\sigma^2) \qquad [2]$$

The model was cross-validated with different degrees of smoothing (SPAN) to determine the optimal SPAN based on the minimum residual sum of the root mean square error (RMSE). The evaluation of the GAM results was done following Swartzman et al. (1992) and MacKenzie and Schiedek (2007): The trend of the GAM model was drawn with 95% confidence limits. If a horizontal line could be drawn between the 95% confidence area of the fitted trend, the results of the GAM model was judged as no changes over time (P>0.05).

In addition, the whole fish data set (including all species) was analysed, whereby the present range of trophic position (TP) of the various species (2010 -2019) as described by Poiesz et al. (2020) was taken as reference. For all years and all species, the estimates of TP were compared with the reference period and scored as (1) above, (2) within or (3) below the 2010 – 2019 range. Next, trends in these scores over time were analysed per 5-year period.

All computations and analyses were done in R (R Core Team, 2021). The graphics were made using the ggplot package (Wickham 2009).

3. Results

3.1. Fish data

The NIOZ archive contained information about 7031 stomachs of 43 fish species over the years 1932 – 1979. Data for the years 1980 – 2019 included information about another 5217 stomachs of 60 fish species, in total information about 12248 stomachs of 64 fish species. Records were not evenly distributed but clustered both with respect to years as well as fish species. Also, records of some species were only present in the 1940 – 1960’s (skates and shark species), records of bass *Dicentrarchus labrax* (bass) only appeared in the samples in recent times and for some species only few records were available (see Supplementary materials Table S1). The archive data cluster around a few intervals: period 1947-1951; period 1962 -1969; period 1975-1981; period 2005-2009 and the reference period 2010-2019 (Fig 2A).

In total 117 different prey items were described over the years (Table 1). For detailed information see Supplementary materials Table S2. Number of species identified did not show a trend for the various Classes except for slightly higher number Pisces and Polychaetes in recent years (Fig 2B,C,D,E).

For the analysis, fish species from the various functional groups and guilds were selected (Table 2). Trends with GAM in stomach content could be analysed for 15 species and trends in trophic position could be determined in 16 species.

3.2. Stomach content

3.2.1. All data

Within the reference period (2010-2019), Malacostraca were the most important Class of prey in the stomachs of the analysed Wadden Sea fish species based on the mean relative occurrence, followed by Pisces, Polychaetes and Bivalves (Fig 3). The various periods each showed a larger interannual variability of prey mean relative occurrence than the reference period. During the period 2005-2009, the relative mean occurrence of the various prey classes was within the range of the reference period. In the period 1975-1981, more Polychaetes and more Bivalves were found as prey. During the period 1962 -1969, also more Polychaetes were found as prey but less Pisces. The period 1947-1951 displayed a large variability: some years had more Pisces while other years had hardly any Pisces but more Malacostraca as prey (Fig 3).

The Malacostraca prey (3706 records) mainly consisted of the family Crangonidae (2156 records, brown shrimps and other shrimps) and furthermore Copepods (415 records). Pisces (2601 records) were partly unidentified species (772 records) and furthermore Clupidae (394 records, mainly herring) and Gobiidae (339 records, mainly sand goby). In addition, there were 533 records of Callionymidae prey, however this record is doubtful since it was based on a single observation of 512 prey items in one year (1949). The Polychaetes (2917 records) mainly referred to Annilida (2334 records) and furthermore Phyllodocidae (345 records, mainly *Lanice* spec. and *Nereis* spec.). The Bivalvia prey (1831 records) were mainly unidentified (949 records) and other Ensis spec. (829 records). For detailed information see Supplementary material Table S2.

3.2.2. Individual species

The group of pelagic species (Fig 4A) contained one (near)resident species (garfish *Belone belone*), two juvenile marine migrants (herring *Clupea harengus* and sprat *Sprattus sprattus*) and two marine seasonal visitors (scad *Trachurus trachurus* and smelt *Osmerus eperlanus*). Garfish mainly consumed Pisces (herring and to a lesser extent sandeel) but also regularly Malacostraca (mainly brown shrimp). Prey items for herring were mainly Malacostraca (mainly Copepods and to a lesser extent Gammarus, Corophium and Mysidae) and some Pisces (herring and sandeel), Polychaete, (mixture of species) and Bivalves (razor clams). Sprat mainly consumed Malacostraca (mainly consisting of Copepods and to a lesser extent shore crab and brown shrimp). For scad, main prey items were Malacostraca (mainly brown shrimp and shore and swimming crabs) and Pisces (mainly herring and sandeel). Malacostraca (mainly shrimps and swimming crabs and some Copepods) and Pisces (herring and various goby species) were also the main prey items of smelt.

Table 2 Overview of selected fish species from the NIOZ Archive, together with functional group and guild. (Near)-resident: Near-resident or resident species; JMM: juvenile marine migrants; MSV: Marine seasonal visitor. For each species, total number of stomachs and number of years with observations, split up into unpublished data (1932 - 2009) and reference data (2010-2019 after Poiesz et al. 2020), are listed. Type of analysis is indicated by X.

				Number of stomachs			Number of years with observations			Stomach content		Trophic position	
Scientific name	Common name	Functional group	Guild	Total	1932-2009	2010-2019	Total	1932-2009	2010-2019	Composition	GAM	Estimate	GAM
Belone belone	Garfish	Pelagic	(Near)-resident	32	10	22	16	8	8	x		x	
Clupea harengus	Herring	Pelagic	JMM	243	49	194	20	10	10	x	x	x	x
Sprattus sprattus	Sprat	Pelagic	JMM	51	20	31	15	7	8	x	x	x	x
Trachurus trachurus	Scad	Pelagic	MSV	109	29	80	20	10	10	x	x	x	x
Osmerus eperlanus	Smelt	Pelagic	MSV	120	14	106	15	7	8	x	x	x	x
Merlangius merlangus	Whiting	Benthopelagic	MSV	220	90	130	23	13	10	x	x	x	x
Trisopterus luscus	Bib	Benthopelagic	MSV	147	93	54	22	13	9	x	x	x	x
Gadus morhua	Cod	Benthopelagic	MSV	119	77	42	19	13	6	x	x	x	x
Anguilla anguilla	Eel	Benthopelagic	MSV	13	10	3	7	6	1	x		x	x
Ciliata mustela	Five-bearded rockling	Benthic	(Near)-resident	239	88	151	19	9	10	x	x	x	x
Platichthys flesus	Flounder	Benthic	(Near)-resident	456	182	274	28	18	10	x	x	x	x
Myoxocephalus scorpius	Bull-rout	Benthic	(Near)-resident	156	103	53	23	14	9	x	x	x	x
Zoarces viviparus	Viviparous blenny	Benthic	(Near)-resident	144	134	10	16	13	3	x	x	x	x
Pomatoschistus minutus	Sand goby	Benthic	(Near)-resident	133	97	36	14	7	7	x	x	x	x
Pleuronectes platessa	Plaice	Benthic	JMM	1048	942	106	33	24	9	x	x	x	x
Solea solea	Sole	Benthic	JMM	59	39	20	17	12	5	x	x	x	x
Limanda limanda	Dab	Benthic	MSV	1260	1224	36	29	21	8	x	x	x	x

The group of benthopelagic species (Fig 4B) only contained four marine seasonal visitor species (whiting *Merlangius merlangus*, bib *Trisopterus luscus*, cod *Gadus morhua* and eel *Anguilla anguilla*). Whiting focused on Pisces (mainly herring, some sandeel and various goby species), Malacostraca (mainly shrimps, to a lesser extent crabs and some Mysis) and from 2000 on Autobranchia (*Ensis* spec.). Bib mainly consumed Malacostraca (shrimps, crabs and some Mydidae) and some Pisces (herring and to a lesser extent sand goby and sandeel). Main prey items for cod were Malacostraca (mainly brown shrimp and some shore crabs), some Pisces (mainly herring and some sandeel and goby species) and Polychaeta. Eel mainly preyed upon Polychaeta and Malacostraca (brown shrimps and shore crabs).

The group of demersal species (Fig 4C) contained five (near)-resident species (five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus*, bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*), two juvenile marine migrant species (plaice *Pleuronectes platessa* and sole *Solea solea*) and a marine seasonal visitor species (dab *Limanda limanda).* Five-bearded rockling focussed on Malacostraca (mainly brown shrimp but also crabs) and in recent years sometimes on Pisces (mainly herring and also goby species). Flounder consumed a variety of prey items but especially Polychaeta, Pisces (mainly herring and some goby species), Malacostraca (mainly brown shrimps and to a lesser extent *Corophium* and shore and swimming crabs) and some Bivalves. Bull-rout preyed mainly on Malacostraca (brown shrimp and shore and swimming crabs) and to a lesser extent on Pisces (mainly herring). Main prey items of viviparous blenny were Malacostraca (Amphipods, brown shrimp and some crabs) and some Polychaeta, Pisces (herring) and Bivalves. Sand goby preyed especially upon Malacostraca (Copepods, Amphipods, small shrimp and shore crabs) and also on some Polychaeta and Pisces (herring). Plaice consumed a variety of prey species, especially Polychaeta, Malacostraca (mainly shrimps and shore and swimming crabs and some Amphipods and Mysis), Bivalves and Caenogastropoda (Hydrobia). Sole focused on Polychaeta and Malacostraca (mainly shrimps and crabs and some Mysis). Dab consumed a variety of prey items with a focus on Polychaeta, Pisces (mainly herring and furthermore some sandeel), and Malacostraca (mainly brown shrimps and furthermore shore and swimming crabs).

For the group of pelagic species, for all prey items (except for one year for the occurrence of Malacostraca in the diet of herring) for which a GAM with 95% confidence limits could be calculated, a horizontal line could be drawn between the 95% confidence limits of the fitted trend, implying that the frequency of occurrence had not changed over time (Supplementary material Fig S1A and Table 3). For three benthopelagic species (whiting, bib and cod) a GAM with 95% confidence limits could be calculated for the Malacostraca and Pisces and in all cases a horizontal line could be drawn between the 95% confidence limits of the fitted trend, implying that the frequency of occurrence of Malacostraca and Pisces had not changed over time for whiting, bib and cod (Supplementary material Fig S1B and Table 3). For the group of demersal species, for all prey items (except for the occurrence of Bivalves in the diet of plaice) for which a GAM with 95% confidence limits could be calculated, a horizontal line could be drawn between the 95% confidence limits of the fitted trend, implying that the frequency of occurrence had not changed over time (Supplementary material Fig S1C and Table 3).

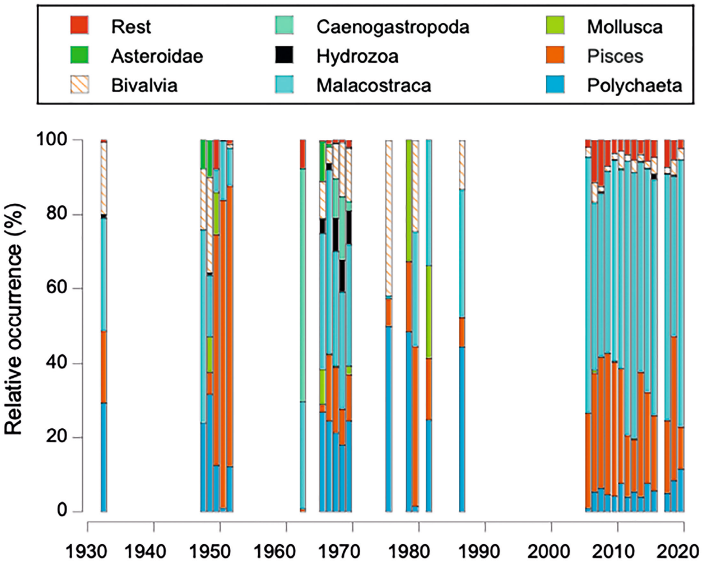


Figure 3 Relative mean occurrence (%) of the most abundant prey classes in the stomachs of Wadden Sea fish species within the NIOZ archive (1932 – 2019). Only years with al least 50 observations are listed.

All GAM parameters of the various trends in prey occurrence of the various fish species [smoother span, number of observations, number of parameters, standard error, smoother matrix, effective degrees of freedom (edf) and the p-value] are presented in Supplementary material Table S3.

3.3. Trophic position

For all individual years of the reference period 2010-2019, estimated trophic levels of the stomach contents based on a rerun with trophic values of identified prey Class were significantly related with original estimates based on trophic values of identified prey Species (Fig 5).

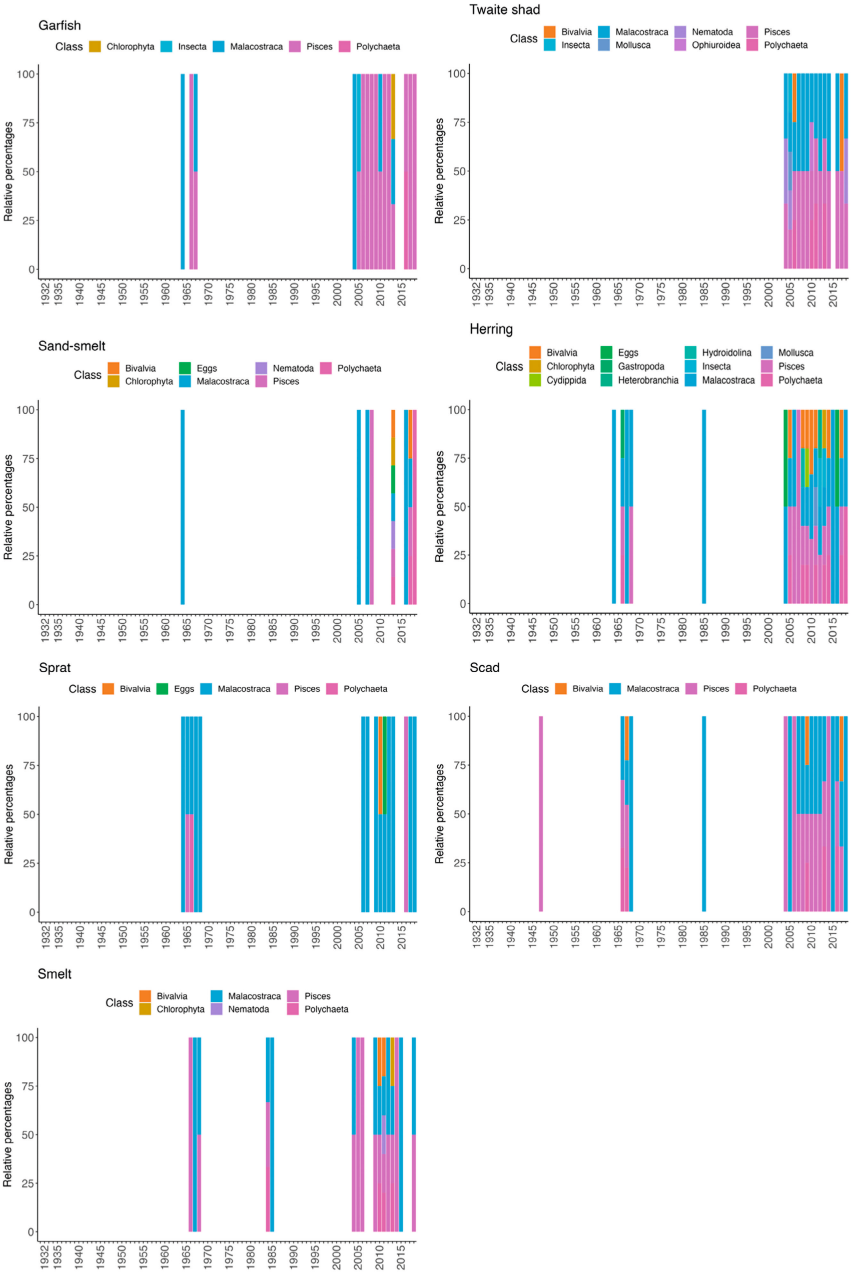


Figure 4A Mean occurrence of various prey items in the stomachs of the selected pelagic species; the (near)-resident species garfish *Belone belone*, twaite shad *Alosa fallax* and sand-smelt *Atherina presbyter*; the juvenile marine migrants herring *Clupea harengus* and sprat *Sprattus sprattus* and the marine seasonal visitors scad *Trachurus trachurus* and smelt *Osmerus eperlanus*.

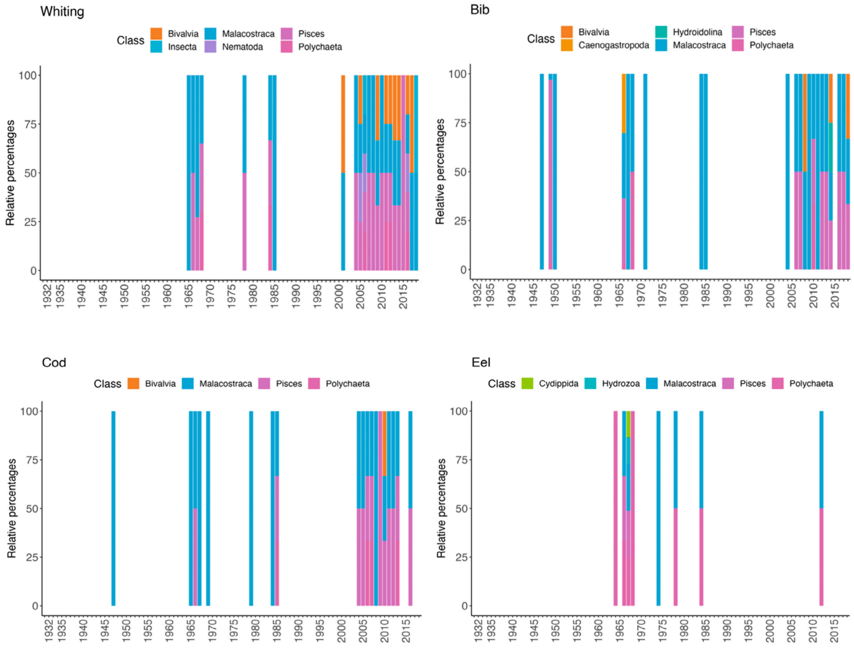


Figure 4B Mean occurrence of various prey items in the stomachs of the selected benthopelagic species; the marine seasonal visitor species (whiting *Merlangius merlangus*, bib *Trisopterus luscus,* cod *Gadus morhua* and eel *Anguilla Anguilla*.

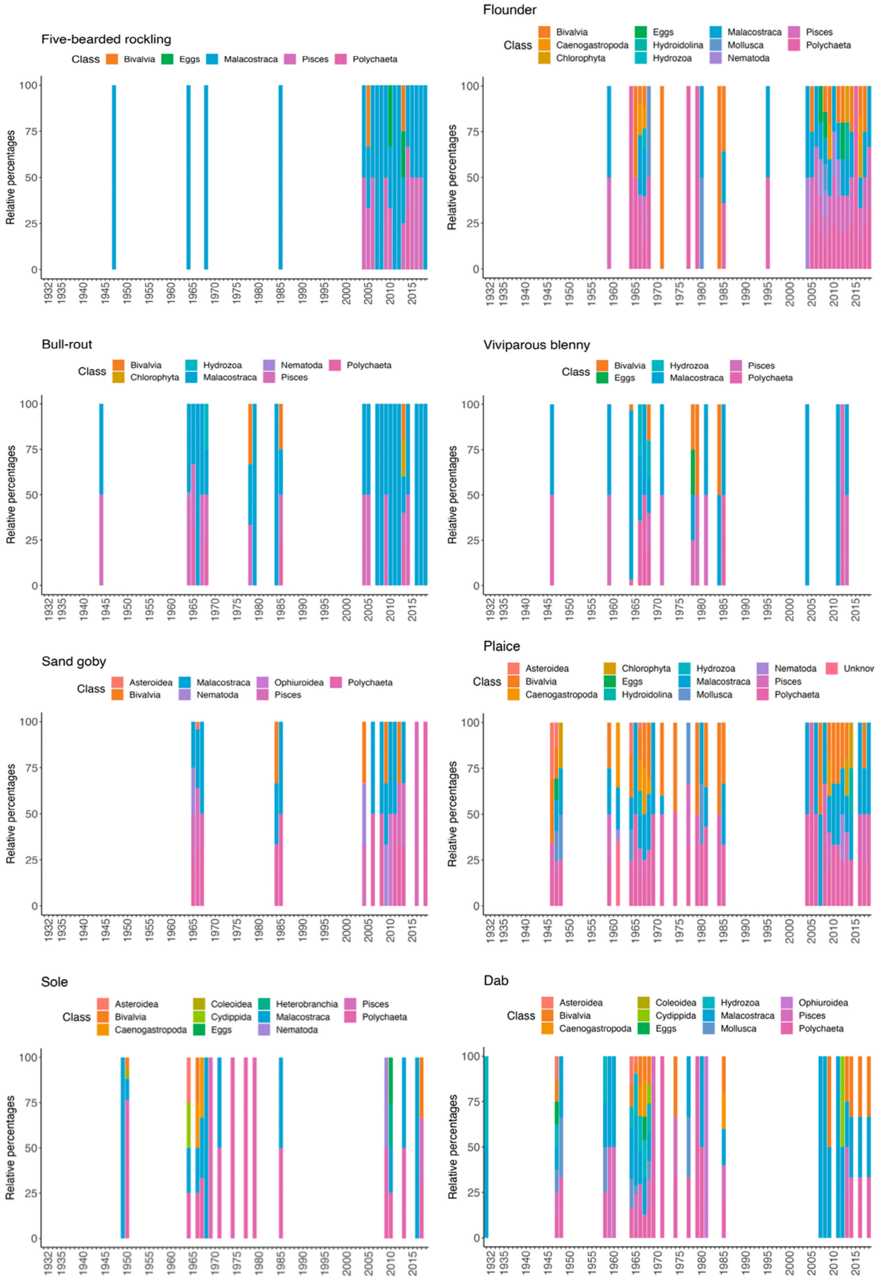


Figure 4C Mean occurrence of various prey items in the stomachs of the selected benthic species; the (near)-resident species five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus*, bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*; the juvenile marine migrant species plaice *Pleuronectes platessa* and sole *Solea solea* and the marine seasonal visitor species dab *Limanda limanda*, thick-lipped grey mullet *Chelon labrosus* and lesser weever *Echiichthys vipera*.

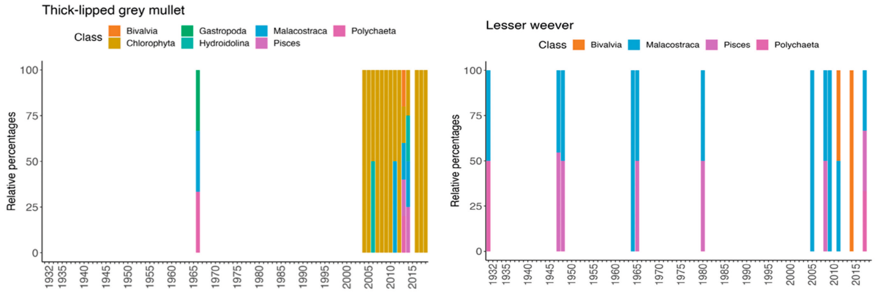


Figure 4C Mean occurrence of various prey items in the stomachs of the selected benthic species; the (near)-resident species five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus*, bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*; the juvenile marine migrant species plaice *Pleuronectes platessa* and sole *Solea solea* and the marine seasonal visitor species dab *Limanda limanda*, thick-lipped grey mullet *Chelon labrosus* and lesser weever *Echiichthys vipera*. *(continued)*

3.2.1. All data

Variability in trophic position of the different prey species was low for most Classes, except for Malacostraca (23%) and Pisces (38%) (Table 1). For the reference period (2010-2019) in almost all fish species, the estimated trophic position showed variation over a range of ~ 2 units (Table 4).

The estimated mean trophic position *(TP)* of the various fish species over the years can be found in Supplementary material Table S4. In the period 2005 – 2009, estimated mean trophic position were within those of the period 2010-2019 (Fig 6). Between 1970 and 1990, the percentage of species with estimates of trophic position within the reference range was lower, around 50 – 60 % and a higher percentage of species had estimates below the reference range compared with above the range (Fig 6). During the period 1945-1950 the percentage of species with estimates respectively below and above the reference range were almost similar (Fig 6).

3.2.2. Individual species

In 16 species, enough data were present to apply General Additive Models (GAMs) to visualise and analyse trends over time (Fig 7ABC).

In all pelagic (twaite shad, herring, sprat, scad and smelt), benthopelagic (whiting, bib, cod and eel) and demersal species (five-bearded rockling, flounder, bull-rout, viviparous blenny, sand goby, plaice, sole and dab), a horizontal line could be drawn between the 95% confidence limits of the fitted trend, implying no change over time (Fig 7ABC). All GAM parameters of the various trends in trophic position of the various fish species [smoother span, number of observations, number of parameters, standard error, smoother matrix, effective degrees of freedom (edf) and the p-value] are presented in Supplementary material Table S5.

Table 3 Changes over time in main prey groups (grey) of selected fish species from the NIOZ Archive 1931-2019. (Near)-resident: Near-resident or resident species; JMM: juvenile marine migrants; MSV: Marine seasonal visitor. Significance of the GAM trend is indicated (n.s.: not significantly deviating from horizontal line or $P<0.05$). Only years with more than 5 observations are included.

Scientific name	Common name	Functional group	Guild	Algae	Bivalves	Malacostraca	Pisces	Polychaeta
Clupea harengus	Herring	Pelagic	JMM		n.s.	P<0.05	n.s.	n.s.
Sprattus sprattus	Sprat	Pelagic	JMM			n.s.		
Trachurus trachurus	Scad	Pelagic	MSV			n.s.	n.s.	
Osmerus eperlanus	Smelt	Pelagic	MSV			n.s.	n.s.	
Merlangius merlangus	Whiting	Benthopelagic	MSV			n.s.	n.s.	
Trisopterus luscus	Bib	Benthopelagic	MSV			n.s.	n.s.	
Gadus morhua	Cod	Benthopelagic	MSV			n.s.	n.s.	
Ciliata mustela	Five-bearded rockling	Benthic	(Near)-resident			n.s.	n.s.	
Platichthys flesus	Flounder	Benthic	(Near)-resident		n.s.	n.s.		
Myoxocephalus scorpius	Bull-rout	Benthic	(Near)-resident			n.s.	n.s.	
Zoarces viviparus	Viviparous blenny	Benthic	(Near)-resident			n.s.		
Pomatoschistus minutus	Sand goby	Benthic	(Near)-resident			n.s.		n.s.
Pleuronectes platessa	Plaice	Benthic	JMM		P<0.05	n.s.		n.s.
Solea solea	Sole	Benthic	JMM			n.s.		n.s.
Limanda limanda	Dab	Benthic	MSV			n.s.	n.s.	n.s.

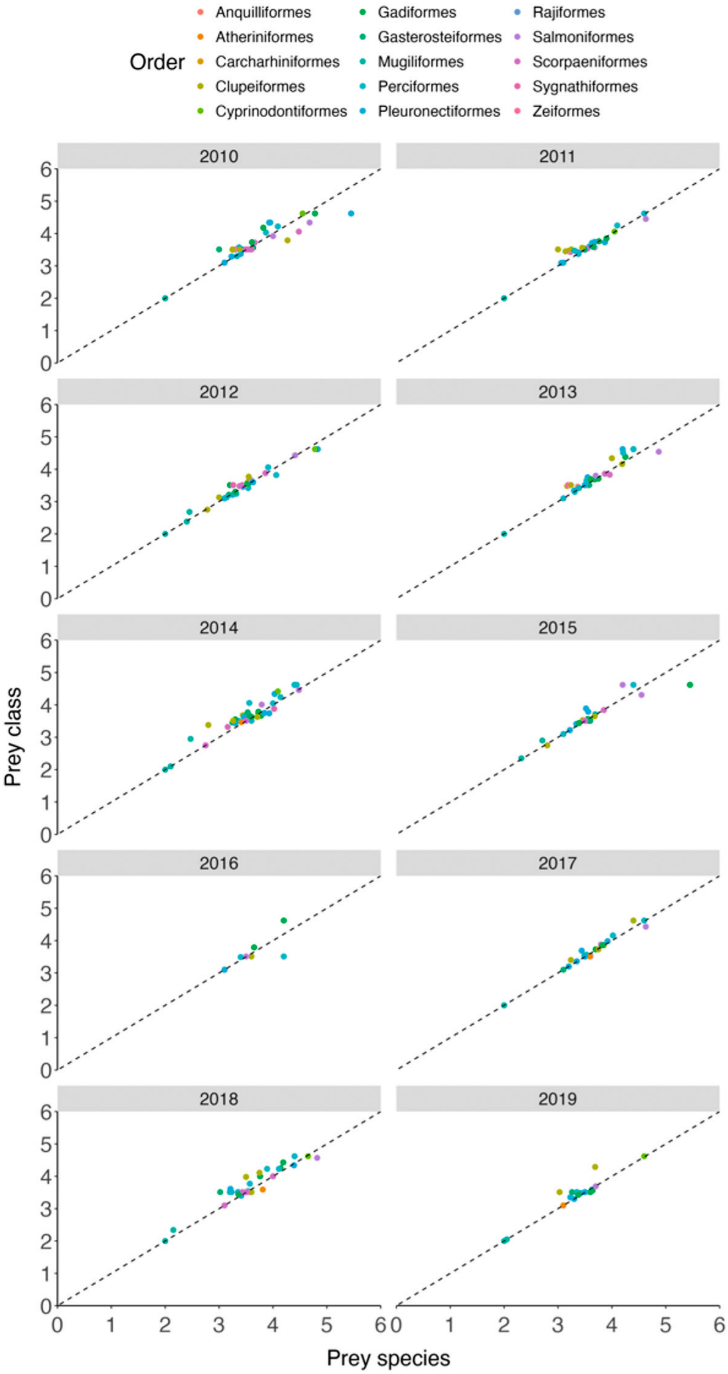


Figure 5 Comparison of estimated TP values of individual fish species per 5-year period with the range of TP of the reference period 2010 – 2019.

Table 4 Size frequency distribution of estimated trophic position (TP) of individual fish for the period 2010 – 2019.

Scientific name	Common name	Trophic position (TP)									
		1.00-1.49	1.50-1.99	2.00 -2.49	2.50-2.99	3.00-3.49	3.50-3.99	4.00-4.49	4.50-4.99	5.00-5.49	Total
Agonus cataphractus	Hooknose				1		28	1	2		32
Alosa fallax	Twaite shad					7	138	23	33		201
Ammodytes tobianus	Sandeel					3	6				9
Anguilla anguilla	Eel					1	8				9
Aphia minuta	Transparent goby										
Arnoglossus laterna	Scaldfish						24	1			25
Aspitrigla cuculus	Red gurnard										
Atherina presbyter	Sand-smelt			1	1	6	30	2	2		42
Belone belone	Garfish					2	2	1	26		31
Callionymus lyra	Dragonet						1		1		2
Callionymus reticulatus	Reticulated dragonet					1					1
Chelon auratus	Golden grey mullet	1		88	1	4	5				99
Chelon labrosus	Thick-lipped grey mullet			55	2	2	16	2	5		82
Chelon ramada	Thin-lipped grey mullet			3							3
Ciliata mustela	Five-bearded rockling				3	3	390	17	25		438
Clupea harengus	Herring			8	1	26	220	12	33		300
Cyclopterus lumpus	Lumpsucker				1		26	2			29
Dicentrarchus labrax	Bass			3	1	52	446	33	100		635
Dipturus batis	Skate										
Echiichthys vipera	Lesser weever					2	3	1			6
Engraulis encrasicolus	Anchovy						2				2
Eutrigla gurnardus	Grey gurnard						1				1
Gadus morhua	Cod					1	67	10	10		88
Gasterosteus aculeatus	Stickleback			2	1	11	58		2	1	75
Gobius niger	Black goby										
Hyperoplus lanceolatus	Greater sandeel						1		9		10

Table 4 Size frequency distribution of estimated trophic position (TP) of individual fish for the period 2010 – 2019. *(continued)*

Scientific name	Common name	Trophic position (TP)									
		1.00-1.49	1.50-1.99	2.00 -2.49	2.50-2.99	3.00-3.49	3.50-3.99	4.00-4.49	4.50-4.99	5.00-5.49	Total
Limanda limanda	Dab					11	27	1	3		42
Liparis liparis	Sea-snail						86	2	0		88
Lipophrys pholis	Shanny								1		1
Melanogrammus aeglefinus	Haddock										
Merlangius merlangus	Whiting				1	19	170	24	25		239
Microstomus kitt	Lemon sole					1					1
Mustelus mustelus	Smooth hound										
Myoxocephalus scorpius	Bull-rout				1	2	100	12	3		118
Neogobius melanostomus	Round goby						7		1		8
Osmerus eperlanus	Smelt			1		11	110	13	33		168
Pholis gunnellus	Butterfish					2	5				7
Phrynorhombus norvegicus	Norwegian topknot										
Platichthys flesus	Flounder			2	2	114	276	14	49		457
Pleuronectes platessa	Plaice			1	2	66	39	3	10		121
Pollachius pollachius	Pollack						46	13	7		66
Pollachius virens	Saithe				1	2	6	7	8		24
Pomatoschistus lozanoi	Lozano's goby					1	10				11
Pomatoschistus microps	Common goby					1	4				5
Pomatoschistus minutus	Sand goby					14	22		4		40
Salmo salar	Salmon										
Salmo trutta	Sea trout					3	47	8	236		294
Sardina pilchardus	Pilchard			4			14		3		21
Scomber scombrus	Mackerel						5	6	8		19
Scophthalmus maximus	Turbot				1	2	58	11	19		91
Scophthalmus rhombus	Brill						13	1	10		24
Solea solea	Sole				1	16	6		5		28

Table 4 Size frequency distribution of estimated trophic position (TP) of individual fish for the period 2010 – 2019. *(continued)*

		Trophic position (TP)									
Scientific name	**Common name**	**1.00-1.49**	**1.50-1.99**	**2.00 -2.49**	**2.50-2.99**	**3.00-3.49**	**3.50-3.99**	**4.00-4.49**	**4.50-4.99**	**5.00-5.49**	**Total**
Sparus aurata	Gilt-head sea bream						2				2
Sprattus sprattus	Sprat			1		1	37		3		42
Squalus acanthias	Spurdog										
Syngnathus acus	Greater pipefish						18	2	2		22
Syngnathus rostellatus	Nilsson's pipefish						33				33
Taurulus bubalis	Sea scorpion					1	21				22
Trachinus draco	Greater weever										
Trachurus trachurus	Scad					4	35	12	79		130
Trigla lucerna	Tub gurnard				1		19	2			22
Trisopterus luscus	Bib					3	106	2	6		117
Trisopterus minutus	Poor cod					1	3				4
Zeus faber	Dory										
Zoarces viviparus	Viviparous blenny			1		3	4	1	2		11

4. Discussion

4.1. Quality and limitations of the NIOZ archive data

Long-term series are unique and in principle valuable data sets, however a precondition is that the quality and the limitations of the data can be judged and that potential pitfalls can be identified. Wiltshire & Dürselen (2004) carried out a quality control of the Helgoland Reede long-term phytoplankton data archive (1962 – present) and listed a number of typical general problems they came across. The most important issues that can be expected for all long-term series can be summarized as:

- lack of meta-information, especially from the past;
- the mismatch between the original records on paper and the electronical archive;
- outdated taxonomic nomenclature and synonyms;
- different procedures over time;
- different investigators over time with different taxonomic knowledge.

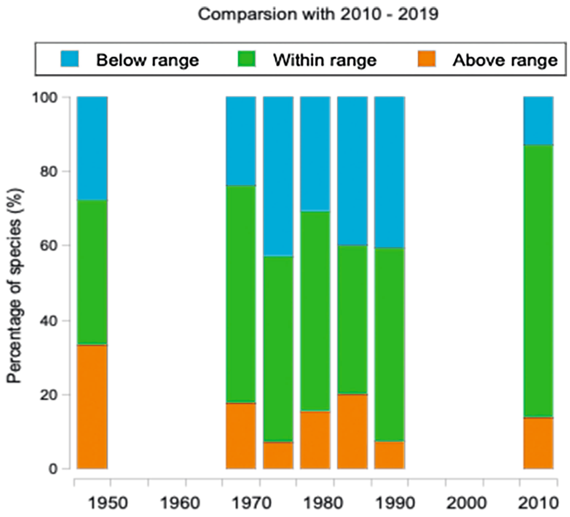


Figure 6 Comparison of estimated mean TP values of all fish species per 5-year period to the reference period TP range (2010 – 2019). For more information see text.

Also, the NIOZ archive data suffers from some of these problems. The NIOZ archive also lacks meta-information with respect to information about potential digestion between time of catch and of stomach analysis. However, most of the records originate from NIOZ courses where fish were dissected immediately after being caught. Stomach content of rare fish species from fishermen might have suffered from digestion: often these stomachs were empty or could not be identified. The NIOZ archive did not suffer from a mismatch between the original records on paper and the electronical archive, because the data were never electronically archived in the past. The problem of outdated taxonomic nomenclature and synonyms occurred but was solved by using WoRMS (http://www.marinespecies.org) for checking species, family, genus and class name of all fish and prey records. As far as we can check in the records, all analyses have always been supervised and/or carried out by qualified NIOZ staff with taxonomic knowledge.

Most striking are the differences in amount of data and in level of taxonomic identification of stomach content over time. From 1980 onwards, fish were collected regularly in spring and summer and until 1980 only randomly as part of NIOZ courses and landings from fishermen. This means that despite the more than 12.000 records of stomach content analysis for the fish community of the Wadden Sea, the dataset shows a large patchiness and variability both with respect to the years in which data were collected and in the fish species analysed. Surprisingly, level of taxonomic identification of prey over time hardly affected the estimate of the trophic position. A sensitivity analysis for the reference period 2010-2019 showed that estimated trophic levels based on prey Class were significantly related with original estimates based on prey Species.

By interpreting the results of the analysis of the NIOZ archive data, these restrictions should be kept in mind.

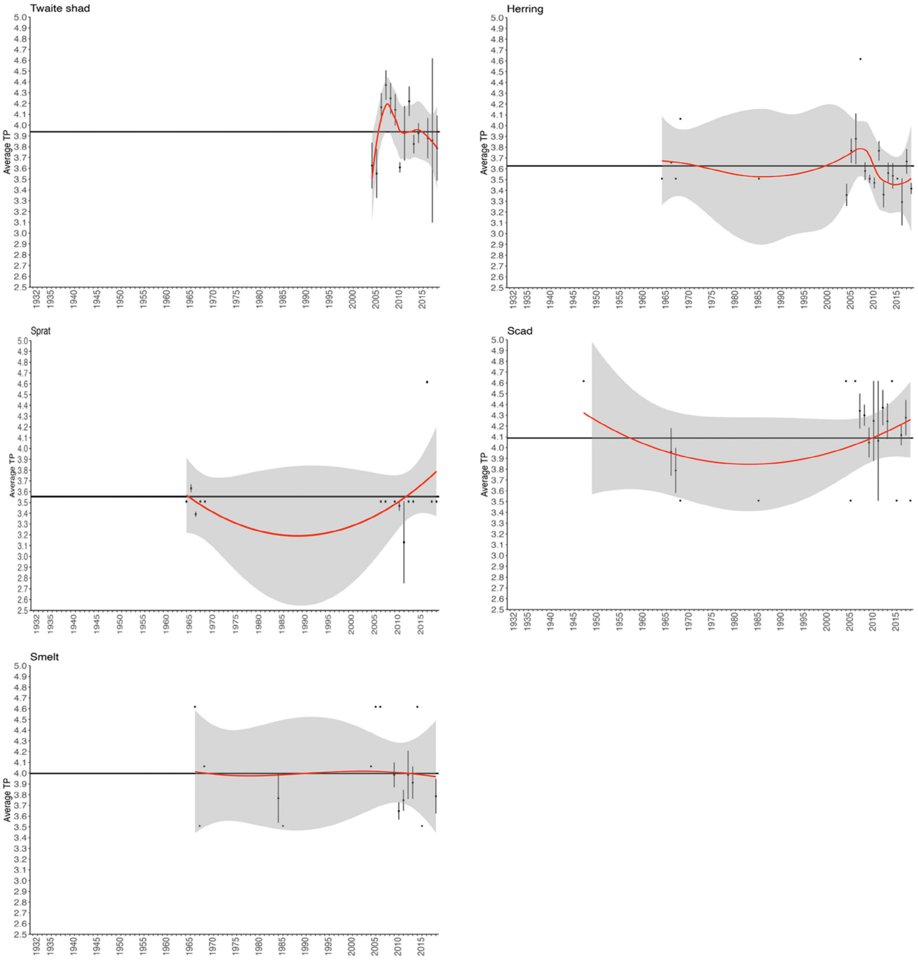


Figure 7A Mean trophic position (–) of the selected pelagic species; the (near)resident species twaite shad *Alosa fallax*; the juvenile marine migrants herring *Clupea harengus* and sprat *Sprattus sprattus* and the marine seasonal visitors scad *Trachurus trachurus* and smelt Osmerus eperlanus.

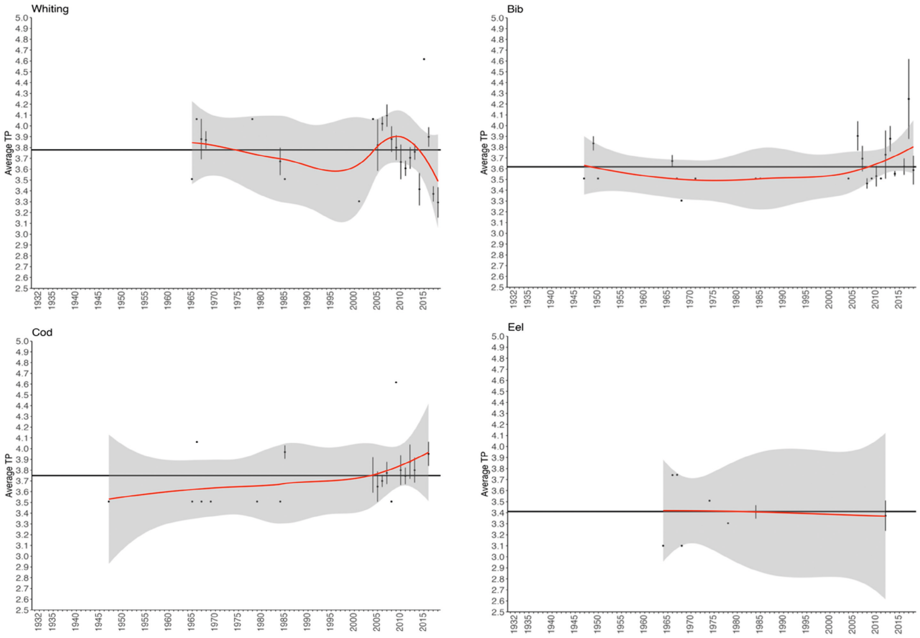


Figure 7B Mean TP (-) of the selected benthopelagic species; the marine seasonal visitor species whiting *Merlangius merlangus*, bib *Trisopterus luscus*, cod *Gadus morhua* and eel *Anguilla Anguilla.*

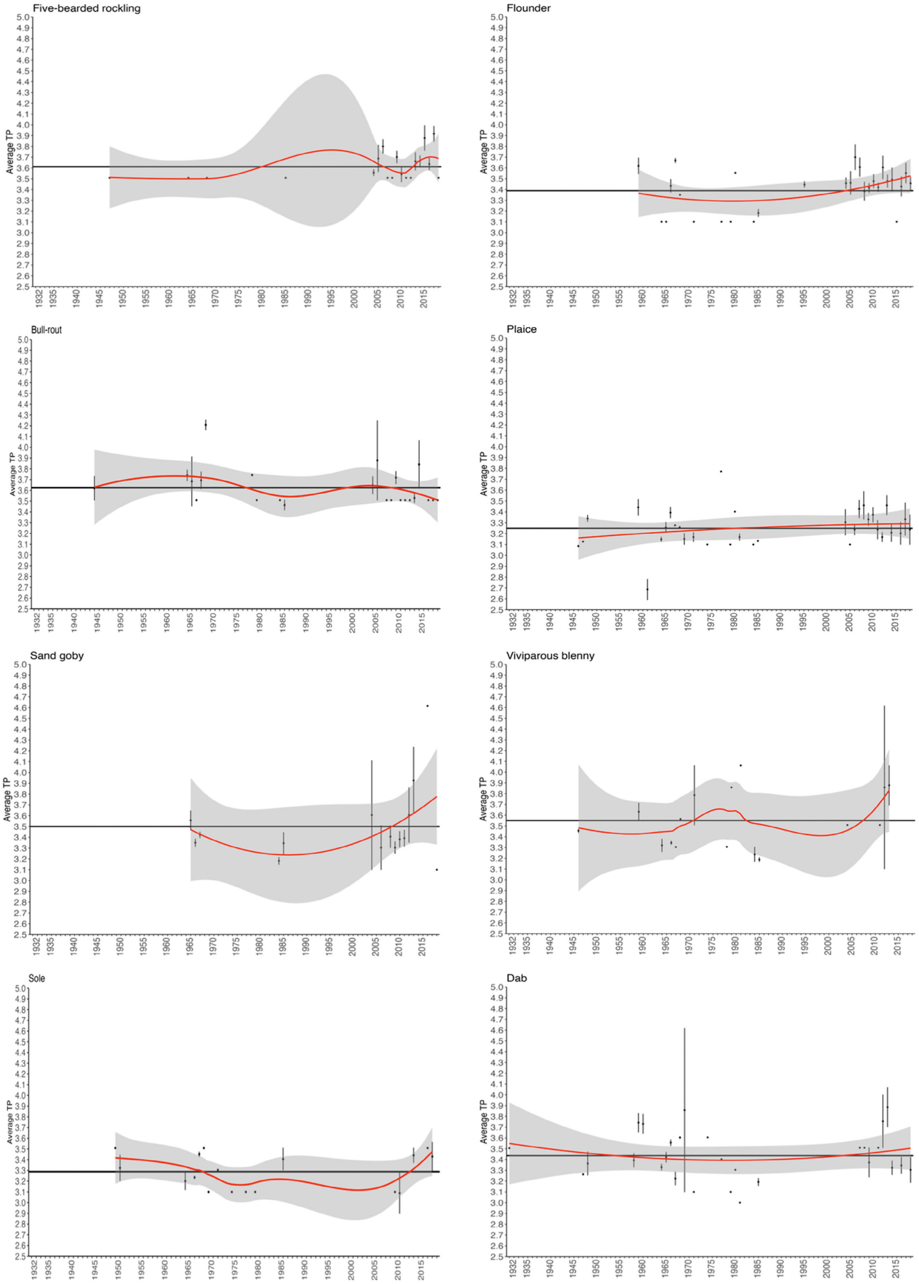


Figure 7C Mean TP (-) of the selected benthic species; the (near)-resident species five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus,* bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*; the juvenile marine migrant species plaice *Pleuronectes platessa* and sole *Solea solea* and the marine seasonal visitor species dab *Limanda limanda.*

4.2. Wadden Sea baseline

What would be a realistic baseline for the Wadden Sea system and in particular for its fish fauna is open for debate. The Wadden Sea has been under the influence of anthropogenic stress for centuries (see for instance Lotze 2005, 2007). Stress factors caused by human-induces activities (overfishing and pollution events), could theoretically be reduced or stopped. This, however, might be not pragmatic. However, other factors such as habitat loss are even more difficult to reverse. The last extensive habitat loss in the western Dutch Wadden Sea took place in the Marsdiep tidal basin in the western part in 1932 with the exclosure of the Zuiderzee estuary by the Afsluitdijk and in the eastern Dutch Wadden Sea in 1964 with the exclosure of the Lauwers (Wolff 1983). This means that for the Marsdiep tidal basin a baseline before 1932 is unrealistic with respect to any analyses with more recent data, including the present situation. The low fishing pressure and low level of pollution (nutrients, chemicals) during the second world war would plea for a realistic baseline around 1945 for the Wadden Sea system.

Quantitative information about the Dutch Wadden Sea system for the period around 1945 is scarce except for water temperature and salinity data (van Aken 2008a,b) and remains fragmentary until the beginning of the 1970s, despite the start of nutrient measurements (phosphorus) from 1949 onwards (Postma 1954) and primary production estimates (Postma and Rommets 1970) and demersal fish surveys in 1963-1965 (Creutzberg and Fonds 1971). Only for the last half century from the 1970's onwards more systematic information is available with presently time series about various abiotic and biotic ecosystem components such as water temperature and salinity, primary production, the benthic community, fish fauna, wading birds and marine mammals for various parts of the Wadden Sea. For an overview see the various quality status reports of the Wadden Sea (https://qsr.waddensea-worldheritage.org/).

The present study contains information on trophic structure based on stomach content dating back to the early 1930's. The Wadden Sea ecosystem in the 1930's will have been a system with lower nutrient concentrations (van der Veer et al. 1989, van Raaphorst & van der Veer 1990, van Raaphorst & de Jonge 2004) but nevertheless a system with a higher fish abundance compared to the present ecosystem. A higher fish abundance in the past is supported by the fact that, before and after the second world war, there was a profitable commercial fyke net fishing in the area. However, catches and profitability decreased rapidly until the last fishing company was terminated in 1966 and taken over by NIOZ to start the long-term monitoring series (van der Veer et al. 2015).

The much and varying variability in the stomach content for any given species within the period 1930-2019 raises the question whether stomach content data of fish species is absent in particular years and decades because the fish species were absent or rare in the ecosystem, or because they were simply not targeted during that time. For most species, missing data indicate that they were not targeted: from the 1980's onwards, stomach content data were collected from a long-term monitoring programme of the fish fauna with a passive fish trap near the entrance of the Wadden Sea in spring and autumn and during that period no species went extinct and common species were caught almost every year (Poiesz et al. 2020). Only most of the skate and shark species disappeared from the Wadden Sea from the 1960's onwards, similar as in other areas (Walker and Heessen 1996, Dulvy and Reynolds 2002, Reynolds et al. 2005, Heessen et al. 2015, Bom et al. 2020, Poiesz et al. 2021b).

4.3. Prey consumption

A variety of sources are available for the reconstruction of the fish food web structure, ranging from anecdotal and semi-quantitative information about species composition (see for instance Roberts, 2007) to quantitative analysis of archaeological remains such as of bones and otoliths. The latter can include stable isotope analysis (Fry, 2006, Middelburg, 2014, Phillips et al. 2014, Tsutaya et al. 2021), genetics, age and growth analyses (see for example Bolle et al. 2004, Cuvelliers et al. 2007) and stomach content analysis [such as deriving trophic structure and predator–prey interactions (Hynes, 1950, Baker et al. 2014)]. Stomach content analysis provides information about recently ingested prey items only, while especially regurgitation and digestion are factors that may cause prey items to be missed or overlooked. The extended period of sampling may have partly overcome these limitations, however, for rare species an insufficient number of stomachs may have been sampled to cover all possible prey species (Karachle and Stergiou 2017, Mulas et al. 2015).

Recent studies in two different parts of the Wadden Sea reveal that, although most of the Wadden Sea fish species are rather omnivorous, their food requirements are fuelled by a few key prey species (Kellnreitner et al. 2012, Poiesz et al. 2020). This omnivorous feeding behaviour can also be recognized in the stomach content compositions of the Wadden Sea fish fauna over the last half century. Interannual variations in stomach composition do occur due to variations in the level of detail of the stomach content analysis over the years as well as variations in prey abundance. Nevertheless, a few groups, Bivalvia/Autobranchia, Polychaeta, Malacostraca (mainly Decapoda: shrimps and crabs) and Pisces, were the main prey items from the 1930's onwards to recent decades. A few key species as main pathways of energy flow to higher trophic levels might be a general characteristic for estuarine systems; it has been described for other areas also, such as Amphipods and Copepods in the French Chanche estuary (Selleslagh et al. 2012).

Trends in prey occurrence in the stomachs could be determined for some prey items in some individual fish species. However, the analysis was hampered by large patchiness and variability in the data and in the variability in the level of detail of the stomach content analysis. In all fish species that could be analysed, prey occurrence showed fluctuations over time. The most important prey species of the Malacostraca, the brown shrimp and the shore crab, both showed large interannual fluctuations in the Dutch Wadden Sea, with a general increase in both species over a 40 yr period (Tulp et al. 2012). Similar fluctuations were observed in secondary production of intertidal bivalves and polychaetes, however without a clear trend over time (Beukema and Dekker 2022). Also, the Wadden Sea fish community showed strong interannual fluctuations in abundance, added to a clear decline from the 1980s until the early 2000s (Tulp et al. 2008, van der Veer et al. 2015). Herring, the most important fish prey species, showed strong variation among years and fluctuated in abundance within one order of magnitude (van der Veer et al. 2015). Therefore, the fluctuations in stomach content composition partly reflects interannual variability in absolute and relative abundance of the most important prey groups.

The large patchiness and variability in the data resulted in large confidence intervals of the GAM smoother over time. Despite the large fluctuations in prey occurrence in the stomachs of the various fish species, hardly any significant differences between years were found (except for the occurrence of Pisces in the diet of smelt and sand-smelt and the occurrence of Malacostraca in the diet of sole and dab). Furthermore, no trends in prey occurrence over time were found in the various species analysed. This means that amphipod crustaceans, brown shrimps and crabs, juvenile herring and gobies and to a lesser extent bivalves and polychaetes are not only the key prey species presently (Poiesz et al. 2020) but already had a pivotal position in the fish food web in the past, at least from the 1930's onwards (this study).

4.4. Trophic position

The large patchiness in the data for all Wadden Sea fish species with respect to years of sampling, results in a mozaik of snapshots of trophic positions of individual species over time and in a number of species with enough data to apply General additive models (GAMs) to visualise and analyse trends over time. The analysis of the complete data set and the analyses of the individual species both indicated that trophic positions during the period 1930-2010 were variable but did not significantly differ from those in the present reference period (as described in Poiesz et al. 2020). The variability in individual stomach contents, and hence in the estimates of trophic position, illustrates the omnivorous character of most of the fish species in the Wadden Sea: current day estimations of trophic position vary by 2 units for most fish species (Poiesz et al. 2020, Table 3). It cannot be excluded that the present dataset with high sampling variability might be not robust enough to identify trends over time for these fish species with an inherent large individual variability in trophic position.

On the other hand, network analyses indicate that estuaries are rather stable systems, where a few species such as for instance clupeids, flatfish and gobies are able to cope with the inherent cyclical and seasonal perturbations: those species are robust and are responsible for a stable system (Lobry et al. 2008). In the western Dutch Wadden Sea there are also no trend indications in the number of species caught over the period 1960 – 2010 (van der Veer et al. 2015). The fact that in this study no trend in trophic position was found in species belonging to different modes of life (pelagic, benthopelagic and demersal) and guild (near-resident, juvenile marine migrant or seasonal visitor) might imply that this could also hold true for the other species not analysed in this study.

Although estuaries might be rather stable systems, serious impacts of anthropogenic stress have nevertheless been documented for many of these systems (see for instance Kennish 1991, 2002, Chapman and Wang 2000), including the Wadden Sea (Lotze 2005, 2007). With respect to the fish fauna, this has led to the disappearance of most skate and shark species in the area, causing a loss of biodiversity in the Wadden Sea from the 1960's onwards, similar to those reported in other areas (Walker and Heessen 1996, Dulvy and Reynolds 2002, Reynolds et al. 2005, Heessen et al. 2015, Bom et al. 2020, Poiesz et al. 2021b). Before the 1960s, the Wadden Sea fish community did include skate and sharks, top predators with a relatively high trophic position.

The present study does not indicate any changes in trophic position of individual species in the western Dutch Wadden Sea over the last 80 years. This may be different at the community level. Although fish species composition in the western Wadden Sea has shown to be rather robust, species composition does show some interannual variation (van der Veer et al. 2015). Some species have also disappeared in the past, such as most of the skate and shark species. Furthermore, year-to year fluctuations in the relative abundance of the various fish species (Tulp et al. 2008, van der Veer et al. 2015) will be reflected in interannual variations in the trophic structure of the fish community. In the western Wadden Sea, the trophic structure of this community showed indeed some fluctuations from 1980 to 2011. For both the demersal and benthopelagic fish fauna the trophic position remained the same, while for pelagic fish the mean fell from about 3.9 to 3.1., mainly due to the decrease in abundance of predatory pelagic fish such as cod and garfish (van der Veer et al. 2015).

The 10-fold decrease in total biomass of the catches of both pelagic and demersal species from 1980 to 2011 (van der Veer et al. 2015) illustrates the degradation of the trophic role of the fish community at the ecosystem level in the western Wadden Sea. To what extent this has affected ecosystem functioning is unclear. In the North Sea, the depletion of demersal fish species in the period 1973-2000 appears to have released the benthos from "top-down" biomass control, leading to an increase in benthic production and invertebrates (Heath 2005). To what extent the trophic structure of the fish community in the western Wadden Sea are a reflection of a more general pattern also in the other tidal basins of the Wadden Sea is unclear. The fact that most species are omnivorous and species composition appears to be largely the same at a large scale (Kühl and Kuipers 1983, Kellnreiter et al. 2012, Meyer et al. 2016, Poiesz et al. 2020) might suggest a general pattern in trophic position of the fish species in the

Wadden Sea. However, the fact that local and interannual differences were found in the abundance of demersal fish in the western, central and eastern part of the Wadden Sea and in its coastal regions (Tulp et al. 2017) implies that at the community and ecosystem level the trophic structure of the fish community may differ to some extent.

4.5. Conclusive remarks

In this study, trends in prey species consumed and in trophic position were analysed and by means of stomach content information compared to the present situation (2010-2019) for 18 omnivorous fish species in the western Dutch Wadden Sea. Prey consumption of different fish species showed variability over time, but without a change over time. Also, in all 18 species, no significant change in mean trophic position over time could be found. Despite the general decrease in fish abundance in the area (van der Veer et al. 2015). The present study does not indicate any changes in trophic position of individual species in the western Dutch Wadden Sea over the last 80 years despite the serious level of anthropogenic stress (pollution, eutrophication events, climate change) and the decrease in fish abundance in the area.

Acknowledgements

Thanks are due to all our colleagues who assisted in the collection and analyses of the samples, especially Rob Dapper, Ewout Adriaans, Willem Jongejan, Sieme Gieles and Marco Kortenhoeven. All recent fish sampling and handling was done under CCD project number: AVD8020020174165.

Supplementary materials

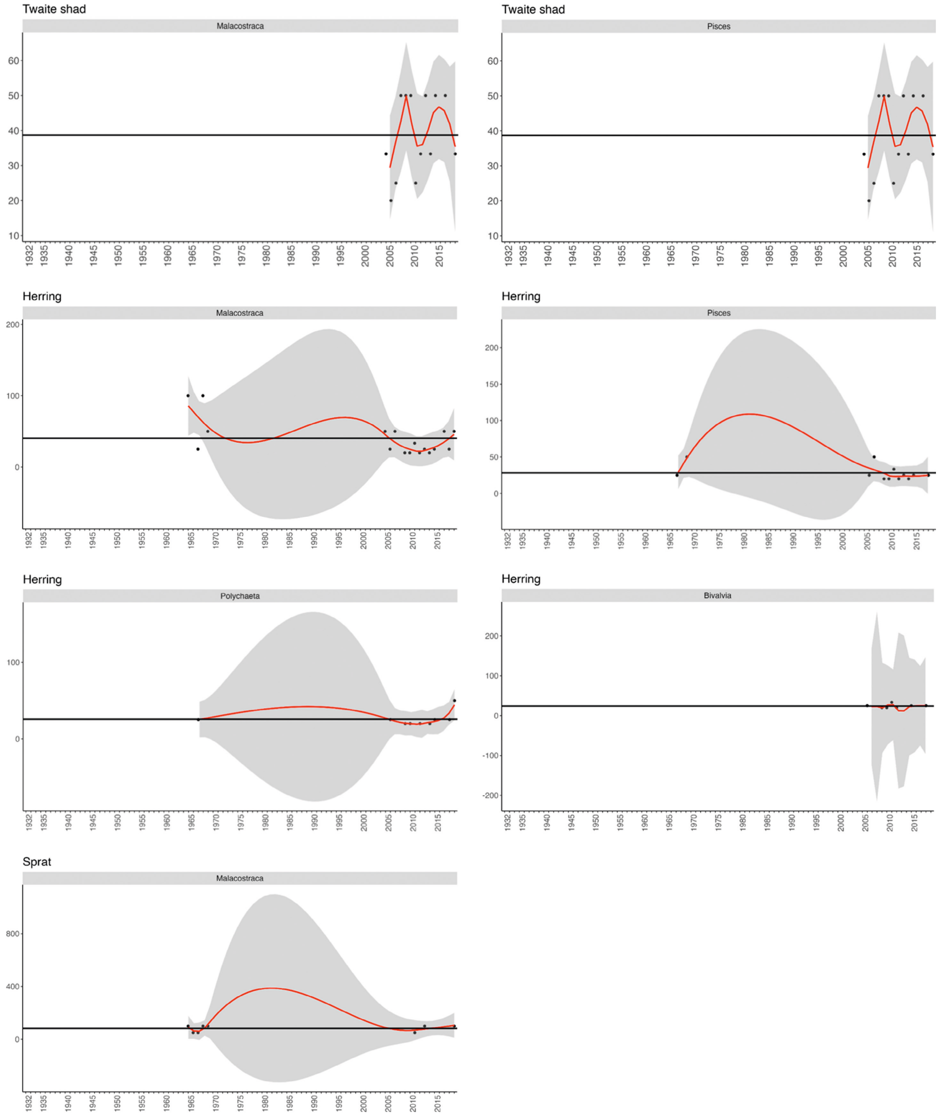
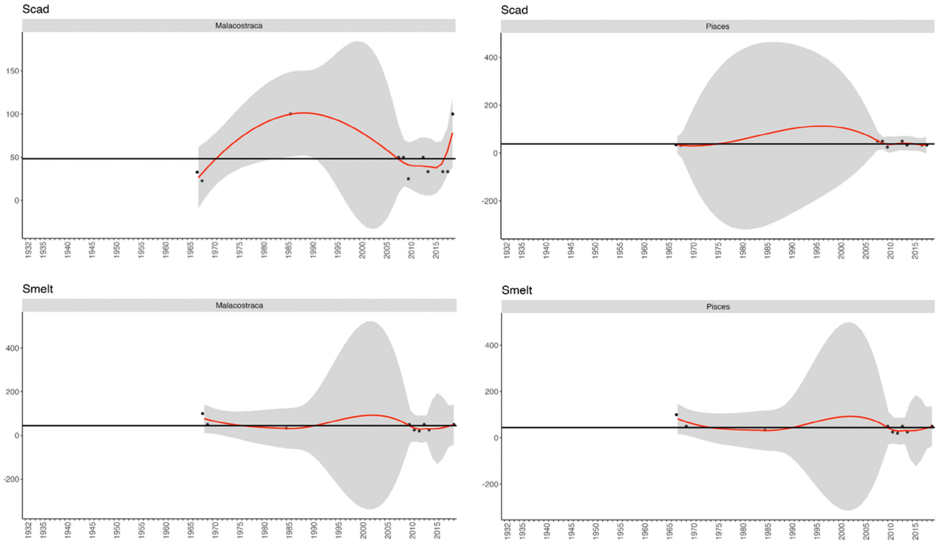


Figure S1A Trends in the occurrence of the main prey classes (%) of the selected pelagic species; the (near)-resident species twaite shad *Alosa fallax*; the juvenile marine migrants herring *Clupea harengus* and sprat *Sprattus sprattus* and the marine seasonal visitors scad *Trachurus trachurus* and smelt *Osmerus eperlanus*.



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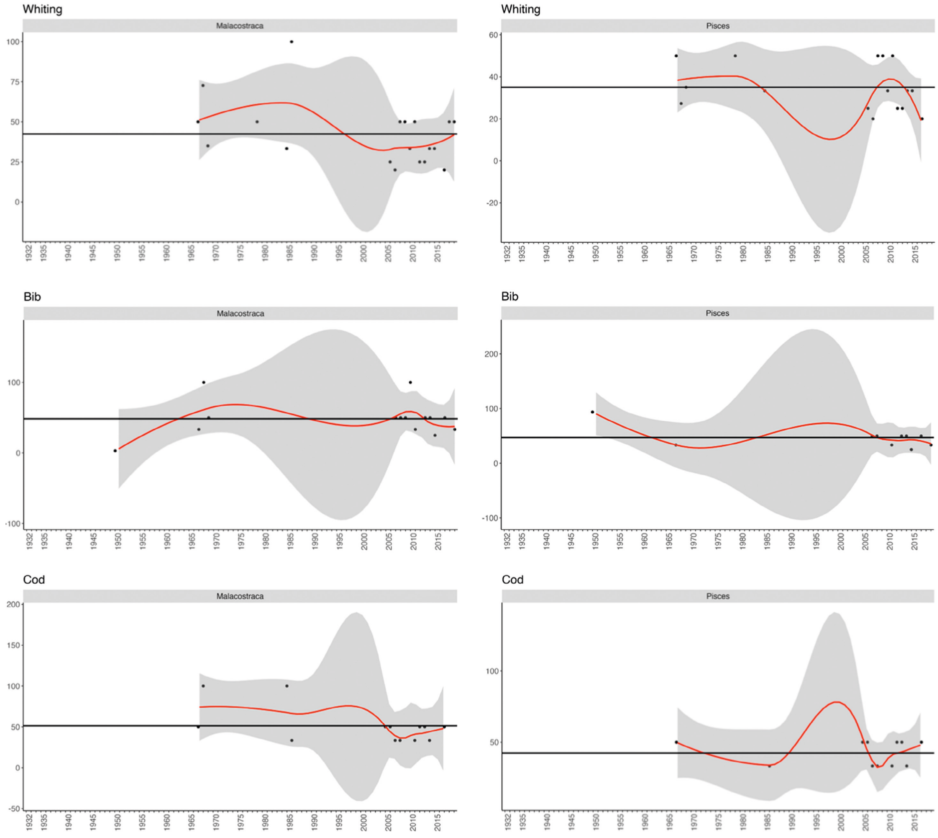

Figure S1B Trends in the occurrence of the main prey classes (%) of the selected benthopelagic species; the marine seasonal visitor species whiting *Merlangius merlangus*, bib *Trisopterus luscus* and cod *Gadus morhua*.

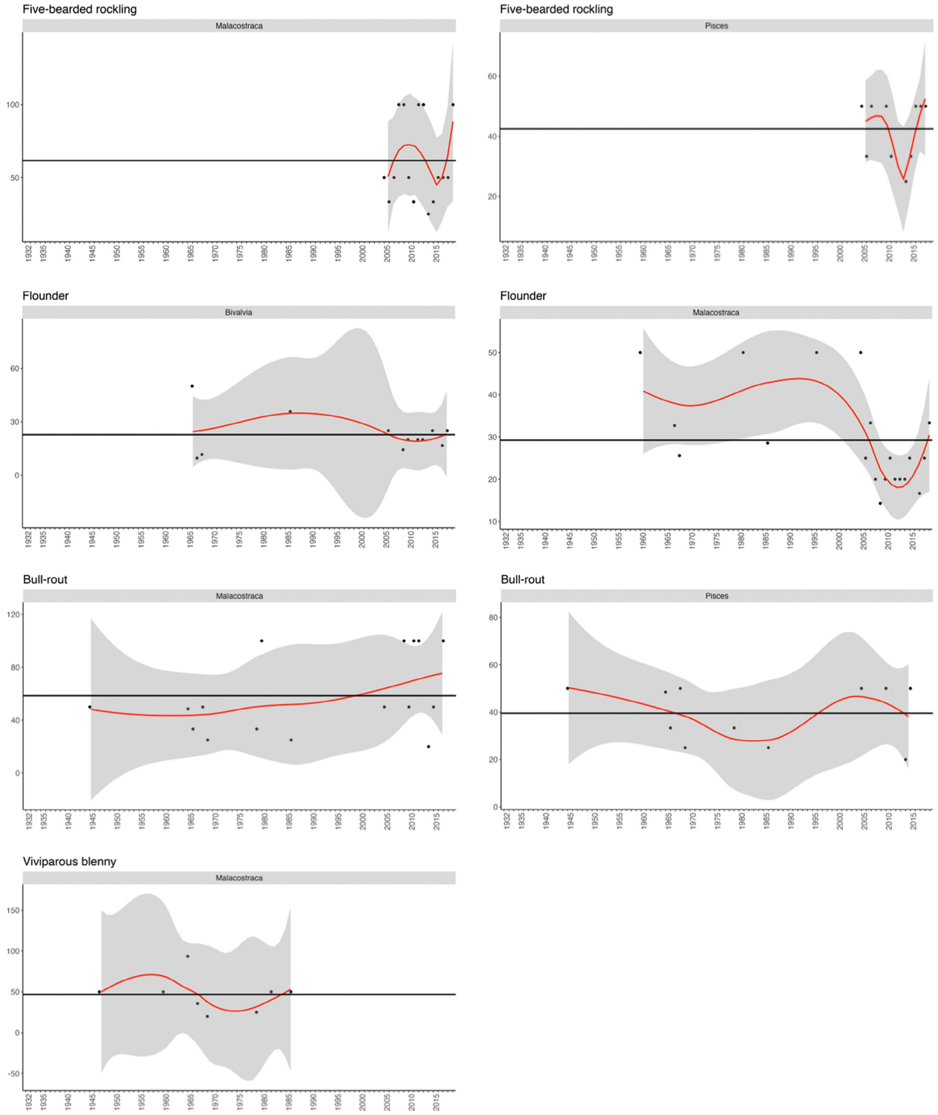

Figure S1C Trends in the occurrence of the main prey classes (%) of the selected benthic species; the (near)-resident species five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus*, bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*; the juvenile marine migrant species plaice *Pleuronectes platessa* and sole *Solea solea* and the marine seasonal visitor species dab *Limanda limanda and* sand-smelt *Atherina presbyter* and thick-lipped grey mullet *Chelon labrosus*.

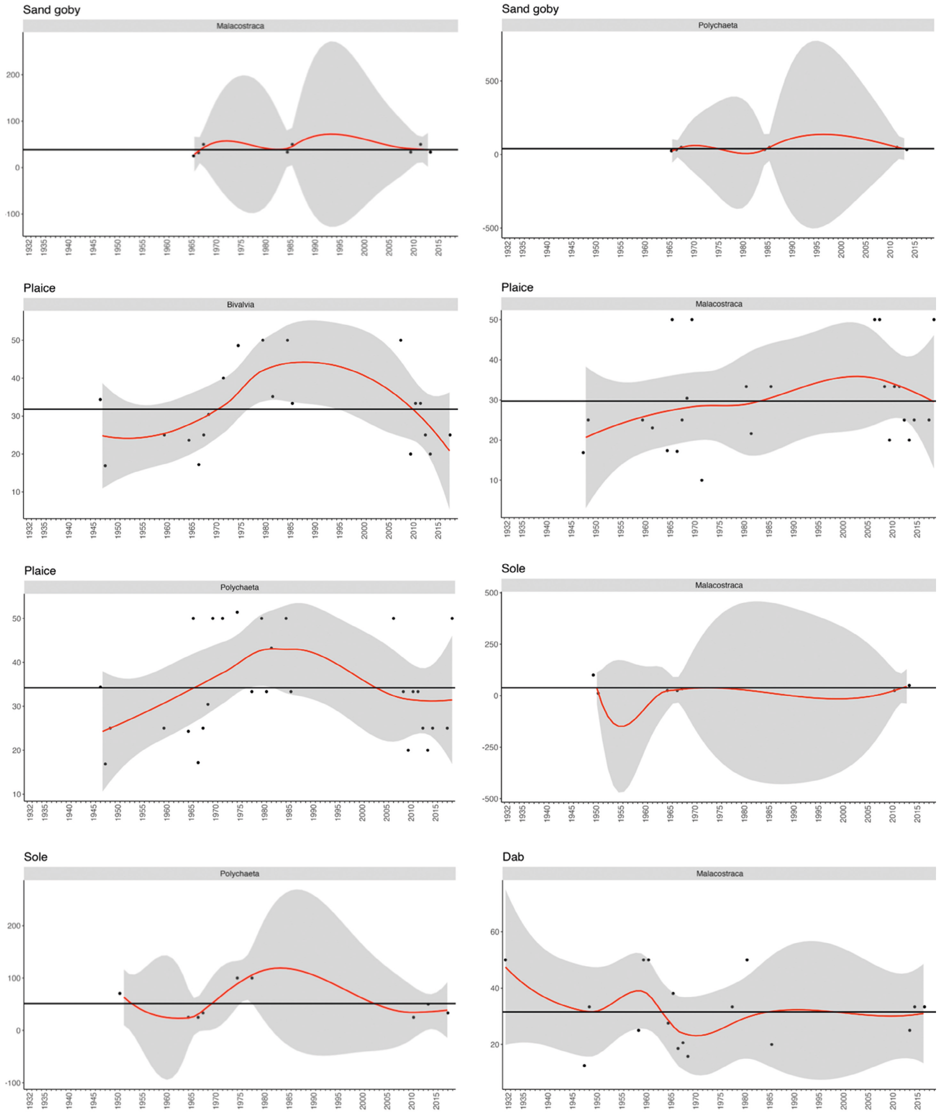

Figure S1C Trends in the occurrence of the main prey classes (%) of the selected benthic species; the (near)-resident species five-bearded rockling *Ciliata mustela*, flounder *Platichthys flesus*, bull-rout *Myoxocephalus scorpius*, viviparous blenny *Zoarces viviparus* and sand goby *Pomatoschistus minutus*; the juvenile marine migrant species plaice *Pleuronectes platessa* and sole *Solea solea* and the marine seasonal visitor species dab *Limanda limanda and* sand-smelt *Atherina presbyter* and thick-lipped grey mullet *Chelon labrosus. (continued)*

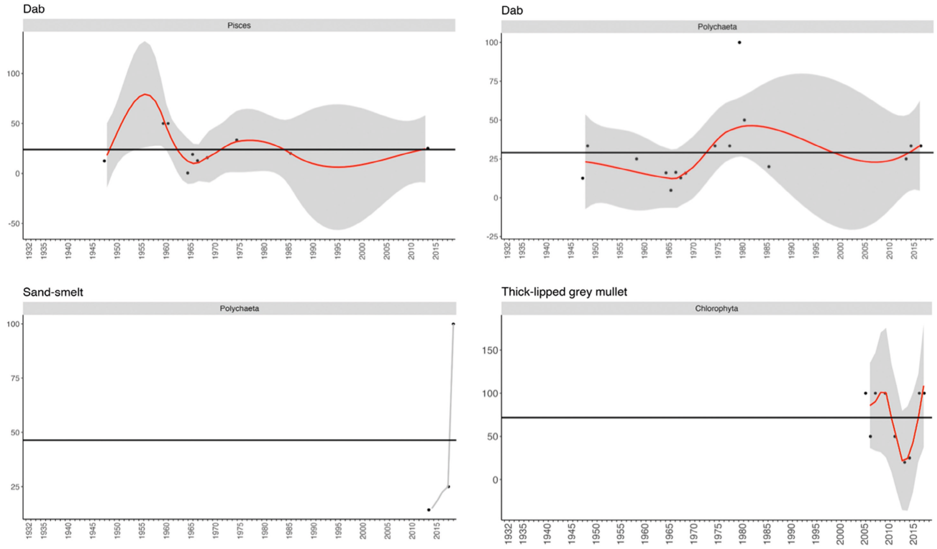

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Table S1 Overview of stomach content information of the NIOZ archive (1932-2019). For each species, total number of stomachs with content per year are listed.

Table S2 Number of prey items found in the stomachs of the Wadden Sea fish over the years 1932 – 2019.

Table S3 Overview of prey items found in the stomachs of the various fish species of the NIOZ archive between 1931-2019, together with trophic position according to FishBase (2022).

Table S4 Mean annual trophic position (TP) of the various fish species of the NIOZ archive between 1931-2019, based on stomach content composition.
Colour code values in 2010 - 2019: 🟦 Below range 🟩 Within range 🟧 Above range
All calculations based on at least 10 observations.

Common name	1947	1948	1949	1950	1960	1962	1965	1966	1967	1968	1981	1985	1986	
Bass														
Bib				🟧 3,8					🟧 3,6					
Bull-rout							🟧 3,7						🟦 3,4	
Cod													🟧 4,0	
Dab		🟦 3,3			🟩 3,7		🟦 3,3	🟩 3,5	🟩 3,6				🟦 3,2	
Dragonet		3,4					3,3	3,2						
Five-bearded rockling														
Flounder					🟩 3,6			🟦 3,1						
Golden grey mullet														
Haddock							3,4							
Herring														
Lesser weever		4,6									4,5			
Lozano's goby									3,4	3,4				
Plaice		🟦 3,1				🟦 2,7	🟦 3,1	🟩 3,2					🟦 3,1	
Pollack														
Poor cod				3,7										
Red gurnard			4,6											
Sand goby									3,3	3,4				
Sand-smelt														
Sandeel							3,4	3,9					3,5	
Scad														
Sea trout														
Sea-snail														
Shore crab														
Smelt														
Sole				3,5			3,2							
Sprat								3,6						
Stickleback														
Thick-lipped grey mullet														
Tub gurnard		4,5							3,6					
Turbot														
Twaite shad														
Viviparous blenny	3,5						3,3						3,2	
Whiting												🟩 3,6		

🟦 Below range		2				1	2	1					3	
🟩 Within range					2			2	1			1		
🟧 Above range				1			1		1				1	

	1996	2005	2006	2007	2008	2009		2010	2011	2012	2013	2014	2015	2017	2018	2019	min	max
		3,9	3,7	3,7	4,1	3,8		3,9	3,7	3,6	3,8	3,6	3,8	3,5	3,9	3,6	3,5	3,9
				3,9		3,5			3,6				3,5	3,6			3,5	3,6
		3,7						3,7	3,5			3,6					3,5	3,7
		3,8		3,6						3,7	3,9						3,7	3,9
												3,8	3,3				3,3	3,8
		3,5	3,7	3,7	3,5			3,6	3,5			3,7	3,6	3,6	3,8		3,5	3,8
	3,5		3,4	3,7	3,6	3,4		3,4	3,5	3,4	3,7	3,4	3,5	3,5	3,6	3,5	3,4	3,7
				2,0		2,0			2,0			2,1	2,4	2,0			2,0	2,4
		3,4	3,7					3,5	3,5	3,8	3,4	3,5	3,6		3,7	3,4	3,4	3,8
						3,5		3,3	3,4			3,5	3,2				3,2	3,5
										3,6							3,6	3,6
												3,5					3,5	3,5
						4,4		4,2			4,4	4,2		4,1	4,3	3,5	3,5	4,4
		4,5	4,4	4,4		4,4		4,5	4,5	4,4	4,5	4,4	4,4				4,4	4,5
		3,5							3,6	3,5							3,5	3,6
												3,9					3,9	3,9
								4,0	3,6	3,8		3,9					3,6	4,0
															3,2		3,2	3,2
										3,2		3,8					3,2	3,8
			2,0		2,0					2,6		2,4					2,4	2,6
									3,8			3,9					3,8	3,9
				4,2		4,3			3,6		4,2	3,8					3,6	4,2
				4,0	4,1					3,6	3,7	3,7	3,4	3,9			3,4	3,9
				1	1	2												
	1	7	5	4	1	5												
				4	2	1												

Table S5 Overview of the relationship between mean occurrence of the various prey items (PO) in the stomachs of selected fish species and year (Year) according to PO ~ s. (Near)-resident: Near-resident or resident species; JMM: juvenile marine migrants; MSV: Marine seasonal visitor. Only years with a minimum of 5 observations are included.

PART IV

General discussion

9

Synthesis

9.1 The present Wadden Sea fish food web structure

The study of the present fish food web structure was based on material collected at a single spot by means of a passive gear (a kom-fyke) in the western Wadden Sea over a period of almost a decade (2010-2018). This kom-fyke is an efficient tool to collect fish material, and it is in use for fish monitoring in the western Wadden Sea from 1960 onwards (van der Veer et al. 2015). It has a leader of 200m running from the beach towards deeper waters with two chambers and a fyke at the end. Fish swimming against the leader are guided towards the two chambers (the so-called 'kom') and from there collected into the fyke. A kom-fyke is collecting both demersal, benthopelagic and pelagic fish with a high daily frequency. However, this type of gear also has its limitations: it is labour intensive; it is restricted to relatively shallow waters; it collects only actively migrating fish species and it collects only individuals larger than the mesh size of the net.

Despite these limitations, most of the Wadden Sea fish species can be collected by means of a kom-fyke. In total 82 fish species were caught over the period 1960 – 2011 (van der Veer et al. 2015). While this study covered only a decade, it still caught 54 different fish species and these species include the most common and abundant fish species recorded in the area (Witte & Zijlstra 1983). However, it cannot be excluded that some fish species or life stages are not fully sampled due to the combination of data collecting at a single spot; in shallow waters; and for only a decade.

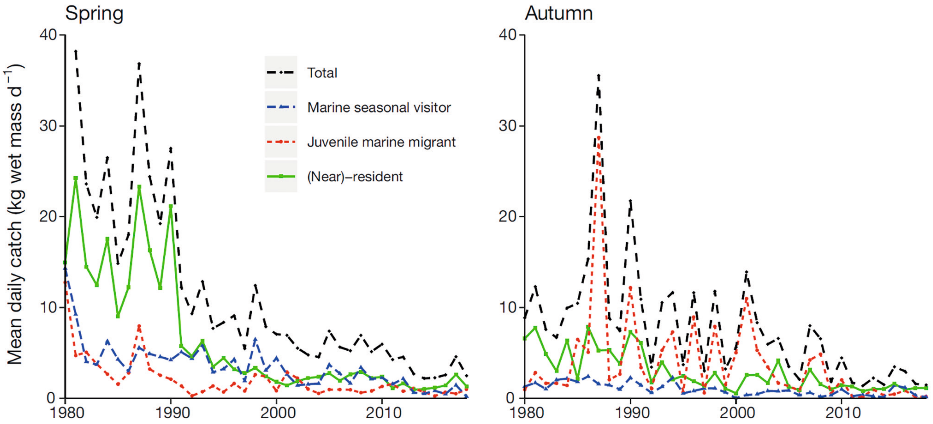

Figure 1 Mean daily fyke catch (kg wet mass d^{1}; total and for different guilds separately) in spring and autumn.

This study was carried out after a period in which monitoring programmes showed a strong decrease in fish biomass in the Wadden Sea from the 1980s to around 2010 (Tulp et al. 2008, van der Veer et al. 2015). The kom-fyke catches showed even a 10-fold decrease in total daily catch biomass of both pelagic and demersal species from 1980 to the present (van der Veer et al. 2015). During the period of this study, there was some fluctuations in the mean total daily catch in the kom-fyke, however without a further decrease in any of the different guilds or functional groups (Fig 1). This means that based on the kom-fyke monitoring, the Wadden Sea fish food web presented in this study (2010-2018) seems to reflect a period of relatively stable fish biomass in the area.

Different approaches were combined for the analysis of the Wadden Sea fish food web. Trophic position (TP) of the various fish species was based directly on the stable nitrogen isotope values ($\delta^{15}N$) relative to baseline of the pelagic and benthic primary production and indirectly calculated from the stomach composition based on fixed trophic positions of the prey items. In addition, stomach content composition was used to identify the various predator-prey interactions. Results can be summarized as follows:

- Stable isotope values of the various fish species showed that they were not different between immigrating fish in spring and emigrating fish in autumn, suggesting a similar trophic niche of the various fish species in the coastal zone and inside the Wadden Sea. This means that the fish food web based on kom-fyke catches near the entrance of the Wadden Sea actually also represent the situation in the coastal zone.
- For all fish species, estimated trophic position based on stomach content differed from TPs based on stable isotopes, whereby estimated TPs based on isotope values were lower than those based on stomach content composition. Stomach content analysis might have missed smaller and fast digestible prey species with low trophic positions. Also, the estimate of trophic position from stable isotope values is based on a fixed similar enrichment with increasing trophic position for all species (van der Zanden et al. 1997, Post 2002), although this trophic fractionation might be species-specific (Vesely et al. 2024).
- Both the estimates based on stomach content and those on stable isotopes, indicate that the present fish food web still covers a range from various trophic levels from algae consumers (TP=2) to top predators (TP around 4).
- Stomach content analysis indicates that the fish fauna comprises of a mix of opportunistic feeders and more specialized fish species. The opportunistic feeders consuming on a variety of prey items and especially a few key prey species are consumed by many fish species and fuel the fish food web: amphipod crustaceans, brown shrimps, juvenile herring and gobies. The role of juvenile herring and gobies is two-fold: they are abundant and important as fish predators but also as prey items for other fish species.
- Due to the pivotal position of a few key prey species in combination with the opportunistic behaviour of most fish species, intra- and interspecific competition cannot be excluded as it is also indicated by the overlap in diet of many fish species of different groups (semi-resident, juvenile marine migrants, seasonal migrants). Food competition nowadays might be lower than in the past educed competition due to the lower fish biomass nowadays (Tulp et al. 2008, van der Veer et al. 2015).

9.2. Spatial variability in Wadden Sea fish food web structure

The comparison of the Wadden Sea fish food web structure simultaneously in different tidal basins indicated some spatial variability. eDNA sampling of the fish community revealed a pattern with on the one hand common (core) species being found everywhere and on the other hand spatial differences with respect to rare (transient) species. Stomach content analysis showed that a small group of key species are fuelling the Wadden Sea fish food web. Copepods and brown shrimp are the most important key species, followed by mysid shrimp, shore crab and herring. However, the importance of mysid shrimp, shore crab and herring showed spatial variability between Marsdiep and Ems.

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). Spatial variability in trophic structure could only be analysed for a few fish species. For these species, estimates of absolute trophic positions based on compound-specific stable isotopes indicated a low spatial variability. Also a comparison of relative trophic positions showed a significant correlation for most fish between different basins, indicating a large spatial similarity in trophic structure.

The few data about the absolute trophic position of some Wadden Sea epibenthic and fish species indicate a range in trophic positions from at least 2 – 4 (Table 1). Bass (*Dicentrarchus labrax*) and viviparous blenny (*Zoarces viviparus*) are top predators in the system with a trophic position of respectively 4.0 and 3.8. The trophic positions of the key prey species have a strong impact on the fish food web. Two of the main key prey species, the shore crab (*Carcinus maenas*) and the brown shrimp (*Crangon crangon*) have already a trophic position of respectively 3.2 and 3.5 (Table 1). This relatively high trophic position reflects their omnivore behaviour. Both species consume a variety of prey. For brown shrimp Mysids and amphipods and copepods together constituted the dominant prey (Oh et al. 2001, Hostens & Mees 2003) and for shore crab *Crangon crangon* and *Hidiste diversicolor* (Beata et al. 2006). Mysids and amphipods, copepods and the brown shrimp are even more important as prey species in the Wadden Sea fish food web than indicated by the stomach content information of the fish species because they are also important food items for the other key prey species, the brown shrimp and the shore crab.

Mysids and amphipods, copepods, brown shrimps and shore crabs are common and abundant species inside the Wadden Sea (Beukema 1976, Roast et al. 1998) and also in the coastal zone (Beyst et al. 2001, Oliveira et al. 2023. Also, in other Wadden Sea tidal basins calanoid copepods, brown shrimps and mysid shrimps have been described as the most abundant prey items for the fish community, such as in the Sylt-Rømø tidal basin in the northern Wadden Sea Kellnreitner et al. 2012). For brown shrimps, clear spatial and temporal variability in abundance has been found both inside the Wadden Sea as well as in the coastal zone (Kuipers & Dapper 1984, Siegel et al. 2005, Campos et al. 2010, Tulp et al. 2012) and similar variability might be assumed for the other key prey species. Such spatial and temporal variability in key prey species abundance due to location specific differences in hydrography and geomorphology, might explain the observed differences in relative importance of the various prey items in the various studies in the Wadden Sea

Overall, there seems to be a large-scale similarity in fish food web structure due to a similarity in the key prey species and in the fish abundant components of the fish community. Some spatial variability occurs due to a combination of differences in local fish community (especially more rare species) and local and interannual differences in predator and prey abundances.

Table 1 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	TP	S.E.
Prey items				
	Carcinus maenas	Shore crab	3.2	0.1
	Crangon crangon	Brown shrimp	3.5	0.1
Predatory fish				
	Solea solea	Sole	3.0	0.1
	Pleuronectes platessa	Plaice	3.2	0.1
	Platichthys flesus	Flounder	3.4	0.1
	Clupea harengus	Herring	3.4	0.9
	Osmerus eperlanus	Smelt	3.8	0.1
	Zoarces viviparus	Viviparous blenny	3.8	0.1
	Dicentrarchus labrax	Bass	4.0	0.1

9.3. Past Wadden Sea fish food web structure

In this study reflections about the past Wadden Sea fish food web are restricted to the last century for two reasons. Firstly, the last extensive habitat losses in the area took place in the western Dutch Wadden Sea in the Marsdiep tidal basin with the exclosure of the Zuiderzee estuary by the Afsluitdijk in 1932 and in the eastern Dutch Wadden Sea in 1964 with the exclosure of the Lauwers (Wolff, 1983). Secondly, most quantitative information is restricted to the last century only.

Until the construction of the Alsluitdijk, there was a profitable commercial fishery in the western Wadden Sea and Zuiderzee on herring (a special spring race the Zuiderzee herring), anchovy, flounder, smelt, eel and brown shrimp (Fig 2, Table 2). This fishery collapsed with the closure of the Zuiderzee and the transformation of the area into a freshwater basin (Lake IJssel). It was hoped that the Zuiderzee herring and the anchovy would shift their spawning grounds towards the Waddenzee, but this was not the case and both species disappeared almost completely from the Wadden Sea. Nowadays, herring in the western Wadden Sea belongs to another race (Wolff 2000, Lotze 2005). With the construction of the fish migration river through the Afsluitdijk, a direct connection between the estuarine Wadden Sea and the freshwater Lake IJssel will be restored, however, at a much smaller scale than the semi-open barrier in the Easter Scheldt. It is unlikely that this will result in the situation before the closure with for instance a reappearance of the Zuiderzee herring.

The lack of quantitative information from before the closure of the Alsluitdijk means that quantitative reflections about the Wadden Sea fish food web can only focus on roughly the last century. The low fishing pressure and low level of pollution (nutrients, chemicals) during the second world war due to restricted activities would plea for a baseline around 1945.

Figure 2 Archive pictures of commercial fisheries around 1930-1940 along the coastline of the Marsdiep in the western Wadden Sea with beach seine for herring (top picture) and fish in general by kom-fyke (bottom picture). (Pictures from collection of Maarten Stoepker and Anelfo archive).

Insight in and conclusions about the past Wadden Sea fish food web is based on two quantitative studies, both based on estimates of trophic position based on stomach content information from the NIOZ archive:

- An analysis of the former trophic position of sharks and skates, species that were present in the coastal zone of the Netherlands and have disappeared nowadays;
- An analysis of the past trophic position of various fish species that nowadays are still present in the area.

Table 2 Quantity (1000 kg) and total value (1000 Dutch guilders) of commercial fish caught in the former Zuiderzee (Lake IJssel) in the years before and after the enclosure by the Afsluitdijk. After Redeke (1922, 1941).

Year	Herring	Anchovy	Flounder	Eel	Smelt	Garfish	Shrimps	Total value
1925	8987	1384	1067	756	1478	6	1910	3612
1926	6263	3326	709	714	802	23	1588	3499
1927	9643	882	711	602	711	33	2541	2537
1928	12387	139	1008	694	1273	22	2117	2507
1929	10621	397	1686	858	752	4	2048	2629
1930	11246	5428	3647	838	1285	2	2313	4808
1931	12106	3578	2321	941	1349	3	1588	3094
1932	9985	378	1273	1048	476	9	589	1788
1933	12		1265	2125	337		50	1470
1934			1124	2688	447			1662
1935	0.1		232	1970	317			1068
1936			48	2405	271			1091
1937			43	3595	130			1315
1938			25	2588	209			1285

The fact that the Wadden Sea fish community has lost most of its shark and skate species means that the fish food web structure must have been more complex in the past. The various shark and skate species belonged to different functional groups: (near)-residents, juvenile marine migrants and marine seasonal visitors. The various functional groups had different diet preferences: the (near-)resident and juvenile marine migrant species were demersal while the marine seasonal visitors species were pelagic. Most of the species were generalist predators. Not only their diet showed overlap for especially fish and crustaceans, but also their diet indicated competition with other fish species for at least fish and crustaceans. Estimates of their trophic position indicated that it ranged from 3.2 for thornback ray to even 4.6 for the tope shark. It is clear that with the disappearance of shark and skate species, the Wadden Sea food web has also lost some of its top predators.

Although the NIOZ archive contained 7031 stomach records of 43 species over the period 1932-1979, records were not evenly distributed but clustered both with respect to years as well as to fish species. Only for 18 fish species, stomach content could be analysed and trophic position could be estimated, whereby the period 2010-2018 is used as the reference period. The stomach content analysis showed a variability in prey occurrence in the stomachs of different fish species over time, but without a significant trend. Even the present fish food web shows fluctuations in relative mean prey occurrence in the order of 10- 20% (Fig 3). Part of the variability will be caused by fluctuations in prey abundance. In some period, larger differences in relative mean prey occurrence occur. It is unclear whether this is caused by differences in predator-prey interactions or whether it reflects a bias due to the fact that it is based on information of only a relatively small set of fish species. The estimates of trophic position showed a variability in the order of 1 unit or even more for all species both within and between years. Surprisingly, no significant trend in mean trophic position over time could be found in any of the 18 species, despite the serious anthropogenic stress (pollution, eutrophication events, climate change) and the decrease in fish abundance in the area during the last 50 years. The present study does not indicate any changes in trophic position of individual common species in the western Dutch Wadden Sea over the last 80 years. However, this conclusion is preliminary and more independent data are required.

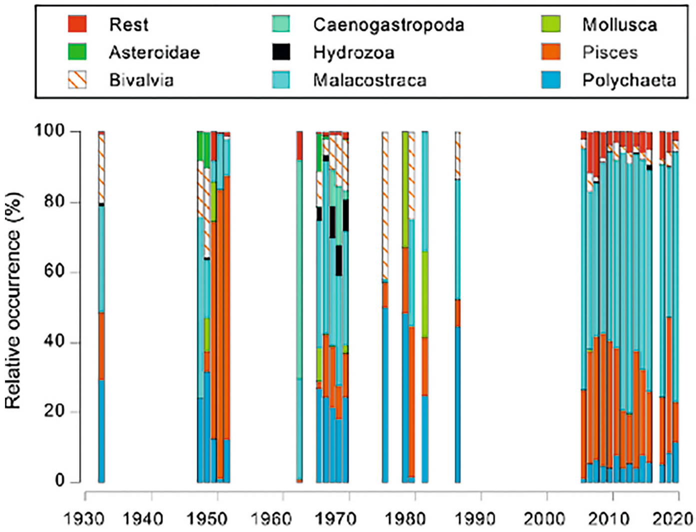

Figure 3 Relative mean occurrence (%) of the most abundant prey classes in the stomachs of the Wadden Sea fish species within the NIOZ archive (1932-2009). Only years with at least 50 observations are listed.

In conclusion, the Wadden Sea fish fauna has undergone changes compared with the present situation, especially with respect to fish abundance, fish species composition and fish food web. The various predator-prey relationships show interannual variability, without presently any indications of significant temporal changes. Fish abundance has strongly decreased even from 1945 to present. After 1945 there was commercial fishing with passive gears along the coastline of the Wadden Sea, until this was no longer profitable in the 1960s. Regulating mechanisms (competition for food, density-dependent growth, mortality) might have been (more) common in the past. Fish species composition has changed over time especially due to the disappearance of most skate and shark species. The disappearance of these species has removed this part of the fish food web. For the other Wadden Sea fish species, a few key prey species are dominant as food. At a large scale, copepods and brown shrimp are most important, followed by mysid shrimp, shore crab and herring. Surprisingly, herring is still a dominant food source despite the disappearance of the Zuiderzee herring after 1932. Predator-prey relationships and hence trophic position show some interannual variability, most likely due to fluctuations in prey and predator abundance. Presently there are no indications of any systematic changes over time. The lower fish biomass in the area has reduced the trophic role of the fish community at the ecosystem. The reduction of trophic structure has also been observed in other coastal areas around the North Sea (Thurstan et al. 2010, McHugh et al. 2011, Frelat et al. 2022, Moore et al. 2023). This might point to general mechanisms in all locations behind these changes.

Human disturbances are expected to have less of an effect on systems with more biological and ecological interactions, than systems which are more stand-alone (Jordán et al. 2024). Consequently, the structure of marine fish food webs are then expected to be able to withstand more disturbances by anthropogenic origin (Albouy et al. 2019). Therefore, in contrast to open-water communities, coastal food webs with more variable temperatures have greater interaction redundancy (e.g. species fulfilling similar roles in an ecosystem, can respectively increase and stabilize processes in ecosystems), which may benefit robustness and decrease the chance a species getting extinct. Our results suggest that coastal marine ecosystems and its fish communities, have more ecological connections (in terms of complexity) and therefore are more resistance to changes and environmental fluctuations (Albouy et al. 2019).

When looking at a larger spatial scale (global), biodiversity patterns and food-web dynamics are not directly scalable to each. In order to conserve species diversity as well as the functional trophic integrity of communities, patterns of biodiversity and food-web characteristics over a large spatial scale must be jointly studied (Blackman et al. 2022). Therefore, studying the whole fish food web both in the present and the past and over a larger spatial scale, helps to understand the importance of knowledge of every species living in a fragile environment such as the Wadden Sea. By answering these questions, a greater understanding of how and why the fish populations in the Wadden Sea changed over time and on a spatial scale will be achieved. Making it possible to predict further changes in either food web structures or shifts in populations. Which in turn might alter the function of the Wadden Sea as a commercially fishing area or even as nursery ground for young fish. Results found in this thesis should thus strengthen the importance of international negotiations in to conserve and use sustainably marine biological resources of areas beyond national borders.

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Summary

Different approaches were combined for the analysis of the Wadden Sea fish food web. Trophic position (TP) of the various fish species was based directly on the stable nitrogen isotope values ($\delta^{15}N$) relative to baseline of the pelagic and benthic primary production and indirectly calculated from the stomach composition based on fixed trophic positions of the prey items. Spatial analyses were performed by means of both stable isotope values and stomach content analyses from two far away locations in the Wadden Sea as well as environmental DNA.

This study focusses on spatial and temporal variability in the Wadden Sea fish food web, whereby the aim is threefold:

[1] a detailed analysis of the present fish food web to fill in the gab of knowledge for the western Dutch Wadden Sea;
[2] an analysis of the spatial variability in the Wadden Sea fish food web;
[3] an analysis of the temporal variability in the Wadden Sea fish food web, focussing on the last century.

The present Wadden Sea fish food web (ad 1) and spatial variability in fish food web (ad 2) will be based on both stomach content and stable isotope analysis analyses for the Marsdiep basin in the western Wadden Sea and the Ems basin in the eastern Wadden Sea as well as environmental DNA for a larger consecutive spatial scale. Temporal variability in the Wadden Sea fish food (ad 3) will focus on the western Wadden Sea and will be based on the NIOZ archive of historical information of stomach content data of Wadden Sea fish species dating back to 1930.

Part I: Western Wadden Sea fish food web structure

In **chapter 3** the food web structure of a coastal fish community (western Dutch Wadden Sea) was studied based on stomach content data from samples collected between 2010 and 2018. Results showed the pivotal position of a few key prey species (amphipod crustaceans, brown shrimps, juvenile herring and gobies) for the coastal Wadden Sea fishes and that the substantial prey overlap in the diet of some predators cannot exclude intra- and inter-specific competition among these predators.

Chapter 4 investigates the trophic structure of the western Wadden Sea fish community by means of stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of 1658 samples from 57 fish species collected between 2012 and 2016. Results of this study showed that the estimated trophic positions based on isotope values were lower than those based on stomach content composition, 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).

Part II: Spatial variability in Wadden Sea fish food web structure

Chapter 5 investigates the spatial variability in the fish community composition by means of monthly sampling of environmental (e)-DNA concentrations at eight stations spread out over the Dutch Wadden Sea from west to east. Results illustrated spatial variability in fish community in the Dutch Wadden Sea with common (core) species being present at all locations and differences between locations with respect to rare (transient) species, most probably due to location specific differences in hydrography and geomorphology.

In **chapter 6** the spatial variability in food web structure for the Wadden Sea fish community was investigated by simultaneously analysing stomach content and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in the Marsdiep and Ems basin in the Dutch Wadden Sea. 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. 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.

Part III: Past Wadden Sea fish food web structure

Chapter 7 focusses on the historical trophic ecology of some divergent shark and skate species in the Dutch coastal North Sea zone. In this study historical dietary data of four species of sharks and skates being in the past (near)-residents, juvenile marine migrants and marine seasonal visitors of the Dutch coastal North Sea zone, were analysed for the period 1946 - 1954. Based on stomach content composition, the trophic position of four of the various shark and skate species could be reconstructed. The analysis indicates that most of the shark and skate species were generalist predators. The calculated trophic positions of shark and skate species indicate that those species were not necessarily at the top of the marine ecosystem food web, but they might have been the top predators of their particular ecological assemblage.

Chapter 8 investigates the temporal variability in fish food web structure of the western Wadden Sea over almost the last century. Information about stomach content composition over the period 1930 – 2019 was analysed to reconstruct long-term trends in trophic position of individual species. In 18 species analysed, no significant trend in mean trophic position over time could be found, despite the serious anthropogenic stress (pollution, eutrophication events, climate change) and the decrease in fish abundance in the area during the last 50 years. The present study does not indicate any changes in trophic position of individual species in the western Dutch Wadden Sea over the last 80 years.

Fish food web structure of the Wadden Sea

The work presented in this thesis provides us with a better understanding of the spatial distribution of several fish species in the Wadden Sea. In **chapter 9** some highlights and results are discussed and put in a broader context. Therefore, studying the whole fish food web both in the present and the past and over a larger spatial scale, helps to understand the importance of knowledge of every species living in a fragile environment such as the Wadden Sea. By answering these questions, a greater understanding of how and why the fish populations in the Wadden Sea changed over time and on a spatial scale will be achieved. Making it possible to predict further changes in either food web structures or shifts in populations. Which in turn might alter the function of the Wadden Sea as a commercially fishing area or even as nursery ground for young fish. Our results should thus strengthen the importance of international negotiations in to conserve and use sustainably marine biological resources of areas beyond national borders.

Summary

Samenvatting

Deze studie werd uitgevoerd na een periode waarin monitoringprogramma's een sterke afname van de visbiomassa in de Waddenzee lieten zien van de jaren 1980 tot en met 2010. De kom-fuik vangsten lieten zelfs een 10-voudige afname zien in de totale dagelijkse vangstbiomassa van zowel pelagische als demersale soorten van 1980 tot heden. Gedurende de periode van deze studie waren er enkele schommelingen in de gemiddelde totale dagelijkse vangst in de kom-fuik, maar zonder een verdere afname in een van de verschillende guilds of functionele groepen. Dit betekent dat op basis van de kom-fuikmonitoringprogramma het visvoedselweb van de Waddenzee dat in deze studie (2010-2018) wordt gepresenteerd, een periode van relatief stabiele visbiomassa in het gebied lijkt weer te geven.

Verschillende benaderingen werden gecombineerd voor de analyse van het visvoedselweb van de Waddenzee. De trofische positie (TP) van de verschillende vissoorten werd gebaseerd op de stabiele stikstofisotopenwaarden ($\delta^{15}N$) ten opzichte van de zogenaamde 'baseline' van de pelagische en benthische primaire producenten en indirect berekend op basis van de maagsamenstelling en de trofische posities van de prooien. Daarnaast werd de samenstelling van de maaginhoud gebruikt om de verschillende predator-prooi interacties te identificeren. Deze studie richt zich op de ruimtelijke en temporele variabiliteit in het visvoedselweb van de Waddenzee, waarbij het doel drieledig is:

[1] een gedetailleerde analyse van het huidige voedselweb van de vissen om de missende kennis over de West-Nederlandse Waddenzee op te vullen;
[2] een analyse van de ruimtelijke variabiliteit in het voedselweb van de Waddenzee;
[3] een analyse van de temporele variabiliteit in het voedselweb van de Waddenzee, met de focus op de afgelopen eeuw.

Het huidige visvoedselweb van de Waddenzee (ad 1) en de ruimtelijke variabiliteit in het visvoedselweb (ad 2) zullen gebaseerd zijn op zowel maaginhoud- als stabiele isotopenanalyse voor het Marsdiepbekken in de westelijke Waddenzee en het Eemsbekken in de oostelijke Waddenzee. Tijdelijke variabiliteit in het visvoedsel van de Waddenzee (ad 3) zal zich richten op de westelijke Waddenzee en zal gebaseerd zijn op het NIOZ-archief met historische informatie over maaginhoudgegevens van vissoorten in de Waddenzee die teruggaan tot 1930.

Part I: Western Wadden Sea fish food web structure

In **hoofdstuk 3** werd de structuur van het voedselweb van een kustvisgemeenschap (de westelijke Nederlandse Waddenzee) bestudeerd op basis van maaginhoudgegevens van samples die tussen 2010 en 2018 werden verzameld. Resultaten toonden de centrale positie van een paar belangrijke prooisoorten (vlokreeftachtigen, bruine garnalen, jonge haring en grondels) voor de kustvissen van de Waddenzee. Daarnaast kon de aanzienlijke prooioverlap in het dieet van sommige vissen intra- en interspecifieke concurrentie tussen deze roofdieren niet uitsluiten.

Hoofdstuk 4 onderzoekt de trofische structuur van de visgemeenschap van de westelijke Waddenzee door middel van stabiele isotopenanalyse ($\delta^{13}C$ en $\delta^{15}N$) van 1658 samples van 57 vissoorten verzameld tussen 2012 en 2016. De trofische nicheruimte van benthopelagische soorten was het kleinst en overlapt met die van de pelagische en benthische soorten. Wat betreft het gebruik van het gebied, was de trofische nicheruimte kleiner voor jonge mariene migrerende soorten (kraamkamer soorten) en overlapte deze met die van de (near)resident soorten en mariene seizoensbezoekers. Potentieel is de trofische competitie het hoogst voor de functionele groep van benthopelagische soorten en de guild van jonge mariene migrerende soorten (kraamkamer soorten).

Part II: Spatial variability in Wadden Sea fish food web structure

Hoofdstuk 5 onderzoekt de ruimtelijke variabiliteit in de samenstelling van de visgemeenschap door middel van maandelijkse bemonstering van (e)-DNA-concentraties op acht stations verspreid over de Nederlandse Waddenzee van west naar oost. Gedurende een jaar werden op alle locaties twintig soorten geïdentificeerd. De resultaten uit deze studie illustreerden de ruimtelijke variabiliteit in de visgemeenschap in de Nederlandse Waddenzee, waarbij algemene (kern)soorten op alle locaties aanwezig waren en er verschillen waren tussen locaties met betrekking tot zeldzame (tijdelijk aanwezige) soorten, hoogstwaarschijnlijk vanwege locatie specifieke verschillen in hydrografie en geomorfologie.

In **hoofdstuk 6** werd de ruimtelijke variabiliteit in de structuur van het voedselweb voor de visgemeenschap in de Waddenzee onderzocht door gelijktijdig de maaginhoud en stabiele bulk isotopen ($\delta^{13}C$ en $\delta^{15}N$) te analyseren in het Marsdiep en het Eemsbekken in de Nederlandse Waddenzee. De waargenomen ruimtelijke variabiliteit in prooivoorkeuren was hoogstwaarschijnlijk het resultaat van lokale verschillen in predator- en prooiovervloed. Hoewel schattingen op basis van bulkstabiele isotopen de absolute trofische niveaus in beide bekkens onderschatten, kunnen ze wel worden gebruikt voor de analyse van relatieve trofische posities van vissoorten. Relatieve trofische posities toonden een significante correlatie voor de meeste vissen tussen het Eemsbekken en het Marsdiep-bekken, wat ook duidt op een grote ruimtelijke gelijkenis in trofische structuur.

Part III: Past Wadden Sea fish food web structure

Hoofdstuk 7 richt zich op de historische trofische ecologie van enkele uiteenlopende haaien- en roggensoorten in de Nederlandse kustzone van de Noordzee. In deze studie werden historische dieetgegevens van vier soorten haaien en roggen geanalyseerd van de Nederlandse kustzone van de Noordzee geanalyseerd voor de periode 1946 –

1954. De resultaten laten zien dat de meeste haaien- en roggensoorten generalistische predatoren waren. De berekende trofische posities van haaien- en roggensoorten geven aan dat die soorten niet noodzakelijkerwijs bovenaan het voedselweb van het mariene ecosysteem stonden, maar dat ze mogelijk de toproofdieren van hun specifieke ecologische niche waren.

Hoofdstuk 8 onderzoekt de temporele variabiliteit in de structuur van het voedselweb van vissen in de westelijke Waddenzee gedurende bijna de afgelopen eeuw. Informatie over de samenstelling van de maaginhoud gedurende de periode 1930-2019 werd geanalyseerd om langetermijntrends in de trofische positie van individuele soorten te reconstrueren. Bij alle 18 soorten kon geen significante trend in gemiddelde trofische positie in de loop van de tijd worden gevonden, ondanks de antropogene stress (vervuiling, eutrofiëring, klimaatverandering) en de afname van de visovervloed in het gebied gedurende de laatste 50 jaar. De huidige studie geeft geen veranderingen aan in de trofische positie van individuele soorten in de westelijke Nederlandse Waddenzee gedurende de laatste 80 jaar. Op gemeenschapsniveau varieert de trofische structuur als gevolg van interannuele schommelingen in de soortensamenstelling en jaarlijkse schommelingen in de relatieve overvloed van de verschillende vissoorten. Op ecosysteemniveau is de trofische rol van de visgemeenschap gedegradeerd als gevolg van de afname van de totale visbiomassa in het gebied.

Fish food web structure of the Wadden Sea

Het werk dat in dit proefschrift wordt gepresenteerd, geeft ons een beter begrip van de ruimtelijke verspreiding van verschillende vissoorten in de Waddenzee. In **hoofdstuk 9** worden enkele hoogtepunten en resultaten besproken en in een bredere context geplaatst. Het bestuderen van het hele visvoedselweb, zowel in het heden als in het verleden en op een grotere ruimtelijke schaal, helpt om het belang van kennis van elke soort die leeft in een kwetsbare omgeving zoals de Waddenzee te begrijpen. Door de verschillenden vragen te beantwoorden, zal er meer kennis en begrip ontstaan over het hoe en waarom de vispopulaties in de Waddenzee op een temporale en spatiale schaal zijn veranderd. Hierdoor wordt het mogelijk om verdere veranderingen in voedselwebstructuren of verschuivingen in populaties te voorspellen. Wat op zijn beurt de functie van de Waddenzee als commercieel visgebied of zelfs als kraamkamer voor jonge vissen zou kunnen veranderen. Onze resultaten moeten dus het belang van internationale onderhandelingen over het behoud en het duurzame gebruik van mariene biologische hulpbronnen in gebieden buiten de landsgrenzen benadrukken.

Acknowledgement/Dankwoord

Na vier intensieve jaren en bijna drie jaren waarin ik naast mijn promotie nog andere zaken had lopen, heb ik dan eindelijk het punt bereikt dat ik mag zeggen dat het af is! Na al die jaren heb ik kennis opgebouwd, eigenschappen ontwikkeld en veel nieuwe mensen leren kennen die mij als wetenschapper en persoon hebben doen groeien.

Mijn masterstudie in Amsterdam heb ik in 2014 afgerond, waarna ik direct een baan aangeboden kreeg als kwaliteitsmanager bij Vreugdenhil Dairy Foods in Gorinchem. Een erg leuke en leerzame baan, waar ik als jong broekie het echte werkende leven heb mogen ervaren en uiteindelijk leidinggaf aan grote projecten. In mijn gedachtes wist ik dat ik op een gegeven moment, wanneer er een mogelijkheid langs zou komen, ik weer terug de wetenschap in wilde. In 2016 kwam er een vacature uit bij het NIOZ op Texel voor research assistant met een focus op vispopulaties van de Waddenzee. Dit zag ik als de uitgelezen kans om (via een omweg weliswaar) weer terug de wetenschap in te gaan en uiteindelijk een PhD te gaan doen. Gelukkig werd ik aangenomen en vertrok ik naar Texel om aan een nieuw avontuur te beginnen! Na een aantal maanden als research assistant gewerkt te hebben verscheen er een vacature voor de positie van PhD onder Henk van der Veer met als onderwerp “vispopulaties in de Waddenzee en Noordzee”. Voor deze functie werd ik aangenomen en zo begon mijn wetenschappelijke carrière iets eerder dan ik had gepland.

Mijn tijd als PhD’er heb ik als zeer uitdagend en interessant ervaren en ik heb het gehele traject met veel plezier doorlopen. De mensen op het NIOZ die mij dag en nacht hebben gesteund, ben ik nog steeds dankbaar. Henk, Hans, Marco, Sieme en ik als ‘harde fuik kern’ hebben een mooie tijd beleefd op en rond de fuik en in het snijlab! De dagen op de oude vlet waarbij we de fuik gingen legen, waren dagen die ik me als de dag van gisteren kan herinneren. Ik dacht: “dit is gewoon mijn kantoor”…hoe mooi kan het leven zijn! Uiteraard denk je dat niet meer als het koud is, regent en enorm hard waait, maar goed, die dagen horen er ook bij.

Henk als dagelijkse begeleider en mentor, kan ik wel zeggen dat we er samen iets moois van hebben gemaakt. In het begin van mijn PhD kon ik mij niet voorstellen hoe het zonder jou zou zijn. Echter, na een paar jaar had ik wel eens de gedachte “wat fijn dat hij er een dagje niet is?”. Nee, zonder dollen; mede dankzij Henk kan ik wel zeggen dat ik mijn PhD heb gehaald. Jouw enthousiasme hebben mij ook de kracht gegeven om door te gaan.

Hans, wat hebben wij veel meegemaakt samen. Tot uren samen brainstormen op kantoor naar uren op het snijlab, waarbij we de magen van talloze vissen hebben bekeken. In de laatste jaren (en ook tijdens de grote lock-down vanwege de coronacrisis in 2020), hebben wij samen op kantoor en virtueel ongeveer 15000 otolieten van botten, tongen en voornamelijk schollen geteld. Ik moet eerlijk bekennen dat mijn dromen op een gegeven moment gedomineerd werden door de dagringen van otolieten. Tenslotte wil ik zeggen dat in al die jaren dat we kantoorgenoten waren, Hans voor mij een grote steun en toeverlaat is geweest.

Marco en Sieme, wat hebben wij veel gelachen en veel avonturen beleefd met z'n drieën. Door weer en wind en op de mooie dagen hebben we samen genoten van alles wat de Waddenzee te bieden heeft. Eerst op het water, vervolgens het uitzoeken en tellen van allerlei soorten vissen, waarna we ons te goed deden aan een welverdiend kopje koffie met een prachtig uitzicht op de haven en de Waddenzee. Onze gesprekken en vriendschap hebben mij als persoon gevormd en er mede voor gezorgd dat mijn (soms) eenzame dagen op de Potvis niet zo eenzaam meer waren. Dank jullie wel!

Naast de mensen die ik tijdens mijn PhD heb ontmoet, zijn er natuurlijk ook mensen geweest die mij achter de schermen altijd gesteund hebben. De vier personen die mij van jongs af aan hebben gesteund en gestimuleerd in het bereiken van mijn doel zijn uiteraard mijn ouders en boer en zus. Pap en mam en Martin en Frederike, zonder jullie was ik nooit zo ver gekomen. Door jullie hulp en steun heb ik kunnen verwezenlijken wat ik altijd voor ogen had. We waren er voor elkaar in de mooie en minder mooie momenten, maar uiteindelijk hebben we het gered! Ik wil jullie bedanken voor alles wat jullie voor mij hebben gedaan.

Dankzij mijn vriendenkring van de laatste jaren in Kudelstaart, waarbij ik vooral denk aan Andreas en Esther, heb ik ontspanning en stimulans ervaren die ik nodig had. Jullie interesses en goede gesprekken hebben mij net dat steuntje in de rug gegeven.

Als laatste wil ik de persoon bedanken die mijn leven in de laatste fase van mijn PhD verrijkt heeft. Jan-Willem, jij hebt mijn leven zin gegeven op een manier waarvan ik dacht dat het niet bestond. Jij bent de meest welkome aanvulling op mijn leven en jij hebt mij de tijd gegeven en het geduld gehad om mijn doel te bereiken. Ik wil je bedanken voor ons prachtige gezinnetje dat we nu hebben met de twee meiden en onze twee zonen: Nickolay en Michaël.

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