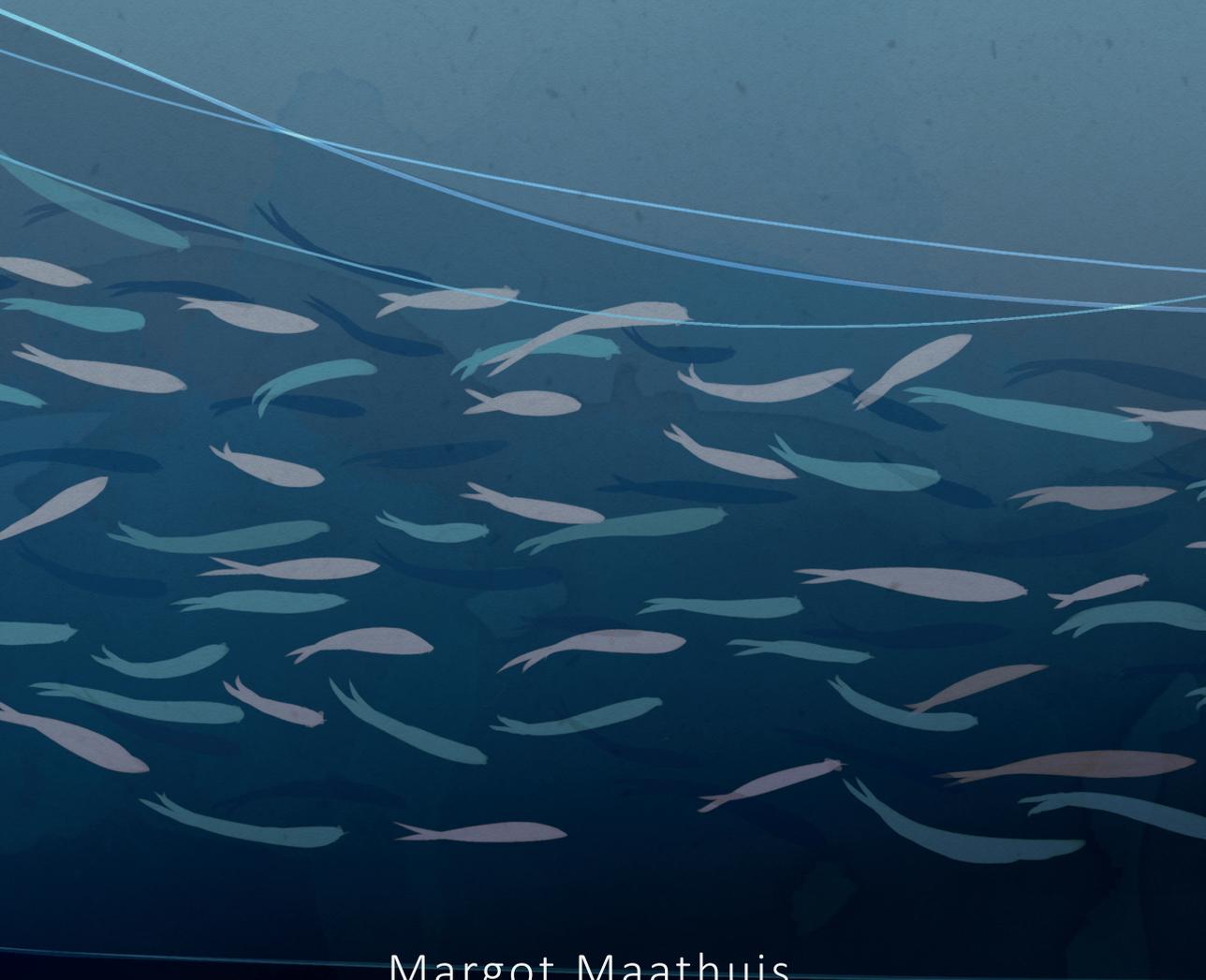


PELAGIC FISH IN SHALLOW WATERS

The ecology of small pelagic fish
in the Dutch Wadden Sea



Margot Maathuis

Propositions

1. Treating small herring and sprat as "*clupeids*" is a generalization that overlooks differences in their ecological roles.
(this thesis)
2. A key role of small pelagic fish in shallow, dynamic ecosystems is their contribution to energy transfer between benthic and pelagic habitats.
(this thesis)
3. CO₂ emissions should be considered in study design.
4. The focus on concrete deliverables in project proposals leads to the omission of necessary pilot studies.
5. Marine conservation areas are currently insufficiently protected against human use.
6. Real progress towards sustainable travel requires international government regulations over sole reliance on consumer actions.

Propositions belonging to the thesis, entitled

Pelagic fish in shallow waters

The ecology of small pelagic fish in the Dutch Wadden Sea

Margot A.M. Maathuis

Wageningen, 17 April 2025

Pelagic fish in shallow waters

The ecology of small pelagic fish in the Dutch Wadden Sea

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Pelagic fish in shallow waters

The ecology of small pelagic fish in the Dutch Wadden Sea

Margot A.M. Maathuis

Thesis

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Chapter 1

General Introduction

1.1 Pelagic fish in shallow waters?

The open ocean, with its clear blue depths ('the *pelagic zone*') and occasional large schools of fish driven by predators like dolphins, tuna, and sharks, is the familiar image of small pelagic fish depicted by documentaries. However, as we move closer to the coast, often shaped by dynamic tidal forces, the waters become shallower and murkier. Despite their name, small *pelagic* fish (SPF) also inhabit these shallow coastal areas, which play a crucial role in their life cycle. Paradoxically, even though such coastal zones are closer to land than the deep ocean, studying small pelagic fish here proves to be equally, if not more, challenging. Which often leaves coastal areas under-investigated for SPF (Brehmer et al., 2006; David et al., 2022).

The open ocean and coastal zones are interconnected, with many marine fish species migrating back and forth to complete their life cycles, using coastal areas as feeding grounds, nursery areas, spawning sites, or migration corridors (Figure 1). Coastal regions support large numbers of fish that take advantage of these beneficial habitats that provide food, shelter and benign temperatures (Beck et al., 2001; Elliott & Hemmingway, 2002). For instance, in the north-east Atlantic, 44% of commercially exploited fish species use coastal habitats during at least one life stage (Seitz et al., 2014). The use of coastal habitat during various life stages can be classified into ecological guilds, such as resident, diadromous (migrants), marine juvenile, marine seasonal, and marine adventitious fish (Elliott & Dewailly, 1995). Residents spend their entire lives in coastal waters, relying on these areas for all life stages. In contrast, diadromous fish use coastal areas mainly as migratory route. Marine juveniles inhabit coastal areas to develop and grow. Marine seasonal fish visit coastal areas during specific times of the year for activities like feeding or spawning. Lastly, marine adventitious species occasionally appear in coastal waters but do not rely on them for any specific life stage.

The life history of a species is shaped by trade-offs between growth, reproduction, and survival (Pecuchet et al., 2017), and throughout their life cycle fish rely on different habitats to optimize conditions for these processes and to maximize fitness. This strategy is well explained by Harden Jones' migration triangle, which emphasizes the interconnected use of distinct habitats for feeding, spawning, and nursery functions (Harden Jones, 1968). According to this concept, fish migrate between these habitats to optimize energy intake during feeding, ensure safe and suitable conditions for reproduction, and provide their offspring with environments that maximize early life stage survival (Harden Jones, 1968). Eggs and early larval fish are primarily transported passively by ocean currents, while older life stages may actively migrate through swimming or selective tidal transport, using tidal currents to navigate between habitats (Gibson, 2003; Bolle et al., 2009).

While coastal areas are widely recognized for their high ecological value, they are also economically valuable and often densely populated, making them particularly vulnerable to anthropogenic stressors. Stressors include coastal development, pollutants, eutrophication, anoxia, invasive species, and (indirect) impacts of fishing (Brown et al., 2018). An assessment of 12 coastal areas worldwide, from the onset of human settlement to present, reveals that human activities have significantly altered coastal ecosystems, which has resulted in severe depletion of species abundance and degradation of habitats (Lotze et al., 2006; Nichols et al., 2019). Especially ecosystems with prolonged human impact exhibit significant degradation, with the Adriatic, Wadden, and Baltic Seas being among the most affected (Lotze et al., 2006).

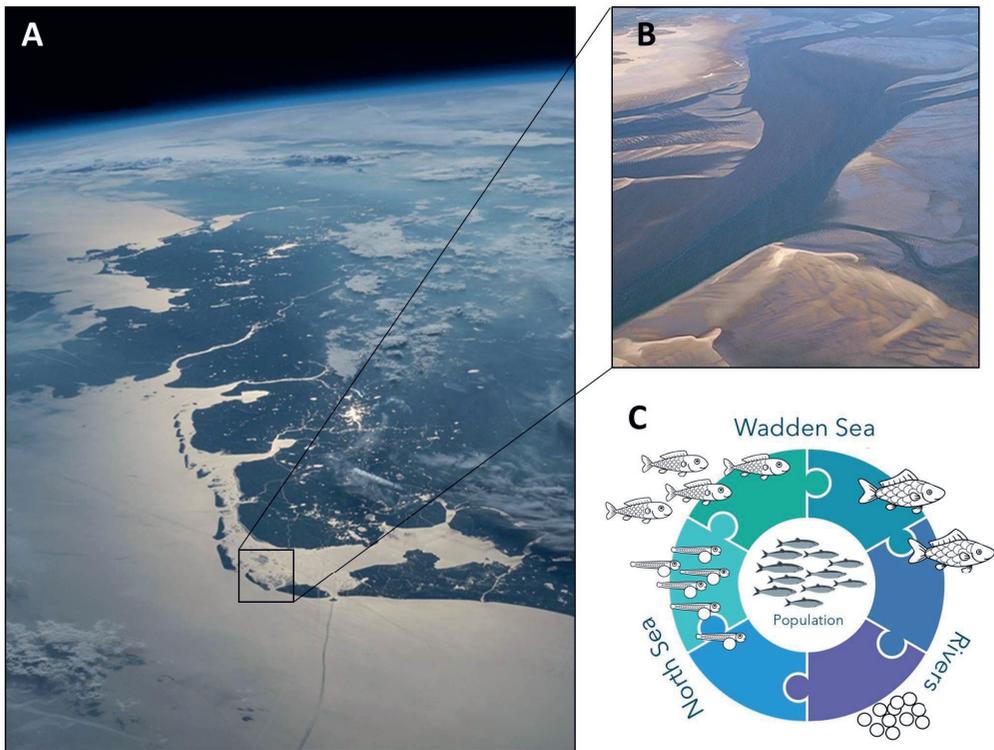


Figure 1. A) Image from the International Space Station by NASA astronaut Jeff Williams, showing part of Europe and illustrating the interconnectedness of water masses (source: NASA). B) Fish migrating between offshore and coastal waters experience changing conditions, such as shallower water near the coast, as shown in this aerial view of the Wadden Sea and its tidal flats (source: Foto Fitis). C) Schematic of a fish's life cycle, emphasizing the need for multiple habitats.

1.2 Wadden Sea

The Wadden Sea, the world's largest unbroken system of gullies and intertidal flats, stretches along the coasts of the Netherlands, Germany, and Denmark. Spanning approximately 500 km of coastline, it is separated from the North Sea by a row of barrier islands and sandbanks, with deep tidal channels providing the connection between the two. Within the Wadden Sea, these channels branch into numerous gullies and creeks, with two-thirds of the sea floor exposed at low tide. It is designated as a UNESCO World Heritage site, Natura 2000 site, and Ramsar site of international importance. The area is renowned for its significance to birds, especially wading species, serving as a critical stopover for migratory waterbirds along the East Atlantic Flyway (van Roomen et al., 2022) and as a breeding ground for species such as gulls and terns (Koffijberg et al., 2022).

Public and scientific attention, as well as management (i.e. encompassing species conservation, area planning, climate adaptation, human activities), predominantly focus on birds in the Wadden Sea. However, it is also a crucial nursery for juvenile fish in the North Sea ecoregion. While the nursery function for bottom-dwelling species in the Wadden Sea has been extensively studied over the past 60 years (e.g. van der Veer et al., 2011, 2022; Tulp et al., 2017), the assemblage of SPF has received little attention. However, Couperus et al. (2016) showed that SPF are the dominant component of the overall fish biomass in the Wadden Sea. Furthermore, SPF are an important food source for fish, piscivorous birds and marine mammals (e.g. Dänhardt & Becker 2011b; Poiesz et al., 2021; Ransijn et al., 2021). Consequently, the lack of knowledge on SPF hampers a comprehensive understanding of the Wadden Sea ecosystem.

The Wadden Sea is a heavily modified ecosystem (de Jonge et al., 1993; Lotze, 2005). Since its origin 7,500 years ago, humans have interacted with it through exploitation, habitat alteration, and pollution (Lotze, 2005). For the Dutch Wadden Sea, the most significant change was the construction of the Afsluitdijk in 1932, which transformed the Zuiderzee into the freshwater Lake IJssel. This damming caused substantial changes in water currents and habitat availability (de Jonge et al., 1993), leading to major shifts in the fish community (Redeke, 1939). The Zuiderzee supported a thriving fishery, with annual catches of Atlantic herring (*Clupea harengus*, hereafter herring) reaching 10,000 tons and European anchovy (*Engraulis encrasicolus*, hereafter anchovy) catches of 2,000 tons (Postuma & Rauck, 1978). After the construction of the Afsluitdijk, large spawning populations of anchovy and herring disappeared (Boddeke & Vingerhoed, 1996). Nowadays, two sluice complexes are the only connections between the estuarine Wadden Sea and the freshwater inland. Excess freshwater is discharged during low water, resulting

in a short migration window, an abrupt transition between salt- and freshwater, and a high water velocity (Griffioen & Winter, 2014).

Zijlstra (1978) attempted to classify the function of the Wadden Sea for the 102 fish species present at that time. For herring and European sprat (*Sprattus sprattus*, hereafter sprat), he noted that the Wadden Sea is probably a nursery, though this was not clearly established. Furthermore, he classified anchovy as a scarce near-resident species leaving the cold temperatures in winter, while European smelt (*Osmerus eperlanus*, hereafter smelt) was classified as seasonal winter visitor during its adult stage (Zijlstra, 1978). However, it remained unclear whether smelt were merely flushed out of Lake IJssel or intentionally migrated to the Wadden Sea. Moreover, Zijlstra's uncertain classifications, made nearly 50 years ago, along with the lack of information on the geographical origin of SPF in the Wadden Sea, highlight the need for updated and targeted studies to better understand the Wadden Sea's role in their life cycle.

1.3 Fish monitoring in the Wadden Sea

In the Dutch Wadden Sea, structural monitoring of the fish fauna is carried out through four sampling programs. The oldest is the Marsdiep-fyke program, running since 1960 at the southern tip of Texel (e.g. van der Veer et al., 2015). This fyke is emptied daily in spring (late March to June) and autumn (September to early November). Since 1970, the annual Demersal Fish Survey (DFS) has been in place, covering the entire Dutch Wadden Sea and focusing on juvenile sole and plaice, information that is used in stock assessments (e.g. Tulp et al., 2017; ICES 2023). This survey, conducted each September with a 3 m bottom trawl, is effective for demersal fish but suboptimal for SPF, as it only captures those SPF near the bottom or during brief periods in the water column. Since 2001, a fyke program at Kornwerderzand, a sluice in the Afsluitdijk, has targeted diadromous species, sampling twice a week in spring and autumn (Griffioen & Winter, 2014). Additionally, since 2006, a stow net survey has been conducted in the Ems estuary at three locations, sampling once in spring and autumn (Jager et al., 2019). The mentioned sampling programs are not specifically targeted at SPF, often using suboptimal gear or covering only small areas or short time periods. Besides structural fish monitoring, shorter-term studies on SPF in the Dutch Wadden Sea have been conducted, such as those on early life stages of herring (Corten & van de Kamp, 1979) and anchovy (Boddeke & Vingerhoed, 1996). More recently, Couperus et al. (2016) quantified SPF abundances in the Marsdiep, highlighting their importance in terms of biomass and demonstrating that technical challenges of acoustic techniques in shallow water can be addressed.

There is a similar gap in SPF monitoring in the German and Danish Wadden Seas. In Denmark, structural fish monitoring is limited to glass eels and the tagging of diadromous fish in rivers (Tulp et al., 2022). In Germany, estuaries such as the Elbe and Weser are monitored with stow nets once or twice a year, and an annual Demersal Young Fish Survey (DYFS) using a beam trawl is conducted each September (Tulp et al., 2022; ICES 2023). Additionally, the Sylt-Rømø Bight has been monitored monthly with a mini bottom trawl since 2007 (Odongo et al., 2024). Lastly, targeted stow net surveys for small pelagic fish were conducted at Jade Bay each spring from 2005, but unfortunately stopped in 2017 (Dänhardt & Becker, 2011a).

Despite these efforts, there still is a lack of long-term, continuous monitoring with detailed temporal and spatial data on SPF in the Wadden Sea. This gap does not only apply to SPF but extends to fish monitoring in habitats other than gullies, such as salt marshes, shellfish beds, and to monitoring of larger fish species like tope shark, mullet and sea bass. To address these deficiencies, the Swimway-Wadden Sea program has been initiated.

1.4 Swimway-Wadden Sea

Time trends in the Wadden Sea fish fauna have raised concerns about the ecosystem's functioning (e.g. van der Veer et al., 2011; 2015; Tulp et al., 2022). After peaking in the mid-1980s, total fish biomass sharply declined from 1980 to 2000, after which it became relatively stable (Tulp et al., 2017). Additionally, the size structure of the fish community changed, with most declines in the larger size classes (Tulp et al., 2017). However, the exact causes and trends for specific species remain unclear. To address these concerns, Trilateral Fish Targets were established in the Wadden Sea Plan (CWSS 2010), and later refined into the Trilateral Swimway Action Programme (CWSS 2019). Their overall objective is to ensure no human-induced bottlenecks in the Wadden Sea for fish populations or their ecosystem functions, aiming for viable stocks and natural reproduction of typical Wadden Sea fish species.

The Dutch Swimway-Wadden Sea program emerged from the Trilateral Swimway Action Program, with a central focus on the life cycle approach. It includes five PhD studies and one postdoctoral study that incorporates the knowledge gained into theoretical models. This thesis is the result of one of the PhD studies within the program. The other PhD studies focus on the importance of salt marshes for fish (Hannah Charan-Dixon), the role of shellfish beds for fish (Maryann Watson), the future prospects for fish populations in the Wadden Sea (Bass Dye) and the migration patterns of large fish in the Wadden Sea (Jena Edwards; Figure 2).

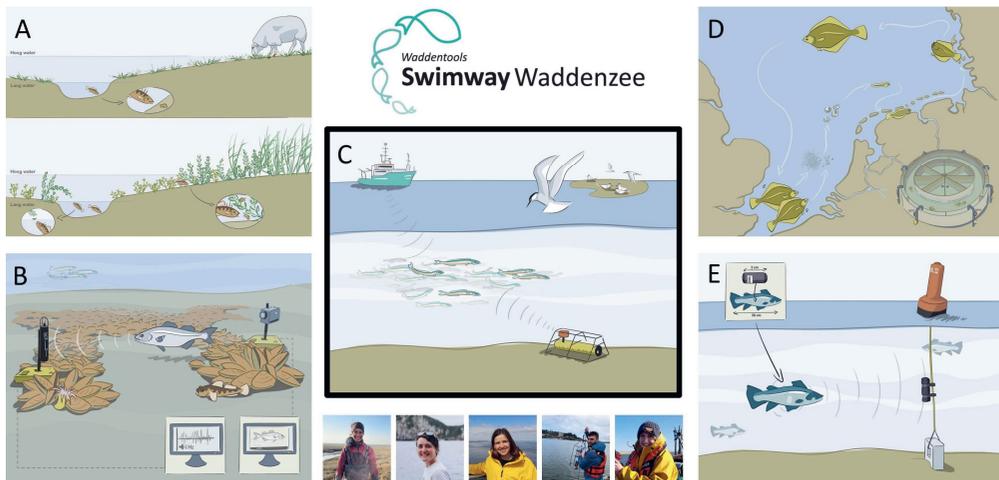


Figure 2. Overview of the Swimway-Wadden Sea PhD study topics. Each image represents a PhD project, with the central image depicting the subject of my thesis. A) Hannah Charan-Dixon, B) Maryann Watson, C) Margot Maathuis, D) Bass Dye, and E) Jena Edwards. The pictures at the bottom follow the same order. Drawings by Horst Wolter.

1.5 Role in food web

SPF are often referred to as forage fish due to their importance as a food source for e.g. birds and marine mammals (e.g. Pikitch et al., 2012; Engelhard et al., 2014; Ouled-Cheikh et al., 2022). Given the Wadden Sea's importance for bird populations, conservation efforts and policies often focus on improving conditions for birds. To inform these policies, the primary interest in studying SPF is their role as food source for piscivorous birds. This is especially important for breeding birds, such as the sandwich tern and common tern, as aligned food availability with the needs of these predators is essential for their survival and successful reproduction (Stienen et al., 2000; Dänhardt & Becker, 2011b). While management strategies typically target higher trophic levels, understanding changes in predator populations requires a comprehensive understanding of the base of the food web.

SPF transfer energy from zooplankton to larger predators, making them vital to marine food webs, especially since zooplankton and SPF can occur in large quantities. Zooplankton communities exhibit strong responses to environmental changes (Martens & van Beusekom, 2008; Richardson, 2008), which in turn impacts zooplanktivorous SPF (Heneghan et al., 2023). However, just like information on SPF, zooplankton knowledge in the Dutch Wadden Sea is still limited. Most studies

on the Wadden Sea food web have focused on the macrobenthic community (e.g. Christianen et al., 2017; Jung et al., 2020). In addition, in the western Dutch Wadden Sea, two studies have investigated the fish community's food web through visual stomach inspection (Poiesz et al., 2020) and stable isotope analysis (Poiesz et al., 2021). Here, it was concluded that only a few key species, including juvenile herring, are preferred prey for many fish species, highlighting the crucial role of herring in the Wadden Sea's food web.

1.6 Study species

This thesis targets several species, here I will shortly introduce their main characteristics (Figure 3).

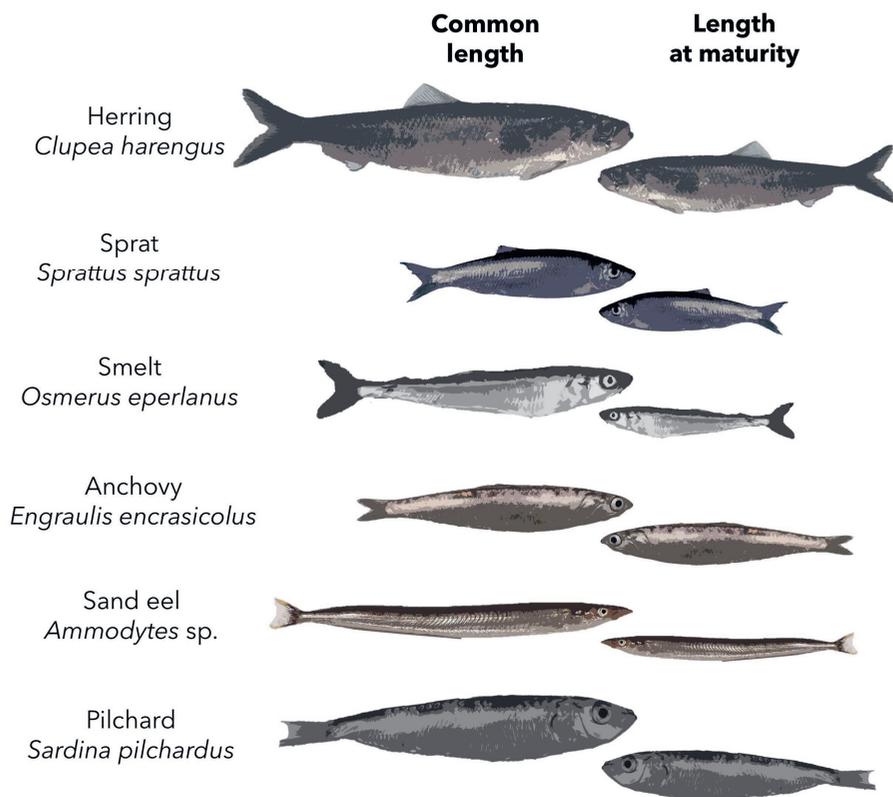


Figure 3. Main small pelagic fish species in the Dutch Wadden Sea. Images are scaled relative to each other: the left side shows the common maximum adult length of each species, while the right side depicts their approximate length at maturity.

Atlantic herring, *Clupea harengus* (Linnaeus, 1758)

Herring is numerically and commercially one of the most important species in the north-east Atlantic (Seitz et al., 2014). They are distributed across the North Atlantic, from the Bay of Biscay to the Barents Sea, and also inhabit less saline environments. Herring are migratory fish, using distinct grounds for feeding, overwintering, and spawning. Their life history characteristics are highly variable among populations, involving multiple spawning grounds and distinct spawning periods (Mcquinn, 1997; Payne, 2010). Herring are determinate single-batch spawners that deposit eggs on suitable substrates (Petitgas et al., 2010; Frost & Diele, 2022). In the North Sea, they typically reach maturity at ~26 cm at an age of 2-3 years (Heessen et al., 2015). The largest herring reported is 43 cm, with a common size of up to 30 cm (Heessen et al., 2015). Herring have been a significant target in fisheries for a long time. In the Netherlands, it is the second most landed species, with 132 million kilos reported in 2022 (CBS, 2022). Overexploitation led to a collapse in their abundance in the 1970s, but after targeted fisheries management their numbers have generally recovered since then.

European sprat, *Sprattus sprattus* (Linnaeus, 1758)

Sprat are widely distributed across the shelf waters of Europe and North Africa, from Norway to Morocco. They tolerate a broad range of salinities and are abundant in estuarine habitats. Sprat generally live up to 5 years and rarely exceed 16 cm in length (Peck et al., 2012a). They exhibit regional differences, with genetic variations and recognized subspecies (Whitehead, 1985; McKeown et al., 2020; Saltalamacchia et al., 2022). In the North Sea, sprat commonly reach up to 15 cm, with a peak length of around 11 cm (Heessen et al., 2015). Peak spawning in the North Sea occurs between May and August and may be triggered by temperature (Baumann et al., 2009). Sprat are indeterminate multiple-batch spawners, releasing eggs into the water (Petitgas et al., 2010). They generally spawn for the first time at age 1 or 2, around 10 cm in length (Heessen et al., 2015). Fisheries mainly target sprat in the North Sea and Baltic Sea, primarily for the fishmeal industry.

European smelt, *Osmerus eperlanus* (Linnaeus, 1758)

Smelt populations can be either anadromous (migrating) or landlocked (resident) (Tulp et al., 2013). Anadromous smelt are found along the estuarine northern European coastline, while landlocked smelt inhabit many lakes in northern Europe. Anadromous smelt migrate from coastal areas upstream to spawn, and after hatching in freshwater, the juveniles slowly drift downstream to marine waters. This species exhibits high plasticity, resulting in widely varying life history parameters among populations. Anadromous smelt can live up to 8 years and typically reach up to 24 cm in length, while landlocked smelt have shorter lifespans and remain smaller (Heessen et al., 2015). In lakes, they mature by the end of their first year,

whereas anadromous smelt generally mature at age 2 or 3 (Heessen et al., 2015). Smelt are determinate single-batch spawners, forming dense spawning aggregations in late winter or early spring and depositing eggs on suitable substrates (Belyanina, 1969). Smelt are targeted by local fisheries; however, in Lake IJssel, fishing has been closed for some years to preserve smelt as a food source for birds (Keller et al., 2020).

European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758)

The anchovy is a southern species found along the Atlantic coasts from Africa to Scotland, including the Mediterranean and Black Seas. Anchovy abundance in the North Sea fluctuates annually, but there has been an increase in populations since the 1990s, likely due to warmer summers and milder winters (Alheit et al., 2012; Petitgas et al., 2012). Genetic analysis reveals that North Sea and English Channel anchovies form a distinct population from those in the Bay of Biscay (Petitgas et al., 2012). In the North Sea, anchovies commonly reach lengths up to 18 cm, with the oldest individuals reaching 5 years (Heessen et al., 2015). Most anchovies reach maturity at age 1 (Heessen et al., 2015). They are indeterminate multiple-batch spawners, releasing eggs every 3-5 days. Spawning occurs in inshore waters, with water temperature and salinity being key factors (Ibaibarriaga et al., 2007). Anchovy is an important commercial species, especially in the Bay of Biscay, Mediterranean, and Black Sea.

Sandeel, *Ammodytes* sp.

Although they spend most of their life buried in the sand, sandeels are considered SPF as they form dense schools when foraging in pelagic waters during daylight in spring, and during the spawning season (van Deurs et al., 2013). In this thesis, identification is generally kept at the genus level due to field identification difficulties. The two species included are the common sandeel (*Ammodytes tobianus*, Linnaeus, 1758) and the lesser sandeel (*Ammodytes marinus*, Raitt, 1934). *A. marinus* typically has a more offshore distribution, so most catches in the Wadden Sea are likely *A. tobianus*. Sandeels are distributed across the eastern North Atlantic, from northern France to the Barents Sea, with local distribution closely linked to sediment type and water velocity (Tien et al., 2017). *A. tobianus* rarely live beyond 3 years, with all fish maturing by age 2, and some even at age 1 (Heessen et al., 2015). In the North Sea, sandeels reach lengths of up to 28 cm (Heessen et al., 2015). Sandeels deposit their eggs on the seafloor, with *A. marinus* spawning from November to February, and *A. tobianus* spawning both in spring and autumn, though more frequently in spring. In the western North Sea, sandeels are targeted by fisheries primarily for fishmeal and oil.

European pilchard, *Sardina pilchardus* (Walbaum, 1792)

Pilchard, sometimes referred to as sardine, are found along the Atlantic coast, including the Mediterranean. They re-invaded the North Sea around 1990, likely in response to warmer temperatures associated with the strengthening of the North Atlantic Oscillation in the late 1980s (Alheit et al., 2012). Life history characteristics vary across their distribution. In the English Channel, pilchard reach maturity at approximately 15 cm in length, with maximum sizes and ages reported at 27 cm and 12 years, respectively (Silva et al., 2006). Spawning occurs from May to November, with a main peak in May and June (Heessen et al., 2015). Pilchard are indeterminate, serial batch spawners, releasing their pelagic eggs at intervals of 7-10 days (Heessen et al., 2015). Fisheries primarily target pilchard in southern Europe and off the West African coast, and during years of high abundance, in the Western Channel.

1.7 Challenges on studying SPF

Studying SPF in the shallow Wadden Sea presents several challenges. In the following sections, I outline these challenges, define the thesis objectives and describe the approaches used in this thesis to address them.

- SPF are highly dynamic in time and space

Due to their short generation times, schooling behaviour, tight coupling to lower trophic levels, and sensitivity to climate variability, SPF populations show considerable variation in abundance and spatial distribution (Alheit & Peck, 2019; Peck et al., 2021). With global climate change driving shifts in fish abundance, distribution, and phenology (Rijnsdorp et al., 2009; Sydeman et al., 2015), these variations are likely to increase. Consequently, detailed studies with high-resolution, long-term coverage are essential, addressing both fine-scale and broad-scale spatial dimensions.

- Shallow murky waters of the Wadden Sea

In areas with low visibility, cameras are ineffective for studying fish. This is particularly true in the highly dynamic Wadden Sea, where we recorded an average visibility of less than one meter. Furthermore, shallow waters complicate acoustic techniques due to significant reflections from the nearby seabed and surface (Thorne, 1998). In addition, vertically oriented transducers face a hydroacoustic blind zone of 2 to 5 m, reducing the effective sampling volume in shallow environments (Simmonds & MacLennan, 2005).

- SPF are small and feed on tiny, fragile prey

One way to study fish movement and habitat use is through telemetry, which uses tracking devices (acoustic tags) to record the movement and behaviour of

individual fish. Most SPF in the Dutch Wadden Sea are juveniles weighing less than 3 grams, and tags must be under 2% of a fish's body weight to ensure the tag does not significantly affect its behaviour or physiology. As a result, telemetry is generally not feasible for these small fish (Edwards et al., 2024). SPF are small, but their prey is even smaller and more delicate, making traditional macroscopic methods challenging for studying their diet (Ershova et al., 2021).

1.8 Thesis objectives

Despite the ecological importance of SPF in the Wadden Sea, significant knowledge gaps exist regarding their ecology. Basic information on their abundance, timing, and reasons for inhabiting the Wadden Sea is lacking. Additionally, the role of SPF as prey and predator in the Dutch Wadden Sea is not well understood. There is no information on the diet preferences of SPF or zooplankton dynamics in this area. Furthermore, from the perspective of SPF being food for larger predators, it is unclear what the food landscape of SPF looks like and how this relates to the foraging locations and food choices of terns.

Therefore, two main questions were posed:

- i) What is the function of the Wadden Sea in the life cycle of small pelagic fish?*
- ii) What is the role of small pelagic fish in the food web of the Wadden Sea?*

The main objective of this thesis is to generate fundamental knowledge about SPF temporal and spatial distribution and habitat use to identify the role of the Wadden Sea in the life cycle of SPF, and to determine their trophic interactions, both as predator and prey.

1.9 Study approaches

To overcome the challenges of studying SPF in the Wadden Sea, and to gain insights into their spatiotemporal dynamics and trophic interactions, this PhD study employed three main approaches:

- Fishing

Fishing is a long-established method for studying marine life, providing direct data on species composition, abundance, and biological characteristics (like size, weight and maturity). I used two types of fishing surveys: a monthly stow net survey and a seasonal trawl survey. The stow net, which covered the full water column (up to 7 m deep), was deployed monthly at fixed locations in four inlets. The semi-pelagic trawl net, with a vertical opening of approximately 5 m, was deployed while cruising in deeper inlets, allowing for fishing at various locations.

- Active acoustic techniques

Acoustic methods are effective for studying marine ecosystems, as they enable rapid sampling with high spatial and temporal coverage using non-invasive techniques (Simmonds & MacLennan, 2005; Benoit-Bird & Lawson, 2016). It involves emitting sound waves into the water and measuring the reflected signals. The signal strength reveals the type and size of objects, while the time it takes for the signal to return indicates their depth in the water column (Benoit-Bird & Lawson, 2016). This approach allows for the study of fish density, distribution, and behaviour, though it does not provide detailed biological information, such as species identification, exact length, or weight. I used active acoustics in two ways: deploying a stationary echosounder (WideBand Autonomous Transceiver (WBAT)) to continuously monitor fish passing a single location, and conducting an acoustic survey to study SPF dynamics across the Dutch Wadden Sea.

- DNA metabarcoding

DNA metabarcoding is widely used for detecting invasive species, assessing biodiversity and studying diet composition (Pompanon et al., 2012; van der Loos & Nijland, 2020). This method detects species presence and approximate abundance within a community by analysing genetic material from bulk samples. It allows for the simultaneous identification of many taxa through high-throughput sequencing, followed by comparison of obtained sequences with a database for species identification. I applied DNA metabarcoding to analyse prey DNA from the stomach contents of herring and sprat. Additionally, it was used to study zooplankton community composition to determine available prey.

The different methods applied vary in the spatiotemporal scales (Figure 4). The acoustic survey covered the broadest spatial area, including all deeper inlets of the Dutch Wadden Sea, but was conducted only twice. The stow net survey, which also includes the diet samples, focused on four inlets and offers more detailed temporal data, being conducted monthly. The stationary echosounder provided the highest temporal resolution, recording data every 90 min for a full year, but is limited to a single location in the most western inlet.

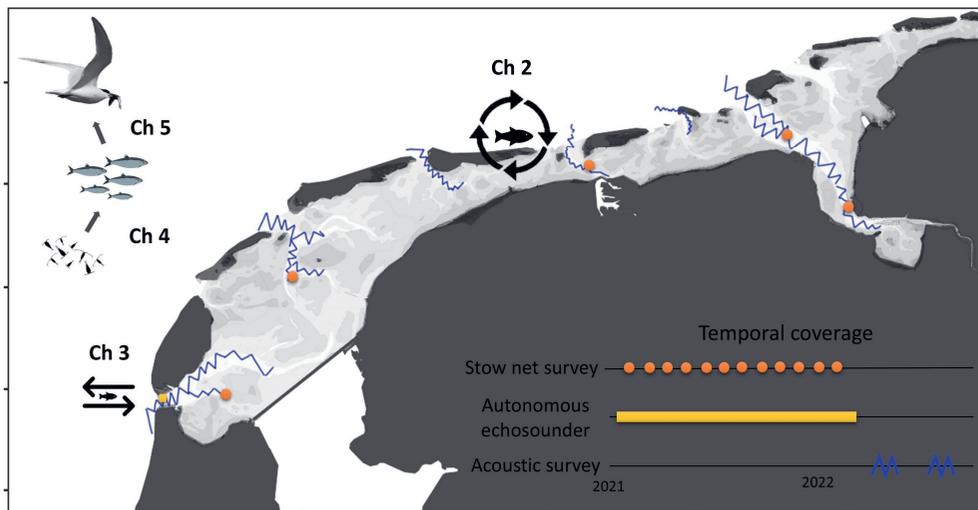


Figure 4. The field sampling methods used in this thesis, illustrating their variation across spatiotemporal scales. Furthermore, the main topic of each research chapter is indicated.

1.10 Outline of the thesis

This thesis aims to provide a comprehensive understanding of SPF in the Wadden Sea by exploring the topic from two perspectives, aligning with the two main questions: i) *what is the function of the Wadden Sea in the life cycle of SPF*, and ii) *what is the role of SPF in the food web of the Wadden Sea?* The first two research chapters investigate the life cycle of different SPF species, addressing questions such as: *which* species are present, *why* do they inhabit the Wadden Sea, and *when* and in *what* densities are they found. The subsequent two research chapters examine trophic relationships within the Wadden Sea, focusing on SPF's role in the food web and their interactions with other species, with questions including: *what* do they eat, *what* is the biomass of SPF present, and *where* are they located?

It is generally stated that the Wadden Sea is a nursery area for fish, but recent data of habitat use, and the spatiotemporal distribution of specifically SPF are lacking.

Chapter 2 addresses the function of the Wadden Sea in the life cycle of SPF, to study which life cycle stages of pelagic fish are present in the Dutch Wadden Sea, and at which spatial and temporal scale. Furthermore, the Wadden Sea is not an isolated waterbody, yet the connectivity for SPF between the North- and the Wadden Sea is poorly understood. Roles of tidal flow, seasonality and wind speed on the exchange of SPF between these waterbodies are unknown. Therefore, **Chapter 3** provides a detailed study of SPF movement between the North Sea and

the Wadden Sea, focusing on temporal variations in density, vertical distribution, and movement behaviour.

In the subsequent two research chapters, multiple trophic levels are studied, from zooplankton to birds. Detailed studies on the year-round diet composition of herring and sprat in the Wadden Sea, as well as recent data on zooplankton dynamics, are currently lacking. Therefore, **Chapter 4** examines the diet of herring and sprat in relation to zooplankton dynamics. **Chapter 5** then focuses on SPF as food for birds, specifically the common tern (*Sterna hirundo*), investigating the SPF food landscape in the Wadden Sea. This includes SPF biomass estimates, energy density, and spatial data of foraging common terns.

Finally, **Chapter 6** places the findings in a broader context, revisiting the study's main objectives and highlighting key insights. This chapter also emphasizes surprising observations, compares the field methods used, provides recommendations for monitoring SPF in Dutch coastal waters, and suggests directions for future research.



Chapter 2

The function of the Wadden Sea in the life cycle of small pelagic fish

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Abstract

Most fish species require different habitats to complete their life cycle, with coastal areas often playing a crucial role as nursery areas for juveniles. The Wadden Sea is an important nursery area for juvenile fish in the North Sea ecoregion. Despite extensive research on the nursery function of the Wadden Sea for demersal species, its role in the life cycle of small pelagic fish (SPF) remains largely unknown. This limits our understanding of the Wadden Sea ecosystem, as SPF are the dominant component of the overall fish biomass and serve as important food source for higher trophic levels. We studied the role of the Wadden Sea in the life cycle of Atlantic herring (*Clupea harengus*), European sprat (*Sprattus sprattus*), European smelt (*Osmerus eperlanus*) and European anchovy (*Engraulis encrasicolus*) through monthly stow net and seasonal trawl surveys. Our study showed that SPF use the Dutch Wadden Sea primarily as juvenile habitat, with herring being the dominant marine juvenile representative. Length frequency distributions and genetic analysis revealed that the juvenile herring originated predominantly from southwestern waters, such as the English Channel. Additionally, the Wadden Sea still provides spawning grounds for herring and anchovy, with no substantial spawning observed for sprat. While smelt can complete nearly its entire life cycle in the Wadden Sea, it depends on connectivity to nearby freshwater for spawning. In summary, the Wadden Sea functions as a nursery for juveniles, and to a lesser extent as spawning grounds for adults. A thorough understanding of these functions is crucial for identifying bottlenecks and implementing effective conservation and management strategies.

1. Introduction

Most fish migrate between habitats to complete their life cycle (Harden Jones, 1968), with coastal areas often playing crucial roles, serving as nurseries, feeding, and/or spawning areas (Beck et al., 2001; Nagelkerken et al., 2015). The Wadden Sea, the largest unbroken system of gullies and intertidal flats in the world, is an important nursery area for juvenile fish in the North Sea ecoregion (Zijlstra, 1978; van der Veer et al., 2011). Eggs and larvae of species spawning in the North Sea are transported with currents and tides towards the coast, where they spend their early life stages, before returning to the deeper waters of the North Sea at a later stage (Bolle et al., 2005; Bolle et al., 2009; Dickey-Collas et al., 2009).

While the nursery function for bottom-dwelling species in the Wadden Sea has been extensively studied in the past (van der Veer et al., 2011, 2022; Tulp et al., 2017), the assemblage of small pelagic fish (SPF) has received little attention to date. This lack of attention hampers the understanding of the Wadden Sea ecosystem, given that SPF are considered to be the dominant component of the overall fish biomass in the Wadden Sea (Couperus et al., 2016). However, information on the habitat use of SPF throughout their life cycles is unavailable, leaving the role of the Wadden Sea for each life stage unclear.

The Wadden Sea has been significantly impacted by large-scale infrastructure projects (Redeke, 1939; de Jonge et al., 1993). For instance, the Wadden Sea had an open connection to the Zuiderzee until the Afsluitdijk dam was built in 1932, closing off a large water body. This caused substantial changes in water currents and habitat availability (de Jonge et al., 1993) and resulted in major shifts in the fish community (Redeke, 1939), including the disappearance of large spawning populations of European anchovy (*Engraulis encrasicolus*, hereafter anchovy) and Atlantic herring (*Clupea harengus*, hereafter herring) (Boddeke & Vingerhoed, 1996). Until now, it was unclear whether the Wadden Sea still serves as spawning habitat for SPF.

Nowadays, the most abundant SPF in the Wadden Sea are herring and European sprat (*Sprattus sprattus*, hereafter sprat) (Couperus et al., 2016; Maathuis et al., 2024a). Together with European smelt (*Osmerus eperlanus*, hereafter smelt), they form a crucial component of the food web, providing the trophic link between zooplankton and piscivorous fish, birds, and sea mammals (Dänhardt & Becker, 2011a). Furthermore, herring and sprat populations in the North Sea support important fisheries. Yet, the geographical origin and timing of spawning of SPF occurring in the Wadden Sea remained uncertain, particularly for herring, with its complex life history, involving multiple spawning grounds and distinct spawning periods (McQuinn, 1997; Payne, 2010). Drift modelling of Dickey-Collas et al.

(2009) indicated that larvae in the Wadden Sea originate from the Downs winter-spawning herring population, which spawns in the English Channel. However, variations in herring body lengths and densities observed throughout the year indicate a mix of spawning origins (Maathuis et al., 2024a). Therefore, despite herring's dominance in the Wadden Sea, it remains unclear from which spawning populations they originate.

The function of the Wadden Sea in the life cycle of SPF was examined using four key species currently or historically dominant or with a significant role in the food web: herring, sprat, smelt, and anchovy. For each species, two hypotheses were tested: i) the Wadden Sea serves as a juvenile habitat, with variations in spatial distribution among species, corresponding to their origin or life history; and ii) the Wadden Sea functions as a spawning ground (excluding smelt). In addition, a third hypothesis was formulated specifically for herring: iii) herring in the Wadden Sea originate from the Downs winter-spawning population.

Therefore, abundance, body length, and maturity were measured, incorporating temporal and spatial variation over a two-year period, using two fishing methods with different temporal and spatial coverage. In 2021-2022, a monthly stow net survey was conducted focusing on seasonal variation in three geographically distinct Wadden Sea areas. In 2022, a trawl survey was carried out in May and October in all tidal inlets and connecting channels, with a higher spatial resolution. Furthermore, genetic analyses of herring were conducted to identify the populations present in the Wadden Sea, providing insights into their geographical origin and spawning period.

2. Methods

2.1 Study area

The Wadden Sea is a large interconnected system of gullies, intertidal sand flats, mud flats, and salt marshes. It borders the North Sea coasts of the Netherlands, Germany and Denmark and is characterised by strong tidal currents. Although most of the Wadden Sea is shallow, with depths typically below 15 m, the inlets between the barrier islands connecting the Wadden Sea to the North Sea are up to 40 m deep. This study was done in six inlets, from west to east: Marsdiep, Vliestroom, Borndiep, Westgat, Lauwers, and the Ems-Dollard (Figure 1). The inlets were grouped to represent three main areas: West (Marsdiep and Vliestroom), East (Borndiep, Westgat and Lauwers) and Ems-Dollard, in accordance with Tulp et al.

(2017), and supported by a basin cluster analysis based on ecotopes (Baptist et al., 2019b). The western area is the deepest, the eastern area is the most saline, and the Ems-Dollard is an estuary characterized by high turbidity levels and lower salinity.

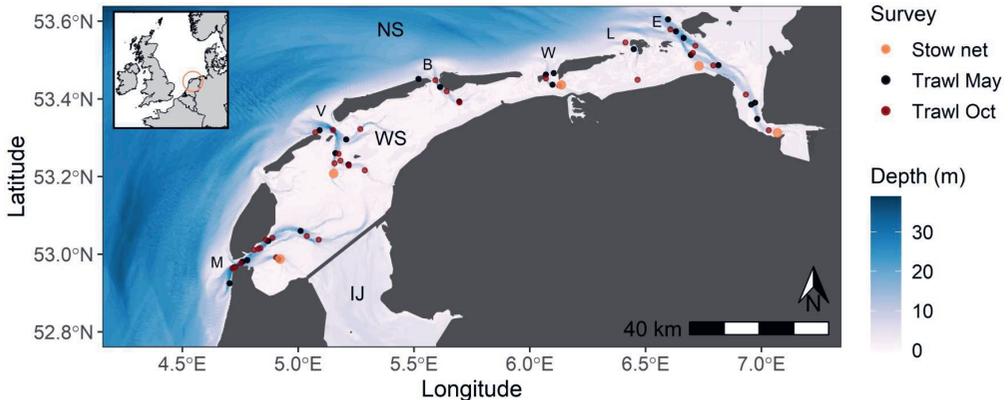


Figure 1. Map of the study area showing Wadden Sea (WS), North Sea (NS) and Lake IJssel (IJ). Coloured circles represent sampling locations of the two surveys, with the six inlets denoted by their initial letters (M = Marsdiep, V = Vliestroom, B = Borndiep, W = Westgat, L = Lauwers, and E = Ems-Dollard). Stow net fishing occurred monthly in 2021-2022, and trawls were taken in May and Oct 2022.

2.2 Field sampling

Fish community composition and fish size distribution were assessed using two survey methods: a monthly stow net survey, and a seasonal trawl survey.

2.2.1 Monthly stow net survey

Each month from 22-3-2021 until 3-3-2022 fishes were caught in four inlets (Marsdiep, Vliestroom, Westgat and Ems-Dollard, Table 1) via stow net fishing. This is a passive fishing method using water currents driving fish into the net, held open by two 8 m beams. The nets were positioned vertically in the water during high current speeds at both incoming and outgoing tide during daytime. Weather permitting, nets were deployed on both sides of the ship and treated as a single haul. The nets covered the entire water column (average depth 5.3 m), with a stretched mesh size of 20 mm in the codend. Polyethylene codends were used in all months except April when, due to miscommunication, a nylon net with the same mesh sizes, but including hoops, was used. This resulted in catchability differences,

where in April, fish smaller than 9 cm were hardly caught (supplementary Figure S1). However, we chose to include the April data in the analysis, as no alternative data were available. During some hauls, jellyfish (mainly *Mnemiopsis leidyi*), Bryozoa (*Electra pilosa*), or sea lettuce (*Ulva lactuca*) partially clogged the net, mainly between May and November. Fixed fishing locations were used, with backup sites designated for stormy weather (Figure 1). Typically, these sites were near each other, except in the Ems-Dollard, where fishing occurred for 6 months in the Dollard (southern site Figure 1), and for 5 months in the Westereems (northern site Figure 1), located about 30 km northwest of the Dollard location. The Ems-Dollard is an open estuary, therefore the conditions in the Dollard, closer to the Ems river, differ from the Westereems. Not all inlets could always be fished, owing to adverse weather conditions, particularly in autumn and winter (Table 1). Altogether, 75 hauls were conducted, with an average haul duration of 48 min.

Flow velocity was measured throughout each haul, using two flow meters (General Oceanics, USA), positioned approximately 10 m behind the vessel to avoid interference with the net. Mean flow velocity and net opening area were used to calculate catch per unit effort (CPUE) as catch rate in numbers per 10000 m³ water. Fish total length was measured to the nearest mm. Additionally, clupeid post-larvae were regularly caught but too small for accurate field identification, likely comprising a mixture of juvenile herring, sprat, and pilchard (*Sardina pilchardus*). Most were identifiable from 5 cm in length, with nearly all identifiable above 6 cm. Visibility, water temperature and salinity were measured during stow net sampling using a Secchi-disk and a Valeport mini CTD (Valeport Ltd, United Kingdom). Water temperature ranged from 5 °C in February up to 21 °C in July, visibility ranged between 0.2 m and 2.2 m, and salinity fluctuated between 14 and 31, see supplementary Figure S2 for trends per area.

2.2.2 Seasonal trawl survey

Daytime trawl hauls were conducted over two fortnight periods in May and October 2022, across the six inlets (Figure 1; Table 1). The trawl hauls were part of a hydro-acoustic survey covering all deeper inlets of the Dutch Wadden Sea, using a commercial fishing vessel. A semi-pelagic otter board trawl net, with a 17 m horizontal by 5 m vertical opening (based on sewing pattern dimensions) and a 10 mm mesh codend lining, was towed behind the vessel. Fishing speed averaged 3 knots through water, with hauls mainly executed in midwater, between 5 and 10 m depth. The average depth of the water column during fishing was 16.6 ± 7 m. In total, 50 hauls were conducted, with an average duration of 14 min. Haul duration was used to calculate CPUE, expressed as fish numbers per minute trawling. Fish total length was measured to the nearest mm.

Table 1. Overview of sampling periods and sample sizes of hauls and individual fish used for final analysis. Abbreviations - Inlets: M = Marsdiep, V = Vliestroom, B = Borndiep, W = Westgat, L = Lauwers and E = Ems-Dollard; species: Her = herring, Spr = sprat, Osm = smelt and Ane = anchovy; * indicates fishing was conducted at the backup site Dollard instead of Westereems, and ** indicates a different net was used.

Survey	Sampling period	Number of hauls per inlet						Number of fish for maturity assessment				DNA
		M	V	B	W	L	E	Her	Spr	Osm	Ane	Her
Stow net	22-25 Mar '21	2	2	-	2	-	2	47	39	13	0	0
	26-30 Apr '21**	2	2	-	2	-	2*	34	17	45	2	0
	18-20 May '21	2	2	-	2	-	-	17	13	35	10	0
	21-24 Jun '21	2	1	-	2	-	2	24	36	42	0	0
	25-28 Jul '21	2	2	-	2	-	2*	27	23	77	8	0
	23-26 Aug '21	-	2	-	2	-	1	27	7	53	0	0
	19-22 Sep '21	1	1	-	2	-	1	24	25	38	10	0
	24 Oct-3 Nov '21	1	2	-	2	-	2*	48	40	65	0	0
	22-25 Nov '21	-	1	-	2	-	1*	37	22	49	0	0
	13-16 Dec '21	1	2	-	2	-	2*	76	48	73	0	0
	23-25 Jan '22	-	1	-	2	-	2*	61	39	50	0	0
1-3 Mar '22	-	-	-	2	-	2	63	30	15	0	0	
Trawl	9-18 May '22	6	4	3	3	1	8	120	36	19	174	17
	17-26 Oct '22	6	6	3	2	2	6	99	31	40	2	19
Sums	Mar '21-Oct '22	35	38	6	29	3	33	704	406	614	206	36

2.3 Maturity assessment

Herring, sprat, smelt, and anchovy were collected during both surveys for assessment of fish maturity. For each area, up to 5 individuals per cm size class per month were collected. Under our permit for animal experiments, the minimum size for collecting herring, smelt, and anchovy was restricted to 8 cm, and to 6 cm for sprat. The maturity assessment followed the ICES M6 scale, where stages 1 to 4 represent immature, maturing, spawning, and spent, respectively (ICES, 2014). Stages 5 (omitted spawning) and 6 (abnormal gonad) were not observed. In total, 704 herring, 406 sprat, 614 smelt and 206 anchovy were assessed for maturity (Table 1).

2.4 Genetic assessment of herring

Fin tissue clips were collected from 36 herring during the trawl survey, 17 in May and 19 in October 2022, covering all observed sizes and all areas. Genotyping was performed using 59 single-nucleotide polymorphism (SNP) markers across 20

chromosomes to assign individuals to distinct herring populations, defined by spawning grounds and spawning seasons, following the procedure described by Seljestad et al. (2024). For nine individuals, > 25% of the SNPs were missing due to low DNA quality genotypes. However, the most informative markers to discriminate herring populations relevant for this study were genotyped and thus these nine samples were not removed from further analysis. For assignment the R-package rubias (Moran & Anderson, 2019) was used, estimating individual posterior assignment probabilities by a fully Bayesian model conditional on the reference allele frequencies with a parametric bootstrapping correction (2000 MCMC iterations, 100 burn-in, 100 bootstraps). Bootstrap corrected posterior means of membership in each grouping were used to assign individuals to their most likely baseline population. We used the established baseline by Seljestad et al. (2024) for the assignment analysis.

2.5 Data analysis

Data analysis was conducted in R (version 4.3.1; R Core Team, 2023) and RStudio (version 2023.06.1; RStudio Team, 2023). CPUE data were aggregated per cm class (rounded down to the nearest integer) per haul. Two stow net surveys were delayed due to bad weather; however, to maintain consistency in data analysis, the period 24 October to 3 November 2021 was designated as October, and 1 to 3 March 2022 as February. To depict seasonal and spatial variation in SPF species and length composition while ensuring visibility of all species, CPUE data required fourth root transformation due to significant differences in catch rates, after which CPUE data were averaged per season and area. Furthermore, species-specific length frequency distributions were generated by averaging square root transformed CPUE data per bimonthly period and subsequently back transformed. Then proportions of maturity stages were calculated per cm class, per bimonthly period, and applied to the length frequency distributions. To provide an overview of the proportion of each maturity stage per length class, data from all months and locations were pooled. Both surveys were combined, and since we aimed for representative maturity sampling, no additional adjustments for catchability were made.

Length frequency distributions were used to identify the arrival of new year classes, with a notable reduction in sizes indicating the presence of young-of-the-year (YOY) individuals. To investigate spatial variation and potential origins of YOY herring using stow net catch data, average lengths and CPUEs were calculated per month per inlet for YOY herring (defined as herring smaller than 8 cm), and the two months of highest YOY influx were further examined. The 8 cm threshold was selected to capture the first peak of small herring observed in the monthly length frequency

plots of herring (see supplementary Figure S3). This analysis was not conducted for the other species because of insufficient data (spatially or temporally).

Ethical statement:

All sampling was performed in accordance with Dutch law concerning animal welfare. The protocol was approved by the Animal Ethical Commission of Wageningen UR (experiment code: 2020.D-0026.001; application: 40100202010984).

3. Results

3.1 Seasonal and spatial species composition

Herring was the most abundant SPF species in the Dutch Wadden Sea, followed by sprat (Figure 2). Additionally, smelt, anchovy and clupeid post-larvae were observed regularly. Two other SPF species were only briefly observed: sandeel (*Ammodytes* sp.), typically buried in the sand for most of the year, was frequently caught in May, while pilchard was frequently caught in September. Clupeid larvae appeared in January and numbers peaked in May and June, before they were large enough to be identified. A spatiotemporal pattern in clupeid larvae abundance was present: increasing from west to east from spring to summer. Herring maintained high abundances year-round in all inlets. Sprat showed highest abundances in the West, except in spring 2021, where small sprat were most abundant in Ems-Dollard. Smelt was most prevalent in the Ems-Dollard and in the East. Smelt abundance in the Ems-Dollard varied with sampling location and was notably higher when sampled in the Dollard compared to the Westereems. Anchovy was present in all inlets, in low numbers throughout the year.

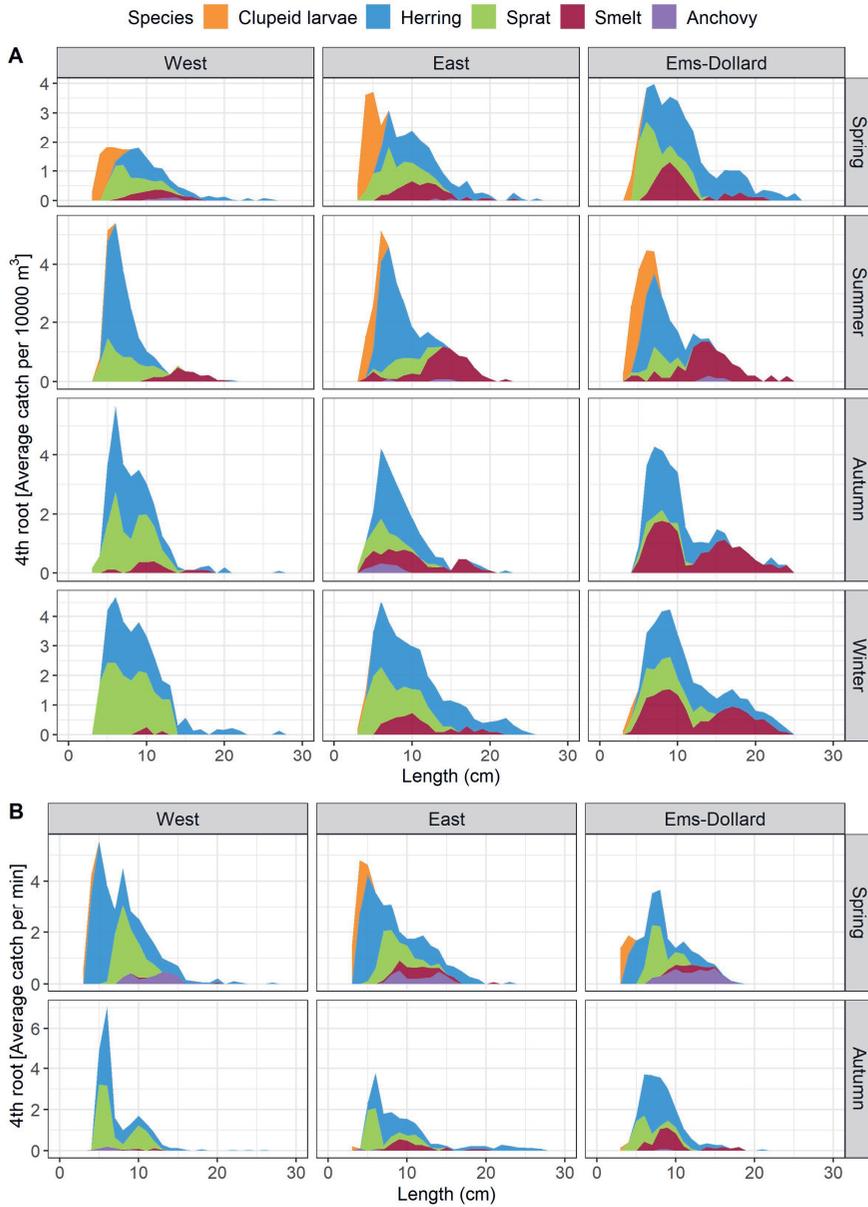


Figure 2. Cumulative length frequency distributions of five species across different areas and seasons for A) stow net survey (2021-2022) and B) trawl survey (2022). The Y-axes denote fourth root transformed CPUEs, measured per 10000 m³ (A) or per minute trawling (B). The colours denote different species, with clupeid larvae being unidentified post-larvae of the family Clupeidae.

3.2 Herring

Herring abundance peaked in July and August. Herring sizes ranged from 4 to 27.7 cm, with the majority smaller than 12 cm. Individuals larger than 12 cm were observed from November to April (Figure 3A, upper panel). Based on the stow net survey, the influx of YOY herring in 2021 began in June, reaching its peak in July and August. However, during the 2022 trawl survey, high abundances of herring around 5.5 cm were already observed in May. So the primary influx of YOY occurred early summer, but 5-6 cm herring were continuously caught until January.

Maturity estimates revealed that most herring were juvenile, from June until September even exclusively, while in other months individuals larger than 18 cm were also observed to be maturing, spawning, or spent (Figure 3A, middle panel). Out of 704 individuals, 39 were found to be spawning, with 31 herring spawning in October, 1 in March, 6 in April, and 1 in May. The mean size of spawning individuals was 24.1 cm, with the smallest spawning individual measuring 19.5 cm. All spent individuals were larger than 21 cm, and primarily observed in April. Spawning herring were observed in several inlets (Figure 3A, lower panel), with one notable observation in the Lauwers inlet, where 25 of the 31 herring spawning in October were caught.

The mean length of YOY herring was lowest in the western area in most months, especially during summer (Figure 4A). CPUE per month shows that initially, the abundance of YOY increased in the West, followed by the East, and later in the Ems-Dollard area (Figure 4B). This pattern suggests that YOY herring originate from southwestern waters. However, in the trawl data of May 2022, the west-east gradient is less apparent, as the Lauwers inlet (East area) shows the highest CPUE of YOY herring, with a mean total length of 5.2 ± 0.2 cm.

The suggestion that herring originate from southwestern waters is supported by genetic analysis, indicating that 28 out of 36 herring were winter-spawning Downs herring, and 6 were autumn-spawning herring (mainly North Sea autumn-spawning (NSAS) herring, Table 2). Additionally, in May, two spring-spawning herring were identified, which were assigned as western Baltic spring-spawning (WBSS) herring but with low posterior assignment probabilities (< 80%). In May, herring measuring 8 cm were identified as NSAS, while the herring of 5-6 cm and 11-16 cm belonged to the Downs population. In October, all fish below 14 cm were classified as Downs, while those above 23 cm comprised a mixture of different populations.

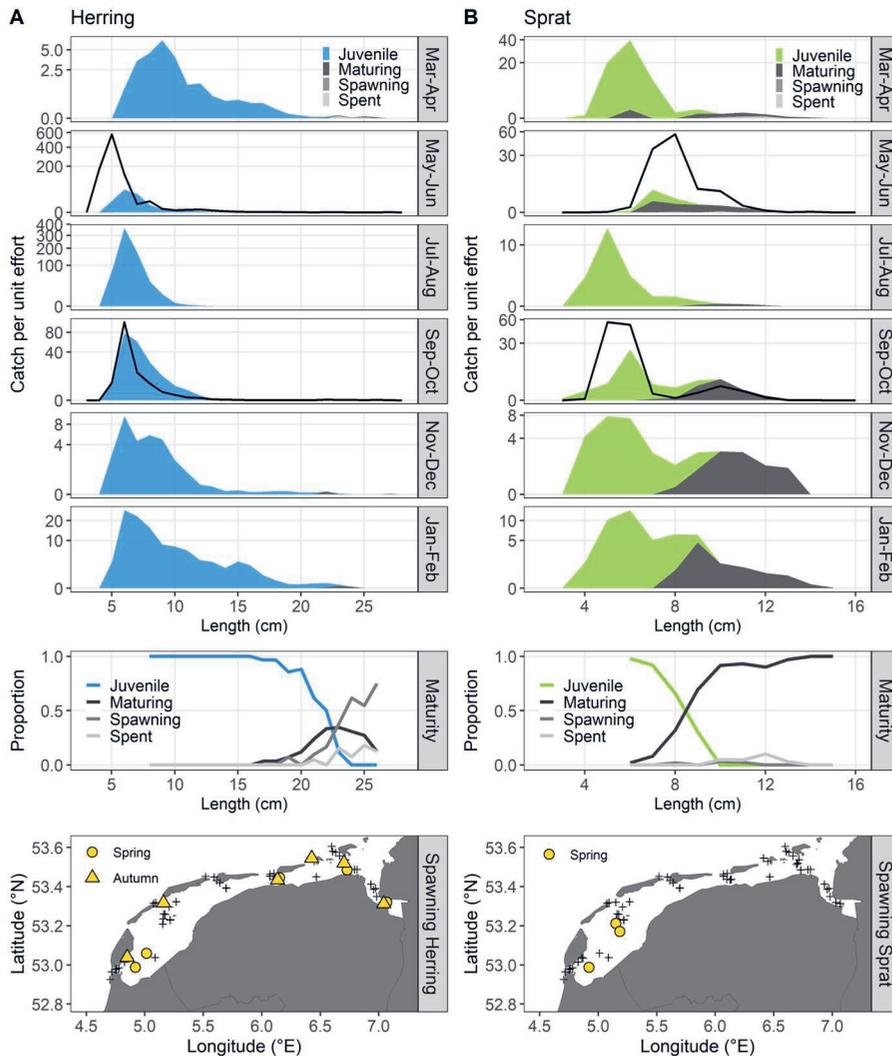


Figure 3. Upper panels: length frequency distributions of small pelagic fish in the Dutch Wadden Sea captured by the stow net survey (coloured areas) per bimonthly period from March 2021 to February 2022, combined with data from the trawl survey in May and October 2022 (black line). The square root scaled Y-axes represent average CPUEs, with the stow net survey measured numbers per 10000 m³ and the trawl survey per minute. Middle panels: proportions of small pelagic fish in specific maturity stages, averaged over both surveys, all months and locations. Lower panels: maps displaying locations where spawning individuals were observed during both surveys, with shapes denoting the period caught, and hauls without spawning individuals marked with '+'. Panels are arranged identically for each species: A) *Clupea harengus*, B) *Sprattus sprattus*, C) *Osmerus eperlanus*, and D) *Engraulis encrasicolus*.

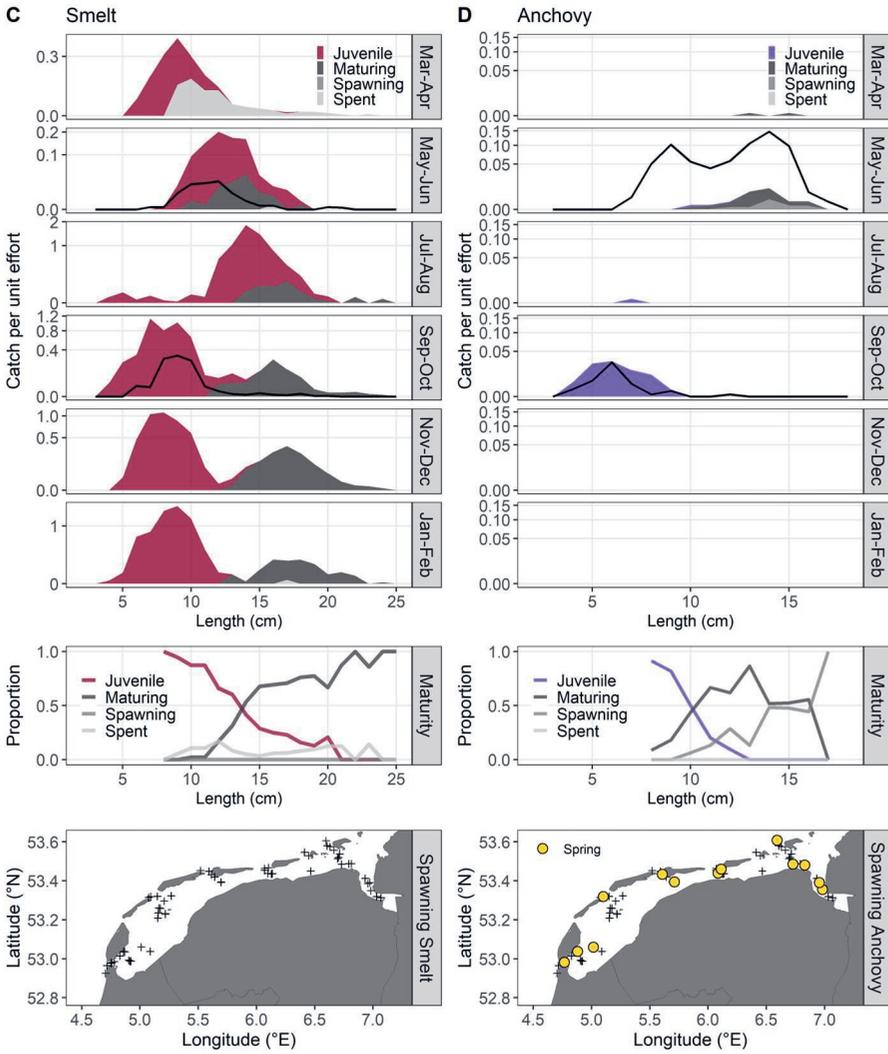


Figure 3. Continued from page 34.

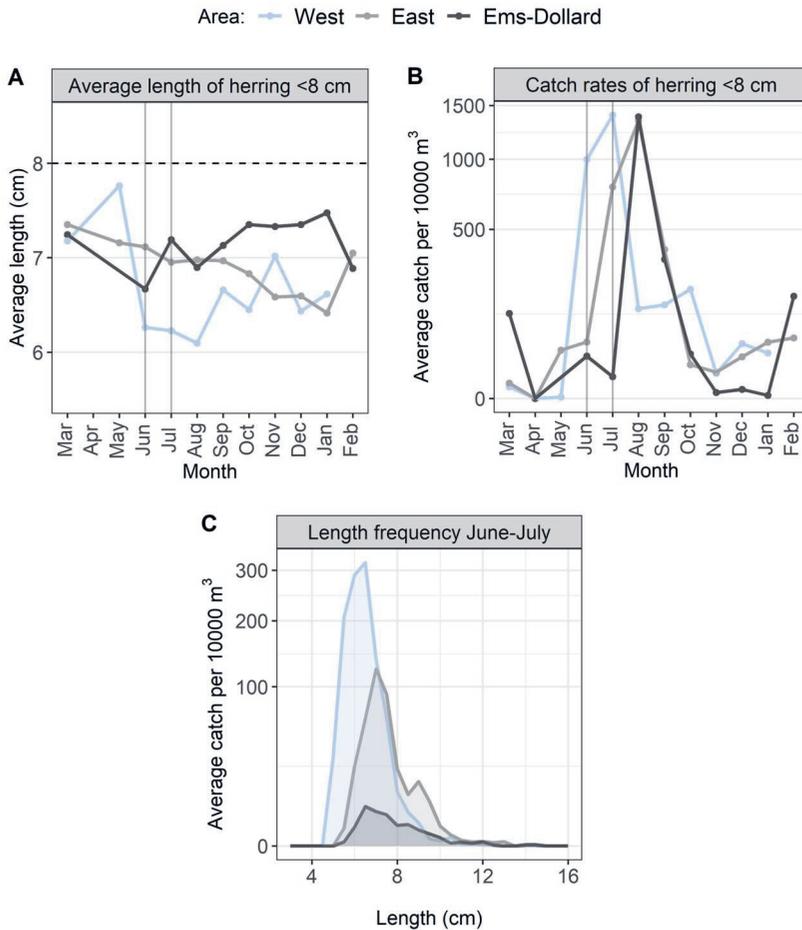


Figure 4. A) Average length of herring smaller than 8 cm per month, with colours indicating area. B) Herring catch per unit effort (CPUE) per month and C) length frequency for herring in the main influx period (June and July) across three areas. CPUE is measured per 10000 m³, and figure is based on monthly stow net data.

Table 2 (page 37). Spawning ground population classification of 36 herring based on genetic analysis and stock assignment model. Area indicates catch location (ED = Ems-Dollard). Maturity stage is indicated as: juvenile (Juv), maturing (Mat) or spawning (Spa). Population shows the most likely population to which the individual belongs: Downs winter-spawners (Downs), North Sea Autumn Spawners (NSAS), Western Baltic Spring Spawners (WBSS), Baltic Autumn-Spawning herring (BASH). Assignment probability that an individual is assigned to the given baseline population and missing SNPs are provided.

Month	Length (cm)	Area	Maturity	Population	Probability	Missing SNPs
May	5.3	West	Juv	Downs	1.00	0
	5.3	East	Juv	Downs	0.97	1
	5.6	East	Juv	Downs	1.00	2
	5.7	West	Juv	Downs	0.99	0
	5.8	West	Juv	Downs	0.99	0
	8.1	ED	Juv	NSAS	0.98	0
	8.3	ED	Juv	NSAS	1.00	2
	8.8	ED	Juv	NSAS	1.00	0
	11.1	ED	Juv	Downs	1.00	8
	11.7	ED	Juv	Downs	0.91	5
	11.9	ED	Juv	Downs	1.00	22
	13.2	ED	Juv	Downs	1.00	2
	13.2	ED	Juv	Downs	1.00	23
	13.7	ED	Juv	Downs	1.00	0
	16.3	ED	Juv	Downs	1.00	36
	22.5	West	Spa	WBSS	0.63	13
22.7	West	Spa	WBSS	0.80	19	
Oct	5.8	West	Juv	Downs	1.00	16
	6.0	West	Juv	Downs	1.00	3
	6.2	West	Juv	Downs	1.00	4
	6.3	West	Juv	Downs	1.00	10
	8.5	East	Juv	Downs	0.93	3
	8.6	West	Juv	Downs	1.00	35
	8.9	East	Juv	Downs	1.00	5
	8.9	East	Juv	Downs	1.00	4
	13.2	West	Juv	Downs	0.98	19
	13.3	East	Juv	Downs	1.00	2
	13.3	East	Juv	Downs	1.00	16
	13.9	West	Juv	Downs	1.00	36
	23.0	West	Spa	BASH	1.00	1
	24.7	West	Mat	NSAS	1.00	2
	24.7	East	Spa	Downs	1.00	2
	26.7	East	Spa	Downs	0.99	7
	26.9	East	Spa	Downs	1.00	0
26.9	West	Spa	NSAS	0.98	0	
27.3	East	Spa	Downs	1.00	2	

3.3 Sprat

Sprat were present year-round. Their sizes ranged from 4 to 15.2 cm, with the majority being smaller than 8 cm (Figure 3B, upper panel). Multiple peaks across the different lengths were observed, notably visible in the monthly length frequency distribution (supplementary Figure S3). In June, the peak of small sprat occurred at 8 cm, while in July, it occurred at 5 cm, suggesting the presence of a new year class. Sprat smaller than 5 cm were consistently observed from July until February. A second peak of YOY emerged in December, although with lower CPUEs than the July peak. The influx of sprat measuring 4-5.5 cm occurred locally, with the highest CPUEs observed in Marsdiep in July, and in Vliestroom in December. The sprat cohort entering the Wadden Sea in July showed a 2 cm increase in length frequency by September, while sprat entering in December reached only 6 cm by March.

Most sprat in the Dutch Wadden Sea were juveniles (Figure 3B, middle panel). However, the first sprat began to mature at 6-7 cm, with 90% reaching maturation by 10 cm. Out of 406 assessed individuals, only 4 spawning sprat and 12 spent individuals were observed, all between March and July. Spawning sprat were exclusively observed in the western Wadden Sea (Figure 3B, lower panel), while spent sprat were also observed in eastern areas. The average size of spawning and spent individuals was 11.5 cm, ranging from 8.8 to 12.7 cm.

3.4 Smelt

Smelt abundance varied by location and month, with the highest mean CPUE observed in October. Smelt sizes ranged from 4.2 to 24.7 cm. The length frequency plot shows two peaks from September to February: one representing YOY and another for older individuals, suggesting the presence of multiple year classes (Figure 3C, upper panel). The influx of YOY started in late summer with a few individuals in July and August, yet the main peak occurred in September and October. YOY smelt were primarily observed in the Ems-Dollard and the eastern Wadden Sea, with only five YOY caught in the western Wadden Sea. Furthermore, the trawl survey also showed highest CPUEs for smelt in October, with dominance of the Ems-Dollard area. However, contrary to the stow net survey, smelt smaller than 8 cm were hardly caught during the trawl survey. Monthly length frequency distributions showed an increase in mean body length from around 5 cm upon influx to 7.5 cm by October, followed by stable lengths over the winter months, and from March onwards clear increase of body length again (supplementary Figure S3).

Most smelts were either juveniles or maturing individuals. From September to February, the first CPUE peak comprised solely of juveniles, while the second CPUE peak consisted of maturing individuals (Figure 3C, upper panel). However, from March onward, maturity was less dependent on size, with juvenile, maturing, and spent individuals observed above 10 cm. At 14 cm, the percentage of maturing smelt surpassed the percentage of juvenile smelts (Figure 3C, middle panel). No spawning individuals were observed in the Wadden Sea (Figure 3C, lower panel), and larger fish were absent in February and March (supplementary Figure S3), indicating adults left to spawn in freshwater. Spent individuals were observed in April, with an average length of 15 cm, which were observed in all inlets except Vlietstroom.

3.5 Anchovy

Anchovy presence in the Dutch Wadden Sea was seasonal, with generally modest abundance (Figure 3D, upper panel). The highest densities were recorded in May, observed across all inlets but highest in the Ems-Dollard area. The trawl survey revealed two peaks in length frequency in May: around 9 cm and 14 cm, with the largest individual measuring 17.5 cm. YOY were observed in autumn, albeit only locally: in September 2021 in Westgat and October 2022 in Marsdiep, with sizes starting at 4.3 cm.

In September-October, all anchovies were juvenile, while in May-June, the individuals were mainly maturing or spawning (Figure 3D, upper and middle panel). Spawning was observed exclusively in May and June and occurred in all inlets (Figure 3D, lower panel). The average size of spawning anchovy was 14.4 cm.

4. Discussion

4.1 Wadden Sea as nursery area for SPF

The presence of juvenile SPF, typically smaller than 10 cm, supports the hypothesis that the Wadden Sea serves as a juvenile habitat. Following Beck et al. (2001), there are four requirements for a juvenile habitat to qualify as nursery: if juvenile fish exhibit 1) higher densities, 2) accelerated growth, 3) improved survival, or 4) more successfully transition to adult habitats compared to other habitats. Below, we discuss the four factors outlined in Beck's paper, using our data and those available in literature.

The requirement of higher densities can be addressed using Tulp et al. (2017). They examined the biomass contribution of herring, sprat, and smelt across the Dutch Wadden Sea and along the Dutch coast. They found that smelt and herring predominantly inhabited the Wadden Sea, with smelt most prevalent in Ems-Dollard, while sprat was more abundant along the Dutch coast. Their study was based on a beam trawl survey, using gear that is not optimal for catching SPF. In the International Bottom Trawl Survey (IBTS), located further offshore, sprat is the dominant SPF species, followed by herring and eventually anchovy (Grift et al., 2004; ICES DATRAS database, 2024). Moreover, in coastal acoustic surveys carried out in 2002-2003 in April and June along the Dutch coast, sprat dominated the catches while herring measuring 6-8 cm were almost absent (Grift et al., 2004). This finding was confirmed by a recent coastal survey in June 2023 (unpublished data Wageningen Marine Research), indicating that YOY herring are more bound to shallow waters like the Wadden Sea compared to sprat. Despite the challenges of comparing fish densities between different regions using fishing methods with different catchabilities, it appears that the Wadden Sea supports substantial numbers of herring, anchovy and smelt compared to neighbouring waters. However, for sprat, this is less clear, as its distribution is more offshore. Within the Wadden Sea, comparing the pelagic habitat with salt marshes, herring and smelt occur in both habitats, while sprat and anchovy were mainly observed in deeper water (Friese et al., 2021). In these salt marshes, only small individuals were observed, frequently measuring below 4.5 cm.

Estuaries are typically highly productive because of nutrient inflow (Cloern et al., 2014) and are often warmer than offshore waters during spring and summer (van Aken, 2008), accelerating growth rates (Peck et al., 2012a). For instance, optimal temperatures for the early life stages of sprat range between 18 and 22 °C (Peck et al., 2012a), which is within the range of the water temperatures we observed in the Wadden Sea from June to September, the period of primary influx of YOY sprat. The seasonal shifts in our length frequency data indicate growth for sprat, smelt and herring. However, quantifying exact growth rates by tracking the size of specific cohorts over time was not feasible here due to immigration and emigration within the year.

Juvenile fish typically face high mortality due to predation. A modelling study investigating the survival of juvenile herring in the Scheldt estuary (southern North Sea), revealed that herring migrating to estuaries during their early life stages exhibited a 10-fold increase in survival compared to those remaining at sea, which was primarily attributed to decreased predation pressure in turbid estuarine waters (Maes et al., 2005). In the Wadden Sea, main predators of SPF include seabirds such as terns, and piscivorous fish such as whiting and sea bass (Maes et al., 2005; Baptist

& Leopold, 2010; Cardoso et al., 2015). These predators primarily rely on sight for hunting, with terns often foraging at the boundary between turbid and clear water (Baptist & Leopold, 2010). Turbid waters may increase the survival rates of juvenile fish by reduced predation or increased plankton availability (e.g. Fiksen et al., 2002; Utne-Palm, 2002; Maes et al., 2005). The mean visibility measured during stow net fishing was 0.8 m, relatively low compared to the North Sea (Capuzzo et al., 2015), indicating that SPF can 'hide' in the Wadden Sea. However, the effect of turbidity on fish depends on species and intensity, as high turbidity can also negatively impact foraging success (Whitfield, 2021).

The presence of open connections through inlets suggests unhindered migration between the Wadden Sea and the North Sea (Couperus et al., 2016). However, for the anadromous smelt, obstacles like the Afsluitdijk hinder the connection with freshwater. Determining the contribution of juvenile habitats to adult populations, is essential for assessing the importance of the Wadden Sea on a population level, which requires large-scale sampling (e.g. van der Veer et al., 2024). Additionally, studying otolith microchemistry could potentially reconstruct fish movements and addressing natal origins through analysis of element profiles along otolith axes (Nachón et al., 2020).

In summary, the Wadden Sea exhibits relative high densities of juvenile SPF compared to neighbouring areas, it has the potential for fast growth and increased survival rates, and offers good migration possibilities to contribute to the adult populations (apart from smelt). The Wadden Sea thus qualifies as a nursery area for SPF based on these criteria. Long-term trends show that the nursery function of the Wadden Sea is reduced for several flatfish species since the 1990's (van der Veer et al., 2011, 2022). Yet, there is no historical reference for SPF, making it difficult to assess long-term trends in their nursery function in the Wadden Sea. SPF have not been regularly sampled using pelagic gear, so historical records are limited. However, data from demersal surveys (which are suboptimal for pelagic fish) suggest a pattern of increasing pelagic species densities during the late 20th century, followed by a gradual decrease in recent decennia (Tulp et al., 2017).

4.2 Wadden Sea as spawning habitat for SPF

Spawning grounds vary between species and reproductive strategy. Anchovy and sprat are indeterminate multiple-batch spawners whose eggs are released in the water, while herring and smelt are determinate single-batch spawners that deposit eggs on suitable substrates (Belyanina, 1969; Petitgas et al., 2010; Frost & Diele, 2022). Historically, before the construction of the Afsluitdijk, anchovy, herring and smelt spawned in the Zuiderzee in large numbers, supporting an important fishery

around the 1930s (ICES, 1903; Redeke, 1939; de Jonge et al., 1993). However, the closure of the Afsluitdijk drastically changed the ecosystem: a transition from an estuarine bay to a freshwater lake. This led to the disappearance of herring shortly after closure, and two decades later, anchovy also disappeared (Redeke, 1939; de Jonge et al., 1993; Boddeke & Vingerhoed, 1996; Petitgas et al., 2012). One of our objectives was to study if remnants of these historic spawning aggregations still occur. We observed spawning herring both in spring and in autumn. Spawning occurred in various inlets in the Wadden Sea, with a notable concentration in the northern part of the Lauwers inlet during autumn. In May, the highest CPUE for herring was recorded in the Lauwers inlet, with a clear size peak around 5 cm. Additionally, many spent herring were found north of Schiermonnikoog in January 2024 (unpublished results Wageningen Marine Research). These findings suggest a potential local spawning aggregation north of Lauwers, with subsequent juvenile development occurring in the Lauwers inlet. Additional surveys for eggs and larvae, complemented with genetic or microchemistry analysis, are necessary to confirm this.

Genetic analysis showed that spawning herring in October included both autumn-spawning and winter-spawning populations, with the latter group found in the Lauwers inlet. Two spring-spawning individuals revealed low assignment probabilities, suggesting they may belong to a population not represented in the baseline data. These two individuals, currently assigned to WBSS, may actually belong to a local coastal population. Historically, herring spawned in the Zuiderzee from mid-April until late May, providing a spawning ground with shallow brackish water, where eggs adhered to a substrate of firm sand and mud (ICES, 1903). Generally, herring attach adhesive eggs to coarse seabed substrates or aquatic vegetation such as coarse sand, gravel, small rocks, shells, kelp, and other macroalgae, in habitats with strong currents to improve oxygenation and prevent sediment build-up on eggs (Frost & Diele, 2022). Presently, the largest spring-spawning populations are found in the Norwegian and Baltic Sea. Part of the herring along the UK and Irish coasts also spawn in spring, although these populations are generally smaller (Dickey-Collas et al., 2009; Petitgas et al., 2010). Sightings of spring-spawning herring have been recorded for the German Wadden Sea in the past decade: spawning herring have been observed in the Jade Bay in April, and herring eggs were found on harbour walls in Wilhelmshaven (personal communication A. Dänhardt). Additionally, in May 2013, substantial quantities of herring eggs were discovered on *Fucus* macroalgae in the Hörnumtief and the Holmer Siel (personal communication H. Büttger). To determine the origins of the spring-spawning herring in the Dutch Wadden Sea, and whether they are possible remnants from the old Zuiderzee stock, large-scale sampling of early life stages and genetic analyses is required.

Another species potentially remnant from a Zuiderzee stock is the anchovy. Currently, the main spawning areas for anchovy are located south of the North Sea, in the Bay of Biscay, and the Mediterranean (Bellier et al., 2007). In the Netherlands, spawning occurred historically in the northern part of the Zuiderzee, the upper section of the Dollard, and in the Oosterschelde estuary in June and July (ICES, 1903). Following the closure of the Zuiderzee, smaller anchovy spawning areas persisted in the Oosterschelde estuary, and anchovy eggs were detected again in 1993-1994 in the western Dutch Waddenzee (Boddeke & Vingerhoed, 1996). Water temperature and salinity are key factors in determining suitable spawning conditions for anchovy, with a water temperature of 14 °C as the lower threshold for spawning (Ibaibarriaga et al., 2007). In our study, spawning anchovy were observed at temperatures close to this lower threshold. Although anchovy abundance in the North Sea fluctuates between years (Petitgas et al., 2012), there has been an increase in anchovy populations since the 1990s (Alheit et al., 2012). This increase was likely due to the expansion of suitable thermal windows, as warmer summers and fewer severe winters improved the productivity (Petitgas et al., 2012). Genetic analysis has shown that the North Sea and English Channel anchovy constitute a distinct population from the Bay of Biscay population (Petitgas et al., 2012). Therefore, it is plausible that anchovy spawning in the Wadden Sea represents part of local remnant populations that benefitted from improved thermal conditions in recent decades.

Historically, smelt also spawned in low saline areas of the Zuiderzee (Redeke, 1939). While smelt can complete nearly its entire life cycle in the Wadden Sea, it depends on connectivity to freshwater for spawning. Currently, only two sluice complexes provide connections between the estuarine Wadden Sea and freshwater Lake IJssel. However, excess freshwater is discharged during low water, resulting in a short migration window, a sudden transition between salt- and freshwater, and high water velocity. Microchemistry analysis showed no substantial contribution from the anadromous smelt to the spawning stock of the landlocked population (Tulp et al., 2013), suggesting no substantial contribution to the Lake IJssel population by anadromous smelt. Consequently, the primary spawning locations for anadromous smelt in the Wadden Sea nowadays are likely located upstream in the unobstructed rivers Ems and Elbe (Eick & Thiel, 2014; Jager et al., 2019). This is supported by long-term stow net monitoring in the Ems-Dollard, showing large abundances of YOY smelt in late September upstream in the Ems river (Kopetsch, 2023).

In sprat, approximately 50% reach maturity at a size of 8 cm according to IBTS data in the North Sea (Heessen et al., 2015). In our sample of 137 individuals larger than 8 cm between March and August, only 3% were found to be spawning, and 8% were spent, suggesting that the Wadden Sea is not a primary spawning ground for

sprat. Typically, sprat spawning areas are situated in the German Bight, Southern Bight and English Channel (Baumann et al., 2009; Munk et al., 2024), indicating that sprat generally spawns further offshore.

Concluding, the presence of spawning individuals in the Wadden Sea suggest that the area still provides spawning grounds for herring and anchovy. Meanwhile, limited spawning was observed for sprat and no spawning for smelt.

4.3 The life cycles of SPF using the Wadden Sea

Having identified the role of the Wadden Sea as nursery and spawning areas for the four species, we now examine their entire life cycles. Herring, the dominant juvenile representative, arrives in the Wadden Sea as clupeid post-larvae or YOY and remains as a juvenile to grow and develop. However, the abundance of herring larger than 12 cm was relatively low, indicating that most larger herring leave the Wadden Sea. The primary influx period of YOY herring occurred from late May onwards. Surprisingly, small herring were observed throughout the year, suggesting that the Wadden Sea is used as juvenile habitat by multiple herring populations. Our findings support our third hypothesis that herring in the Wadden Sea originate from the Downs winter-spawning population: 78% of the sampled individuals were Downs herring. This indicates a clear connectivity for herring between habitats in the English Channel and the Wadden Sea. Additionally, a west-east gradient in YOY herring length was observed: size was generally lowest in the western area, and an increase in abundance of small sized herring started initially in the western inlets. Driven by the counterclockwise residual circulation in the North Sea, herring offspring are transported north-eastward (Dickey-Collas et al., 2009), resulting in the initial influx of juveniles into the westernmost inlets of the Wadden Sea. While the primary peak of herring influx can now be attributed to autumn and winter spawners from southwestern waters, the origin of the smaller herring entering around November until January remains unclear.

Like herring, sprat arrives in the Wadden Sea as post-larvae or YOY, remaining to grow and develop. While most individuals were under 10 cm, sizes of up to 15 cm were observed regularly. Given that sprat rarely exceeds 16 cm (Peck et al., 2012a), they can complete a substantial portion of their life cycle in the Wadden Sea. However, the origin of sprat in the Dutch Wadden Sea remains unclear. A study in the German Bight showed that sprat present in August (~7 cm) originated mainly from an area north of the Wadden Sea and were hatched from mid-March to mid-May (Baumann et al., 2009). Multiple cohorts were present, including a new cohort in October (~4 cm), which were born in July (Baumann et al., 2009). This situation is similar to what we observed in the Wadden Sea, indicating that sprat in the

Wadden Sea originate from a wide range of North Sea spawning grounds and periods.

In contrast to herring and sprat, smelt eggs are deposited in freshwater and juveniles enter the Wadden Sea from freshwater areas. The Wadden Sea provides juvenile habitat for smelt, which typically enter at sizes around 5 cm in autumn, and exhibit clear growth throughout the season. Smelt show a distinct spatial distribution, with higher concentrations in the eastern part, close to the freshwater outflow of the river Ems. In the Netherlands, smelt exhibit two distinct life history strategies: a migrating anadromous population and a landlocked freshwater population in Lake IJssel. The level of interchange between these two smelt populations is still unclear. Currently, there is no evidence for a substantial contribution from the anadromous population to the spawning stock of the landlocked population (Tulp et al., 2013). However, large numbers of YOY landlocked smelt flush out with discharge water through the Afsluitdijk sluices into the Wadden Sea, with a peak in October (Tulp et al., 2013). Despite frequent captures of smelt along the Afsluitdijk in fyke monitoring (van Rijssel et al., 2023), we did not observe an influx of YOY freshwater smelt into the Western Wadden Sea. This might be caused by the study locations away from freshwater inlets, further towards the North Sea.

Anchovy used the Wadden Sea only seasonally: they spawned in May and the YOY were briefly present in autumn. Being at the northern part of its distribution range, temperature plays a crucial role for anchovy (Raab et al., 2013). Temperatures between 17 and 19 °C during the growing season had a strong positive effect on the anchovy CPUE the following year, indicating such temperatures are advantageous for growth and increase the overwinter survival of YOY anchovy (Raab et al., 2013). In September, the water temperature in the Wadden Sea was still 17 °C, but dropped quickly thereafter, potentially explaining why YOY anchovy were absent after October. Similar seasonal behaviour was observed in the German Wadden Sea, where adult anchovy were present in June but migrated away to deeper waters after spawning (Alheit et al., 2012).

Our study primarily focused on post larval stages. To fully comprehend the role of the Wadden Sea in the full life cycle of SPF, additional studies are necessary. These should address questions such as whether the YOY are locally produced, and when and where these early life stages occur. Conducting egg and larvae surveys, along with techniques like otolith daily increment analysis will be essential (Baumann et al., 2009). Knowledge on the role of the Wadden Sea within the entire life cycle of SPF is crucial for effective management, both for the sustainability of populations as well as their functioning within the Wadden Sea ecosystem as important prey of higher trophic levels and as predators of zooplankton (Maathuis et al., 2024b).

This study examined the role of a shallow tidal ecosystem in the life cycle of four SPF species. Globally, research on the nursery value of coastal pelagic habitats is limited, with most studies focusing on subtidal soft bottoms, seagrass beds, and mangroves (Ciotti et al., under review), or on SPF in deeper waters due to sampling challenges in shallow depths (Brehmer et al., 2006; David et al., 2022). Yet, shallow coastal waters are widely acknowledged as critical habitats for many migratory pelagic fish species during part of their life cycle (e.g. Chícharo et al., 2012; Polte et al., 2017; David et al., 2022). Notably, in the Bay of Biscay, David et al. (2022) observed significantly higher SPF biomass, as well as larger and denser shoals, in shallow areas (< 20 m) compared to deeper offshore waters. Furthermore, they found higher SPF densities in summer compared to autumn. While we observed year-round use of the Wadden Sea by SPF, our study also revealed seasonal variations in abundance, species composition and size structure. These findings highlight the importance of shallow nearshore habitats for different life stages of different SPF species throughout the year.

Conclusions

Our study represents one of the first dedicated efforts to comprehensively investigate SPF within the Dutch Wadden Sea, both in terms of temporal and spatial coverage. Our findings highlight the Wadden Sea's importance as a juvenile habitat for SPF, and its role in providing spawning grounds, particularly for herring and anchovy. The relative high densities of juvenile SPF compared to neighbouring areas, the potential for fast growth, possible increased survival rates due to favourable turbid conditions and open migration possibilities towards the North Sea, support the qualification of the Wadden Sea as a potential juvenile nursery area for SPF. However, further studies are needed to evaluate the contribution to adult populations compared to neighbouring areas. Our research reveals that multiple cohorts of herring and sprat from various North Sea spawning grounds use the Wadden Sea for growth and development. Genetic analysis indicated that most herring originated from the Downs spawning population. Furthermore, adult herring were observed in spawning stage during both spring and autumn, suggesting potential local spawning aggregations. While smelt can complete nearly its entire life cycle in the Wadden Sea, it relies on freshwater connectivity for spawning. Anchovy, a seasonal visitor, arrived in May to spawn, with YOY briefly appearing in autumn. Additional research on early life stages of SPF is essential for a comprehensive understanding of the role of the Wadden Sea in their life cycles.

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Supplementary material

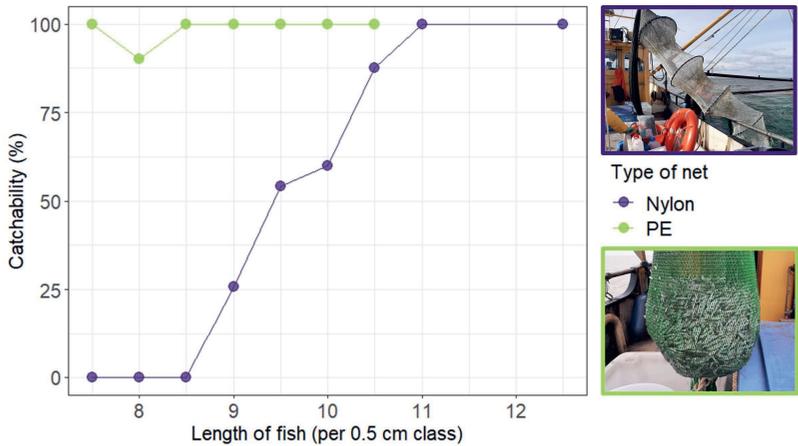


Figure S1. Catchability of the two types of net used in stow net survey. Polyethylene (PE, green) codends were used in all months except April when a nylon net (purple), including hoops, was used. Total sample size was 200 herring and sprat.

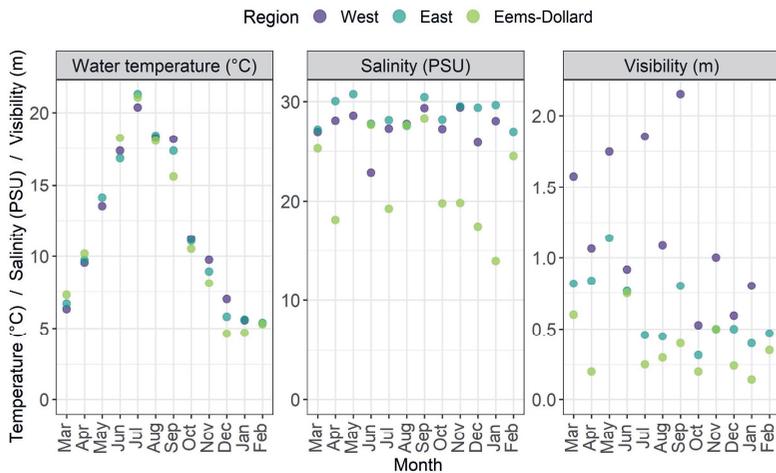


Figure S2. Environmental data from the Dutch Wadden Sea spanning March 2021 to February 2022, including water temperature (°C) and salinity measured by a CTD, and visibility (m) assessed using a Secchi disk. All measurements were conducted during fishing operations as part of the stow net survey. Due to stormy weather conditions, measurements were not taken in all regions every month.

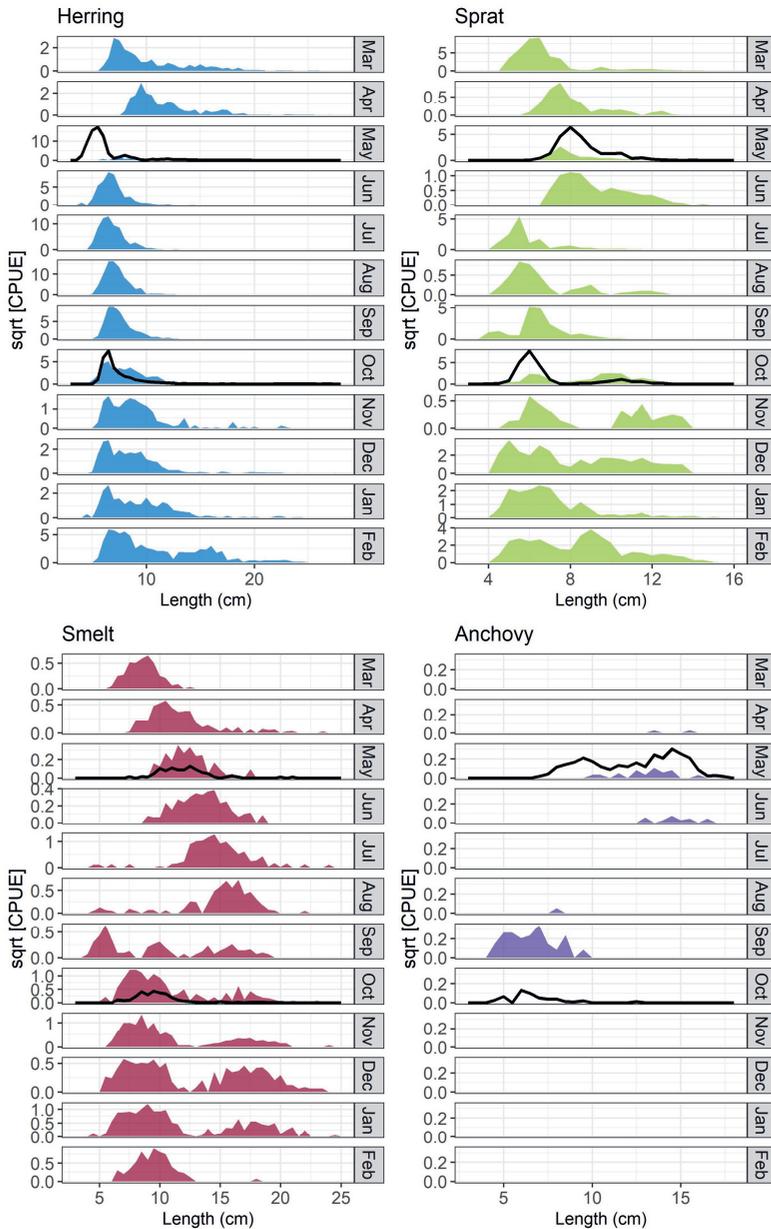


Figure S3. Length frequency distributions of *Clupea harengus*, *Sprattus sprattus*, *Osmerus eperlanus* and *Engraulis encrasicolus* in the Dutch Wadden Sea. Square root transformed catch per unit effort (CPUE) data from March 2021 to February 2022 are shown from the stow net survey (coloured areas), alongside May and October 2022 trawl survey data (black lines). The Y-axes represent CPUE (aggregated per 0.5 cm class), with the stow net survey measured per 10,000 m³ and the trawl survey per minute trawling.



Chapter 3

Resolving the variability in habitat use of juvenile small pelagic fish in a major tidal system by continuous echosounder measurements

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Abstract

Shallow coastal areas are important nurseries for larvae and juveniles of many fish species. However, empirical data on small pelagic fish (SPF) in such regions are lacking, and temporal variability in abundance and habitat use by SPF are unknown. Given the critical role of SPF as a trophic link, their commercial value and their sensitivity to climate variability, there is a need for a quantitative and high-resolution monitoring method. We deployed a bottom-mounted echosounder, combined with a water current profiler, to examine the temporal variation in density, vertical distribution, and movement behaviour of SPF in the Marsdiep, a dynamic tidal inlet between the North Sea and the Wadden Sea. The acoustic setup provided year-round records of fish density distribution in the water column every 90 min. Monthly fish samples were collected to help interpret acoustic observations. Our analyses indicated that the Marsdiep is a migration gateway for SPF from the North Sea into the Wadden Sea, particularly for juvenile herring and sprat. We observed clear seasonality with high fish densities from mid-spring to early autumn. Fish typically used the upper half of the water column. Swimming behaviour was primarily driven by currents. Yet, during outgoing tide SPF, resisted the outflowing current, suggesting efforts to remain in the Wadden Sea, supporting the hypothesis that the Wadden Sea serves as a preferred nursery area for SPF. Our high frequency, multi-seasonal and vertically resolved observations provide unique insight into the use of the Marsdiep by SPF. Furthermore, potential applications of autonomous echosounder moorings are discussed.

1. Introduction

Estuaries and tidal basins around the globe serve as nursery areas for fish (Beck et al., 2001; Baumann et al., 2009), where adult fish either spawn directly, or their larvae drift from further offshore into these areas. Larvae and juvenile fish develop here, and after reaching a certain stage eventually move offshore where they spend the rest of their lives. This pattern also occurs in small pelagic fish (SPF) that provide the trophic link between zooplankton and piscivorous fish, birds and marine mammals. Early life stages of SPF such as Atlantic herring (*Clupea harengus*, hereafter herring) and European sprat (*Sprattus sprattus*, hereafter sprat) drift from the North Sea via tidal inlets into the largest intertidal area in the world, the Wadden Sea (Zijlstra 1978; Dickey-Collas et al., 2009; van der Veer et al., 2015; Couperus et al., 2016). The Wadden Sea is a system of tidal channels and intertidal sand and mud flats, and because of its unique properties, it is listed as a UNESCO World Heritage site. In the Wadden Sea, the biomass of SPF is considered the dominant component of the overall fish biomass; yet, the assemblage of SPF occurring in the Dutch Wadden Sea has hitherto received little research attention (Couperus et al., 2016). We lack empirical data concerning, for instance, SPF quantity, age, growth, and temporal migration patterns in this area. Given the central role of SPF in the Wadden Sea ecosystem, insight into the functioning of the food web has so far been limited by this knowledge gap.

Given their short generation times, tight coupling to lower trophic levels, sensitivity to climate variability and aggregation behaviour, SPF populations are known for significant variations in abundance and spatial distribution (Axenrot et al., 2004; Peck et al., 2021). Particularly in the current era of global climate change, where fish populations experience shifts in abundance, distribution and phenology (Rijnsdorp et al., 2009; Sydeman et al., 2015), the variation of occurrence in time and space is increasing even further. Therefore, the study of SPF occurrence and behaviour requires long-term high-resolution acoustic monitoring. However, tidal channels are highly dynamic, and hence complicated to study (Fraser et al., 2017). Yet, the use of hydroacoustic techniques is the only suitable option for long-term continuous monitoring at such sites, because high-frequency trawling is impractical and destructive, while use of cameras is impossible due to limited visibility.

Active acoustic techniques are widely used for continuous monitoring (Klemas, 2013; Benoit-Bird & Lawson, 2016). Recently, the use of bottom-mounted echosounders has become more widespread, for example, to explore vertical migration and swimming behaviour of mesopelagic fish (Kaartvedt et al., 2009), to assess the impact of noise on SPF around windfarms (Kok et al., 2021), to study overwintering sprat in a Norwegian fjord (Solberg et al., 2012), and to evaluate if a sparse echosounder mooring array could accurately produce fish abundance

indices (De Robertis et al., 2018). In addition, the ecological effects of tidal stream turbines on fish behaviour are often studied using bottom-mounted echo-sounders (Viehman & Zydlewski, 2017; Williamson et al., 2019; Scherelis et al., 2020). Solberg et al. (2012) showed that a bottom-mounted echosounder has several advantages: a stable platform, its non-intrusive nature and its possibilities for long operation times and large data-storage capacity.

Due to the technical challenges imposed by the highly dynamic nature and shallow depth of tidal habitats, knowledge on responses of pelagic fish to dynamic physical conditions is limited. Although most of the Wadden Sea is shallow, with depths typically below 15 m, the inlets between the barrier islands that connect the Wadden Sea to the adjacent North Sea are up to 40 m deep. The largest and westernmost inlet of the Dutch Wadden Sea is the Marsdiep. Herring larvae originating from the English Channel are transported northeast, and a proportion enters the Wadden Sea via this inlet (Dickey-Collas et al., 2009). In addition, tidal inlets are important as feeding areas for predators, as demonstrated by a study of porpoise distribution in the Marsdiep (IJsseldijk et al., 2015). The study showed that porpoise distribution was driven by tidal forces, likely due to the availability of SPF. Therefore, gaining knowledge of SPF behaviour and occurrence as a major food source in the Wadden Sea ecosystem is needed to understand the occurrence and behaviour of their predators. Furthermore, insight in the (timing of) vertical distribution of SPF in the water column is important for understanding feeding patterns and population dynamics of surface-feeding seabirds (Baptist & Leopold 2010; Dänhardt et al., 2011). Moreover, herring and sprat are commercially important species, and their status is assessed annually using indices based on surveys in the North Sea, conducted during specific periods (ICES, 2022). However, these seasonally static surveys might not capture phenological changes induced by climate change.

This study is the first to continuously sample fish distribution and current speed in a major inlet into the Wadden Sea for an entire year, using a bottom-mounted autonomous echosounder and a current speed profiler, to better understand SPF density, vertical distribution and movement behaviour. The current speed data from the mooring were supplemented with ferry-borne acoustic Doppler current profiler (ADCP) data of the Marsdiep (van der Molen et al., 2022). Fish density and vertical distribution were investigated for their relation to temporal and environmental variables, including date, time of day, tidal cycle, wind speed, wind direction and lunar cycle. Swimming speed and direction of individual fish were compared to speed and direction of water currents to study fish swimming behaviour and examine how they adapt their direction and speed to the surrounding water currents.

The study had three objectives. The first objective was to quantify the temporal variability in fish density in the Marsdiep during an entire year and examine its relation to environmental factors. Many processes in the life cycle of SPF, for instance spawning and life-stage transitions, are influenced by factors including water temperature and prey abundance (Peck et al., 2013) and therefore are bound to specific time periods. Hence, coastal areas frequently exhibit seasonal utilization patterns by early life stages of fish (Guerreiro et al., 2021). For our study area, it is expected that juvenile SPF drift and migrate towards the coastal zone during spring (Baumann et al., 2009; Dickey-Collas et al., 2009; van Walraven et al., 2017b), where they feed and grow, resulting in the highest densities during summer. In autumn, colder Wadden Sea temperatures (van Aken, 2008), cause the fish to migrate towards deeper water, resulting in lower abundances during winter. Accordingly, it is expected that seasonality is the most important predictor for SPF density in the Wadden Sea. The second objective was to investigate the temporal variability in vertical fish distribution. The most critical predictor for vertical fish distribution is expected to be time of day, due to the influence of light intensity on feeding, aggregation behaviour and predator avoidance (Axenrot et al., 2004; Didrikas & Hansson, 2009). The third objective was to study the movement behaviour (i.e. swimming speed and direction) of individual fish in relation to tidal currents. Early life stages of many marine fish are unable to outswim water currents, but there is a significant variation in swimming capacity between species and life stages (Peck et al., 2012b). Critical swimming speeds of 0.2-0.9 ms⁻¹ have been reported for juvenile herring (Moyano et al., 2016); therefore, we expected that the swimming capacity of SPF is insufficient to outswim the strongest currents in the Marsdiep reaching up to 2 ms⁻¹.

2. Methods

2.1 Study location

Hydroacoustic data were collected in the Marsdiep, the westernmost inlet of the Wadden Sea (Figure 1). The Marsdiep is a well-mixed tidal inlet with a salinity of around 30 PSU (Buijsman & Ridderinkhof, 2007). Here, discharged freshwater from Lake IJssel meets water masses from the North Sea that are moving north-eastward along the Dutch coast. The Marsdiep is about 4 km wide and is the deepest inlet of the Dutch Wadden Sea, with a maximum depth of over 40 m. The Marsdiep seafloor consists of medium-sized sand, with sand dunes several meters high (Buijsman & Ridderinkhof, 2007). The tidal currents in this area are strong, reaching a maximum of 2 ms⁻¹, and the average tidal range at Den Helder is approximately 1.4 m. Due to

tidal asymmetry, incoming currents are stronger, whereas the duration of outgoing currents is longer (Buijsman & Ridderinkhof, 2007).

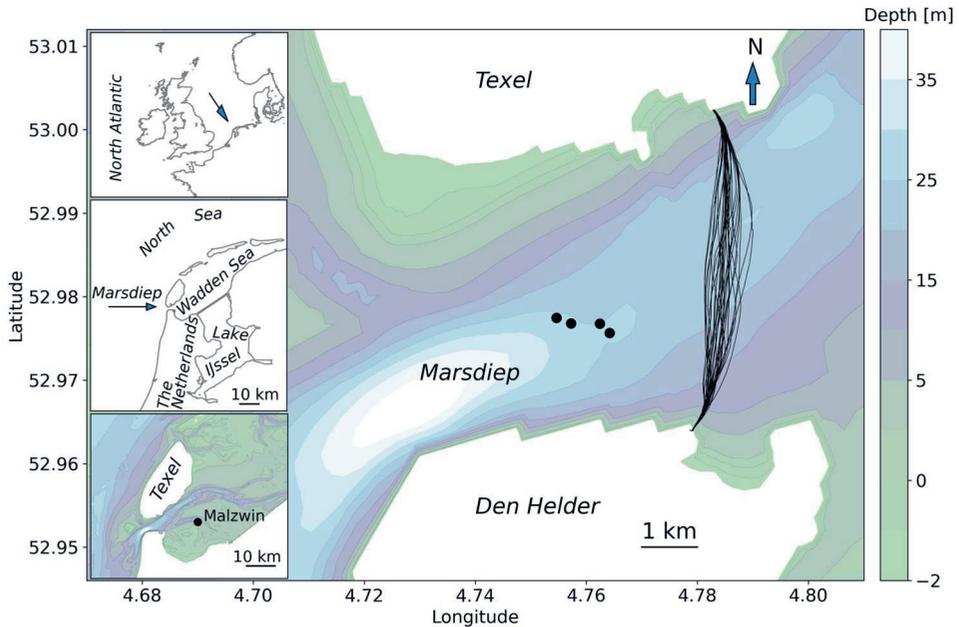


Figure 1. Study area: Marsdiep - the westernmost tidal inlet of the Wadden Sea located between the island Texel and the Dutch mainland. Contours depict depths (m) below mean sea level, black dots indicate the WideBand Autonomous Transceiver (WBAT) locations, and black lines depict a selection of ferry crossing trajectories. Insets give an overview of the wider area, zooming in from top to bottom. The black dot labelled 'Malzwin' indicates the stow net fishing location.

2.2 Acoustic data collection

Data were collected using a bottom-mounted WideBand Autonomous Transceiver (WBAT) echosounder and an ADCP attached to a stainless-steel frame (Figure 2). The battery powered Simrad EK80 WBATs were equipped with a 18° split-beam transducer at 38 kHz, and a 7° single beam at 200 kHz. Only the data collected by the 38 kHz transducer were analysed for this study. The Nortek 2 MHz Aquadopp ADCP collected information on water velocity, temperature, azimuth, pitch and roll. Transducers were positioned 0.9 m (i.e. the height of the frame) above the sea floor.

The total deployment period was 12 months ranging from 18 March 2021 until 20 March 2022, with seven revisits for recovery and deployment operations (supplementary Table S1). All moorings were deployed within 350 m of location

52° 58.60'N and 4° 45.57'E, where bottom depth varied between 26 and 29 m. A pilot test was conducted to test the effect of WBAT position on acoustic measurements of fish density, revealing no significant difference in measured backscatter between the locations (for details, see supplementary Text S1 and supplementary Figure S1). WBAT calibration was carried out 1.5 months before deployment, using a 38.1 mm diameter tungsten sphere (Demer et al., 2015); for details, see supplementary Table S2).

The WBATs were programmed to transmit pulses of 256 μ s in narrowband mode at a ping interval of 0.4 s with a transmission power of 113 W, with the exception of deployments B and C, where a ping interval of 0.25 s was used. Wake-up intervals of 1.5 hours were used with 12 'short' (minimum 72 sec) and 4 'long' (16 min) measurements per day (referred to as 'sampling interval'). Statistical analysis showed that any short recording would generate the same mean backscatter as the longer recordings (see Text S1 for details).

The ADCP mounted on the frame was programmed to wake up every 10 min to measure current speed and direction. However, it was only able to measure the bottom 10 m of the water column. Therefore, additional ADCP data from a nearby ferry-based time series was used to fill the gaps. This ferry-based ADCP dataset consists of measurements during crossings with 30 min intervals in the Marsdiep each day from 06:00 until 21:00 using two Teledyne RD Instruments Workhorse Monitor 1200 kHz ADCPs mounted at the front and rear of the ferry 4.5 m below the sea surface, and measured at 0.5 m depth intervals. The data of both ADCPs were combined and averaged over 100 latitudinal intervals along each crossing (van der Molen et al., 2022). The sampling locations of the two ADCP datasets differed by about 2 km. The frame-mounted ADCP data from March and April 2021 were compared to the ferry ADCP data to evaluate if both produced comparable results. A hydrodynamic model simulation using the model of Duran-Matute et al. (2014) indicated up to 0.2 ms^{-1} faster current speeds at the mooring location in the Marsdiep compared to the ferry crossings, suggesting a need for correction. A conversion factor was derived based on the comparison between the two ADCPs over coinciding measurements in depth and time using a least squares fit. Although the ADCP on the ferry recorded data throughout the entire crossing, only a subset of profiles corresponding to a 200 m section at the centre of the inlet was used for this study.

Sea-level height (cm) data were obtained from Rijkswaterstaat from measurements in the Marsdiep relative to the Amsterdam Ordnance Datum (or 'NAP', <http://waterinfo.rws.nl>). Furthermore, hourly wind speeds and directions for this area were obtained from the Royal Dutch Meteorological Service (KNMI, <https://daggegevens.knmi.nl>).

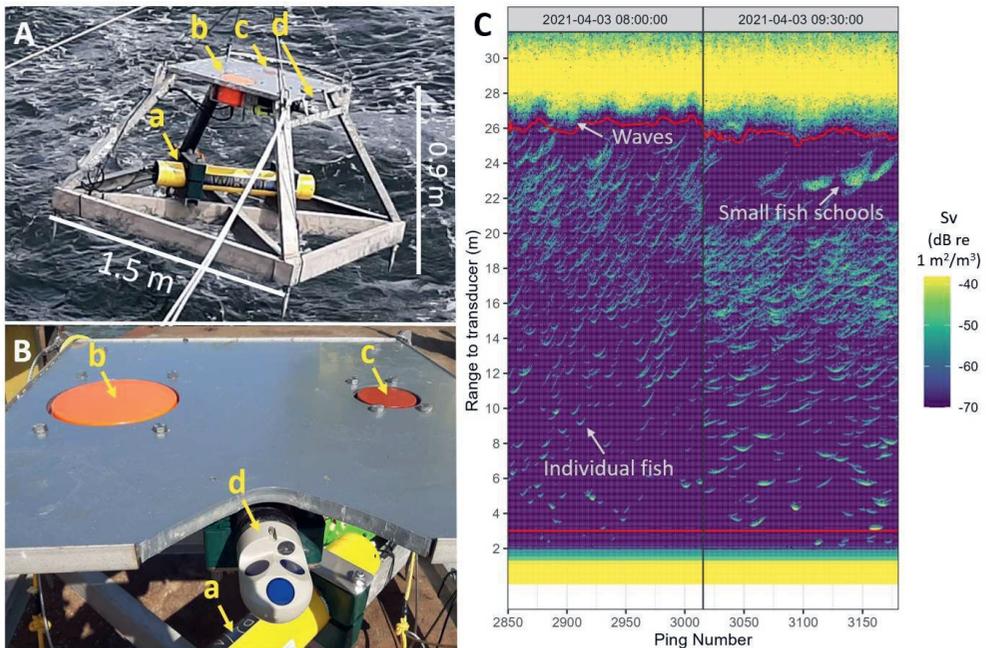


Figure 2. Acoustic mooring setup shown in A) side view and B) top view. The setup includes a WBAT transceiver (a) connected to a 38 kHz split beam (b) and a 200 kHz single beam transducer (c), and an Aquadopp acoustic Doppler current profiler (d). The dimensions of the frame are indicated in white. C) Echogram of two samples taken on 3 April 2021, to illustrate a typical echogram. The y-axis represents the range from the transducer (m), and the x-axis represents the ping number. The red lines indicate the bottom and surface exclusion lines, and the colour represents the volume backscatter (S_v in dB re $1 \text{ m}^2/\text{m}^3$). The yellow objects within the two red lines are recorded traces of fish and fish schools.

2.3 Data collection on fish species composition and size

To help interpret the acoustic observations, fish species composition and fish size distribution were determined during daytime ship-based stow net fishing in the Marsdiep area at location Malzwin (coordinates: $52^\circ 59.22'N$ and $4^\circ 55.29'E$, Figure 1) once a month in March, May, June, July, September, November, and December 2021. Due to bad weather, no samples were taken in April, August and October 2021, and January and February 2022. Stow net fishing is a passive fishing method using water currents. Hence, sampling was done during highest current speeds at both incoming and outgoing tide. Due to strong winds only incoming tide was sampled in September and December, and only outgoing tide in November. The average visibility at the sampling location was 0.9 m, the average depth was 5.5 m,

and the average sampling duration was 44 min. The net covered the entire water column, and had a stretched mesh size of 20 mm. Although gelatinous organisms were also caught, the focus here was solely on fish, which were measured to the nearest mm. By using a flowmeter (General Oceanics, USA) in front of the net, the catch rate per 1000 m³ water was calculated.

2.4 Acoustic data processing

Processing and visualisation of acoustic backscatter data was done using Echoview® 13 (Echoview Software Pty Ltd, Hobart, Australia). Processing included data calibration, echogram cleaning, individual target detection and tracking. The primary cleaning involved the removal of noise at the surface caused by wind-induced bubbles. The noise boundary was determined by creating lines using 'best bottom candidate' and 'threshold offset' algorithms in Echoview, and further editing these lines manually. Additional sections affected by other noise, such as multiple surface reflections, were masked by manual editing ('bad regions' in Echoview). This procedure was applied to the entire dataset until all significant noise, spikes, ping dropouts and other bad regions were masked. The first 3 m above the transducer were excluded due to transducer nearfield and ringing effects (Simmonds & MacLennan, 2005). All manual steps were carried out by the same person twice to ensure consistency. The observed fish data included different kinds of aggregations, such as densely packed small schools, layered shoals with varying depth and density, individual targets and a few large schools. These formations were collectively used to calculate a numerical density indicator: volume backscatter (S_v). The individual targets were further isolated and identified by a tracking algorithm to assess the behavioural patterns and estimate approximate size of individuals. Further data analysis was done using R (version 4.1.0) and RStudio (version 1.3.959).

2.4.1 Volume backscatter

After cleaning in Echoview, echo integration values were exported using a lower S_v threshold of -65 dB re 1 m²m⁻³. The exported backscatter, as a proxy for SPF density, is considered a quantitative 'ecological unit', rather than being classified into specific species. Jellyfish and ctenophores are present in the area, particularly during summer (van Walraven et al., 2013; 2015). To avoid any influence from their presence on fish density estimates, S_v thresholds were tested ranging from -70 to -50 dB re 1 m²m⁻³. Although stronger thresholds resulted in the removal of more data points, the resulting patterns (e.g. temporal trends and changes in density) remained similar across all tested thresholds, with only changes in the absolute

numbers as the threshold became more stringent (supplementary Figure S2). This additional analysis confirmed that the determined threshold of -65 dB successfully captured the main fish densities while remaining unbiased by noise and untargeted species (such as zooplankton and other gelatinous organisms).

For objective 1, concerning the change in density of fish abundance over time, S_v data collected during each sampling interval were integrated over the water column and treated as one sample. For objective 2, concerning the vertical distribution of fish, S_v data were binned into 1 m vertical cells and averaged over each sampling interval. The nautical area scattering coefficient (NASC, MacLennan et al., 2002) was used as an index for fish density. NASC ($\text{m}^2\text{nmi}^{-2}$) represents the integrated backscatter (S_v) from the observed water column scaled to a square nautical mile area, commonly used as a proxy for fish density in fisheries acoustic surveys. Although NASC is normally used for describing fish density in studies with a spatial component, it also serves as a standard metric of fish density for stationary studies (De Robertis et al., 2018).

2.4.2 Target tracks

For objective 3, concerning the individual movement of fish, single targets and target tracks were exported including target strength (TS), speed and direction. For single target detection, a TS threshold of -68 dB re 1 m^2 was used (detection settings in supplementary Table S3). Prior to track detection, school detection was performed to create polygons, which were used as exclusion masks over the single target echograms to eliminate regions potentially containing overlapping multiple individuals (detection settings in supplementary Table S4). Target tracks were detected automatically using the following criteria: a minimum length of 6 pings, including a minimum of 4 data points (i.e. detected single targets), not exceeding a 0.5 m depth difference from ping to ping and containing not more than 3 consecutive empty pings (detection settings in supplementary Table S5). These parameters were selected based on series of trial-and-error detections followed by visual inspections. Subsequently, the detected single targets and target tracks were exported for the analysis of fish speed and direction. Occasionally, the algorithm may erroneously identify detections from separate fish as part of the same track, which may result in unrealistic speed estimates. To eliminate such false detections, the tracks were filtered based on an upper fish speed threshold over ground (2.5 ms^{-1}). Estimated speeds faster than this were considered false and were eliminated.

2.5 Data analysis

2.5.1 Fish density and vertical distribution

Two types of models were used to analyse volume backscatter in relation to environmental and temporal variables: generalized additive models (GAMs) for fish density analysis and generalized additive mixed models (GAMMs) for fish vertical distribution analysis. Due to the right-skewed distribution, log-transformed NASC was used as the response variable in the models. The models were constructed using the 'gam' and 'gamm' functions from the 'mgcv' package in R (version 1.8-40, Wood, 2017). Data exploration, including outlier detection, assessment of multicollinearity and examination of relationships between NASC and environmental/temporal variables, followed the protocol of Zuur et al. (2010). The models assumed a Gaussian distribution with an identity link function and were visually assessed for meeting the assumptions of homogeneity of variance and normality of residuals.

A GAM was used to determine how much variability in fish density could be explained by nonlinear effects of date, tidal cycle, time of day, temperature, windspeed, wind direction and lunar cycle. Tidal cycle was defined as time (hours) after low water slack tide, and lunar cycle was defined as time (hours) after full moon; the variance inflation factor confirmed that these two variables were not colinear. Because temperature correlated strongly with date, the former was removed from the model. After removing 5% poor-quality observations due to surface exclusion and bad regions, the final dataset comprised 5307 samples to be included in the analysis. Tidal cycle, time of day, wind direction and lunar cycle were included using cyclic cubic regression splines, and date was included using a cubic spline smoother. Windspeed was included as a linear effect. Date and tidal cycle were also included as an interaction term using a tensor spline. We used a basis dimension (k) of 4 for all variables except for date, here we increased k to 7 to capture the strong seasonal variation present in the data. The dredge function in the 'MuMIn' (multi-model inference) package (Bartón, 2022) was applied for model selection to determine the order of importance of the explanatory variables based on Akaike's information criterion (AIC, e.g. Burnham & Anderson, 2004). Here, explanatory variables were removed from the full model to observe the impact of their removal on model AIC.

A GAMM was used to analyse the vertical distribution of fish density in response to nonlinear effects of date, time of day and tidal cycle. The water column was divided into samples of 1 m height ('layers'), and since the vertical samples were not independent and multiple samples belonged to the same sampling interval, we used a GAMM with sampling interval as a random effect. After removing 36% poor-

quality observations due to surface exclusion and bad regions, the final dataset comprised 78946 samples to be included in the analysis, corresponding to 3571 sampling intervals. The NASC dataset contained approximately 1.2% zero values; to account for zeros before log-transforming the data, they were replaced by the smallest positive value present, divided by two. All explanatory variables were added to the model as interactions with depth. The four partial effects (depth, date, time of day and tidal cycle) were included individually as well. All the interactions were included as tensor smoothing splines, with time of day and tidal cycle included using a cyclic cubic regression spline, and depth and date were included using a cubic spline. No model selection was conducted.

2.5.2 Fish movement relative to tidal currents

Fish speed and swimming direction were compared to current speed and direction to study fish movement behaviour. Fish speed and swimming direction in the horizontal plane were calculated by Echoview based on the range, time and angular position of subsequent targets belonging to the same fish track. Periods of fish tracks that corresponded with available ferry ADCP data were selected. Therefore, the analysis was restricted to daytime (06:00-21:00 h) in the period March until December 2021.

Data were separated into incoming tide (eastward into the Wadden Sea) and outgoing tide (westward into the North Sea). Because we were interested in the differences in fish swimming behaviour between the tidal phases, data from slack tides were removed by excluding periods with low water speeds ($< 0.5 \text{ ms}^{-1}$).

We aimed to study fish swimming behaviour and examined how fish adapt their direction and speed to surrounding water currents. To quantify swimming effort, fish speed through water was calculated with the following equation:

$$v_{fw} = \text{sign}(v_{fg}) * (v_{fg} - v_w) \quad (\text{Eq. 1})$$

Where v_{fw} is the fish speed through water, v_{fg} is the fish speed over ground as measured by the echosounder and v_w is the speed of the water current as measured by the ADCP, all in ms^{-1} . The sign of v_{fg} specifies whether the fish is directed inward (plus) or outward (minus). The multiplication by the direction is added to the formula to scale the outcomes, and to be able to compare the speeds of the two tidal phases.

Upon initial examination, we observed that most fish movements were aligned with the mainstream currents in the inlet. To explore how swimming effort was affected by currents, we excluded fish movements in non-mainstream directions based on

the frequency of directed swimming at various azimuth angles. Specifically, we eliminated the angles in which fish swam less frequently, i.e. less than the 66th percentile. Data were binned into 4 m vertical intervals, and we focused on the depth interval 15-19 m, one of the well sampled depth bins. Bins at closer range to the transducer suffered from the low sampling volume where individual fish were less likely tracked (i.e. duration of ensonification in the beam is too short). Conversely, in highly expanded beams farther away from the transducer, multiple targets were more likely to overlap, making it difficult to track individual targets. For the seasonal pattern, we calculated the median fish speed through water per week for both tidal phases.

A total of 1198229 target tracks were detected after applying the speed threshold filter. To accurately measure fish speed and exclude jellyfish, we applied a TS threshold. To determine the appropriate threshold value, we estimated fish size using a TS-length equation specific to herring and sprat:

$$TS = 20\log_{10}(L) - 67.8 \quad (\text{Eq. 2})$$

where TS is the target strength (dB), and L is the fish length (cm) (Didrikas & Hansson, 2004). This allowed us to establish which TS values were associated with fish in our study area. Based on the multiple TS peaks in the fish-tracking dataset, two main categories were identified: group 1, consisting of clupeid fish (TS > -60 dB), and group 2, consisting of gelatinous organisms (TS ≤ -60 dB). Therefore, we established a TS threshold of -60 dB for the analysis on fish speeds. This TS threshold in combination with the depth bin threshold resulted in a sample size of 194628 observations for studying fish speed over ground. Furthermore, we obtained current speed data from 6536 ferry crossings, which we used to match with the fish data, resulting in 50759 samples that enabled us to calculate fish speed through water. In addition, a non-parametric unpaired Wilcoxon rank sum test was performed to determine whether there was a significant difference in the mean fish speed through water between incoming and outgoing currents.

3. Results

3.1 Patterns in fish density and catch data

Fish density in the Marsdiep inlet varied between NASC values of 5 m²nmi⁻² to a maximum of around 100000 m²nmi⁻² (Figure 3). The variation in NASC was particularly high from January 2022 onwards. The observed NASC values increased towards summer, characterized by a first peak around June and a second peak in September, followed by a decline.

The results of the fish catches at location Malzwin in the Marsdiep indicated that Atlantic herring was the dominant SPF, followed by European sprat (Figure 4A). From March until June, small clupeid post-larvae (i.e. < 5 cm) were caught, which could not be identified to species level and were likely a mix of herring, sprat and pilchard. Herring and sprat were present year-round, with a peak of herring in June and July, and a peak of sprat in March. Clupeidae (i.e. the family that includes herring, sprat and pilchard, among others) made up 99% of the catches in the Marsdiep, with herring accounting for 66.7%, sprat for 23.1% and clupeid post-larvae for 9.4%. Other species such as smelt (*Osmerus eperlanus*), sandeel (*Ammodytes* sp.), whiting (*Merlangius merlangus*) and Nilsson's pipefish (*Syngnathus rostellatus*), were also observed regularly, although their overall contribution was only approximately 1%. Therefore, the acoustic backscatter, addressed in this study as an SPF ecological unit, mostly represents clupeid species.

The SPF community in the Marsdiep was dominated by small herring and sprat, with overall mean sizes of 6.4 and 7.3 cm, respectively. The size distributions show seasonal variations, with larger individuals (> 10 cm) being primarily observed in May and October for herring, and November and December for sprat (Figure 4B). In June and July, herring of about 5 cm dominate the distribution, whereas small sprat dominated the distribution in July and September. No clear temporal increase in size was observed, as small individuals (5-6 cm) were consistently present from June until December. In addition to our catch data, the length of SPF was estimated from acoustic data using the TS distribution. The mean TS of detected target tracks from March to December showed three distinct peaks (Figure 5A). The largest peak, at -49.5 dB, corresponds to clupeid fish measuring approximately 8.2 cm (Eq. 2). Additionally, smaller peaks at -57 and -63 dB were observed. The former peak corresponds to clupeid size of about 3.5 cm, while the latter peak is probably dominated by gelatinous organisms. Minimal seasonal variations were observed in the primary TS peak, whereas TS values below -55 dB displayed seasonal variations (Figure 5B). However, in winter, a different distribution was observed, with the largest peak being -46.5 dB, which corresponds to clupeid fish measuring approximately 11.6 cm.

The GAM, which describes factors affecting the variability in fish density, explained 36.1% of the deviance in the NASC data (Model 1 in Table 1). All variables were significant ($P < 0.001$), highlighting the complexity of the system. Model selection showed that the two partial effects of the interaction term could be removed, as the delta AIC was < 2. This indicated that most of the information was captured by the interaction of date and tidal cycle, rather than by the factors separately. Subsequently, the first environmental variable to be removed from the model was

lunar cycle, resulting in a 40.5 AIC increase. This was followed by wind direction (+43.0), windspeed (+71.3), time of day (+284.9), and finally, the interaction of date and tidal cycle (+792.9). These findings suggest that the interaction of date and tidal cycle was the most influential environmental factor driving fish density in the Marsdiep inlet.

The GAM showed seasonality in fish densities, and mean NASC values depend on the phase of the tidal cycle (Figure 6). NASC densities at high water slack tide and outgoing tide showed a similar seasonal pattern, with highest NASC values observed around mid-September. For incoming tide and low water slack tide, the peak in NASC was reached around mid-May. In general, highest fish densities were observed from mid-spring until the beginning of autumn. However, at low water slack tide, NASC values increased from December onwards. In addition, fish densities were highest at incoming tide and at high water slack tide for most of the year. Furthermore, densities were lower during daytime, especially around noon. Wind speed and direction both affected fish density, with higher windspeeds resulting in lower NASC values. The highest NASC values were observed for a wind direction of 60° , aligned with the geographical orientation of the Marsdiep. The model showed that the highest NASC values occurred around new moon.

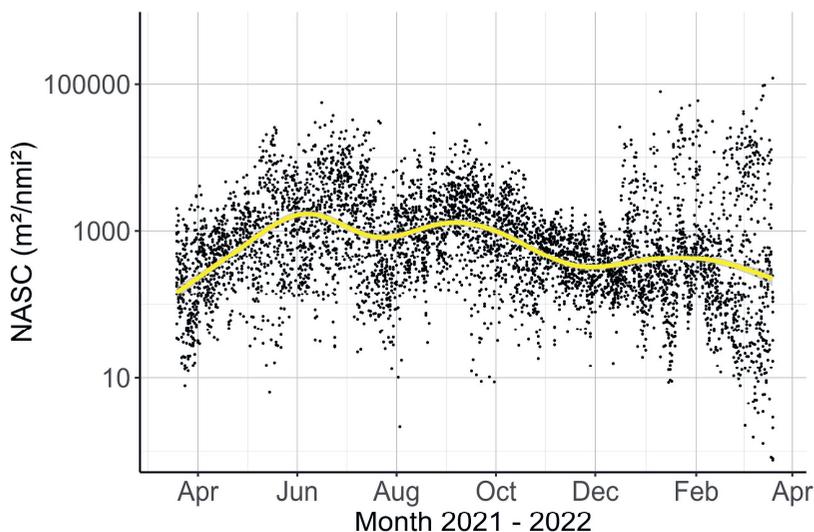


Figure 3. Fish density values in the Marsdiep over the course of one year. Black dots show the observed fish density, presented as nautical area scattering coefficient (NASC, in $\text{m}^2\text{nmi}^{-2}$) on a log scale, and the yellow line shows a generalized additive model smoother.

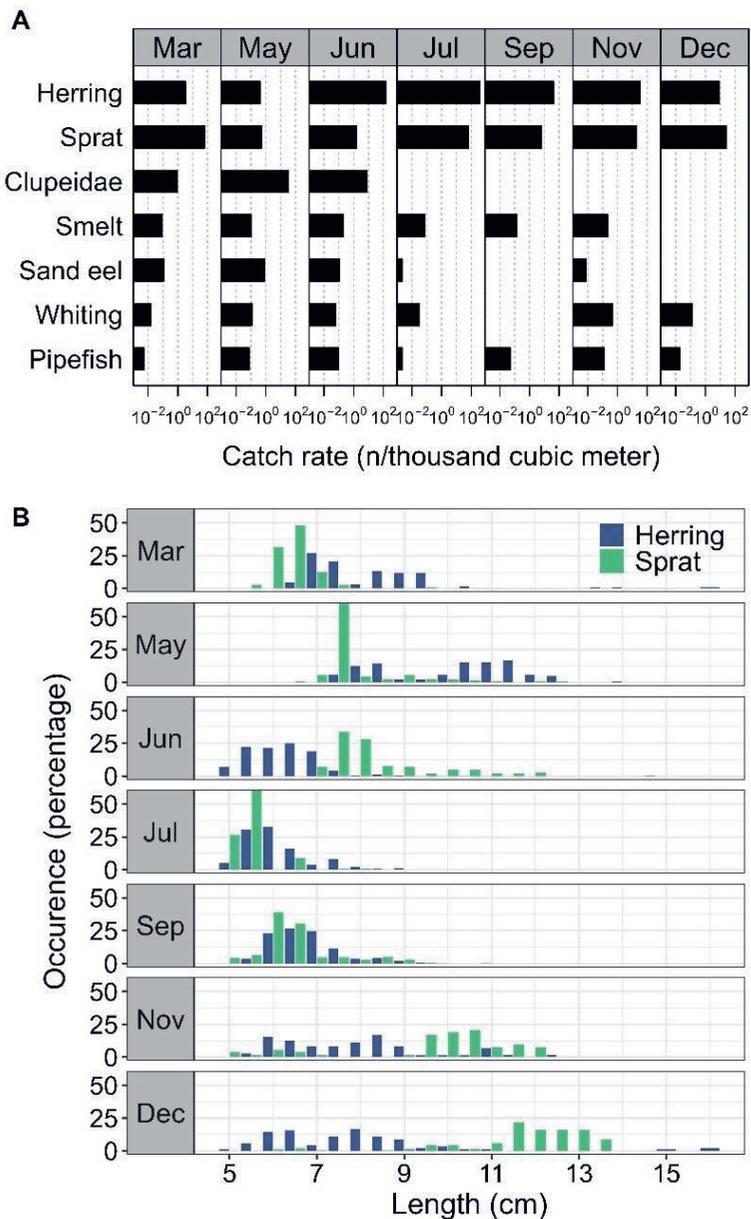


Figure 4. A) Fish catch compositions plotted on a log scale; fish are listed in order of decreasing abundance. Scientific names of the species from top to bottom: *Clupea harengus*, *Sprattus sprattus*, unidentified post-larvae (< 5 cm) of the family *Clupeidae*, *Osmerus eperlanus*, *Ammodytes* sp., *Merlangius merlangus* and *Syngnathus rostellatus*. B) Distribution of size classes (in 0.5 cm intervals, minimum length 5 cm) within the total catch of herring and sprat in the Marsdiep inlet (location Malzwin) from March until December 2021.

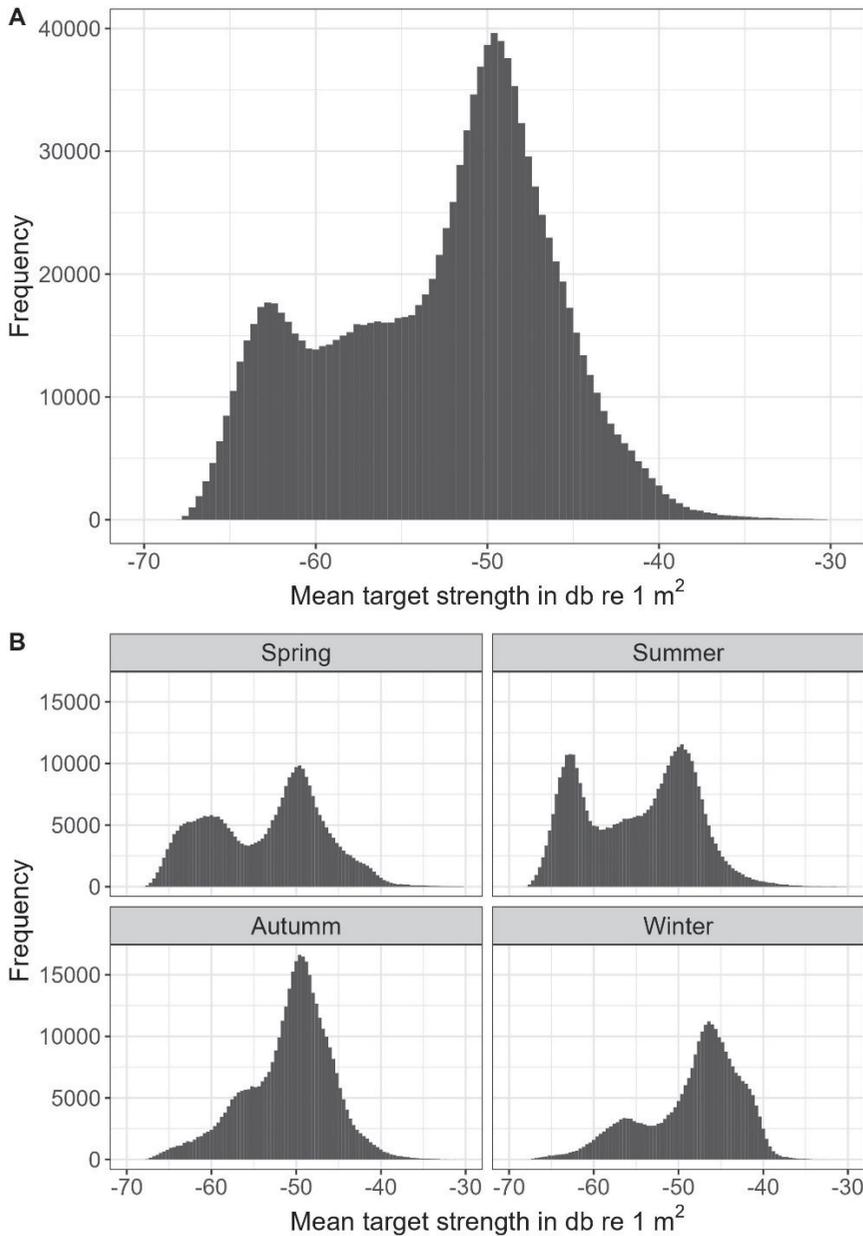


Figure 5. Mean target strength (dB re 1 m²) of echo traces in the Marsdiep. A) Over the period March until December 2021, corresponding to the dataset utilized for the movement analysis, and B) over the period March 2021 until March 2022, divided by season.

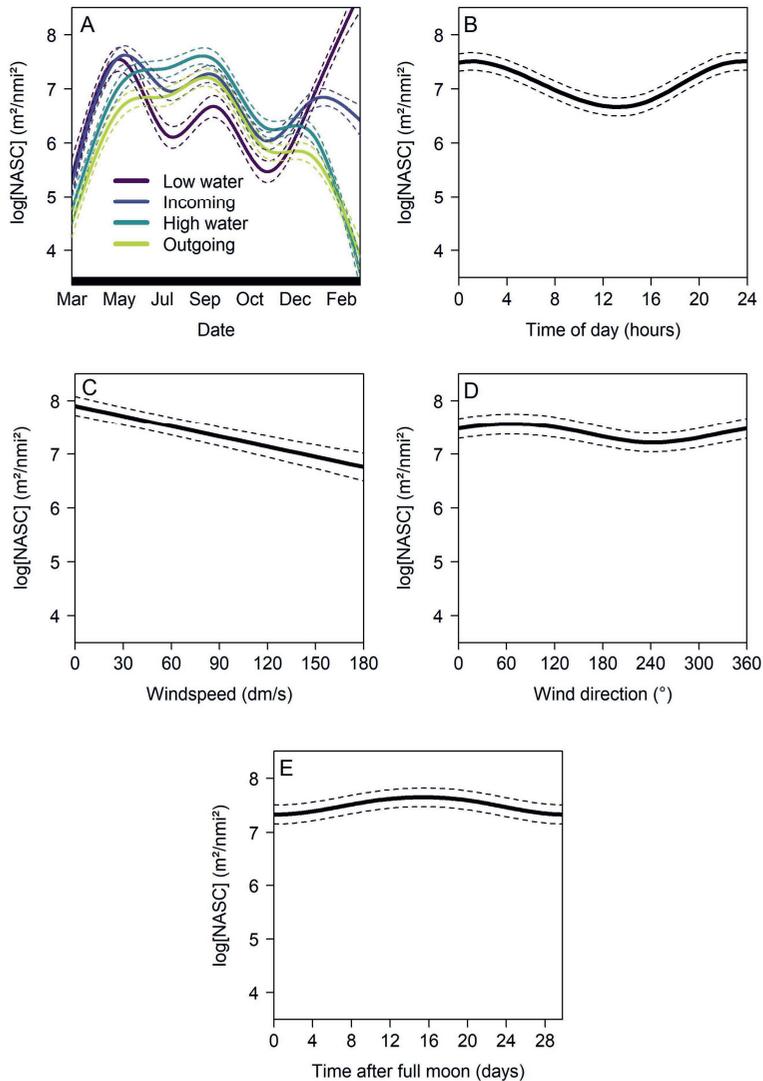


Figure 6. Generalized additive model for fish density, presented as log-transformed nautical area scattering coefficient (NASC in m^2/nmi^2). Smoothers for A) the interaction of date and tidal cycle, B) time of day, C) windspeed, D) wind direction and E) lunar cycle. The dashed lines present the 95% confidence intervals, and different colors in A) indicate the different phases in the tidal cycle: low water slack tide, incoming tide, high water slack tide and outgoing tide. This figure is based on a set of median values used for plotting: time = 00:00 h, date = 20 September 2021, tide = 6 (i.e. high water slack tide), windspeed = 50 dm/s^{-1} (Beaufort ~ 3), wind direction = 220° (SW, dominant wind direction in the Netherlands) and lunar cycle = full moon.

Table 1. Generalized additive model (GAM) and generalized additive mixed model (GAMM) results of the two models describing 1) fish density in the total water column and 2) vertical distribution of fish density. EDF: effective degrees of freedom, NASC: nautical area scattering coefficient ($\text{m}^2\text{nmi}^{-2}$), α : intercept; te : tensor product interactions; s : smooth function; i : sample; ϵ : residuals; N : normal distribution; σ standard deviation; β : random effect for sampling interval.

Model	Model formula	Type	Terms	EDF	F	p
1	$\text{Log}(\text{NASC}) = \alpha + te(\text{date}, \times \text{tide}_i) + s(\text{date}_i) + s(\text{tide}_i) + s(\text{time}_i) + \text{windspeed} + s(\text{wind direction}_i) + s(\text{lunar cycle}_i) + \epsilon_i$ $\epsilon_i \sim N(0, \sigma^2)$	GAM	Date \times tidal cycle	10.9	72.3	<0.001
			Date	5.9	139.2	<0.001
			Tidal cycle	1.7	6.0	<0.001
			Time	2.0	146.5	<0.001
			Wind speed	-0.006 ± 0.0007^a	<0.001	
			Wind direction	1.9	22.4	<0.001
			Lunar cycle	1.9	21.3	<0.001
Full model: 36.1% deviance explained; intercept 6.7 ± 0.04 ; $p < 0.001$; $\epsilon_i \sim N(0, 1.26^2)$						
2	$\text{Log}(\text{NASC}) = \alpha + s(\text{depth}_i) + s(\text{date}_i) + s(\text{time}_i) + s(\text{tide}_i) + te(\text{depth}_i, \times \text{date}_i) + te(\text{depth}_i, \times \text{time}_i) + te(\text{depth}_i, \times \text{tide}_i) + \beta + \epsilon_i$ $\beta \sim N(0, \sigma_{\text{sampling interval}}^2)$ $\epsilon_i \sim N(0, \sigma^2)$	GAMM	Depth	3.0	574.6	<0.001
			Date	5.8	109.8	<0.001
			Time	2.0	167.0	<0.001
			Tidal cycle	1.9	12.6	<0.001
			Depth \times date	14.9	70.3	<0.001
			Depth \times time	8.0	35.6	<0.001
			Depth \times tidal cycle	7.6	104.9	<0.001
Full model: adjusted $R^2 = 0.22$; $\beta \sim N(0, 1.49^2_{\text{sampling interval}})$; $\epsilon_i \sim N(0, 1.57^2)$						

^a As windspeed is included as a non-smoothed linear effect, the values here represent the estimate of the intercept and the standard error.

3.2 Vertical distribution

The highest fish densities occurred at depths ranging from 7 to 13 m, with variations across temporal scales such as daily, seasonal and tidal cycles (see Figure 7 for interactions of temporal variables with depth, and supplementary Figure S3 for partial effects individually). Rather than displaying all months, we depicted a subset of periods: three consecutive months in spring (March, April, May), the crucial period for fish-eating birds, and two other periods: the beginning of autumn (September) and mid-winter (January). The depth distribution pattern varied by season, with the peak at around 10 m depth being least pronounced in March and most pronounced in January, with intermediate patterns in the other months. Further investigation into the diurnal variation in fish density showed that during the day, fish densities were lower towards the surface. At night, fish density was higher compared to daytime, and fish were more evenly distributed up to a depth of about 13 m, after which density declined sharply. Additionally, our results indicate that during outgoing tide, fish density was relatively uniform over the water column, whereas during other phases of the tidal cycle, there was a clear peak around 10 m.

The GAMM showed that all tested variables had a significant effect ($P < 0.001$) on NASC (Model 2 in Table 1). The adjusted R^2 of the model was 0.22. Validation confirmed that the model assumptions were met, although the replacement of zeroes with half of the observed minimum NASC values created some unwanted patterns in the residuals. The small number of replacements meant that the interpretation of the model was not compromised.

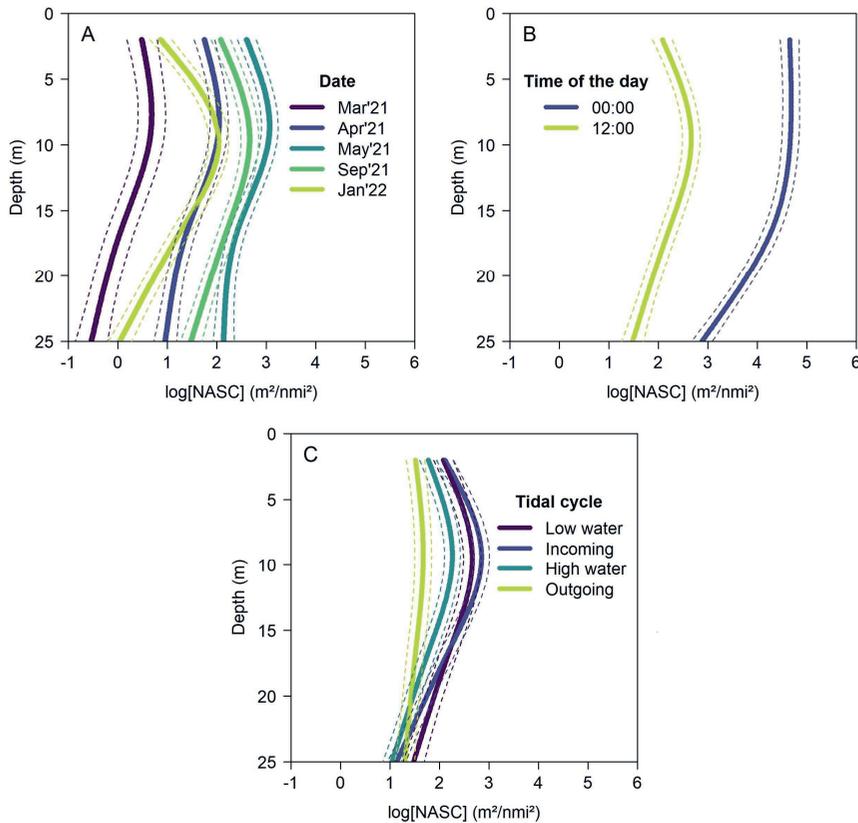


Figure 7. Generalised additive mixed model showing the vertical distribution of fish, presented as log-transformed nautical area scattering coefficient (NASC in m^2nmi^{-2}) with depth (m). Smoothers for the interactions of depth with A) date, B) time of day and C) tidal cycle. The dashed lines present the 95% confidence intervals, and different colors indicate different months of the year (A), noon or midnight (B) and phases of the tidal cycle (C). This figure is based on a set of median values used for plotting: time = 12:00 h, date = 20 September 2021, tide = 0 (i.e. low water slack tide). Due to surface interference, there are no data for the upper 2 m. The smoothers of the partial effects can be found in supplementary Figure S3.

3.3 Individual target tracks: fish movement related to current speed

Current speeds ranged between 0 and 2 ms⁻¹ (Figure 8A). The main current directions were 30° and 210°, representing incoming and outgoing tides, respectively. The absolute fish speed over ground ranged between 0 and 2.5 ms⁻¹, with 1.3 ms⁻¹ (4.7 kmh⁻¹) being the absolute mean calculated over the full study period (Figure 8B). The mean directions estimated by the tracked targets aligned well with the main current directions, with an approximate deviation of only 10°, suggesting that the fish orient themselves to the direction of the current flow.

Fish speed through water revealed swimming behaviour in relation to the current. A positive speed indicated swimming with the current, a zero value indicated swimming against the current to maintain position, and a negative speed indicated movement in the opposite direction of the current. During both tidal phases, fish typically swam with the current and had a positive speed through water (Figure 8C). Incoming currents resulted in significantly faster fish speeds through water compared to outgoing currents, with means of 0.42 and 0.18 ms⁻¹ respectively ($W = 117961711$, $n_1 = 23980$, $n_2 = 23532$, $P < 0.001$). Negative fish speeds through water were observed more frequently during outgoing currents. Furthermore, fish speeds through water were highest during summer months (Figure 8D).

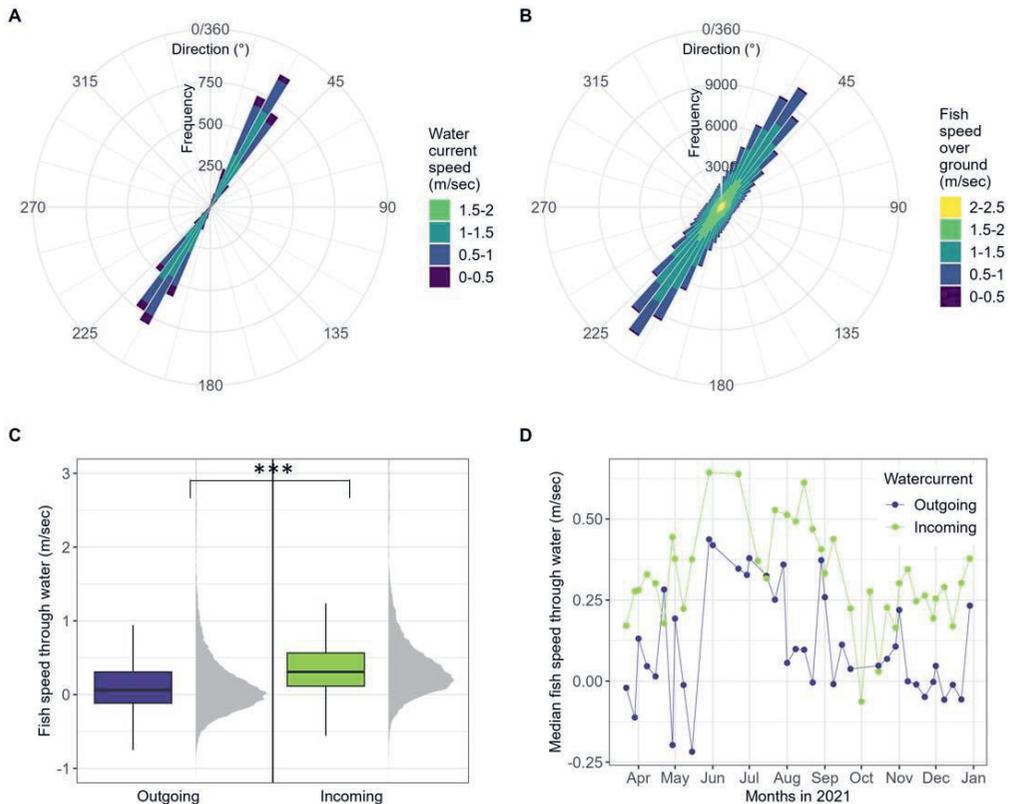


Figure 8. A) Water current speed and direction and B) fish speed over ground and direction. The length of each 'spoke' represents the frequency of directions, and the colours indicate the speed (ms^{-1}). C) Distribution (boxes show minimum, 1st quartile, median, 3rd quartile and maximum) of fish speed through water (ms^{-1}) at outgoing tide and incoming tide. Asterisks indicate a significant difference in fish speed through water between outgoing and incoming current (Wilcoxon rank sum test, with $W = 117961711$, $n_1 = 23980$, $n_2 = 23532$, $P < 0.001$). D) Median fish speed through water (ms^{-1}) over the season, split between outgoing tide and incoming tide; only data points that include > 100 individual tracks are displayed. Figure is based on data from March until December 2022 of depths between 15 and 19 m.

4. Discussion

4.1 Patterns in fish density related to fish catches

The scope of this study was to study temporal variability in SPF density, vertical distribution and movement behaviour in a major tidal inlet of the Wadden Sea. The highest fish densities were observed from mid-spring until the beginning of autumn. Catch data from location Malzwin suggest that this increase can be attributed to the movement of juvenile herring and sprat from the North Sea into the Wadden Sea. Interestingly, no clear temporal increase in size was observed, which would be expected if a single cohort was present and exhibited growth over time. Furthermore, herring in March and May were larger compared to June and the remainder of the year, when the distribution was dominated by herring measuring 5-6 cm. This suggests that juvenile herring from multiple origins pass through the Marsdiep at different times in the year and that the Marsdiep is a suitable habitat for the small size classes of clupeids.

In the North Sea, four large herring spawning components have been described based on their spawning ground and timing. The Orkney-Shetland component spawns first in August/September, followed by the Buchan and Banks component in September/October, and the Downs component spawns latest in December/January (Payne, 2010). A modelling study showed that larvae of Downs herring drift from the English Channel north-eastward before arriving in the Wadden Sea after mid-April, although annual variation in timing and abundance is high (Dickey-Collas et al., 2009). The size distribution of herring in the Marsdiep indicated a new year-class entering in June, potentially corresponding to juvenile Downs herring. However, the observed increase in density already started in April, but the size of herring (7-8 cm) was too large to be attributed to juvenile Downs herring (Heath et al., 1997). Hence, this indicates the involvement of another spawning component. Furthermore, the presence of small individuals later in the year suggests the possibility of yet another batch of herring arriving. These individuals do not necessarily have to belong to one of the large components mentioned above, but may belong to smaller coastal stocks, which are known to be present in the whole north-east Atlantic. Hence, it remains unclear which specific herring spawning components use the Wadden Sea for their juvenile stages, necessitating future studies utilizing techniques such as DNA discrimination (Kongsstovu et al., 2022; Bekkevold et al., 2023). Instead of the Wadden Sea being an area where herring of a certain spawning component grows up, the results indicate that it is a suitable area for herring of a certain size range that may originate from different spawning components. Additionally, the increase in NASC can be partly explained by migration of juvenile sprat. Adult sprat have a longer spawning

period that typically ranges from March to August, but that can begin as early as January in the English Channel when the temperature threshold of 6°C is reached, and progressively later in more north-eastern areas such as the German Bight (Alshuth, 1988; Baumann et al., 2009).

The seasonal fish density pattern was influenced by the phase of the tide. The primary peak around mid-May was mostly driven by high fish densities at low water slack tide and incoming tide. Fish densities were higher at night, similar to Scherelis et al. (2020), which may be due to the presence of larger size classes, nocturnal species (Maes et al., 1999; Didrikas & Hansson, 2009) or small-scale migration, both horizontally and vertically.

No other recent studies have conducted year-long, high-temporal-resolution monitoring of pelagic fish in the Wadden Sea. Rademaker et al. (2024) modelled weekly catches of herring in the Marsdiep during spring and autumn based on fyke observations at the southern tip of Texel to study herring movement into the Wadden Sea. They found that, during the past 39 years, the time of year was the most important factor affecting herring catches, alongside lunar illumination and relatively low water temperatures in the North Sea. The highest captures were recorded consistently between July and October, which is similar to our findings, although over time there has been a relative increase in catches earlier and a relative decrease in captures later in the year, indicating a small shift in phenology. Also, Couperus et al. (2016) found the highest fish densities at incoming tide during their surveys in May and October in the Marsdiep.

March 2022 recordings showed more extreme fish densities compared to March 2021, suggesting large annual variation. Such variation is expected because herring experience high variability in recruitment (Payne et al., 2009; Burbank et al., 2022); for instance due to interannual variations in year-class strength (Nash & Dickey-Collas, 2005) and fluctuations in larval transport resulting from variations in hydrodynamical circumstances (Dickey-Collas et al., 2009). Collecting long-term continuous information on the early life stages of SPF could contribute to fisheries management of herring and sprat in the North Sea. By identifying interannual changes in juvenile abundance and timing, it could provide crucial information on variation in recruitment and year-class strengths, additional to existing indices based on research vessel surveys carried out in specific periods of the year (ICES, 2022). Continuous monitoring is especially relevant for capturing changes in the timing of migration and nursery use. Phenological shifts due to temperature changes have been reported for a wide range of taxa, including fish larvae (Asch, 2015) and zooplankton (Heneghan et al., 2023). Experiments on Downs herring demonstrated faster development in warmer waters, and lower fertilization and hatching rates (Toomey et al., 2023). Given the expected increase in sea surface

temperature, it is crucial to closely monitor potential shifts in timing to inform fisheries management.

4.2 Patterns in vertical distribution of fish

Our study showed extensive temporal variation in the vertical distribution of fish. We expected that time of day would be the primary driver of vertical fish distribution. That was indeed confirmed, but tidal cycle and seasonality also significantly affected the vertical distribution. Our findings are consistent with those of Williamson et al. (2019), who reported that the tidal cycle has an impact on the vertical distribution of the SPF community in Scotland.

Herring and sprat are known to exhibit diel vertical migration (DVM) in several regions, with individuals schooling at deeper depths during daylight hours, in shallower depths after dawn and before dusk, and becoming more dispersed during darkness (Blaxter & Parrish, 1965; Nilsson et al., 2003; Solberg & Kaartvedt, 2017; Whitton et al., 2020). However, there are also cases of juvenile herring exhibiting an inverse DVM pattern, where they move to shallower depths to evade predators that follow a DVM pattern (Jensen et al., 2011). Although small schools were frequently observed (see example in Figure 2), we did not often observe the formation of large schools in this area, which might explain why we did not observe DVM behaviour as clearly as theory describes. Yet, we noticed that the fish tended to swim at greater depths during the day and became more dispersed at night. This observation is in line with results of an acoustic mooring study on pelagic fish in a shallow (~20 m) coastal bay in the Baltic Sea, where the median depth of fish was 10.7 m by day and 4.8 m by night (Didrikas & Hansson, 2009). In addition, our findings of highest fish densities between 7 and 13 m are similar to those of a daytime ship-based acoustic survey in the Marsdiep (Couperus et al., 2016). The reason for not observing large schools remains unclear, but it could be attributed to the dynamic environment and high turbidity. Turbidity has a negative effect on prey capture (Ortega et al., 2020), which may reduce the need for fish to hide in vast schools.

Several hypotheses have been proposed to explain the vertical distribution of fish, including food availability, predator avoidance and bioenergetics (Cardinale et al., 2003). In the case of the Marsdiep, the water column is usually vertically well-mixed due to wind and strong tidal currents (Buijsman & Ridderinkhof, 2007). Hence, we do not expect food availability, salinity or water temperature to be the main driving factors of vertical fish distribution. Instead, predator avoidance may play a significant role, as fish tend to avoid swimming near the surface to evade diving birds, such as terns (e.g. sandwich tern *Sterna sandvicensis*: Baptist & Leopold,

2010, common tern *Sterna hirundo*: Dänhardt & Becker, 2011a) and gulls (e.g. lesser black backed gull *Larus fuscus*: Baptist et al., 2019a). Our findings of lower densities of fish towards the surface during daytime support this hypothesis. Meanwhile, the probability of prey capture by terns is also affected by water transparency (Baptist & Leopold, 2010), which was not considered in our analysis. Furthermore, terns and gulls typically dive to relatively shallow depths (e.g. lesser black backed gulls dive within 0.6 m of the sea surface (Baptist et al., 2019a)). Unfortunately, our method did not cover the zone specifically relevant for diving seabirds. Therefore, future studies should aim to sample as close to the surface as technically possible and include turbidity as a covariate to better understand the relationship between visual predators and the distribution of SPF.

4.3 SPF use the Marsdiep as a gateway to the Wadden Sea

The direction and speed of currents in the Marsdiep are primarily governed by tides. Currents reach high speeds through the inlet and consequently exert significant control over the movement of fish in the area. Our study showed that fish tended to swim with the current, rather than trying to maintain their geographical location by swimming into the current. This is consistent with our expectation, as swimming against a strong current requires higher energy expenditure and results in a higher metabolic cost. Interestingly, during outgoing tide, fish showed a significantly lower speed through water compared to incoming tide. The high positive fish speeds through water observed during incoming tide probably indicate the migration of juvenile herring and sprat towards the Wadden Sea. Conversely, the lower fish speeds and frequent negative values during outgoing tide suggest that fish resist, and at times swim against the current, when pushed out of the Wadden Sea. This swimming behaviour may indicate that SPF try to remain within the Wadden Sea because it is a more preferred nursery area than the nearby North Sea. Together with the observation of higher fish densities during incoming tide throughout most of the year, this suggests that the Marsdiep inlet serves as a gateway to the Wadden Sea for SPF.

In the dynamic environment of the Marsdiep, mean fish swimming speeds of 0.42 ms^{-1} during incoming tide and 0.18 ms^{-1} during outgoing tide were observed. These values are slightly higher than those reported for juvenile herring in Himmerfjärden Bay in the Baltic Sea (Sweden), which ranged from 0.1 to 0.26 ms^{-1} (Didrikas & Hansson, 2009). The observed higher fish swimming speeds during summer months may likely be attributed to higher water temperatures, which affect the metabolism of cold-blooded fish significantly (Beamish, 1978). In addition, it is plausible that changes in fish community, such as species composition and size,

resulted in different swimming capabilities, which consequently resulted in seasonally different swimming speeds.

Combining continuous data on fish density and swimming behaviour across tidal phases suggests that SPF mainly immigrate into the Wadden Sea via this inlet. However, to study whether there is indeed a net influx of SPF into the Wadden Sea, the acoustic setup needs to be extended to cover multiple inlets, preferably at different positions within a single inlet as well. A probable hypothesis is that the prevailing north-eastward current in the adjacent North Sea causes SPF to enter the Wadden Sea through the Marsdiep, and exit through the easterly inlets. A WBAT array in multiple inlets is needed for a more comprehensive understanding of SPF migration patterns.

4.4 Study limitations on fish movement behaviour

The analysis on fish movement behaviour focused primarily on daytime observations due to the availability of the ferry ADCP data. Most SPF are visual feeders, so fish swimming speed and direction relative to the currents may differ at night when fish are presumably less active (Didrikas & Hansson, 2009). Additionally, during daytime, SPF tend to form schools or swim nearer to each other (Blaxter & Parrish, 1965), making it difficult to acoustically detect individual tracks. To prevent false detections in fish aggregations (i.e. consisting of multiple fish), all school-like echo traces were initially masked from the data before using the automatic algorithm to detect single targets and target tracks. This approach ensures the reliability and objectivity of the detected tracks. However, the inability to detect individual tracks within aggregations potentially introduced a bias towards free-swimming objects, which may not provide a representative sample of the entire fish community. Consequently, it was necessary to understand what portion of observed targets could be gelatinous organisms and to subsequently exclude them. Therefore, we classified targets based on the TS distribution and calculated corresponding fish sizes (Eq. 2). Since the swim bladder contributes significantly to the TS, the TS-length equation cannot accurately estimate the size of fish larvae lacking a swim bladder. The peak in the TS distribution at -63 dB is likely caused by jellyfish and ctenophores, and therefore we excluded gelatinous organisms using a TS threshold of -60 dB. The main candidate species for this gelatinous group, as also observed in our stow net catches, are *Mnemiopsis leidyi*, *Pleurobrachia pileus*, *Aurelia aurita*, *Cyanea* spp. and *Rhizostoma octopus* (van Walraven et al., 2013; 2015; van Walraven, 2016). In absence of reported TS values for these specific species, we assumed their TS values to be in the range of other jellyfish species reported in the literature (Brierley et al., 2004; De Robertis et al., 2018; Yoon et al., 2018).

The dominant length of clupeids in the Marsdiep based on the mean TS was 8.2 cm, which is about 1-2 cm larger than the catch data showed. This variation could be attributed to several factors, including the specific TS-length equation applied, the 11 km distance between the sample locations, the catchability of the net and limited availability of catch samples compared to the continuously available TS samples. However, both catch data and the TS analysis consistently indicated the dominance of small fish in the area without clear size progression over time, and generally larger fish during winter.

The location of the acoustic fish observations by the WBAT and the current measurements by the ferry ADCP were approximately 2 km apart. Although this distance may be negligible on an oceanic scale, it could impact the accuracy of the established relationships due to the dynamic topography of the Wadden Sea and its inlet structure. This analysis comparing the speed and direction of currents with fish movement provides a general overview based on averages of large numbers of observations. However, the approach may have masked finer features. Future studies could investigate fish speed and direction at different phases of the tidal cycle and at various depths, considering the fluctuation of current speeds caused by shear stress. This could provide insights into whether SPF in the Marsdiep use selective tidal transport (i.e. migrations in synchrony with the tidal cycle (Gibson, 2003)). Future studies could also consider discriminating between species and sizes, for instance using multiple frequencies (Demer et al., 1999) or physics-based scattering models (Yoon et al., 2021), and incorporating fish tilt and the effect of pressure on swim bladder shape and compression (Ona, 2001; Gorska & Ona, 2001).

4.5 Use of continuous echosounders in dynamic tidal inlets

We describe the fish density patterns in the Marsdiep based on measurements over a relatively small surface area: the diameter of the WBAT beam at the surface is around 7.5 m. Yet, by conducting a small pilot experiment with four WBATs, we showed that although the measured area of one echosounder is small, it still provides an accurate signal for a larger area. This finding is in line with a previous study by De Robertis et al. (2018), who found that deploying three bottom-mounted echosounders at the right location can provide an index of abundance comparable to a spatial survey. Unfortunately, our setup did not allow for sampling the bottom 4 m, and due to surface interference and air entrainment, the top 2 m were also absent. This means that if fish were distributed close to the surface or bottom during certain periods, they would not be representatively picked up by our equipment. While acoustic sampling near the surface is technically limited, future studies could incorporate the use of multiple instruments concurrently, both upward and downward looking, to include the bottom portion.

The initial intention of this study was to collect data until June 2022. Unfortunately, the echosounder system became unusable after March 2022, being buried under the sand due to fast-moving sand waves of over 4 m height, illustrating the highly dynamic nature of the area. We recommend using this setup in tidal inlets, but our study indicates that local dynamic circumstances can complicate its implementation, and the exact location and retrieving system (either free standing or moored to e.g. a buoy) should be adjusted to local circumstances. After an initial trial and error phase, this method can be highly cost-effective. While it required a substantial time investment to accumulate the essential experience, both in the field and in the analysis, we see opportunities to automate aspects of the analysis, such as data cleaning. If specific tasks in the process can be automated, this method has the potential to be an efficient option for continuous monitoring, potentially replacing, or at least adding to, costly ship-based surveys.

Conclusions

The Marsdiep is a complex tidal inlet, where multiple factors govern the abiotic circumstances and hence affect fish occurrence. Date, time of day, tidal cycle, wind and lunar cycle were all important factors for explaining the variation in either density or vertical distribution of SPF. Our analyses indicate that the Marsdiep inlet serves as a migration gateway for SPF moving from the North Sea into the Wadden Sea, particularly for juvenile herring and sprat. We observed a clear seasonality with a peak in fish density from mid-spring to early autumn. The presence of small juveniles from June until December suggests that clupeids from multiple spawning components pass through the Marsdiep at different times in the year. Fish were typically distributed between 7 and 13 m depth during daytime. At night, fish were more evenly distributed up to a depth of 13 m, below which their density declined sharply. Fish swimming behaviour was primarily driven by currents, yet we observed more resistance against the current during outgoing tide. This suggests that fish are trying to remain in the Wadden Sea, supporting the hypothesis that the Wadden Sea serves as nursery area for SPF. Furthermore, we demonstrated that our acoustic mooring setup is effective for collecting high-quality continuous time series data on fish density, vertical distribution and swimming behaviour in dynamic tidal inlets. This study showed that continuous acoustic data on SPF can enhance the understanding of the local ecosystem. Additionally, by identifying densities and conditions of juveniles of commercial species, it could also contribute to fisheries management on a regional scale by detecting (inter)annual changes in juvenile abundance and timing, aiding in the understanding of recruitment variability and year-class strengths.

Acknowledgements

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Supplementary materials

1. Supplementary analyses

Text S1. Details of the statistical tests mentioned in section 2.2 on acoustic data collection.

a. Effect of specific location on backscatter?

A pilot test was conducted to test the effect of WBAT position on acoustic measurements of fish density. In this pilot test, four WBATs were deployed at three positions (see Table S1 for coordinates), recording for 5 days (Figure S1). The results of an ANOVA test on log-transformed NASC values (nautical area scattering coefficient in $\text{m}^2\text{nmi}^{-2}$) showed no significant difference in the measured backscatter between locations ($F(3,312) = 1.69, P = 0.17$).

b. Effect of sampling interval (short vs. long) on backscatter?

Since there are no established guidelines for the minimum necessary duration of representative recordings, a combination of short and long cycles was used, with wake-up intervals of 1.5 hours. Specifically, 12 'short' and 4 'long' measurements were taken per day, with short cycles lasting minimally 72 sec and long cycles lasting 16 min. This approach was chosen to enhance battery life while allowing for testing the effect of recording duration on sampling bias. To evaluate sampling bias, the longer recordings were split into 13 smaller groups and treated as independent sets of measurements. Statistical analysis, using ANOVA, showed that the mean backscatter did not differ significantly between the groups ($P > 0.1$). This suggests that any short recording would generate the same mean backscatter as the longer recordings.

2. Supplementary figures

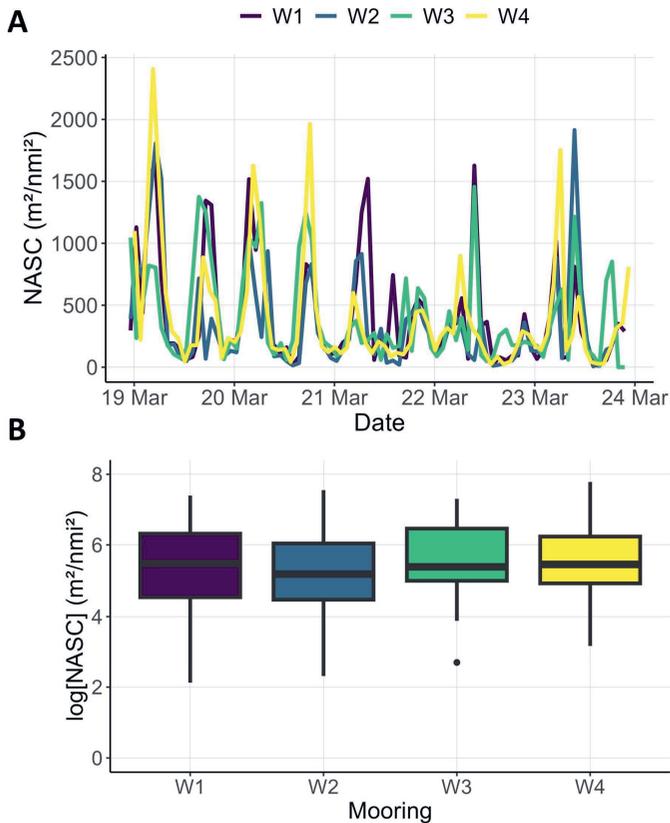


Figure S1. A) WBAT echosounders, at different positions show similar NASC values (nautical area scattering coefficient in m^2/nmi^2) over time. The colours represent the four different WBATs and coordinates can be found in Table S1. B) Boxplots showing the distribution (i.e. minimum, 1st quartile, median, 3rd quartile and maximum) of log transformed NASC data of the 5-day period for the different WBATs. ANOVA test showed no significant difference ($F(3,312) = 1.69$, $P = 0.17$).

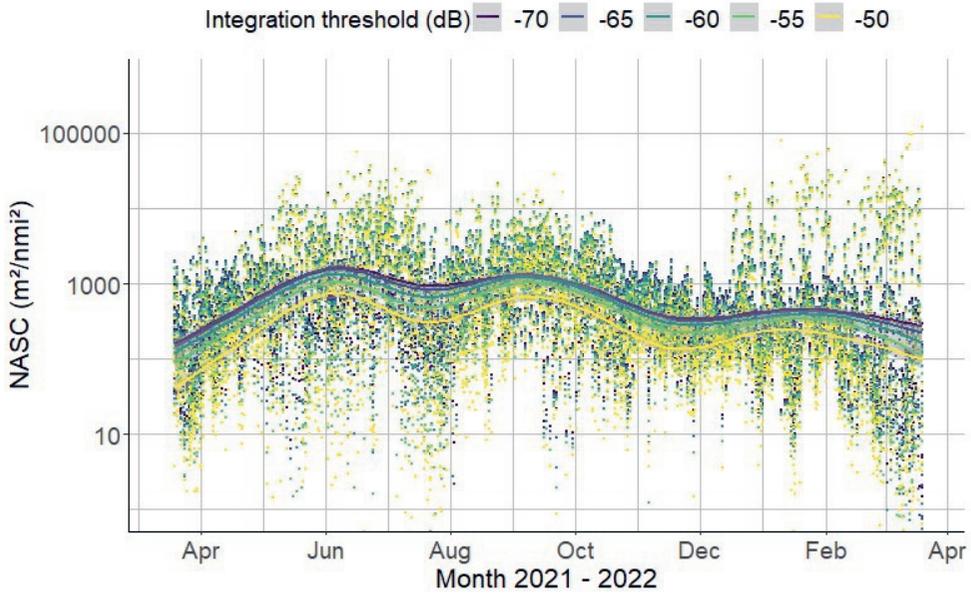


Figure S2. Fish density over the course of one year using different lower volume backscatter integration thresholds ranging from -70 to -50 dB re $1 \text{ m}^2\text{m}^{-3}$, represented by the different colours. The observed fish density is presented as the NASC (nautical area scattering coefficient in $\text{m}^2\text{nmi}^{-2}$) on a logarithmic scale. The GAM smoothers show the overall trend for each threshold value.

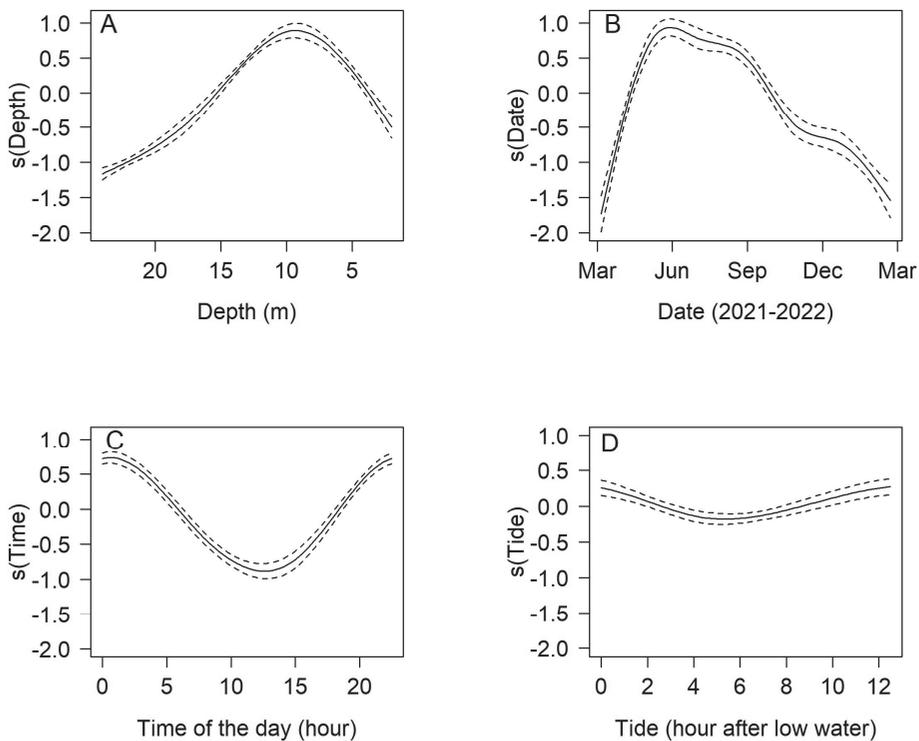


Figure S3. Smoothers of the partial effects of the GAMM showing vertical fish distribution: depth (A), date (B), time of day (C), and tidal cycle (D). The figures for the interactions are given in the main text. The y-axis presents the partial effect of the smooth terms on the NASC values (nautical area scattering coefficient in $\text{m}^2\text{nmi}^{-2}$), and the dotted lines present the 95% confidence intervals.

3. Supplementary tables

Table S1. Information about the four deployments for testing the impact of location (1 to 4), and the seven deployments (A to G) conducted during the study period. Deployment 4 is the initial phase of deployment A. Pitch and roll values varied over time owing to the dynamics of the study site, so values given here are median values per deployment period, and pitch and roll were not collected for deployments 1 to 3.

ID	Start	End	Depth (m)	WB AT	Coordinates	Pitch (°)	Roll (°)	Ping interval (s)	Sampling interval duration (s)
1	19-03-21	23-03-21	29.0	W1	052°58,605'N 004°45,410'E	NA	NA	0.4	Short: 72 Long: 957
2	19-03-21	23-03-21	23.0	W2	052°58,739'N 004°45,742'E	NA	NA	0.4	Short: 72 Long: 957
3	19-03-21	23-03-21	27.5	W3	052°58,540'N 004°45,877'E	NA	NA	0.4	Short: 72 Long: 957
4A	19-03-21	15-04-21	28.5	W4	052°58,607'N 004°45,436'E	-7	1	0.4	Short: 72 Long: 957
B	15-04-21	20-05-21	26.5	W4	052°58,539'N 004°45,851'E	11	0	0.25	Short: 72 Long: 957
C	20-05-21	29-09-21	27.0	W2	052°58,605'N 004°45,747'E	-1	15	0.25	Short: 147 Long: 957
D	29-09-21	13-10-21	26.5	W1	052°58,605'N 004°45,747'E	8	-2	0.4	Short: 147 Long: 957
E	13-10-21	02-12-21	26.5	W1	052°58,605'N 004°45,747'E	11	-1	0.4	Short: 147 Long: 957
F	02-12-21	08-03-22	27.5	W1	052°58,648'N 004°45,278'E	1	1	0.4	Short: 147 Long: 957
G	08-03-22	20-03-22	26.0	W2	052°58,607'N 004°45,436'E	-1	10	0.4	Short: 147 Long: 957

Table S2. Details on the calibration of the 38 kHz transducers used in this study. Calibration was carried out in the harbour of IJmuiden (the Netherlands).

WBAT	Calibration date	Salinity (ppt)	Water temperature (°C)	38 kHz gain	Minor-axis 3 dB beam angle (°)	Major-axis 3 dB beam angle (°)
W1	02/03/2021	23.1	6.6	20.634	18.08	18.02
W2	24/02/2021	22.3	6.0	20.414	17.77	18.43
W3	23/02/2021	23.0	6.2	20.460	17.95	18.14
W4	02/03/2021	23.1	6.6	20.860	19.18	17.88

Table S3. Detection settings for automatic single target detection in Echoview.

Setting	Value
TS threshold (compensated TS) (dB re 1 m ²)	-68
Pulse length determination level (dB re 1 W)	6
Minimum normalized pulse length	0.5
Maximum normalized pulse length	1.8
Maximum beam compensation (dB re 1 m ²)	9
Maximum st dev. of minor-axis angles (degrees)	1.2
Maximum st dev. of major-axis angles (degrees)	1.2

Table S4. Detection settings for automatic fish school detection in Echoview.

Setting	Value
Mode	Ping time
Minimum total school height (m)	1.8
Minimum candidate length (s)	3
Minimum candidate height (m)	1.2
Maximum vertical linking distance (m)	0.5
Maximum horizontal linking distance (s)	0.5
Minimum threshold (dB)	-55

Table S5. Detection settings for automatic target track detection in Echoview.

Setting	Value
Mode	4D
Track detection algorithm gain values (range from 0-1)	
- Alpha major axis	0.5
- Alpha minor axis	0.5
- Alpha range	0.3
- Beta major axis	0.5
- Beta minor axis	0.5
- Beta range	0.3
Track acceptance	
- Minimum number of single targets per track	4
- Minimum number of pings in track	6
- Maximum gap between single targets (pings)	3



Chapter 4

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

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Abstract

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

1. Introduction

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

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

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

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

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

This study investigated the diet composition of herring and sprat throughout an entire year in the Dutch Wadden Sea using DNA metabarcoding targeting the Cytochrome Oxidase Subunit I (CO1) gene and 18S ribosomal RNA regions.

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

2. Methods

2.1 Fish and zooplankton sampling

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

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

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

Additionally, zooplankton samples were collected using a centrifugal pump (pump rate: ~80 l/min) deployed on the ship during fishing operations to capture concurrent prey communities and fish stomach contents. For each zooplankton sample, 100 l of sea water were pumped from a depth of 2-3 m and sieved over a 200 µm plankton net. A total of 66 zooplankton samples were collected: 22 for DNA analysis and 44 for image analysis, corresponding to 1 DNA sample and 2 image analysis samples per fishing location. Zooplankton samples for DNA analysis were preserved in 96% ethanol and stored at -20°C, and samples for image analysis were preserved in 4% formalin and stored at room temperature.

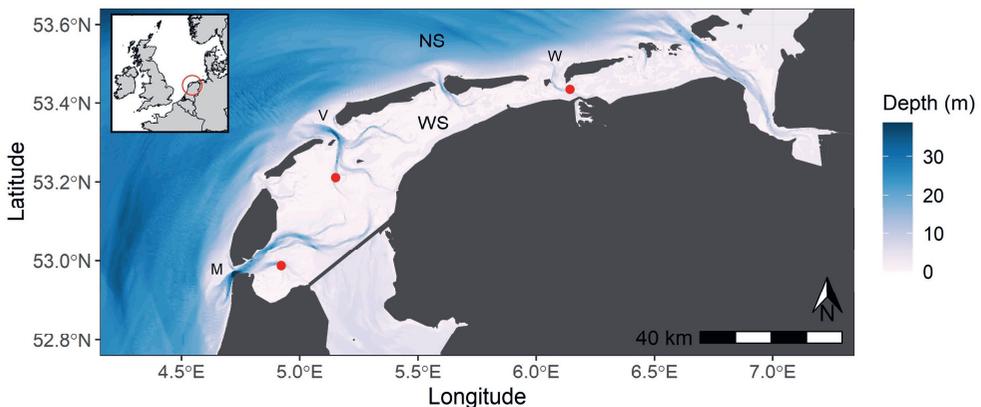


Figure 1. Map of the Dutch Wadden Sea (WS), adjacent to the North Sea (NS), with colours representing bathymetry. The inlets are denoted by their initial letters (M = Marsdiep, V = Vliestroom, W = Westgat), land area is depicted in dark grey, and sampling locations are marked with red circles.

2.2 Fish stomach content sampling

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

2.3 DNA extraction, amplification, and library preparation

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

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

respectively. Both protocols were extended with the EXP-PBC096 kit, according to manufacturer's protocol.

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

After quantifying DNA concentrations of barcoded amplicons, samples were pooled in equimolar concentrations and cleaned using Agencourt Ampure XP beads (Beckman Coulter Inc., IN, USA). Pooled products were further processed using SQK-LSK110 and SQK-LSK114 sequencing kits, according to manufacturer's protocol. DNA concentrations were determined using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). DNA extractions, PCR reaction preparations and library preparations were performed inside a UV cabinet to prevent contamination.

2.4 MinION nanopore sequencing

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

2.5 Bioinformatic analysis

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

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

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

identities, all identified species were considered reliable and species names were combined, or the record was downgraded to a genus level identification.

2.6 Image based processing of zooplankton samples

Samples containing high densities of different-sized material, were size fractioned using 200, 300 or 500 μm sieves. Fractions with high zooplankton densities were further subsampled using a Motoda box splitter. Clumps of jellyfish or other large material were manually taken out and thoroughly rinsed with seawater to retain attached plankton. Full samples, size fractions, or subsamples were placed in an acrylic tray (dimensions 24.5 x 15.8 x 3.0 cm) to be scanned using an Epson Perfection V850 scanner at 3200 dpi. Captured images were processed using Zooprocess (Gorsky et al., 2010) to isolate individual zooplankton objects and to extract morphological features for each object. Subsequently, the processed images, associated metadata, and morphological features were uploaded to Ecotaxa (Picheral et al., 2022). Annotation of the images was performed in Ecotaxa, aided by its supervised machine learning feature and a learning set consisting of manually classified objects from 30 randomly selected samples of our area. The objects were classified into nine main taxonomic groups: Annelida, Bivalvia, Cirripedia, Copepoda, Foraminifera, Gastropoda, Malacostraca, Ostracoda, and an 'other' category encompassing Echinodermata, Daphnia, Dinoflagellates, Bryozoa larvae, and Ctenophora. Additionally, a class was created for artefacts, including bubbles and detritus. Automatically generated annotations were manually verified, corrected, and subsequently exported from Ecotaxa.

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

2.7 Data analysis

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

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

Data filtering and statistical analysis of metabarcoding results were facilitated by R package Phyloseq v1.44.0 (McMurdie & Holmes, 2013). Rarefaction curves were generated and read count per sample was plotted against observed number of clustered amplicon sequence variants (ASVs, supplementary Figures S2-S4). Correcting for rarefaction is a topic of significant debate (McMurdie & Holmes, 2014; Cameron et al., 2021). Subsampling for rarefaction was applied, given the positive trend observed in sample read count against ASV diversity. Notably, runs from the first batch were sequenced twice as long, resulting in twice the ASV diversity. Read counts and sample sizes at each stage are detailed in supplementary Tables S2 and S3 for CO1 and 18SV4, respectively. To homogenize prey diversity across all samples, read counts were standardized to 24000 reads for all runs using Phyloseqs 'rarefy_even_depth' function. Consequently, 7 CO1 and 11 18SV4 samples were excluded owing to insufficient read counts.

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

Presence or absence of prey per sample was used to calculate the frequency of occurrence at species and genus levels to classify the 10 most observed prey per

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

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

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

$$E_i = (r_i - p_i)/(r_i + p_i) \quad (\text{Eq. 2})$$

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

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

3. Results

3.1 Stomach fullness and condition

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

3.2 Diet composition and seasonal variation

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

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

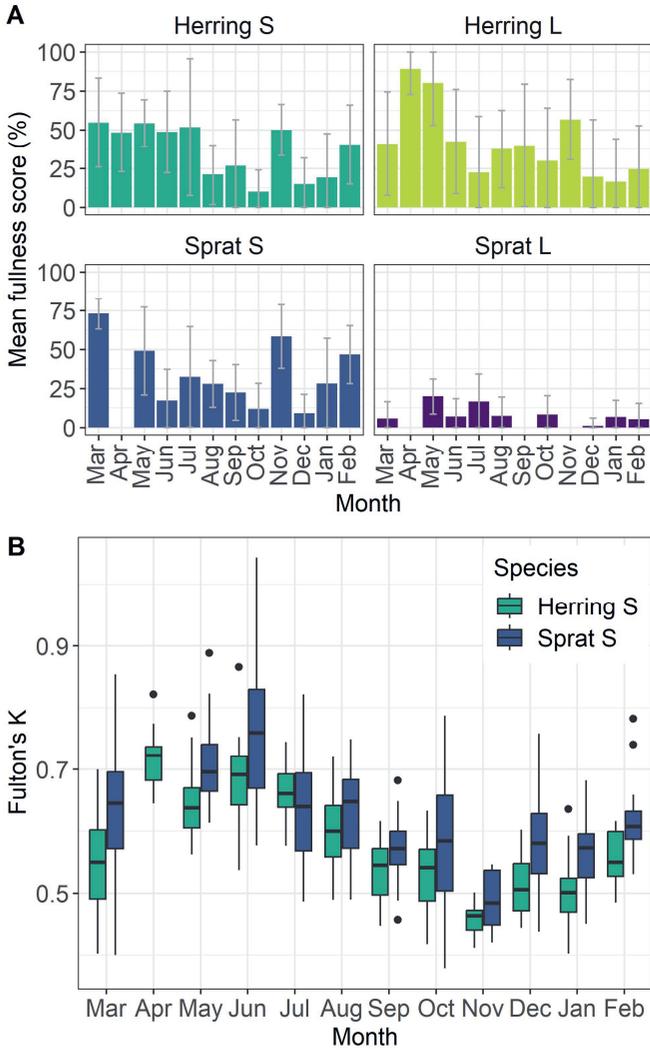


Figure 2. A) Average stomach fullness of the four predator groups per month, with error bars indicating the standard deviation. Note that there are no observations for sprat in April and for large sprat in November. B) Boxplots of Fulton's K for small herring and sprat per month. Boxes show minimum,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

bifilosa was positioned between winter months, and *A. tonsa* was positioned between late summer months, corresponding with their seasonal occurrence.

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

On average, copepods made up about 35% of the reads in the diet of the studied herring and sprat, yet this varies widely per size class (Figure 5). The copepod fraction was largest in small sprat (CO1: 57%, 18SV4: 49%) and small herring (CO1: 41%, 18SV4: 38%), followed by large sprat (CO1: 36%, 18SV4: 34%), and large herring (CO1: 12%, 18SV4: 17%). Stomachs of larger fish, especially large herring, contained higher percentages of pelagic invertebrates, like mysids and fish. Both herring and sprat exhibited regular consumption of (larvae of) benthic invertebrates. This group encompassed various species of Amphipoda, Decapoda, and Gastropoda, such as *Corophium volutator*, *Carcinas meanas*, and *Peringia ulvae* (supplementary Table S6). Most benthic invertebrates observed in the diet were likely (planktonic) larval stages of species that are considered benthic as adults. Metabarcoding lacks resolution to specify life stages, but larvae were regularly observed macroscopically, as well as some benthic adult individuals.

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

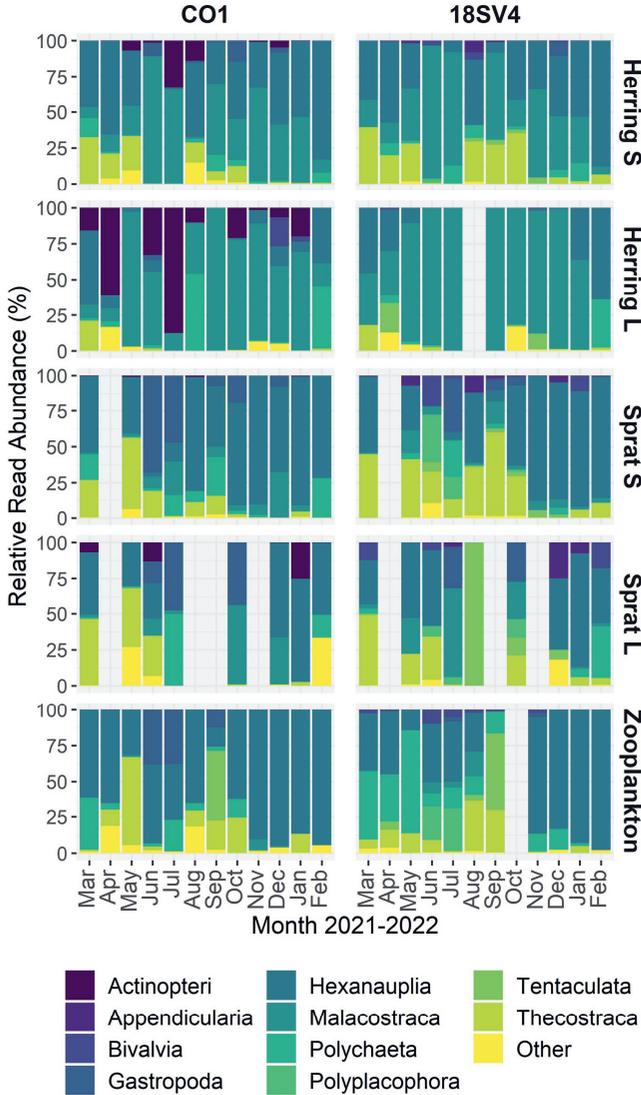


Figure 3. Relative Read Abundances (RRA) indicating diet composition of the four predator groups and the community composition of the zooplankton samples at class level of CO1 (left) and 18SV4 (right). The bars represent different months, with empty bars indicating either no samples could be collected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

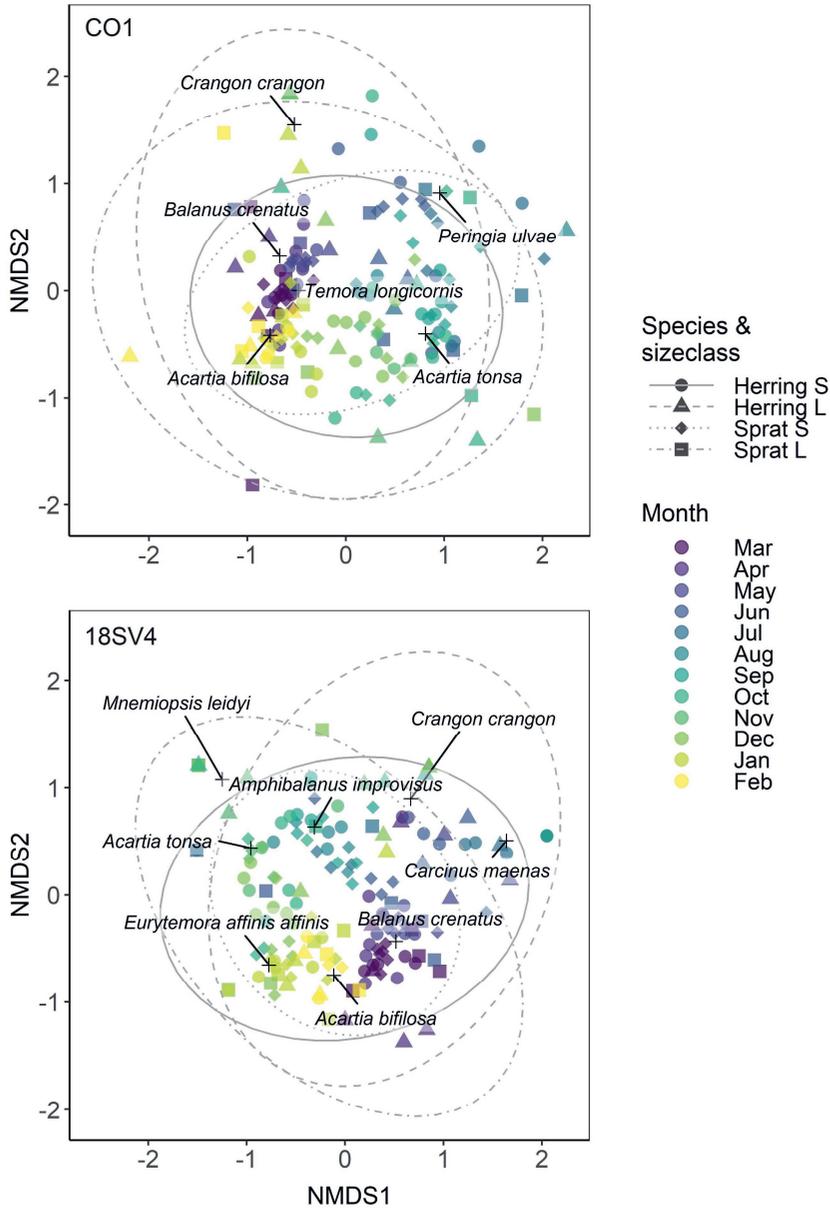


Figure 4. Nonparametric multidimensional scaling (NMDS) plots illustrating fish diet composition based on Bray-Curtis distances of stomach content using CO1 (top) and 18SV4 (bottom). Each sample is represented as a single symbol, with colour indicating month and shape indicating predator group. Prey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

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

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

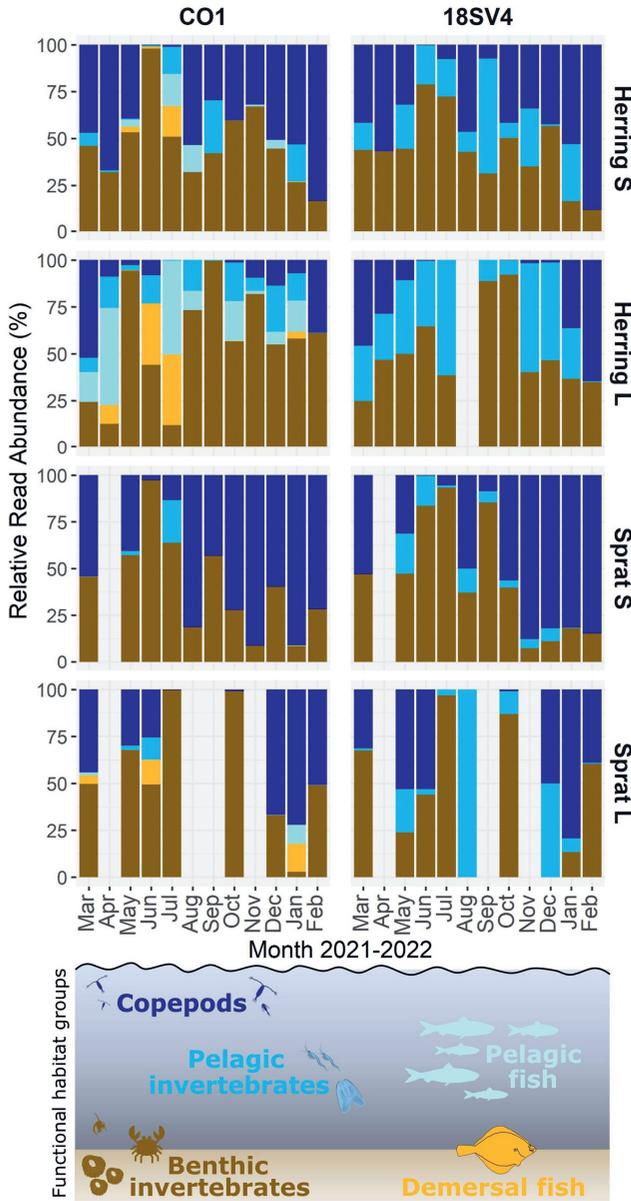


Figure 5. Diet composition of the four predator groups, categorized in functional habitat groups. Coloured bars illustrate relative read abundance (RRA) of functional habitat groups of CO1 (left) and 18SV4 (right). Empty bars indicate that no samples were collected or that samples did not pass quality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

Table 2. Frequency of occurrence (%) of preyed fish species in the four predator groups (Her = herring, Spr = sprat), using marker CO1. Species are listed if observed at least three times, and asterisks indicate that no results can be provided because host sequences were excluded. Months of occurrence in predator stomachs are indicated on the right side (grey = presence and white = absence).

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

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

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

3.3 Seasonal patterns in zooplankton

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

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

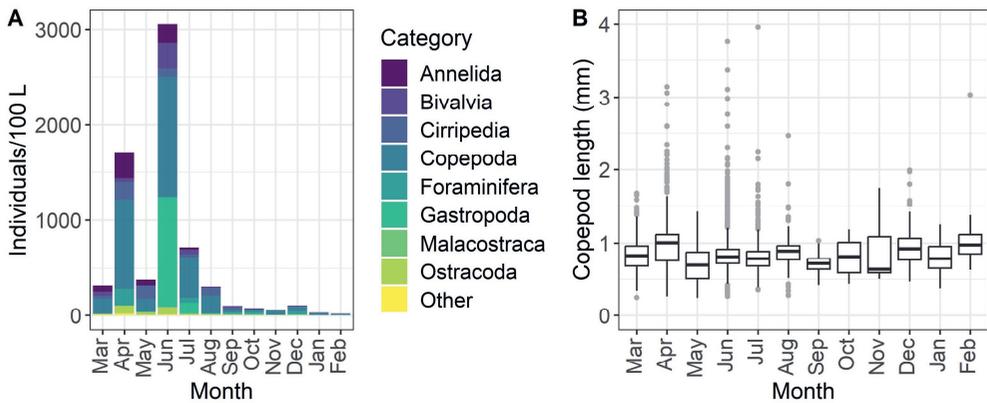


Figure 6. Zooplankton image analysis results per month: A) abundance and B) copepod length. Colours indicate taxonomic categories. Boxes show minimum, 1st quartile, median, 3rd quartile, and maximum values.

3.4 Intra- and interspecific variation

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

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

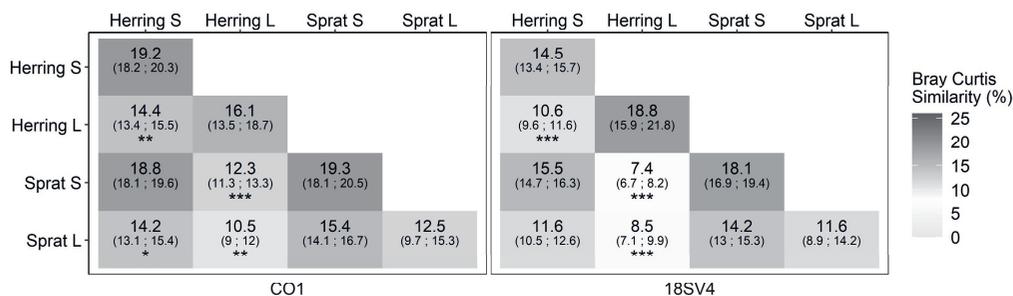


Figure 7. The Bray-Curtis similarity index (BCI) presented in percentage, between and across each predator group, with 95% confidence intervals between brackets. A low BCI indicates low niche overlap. Asterisks indicate statistical significance (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

3.5 Selective feeding behaviour

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

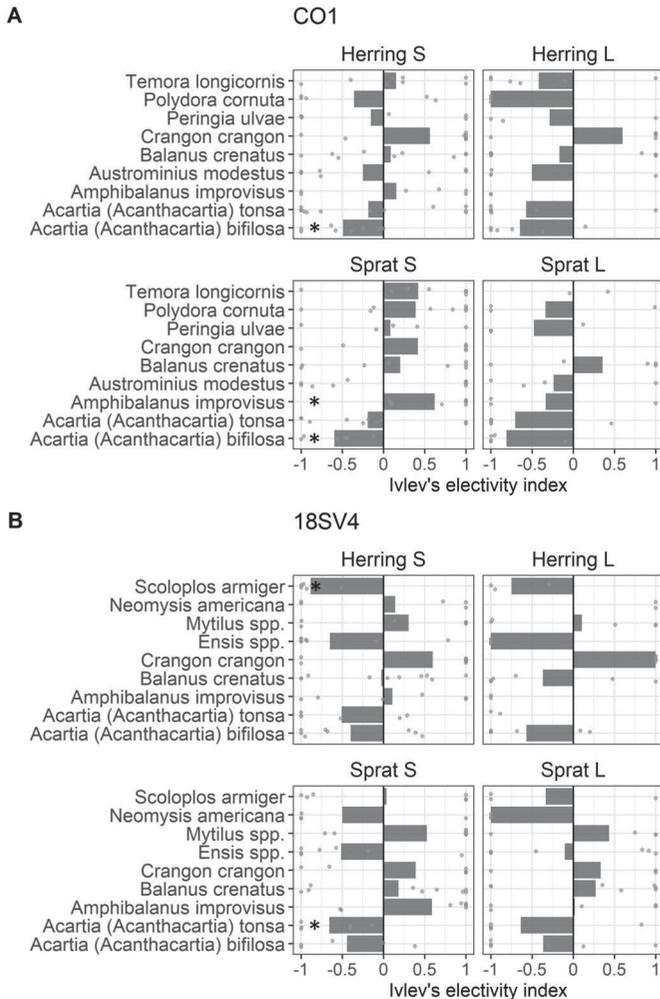


Figure 8. Ivlev's electivity index for the four predator groups for A) CO1 and B) 18SV4. Negative values indicate avoidance, positive values indicate selection. Bars indicate means and grey points indicate individual samples. Means are only given if $n \geq 3$. Asterisks indicate $P < 0.01$, obtained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

4. Discussion

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

4.1 No single diet method is perfect

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

DNA metabarcoding is a well-established tool for describing the diversity of (pelagic) communities and has been utilized in diet studies for over a decade. One of the major advantages of DNA metabarcoding is the high resolution in identification of degraded prey items and soft bodied taxa (Ershova et al., 2021; Novotny et al., 2022). This was especially relevant for sprat, which showed more degraded prey in their stomach than herring. The application of two markers enhanced the overall completeness of prey species identification. The degree of detail achieved in this study, both taxonomically and temporally, is necessary to understand processes operating at the base of the food web. However, identifying trophic interactions through DNA metabarcoding has its limitations. With the rapid expansion of reference databases and numerous studies on the impact of technical choices to minimize bias, such as marker selection and bioinformatic approaches (e.g. Alberdi et al., 2018; van der Loos & Nijland, 2020), the primary remaining issue is establishing the quantitative value of the proportion of reads. The RRA is

impacted by factors such as PCR bias and variable gene copy numbers among tissues and taxa, and is therefore not directly quantitatively equal to prey abundance (Lamb et al., 2019). Furthermore, the developmental stages of prey may impact RRA, particularly in the CO1 marker, as mitochondrial activity is generally higher during growth (Duke & Burton, 2020).

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

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

4.2 Seasonal variation in stomach fullness and fish condition

Herring and sprat stomach fullness varied monthly, showing no clear seasonal pattern. Large sprat exhibited exceptionally low stomach fullness throughout the year. Falkenhaug & Dalpadado (2014) reported decreasing fullness with increasing sprat size, and reduced feeding activity of large sprat during winter. Surprisingly, we also observed low values in spring and summer: maturing sprat typically intensify feeding during spring to meet the energy demands of reproduction. The notable prevalence of empty stomachs in our study may indicate a lack of feeding flexibility or the absence of suitable prey for adult sprat in the Wadden Sea. Yet, sprat often displays relatively low stomach fullness in the field, as compared to laboratory conditions (Peck et al., 2012a). An alternative explanation is that adult sprat primarily feed at night (Bernreuther et al., 2013). With fast prey degradation

and fast gastric evacuation rates, e.g. estimated for herring at 1.75 h (Darbyson et al., 2003), nocturnal feeding fish will have empty stomachs during the day. However, our study setup, limited to one sample per day during daylight, did not allow to study the effect of sampling time on stomach fullness.

Given the priority of growth over energy storage in juvenile fish, Fulton's K values remained low for small herring and sprat. Condition varied seasonally, with higher Fulton's K values observed from April to July, the months of elevated zooplankton abundance. A study in the Baltic Sea on herring and sprat similarly identified a positive correlation between condition and total zooplankton biomass (Casini et al., 2006).

4.3 Local and temporal variation in diet composition

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

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

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

4.4 Intra- and interspecific interactions

Diets of small herring and sprat did not differ significantly, implying potential interspecific competition during periods of limited food availability. This observation might partly explain their generalist diet, as high abundances of clupeids in the Wadden Sea necessitate the use of all available resources. In the Dutch Wadden Sea, juvenile herring and sprat exhibit highest densities from mid-spring to early autumn (Maathuis et al., 2024a). This peak coincides with high zooplankton densities, providing favourable conditions for juvenile fish growth and development. Despite winter's reduced zooplankton availability, lower fish densities may alleviate resource scarcity.

In contrast, while no significant difference in diet was observed between the two size classes of sprat, as also noted by Falkenhaug & Dalpadado (2014), herring displayed an ontogenetic shift consistent with observations in the Baltic Sea (Casini et al., 2004). The primary difference in diet between small and large herring was the increased consumption of mysids and fish in large herring. Cannibalism in herring is well-documented (Corten, 2013), and previous studies also noted herring feeding on sprat eggs and larvae (Karaseva et al., 2013), indicating intra-guild predation, an important factor in the dynamics of SPF (Irigoiien & de Roos, 2011). Particularly in April, large herring were observed consuming clupeid (post-)larvae. While this could potentially impact local early life stage mortality, the sheer abundance of early life stage clupeids largely outnumbers large herring, mitigating

its overall influence. Most macroscopic diet studies on herring and sprat do not mention fish larvae as a significant part of their diet, which may be attributed to the rapid digestion rates of early life stages, losing morphological characters required for visual identification (Legler et al., 2010). Furthermore, many prey fish species were only seasonally part of their diet, probably in their early pelagic stages (eggs or larvae), for instance sole (*Solea solea*), plaice (*Pleuronectes platessa*), and pilchard (*Sardina pilchardus*).

4.5 Herring and sprat show opportunistic feeding behaviour

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

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

4.6 Wider implications

This study revealed that herring and sprat exhibited a generalist diet, consuming the diverse prey community available in the Wadden Sea without strong prey selection. This suggests resilience to future changes; if one prey species declines, they can likely adapt to shifts in community composition. This adaptability is already evident in their seasonally diverse diet, with different species consumed each season. Being an opportunistic, generalist mesopredator, provides an advantage in adapting to ecosystem changes, allowing for rapid adjustment to novel conditions (Olin et al., 2022). Ecosystem models used for predicting future scenarios or carbon fluxes, often rely on broad assumptions regarding diet composition due to the lack of accurate and representative field data. Understanding interactions at the base of the food web is crucial for various management implications, including developing or refining ecosystem models and studying fisheries impact. By elucidating predator-prey relationships and providing detailed data on trophic dynamics for these key species, this study significantly enhances our understanding of ecosystem functioning.

Conclusions

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

Acknowledgements

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Supplementary materials

1. Supplementary figures

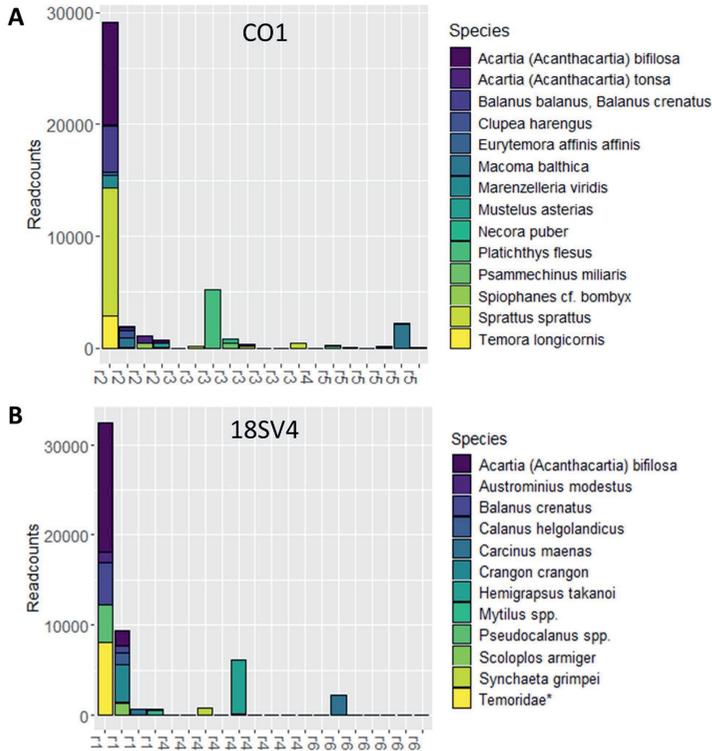


Figure S1. Read counts of the negative control samples for A) CO1 and B) 18SV4. The names of the samples indicate the run ID: r1 and r2 belong to batch one and r3 until r6 belong to batch two. The taxa are filtered to include only the species with a minimum occurrence of 300 reads in the entire dataset.

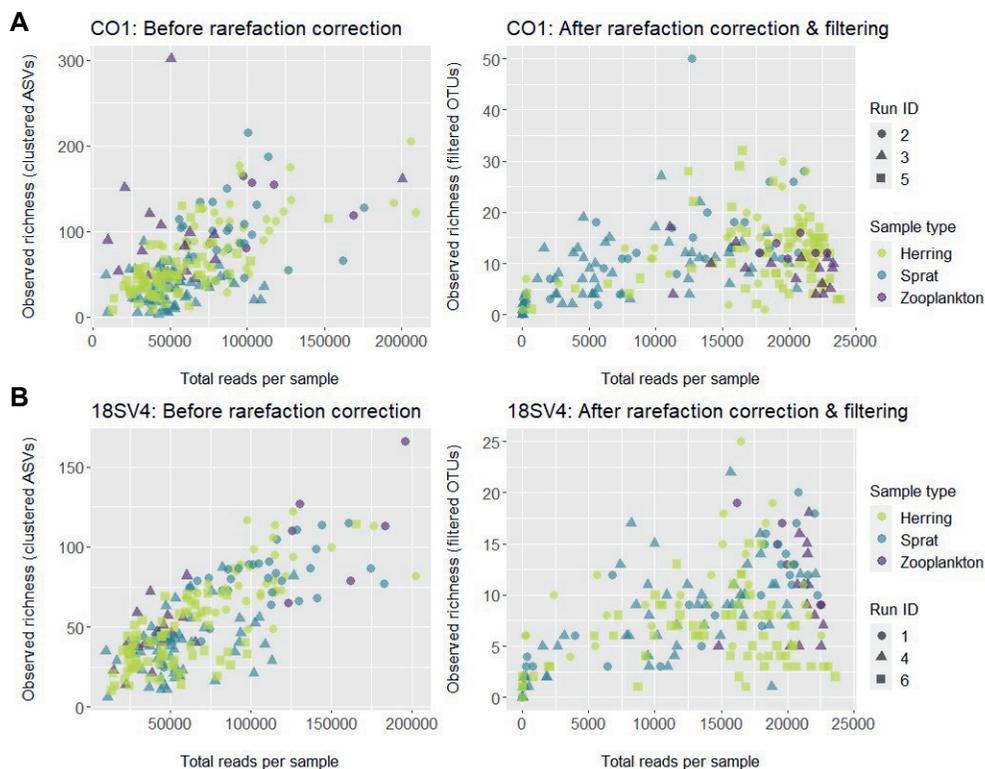


Figure S2. The correlation of richness and the total read count per sample for the A) CO1 samples and B) 18SV4 samples. The left panels depict richness in number of clustered amplicon sequence variants (ASVs), and the right panels depict richness in number of filtered operational taxonomical units (OTUs). The left panels show the data before correction for rarefaction. The panels on the right show the data after pre-processing, so after rarefaction correction and filtering. The colours indicate the sample types and run IDs are distinguished by shape.

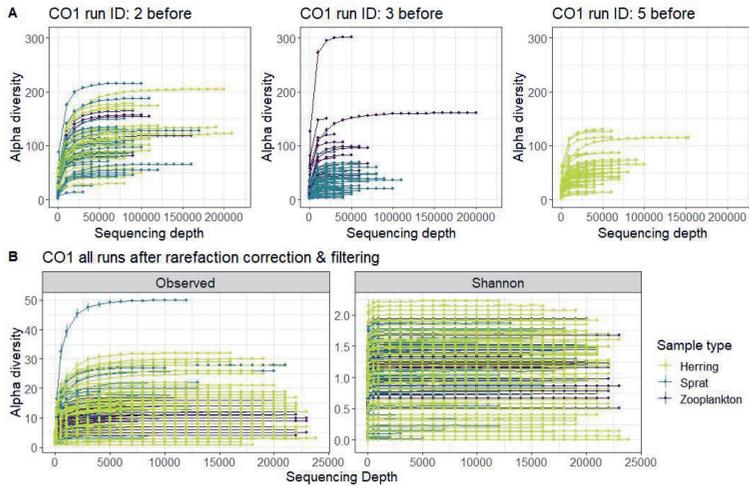


Figure S3. Rarefaction curves of the CO1 samples A) before rarefaction and B) after rarefaction correction and filtering. To ease comparison, panel A shows the three runs separately. In panel B, the left side depicts the rarefaction curves for the number of observed species, and rarefaction curves for the Shannon index are depicted on the right side. The colours indicate the sample types.

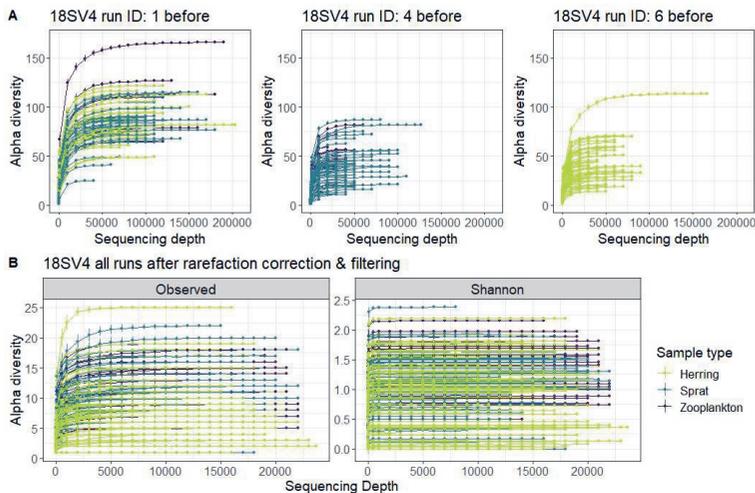


Figure S4. Rarefaction curves of the 18SV4 samples A) before rarefaction and B) after rarefaction correction and filtering. To ease comparison, panel A shows the three runs separately. In panel B, the left side depicts the rarefaction curves for the number of observed species, and rarefaction curves for the Shannon index are depicted on the right side. The colours indicate the sample types.

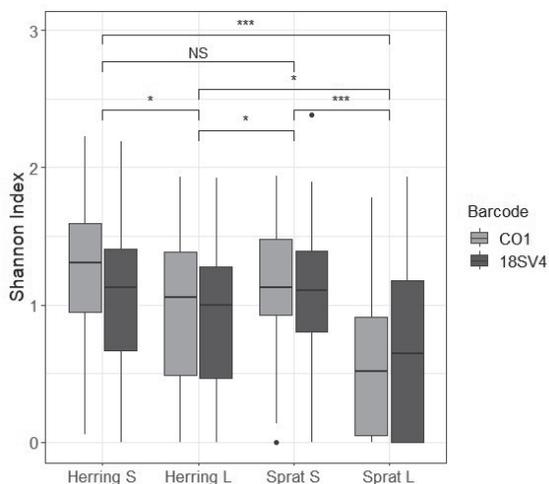


Figure S5. Boxplots showing alpha diversity of fish stomach contents: Shannon index values for the four predator groups per marker. Boxes show minimum, 1st quartile, median, 3rd quartile and maximum values. There was no statistical difference between the markers per predator group ($F(1) = 1.87, P = 0.17$). Significance is indicated with asterisks (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

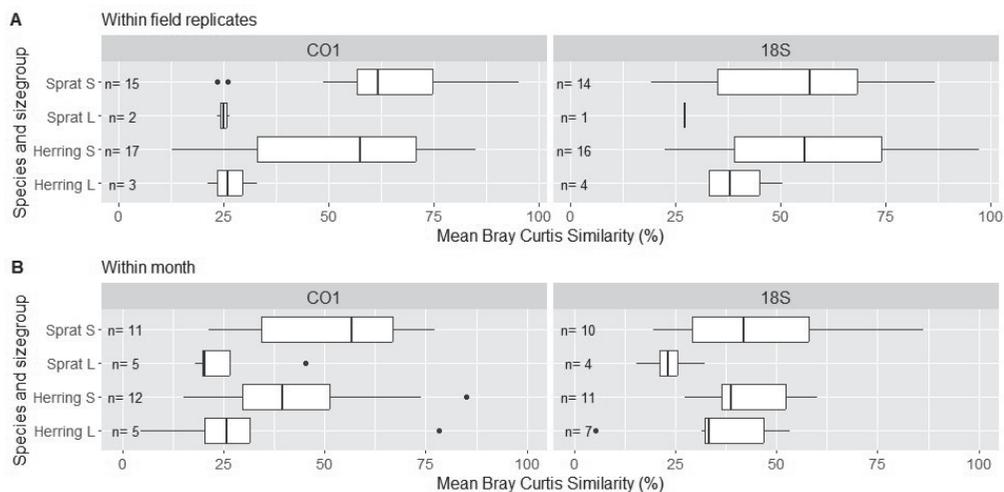


Figure S6. Boxplots of the Bray Curtis similarity ($((1 - \text{Bray Curtis distance}) * 100)$) for the different predator groups and markers for A) within field replicas and B) within months (including two different regions). Boxes show minimum, 1st quartile, median, 3rd quartile and maximum values, and sample sizes are provided.

2. Supplementary tables

Table S1. Overview of collected and analysed samples. Numbers represent samples per group (species and size), per sample type and month. The 'Fish' rows note the number of collected fish. The 'DNA' rows note the number of pooled DNA samples, while the 'CO1' and '18SV4' rows correspond to the number of samples remaining for analysis after corrections and filtering. The 'Image' row notes the sample size for image analysis of zooplankton.

Group	Type	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Sum
Herring small	Fish	27	30	29	20	30	19	29	30	15	30	30	15	304
	DNA	6	6	6	4	6	4	6	6	3	6	6	3	62
	CO1	6	6	6	4	6	4	6	6	3	6	5	3	61
	18SV4	6	6	6	4	5	4	5	5	3	6	6	2	58
Herring large	Fish	14	25	15	10	10	4	10	20	15	19	29	15	186
	DNA	3	5	3	2	2	1	2	4	3	4	6	3	38
	CO1	3	4	3	2	2	1	1	4	2	4	5	3	34
	18SV4	3	3	3	2	1	0	1	3	3	3	6	3	30
Sprat small	Fish	26	0	30	29	30	17	30	25	15	30	30	15	277
	DNA	6	0	6	6	6	4	6	5	3	6	6	3	57
	CO1	6	0	6	5	4	3	6	5	3	6	6	3	53
	18SV4	6	0	6	6	4	3	6	5	3	6	6	2	53
Sprat large	Fish	30	0	5	25	9	10	0	15	0	24	25	15	158
	DNA	6	0	1	5	2	2	0	3	0	5	5	3	32
	CO1	5	0	1	4	2	0	0	2	0	3	4	3	24
	18SV4	4	0	1	4	2	1	0	2	0	4	4	2	24
Meso-zoo-plank-ton	Image	4	4	4	4	4	4	4	4	4	4	4	4	44
	DNA	2	2	2	2	2	2	2	1	2	2	2	1	22
	CO1	2	2	2	2	2	2	2	1	2	2	2	1	22
	18SV4	2	2	2	2	2	2	1	0	2	2	2	1	20

Table S2. Read counts and sample sizes for distinct runs of CO1 samples at different stages of data processing.

Run ID	Before rarefaction correction			After rarefaction correction	After filtering: all samples			Further filtering: >100 reads
	Mean read count per sample	Mean observed ASVs per sample	Total number of samples & read count	Total number of samples & read count	Mean read count per sample	Mean observed OTUs per sample	Total number of samples & read count	Total number of samples & read count
2	93285	101	213; 13913319	206; 4944000	14117	13	206; 2861664	194; 2694101
3	52078	47			9990	8		
5	48940	48			18363	12		

Table S3. Read counts and sample sizes for distinct runs of 18SV4 samples at different stages of data processing.

Run ID	Before rarefaction correction			After rarefaction correction	After filtering: all samples			Further filtering: >100 reads
	Mean read count per sample	Mean observed ASVs per sample	Total number of samples & read count	Total number of samples & read count	Mean read count per sample	Mean observed OTUs per sample	Total number of samples & read count	Total number of samples & read count
1	110650	83	211; 15006698	200; 4800000	13892	10	200; 2694465	185; 2694247
4	52655	40			12612	8		
6	48736	38			13987	6		

Table S4. Overview of prey species, with taxonomical information, categorized into their respective functional habitat groups (inv. = invertebrates). The 2nd and 3rd column indicate whether a species was identified by the CO1 marker (C), and 18SV4 marker (S), respectively. In instances where specific species could not be identified to single name, species names were combined (see methodology). This list includes prey taxa with a RRA of at least 1% in one of the samples.

Prey species name	C	S	Genus	Family	Class	Phylum	Functional group
<i>Acartia biflosa</i>	1	1	<i>Acartia</i>	<i>Acartiidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Acartia tonsa</i>	1	1	<i>Acartia</i>	<i>Acartiidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Acartia clausi</i>	0	1	<i>Acartia</i>	<i>Acartiidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Alitta succinea</i>	0	1	<i>Alitta</i>	<i>Nereididae</i>	Polychaeta	Annelida	Benthic inv.
<i>Alitta succinea</i> , <i>Hediste diversicolor</i>	1	0	<i>Alitta</i> , <i>Hediste</i>	<i>Nereididae</i>	Polychaeta	Annelida	Benthic inv.
<i>Ammodytes tobianus</i>	1	0	<i>Ammodytes</i>	<i>Ammodytidae</i>	Actinopteri	Chordata	Pelagic fish
<i>Amphiascopsis cinctus</i>	1	0	<i>Amphiascopsis</i>	<i>Miraciidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Amphibalanus improvisus</i>	1	1	<i>Amphibalanus</i>	<i>Balanidae</i>	Thecostraca	Arthropoda	Benthic inv.
<i>Amphibalanus improvisus</i> , <i>Balanus renatus</i>	1	0	<i>Amphibalanus</i> , <i>Balanus</i>	<i>Balanidae</i>	Thecostraca	Arthropoda	Benthic inv.
<i>Aora gracilis</i>	1	1	<i>Aora</i>	<i>Aoridae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Arenicola marina</i>	1	1	<i>Arenicola</i>	<i>Arenicolidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Atherina presbyter</i>	1	0	<i>Atherina</i>	<i>Atherinidae</i>	Actinopteri	Chordata	Pelagic fish
<i>Aurelia aurita</i>	1	0	<i>Aurelia</i>	<i>Ulmaridae</i>	Scyphozoa	Cnidaria	Pelagic inv.
<i>Austrominius modestus</i>	1	1	<i>Austrominius</i>	<i>Elminidae</i>	Thecostraca	Arthropoda	Benthic inv.
<i>Balanus crenatus</i>	1	1	<i>Balanus</i>	<i>Balanidae</i>	Thecostraca	Arthropoda	Benthic inv.
<i>Bathyporeia pelagica</i>	1	0	<i>Bathyporeia</i>	<i>Pontoporeiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Bathyporeia sarsi</i>	1	1	<i>Bathyporeia</i>	<i>Pontoporeiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Calanus helgolandicus</i>	0	1	<i>Calanus</i>	<i>Calanidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Caprella equilibra</i>	0	1	<i>Caprella</i>	<i>Caprellidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Caprella mutica</i>	1	0	<i>Caprella</i>	<i>Caprellidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Carcinus maenas</i>	1	1	<i>Carcinus</i>	<i>Carcinidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Centropages spp.</i>	0	1	<i>Centropages</i>	<i>Centropagidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Centropages typicus</i>	0	1	<i>Centropages</i>	<i>Centropagidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Chelon auratus</i> , <i>Chelon ramada</i>	1	0	<i>Chelon</i>	<i>Mugilidae</i>	Actinopteri	Chordata	Pelagic fish
<i>Corophium volutator</i>	1	0	<i>Corophium</i>	<i>Corophiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Crangon crangon</i>	1	1	<i>Crangon</i>	<i>Crangonidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Crassikorophium bonellii</i>	0	1	<i>Crassikorophium</i>	<i>Corophiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Cumopsis goodsir</i>	1	0	<i>Cumopsis</i>	<i>Bodotriidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Cyanea lamarckii</i>	0	1	<i>Cyanea</i>	<i>Cyaneidae</i>	Scyphozoa	Cnidaria	Pelagic inv.
<i>Echinocardium cordatum</i>	1	0	<i>Echinocardium</i>	<i>Loveniidae</i>	Echinoidea	Echinodermata	Benthic inv.
<i>Electra pilosa</i>	1	1	<i>Electra</i>	<i>Electridae</i>	Gymnolaemata	Bryozoa	Benthic inv.
<i>Ensis ensis</i> , <i>Ensis leei</i> , <i>Ensis siliqua</i>	0	1	<i>Ensis</i>	<i>Pharidae</i>	Bivalvia	Mollusca	Benthic inv.
<i>Eteone longa</i>	0	1	<i>Eteone</i>	<i>Phyllodocidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Eudendrium racemosum</i>	0	1	<i>Eudendrium</i>	<i>Eudendriidae</i>	Hydrozoa	Cnidaria	Benthic inv.
<i>Euplokamis brunnea</i> , <i>Pleurobrachia pileus</i>	0	1	<i>Pleurobrachia</i>	<i>Pleurobrachiiidae</i>	Tentaculata	Ctenophora	Pelagic inv.
<i>Eurytemora affinis affinis</i>	1	1	<i>Eurytemora</i>	<i>Temoridae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Euterpina acutifrons</i>	1	0	<i>Euterpina</i>	<i>Euterpinidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Eutonina indicans</i>	1	0	<i>Eutonina</i>	<i>Eirenidae</i>	Hydrozoa	Cnidaria	Pelagic inv.
<i>Gammarus crinicornis</i>	1	0	<i>Gammarus</i>	<i>Gammaridae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Gammarus locusta</i>	0	1	<i>Gammarus</i>	<i>Gammaridae</i>	Malacostraca	Arthropoda	Benthic inv.

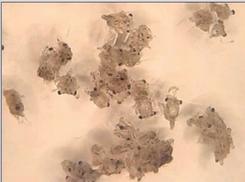
Prey (species name)	C	S	Genus	Family	Class	Phylum	Functional group
<i>Gammarus tigrinus</i>	1	1	<i>Gammarus</i>	<i>Gammaridae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Gastrosaccus spinifer</i>	0	1	<i>Gastrosaccus</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Harpacticus flexus</i>	1	0	<i>Harpacticus</i>	<i>Harpacticidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Harpacticus sp.</i>	0	1	<i>Harpacticus</i>	<i>Harpacticidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Hemigrapsus sanguineus</i>	1	0	<i>Hemigrapsus</i>	<i>Varunidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Hemigrapsus takanoi</i>	1	1	<i>Hemigrapsus</i>	<i>Varunidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Hippolyte varians</i>	1	1	<i>Hippolyte</i>	<i>Hippolytidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Hyperia galba</i>	0	1	<i>Hyperia</i>	<i>Hyperiidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Idotea balthica</i>	1	0	<i>Idotea</i>	<i>Idoteidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Jassa herdmani</i>	1	0	<i>Jassa</i>	<i>Ischyroceridae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Liocarcinus holsatus</i> , <i>Polybius henslowii</i>	1	0	<i>Liocarcinus</i> , <i>Polybius</i>	<i>Polybiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Liocarcinus marmoratus</i>	1	0	<i>Liocarcinus</i>	<i>Polybiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Liparis liparis</i>	1	0	<i>Liparis</i>	<i>Liparidae</i>	Actinopteri	Chordata	Demersal fish
<i>Macoma balthica</i>	1	0	<i>Limecola</i>	<i>Tellinidae</i>	Bivalvia	Mollusca	Benthic inv.
<i>Magelona johnstoni</i>	1	0	<i>Magelona</i>	<i>Magelonidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Marenzelleria viridis</i>	1	1	<i>Marenzelleria</i>	<i>Spionidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Melita palmata</i>	1	0	<i>Melita</i>	<i>Melitidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Merlangius merlangus</i>	1	0	<i>Merlangius</i>	<i>Gadidae</i>	Actinopteri	Chordata	Demersal fish
<i>Mesopodopsis slabberi</i>	1	0	<i>Mesopodopsis</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Microarthridion fallax</i>	1	0	<i>Microarthridion</i>	<i>Tachidiidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Microprotopus maculatus</i>	1	0	<i>Microprotopus</i>	<i>Microprotopidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Mnemiopsis leidyi</i>	0	1	<i>Mnemiopsis</i>	<i>Bolinopsidae</i>	Tentaculata	Ctenophora	Pelagic inv.
<i>Monocorophium acherusicum</i>	1	0	<i>Monocorophium</i>	<i>Corophiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Monopseudocuma gilsoni</i>	1	0	<i>Monopseudocuma</i>	<i>Pseudocumatidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Mytilus spp.</i>	0	1	<i>Mytilus</i>	<i>Mytilidae</i>	Bivalvia	Mollusca	Benthic inv.
<i>Necora puber</i>	1	0	<i>Necora</i>	<i>Polybiidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Neomysis americana</i>	1	1	<i>Neomysis</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Neomysis americana</i> , <i>Praunus flexuosus</i>	1	0	<i>Neomysis</i> , <i>Praunus</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Nephtys cirrosa</i>	1	0	<i>Nephtys</i>	<i>Nephtyidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Nototropis swammerdamei</i>	1	1	<i>Atylus</i>	<i>Atylidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Obelia bidentata</i>	1	1	<i>Obelia</i>	<i>Obeliidae</i>	Hydrozoa	Cnidaria	Benthic inv.
<i>Obelia longissima</i>	1	0	<i>Obelia</i>	<i>Obeliidae</i>	Hydrozoa	Cnidaria	Benthic inv.
<i>Oikopleura dioica</i>	0	1	<i>Oikopleura</i>	<i>Oikopleuridae</i>	Apendicularia	Chordata	Pelagic inv.
<i>Osmerus eperlanus</i>	1	0	<i>Osmerus</i>	<i>Osmeridae</i>	Actinopteri	Chordata	Pelagic fish
<i>Pagurus bullisi</i> , <i>Tomopagurus merimaculosus</i>	0	1	<i>Pagurus</i> , <i>Tomopagurus</i>	<i>Paguridae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Paracalanus parvus</i>	1	1	<i>Paracalanus</i>	<i>Paracalanidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Peringia ulvae</i>	1	1	<i>Peringia</i>	<i>Hydrobiidae</i>	Gastropoda	Mollusca	Benthic inv.
<i>Polycera quadrilineata</i>	1	0	<i>Polycera</i>	<i>Polyceridae</i>	Gastropoda	Mollusca	Benthic inv.
<i>Polydora cornuta</i>	1	1	<i>Polydora</i>	<i>Spionidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Pomatoschistus lozanoi</i>	1	0	<i>Pomatoschistus</i>	<i>Gobiidae</i>	Actinopteri	Chordata	Demersal fish
<i>Pomatoschistus spp.</i>	1	0	<i>Pomatoschistus</i>	<i>Gobiidae</i>	Actinopteri	Chordata	Demersal fish
<i>Praunus flexuosus</i>	1	1	<i>Praunus</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Pseudocalanus elongatus</i>	1	1	<i>Pseudocalanus</i>	<i>Clausocalanidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Pseudodiaptomus marinus</i>	1	1	<i>Pseudodiaptomus</i>	<i>Pseudodiaptomidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Pygospio elegans</i>	0	1	<i>Pygospio</i>	<i>Spionidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Rathkea octopunctata</i>	1	0	<i>Rathkea</i>	<i>Rathkeidae</i>	Hydrozoa	Cnidaria	Pelagic inv.
<i>Rhipidocotyle galeata</i>	0	1	<i>Rhipidocotyle</i>	<i>Bucephalidae</i>	Trematoda	Platyhelminthes	Benthic inv.
<i>Rhizostoma pulmo</i>	0	1	<i>Rhizostoma</i>	<i>Rhizostomatidae</i>	Scyphozoa	Cnidaria	Pelagic inv.
<i>Rhopilema nomadica</i>	0	1	<i>Rhopilema</i>	<i>Rhizostomatidae</i>	Scyphozoa	Cnidaria	Pelagic inv.

Prey (species name)	C	S	Genus	Family	Class	Phylum	Functional group
<i>Sardina pilchardus</i>	1	0	<i>Sardina</i>	<i>Clupeidae</i>	Actinopteri	Chordata	Pelagic fish
<i>Schistomysis kervillei</i>	0	1	<i>Schistomysis</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Schistomysis ornata</i>	1	0	<i>Schistomysis</i>	<i>Mysidae</i>	Malacostraca	Arthropoda	Pelagic inv.
<i>Scoloplos armiger</i>	0	1	<i>Scoloplos</i>	<i>Orbiniidae</i>	Polychaeta	Annelida	Benthic inv.
<i>Semibalanus balanoides</i>	1	0	<i>Semibalanus</i>	<i>Balanidae</i>	Thecostraca	Arthropoda	Benthic inv.
<i>Solea solea</i>	1	0	<i>Solea</i>	<i>Soleidae</i>	Actinopteri	Chordata	Demersal fish
<i>Spisula subtruncata</i>	1	0	<i>Spisula</i>	<i>Mactridae</i>	Bivalvia	Mollusca	Benthic inv.
<i>Sprattus sprattus</i>	1	0	<i>Sprattus</i>	<i>Clupeidae</i>	Actinopteri	Chordata	Pelagic fish
<i>Tachidius discipes</i>	1	0	<i>Tachidius</i>	<i>Tachidiidae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Temora longicornis</i>	1	1	<i>Temora</i>	<i>Temoridae</i>	Hexanauplia	Arthropoda	Copepoda
Eurytemora, Temora	0	1	<i>Temora</i> , <i>Eurytemora</i>	<i>Temoridae</i>	Hexanauplia	Arthropoda	Copepoda
<i>Tonicella marmorea</i>	0	1	<i>Tonicella</i>	<i>Mopaliidae</i>	Poly-placophora	Mollusca	Benthic inv.
<i>Urothoe poseidonis</i>	1	1	<i>Urothoe</i>	<i>Urothoidae</i>	Malacostraca	Arthropoda	Benthic inv.
<i>Yamaguticestus longicollis</i> , <i>Yamaguticestus squali</i>	0	1	<i>Yamaguticestus</i>	<i>Phyllobothriidae</i>	Cestoda	Platyhelminthes	Benthic inv.

Table S5. Frequency of occurrence (FO, %) of top 10 occurring species in the five sample groups for CO1 and 18SV4.

Species	Rank	CO1		18SV4	
		Species	FO (%)	Species	FO (%)
Herring S	1	<i>Temora longicornis</i>	65.6	<i>Acartia bifilosa</i>	46.6
	2	<i>Acartia tonsa</i>	59.0	<i>Paracalanus parvus parvus</i>	41.4
	3	<i>Balanus crenatus</i>	52.5	<i>Amphibalanus improvisus</i>	39.7
	4	<i>Acartia bifilosa</i>	49.2	<i>Oikopleura dioica</i>	36.2
	5	<i>Paracalanus parvus parvus</i>	42.6	<i>Eurytemora affinis affinis</i>	34.5
	6	<i>Harpacticus flexus</i>	39.3	<i>Balanus crenatus</i>	34.5
	7	<i>Euterpina acutifrons</i>	34.4	<i>Acartia tonsa</i>	34.5
	8	<i>Eurytemora affinis affinis</i>	32.8	<i>Pseudocalanus elongatus</i>	34.5
	9	<i>Amphibalanus improvisus</i>	29.5	<i>Calanus helgolandicus</i>	29.3
	10	<i>Pseudocalanus elongatus</i>	29.5	<i>Mnemiopsis leidyi</i>	29.3
Herring L	1	<i>Crangon crangon</i>	38.2	<i>Crangon crangon</i>	46.7
	2	<i>Acartia bifilosa</i>	38.2	<i>Acartia bifilosa</i>	36.7
	3	<i>Sprattus sprattus</i>	35.3	<i>Mnemiopsis leidyi</i>	33.3
	4	<i>Schistomysis ornata</i>	32.4	<i>Schistomysis kervillei</i>	30.0
	5	<i>Praunus flexuosus</i>	32.4	<i>Praunus flexuosus</i>	23.3
	6	<i>Osmerus eperlanus</i>	29.4	<i>Carcinus maenas</i>	23.3
	7	<i>Temora longicornis</i>	26.5	<i>Gastrosaccus spinifer</i>	20.0
	8	<i>Acartia tonsa</i>	23.5	<i>Calanus helgolandicus</i>	20.0
	9	<i>Merlangius merlangus</i>	23.5	<i>Pseudocalanus elongatus</i>	20.0
	10	<i>Mnemiopsis leidyi</i>	20.6	<i>Nototropis swammerdamei</i>	16.7
Sprat S	1	<i>Acartia tonsa</i>	67.9	<i>Balanus crenatus</i>	56.6
	2	<i>Temora longicornis</i>	60.4	<i>Pseudocalanus elongatus</i>	56.6
	3	<i>Paracalanus parvus parvus</i>	58.5	<i>Harpacticus sp.</i>	52.8
	4	<i>Acartia bifilosa</i>	56.6	<i>Acartia bifilosa</i>	50.9
	5	<i>Amphibalanus improvisus</i>	47.2	<i>Amphibalanus improvisus</i>	49.1
	6	<i>Balanus crenatus</i>	45.3	<i>Paracalanus parvus parvus</i>	47.2
	7	<i>Harpacticus flexus</i>	45.3	<i>Oikopleura dioica</i>	45.3
	8	<i>Polydora cornuta</i>	45.3	<i>Mytilus spp.</i>	41.5
	9	<i>Amphiascopsis cinctus</i>	37.7	<i>Temora longicornis</i>	41.5
	10	<i>Pseudocalanus elongatus</i>	37.7	<i>Acartia tonsa</i>	39.6
Sprat L	1	<i>Acartia bifilosa</i>	45.8	<i>Balanus crenatus</i>	45.8
	2	<i>Balanus crenatus</i>	41.7	<i>Acartia bifilosa</i>	45.8
	3	<i>Temora longicornis</i>	33.3	<i>Pygospio elegans</i>	33.3
	4	<i>Eurytemora affinis affinis</i>	29.2	<i>Eurytemora affinis affinis</i>	25.0
	5	<i>Acartia tonsa</i>	29.2	<i>Mytilus spp.</i>	25.0
	6	<i>Pseudocalanus elongatus</i>	25.0	<i>Temora longicornis</i>	25.0
	7	<i>Osmerus eperlanus</i>	25.0	<i>Pseudocalanus elongatus</i>	25.0
	8	<i>Rathkea octopunctata</i>	20.8	<i>Ensis ensis, E. leei, E. siliqua</i>	25.0
	9	<i>Harpacticus flexus</i>	20.8	<i>Harpacticus sp.</i>	25.0
	10	<i>Microprotopus maculatus</i>	16.7	<i>Mnemiopsis leidyi</i>	20.8
Zoo-plankton	1	<i>Acartia bifilosa</i>	63.6	<i>Acartia bifilosa</i>	65.0
	2	<i>Acartia tonsa</i>	63.6	<i>Balanus crenatus</i>	55.0
	3	<i>Electra pilosa</i>	59.1	<i>Pygospio elegans</i>	50.0
	4	<i>Balanus crenatus</i>	45.5	<i>Acartia tonsa</i>	45.0
	5	<i>Temora longicornis</i>	40.9	<i>Temora longicornis</i>	45.0
	6	<i>Paracalanus parvus parvus</i>	40.9	<i>Ensis ensis, E. leei, E. siliqua</i>	45.0
	7	<i>Austrominius modestus</i>	40.9	<i>Electra pilosa</i>	45.0
	8	<i>Obelia dichotoma</i>	40.9	<i>Acartia clausi</i>	40.0
	9	<i>Polydora cornuta</i>	36.4	<i>Scoloplos armiger</i>	35.0
	10	<i>Obelia bidentata</i>	31.8	<i>Amphibalanus improvisus</i>	35.0

Table S6. Pictures of stomach contents of herring and sprat, given here as illustrative examples to complement the results of the DNA analysis. The top section shows examples of a full stomach, where some prey is easily distinguishable, while others would require closer examination. The left section shows pictures of discernible benthic (7x) and pelagic (1x) prey species, captured under various magnifications. The right section depicts fish (larvae) found in the stomachs of herring and sprat. Individual picture captions detail descriptions and specify the respective species of which the stomachs originate.

Example of filled stomachs		
		
Undiscernible prey - herring	Undiscernible prey - sprat	Arenicola & amphipoda - herring
Benthic invertebrate prey (larvae)		Fish prey (larvae)
		
Peringia ulvae - sprat	Bivalve spat - sprat	Unknown fish - herring
		
Corophium volutator - herring	Jassa marmorata - herring	Unknown fish & mysids - herring
		
Carcinus meanas - herring	Amphipod - herring	Clupeidae - herring
		
Various Annelida - herring	Mesopodopsis slabberi - herring	Sandeel & goby - herring



Chapter 5

The small pelagic fish food landscape and its use by common terns *Sterna hirundo* in the Wadden Sea

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Abstract

Small pelagic fish (SPF) play a vital role in marine ecosystems, transferring energy from plankton to higher trophic levels. However, SPF dynamics in shallow coastal areas are often under-studied, despite their importance as crucial prey near breeding sites of fish-eating birds such as common terns (*Sterna hirundo*). This study had two connected objectives: to map the pelagic fish food landscape in the Dutch Wadden Sea and to describe its use by common terns. Two hydro-acoustic fish surveys were conducted in May and October 2022, while tern distribution was assessed using aerial counts and GPS tracking. Prey selection was analysed through camera traps and DNA analysis of faeces. Herring and sprat were the predominant species in the SPF community, with sandeel also abundant in May. Smelt was most common in freshwater-influenced areas. Most fish were below 12 cm, fitting the preferred size range for common terns. SPF energy density ranged from 20.1 to 22.1 kJ/g dry weight, with sprat having the highest values. SPF biomass showed significant spatiotemporal variability across transects, inlets, and seasons. Common tern foraging frequently occurred around the gullies, feeding mostly on herring. A significant correlation was found between SPF biomass and common tern density, indicating food landscape may be a good predictor of tern distribution. This study presents the first integrated biomass estimates of SPF in the Dutch Wadden Sea for both spring and autumn, providing essential data for future energy budget models and management strategies.

1. Introduction

The alignment between prey availability for predators and their food requirements is crucial for predator survival and reproduction. Changes in prey availability are a key driver of population declines in various predators, including seabirds feeding on small pelagic fish (SPF) (Cury et al., 2011; Guillemette et al., 2018; Reynolds et al., 2019; Fayet et al., 2021). SPF play a vital role in marine food webs by transferring energy from plankton to larger predators, including birds, marine mammals, and piscivorous fish (Ouled-Cheikh et al., 2022; Ruzicka et al., 2024).

In Europe, studies on the relation between fish and fish-eating birds have traditionally been concentrated around seabird colonies along the coasts of Norway and the United Kingdom (e.g. Furness and Tasker 2000; Frederiksen et al., 2004; Erikstad et al., 2013; Reiertsen et al., 2014). Far fewer studies have addressed the interaction between SPF and piscivorous birds in the Wadden Sea, the largest unbroken shallow system of gullies and intertidal flats globally. The area is particularly renowned for its importance to wading birds, serving as a crucial stopover site for migratory shorebirds along the East Atlantic Flyway (van Roomen et al., 2022). Besides this role as stopover site, the Wadden Sea is also an important breeding area for fish-eating seabirds such as the lesser black-backed gull (*Larus fuscus*), sandwich tern (*Thalasseus sandvicensis*), and common tern (*Sterna hirundo*) (Koffijberg et al., 2022). Because they acquire fish by plunge-diving, these species rely on fish swimming in the upper water column. They primarily feed on SPF, with abundance and condition of SPF serving as predictors for chick growth and successful breeding (Stienen et al., 2000; Dänhardt & Becker 2011b; Vedder et al., 2019).

Apart from their critical role for birds, SPF also serve as a food source for piscivorous fish such as sea bass (Cardoso et al., 2015) and marine mammals such as the harbour porpoise (Ransijn et al., 2021). SPF are considered high-quality food that cannot easily be replaced by other food types in terms of energy provision (Spitz et al., 2010). Despite their critical role, there is currently no detailed information available on the food landscape of SPF in the entire Wadden Sea, but see Stienen et al. (2000) and Dänhardt & Becker (2011a) for local studies. Here we define 'food landscape' as the spatial distribution of SPF as prey for piscivorous birds, including the species- and size composition and their nutritional values. Hence it does not include the actual prey accessibility which is a result of parameters such as vertical distribution of fish in the water column, water transparency, or fish swimming speed.

SPFs are challenging to study in shallow water owing to sampling difficulties, resulting in the fact that coastal areas are under-sampled (Brehmer et al., 2006).

Also, SPF are highly mobile and their abundances show highly variable dynamics in space and time (Saraux et al., 2014). Typically, SPF are studied using hydro-acoustic vessel-based surveys, covering large areas along predetermined transects, using acoustic backscatter combined with biological information gained from fishing hauls to estimate fish biomass. However, because these surveys primarily focus on adult SPF stocks, they generally take place in areas deeper than 20 m. The Dutch Wadden Sea, in contrast, serves as a juvenile nursery for SPF and is generally shallower than 20 m (Maathuis et al., 2025). Here, no comprehensive area-wide assessment of SPF biomass has been conducted. Coastal areas are, however, crucial habitats for fish-eating birds because of the vicinity to their breeding locations, especially for central place foragers, which deliver single prey items to their chicks, such as terns (Gochfeld & Burger 1996). Coastal areas are also crucial habitats for fish because they spend their early life stages here, therefore the interaction between fish-eating birds and SPF especially in the shallow coastal areas is still a significant knowledge gap.

This study addressed two connected objectives: first, to determine the food landscape for birds feeding on SPF, focusing on the temporal and spatial distribution of SPF species, size, biomass and caloric values; second, to identify the use of this fish food landscape by fish-eating birds, focussing on common terns, being one of the most widespread and abundant fish-eating bird species in the region. This led to the following three questions: i) Which species and sizes contribute to the SPF community in the Wadden Sea, and what is the energetic content of the key SPF species? ii) What is the estimated biomass of small pelagic fish in the Dutch Wadden Sea, both in spring and autumn? iii) How does the distribution of SPF relate to the habitat use and prey choice of fish-eating birds in the Wadden Sea, particularly common terns?

To achieve the objectives of the study, two area-wide hydro-acoustic fish surveys were conducted, one in May - at the start of the tern breeding season, and one in October 2022 - as an integration of growth, migration and mortality of SPF populations over summer, but when most terns have left the area after breeding. SPF community composition, size frequency and length-weight relationships were studied, resulting in the first integrated biomass estimation of SPF in the Dutch Wadden Sea in spring and autumn. Furthermore, the energetic content of the fish was analysed using calorimetry. The distribution of fish-eating birds was recorded during aerial counts simultaneously with the hydro-acoustic survey in May. Moreover, breeding common terns were tracked using GPS data, and their food provisioning was studied using camera traps at their nests and DNA metabarcoding of their faeces.

2. Methods

2.1 Study area

The Wadden Sea is a large interconnected system of gullies, intertidal sand flats, mud flats, and salt marshes, along the northern coasts of the Netherlands, Germany and the western coast of Denmark. It is a dynamic system, characterised by strong tidal currents (Maathuis et al., 2024a). Although most of the Wadden Sea is shallow, with depths typically below 15 m, the inlets between the barrier islands connecting the Wadden Sea to the North Sea are up to 45 m deep. Hydroacoustic data were collected in six inlets, moving from west to east: Marsdiep, Vlietstroom, Borndiep, Westgat, Lauwers, and Ems-Dollard (Figure 1A, see next page).

2.2 Small pelagic fish

2.2.1 SPF community composition and length distribution

Hydro-acoustic vessel-based surveys were conducted over two periods, from 9 to 19 May 2022 and from 17 to 27 October 2022, across all inlets of the Dutch Wadden Sea, except Eierlandse Gat, because this inlet is too shallow. Acoustic data were collected during daytime, using a Simrad EK60 echosounder with a 38 kHz and 200 kHz split-beam transducer, fixed to a towed body on the portside of a commercial fishing vessel. The towed body was positioned about 1 - 1.5 m below sea surface to prevent air bubbles caused by waves from interfering with measurements. Only data from the 38 kHz transducer were analysed for this study. Calibration of the acoustic equipment was performed at the start of each survey using a copper sphere (Demer et al., 2015; supplementary Table S1). Zig-zag transects were cruised at a speed of about 13 km/h.

Fishing hauls using a trawl net were conducted to assess fish community composition and size distribution across the six inlets on locations with relatively high amount of backscatter on the echogram, i.e. fish schools (Figure 1A). A semi-pelagic trawl net with a 17 m horizontal by 5 m vertical opening and a 10 mm mesh codend lining was used. Fishing speed was about 6 km/h with hauls primarily executed in midwater, between 5 and 10 m depth. The average depth of the water column during fishing was 16.9 ± 6.4 m. In total 50 hauls were conducted, averaging 14 min each. As a cross-check to acoustic densities, haul duration was used to calculate catch per unit effort (CPUE), expressed as fish numbers per minute trawling. Total length of fish in the trawl catches was measured to the nearest mm. Individual fish weight was measured for European anchovy (*Engraulis encrasicolus*),

Atlantic herring (*Clupea harengus*), sandeel (*Ammodytes* sp.), European smelt (*Osmerus eperlanus*) and European sprat (*Sprattus sprattus*) to the nearest 0.5 g in May and 0.1 g in October. No weights were measured for European pilchard (*Sardina pilchardus*).

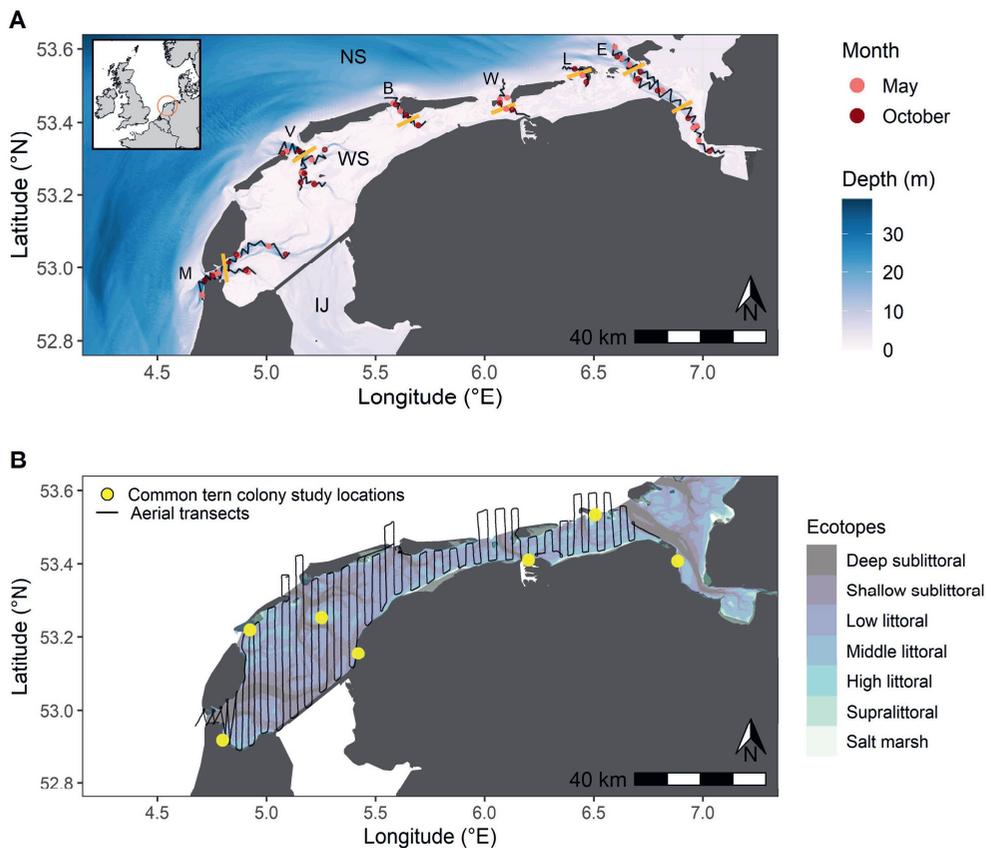


Figure 1. A) Map of the study area showing the Wadden Sea (WS), North Sea (NS), and the freshwater Lake IJssel (IJ), including bathymetry. Black zig-zag lines indicate the acoustic transects, coloured circles represent fish sampling locations, and the six sampled inlets are labelled with their initial letters (M = Marsdiep, V = Vlietstroom, B = Borndiep, W = Westgat, L = Lauwers, and E = Ems-Dollard). Additionally, the orange lines indicate the boundary between strata, marking the transition between the landward and the seaward side of the inlets. B) Map of the study area including ecotopes (Baptist et al., 2019b). Common tern colony study locations are marked with yellow circles and transects flown during the aerial survey are indicated by black lines.

2.2.2 Length-weight relations

Seasonal length-weight relationships of the form $W = aL^b$ were estimated for herring, sprat, smelt, anchovy and sandeel, using linear regression models on log-transformed length L (in cm) and log-transformed weight W (in g). For anchovy and sandeel only few individuals were caught in October and the length-weight relationship could only be estimated for May. The relationships were calculated for the entire size range of fish caught: herring (6-28 cm), sprat (6-14 cm), smelt (6-21 cm), anchovy (7-17 cm), and sandeel (6-18 cm). Since pilchard weights were not measured in our study, Fishbase (<https://www.fishbase.se>, assessed 10 June 2024) was used to obtain the a and b parameters in the length-weight relationships for the species. Linear regression models were used to test differences between months. Initially, an interaction between month and length was included. If the interaction was not significant, month was tested as a main effect. Based on these length-weight relationships, mean weights corresponding to each length class per species were calculated.

2.2.3 Biomass estimates

Processing and visualization of acoustic backscatter data were performed using Echoview® 14 (Echoview Software). Acoustic observations between the first 2.0 to 2.4 m below the transducer were excluded due to transducer ringdown effects (Simmonds & MacLennan 2005). Seabed backscatter was excluded with a line positioned 0.4 m above the bottom, which was generated using the 'best bottom candidate' algorithm in Echoview. This line was manually checked and edited, and sections affected by noise were manually excluded. The echograms displayed various aggregations, including small fish schools, individual targets, and a few large schools. These aggregations were collectively used to calculate a numerical fish density indicator: volume backscatter (S_v). Echogram sections that contained acoustic backscatter from fish were isolated using masks to effectively remove background noise and various other untargeted backscatter. Echo integration values were then exported per 200 m transect interval (referred to as samples), using a lower S_v threshold of -65 dB re $1 \text{ m}^2\text{m}^{-3}$. The nautical area scattering coefficient (NASC in $\text{m}^2\text{nmi}^{-2}$, MacLennan et al., 2002) represents the integrated backscatter from the observed water column, scaled to a square nautical mile area. Samples at the corners of zigzag transects were removed to prevent multiple overlapping observations.

The total distance covered by acoustic measurements in each survey was about 300 km, corresponding to 1550 samples from 144 transects in May and 1682 samples from 151 transects in October. Subsequently, mean NASC values were calculated

per transect (see supplementary Figure S1). Transects were assigned to inlets and further divided into 'strata' (Figure 1A). Two strata were defined per inlet: 'landward' for the side closer to the Dutch mainland and 'seaward' for the side facing the North Sea. The exception was the Ems inlet, which is an estuary and included an additional low salinity stratum called the Dollard.

Trawl fishing data (species composition, length-weight relationships, and length frequency distributions) were used to divide NASC values by species and subsequently calculate fish density and biomass per hectare. Hauls were assigned to strata, excluding 5 of the 50 hauls that were considered not representative. To calculate the percentage weight contribution of specific species to the total sample, six pelagic fish species were selected: anchovy, herring, pilchard, sandeel, smelt, and sprat. Sandeel comprised two species: lesser sandeel (*Ammodytes tobianus*) and Raitt's sandeel (*Ammodytes marinus*), in approximately a 4:1 ratio. Demersal species that were caught in the trawls (e.g., whiting, plaice) were probably caught due to gear approaching the bottom, thus not representative of the acoustic backscatter in the water column and were therefore excluded. Also, pelagic species such as clupeid post-larvae (< 5 cm), greater sandeel, pipefish, and pencil squid were excluded due to low abundances or weak backscattering properties. For presenting pelagic community composition, percentage weight contribution was averaged by stratum. However, for Westgat and Lauwers, there were not enough representative hauls to divide the inlets into a landward and seaward stratum, so each inlet was treated as a single unit.

Using the percentage contribution averaged over strata, NASC values per species per transect were determined for biomass estimation. A correction factor was applied to the weight contribution of sandeel to avoid overestimation, as sandeels lack a swim bladder and do not reflect strongly at 38 kHz (Johnsen et al., 2009; Couperus et al., 2016). Catch data were aggregated per half cm class (rounded down to the nearest half cm), and the proportion of each species per length class per sample was calculated and averaged over the strata. For herring and sprat, hauls with fewer than 25 individuals of a species were not included in the average, as these length frequencies were not representative. Additionally, for presenting length frequency distributions in Figure 3, CPUE was calculated using haul duration, expressed as fish numbers per 10 min of trawling. Data were square root transformed, averaged per inlet per season, and then back transformed.

Acoustic target strength (TS) per length class was calculated using the TS-length equation: $TS = 20 \log(L) - b_{20}$, where b_{20} is a species-specific constant (Foote 1987). For herring and sprat, a b_{20} value of -67.8 dB was applied, suitable for juvenile individuals in relatively shallow conditions (Didrikas & Hansson, 2004;

Maathuis et al., 2024a). A standard b_{20} value of -71.2 dB, commonly used for clupeids in stock assessments (Lusseau et al., 2023), was used for other SPF species lacking species-specific values. For consistency in comparisons with other studies, calculations for herring and sprat were also conducted using the b_{20} of -71.2 dB.

Subsequently, SPF abundance per hectare was determined by computing the area backscattering coefficient (ABC in m^2) based on NASC values per length class per species, and further divided by the backscattering cross section (σ_{bs} , the linear equivalent of TS). Biomass was then calculated by multiplying the abundance of fish by the weight per length class and summing across all length classes. This yielded biomass values (in kg/ha) per transect, which were averaged per stratum and inlet. To obtain total SPF biomass, results for all six species were aggregated. Data analysis was done using R (version 4.3.1) and RStudio (version 2023.06.1).

2.2.4 Energy density

Fish energy density was estimated for herring, sprat, sandeel, and smelt. Herring, sprat, and sandeel were collected in the Dutch Wadden Sea during a stow net survey from April to July 2021 (Maathuis et al., 2025). Smelt were collected during the hydro-acoustic survey in May 2022 (this study). A total of 82 herring, 59 sandeel, 12 smelt, and 78 sprat, ranging in length between 6 to 16 cm, were collected. To measure dry weight, fish were placed in a freeze dryer for a minimum of three nights at -50°C and 0.02 mbar. Subsequently, whole animals (excluding otoliths for sandeels) were ground into powder and compressed into a pill of approximately 0.5 g dry weight. If dry weight of individual fish was less than 0.5 g, benzoic acid was added to reach 0.5 g, with corrections made afterward. The energy density was analysed using an IKAC2000 calorimeter (IKA, Germany). The energy density per g wet weight was calculated using the ratio dry weight to wet weight. Linear regression models were used for each species to test whether energy density varied significantly with fish length. Because significant differences were found for sandeel and smelt, only fish between 10.5 and 13.5 cm were included in the subsequent analysis, which involved a Kruskal-Wallis test followed by pairwise Wilcoxon tests with Bonferroni correction to evaluate interspecies differences.

Subsequently, energy density and biomass estimates were multiplied to estimate the energy available per inlet, specifically calculated here for May, at the start of the common tern breeding season. Biomass values for herring, sandeel, sprat, and smelt in the 4 to 12 cm size range were selected as these were included in the terns' diet.

2.3 Common terns

The common tern is a widespread piscivorous bird in the Dutch Wadden Sea. Yet, counts from 1991 to 2017 show an annual population decline of 5% here (Koffijberg et al., 2021). Common terns breed in small to medium-sized colonies on islands, saltmarshes, wetlands (both natural and artificial), and flat rooftops behind mainland dykes. The breeding season runs from April to July, with the main egg laying period in mid-May for experienced breeders (Ludwigs & Becker 2002) and a chick rearing period of about 25 days from mid-June to mid-July (Becker & Ludwigs 2004). As a central place forager, the species delivers single fish to chicks after foraging trips that can extend several tens of kilometres from the colony (Militão et al., 2023; Kralj et al., 2024).

2.3.1 Counts

To describe the spatial distribution across the Dutch Wadden Sea of (foraging) common terns and other piscivorous birds, aerial transect surveys were conducted on 12 and 13 May 2022, simultaneously with the acoustic fish survey. The entire Dutch Wadden Sea was surveyed, except for the Ems Dollard estuary (Figure 1B). Counts were made from a Cessna 172 aircraft at an average altitude of 75 m, recording the number and behaviour of common terns. Transects were spaced 2.5-3 km apart, with no overlap between areas counted. Most counts took place around low tide, with wind speeds of 4-5 Bft and minimal cloud cover. The effective strip width for common terns was 168 m (Manche et al., 2023). To analyse the relationship between common tern distribution and fish distribution, we created a 168 m buffer around the aerial transects and selected those parts of the buffer that were within 2.5 km of the acoustic fish transects. The number of foraging common terns within these remaining buffers was divided by its area to calculate bird density. This was done per stratum. A linear model was then used to test whether common tern density correlated with SPF biomass. Furthermore, ground-based counts of breeding colonies and high-tide roosts of common terns in May and June 2022 were acquired from a standardized long-term bird count carried out by Sovon Dutch Centre for Field Ornithology (Hornman et al., 2012; Vergeer et al., 2023).

2.3.2 Habitat use

During the breeding seasons of 2021 and 2022, a total of 44 common terns from seven colonies (Figure 1B, range 1-14 terns per colony) were tracked using Pathtrack nanoFix GEO RF tags (3.6 g; Pathtrack Ltd, United Kingdom). Adult terns were caught on their nests during the last week of egg incubation, using walk-in

traps or spring traps. Tags were attached with a leg-loop harness. One tag was lost before data collection began, and the remaining 43 tags provided an average of 27 days of GPS data per tag (range 1-71 days). With GPS location intervals of 40 min, individual foraging trips could not be distinguished. Instead, we analysed the distribution of GPS locations per habitat type for each colony, excluding GPS locations inside the colonies. The averages of these habitat distributions were then used as indicator for habitat preference of common terns in the Dutch Wadden Sea. Areas were classified into the North Sea, Wadden Sea, and inland areas (both waterbodies and land), with the Wadden Sea further divided into six ecotopes based on water depth and current dynamics (Baptist et al., 2019b; Figure 1B).

2.3.3 Prey choice

Prey choice, in terms of prey size and type, was studied using two methods: by means of camera traps and by DNA metabarcoding of faeces. Camera traps were placed at common tern nests in seven colonies in 2021 and 2022, capturing images of prey fed to chicks up to 10 days old. This yielded 1620 observations of prey, of which species and length were recorded, with prey length measured relative to the adult's bill length (average 3.6 cm; Baker 2016). Identifying prey species from images was challenging due to image quality and small visual differences between species. Herring, sprat and smelt were grouped together as "herring-like" prey items. Additionally, DNA analysis was conducted on tern faeces for detailed prey identification. In June and July of 2022, we collected 25 faecal samples from young chicks (5-12 days old), 25 from older chicks (12+ days), and 1 from adult common terns across five colonies. Samples were grouped by location and age class, and relative read counts were averaged within each age group. DNA metabarcoding was conducted using the MiFish mitochondrial 12S primer (Miya et al., 2020). DNA was extracted using a chloroform-isoamyl alcohol method and PCR amplification was done in triplicate. Controls indicated no significant DNA degradation, contamination or PCR inhibition. Sequencing was performed on an Illumina MiSeq platform (Illumina, United States). Sequencing and bioinformatic analysis were both carried out by BaseClear (BaseClear B.V., the Netherlands).

All sampling was performed in accordance with Dutch law concerning animal welfare. The fish protocol was approved by the Animal Ethical Commission of Wageningen UR (experiment code: 2020.D-0026.001; application: 401002020 10984). The bird protocol was approved under permit AVD25000202010465. The datasets generated and analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

3. Results

3.1 SPF community composition

Herring was the most abundant SPF species in the Dutch Wadden Sea, followed by sprat (Figure 2). In May, there was a distinct difference in the herring-to-sprat ratio between the landward and seaward side of the inlets, with more herring on the landward and more sprat on the seaward side. This pattern was less clear in October, when also sprat contributions decreased towards the east. Sandeel was frequently caught in May, contributing up to 20% in weight, primarily on the landward sides of Marsdiep and Borndiep. Smelt exhibited an easterly distribution in both months, mainly present in the Dollard, Westgat, and landward side of Borndiep. Pilchard was nearly absent in May but was frequently caught in October, more on the outside of the inlets than on the Wadden Sea side. Anchovy did not substantially contribute to the catch in October but was occasionally present in May, mainly in the Dollard, where it made up 20% of the total weight.

3.2 SPF length distributions

The SPF community in the Dutch Wadden Sea was predominantly composed of small individuals, with most lengths below 12 cm in May and below 15 cm in October. In May, herring was the smallest SPF species present, peaking in abundance around 5.5 cm, with the highest CPUE in the Lauwers inlet. Sprat abundances peaked around 7.5 cm in size, while length of sandeel, anchovy, and smelt displayed was more variable, ranging from 8 to 15 cm. In October, sprat was the smallest SPF species, though the size differences between species were less pronounced than in May. Sprat showed a main peak in CPUE around 5.5 cm and a smaller peak around 10 cm. Pilchard had a similar size distribution to sprat. Herring averaged around 6 cm, increasing to 7 cm towards the eastern inlets, while most smelt ranged between 7 and 11 cm. In both months, Marsdiep, Vliestroom, and Borndiep exhibited similar length-frequency distributions, while other inlets showed more variation. Generally, the sizes of SPF in the Wadden Sea were similar to the sizes of prey fish fed to common tern chicks, which ranged from 2.7 to 12.6 cm, with a mean size of 5.6 ± 1.7 cm (see supplementary Figure S2).

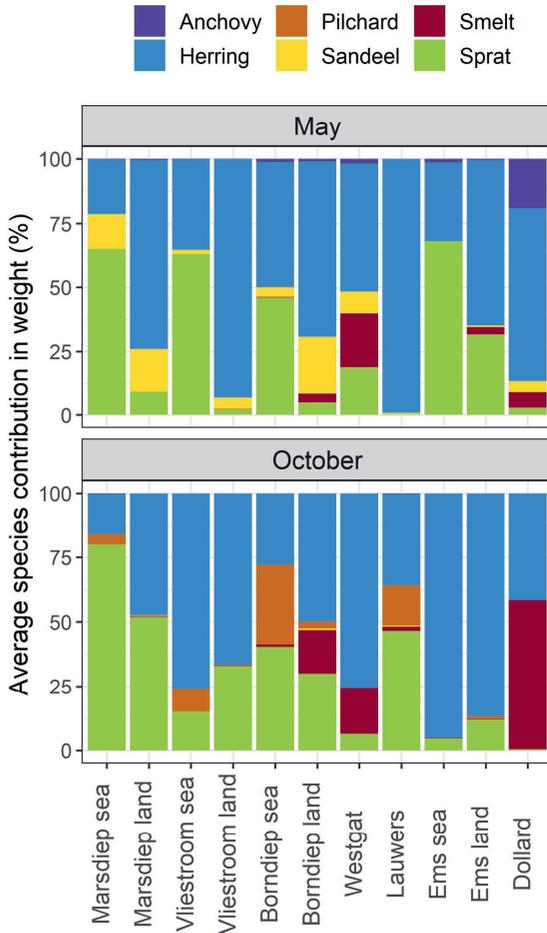


Figure 2. Average percentage contribution by weight of six small pelagic fish species across different inlets and strata in the Dutch Wadden Sea. The top panel represents data from May 2022, and the bottom panel represents October 2022. Species are differentiated by colour. Inlets on the x-axis are ordered from west to east, and landward ('land') and seaward ('sea') strata are presented if applicable.

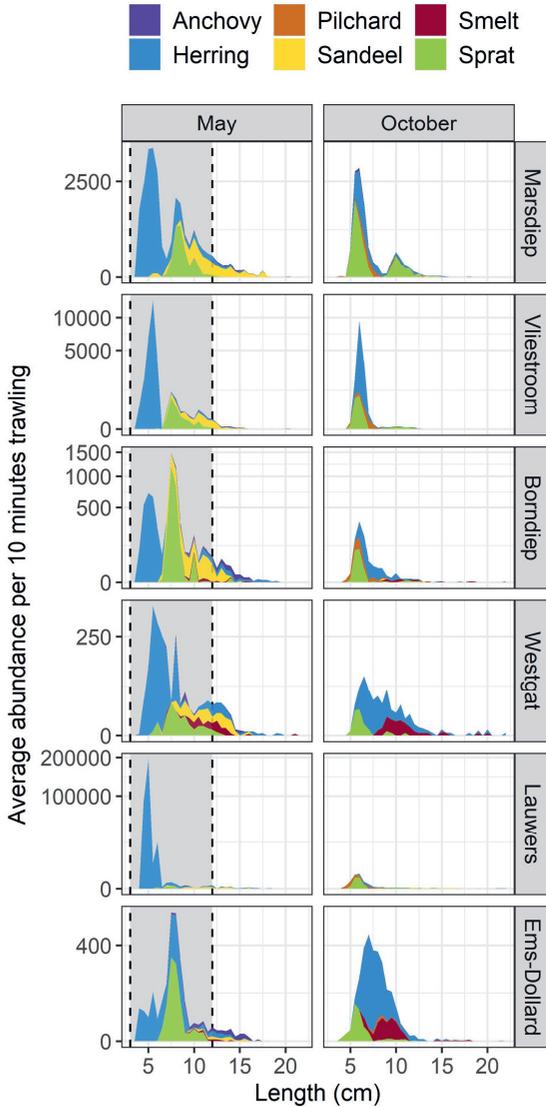


Figure 3. Cumulative length-frequency distributions of six small pelagic fish species across six inlets in May (left) and October (right) 2022. The Y-axes are square root scaled, representing average catch per unit effort in numbers per 10 min trawling. Species are distinguished by colour. The grey squares indicate the prey size fed to common tern chicks.

3.3 SPF length-weight relations

Most species exhibited lower weights for their length in October compared to May, suggesting that fish were in slightly better condition in May. Specifically, herring showed this trend most notably between 8 and 20 cm, smelt across the entire range, and sprat from 10 cm onwards (supplementary Figure S3). Sandeel consistently had lower weights for the same lengths compared to other species. The a parameter, ranging from 0.002 to 0.008, differed significantly between May and October for herring, sprat, and smelt (Table 1). Additionally, the b parameter, which was above 3 for all species except sprat in May, was significantly different for sprat and smelt.

Table 1. Length-weight relationships ($W = aL^b$) for small pelagic fish in the Wadden Sea, measured in May and October 2022, including sample sizes (n). Linear models tested differences between months, starting with an interaction between month and length. If non-significant, month was tested as a main effect. For anchovy and sandeel, the relationship was only estimated for May due to low October catches.

Species	n		P value interaction	P value month	Adj R ²	a		b	
	May	Oct				May	Oct	May	Oct
Herring	324	403	0.9754	<0.0001	0.99	0.0036775	0.0032747	3.25068	
Sprat	254	226	<0.0001	<0.0001	0.95	0.0083651	0.0041255	2.88363	3.199316
Smelt	87	125	<0.0001	<0.0001	0.99	0.0041576	0.0020340	3.15730	3.414154
Anchovy	175	5	NA	NA	0.98	0.0027795		3.31147	
Sandeel	236	30	NA	NA	0.94	0.0026296		3.04063	

3.4 SPF biomass

Herring exhibited the highest biomass, particularly in May (Figure 4). Biomass levels were generally higher in May than in October, except for smelt. Species distributions varied seasonally; for example, high herring biomass was observed in the Lauwers inlet on the landward side in May, but not in October, and the opposite occurred in sprat. Additionally, sandeel had a clear westerly distribution in May, with biomasses up to 15 kg/ha in the Marsdiep, but its biomass remained below 1 kg/ha everywhere in October. Clear differences were also noted between the seaward and landward side of the inlets in May: sprat was generally more abundant on the seaward side of the inlets, whereas herring showed higher biomass on the landward side. Anchovy biomass was below 1 kg/ha in all areas in October, with notable presence in the Marsdiep, Borndiep, and in the Ems-Dollard in May (supplementary Figure S4). In contrast, pilchard biomass remained under 1 kg/ha

in May, but showed a clear increase in October, reaching a maximum of 17 kg/ha on the seaward side of the inlets, particularly in the Marsdiep, Vliestroom, Borndiep, and Lauwers (supplementary Figure S4).

In May, total fish biomass decreased from west to east, primarily due to reductions in herring, sandeel, and sprat biomass (Figure 5, supplementary Table S2). In contrast, smelt biomass generally increased eastward, with the highest values in Westgat and Ems-Dollard. Despite this, Westgat consistently had the lowest biomass of SPF in both months. The mean SPF biomass in May was highest in Marsdiep, followed by Lauwers, Vliestroom, Borndiep, Ems-Dollard, and Westgat, with values of 330, 317, 134, 123, 71, and 17 kg/ha, respectively (supplementary Table S2). Large standard deviations indicated significant variation between transects. Due to their small sizes and low weights, high abundances were observed; for example, in Marsdiep in May, a mean estimated SPF biomass of 330 kg/ha corresponds to 207,495 SPF/ha. In October, the west-east pattern was absent, with all regions showing similar biomass levels, ranging between 11 and 37 kg/ha. When using a TS value of -71.2 dB to calculate biomass for herring and sprat, the biomass of these species approximately doubles (supplementary Table S2).

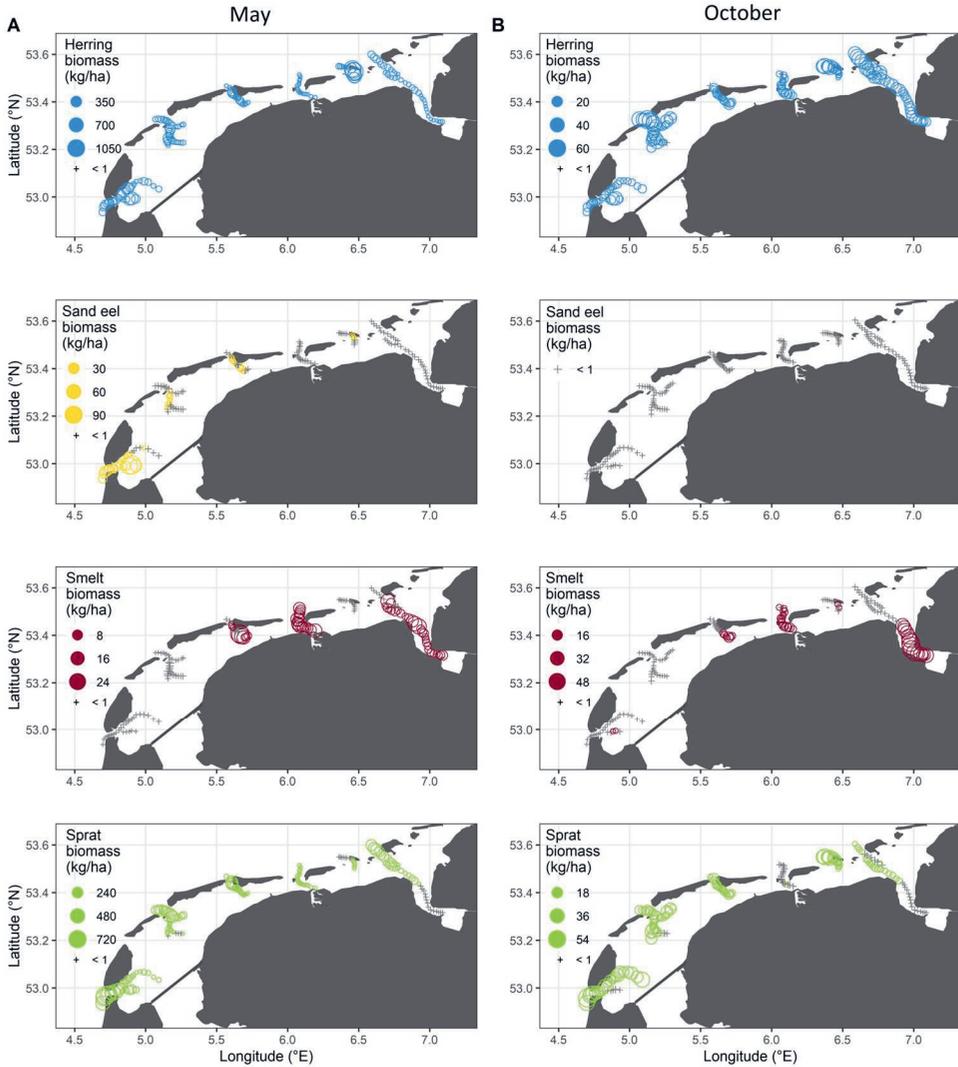


Figure 4. Distribution of biomass for four species of small pelagic fish in the Dutch Wadden Sea in May (A) and October 2022 (B). The diameter of each circle corresponds to the biomass value in kg/ha, while grey crosses indicate biomass values less than 1 kg/ha. Note the distinct biomass ranges displayed in each plot that differ between species and seasons.

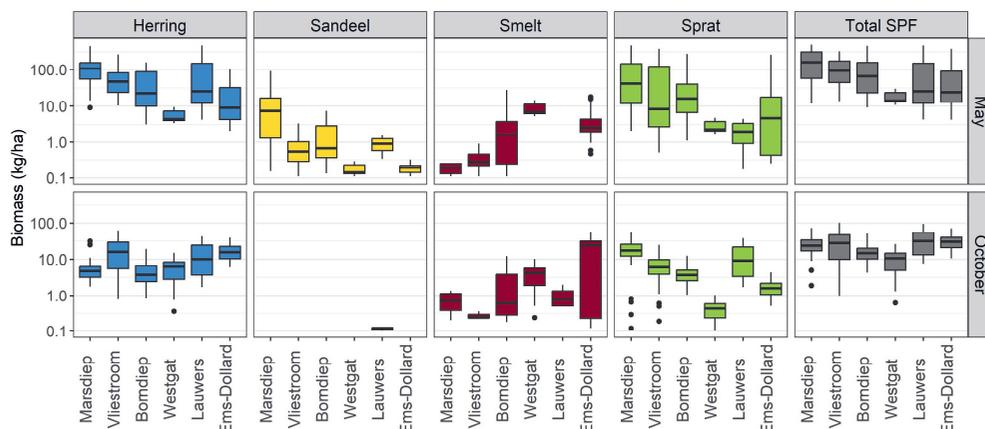


Figure 5. Biomass (kg/ha) for four species of small pelagic fish (SPF) in the Dutch Wadden Sea in May and October 2022, along with the total biomass of SPF (pilchard and anchovy included). The observations used for the boxplots are individual samples, the y-axis is logarithmic, and boxes show the minimum, 1st quartile, median, 3rd quartile, and maximum values.

3.5 SPF Energy density

The average energy density of SPF in the Dutch Wadden Sea ranged from 20.1 to 22.1 kJ/g dry weight, corresponding to 4.5 to 5.7 kJ/g wet weight (Table 2). Energy density did not differ significantly across lengths for herring and sprat, but did for smelt (linear regression, $P = 0.001$, adjusted $R^2 = 0.064$) and sandeel (linear regression, $P = 0.020$, adjusted $R^2 = 0.076$; Figure 6A). Energy density of smelt increased with size, while the opposite occurred in sandeel. Sprat showed highest energy density values but also the largest variation. Species differences were significant (Kruskal-Wallis test, $H_3 = 26.20$, $P < 0.001$). Pairwise comparisons revealed that herring had significantly lower values compared to the other species (Wilcoxon rank sum test, $P < 0.002$), while no significant differences were observed between the other species. The energy potentially available for common terns in the Wadden Sea during the breeding season, calculated by multiplying energy density by the biomass estimates of suitable species under 12 cm from May, shows a decrease towards the east, with the exception of Lauwers inlet. However, there were substantial variations between individual transects.

Table 2. Energy density (in kJ/g) for dry weight and wet weight of four small pelagic fish species in the Dutch Wadden Sea, collected from April to July, with sizes ranging from 6 to 16 cm. Average values are given, including standard deviation and 'n' indicates the sample size.

Species	n	Energy density dry weight (kJ/g)	Energy density wet weight (kJ/g)
Herring	82	20.13±1.34	4.52±0.70
Sandeel	59	21.15±0.88	5.29±0.56
Smelt	12	21.88±0.84	4.94±0.44
Sprat	78	22.12±2.33	5.67±1.70

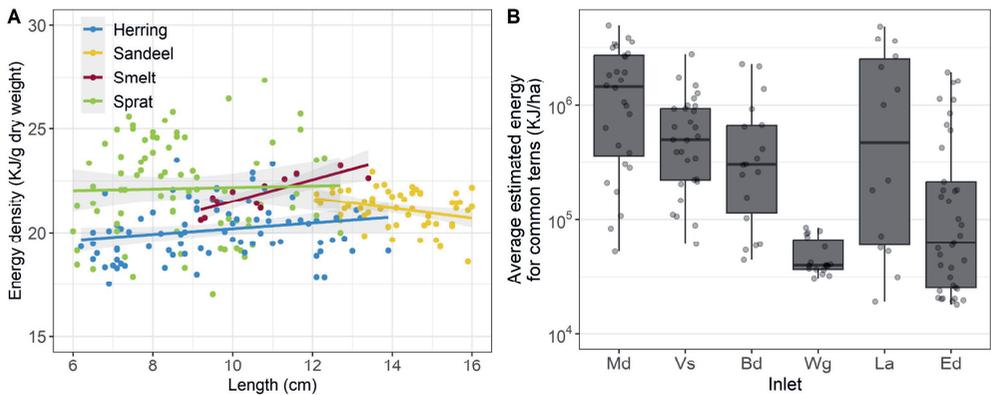


Figure 6. A) Energy density (kJ/g dry weight) across lengths for four small pelagic fish species. Points represent individual data, and solid lines show linear regression fits for each species, with shaded areas representing 95% confidence intervals. B) Estimated energy of suitable small pelagic fish available for common terns (i.e. herring, sprat, smelt and sandeel below 12 cm) per inlet in the Dutch Wadden Sea. From left to right: Marsdiep, Vliestroom, Borndiep, Westgat, Lauwers and Ems-Dollard. Note that the y-axes do not start at 0.

3.6 Foraging by common terns

During the aerial survey, 531 common terns were counted, of which 40% were foraging (Figure 7A), 17% were flying, some with fish in their bills, and 43% were resting. Foraging and flying frequently occurred in the studied inlets (note that no bird data is available for Ems-Dollard). Ground-based counts of breeding colonies and high-tide roosts in May and June 2022 indicated circa 4600 common terns in the Dutch Wadden Sea area (Manche et al., 2023). Within the buffer around the fish survey tracks, 124 common terns were counted. A significant correlation was found in May between the density of foraging common terns and that of suitable SPF biomass within the same area (linear regression, $P = 0.0045$, adjusted $R^2 = 0.61$; Figure 7B). For instance, both the Lauwers landward side and Marsdiep seaward side had high fish biomass and high tern density, whereas Westgat exhibited low densities of both terns and fish.

Examining GPS position data from tracking devices revealed that common terns were often located above or near the major gullies during the breeding season (Figure 8A; 8B). On average, deep sublittoral and sublittoral areas accounted for 32.7% of the GPS locations, though this varied by colony. DNA metabarcoding analysis of common tern faeces showed that herring was the primary food given to chicks, regardless of their life stage, followed by sprat, sandeel, gobies (*Gobiidae*), and the freshwater species perch (*Perca fluviatilis*) and bream (*Abramis brama*) (Figure 8C). Adults primarily fed on herring, with only minor contributions from sprat and sandeel. Camera trap images confirmed the importance of 'herring-like' prey, showing consistent results across both years and all colonies (overall proportion 75%, per-colony range 58%-84%).

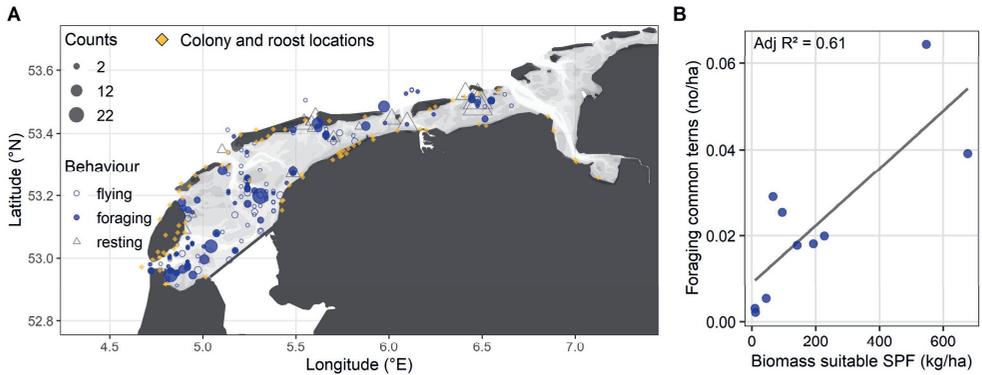


Figure 7. A) Aerial counts of common terns in the Dutch Wadden Sea in May 2022, categorized by behaviour. Note that the Ems-Dollard inlet was not included in the aerial survey. Colony and high-tide roost locations are marked in yellow. B) Average biomass of SPF suitable for common terns (i.e., herring, sprat, sandeel, and smelt in the 3-12 cm size range) plotted against the density of foraging common terns. Each point represents a stratum and the grey line shows the linear regression.

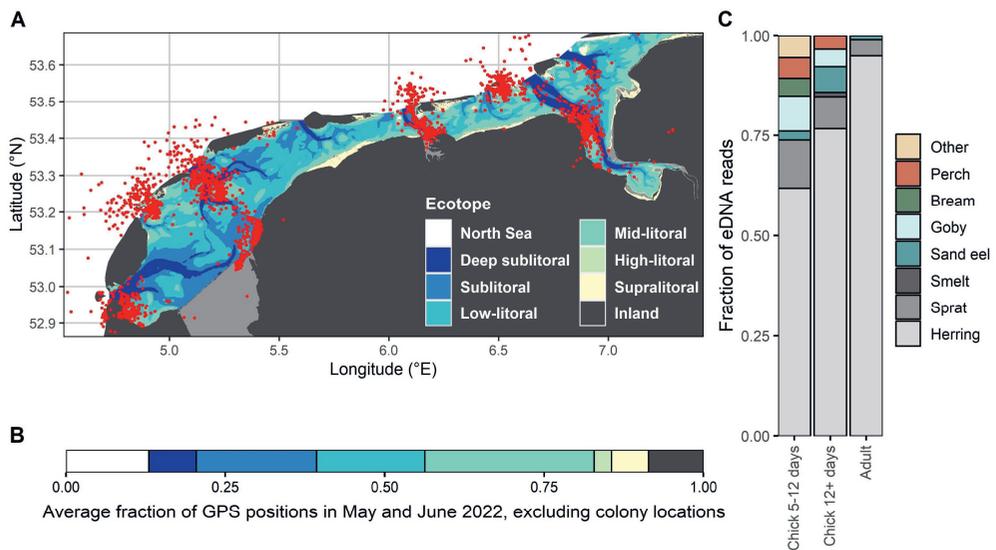


Figure 8. A) Example of GPS locations of common terns in the Wadden Sea during the breeding season. Per colony, 750 random GPS points of different birds are shown. B) Mean habitat use of all common terns from the seven breeding locations, shown as the fraction of GPS points per habitat, excluding points within the colonies. C) Diet composition of common terns in 2022 based on DNA metabarcoding analysis of chick and adult faeces.

4. Discussion

This study had two objectives: first, to describe the pelagic fish food landscape for birds in a large tidal area, and second, to describe its use by common terns. Below we first discuss our findings on SPF, then focus on their predators, and conclude with the implications for management.

4.1 SPF distribution, biomass and energy density

Acoustic data and targeted trawl catches revealed that herring and sprat dominated the SPF community in the Dutch Wadden Sea, with sandeel being present primarily in May. Smelt was more abundant in the eastern inlets. Additionally, anchovy and pilchard showed lower abundances, and were mainly present in spring and autumn, respectively. Most SPF were smaller than 12 cm, fitting the observed prey size range for common terns' prey (3-13 cm). SPF biomass showed significant spatial variability both locally (between transects) and regionally (between inlets), with a west-east decline in May and a more even distribution in October across all inlets but lower overall biomass. The fish biomass in May was an order of magnitude larger than in October, suggesting a strong seasonal pattern. Furthermore, the distribution pattern in May showed a distinction between sprat prevailing in the inlets and herring more within in the 'inner' Wadden sea.

For the Dutch Wadden Sea, no comprehensive area-wide assessment of SPF biomass has ever been conducted. A survey in 1971-1975 using demersal gear, suboptimal for sampling pelagic fish, found more sprat than herring, with greater abundances in spring than in autumn (Dankers & de Veen 1978). In contrast, our current findings indicate higher herring abundance compared to sprat, though autumn biomass remains relatively low, similar to the findings half a century ago (Dankers & de Veen 1978). The lower biomass in autumn is likely driven by large young-of-the-year (YOY) mortality and emigration. Additionally, the large annual variability in recruitment observed for most SPF stocks could contribute to these differences, possibly reflecting a good cohort for one species but not the other (e.g. Voss et al., 2012; Burbank et al., 2022). More recently, a study focusing on the western inlet of the Dutch Wadden Sea, Marsdiep, reported clupeid biomass in May ranging from 184 to 411 kg/ha and between 23 and 72 kg/ha in October (Couperus et al., 2016). Additionally, they estimated 9 kg/ha of sandeel in May, compared to none in October. Our results are very comparable (i.e., the same of order of magnitude), considering methodological differences, such as Couperus et al. (2016) using a b_{20} value of -71.2 dB for clupeids. Our biomass estimates are likely conservative for two reasons. Firstly, we used a high b_{20} value (-67.8 dB, Didrikas & Hansson 2004) in the TS-length relationship to convert acoustic backscatter from herring and sprat into biomass. Published b_{20} values for clupeids are often lower, such as the ICES standard of -71.2 dB used for herring and sprat stock assessments

(Lusseau et al., 2023). Because we dealt with juveniles in shallow conditions, we chose -67.8 dB. Using -71.2 dB would have increased biomass estimates by ~54%. Secondly, our acoustic sampling methodology could not sample fish in the top 3-4 m of the water column due to the depth of the towed body and transducer ringdown effects (Simmonds & MacLennan 2005). We observed fish close to the surface, especially in shallower areas, but they were not acoustically detected and thus excluded from the biomass estimate. Stow net sampling of the top 5 m in the German Wadden Sea has shown that sprat, smelt, and herring often occupy surface waters (Dänhardt & Becker, 2011a). An alternative approach, using a bottom-mounted echosounder aimed upward, could help detect fish closer to the surface, but even this method misses the upper few meters due to interference from waves (Maathuis et al., 2024a).

Although only two short periods were sampled, we now have a better knowledge about SPF spatial and temporal distribution in the Wadden Sea in Spring and Autumn. The SPF distribution is influenced by environmental factors (e.g. Saraux et al., 2014; Pennino et al., 2020). For instance, SPF distribution is likely linked to the tidal movement of water masses between of the North Sea and Wadden Sea (Couperus et al., 2016). Furthermore, a continuous measuring stationary echosounder in the most western inlet of the Dutch Wadden Sea showed that tidal cycle combined with seasonal effects influenced fish density (Maathuis et al., 2024a). Therefore, the next step would be to assess the drivers behind the spatiotemporal patterns of SPF in the Wadden Sea by conducting repeated, longer surveys including enough resolution in factors such as tide, distance to the North Sea, water transparency, salinity, and temperature.

In addition to prey biomass and distribution, the quality of food is crucial for predators, as it directly impacts their ability to meet energy demands and to reproduce successfully. Energy density, a proxy for prey quality, varies widely among prey species. For forage species in the Bay of Biscay, energy density ranged from 2 to 10 kJ/g wet weight, with SPF considered high-quality prey (Spitz et al., 2010). Species-specific energy density values are needed for bioenergetic models, but they are generally scarce (Johnson et al., 2017; Weil et al., 2019). In our study, we found average energy density values varying between 4.5 and 5.7 kJ/g wet weight, with sprat having the highest caloric value per g but also the largest variation. Despite the general scarcity of energy density values, some data are available for the North Sea (Pedersen & Hislop, 2001) and the Wadden Sea (Massias & Becker, 1990; Stienen & Brenninkmeijer, 2002). Pedersen & Hislop (2001) reported energy density values ranging from 3.9 to 5.2 kJ/g for herring (6-15 cm), 5.8 to 6.4 kJ/g for sprat (4-12 cm), and 6.2 kJ/g for sandeel (12-15 cm). These values are broadly consistent with our findings, although Pedersen & Hislop (2001)

reported slightly higher energy density values for sandeel and sprat. Similarly, Stienen & Brenninkmeijer (2002) found slightly higher energy density values for sandeel compared to our results. Because sandeels have lower energy density than clupeids at equal length, Stienen & Brenninkmeijer (2002) showed that sandwich terns relying solely on sandeels could not provide sufficient energy for their chicks to survive.

Energy density is primarily determined by lipid content, which varies seasonally in herring and sprat (Røjbek et al., 2014). In addition, herring and sprat under 10 cm in the Wadden Sea show seasonal variation in condition, with best conditions found in June (Maathuis et al., 2024b), corresponding with our finding that fish were in slightly better condition in May than in October. We measured energy density from April to July, roughly coinciding with the breeding period of common terns. Therefore, the energy density values for October might differ, but seasonal variations in energy density are typically linked to fish reproductive cycles and hence not so pronounced in small, immature individuals like most SPF in our study (Hislop et al., 1991).

4.2 Common terns preying on SPF

Where and when animals forage depends on the spatiotemporal distribution and availability of their prey. The GPS-tracked common terns spent a substantial part of their foraging time above gullies rather than above intertidal areas, unlike black-headed gulls (van Bemmelen et al., 2024). Similarly, the aerial survey showed concentrations of feeding terns near gullies. The shallow depth in intertidal areas prevented these areas being covered by the acoustic survey, so SPF biomass could not be estimated on the intertidal mudflats. Thus, whether SPF remain mainly concentrated in gullies or also inhabit intertidal areas during high tide remains unresolved. Given that forming schools in deeper areas provides protection against predators, it is plausible that SPF concentrated in the subtidal regions. However, to be captured by plunge diving terns, fish need to be available in the upper water layer. Near-surface availability of forage fish may be greatly enhanced by turbulence in the water arising from natural or anthropogenic structures, and terns are known to take advantage of this (Lieber et al., 2021). Extrapolation of the aerial survey data suggests an estimate of around 3200 common terns foraging in the Wadden Sea, which aligns closely (115%) with what would be expected if about half of the breeding population remained at the colony during the survey (Manche et al., 2023). This indicates that the majority of terns were foraging within the Wadden Sea and its inlets, with limited activity in the North Sea or in freshwater inland areas (Manche et al., 2023).

Most of the prey brought to the chicks were herring or sprat. While photos at the nests could not distinguish between these closely related species, DNA analyses revealed that herring was fed to the chicks more frequently, despite sprat's higher energy density. The distribution of sprat further away from the colonies, many of which are located relatively close to or on the mainland, is a likely reason for this discrepancy.

Our fish study covers the main egg laying period for common terns (Ludwigs & Becker 2002). Hatching of the eggs happens about three weeks later. Since chick diet varies with age, requiring progressively larger fish (Stienen et al., 2000), fish of appropriate sizes must be available throughout the chick-rearing period. In mid-May, fish present were mostly below 12 cm, with peaks around 5.5 cm for herring and 7.5 cm for sprat. Over the next three weeks, fish sizes are expected to increase slightly due to growth. Additionally, more YOY herring arrive in the Wadden Sea in this period, while the influx of YOY sprat peaks in July (Maathuis et al., 2025). The presence of varying sized fish is crucial for chick development. Larger fish cannot be swallowed well by the smallest chicks, but smaller fish contain too little energy to be profitably transported to chicks except over very small distances, and hence may not allow the parents to supply enough food for optimal growth (Klaassen et al., 1992; Stienen & Brenninkmeijer, 2002). In May, all size classes needed for differently aged chicks were present. Whether or not they were abundant enough requires intensive field studies consisting of more frequent sampling of both length-structured fish abundance, colony observations of prey delivery by the parents, and reproductive output.

A significant correlation between common tern density and suitable SPF biomass (i.e., herring, sprat, sandeel, and smelt in the 3-12 cm size range) was found, indicating potential fish availability may be a good predictor of tern distribution. Studies on piscivorous seabirds demonstrate that spatial scale matters (Fauchald, 2009; Fauchald et al., 2011). On a large scale (e.g., North Sea or Wadden Sea wide), seabird distributions are generally linked to prey availability, as seen in our study. However, on a local scale, SPF distribution may not predict seabird distribution well due to the effect of additional (environmental) factors. Prey accessibility is likely one of these factors, particularly for diving seabirds that can only access upper half meter of the water column. Unfortunately, acoustic ship-based surveys miss this upper section. However, it was shown in the German Wadden Sea that high SPF densities at greater depths also result in increased SPF near the surface (Dänhardt & Becker, 2011a). Factors such as predator presence, water transparency, and SPF abundance likely affect vertical fish distribution and, consequently, prey accessibility to terns. Furthermore, tern distribution is also determined by the

location of breeding sites, which are limited to areas with bare sand, minimal vegetation, and protection from predators.

4.3 Management implications

The designation of the Wadden Sea as a World Heritage Site is largely due to its unique natural dynamics, creating pioneer habitats that vary annually in location and appearance. Exactly this variability is crucial for breeding terns, such as common terns, sandwich terns, Arctic terns (*Sterna paradisaea*), and little terns (*Sternula albifrons*)—species for which the Wadden Sea is assigned as a Natura 2000 site. For breeding, tern species prefer pioneer habitats—bare soil with little or no vegetation, typically washed over in winter and free from predators like gulls and red foxes (Gochfeld & Burger, 1996). As vegetation succession occurs and predators discover these areas, the sites become less suitable for breeding, prompting terns to move to a new pioneer habitat the next year. However, such natural dynamic situations have become rarer due to embankment and coastal defence structures, sea level rise, and increased predation pressure (van de Pol et al., 2010; Koffijberg et al., 2022). Consequently, initiatives have been taken to provide artificial breeding locations throughout the Wadden Sea, such as the Dutch program 'Wij & Wadvogels' and in Lake Markermeer ('Markerwadden'). The success of such artificial sites depends on sufficient breeding output. Terns deliver single prey items to their chicks, so the distance between breeding locations and feeding grounds is an important determinant of prey delivery rate (Gochfeld & Burger, 1996). If prey is only available at distant sites, artificial breeding locations can become ecological traps, offering attractive breeding habitat but not enabling parents to meet the chicks' energy demands for growth.

Therefore, understanding fish distribution relative to colony distance is vital for optimizing artificial breeding site placement, but data to do so were previously lacking. Our study uniquely combined data on prey biomass, distribution, length, energy density, with information on predator distribution and food choice. This information enables us in a follow up study to evaluate the potential of new colony locations based on the energy budget of common terns. Our current findings suggest that such potential is greatest in the vicinity of the tidal inlets and outer parts of the major gullies, particularly in the western parts of the Dutch Wadden Sea. This study provides a snapshot of the SPF abundance in the Wadden sea system as a detailed baseline for better understanding of the system. However, as in the case of most monitoring studies, acoustic surveys can provide a more reliable picture when conducted consistently over a period of multiple years of time.

Therefore, it is essential to continue such monitoring efforts in the area to better resolve the dynamics of the area for a better management.

SPF often exert bottom-up control on predator populations (Cury et al., 2000), so in addition to informing the placement of (new) artificial breeding sites for common terns, this information is also relevant for other predator species. For instance, it was shown that small herring is a key prey species within the Wadden Sea fish community (Poiesz et al., 2020). Furthermore, harbour porpoises (*Phocoena phocoena*) feed on clupeidae and sandeels (Ransijn et al., 2019), while harbour seals (*Phoca vitulina*) primarily eat sandeels and demersal fish, with some herring as well (Kavanagh et al., 2010; Aarts et al., 2019). Additionally, the Wadden Sea also supports large numbers of overwintering piscivorous birds, such as great cormorant (*Phalacrocorax carbo*), red-breasted merganser (*Mergus serrator*) and grebes Podicipedidae, which are typically found near the gullies (Schekkerman et al., 2015). While management efforts typically focus on higher trophic levels, understanding changes in predator species requires a clear understanding of the dynamics in the lower levels of the food web.

Acknowledgements

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Supplementary materials

1. Supplementary figures

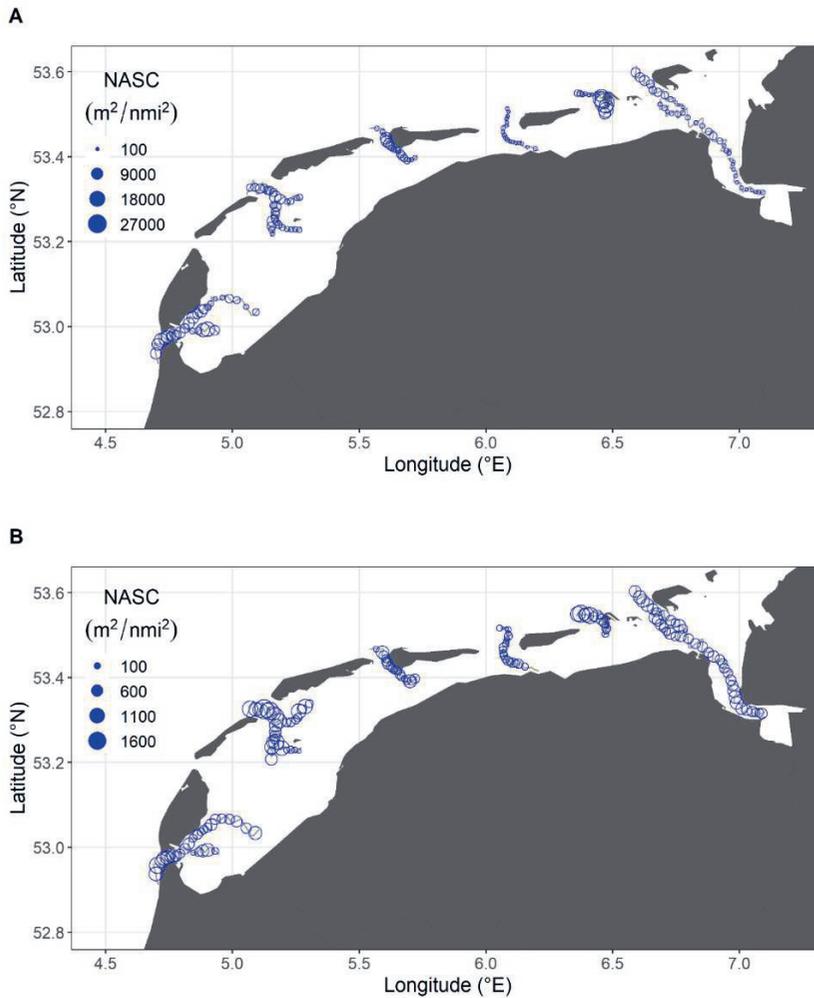


Figure S1. Distribution of acoustic backscatter in the Dutch Wadden Sea for May (A) and October 2022 (B). The diameter of each circle represents nautical area scattering coefficient (NASC) values in m^2/nmi^2 . Note the distinct NASC ranges displayed in each panel.

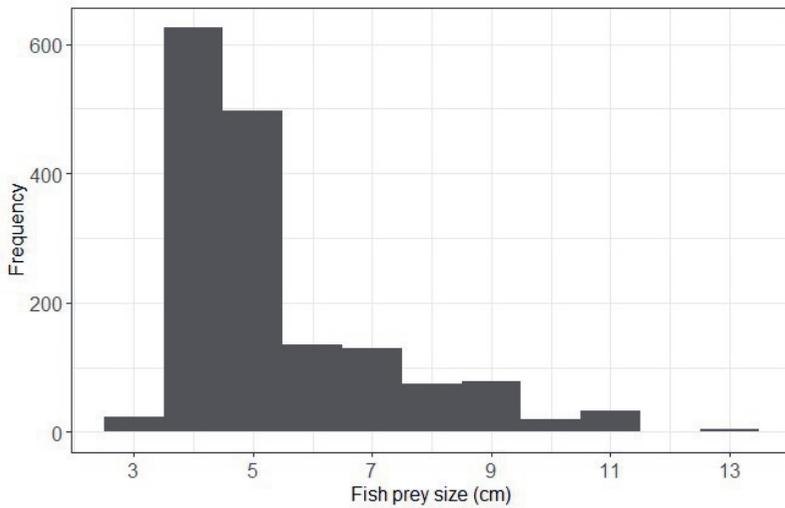


Figure S2. Distribution of prey fish sizes observed in common terns feeding their chicks in the Dutch Wadden Sea (n = 1620).

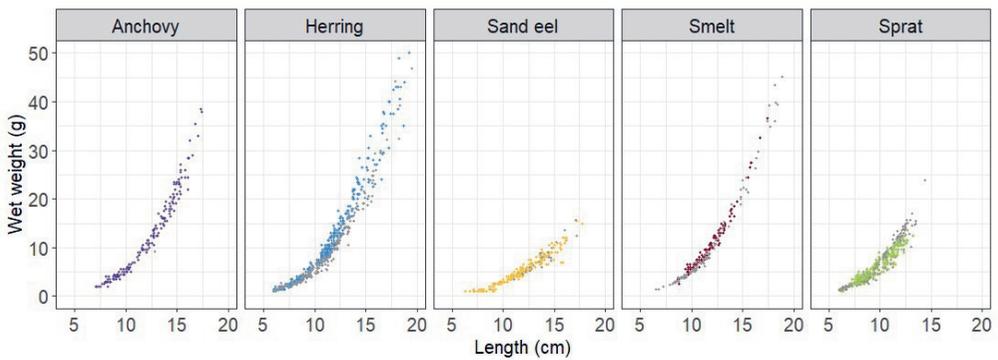


Figure S3. Length-weight plots for five small pelagic fish species in the Wadden Sea, measured in May and October 2022. Coloured points represent measurements from May, while grey points show those from October.

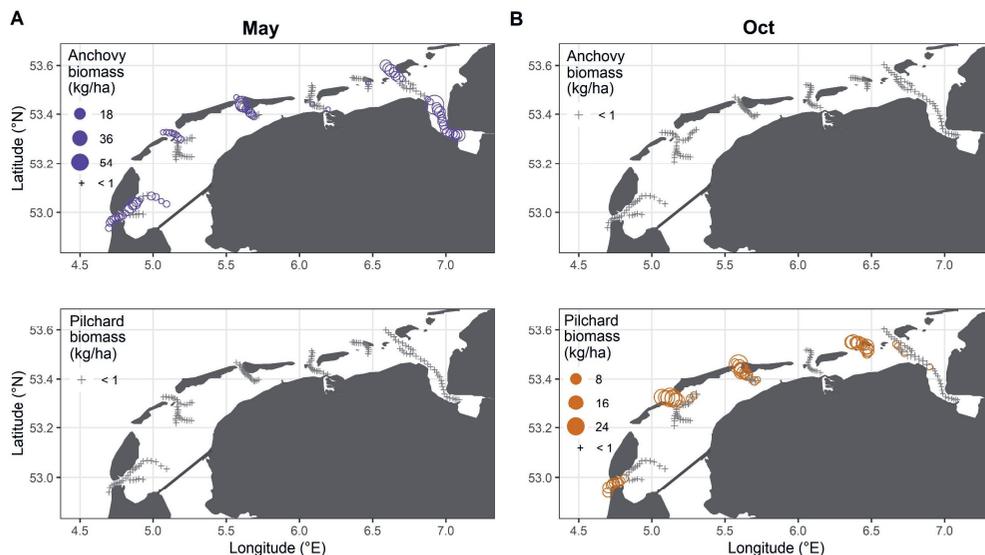


Figure S4. Distribution of biomass for anchovy and pilchard in the Dutch Wadden Sea in May (A) and October 2022 (B). The diameter of each circle corresponds to the biomass value in kg/ha, while grey crosses indicate biomass values less than 1 kg/ha. Note the different ranges in biomass per plot.

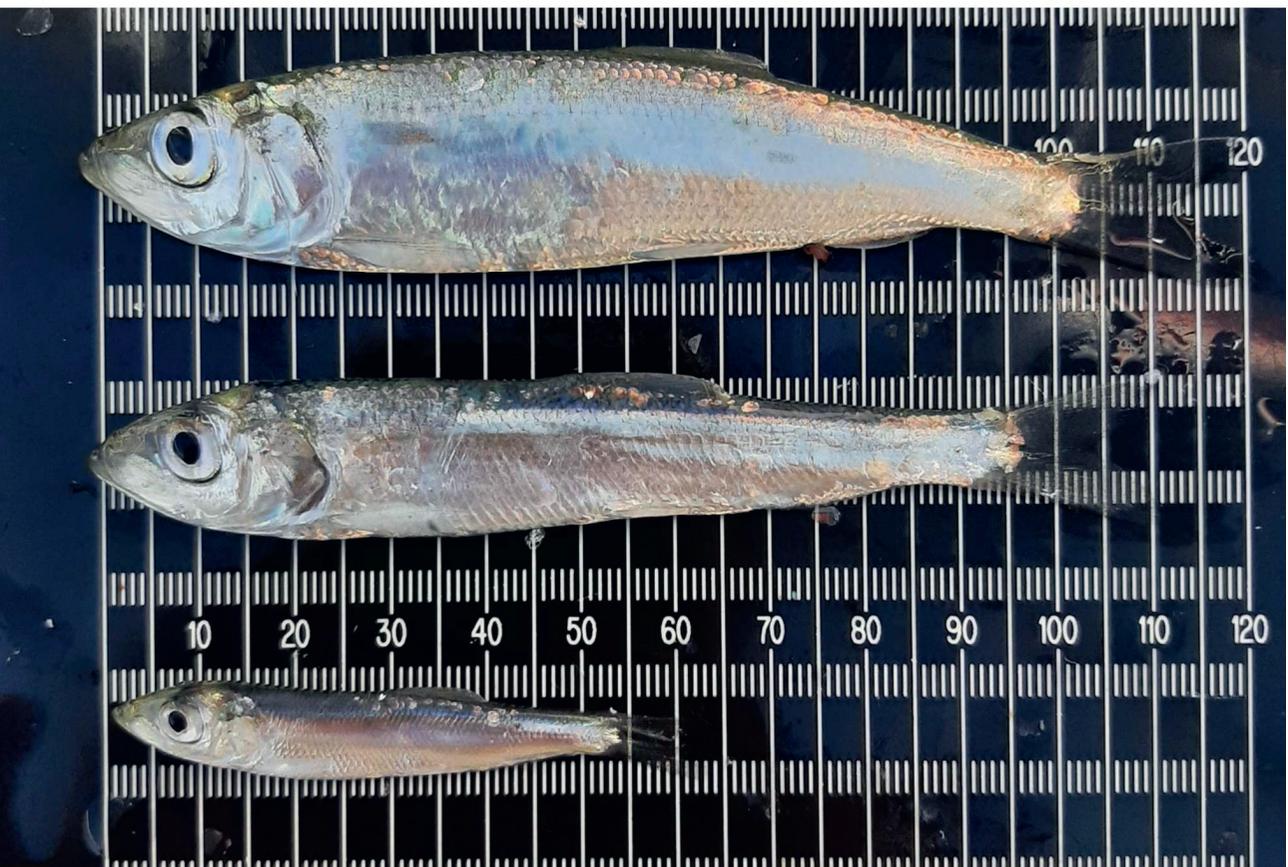
2. Supplementary tables

Table S1. Details on the calibration of the 38 kHz transducer of the towed body, carried out in the harbour of Den Helder (the Netherlands).

Calibration date	Salinity (ppt)	Water temperature (°C)	38 kHz gain	Minor-axis 3 dB beam angle (°)	Major-axis 3 dB beam angle (°)
06/05/2022	29.1	12.8	23.49	6.85	7.10
14/10/2022	30.0	13.5	24.03	6.92	6.97

Stratum	Month	Biomass in kg/ha ± standard deviation						Biomass using -71.2 db			
		Anchovy	Herring	Pilchard	Sand eel	Smelt	Sprat	Total	Herring	Sprat	Total
Marsdiep in	May	4.8 ± 5.9	146.5 ± 141.5	0	13 ± 27	0.1 ± 0.1	37 ± 37.8	201	320.6 ± 309.6	80.9 ± 82.8	419
Marsdiep in	Oct	0 ± 0	7.3 ± 8	0 ± 0	0 ± 0	0.2 ± 0.4	12.9 ± 10.2	20	15.9 ± 17.4	28.1 ± 22.3	44
Marsdiep out	May	4.1 ± 1.5	112 ± 41.2	0	14.6 ± 5.4	0	431.7 ± 158.8	562	245.1 ± 90.1	944.5 ± 347.4	1208
Marsdiep out	Oct	0.2 ± 0.1	4.9 ± 2.2	4 ± 1.8	0	0	28.1 ± 12.6	37	10.8 ± 4.9	61.6 ± 27.6	77
Vliestroom in	May	0.5 ± 0.6	71.6 ± 67.7	0	0.9 ± 0.9	0.2 ± 0.2	23.4 ± 53.9	97	156.7 ± 148.2	51.2 ± 117.9	210
Vliestroom in	Oct	0	13.2 ± 10.8	0.5 ± 0.7	0	0 ± 0.1	7.5 ± 7	21	28.9 ± 23.7	16.5 ± 15.3	46
Vliestroom out	May	2 ± 1.1	58.6 ± 31.4	0	0.6 ± 0.3	0	169.5 ± 91	231	128.1 ± 68.8	370.9 ± 199.1	502
Vliestroom out	Oct	0 ± 0	39.4 ± 12	16.8 ± 5.1	0	0	8.6 ± 2.6	65	86.3 ± 26.3	18.7 ± 5.7	122
Borndiep in	May	3.6 ± 3.1	49.6 ± 42.6	0	3.4 ± 3	13 ± 11.2	6.4 ± 5.5	76	108.5 ± 93.3	14 ± 12.1	143
Borndiep in	Oct	0	7.2 ± 5.6	1.2 ± 0.9	0 ± 0	4.6 ± 3.6	4.2 ± 3.3	17	15.8 ± 12.2	9.2 ± 7.1	31
Borndiep out	May	8.4 ± 9.4	52.4 ± 58.5	0 ± 0	0.9 ± 1	0.5 ± 0.6	90.1 ± 100.7	152	114.5 ± 128	197.2 ± 220.2	322
Borndiep out	Oct	0	3.8 ± 2.6	9.8 ± 6.9	0	0.3 ± 0.2	4.6 ± 3.2	19	8.2 ± 5.7	10 ± 7	28
Westgat in	May	0.7 ± 0.3	5.7 ± 2.3	0	0.2 ± 0.1	8.8 ± 3.5	2.9 ± 1.2	18	12.5 ± 5	6.4 ± 2.5	29
Westgat in	Oct	0	8.6 ± 3.4	0	0	5.8 ± 2.3	0.6 ± 0.2	15	18.9 ± 7.5	1.3 ± 0.5	26
Westgat out	May	0.6 ± 0.2	5 ± 1.7	0	0.2 ± 0.1	7.7 ± 2.6	2.6 ± 0.9	16	11 ± 3.8	5.6 ± 1.9	25
Westgat out	Oct	0	3.6 ± 3.2	0	0	2.4 ± 2.2	0.2 ± 0.2	6	7.9 ± 7	0.5 ± 0.5	11
Lauwers in	May	0.7 ± 0.3	674.8 ± 276.2	0	1 ± 0.4	0	2.8 ± 1.2	679	1476.2 ± 604.2	6.2 ± 2.5	1484
Lauwers in	Oct	0.1 ± 0	3.5 ± 2.2	8.1 ± 5.1	0	1 ± 0.6	3.5 ± 2.2	16	7.7 ± 4.8	7.7 ± 4.8	25
Lauwers out	May	0 ± 0.1	45 ± 72.8	0	0.1 ± 0.1	0	0.2 ± 0.3	45	98.4 ± 159.3	0.4 ± 0.7	99
Lauwers out	Oct	0	21.8 ± 13.2	7.2 ± 4.4	0.1 ± 0	0	19.3 ± 11.7	48	47.7 ± 28.9	42.2 ± 25.6	97
Dollard	May	12.9 ± 13.1	7.2 ± 7.3	0	0.1 ± 0.1	4 ± 4.1	0.5 ± 0.5	25	15.7 ± 15.9	1.2 ± 1.2	34
Dollard	Oct	0 ± 0	10.8 ± 3.9	0	0	32.7 ± 11.7	0.1 ± 0	44	23.7 ± 8.5	0.1 ± 0	57
Eems in	May	0.5 ± 0.5	20 ± 21.1	0	0.1 ± 0.1	4.5 ± 4.8	15.6 ± 16.4	41	43.8 ± 46.1	34.1 ± 35.9	83
Eems in	Oct	0	19.4 ± 7.2	0.6 ± 0.5	0	0.1 ± 0	1.9 ± 1	22	42.5 ± 15.8	4.2 ± 2.1	47
Eems out	May	13.6 ± 4	70.6 ± 20.8	0	0.1 ± 0	0	185.6 ± 54.8	270	154.3 ± 45.6	406 ± 119.9	574
Eems out	Oct	0	29.4 ± 10	0 ± 0	0	0.2 ± 0.1	1.2 ± 0.4	31	64.4 ± 21.8	2.7 ± 0.9	67

Table S2. Biomass estimates (kg/ha) for six small pelagic fish species in the Dutch Wadden Sea in May and October 2022. "Land" indicates the landward and "sea" indicates the seaward substratum. The last three columns present results using a b_{20} value of -71.2 dB for herring and sprat.



Chapter 6

General discussion

Despite small pelagic fish (SPF) being targets of fisheries for centuries and being the focus of numerous studies in the north-east Atlantic, there is surprisingly little known about SPF in the Wadden Sea. The objective of this thesis was to generate fundamental knowledge about SPF temporal and spatial distribution to identify the role of the Wadden Sea in the life cycle of SPF, and to determine their trophic interactions with other species, both as predator and prey. As SPF play an important role transferring energy from lower to higher trophic levels, this knowledge ultimately contributes to a better understanding of the ecosystem functioning of the Wadden Sea. In this final chapter I will synthesize the results, highlight the main findings, put the results in a larger perspective and give suggestions for future studies.

6.1 Synthesis of research chapters

Chapter 2 presents the results of two fishing surveys: a monthly stow net survey and a seasonal trawl survey, examining the role of the Wadden Sea in the life cycle of herring (*Clupea harengus*), sprat (*Sprattus sprattus*), smelt (*Osmerus eperlanus*), and anchovy (*Engraulis encrasicolus*). This study revealed high densities of juvenile SPF present in the Wadden Sea, highlighting its main function as a juvenile nursery habitat for SPF. In addition to its main role as a juvenile nursery, the Wadden Sea functions as a spawning ground for herring and anchovy. Juvenile herring was the most dominant SPF in the Dutch Wadden Sea. The primary influx of herring occurred in summer, continuing throughout the year in lower numbers. Length frequency distributions and genetic analysis confirmed that most young-of-the-year (YOY) herring originated from southwestern waters, such as the English Channel. The majority were identified as winter-spawning Downs herring, with some North Sea autumn spawners also present. Like herring, sprat arrived in the Wadden Sea as post-larvae or YOY, remaining to grow and develop. They likely originate from a wide range of North Sea spawning grounds and periods, though their specific origin remains unclear. No substantial spawning was observed for sprat. Smelt spawn in freshwater, after which YOY smelt enter the Wadden Sea at around 5 cm in autumn and grow throughout the season. The observed size range indicates that smelt can complete nearly their entire life cycle in the Wadden Sea but rely on nearby freshwater for spawning. Lastly, anchovy used the Wadden Sea only seasonally, with spawning individuals present in spring and YOY observed in autumn.

In **Chapter 3**, a stationary echosounder was deployed on the bottom of the Wadden Sea, providing acoustic data at a high temporal resolution, on a local scale. The results revealed that the Marsdiep inlet serves as a migration gateway for SPF between the North Sea and the Wadden Sea, particularly for juvenile herring and

sprat. There was a clear seasonality, with the highest fish densities occurring from mid-spring to early autumn. Fish generally occupied the upper half of the water column, and their movement was largely driven by currents. Interestingly, during outgoing tide, SPF resisted the outflowing current, suggesting efforts to remain in the Wadden Sea, which further confirms the nursery function for these fish.

SPF play a crucial role as an intermediate trophic level, significantly shaping the structure of marine ecosystems (Cury et al., 2000). They often form the 'waist' in marine food webs, with only a few SPF species connecting the biodiverse lower and higher trophic levels (Cury et al., 2000). Typically, SPF species exhibit opportunistic feeding on a variety of prey (Peck et al., 2021), which we also observed when studying the diet of herring and sprat in the Wadden Sea using DNA metabarcoding (**Chapter 4**). Their diet composition showed clear seasonal variation, including significant amounts of (larvae of) benthic prey and fish (post-) larvae, alongside their typically assumed copepod prey. This highlights the essential role of herring and sprat in energy transfer within the Wadden Sea, not only bridging plankton to larger predators but also connecting benthic to pelagic habitats.

Considering SPF's role in supporting upper trophic levels, a global review found that SPF contribute on average to 22% of seabird production, 15% of mammal production, and 34% of total fisheries catch (Ruzicka et al., 2024). Observations suggest that SPF often exert bottom-up control on predator populations (Cury et al., 2000), underscoring the importance of understanding the food landscape created by these fish. In the Wadden Sea, primary SPF predators include other fish species and birds such as terns (Dänhardt et al., 2011; Poiesz et al., 2020). Our research, combining acoustic surveys to study SPF distribution and biomass with the prey choice and habitat use of common terns (*Sterna hirundo*), revealed that tern distribution and foraging preferences largely overlap with the SPF landscape (**Chapter 5**). Common terns primarily foraged around deeper gullies, with clupeidae, especially herring, forming most of their diet. The available fish sizes matched the prey range suitable for common terns. Yet, distribution and peak size varied by species: in May, herring showed highest abundance at ~5 cm, sprat at ~7.5 cm, smelt at ~10 cm, and sandeel (*Ammodytes* sp.) at ~11 cm, with sprat showing the highest energy density. SPF biomass showed notable spatial variability both locally (between transects), regionally (between inlets) and seasonally, with in May higher biomass in the west than in the east, and in October a more even distribution, despite lower overall biomass. A significant correlation was found between SPF biomass and common tern density, indicating food landscape may be a good predictor of tern distribution on a large scale.

This leads to the following main conclusions on the two primary research questions:

i) What is the function of the Wadden Sea in the life cycle of SPF?

The Wadden Sea primarily functions as a juvenile nursery for SPF and, to a lesser extent, as a spawning ground for adult herring and anchovy (Figure 1A). There is a clear seasonality in SPF abundance, distribution, and size composition in the Wadden Sea, driven by species-specific origin and timing of life cycle events, such as the influx of YOY. Herring and sprat are the dominant SPF species, in which especially YOY herring seem more abundant within the Wadden Sea than along the Dutch North Sea coast, while sprat exhibit a more seaward distribution compared to herring.

ii) What is the role of SPF in the food web of the Wadden Sea?

The two most abundant SPF, herring and sprat, feed opportunistically on a wide variety of prey, exhibiting strong seasonal variation (Figure 1B). Alongside copepods, their diet includes (larvae of) benthic species, facilitating energy transfer between benthic and pelagic habitats. Focusing on SPF as prey, we found that common tern distribution and foraging preferences largely overlap with the SPF landscape, mainly feeding on herring around gullies in the Wadden Sea. Furthermore, most SPF were below 12 cm, fitting the preferred size range for common terns' prey.

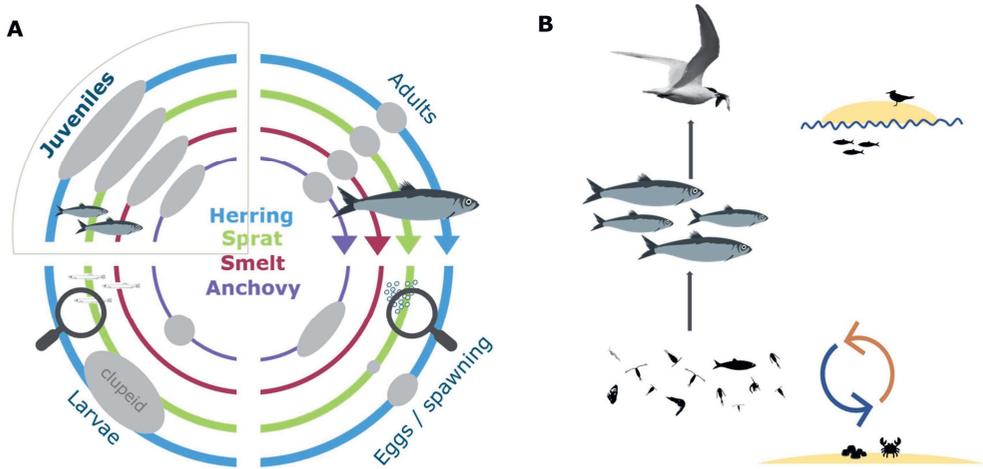


Figure 1. A) The life cycles of key SPF species in the Dutch Wadden Sea—herring, sprat, smelt, and anchovy—represented across four life stages: juveniles, adults, eggs (studied here as spawning individuals), and larvae. The circle's thickness and order denote relative species dominance in the Wadden Sea, while the size of the grey ellipses reflect the relative frequency of observed life stages within each species. The ellipse for clupeid larvae encompasses herring, sprat, and pilchard, as species-level identification was not performed. The juvenile's life stage was most frequently observed, confirming the Wadden Sea's role as a juvenile habitat for SPF. The magnifying glasses highlight gaps in research on early life stages, emphasizing the need for further studies to fully comprehend the role of the Wadden Sea in the full life cycle of SPF. B) SPF in the Wadden Sea serve as key prey for common terns and themselves act as opportunistic predators on a wide variety of prey. The distribution of common terns closely aligns with that of SPF, which is important information to optimize the placement of artificial breeding sites. Herring and sprat feed on copepods, fish larvae, and (larvae of) benthic species, facilitating energy transfer between benthic and pelagic habitats.

6.2 Plasticity of small pelagic fish

SPF are globally important, shaping marine ecosystems and playing a vital role in worldwide food security (Peck et al., 2021). As a result, SPF are studied across the globe, though research topics and knowledge gaps vary by region. In this section, I zoom out to explore the diverse focuses of SPF research. Considering the plasticity of SPF, I demonstrate why local studies are essential to understand their ecology.

Much of the information on SPF has been gathered from upwelling systems, such as those along the eastern coasts of Africa, South America, and North America (e.g. Cury et al., 2000; Bertrand et al., 2008; Fréon et al., 2009). In these highly productive coastal areas, cold, nutrient-rich water from depths of 200-300 m is brought to the surface by intense trade winds and the earth's rotation, fuelling primary production. This, in turn, leads to high fish biomass, with the four main upwelling systems (i.e. the Canary, California, Humboldt, and Benguela Currents) contributing one-fifth of the global marine fish catch (Fréon et al., 2009). Due to their significant socio-economic impacts and the large-scale population oscillations of anchovy and sardine, these ecosystems are well-studied. Research focused here mainly on interactions between SPF species, their prey, and their predators, as well as the effects of fisheries and climate change (Peck et al., 2021).

In temperate marine waters closer to my study area, research has primarily focused on herring and sprat, with the Baltic Sea being particularly well-represented in SPF studies (Peck et al., 2021). However, the Baltic Sea differs significantly from the Wadden Sea, being a semi-enclosed brackish environment with several deep basins and lacking lunar tides. Furthermore, local adaptation in the Baltic Sea has led to genetically distinct populations of herring (Teacher et al., 2013; Guo et al., 2016; Seljestad et al., 2024) and sprat (McKeown et al., 2020). While SPF in the North Sea have also been extensively studied, research here is mostly focused on ichthyoplankton and adults, often related to fisheries. Studies typically examined drivers of recruitment, patterns in stock biomass, or the co-occurrence of SPF species in this transition zone between warmer-water species (e.g., anchovy, pilchard (*Sardina pilchardus*)) and colder-water species (e.g., sprat, herring).

Despite significant worldwide research efforts on SPF, an extensive review underscores the need for more studies on the juvenile stage of SPF (Peck et al., 2021). Numerous studies have highlighted the importance of shallow coastal zones for the early life stages and juveniles of pelagic fish species (e.g. Beck et al., 2001; Polte et al., 2017). However, studies from geographically distant or deeper areas may not be very informative to understand the ecology of SPF in shallow waters, like the Wadden Sea. In this shallow tidal ecosystem, some of our findings contrasted with the usual understanding of SPF. For example, while SPF are generally known to form large schools, tens of meters in diameter, our acoustic

studies mainly detected numerous small aggregations (**Chapters 3 and 5**). This difference in schooling behaviour is likely due to the shallow, turbid, and dynamic conditions in this area. Additionally, we found that herring and sprat had a more diverse diet, including early life stages of benthic prey, compared to the typical zooplanktivorous image of SPF, primarily feeding on copepods (**Chapter 2**). This aligns with a recent study in a shallow Baltic Sea bay, which also found that adult herring seasonally fed on demersal species like gobies and epifauna (Kotterba et al., 2024). Given SPF's wide geographical distribution, encompassing very different environmental conditions, and the potential for genetic differentiation among populations, detailed understanding of region-specific SPF ecology requires region-specific studies.

6.3 Differences between seemingly similar species

Herring and sprat look similar at first glance, and Dutch fishers often use the term '*bliet*' to refer to these small silvery schooling fish, without distinguishing between them. Grouping herring and sprat together as 'clupeids' is also common in bird studies when observations are limited to cameras or binoculars (e.g. Stienen et al., 2000; Dänhardt et al., 2011). Despite visual similarities, herring and sprat have markedly different life history traits, such as maximum size and reproductive strategies. Therefore, distinguishing between herring and sprat is crucial for understanding their ecological roles. In this section, I will provide examples from our observations to illustrate these differences and address some methodological challenges involved in accurately identifying herring and sprat.

While juvenile herring and sprat showed dietary overlap and both fed opportunistically, herring exhibited a size-related shift in diet, with adults consuming more fish post-larvae, a pattern not observed in sprat (**Chapter 4**). Furthermore, both species arrived in the Wadden Sea as post-larvae or YOY to grow and develop; however, herring influx started about 1-2 months earlier than sprat influx (**Chapter 2**). This difference in timing is important for predators, such as breeding terns, as herring were smaller and more abundant during the breeding period compared to sprat (**Chapter 5**). Yet, sprat had a significantly higher energy density than herring (**Chapter 5**). We also found differences in their distribution, particularly in May 2022: sprat were generally more abundant on the outer side of the inlets, whereas herring had higher biomass inside the Wadden Sea (**Chapter 5**). So, juvenile herring seem more bound to shallow waters like the Wadden Sea, whereas sprat are relatively more common along the Dutch coast (**Chapters 2 and 5**).

The distinction between herring and sprat remains a methodological challenge, even within my PhD project. For example, in **Chapter 2**, we grouped all herring, sprat, and pilchard smaller than 5.5 cm as clupeid post-larvae, because field identification was not feasible at such small sizes. Furthermore, using active acoustics alone, an accurate differentiation between herring and sprat was not possible. Echosounders detect fish based on scattering characteristics, such as size, shape, swim bladder, and swimming behaviour, which is quite similar for herring and sprat, especially in the juvenile stages. Therefore, echosounder data typically need to be supplemented with additional methods. In our study, species identification from acoustic data relied on a limited number of catches, which were then extrapolated to other time periods (**Chapter 3**) or the wider area (**Chapter 5**).

Lastly, species identification of juvenile herring and sprat based on otolith shape proved problematic, with interpretations differing between experienced otolith readers in the Netherlands and Norway. Because otolith shape is influenced by both genetic and environmental factors (Cardinale et al., 2004; Berg et al., 2018), it is highly variable among species and populations (Saltalamacchia et al., 2022). Typically, otoliths are read from mature individuals in offshore waters or from Norwegian fjords, serving as our reference. Our findings suggest that local conditions in the shallow and turbid Wadden Sea may affect otolith formation differently compared to reference otoliths from offshore conditions. This highlights the need for comparisons with otolith samples from other coastal regions, eventually complemented with DNA sequencing. Beyond species identification, aging small herring and sprat otoliths also revealed considerable variability among experienced readers. A promising alternative for fish aging involves analysing DNA methylation patterns, reflecting epigenetic modifications linked to aging (Mayne et al., 2023; Piferrer & Anastasiadi, 2023). However, this method requires the development of species-specific epigenetic clocks, which are currently unavailable for the species I studied.

6.4 Understanding full life cycle requires additional knowledge

One of this thesis's main aims was to explore the role of the Wadden Sea in the life cycle of SPF. Our studies identified which species, sizes, and life stages use the Wadden Sea, including the timing of new year-class arrivals and potential spawning events, concluding that the Wadden Sea primarily serves as a juvenile habitat for SPF. However, a comprehensive understanding of its role requires additional knowledge of the entire life cycle, including the early life stages and the origins of the SPF species present in the Wadden Sea. This is for instance important to understand population dynamics of the species under study, because fish recruitment is often determined during the early larval stages (Hjort, 1914; Arevalo

et al., 2023). Moreover, to assess whether the bottlenecks for fish occur within or outside the Wadden Sea, which is the central aim of the Swimway-Wadden Sea project, it is essential to know their natal origin and where they migrate for spawning after leaving the Wadden Sea.

Tracing the spatial origin of the fish we observed in the Wadden Sea can be achieved through various methods. One approach involves counting daily increments in YOY otoliths to determine the hatching day. When combined with sampling the spatiotemporal distribution of eggs and larvae, this method can reconstruct spatial origin (Baumann et al., 2009), though this is time-consuming and technically challenging. Another method is otolith microchemistry, where otoliths reflect the physical and chemical characteristics of ambient water during early development (Campana, 1999). For example, strontium, a marker for salinity gradients, has been used to study smelt connectivity between the Wadden Sea and Lake IJssel (Tulp et al., 2013). Expanding this approach to include additional elements offers greater detail; for example, the 'elemental fingerprinting index' uses 17 chemical elements and has been developed for herring (Moll et al., 2019). This technique proved to be effective for studying herring homing behaviour in the Baltic Sea, with 56-73% of spawning individuals returning to their natal spawning sites (Moll et al., 2022).

We studied the origin for a small sample of herring (i.e. 36 individuals) by genetic alignment analysis, revealing that most herring in the Dutch Wadden Sea belong to autumn- or winter-spawning populations (**Chapter 2**). In addition, we also observed two spring-spawning herring that could not be accurately assigned to any population, as they did not match the available baseline data. Interestingly, the historical Zuiderzee herring were also spawning in spring, drawing a potential connection to the Zuiderzee herring lineage. To determine if the observed spring-spawning herring are indeed closely related to the historic Zuiderzee herring, the first step would be to investigate the existence of museum specimens of Zuiderzee herring, and if available, to use this archival material alongside modern populations for phylogenetic analysis. Aside from our observations, reports indicate spring-spawning herring at various nearby locations, primarily in April. These include the sluices in the Dutch Noordzeekanaal and Brouwersdam (personal communication P. Molenaar; zeevisland.nl), as well as several sites in the German Wadden Sea (personal communication A. Dänhardt; beachexplorer.org). To determine whether these herring represent local populations or are part of a larger 'coastal stock,' it would be valuable to sample additional spring-spawning herring from the aforementioned locations. A follow-up question would be whether these populations are small or contribute significantly to the overall herring stock. Furthermore, we observed an aggregation of spawning herring in October north

of Lauwers and high abundances of YOY herring in May within the Lauwers inlet (**Chapter 2**). To confirm this potential local spawning site, followed by juvenile development here, additional ichthyoplankton surveys are required, preferably complemented by genetic analysis of both spawning adults and YOY.

Finally, while our studies focused on SPF in the Dutch Wadden Sea, future research should ideally compare this area with other coastal zones, including the German and Danish Wadden Seas and North Sea, addressing aspects such as population connectivity, abundance, growth, and mortality. This comparison will highlight the relative importance of the Dutch Wadden Sea and will help complete the understanding of each species' full life cycle by revealing where they spend which life stage.

6.5 Surprising observations highlight new questions

Our sampling efforts revealed several interesting yet surprising observations, which are addressed in this section, along with suggestions for further investigation. These findings nicely illustrate the serendipity in science: while resolving existing questions, surprising observations lead to new research ideas.

One such observation was the identification of two distinct 'types' of herring based on eye size. In some catches, especially in May, a subset of juvenile herring exhibited smaller eyes compared to what we normally observed (Figure 2A). Given that herring exhibit phenotypic plasticity, the variation could be linked to differing environmental conditions, origins, food abundance or dietary composition. Interestingly, similar findings have been reported in the Baltic Sea, where herring feeding on benthic organisms had larger eyes than those consuming zooplankton (personal communication P. Kotterba). A study on Eurasian perch (*Perca fluviatilis*) found that increased dissolved organic matter led to larger eyes, suggesting a sensory adaptation to deteriorating visual conditions (Bartels et al., 2016). However, the extent of this phenomenon in herring remains unclear, as subtle appearance differences can easily go unnoticed. The eye index, which relates eye size to body size (Bartels et al., 2016), could be a valuable tool for categorizing the herring into two types. These types could then be analysed by: i) otolith microchemistry to determine differences in their origins, ii) stomach contents to identify dietary variations, and iii) comparing their overall condition. A follow-up question could investigate whether one type has a potential advantage under certain environmental conditions, which might be explored through laboratory experiments.

Another surprising observation was the high abundance of pilchard post-larvae, identified through myotome counting of clupeid larvae samples. This is unexpected

given the dominance of herring and sprat juveniles in the Wadden Sea and the infrequent catches of pilchard in the region. This small pilot study suggests the need for a dedicated sampling campaign to better understand the post-larval composition, preferably incorporating both myotome counting and DNA analysis for species identification.

Additionally, the impact of parasites on ecological dynamics of SPF warrants further investigation (Moller et al., 1992; Giari et al., 2022). We frequently observed the eye-maggot *Lernaeenicus sprattae* and nematodes. While *L. sprattae*, as its name suggests, is common in sprat, it has not been previously reported in herring. However, we found that of 490 herring sampled for stomach contents, 1.2% were infested with *L. sprattae* (Figure 2B), compared to 15.9% of the sprat. Nematode infestation rates were 21.6% for sprat and 3.3% for herring. At a first glance, we only checked for *L. sprattus* and nematodes, sprat exhibited higher overall parasite prevalence than herring. Since parasites typically drain energy from their hosts, it would be valuable to investigate their impact on fish condition, reproduction, and their relationship with factors such as size, season and location.

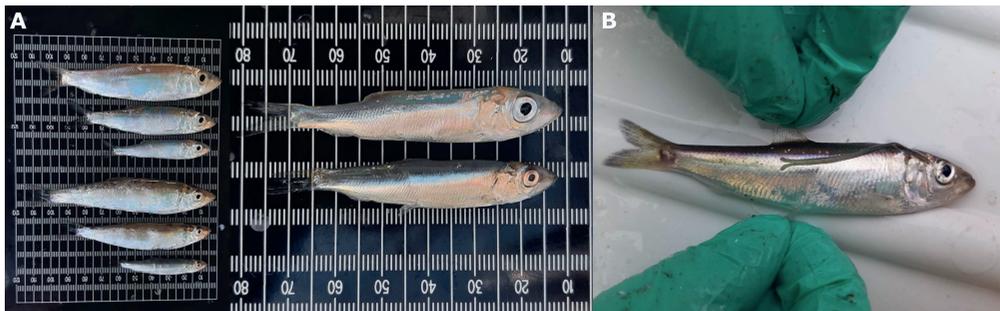


Figure 2. A) Variation in eye-size of herring caught in the Wadden Sea, especially some juveniles appear to have smaller eyes. B) Herring infested with *Lernaeenicus sprattae*, a parasite typically known from sprat, but not previously documented on herring. Pictures taken during the acoustic survey in May 2022 (A) and during the stow net survey in April 2021 (B).

6.6 Long term and detailed monitoring needed to detect changes

A common question I received was whether the fish populations I studied in the Wadden Sea were doing well. It is complicated to answer this question due to the lack of detailed, Wadden Sea wide, long-term data on SPF, collected using pelagic fishing gear. This PhD project offered high-quality and detailed data but spanned a relatively short time frame. Answering such a question is particularly difficult,

given that pelagic fish populations tend to fluctuate substantially in year-class strength and overall abundance due to short generation times and climate-driven variability in prey resources (e.g. Cury et al., 2000; Alheit & Peck, 2019).

In the current era of global change, shifts in abundance, distribution and phenology of fish can be expected (Rijnsdorp et al., 2009; Sydeman et al., 2015; Rademaker et al., 2024). Especially in the north-east Atlantic, where many SPF occur near their latitudinal limits, climate variability profoundly impacts SPF distribution and abundance (Peck et al., 2021). For the Wadden Sea, future rising temperatures and changes in precipitation are expected to result in drier, warmer summers and wetter, milder winters (Hoekstra, 2024). Specifically, from 2001 to 2021, the mean annual water temperature in the western Dutch Wadden Sea has already increased by over 1.0 °C (Dye et al., 2024; Philippart et al., 2024). Furthermore, climate change driven reductions in freshwater input are expected to result in less attraction flow for anadromous fish, decreased nutrient input and combined with higher evaporation, in a higher salinity (Hoekstra, 2024). Further changes anticipated because of climate change include altered circulation patterns and, over the course of several decades, the flooding of tidal flats due to rising sea levels (Hoekstra, 2024).

In addition to climate change, coastal ecosystems like the Wadden Sea are significantly affected by dense human populations and cumulative anthropogenic pressures (Lotze, 2005; Brown et al., 2018). These pressures include increased shipping traffic, dredging, sediment excretion, and the construction of tidal energy installations, such as the recent development of a tidal kite operating in the water column. To mitigate these pressures, conservation measures are in progress, including the Fish Migration River, a project to open the Afsluitdijk to facilitate fish migration through a regulated local opening (Winter et al., 2024). An MSc study by van Elderen (2022) analysed water discharge data from the Kornwerderzand sluices alongside fish catch data, showing that the farther the freshwater plume extends into the Wadden Sea, the more fish, particularly smelt, gather at the sluices. This plume is essential as an attractant and establishes a salinity gradient that should extend sufficiently into the Wadden Sea. However, in warmer and drier summers, reduced freshwater discharge may limit its reach. While this may be disadvantageous for smelt, they are also expected to benefit from improved migration possibilities by the Fish Migration River (Winter et al., 2024).

Given these changing conditions, the Wadden Sea ecosystem is expected to undergo large changes, with shifting temperatures and freshwater input having the greatest impact on SPF. For instance, spatial distributions of SPF will be impacted by temperature increase, because of species- and life stage-specific thermal tolerances (Dahlke et al., 2020). This may result in altered species compositions,

such as a further increase of warm-water species like pilchard (Alheit et al., 2012; Tulp et al., 2022). Another species sensitive to temperature is smelt, with the population in Lake IJssel suffering during warm years, particularly affecting young life stages (Keller et al., 2020). Additionally, ocean warming and changes in food availability may reduce fish body size, as already seen in anchovy in the Bay of Biscay (Taboada et al., 2024) and herring populations off Ireland (Lyashevskaya et al., 2020). This, in turn may impact the food web, for instance for piscivorous birds that depend on high-nutritional prey of the right size to feed their chicks.

By offering a baseline understanding of SPF in the Dutch Wadden Sea, this PhD thesis provides a solid foundation of the present situation. Looking ahead, it serves as a strong starting point, with its value greatly amplified if future studies build on this knowledge and generate comparative data. Detecting changes in community composition, seasonal patterns, trophic interactions, phenology, and distribution requires consistent, long-term, and detailed data collection. This effort demands robust field methods, and a key contribution of this thesis is the experience gained in SPF monitoring techniques in shallow coastal waters, enhancing our ability to study this important group of fish.

6.7 Comparing field methods to study the ecological functioning of SPF in the Wadden Sea

In my PhD study, we employed multiple methods to study SPF, including new and advanced techniques. In this section, I critically review the applied methods, highlighting their strengths and limitations. Following this, I offer practical advice for future monitoring of SPF in the Wadden Sea.

Fishing provides detailed biological information, including species composition, size frequency, maturity and age, which helps in understanding the system's functioning. It also supplies valuable tissues, such as fin clips for genetic analysis and stomach contents for diet studies. However, fishing is an invasive method, leading to the death of many individuals. Furthermore, it offers only local and temporal snapshots of SPF populations, and each net and fishing method has a specific catchability. We used two types of net: i) the stow net is notably effective for shallow areas. It covers the entire water column and captures a broad range of fish species, both demersal and pelagic. However, its use is constrained by anchoring restrictions and specific wind conditions (i.e. right direction relative to the current and speeds < 6 Bft), limiting the locations and instants where it can be successfully applied. ii) The pelagic trawl provides greater flexibility compared to a stow net, allowing for broader coverage and adaptability to varying conditions, but this net only covers part of the water column and cannot be used in water that is

too shallow. Additionally for any survey method, in an area like the Wadden Sea moving from one tidal basin to the next is logistically challenging, especially since the tides dictate which areas can be reached at which tidal phases. Consequently, covering the entire area of the Dutch Wadden Sea in a limited timeframe of one week proved to be extremely challenging.

Another approach is the use of active acoustics through ship-based surveys or stationary echosounders, which are non-invasive methods primarily employed for studying fish biomass, distribution and behaviour. A great advantage of an acoustic survey is that it can quickly and efficiently cover large areas. However, this method is not suitable for very shallow waters (< 6 m) or conditions with wind speeds above 6 Bft. Stationary echosounders, like the WideBand Autonomous Transceiver (WBAT), offer data of high temporal resolution and are cost-efficient. However, they sample only a small area and finding a suitable deployment location is challenging, for instance due to moving sand dunes and bottom-fishing activities. Unfortunately, both acoustic methods suffer from blind zones near the sea surface, primarily due to wave action that generates air bubbles, obstructing detection of fish. Furthermore, echosounders have a blind zone caused by the ringdown effect, which prevents detection of the first meters near the surface when scanning downwards. Furthermore, to obtain biological information, fishing remains necessary. However, recent advancements, such as the biodiversity sensing box (Yu et al., 2024), which includes environmental DNA samplers and hydrophones, could potentially reduce the need for fishing in the future, depending on research questions and the level of detail required.

Each method has its strengths and weaknesses, making the choice of the most suitable approach dependent on the specific research question. We observed significant seasonal variation in SPF abundance and size, emphasizing the need for regular sampling when monitoring SPF. However, fishing is invasive and captures substantial quantities of juvenile fish, which should be considered when planning regular fishing campaigns. Based on my experiences, I recommend a twofold approach to monitor SPF in the Wadden Sea: i) conducting an annual acoustic survey once a year across the entire Wadden Sea, where acoustic measurements are combined with regular trawl fishing to collect biological material and data. This should also include sampling of abiotic factors to better understand how they influence SPF dynamics. Furthermore, the timing of this survey should align with the acoustic MONS ('Monitoring-Onderzoek-Natuurversterking-Soortbescherming') survey along the Dutch North Sea coast in June planned for the coming years. This would correspond with the herring influx period and the bird breeding season; and ii) for more detailed seasonal insights and tracking YOY influx, combining a WBAT with monthly fishing would be ideal. Given the intensity and costs, this could be

carried out once every two to three years, ideally at two locations. Data and experience from Marsdiep and Westereems could serve as a reference. Sampling these deeper inlets would maximize the acoustic sampling volume and provide a comparison of the western and eastern Wadden Sea. Furthermore, an element missing in this PhD project was the inclusion of proper pilot studies for the deployment, retrieval, and data collection of the WBATs, as well as a pilot fishing survey to assess the feasibility of the tight planning and extensive sampling schedule. While experience was gained throughout the process, this resulted for instance in varying WBAT-frame setups. Therefore, a good and realistic monitoring plan needs the inclusion of an initial pilot phase whenever new ideas or equipment are introduced.

In addition to its goal as monitoring tool, this setup would allow for the collection of materials for additional research questions, such as studying the origin of juvenile SPF present in the Wadden Sea. Moreover, as highlighted in Section 6.4, specific questions regarding early life stages would necessitate additional dedicated ichthyoplankton surveys. Lastly, given the critical role of SPF as a major link in the food web, transferring energy from zooplankton to higher predators, I recommend integrative studies of both predator and prey, by sampling across multiple trophic levels. For instance, while knowledge of SPF in the Wadden Sea is limited, even less is known about zooplankton. An integrative approach has proven effective over the decades, for instance in studies of shellfish and shellfish-eating birds in the Wadden Sea (e.g. Kraan et al., 2009; Bakker et al., 2021). Beyond enhancing knowledge of zooplankton and piscivorous predators, such studies will provide a more comprehensive assessment of both bottom-up and top-down forces acting on SPF.

6.8 Management implications and concluding remarks

The 'Trilateral Swimway Action Programme' noted a lack of fundamental understanding of fish ecology in the Wadden Sea (CWSS 2019). It emphasized that advancing knowledge of fish ecology is crucial to achieving its goal of "eliminating human-induced bottlenecks for fish populations in the Wadden Sea". The region's dense population, significant tourism, and economic activities create conflicting stakeholder interests (Kloepper et al., 2022). Insights from studying underrepresented and understudied fish species and habitats through the Swimway-Wadden Sea project contributes to more informed decisions regarding conservation, tourism, and economic development in this region. While not all studies are finished yet, the project has already yielded significant insights. For example, Jena Edwards revealed that mullets are highly mobile, using the entire Wadden Sea driven by the widespread availability of their preferred food. Bass Dye modelled the effects of rising temperatures on various species, showing that these

impacts differ by species, underscoring the importance of species-specific approaches to climate adaptation. Hannah Charan-Dixon demonstrated that the connectivity of salt marsh creeks with the Wadden Sea is an important determinant of habitat quality. Furthermore, Maryann Watson's pioneering use of passive acoustics showed that sound diversity varies by habitat. An additional key contribution is the establishment of a Swimway receiver network, which will continue monitoring fish migration. While the other PhD studies focus on larger migrating fish, the effects of abiotic changes, and fish in salt marshes and around shellfish beds, this PhD study adds another group of fish and habitat type: SPF in the gullies of the Wadden Sea.

Although we gained significant insights into SPF ecology and consequently into the functioning of the ecosystem, translating this knowledge into concrete conservation measures is not straightforward, as tools to steer conditions for pelagic fish are very limited. Improving conditions for migrating, free-swimming pelagic fish, typically not confined to specific substrates or narrow environmental conditions, is difficult due to limited intervention options. However, there are several practical outcomes, along with considerations related to fisheries and bird breeding locations that can inform management, which are discussed here.

For instance, herring spawning conditions may provide a management opportunity due to their reliance on specific environmental factors. Herring reproduction needs habitats with strong currents that enhance oxygenation and prevent sediment accumulation, where adhesive eggs can attach to coarse seabed substrates or, as in the Zuiderzee, to firm sand and mud (ICES 1903; Frost & Diele, 2022). We observed spawning herring in the Wadden Sea (**Chapter 2**), though not in the large quantities known from the Zuiderzee. The first step is to identify and confirm their spawning sites, before taking any further management measures. Furthermore, I recommend compiling all existing local observations of spawning herring to gain a more comprehensive understanding of this phenomenon, including data from the Dutch, German, and Danish Wadden Sea, as well as the Dutch North Sea coasts, similar to the approach taken by Frost & Diele (2022) in Scotland. This could ultimately support the protection of key herring spawning habitats.

Currently, there is no fisheries targeting SPF in the Wadden Sea, although brown shrimp (*Crangon crangon*) fisheries overlap with SPF distribution. Shrimp fishery uses bottom gear, and therefore bycatch rates for SPF are relatively low. However, pressures to make shrimp fishing more sustainable by shifting to pelagic, passive gear (e.g. stow nets) could increase the risk of unwanted SPF bycatch, which should be carefully considered. While SPF are not targeted by fisheries in the Wadden Sea, they are commercially very important further offshore. Since commercially important SPF species spend part of their lives in the Wadden Sea, improved SPF

ecological knowledge, resulting in targeted conservation efforts could potentially enhance commercial stocks offshore.

Furthermore, we have expanded the information available on the prey of breeding terns, such as common terns, sandwich terns (*Thalasseus sandvicensis*), and little terns (*Sternula albifrons*)—species for which the Wadden Sea is assigned as a Natura 2000 site. Their reproductive success relies on the availability of SPF in the right size and condition. Our findings revealed a significant correlation between SPF distribution and common tern density, with herring comprising most of their prey. This insight is crucial when planning new artificial breeding sites, a conservation measure taken nowadays to compensate for the lack of natural breeding locations, due to the lack of natural dynamics. If prey is only accessible at distant locations, such sites risk becoming ecological traps, offering seemingly suitable breeding habitats but failing to support parents in meeting chicks' energy demands for growth. Using the information on SPF distribution, biomass and energy density provided in this thesis, follow-up studies can now explore the energy budget of common terns and calculate the maximum foraging distance to a breeding site. A preliminary analysis by Schekkerman et al. (in prep.) showed that, when a pair of terns raises one chick, distances between 10 and 40 km are feasible, depending on prey type, size and search time. However, with two chicks, this distance is considerably shorter—maximum 20 km. So, our findings indicate that the greatest potential for successful breeding locations is near tidal inlets and the outer sections of major gullies, particularly in the western Dutch Wadden Sea. However, given the rapid variability in SPF dynamics, repeated monitoring is essential.

Lastly, this thesis clearly showed that the Wadden Sea is not an isolated ecosystem; its connectivity with the North Sea is crucial. For example, we showed that the Marsdiep acts as a migration corridor for SPF moving between the North Sea and the Wadden Sea, particularly juvenile herring and sprat (**Chapter 3**). Interestingly, a significant portion of the herring in the Wadden Sea originates from the English Channel, further emphasizing the connectivity between habitats (**Chapter 2**). However, even within the Wadden Sea, many studies, including ours, focus solely on the Dutch, German, or Danish Wadden Sea, with few cross-border investigations. This is a significant limitation, as SPF are migratory species that rely on multiple habitats throughout their life cycle. Therefore, increased cross-border collaboration and a broader, cross-ecosystem approach could enhance our understanding of SPF ecology and put the importance of the Dutch Wadden Sea for SPF into perspective.

In conclusion, SPF have gained significant attention, raising awareness of their ecological role beyond being merely 'bird food'. It's unfortunate that referencing birds is often necessary to justify studying SPF, but I hope this thesis demonstrates their critical role in the ecosystem and raises awareness of the vast numbers of these typically unseen and small fish in the murky waters of the Wadden Sea.

Appendices

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A

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Summary

Pelagic adjective*pe·lag·ic pə-ˈlɑ-jɪk*

"relating to, or living or occurring in the open sea: oceanic"

Studying *pelagic* fish within shallow coastal waters may seem counterintuitive, yet it is the central topic of this thesis. The interconnectedness of open oceans and coastal zones is crucial for many fish species. They depend on coastal areas as feeding grounds, nursery habitats, spawning sites, and/or migration corridors. One such coastal habitat is the Wadden Sea, a shallow, dynamic tidal area characterized by murky waters and strong currents. As outlined in **Chapter 1**, these conditions present challenges for studying small and mobile pelagic fish, a likely reason why this part of the fish fauna has hitherto been understudied. In the Wadden Sea, small pelagic fish (SPF) represent a substantial portion of fish biomass and are a central link in the food web. Therefore, a lack of knowledge about SPF limits our understanding of the ecosystem functioning. Until now, basic information on SPF abundance, timing, and ecological functioning in the Wadden Sea was lacking. Additionally, their roles as predator and prey were poorly understood. Therefore, this thesis addresses two key questions:

- i) What is the function of the Wadden Sea in the life cycle of small pelagic fish?*
- ii) What is the role of small pelagic fish in the food web of the Wadden Sea?*

Chapters 2 and 3 addressed the first research question. We observed high densities of juvenile SPF in the Wadden Sea, highlighting its role as a juvenile nursery habitat, and, to a lesser extent, as a spawning ground for adult herring and anchovy. There was a clear seasonality in SPF abundance, distribution, and size composition in the Wadden Sea, driven by species-specific spatial origin and timing of life cycle events, such as the influx of young-of-the-year (YOY). For example, we demonstrated that most YOY herring originate from southwestern waters, such as the English Channel. Furthermore, YOY herring seemed more abundant within the Wadden Sea, while sprat exhibited a more seaward distribution.

Chapters 4 and 5 addressed the second question by examining the trophic interactions of SPF in the Wadden Sea. **Chapter 4** focused on SPF as predators. We found that herring and sprat feed opportunistically on a wide variety of prey, exhibiting strong seasonal variation in diet composition. Alongside copepods, they consumed (larvae of) benthic species, facilitating energy transfer between benthic and pelagic habitats. Focusing on SPF as prey in **Chapter 5**, we found that common tern spatial distribution and foraging preferences largely overlap with SPF

distribution. Common terns mainly fed on herring around gullies in the Wadden Sea. Furthermore, most SPF were below 12 cm, aligning with the preferred prey size range of common terns. By mapping the food landscape, we provided the first Wadden Sea-wide biomass estimates for SPF in both spring and autumn.

Chapter 6, the general discussion, places the findings of this thesis in a broader context and examines their implications for management. The ecological plasticity of SPF, demonstrated by their wide geographical distribution and adaptability to diverse environmental conditions, emphasizes the need for region-specific studies to fully understand their local ecology. For example, while SPF typically form large schools and primarily feed on copepods, in the shallow Wadden Sea, they form smaller aggregations and show a more benthic-oriented diet. Moreover, despite similar appearance and some shared traits, herring and sprat exhibited distinct differences, underscoring the importance of species-level identification for understanding their ecological roles. This chapter further emphasizes that a comprehensive understanding of the Wadden Sea's role for SPF requires deeper insights into their complete life cycles, including early life stages and the origins of the SPF inhabiting this area. Additionally, comparative studies across the Wadden Sea regions (Dutch, German, and Danish) and the North Sea are recommended to study population connectivity, abundance, growth, and mortality. This will clarify the Dutch Wadden Sea's importance for SPF.

Improving conditions for migrating pelagic fish— which are not typically confined to specific substrates or narrow environmental conditions— is challenging due to the limited intervention options. Yet, several practical outcomes of this PhD study are discussed in the final chapter. For example, I provide recommendations on identifying potential herring spawning habitats and suggest optimal areas for establishing artificial breeding sites for common terns. Throughout this thesis, various methods were used to study SPF, contributing valuable insights into their efficacy and limitations. For monitoring SPF in the Wadden Sea, I recommend combining an annual acoustic survey to track long-term trends, with deploying a stationary echosounder every 2-3 years, alongside monthly fish sampling to monitor seasonal dynamics. Additionally, I emphasize that integrative studies across multiple trophic levels, including prey and predators of SFP, along with the sampling of abiotic factors, are crucial for understanding ecosystem functioning and assessing both bottom-up and top-down forces acting on SPF.

In conclusion, this thesis highlights the essential roles of SPF in ecosystems, and raises awareness on the vast abundance of these typically unseen fish in the murky shallow waters of the Wadden Sea.



Samenvatting

Pelagisch bijvoegelijk naamwoord*pe·lag·isch pe'la:yis*

"in open water, zee, meren, vrij van de bodem levend"

Het onderzoeken van *pelagische* vis in ondiepe kustwateren lijkt misschien tegenstrijdig, maar dit is precies wat ik de afgelopen jaren heb gedaan. De verbinding tussen de open oceaan en de kustzones is cruciaal voor veel vissoorten. Ze zijn afhankelijk van kustgebieden voor voedsel, voortplanting of als onderdeel van hun migratieroute. Het kustgebied dat ik bestudeerd heb is de Waddenzee, een ondiep, dynamisch getijdengebied met troebel water en sterke stromingen. Zoals besproken in **Hoofdstuk 1**, vormen deze omstandigheden flinke uitdagingen bij het onderzoek naar kleine, mobiele pelagische vissen, één van de redenen waarom dit deel van de visfauna tot nu toe onderbelicht is gebleven. In de Waddenzee vormt kleine pelagische vis (KPV) een groot deel van de visbiomassa en speelt het een centrale rol in het voedsel web. Een gebrek aan kennis over KPV beperkt daarom ons begrip van het functioneren van het Waddenzee-ecosysteem. Basisvragen zoals hoeveel, welke soorten, wanneer en waarom ze naar de Waddenzee komen, waren tot nu toe onbeantwoord. Ook hun rol in het voedsel web, zowel als predator als prooi, was nog niet duidelijk. In dit proefschrift richt ik me daarom op twee kernvragen:

- i) Welke functie heeft de Waddenzee in de levenscyclus van kleine pelagische vis?*
- ii) Wat is de rol van kleine pelagische vis in het voedsel web van de Waddenzee?*

Hoofdstukken 2 en 3 richten zich op de eerste onderzoeksvraag. We zagen hoge dichtheden jonge KPV, wat bevestigt dat de Waddenzee met name een opgroeigebied is voor juveniele vis. Daarnaast is het gebied, zij het in mindere mate, ook een paaigrond voor volwassen haring en ansjovis. Gedurende het jaar was er een duidelijke variatie in aantallen, verspreiding en groottesamenstelling van KPV in de Waddenzee. Dit komt doordat de soorten van verschillende plekken afkomstig zijn en de timing van paai, en daarmee de instroom van jonge vis, varieert. Zo toonden we bijvoorbeeld aan dat de meeste juveniele haring afkomstig zijn uit wateren in zuidwestelijke richting, zoals het Kanaal. Bovendien was juveniele haring meer aanwezig in de hele Waddenzee, terwijl sprout meer voorkwam in de zeegaten.

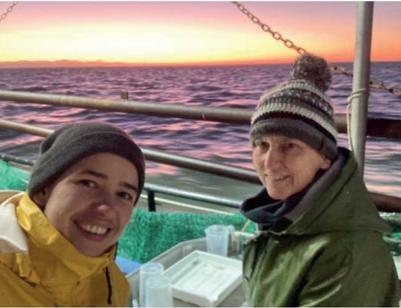
Hoofdstukken 4 en 5 behandelen de tweede vraag door de voedselrelaties van KPV in de Waddenzee te onderzoeken. **Hoofdstuk 4** richt zich op KPV als predator en toonde aan dat haring en sprout opportunistisch eten, met duidelijke seizoensgebonden verschillen in dieet. Hierbij aten ze naast copepoden ook (larven van) bodemgebonden soorten. Zo dragen ze bij aan de energiestroom van

de bodem naar het open water en uiteindelijk naar hogere trofische niveaus. In **Hoofdstuk 5** hebben we KPV als prooi onderzocht en laten we zien dat de ruimtelijke verspreiding en foerageervoorkeuren van visdieven grotendeels overlappen met de verspreiding en afmetingen van KPV. Visdieven foerageerden vooral bij geulen, waar ze voornamelijk haring aten. Door het voedsellandschap in kaart te brengen, maakten we de eerste schattingen van de KPV-biomassa in de Waddenzee voor het voor- en najaar.

Hoofdstuk 6, de algemene discussie, plaatst de bevindingen van dit proefschrift in een bredere context en bespreekt de gevolgen voor het beheer van de Waddenzee. De ecologische flexibiliteit van KPV, zichtbaar in hun wijde verspreiding en aanpassingsvermogen, maakt duidelijk dat regio-specifiek onderzoek nodig is. Zo staan KPV algemeen bekend om het vormen van grote scholen en het eten van copepoden, maar in de ondiepe Waddenzee vormen ze juist kleinere schooltjes en eten ze ook bodemgebonden soorten. Hoewel jonge haring en sprot in uiterlijk veel op elkaar lijken, vertonen ze duidelijke ecologische verschillen, wat het belang van soortspecifieke identificatie onderstreept. Verder benadrukt dit hoofdstuk dat een volledig begrip van de rol van de Waddenzee voor KPV meer inzicht vereist in hun hele levenscyclus, inclusief vroege levensstadia en herkomst. Vergelijkende studies tussen de Waddenzee-regio's (Nederland, Duitsland, Denemarken) en de Noordzee zijn nodig om aantallen, groei, sterfte en de verbinding tussen populaties te onderzoeken. Dit zal ook het belang van de Nederlandse Waddenzee voor KPV tegenover de andere gebieden verduidelijken.

Het verbeteren van de omstandigheden voor migrerende pelagische vissen, die doorgaans niet gebonden zijn aan specifieke substraten of omgevingsomstandigheden, is moeilijk doordat er qua beheer maar beperkt mogelijkheden zijn om bij te sturen. Toch worden in het laatste hoofdstuk enkele praktische uitkomsten van dit proefschrift besproken. Zo raad ik aan om haringpaaigebieden beter in kaart te brengen en geef ik aanbevelingen voor het creëren van kunstmatige broedplaatsen voor visdieven. In mijn PhD-studie hebben we zowel nieuwe als traditionele methoden ingezet om KPV te onderzoeken, wat ons waardevolle inzichten heeft opgeleverd in zowel hun effectiviteit als beperkingen. Om KPV in de Waddenzee te monitoren, adviseer ik een jaarlijkse akoestische survey om langetermijntrends te volgen, gecombineerd met de plaatsing van een stationair echolood voor continue gegevensverzameling en maandelijkse vismonsters om seizoensgebonden veranderingen te volgen elke 2-3 jaar. Daarnaast benadruk ik het belang van studies die meerdere trofische niveaus onderzoeken, dus zowel prooi als predator, en het meten van abiotische factoren.

Kortom, dit proefschrift benadrukt de belangrijke rol van KPV in het ecosysteem en vestigt de aandacht op de grote aantallen van deze vaak onopgemerkte, maar voor het systeem belangrijke vissen in de troebele, ondiepe wateren van de Waddenzee.



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As a PhD student within Swimway, I was fortunate to be part of a larger group of PhDs and postdocs: **Hannah, Jena, Bass, Maryann, Bram** (yes, for me you were definitely part of this group and I'm happy we could share our WBAT experiences), **Hanna**, and more recently, **Mark**. The contact started off slowly— which wasn't surprising given that everyone was scattered across the world due to corona. But the Tuesday videocalls and later the in-person meetings at Swimway events and other symposia were always enjoyable. It was truly inspiring to see your work and all the exciting results. The writing retreat on Schier with **Hannah, Jena** and **Maryann**, was especially motivating and erg gezellig! The Swimway team was, of course, much larger than just the PhDs and postdocs. A huge thank you for the valuable and inspiring discussions and input from **Anieke, Allert, Erwin, Tom, Klemens**, and **Annabelle**. Ook dank aan **Michaela** voor het organiseren van de financiële kant van mijn project, ook een flinke uitdaging. En **Ingrid**, als wetenschappelijk coördinator en begeleider van meerdere PhD's lag er veel druk op jouw schouders. Hoewel het niet altijd makkelijk was, zorgde je er altijd voor dat alles zo soepel mogelijk verliep en hield je iedereen bij elkaar. Bedankt voor je geduld, organisatie en toewijding! En natuurlijk ook veel dank voor de ondersteuning en input vanuit de Waddenvereniging **Wouter, Belinda** en iedereen van de communicatie, en **Koen** voor het maken van de video's.

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About the author

Margot Maathuis was born on 20 March 1994 in Hengelo (Overijssel), the Netherlands. Though she grew up away from the coast, summer holidays in Zeeland and Texel, along with her passion for diving, fostered her interest in marine biology. In 2015, she obtained her BSc in Biology at the University of Amsterdam, where she continued her studies with a Master's in Limnology and Oceanography. During this time, she joined the NICO expedition, crossing the Atlantic aboard the RV Pelagia. For her two research projects, she studied the food web of Lake Markermeer at the University of Amsterdam and investigated the timing of flat oyster larvae release and their settlement preferences at Wageningen Marine Research in Yerseke. The latter project also marked her first encounter with small pelagic fish when she participated in a stow net survey in the Westerschelde. After completing her MSc in 2018, she worked at Bureau Waardenburg in the aquatic ecology team. In March 2020, she began her PhD at the chairgroup Aquaculture and Fisheries of Wageningen University and Research, and she was based at Wageningen Marine Research in IJmuiden. While finishing her PhD study, she embarked on a sailing journey, heading toward new horizons and exploring new seas and coasts.



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Scientific publications

Maathuis, M.A.M., Manche, P., Couperus, A.S., Parmentier, B., Poos, J.J., Poot, M., Schekkerman, H., Sakinan, S. & Tulp, I. (under review). The small pelagic fish food landscape and its use by common terns *Sterna hirundo* in the Wadden Sea. *Marine biology*.

Maathuis, M.A.M., Berg, F., Couperus, B., Poos, J.J., & Tulp, I. (2025). The function of the Wadden Sea in the life cycle of small pelagic fish. *Estuarine, Coastal and Shelf Science*, 109043.

Maathuis, M.A.M., Couperus, A.S., van der Molen, J., Poos, J.J., Tulp, I., & Sakinan, S. (2024). Resolving the variability in habitat use by juvenile small pelagic fish in a major tidal system by continuous echosounder measurements. *Marine Ecology Progress Series*, 741(SPF2), 169-187.

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- Tack, L.F., Vonk, J.A., van Riel, M.C., de Leeuw, J.J., Koopman, J., **Maathuis**, M.A.M., Schilder, K., van Hall, R.L., Huisman, J. & van der Geest, H. G. (2024). Food webs in isolation: The food-web structure of a freshwater reservoir with armoured shores in a former coastal bay area. *Science of the Total Environment*, 925, 171780.
- Brink, A.M., **Maathuis**, M.A.M., & Kamermans, P. (2020). Optimization of off-bottom spat collectors for restoration and production of the European flat oyster (*Ostrea edulis*) in Dutch coastal waters. *Aquatic Conservation*, 30(11).

WIAS training & supervision plan

With the activities listed, the PhD candidate has met the educational requirements set by the Graduate School of Wageningen Institute of Animal Sciences (WIAS). One ECTS equals a study load of 28 hours.



The basic package (1.7 ECTS)

- WIAS introduction day (2020)
- Scientific integrity course (2020)
- Ethics in Animal Science (2023)

Disciplinary competences (19.2 ECTS)

- Writing research proposal (2020)
- Laboratory animal science: design and ethics in animal experimentation (2020)
- Species specific laboratory animal course on fish (2020)
- Advanced molecular ecology (2021)
- Echoview software: fundamentals (2021)
- Echoview software: target detection and tracking (2022)
- Data exploration, regression, GLS & GAM with introduction to R (2023)

Professional competences (3.2 ECTS)

- Reviewing a scientific manuscript (2020)
- Critical thinking and argumentation (2020)
- Effective behaviour in your professional surroundings (2022)
- Scientific publishing (2023)
- Supervising BSc and MSc students (2023)
- The final touch (2024)

Societal relevance (0.6 ECTS)

- Science communication (2022-2024)

Presentation skills (4 ECTS)

- ICES Small Pelagic Fish Symposium, Lisbon, Portugal (2022)
- Noordzeedagen, Texel, the Netherlands (2023)
- British Ecological Society Annual Meeting, Belfast, Ireland (2023)
- Swimway Wadden Sea Conference, Groningen, the Netherlands (2024)

Teaching competences (5 ECTS)

- Supervising MSc thesis on DNA metabarcoding of diet herring and sprat
- Supervising BSc thesis on smelt otoliths
- Supervising MSc internship on energy density of small pelagic fish
- Lecturing BSc course Aquaculture & Fisheries

Total credits: 33.7 ECTS

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