Universität Rostock

Traditio et Innovatio

Trophic lengthening and its impact on the functional biodiversity of estuarine and brackish ecosystems

Dissertation

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität Rostock

vorgelegt an der

Mathematisch-Naturwissenschaftliche Fakultät

Institut für Biowissenschaften, Universität Rostock

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Geb. 05.10.1996 in Nordhausen

Rostock 2024

https://doi.org/10.18453/rosdok_id00005054

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Jahr der Einreichung: 2024

Jahr der Verteidigung: 2025

Table of Contents

Summary

Ecosystems around the world are under anthropogenic stress. Estuarine systems are particularly affected, as they are important shipping routes, economic centers, recreational areas, fishing grounds and collection basins for eutrophication. This often results in symptoms of change, the worst consequence of which is the collapse of apex- and mesopredators, which are often also of economic or species conservation importance. In addition to the already known causes, changes in functional diversity, at the heart of which are food webs and the associated productivity of ecosystems, came into focus for the loss of species. This dissertation deals with one of the most serious changes in food webs, the addition of further trophic levels, in the following called trophic lengthening. This leads to less mass and energy reaching the higher levels of the food web than some predators need to keep their population stable. Until now, these lengthening up to mesopredators were only theoretical constructs, but in this dissertation they were empirically demonstrated for the first time in a cross-system comparison of three estuarine ecosystems, the Elbe and Oder estuaries and the Baltic Sea. Large seasonal sampling campaigns were carried out covering the entire food webs with samples being analyzed using compound-specific isotope analysis of amino acids. The identified trophic positions in combination with other proxy variables and abiotic factors provided a clear picture of the trophic structures of the food webs of these three ecosystems and their environmental regulation. Trophic lengthening was recently found in the pelagic food web of the central Baltic Sea, triggered by massive filamentous, nitrogen-fixing cyanobacteria blooms, which could not be directly consumed by zooplankton. Interestingly, the lengthening was associated with a change in the dominant nitrogen source for primary production from nitrate to N_2. The microbial assemblage became more influential as flagellates and ciliates formed another trophic level between phytoplankton and mesozooplankton. My work showed that this extension was sustainable enough to lengthen the food web up to pelagic cod while benthic flounders were unaffected. In the Elbe estuary, earlier studies showed that the deepening of the harbor area together with the enormous turbidity formed the basis for heterotrophic processes, while at the same time a change in the dominant nitrogen source for production from nitrate to ammonium takes place between spring and summer. My data show that the heterotrophic area was accompanied by mixotrophic tendencies in the phytoplankton pointing to an intensified influence of the microbial food web in the harbor area. Interestingly, higher trophic positions in smelt and other fish were linked to this sensitive nursery area for smelt, which

suggests site-specific trophic lengthening up to pelagic mesopredators in the Elbe. Notably, the historic dietary connection between smelt and terns could not be confirmed in 2021. Despite eutrophic conditions and changes in the primary nitrogen source between spring and summer, no trophic lengthening was observed in the Odra estuary. Together the results from the three estuaries suggest a general mechanism leading to trophic lengthening in aquatic food webs up to apex- and mesopredators when a change in the nitrogen source for production is accompanied by an increased influence of the microbial system at the base of the food web. With the analysis of compound-specific nitrogen isotopes in amino acids and the calculated trophic positions, an important tool can be used to detect trophic lengthening before mesopredator population collapse is inevitable. This opens a time window for society to react with appropriate measures against loss of biodiversity.

1. Introduction

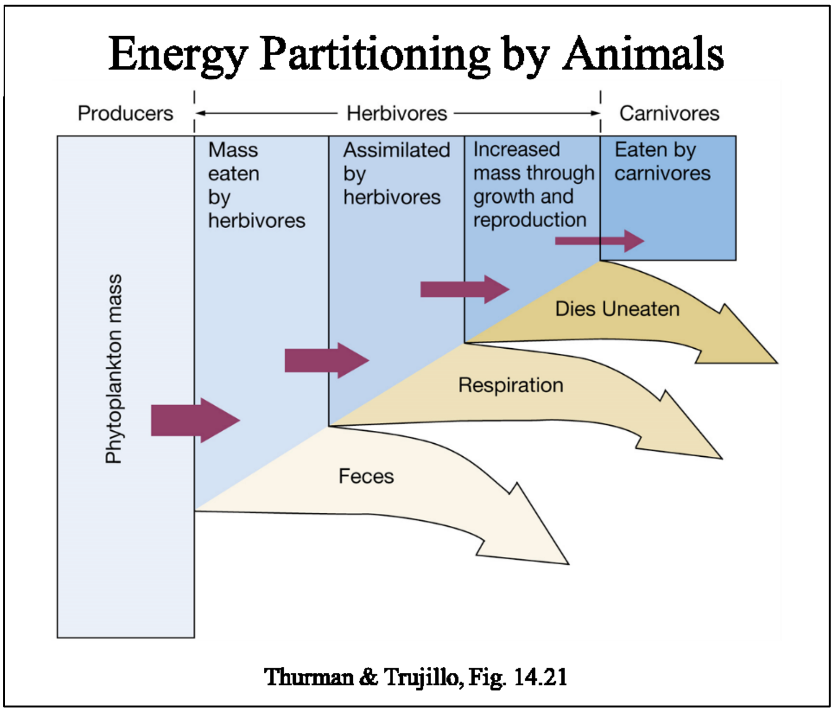
Loss of biodiversity is one of the largest threads to human society. It becomes tangible to anyone whose animals from childhood have diminished. The good news are that once we understand, which factors cause losses of biodiversity in an ecosystem, a society has the chance to take countermeasures. This dissertation was elaborated within the framework of the interdisciplinary BMBF BluEs project, which stands for "Developing estuaries as habitable sustainable ecosystem despite climate change and stress". The BluEs project aimed to provide a comprehensive understanding of the key stressors and critical biological processes that shape the estuaries of the Baltic and North Seas in terms of their potential responses to climate change and other human activities. These included chemical pollution, eutrophication and sediment redistribution. My share in this project was to investigate which environmental factors or anthropogenic stressors regulate the trophic structure of the pelagic and benthic food webs in these estuaries and to which changes in the food webs the collapse of key species such as smelt and Arctic tern in the Elbe estuary or cod in the Baltic Sea are related to.

1.1 Functional diversity and trophic structures of aquatic food webs

Species diminish primarily due to a very low productivity of their population, which calls for investigations of the functional diversity and trophic structures of their food webs as central aspects of their ecology. Functional diversity is described as the range and values of species and traits that influence an ecosystem (Tilman, 2001). Functional diversity is a part of biodiversity, but biodiversity encompasses much more, such as species community structure in an area, all genotypic and phenotypic variations as well as a spatial and temporal component (Tilman, 2001; Tilman et al., 1997). Functional diversity rather refers to the function of an organism in the ecosystem. The functions of the organisms are represented by so-called functional traits (Nock et al., 2016; Tilman, 2001). These represent characteristics of organisms that have an influence on their performance in the ecosystem, these can be physiological (such as photosynthesis or metabolic processes), morphological (egg size, body size, body mass, etc.) but also behavioral (such as hunting strategies, camouflage, escape tactics, etc.) (Nock et al., 2016). If several organisms have a large proportion of the same or similar traits, they can be summarized in functional groups (Tilman, 2001). This makes it possible, for example, to categorize organisms according to the type of energy assimilation, such as chemotrophic or phototrophic organisms (Chapin III et al., 1997; Tilman et al., 1997). But also according to their position in the food web and thus the function they fulfil there, such as autotrophs, herbivores or carnivores (Chapin III et al., 1997; Petchey et al., 2006; Tilman, 2001).

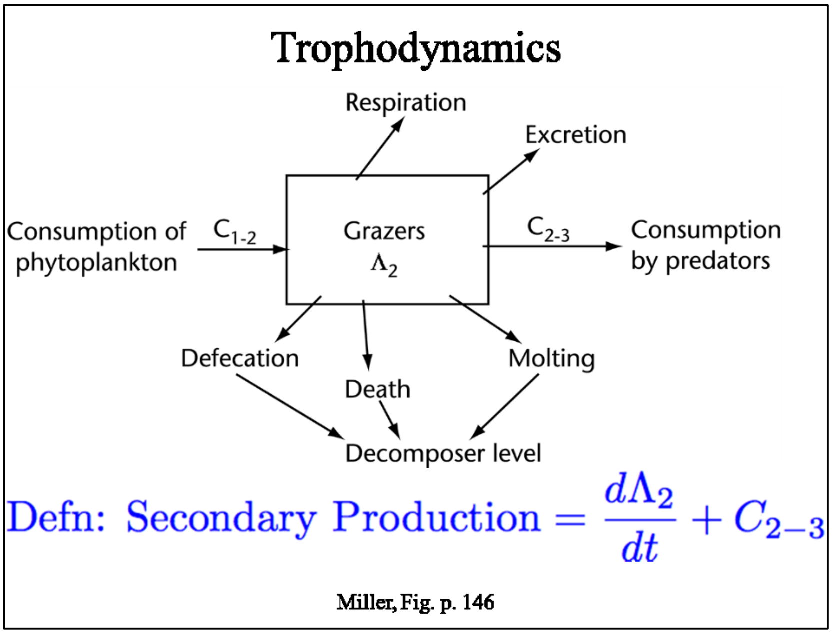
Understanding these individual traits and functional groups of organisms is a crucial point to assess the productivity of an aquatic ecosystem, as the productivity of an organisms is itself a very important functional trait (Danet et al., 2021; Pauly et al., 1995; Pauly et al., 1998; Woodson et al., 2018). The productivity of ecosystems is the rate at which energy and biomass is generated and is generally based on the efficiency of processes (assimilation and dissimilation) of its food webs and the associated efficiency of the transfer of energy between individual functional groups (Eddy et al., 2021; Ryther, 1969; Stock et al., 2017). If we stay with the categorization of traits based on the position of an organism in a food web, we can also speak of so-called trophic positions of an organism, which represent a part of the functional traits (Moosmann et al., 2021). In a food web comprising producers, herbivores and carnivores, all energy runs from the lowest position, the base, to the highest trophic position, the top (Fig. 1A). This process of passing mass and energy from level to level is called trophic transfer. Following the second law of thermodynamics, energy is lost within every trophic transfer, due to the conversion and subsequent release of thermal, chemical or kinetic energy within the involved processes and reactions like respiration, excretion, molting, deamination etc. (Fig. 1B, (Ryther, 1969).

1A



Thurman & Trujillo, Fig. 14.21

1B



Miller, Fig. p. 146

Figure 1A: shows that part of the chemical energy taken in as food by herbivores is excreted as feces and the rest is assimilated. Of the assimilated chemical energy, a large proportion is converted by respiration into kinetic energy to sustain life, and what remains is available for growth and reproduction. Thus, only about 10% of the food consumed by herbivores is available for the next trophic level. Source: Trujillo et al. (2012).

Figure 1B: The place of grazers in the tropho-dynamic scheme can be represented as follows: Secondary production is the rate of change in biomass of grazers, $\Delta\Lambda/\Delta t$, plus the proportion of the increase in biomass that is removed by predation, C2-3. Some would add the non-predatory loss terms of death and molting. Such a determined rate is appropriate for a system in steady state, or for the mean production rate in a seasonal cycle. The problem is to determine this rate for all grazers, or at least only for the dominant grazer. Source: Miller et al. (2012).

In the past, a transfer efficiency of around 10% was generally assumed for aquatic systems (Pauly et al., 1995; Ryther, 1969; Stock et al., 2017). However, it is now clear that this value may well be more variable and may even increase with increasing trophic position (Barneche et al., 2021; Barnes et al., 2010; Mehner et al., 2022). In most cases, however, this 10% transfer efficiency is taken as a standard value, as precise measurements are very difficult if not impossible at the moment (Barnes et al., 2010; Woodson et al., 2020; Woodson et al., 2018).

The energy level of an ecosystem is strongly dependent on its productivity. The distribution also follows the same pattern as that of energy. The highest productivity is concentrated at the base and decreases continuously towards the top (bottom-heavy) (Eddy et al., 2021; Ryther, 1969). Biomass distribution, on the other hand, can also follow other patterns in addition to the classic bottom-heavy distribution. For example, a small amount of producer biomass at the base can still be very productive and thus cause a top-heavy distribution. But as for energy, there are also biomass losses with each trophic transfer in the food web, for example through respiration of CO_2, excretion of NH_4^+, sloppy feeding or by egestion of indigestible food components like silicate or cellulose from diatoms or dinoflagellates that are released in fecal pellets (Figs. 1A, 1B, (Steinberg et al., 2017). This biomass partitioning is thought to result in the pyramid shape of biomass distribution, which is very wide at the base of the primary producers and tapers further with increasing trophic positions towards the top (Woodson et al., 2018). However, recent modeling studies suggest that the biomass of trophic pyramids in aquatic and marine food webs mostly are top-heavy with an increased biomass of certain meso- and apex predators and a small proportion of very productive producers are at the base. Such effects can also occur in combination and thus represent an hourglass form of biomass distribution (Bar-On et al., 2018; Bideault et al., 2021; Woodson et al., 2020; Woodson et al., 2018). Such hourglass forms are probably transitional forms that announce that a collapse is imminent and the upper part of the pyramid will break away, leaving a bottom-heavy pyramid with a large biomass of producers (Nagelkerken et al., 2020). Unfortunately, precise sampling of the biomass of whole aquatic food webs is hardly feasible in most ecosystems and changes in biomasses in a food web often become apparent only when fisheries or tourism are affected by decreasing catches or sights of key species like smelt and terns in the Elbe estuary or of cod in the Baltic Sea (Eero et al., 2020; Scholle et al., 2019).

These two points of energy transfer and productivity are extremely important for the stability of food webs or individual components of food webs (Stock et al., 2017). For example, if too little energy is available, entire branches of food webs can break away. If the producers are not productive enough, it can lead to the loss of organisms or branches in the food web, or if the top-down pressure from too much consumer biomass is too high (Casini et al., 2008; Stock et al., 2017; Woodson et al., 2020; Woodson et al., 2018). In aquatic ecosystems, for example, this can have a huge impact on fish stocks, which are of great interest to fisheries and therefore to humans, but also in terms of species conservation.

The factors causing shifts and changes in the energy and productivity and thus biomass distribution in food webs can be quite diverse but from the above described mechanism of energy flow through a food webs it becomes clear that a key factor is change in food web length, which I will call trophic lengthening in the following. Trophic lengthening means that the general length of a food web increases (Post et al., 2000). This can happen through the introduction of new species and thus also new traits or functional groups, or by changing the food preferences or nutrient sources of already established species (Glibert et al., 2016; Loick-Wilde et al., 2019; Lunau et al., 2013; Swalethorp et al., 2023). Longer food webs are in most cases much less productive (Lalli et al., 1997; Woodson et al., 2018) and it can lead to the organisms at the top levels no longer having enough energy available to maintain their population. Two general problems with trophic lengthening thus are: 1.) an additional level anywhere in the food web reduces the amount of energy and biomass transferred trophic positions above the new level (Ryther, 1969; Stock et al., 2017), and 2.) The robust identification of the trophic position of organisms in the field, which is the prerequisite to identify trophic lengthening per se (Post, 2002).

There have been many theories about the factors that determine the number of trophic levels in a food web. For example Post et al. (2000) hypothesized, that the length of a food web should generally depend on the size of an ecosystem and on the quality and quantity of resources made available. This means, that a smaller ecosystems can maintain only shorter food webs (e.g. three trophic levels like in upwelling systems), larger ecosystems can maintain longer food webs (e.g. six trophic levels like in the open ocean). However, stressors can also cause a lengthening to happen in other ways, that are not related to the given ecosystem size. A change in the nitrogen source, for example, can cause the dominant primary producer of a food web, thus its base, to change (Glibert et al., 2016; Lunau et al., 2013). This also happens, when nutrient ratios change, e.g. from high to low Si:N ratios or from low to high N:P ratios (Penuelas et al., 2020; R. E. Turner et al., 2003; Van Beusekom et al., 2019). In case of a switch from nitrate to ammonium as nitrogen source, the underlying reason is that for a cell it is energetically more advantageous to directly grow on NH_4^+ than on NO_3^- because no reduction is necessary before $NH4^+$ is fixed. Therefore, natural phytoplankton assemblages may compete for NH_4^+ as preferred nitrogen source. Different studies showed that small pico- and nanophytoplankton (0.2 to 20 µm) like dinoflagellates or flagellates may be more successful in utilizing the limited amount of NH_4^+ compared to larger microphytoplankton (20 to 200 µm) like diatoms that in contrast seems to benefit from growing on NO_3^- (Glibert et al., 2016; Wafar et al., 2004) (Fig. 2).

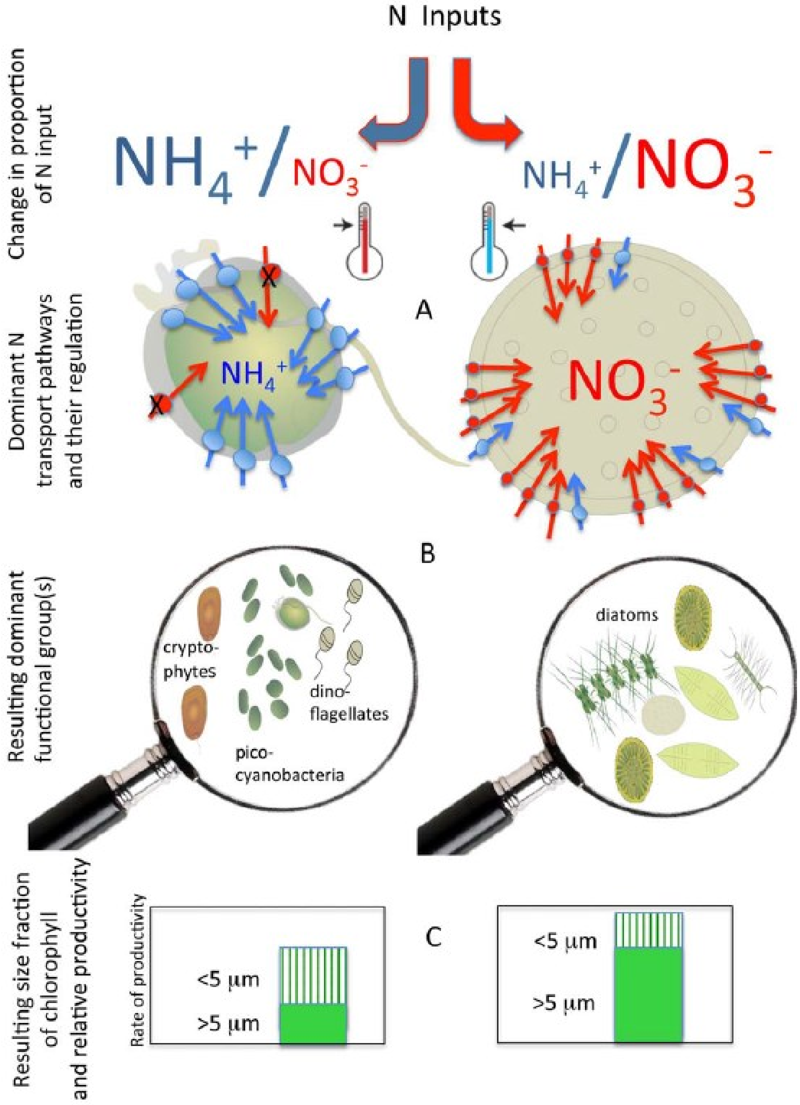


Figure 2: Summary conceptual schematic illustrating of the effect of changes in the proportion of NH4 and NO-3 in the loads of N provided to a natural system. When NH4 is the dominant form, and when waters are warmer, flagellates, cyanobacteria, and chlorophytes among other classes may proliferate, leading to overall productivity dominated by the small size class of algae (e.g.,<5 lm). In contrast, when NO-3 is the dominant form provided, especially under cooler water conditions, diatoms more likely dominate, and the overall production will be more likely dominated by cells of a larger size class (e.g.,>5 lm). Moreover, Chl a yield and total production may be higher than under the NH4 enrichment condition. Source: Glibert et al. (2016).

Thus, a change from a nitrate-driven system to an ammonium-driven system not only alters the composition of the dominant producer, e.g. from diatoms to dinoflagellates, but also the size of the dominant primary producer from large (e.g. >20 µm) to small (e.g. <20 µm). This change in size structure at the base of the food web can affect the entire structure of the subsequent food web, as many planktonic organisms feed size dependent and preferentially 10 times smaller than themselves (Hansen et al., 1997; Kiørboe, 2024;

Rohr et al., 2023). As the new dietary base becomes too small or too large for the established grazer of a food web, a different grazer will take over, which will have a cascading effect on the trophic position of subsequent carnivores as their dietary bases change too.

The microbial system plays an important role here, i.e. flagellates, ciliates, bacteria and also viruses, which can act both downwards on the phytoplankton and upwards on the zooplankton (Steinberg et al., 2017). If, for example, the dominating phytoplankton can no longer be grazed directly by the zooplankton, either because it is too small, too large or otherwise not utilizable, then the role of the microbial system, which takes the nutrients up and passes them on to the zooplankton, increases (Loick-Wilde et al., 2019; Weber et al., 2021). Their omnivory and heterotrophy are key points in the transfer of energy and nutrients (Yamaguchi et al., 2017), because these processes have proven to be more efficient for nutrient recycling. This can be an advantage if the dominant phytoplankton cannot be utilized. For example, phagotrophic (engulfing particulate food) diets of phytoflagellates can ensure a better supply of nutrients (Mitra et al., 2014; Steinberg et al., 2017). The trophic position of such organisms therefore goes beyond that of pure autotrophs (Stoecker et al., 2017). If the zooplankton (especially the mesozooplankton) feeds mainly on these mixotrophic organisms, its trophic position also increases. This effect can be sustainable enough to increase the trophic position of all organisms in the food web and trophic lengthening occurs. However, it has not yet been clarified, whether such a change in trophic position in the plankton compartment is always triggered by a change in nitrogen species or whether a change in TP is always accompanied by a clear reduction in the productivity or biomass of single populations or whole food webs. The latter would be typical for a biological regime shift, which refers to a change in the abundance and composition of organisms across several trophic levels (Dippner et al., 2010; Reid et al., 2001). A biological regime shift can occur smooth, abrupt or discontinuous and is often accompanied by a climatic regime shift (Dippner et al., 2010; Scheffer et al., 2001). A biological regime shift can therefore also result in a trophic lengthening if the changes in species composition and abundance also result in other functional groups or functional traits. For example, an increased proportion of species that have mixotrophic or omnivorous characteristics, as already explained (Steinberg et al., 2017).

In short, there are still many questions that need to be answered in order to better understand, on a large scale, the principles of functional diversity of an ecosystem and, on a small scale, the principles of trophic lengthening. The stressors that can trigger such an event are in many cases caused by humans, either directly or indirectly. Therefore, extremely human-influenced systems are best suited for such an investigation. Therefore, estuarine systems were chosen in this case.

1.2 Estuaries, anthropogenic stress and the Blue Estuaries Project

Estuaries form an important interface between terrestrial/limnic and marine systems. They are generally classified as semi enclosed coastal bodies, with a connection to the open sea, where freshwater rivers and streams meet saltwater streams (Hagy et al., 2013; Pritchard, 1967). This definition is very general and there are various clarifications and variations of the definition (Elliott et al., 2002; Pethick, 1993; Telesh, 2004). Different types of estuaries can also be defined, for example based on morphology, physicochemical conditions or biological parameters (Paturej, 2008). In most cases, estuaries are characterized by the tidal currents, which can vary in strength, so that either the saltwater influence or the freshwater influence is stronger (positive or negative estuaries) (Paturej, 2008; Pritchard, 1952). The degree of mixing of saline and limnic water bodies can also have an influence on the estuarine ecosystems, which are referred to as well-mixed estuaries or stratified estuaries, or an intermediate form. However, salinity and tide do not always have to play a decisive role, as there are also estuarine systems that are strongly characterized by non-tidal limnic waters (lagoons, sounds, etc.) (Paturej, 2008; Pritchard, 1952). Another characterization is the degree of turbidity and therefore light limitation (euphotic or aphotic) (Hagy et al., 2013).

These characteristics of a transitional water body where different water masses (saltwater and freshwater) meet results in a combination of different processes, as organic and inorganic material and nutrients such as nitrate, phosphate or silicate from rivers enter the sea via the estuary (Day Jr et al., 2012). The estuary can serve as a sink for nitrogen and other nutrients, in which various processes such as nitrification, denitrification and re-mineralization take place (Fig 3). Nutrients can also enter the estuarine sediments through sedimentation and only gradually be released again (Day Jr et al., 2012).

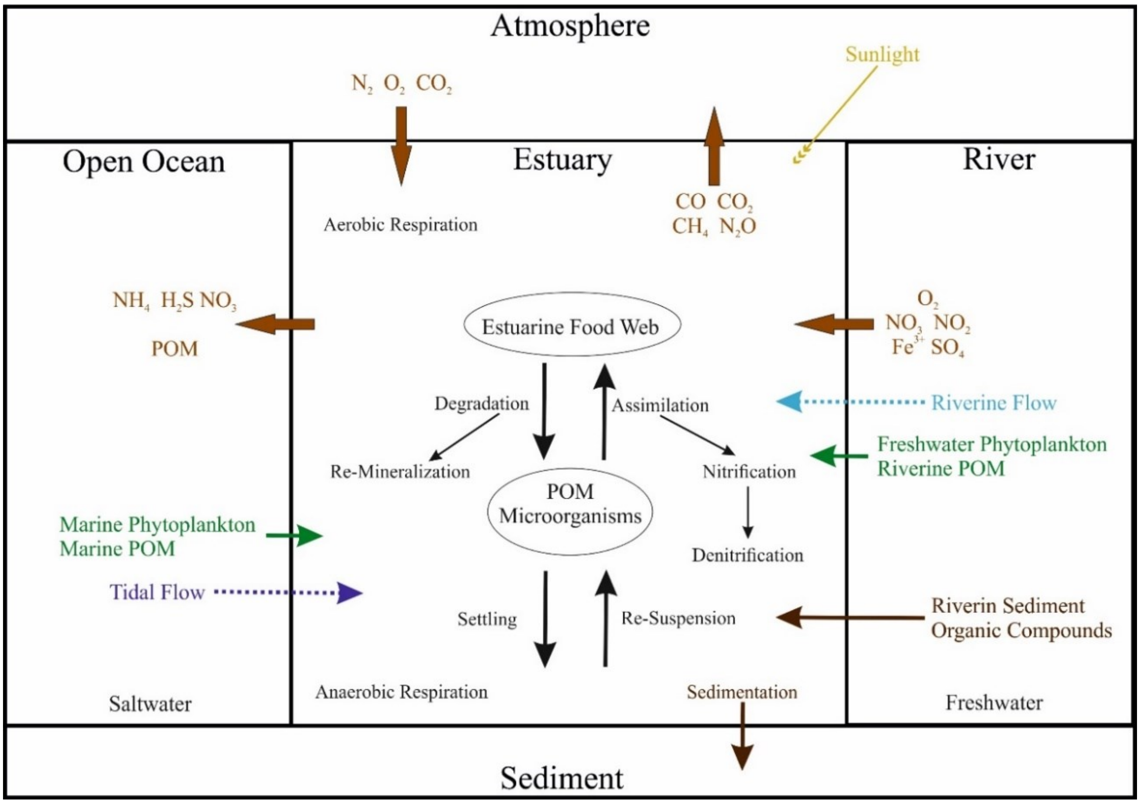


Figure 3: Overview of general substance and material flows in an estuary together with the most important processes in the system and in exchange with the limnic and marine systems bordering the estuary (green = phytoplankton, dark brown = sediment processes, light brown = material flows, blue = water mass flows).

So-called maximum turbidity zones (MTZ) also play a central role in many micro- and macro tidal estuaries (Hagy et al., 2013). This is caused by a strong density gradient between freshwater on the river side and saltwater on the sea side, which leads to a near-bottom flow pattern in combination with a tidal asymmetry of the ebb and flood currents or strong tidal pumping or tidal wave events. The structure and individual morphology of an estuary can also contribute to the size of the MTZ (Bianchi, 2007; Brenon et al., 1999; Jalón-Rojas et al., 2016; Postma, 1967). A lot of SPM is released from the sediment and transported into the water column, which is further distributed by strong vertical mixing (Uncles et al., 1992). This leads to very high turbidity levels in these transitional regions of the estuaries which also play a role in biochemical processes and particle bound reactions and transport of nutrients and pollutants (Etcheber et al., 2007; Jalón-Rojas et al., 2016; Turner et al., 2002). This often leads to changes in the assimilation and dissimilation processes of the food webs in this area. As already mentioned, estuaries can have phototrophic areas in which autotrophic phytoplankton (e.g. diatoms) predominate (Martens et al., 2024) and take over the majority of autotrophic production, which is why the food web is mainly based on freshly synthesized compounds (McCarthy et al., 2007), which are qualitatively better for the upper part of the food web and easier to utilize, resulting in a better transfer efficiency (≤ 10 %).

In contrast, in the highly turbid areas of the MTZ and generally very turbid zones of the estuary, where phototrophy is limited by light, there is often a large proportion of heterotrophic processes that can take place without sunlight (Dodds et al., 2007; Kamjunke et al., 2021). Here processes such as nitrification and denitrification (Fig. 3) and increased uptake of detritus from the microbial food web predominates and degraded detritus rather than newly synthesized organic matter forms the base of the benthic and pelagic food webs (Middelburg et al., 2000). This is critical for food webs because, food stoichiometry determines how efficient food is incorporated by a consumer. Detritus has a high C:N ratio e.g. >10 and is an especially poor dietary source, which leads to increasing respiration and decreasing nitrogen specific net growth efficiency in e.g. copepods (Lalli et al., 1997; Valiela et al., 1995).

But it is not only turbidity and the associated MTZ that can act as a stressor on an estuarine food web. Estuaries are often strongly influenced by humans, as they provide access from the sea to the inland and thus represent important shipping routes. Thus, multiple stressors act on the ecosystem and food webs (Kennish et al., 2014) (Fig. 4).

Figure 4: Overview of a classic estuary and the most important anthropogenic and natural stressors that can affect the ecosystem (Kennish, 2002; Kennish et al., 2014; Kennish et al., 2023).

For example, there are mechanically induced stressors like steadily advancing development of coastal areas, compensation areas and floodplains as well as draining of areas and straightening of river regions. But channel deepening and dredging of harbor areas also have an impact on the estuary. This is mainly due to increased currents and tidal ranges, turbidity (Kennish, 2002; Kennish et al., 2014; Zaikowski et al., 2008), an increase in the MTZ and the release of pollutants through dredged sediments (Rodgers et al., 2020). The introduction of nutrients from the agricultural industry and the associated eutrophication continues to be a major problem, despite the guideline values for nutrient loads for example stipulated by the Water Framework Directive (Chave, 2001). Other stressors include the increasing shipping traffic in the waterways and harbor areas of the estuaries, as well as fishing or general decline of fish stocks (Whitfield, 2021). This leads to problems for organisms in the upper level of the food web due to both the actual shipping traffic, overfishing and bycatch. Last but not least, climate change is a devastating problem, as water temperatures are rising and the frequency of extreme events such as strong storm surges and heat waves is increasing (Kennish et al., 2023).

This dissertation focuses on the influence of stressors on estuarine food webs and unravels if trophic lengthening can be identified and connected to stressors that affect functional traits namely productivity. Three very different types of estuaries were chosen to quantify and compare the range of stressors and their effects on the nitrogen supply and trophic lengthening in their food webs in order to identify a common mechanism leading to trophic lengthening in estuaries. The three investigated estuaries are briefly presented and categorized below.

The Elbe estuary

The Elbe is one of the largest rivers in Germany with a length of 1091 km of which 719 km run through Germany. According to the definitions of Pritchard (1952) and Paturej (2008), the tidal Elbe can be regarded as a positive estuary in terms of saltwater conditions with a mixohaline distribution. In terms of morphology, it is a well-mixed estuary, but this can change temporarily when the tides are turning and stratification can occur at times (Carstens et al., 2004). The estuary of the Elbe and thus the tide influenced area, but begins at the weir in Geesthacht, followed by the port of Hamburg, which is currently the largest port in Germany and the third largest in Europe (handling 8.5 million 20-foot containers per year (Hamburgerhafen, 2020), which leads to a considerable frequency of freight traffic via ships. The Elbe estuary is characterized by salinity fluctuations, strong tides, strong turbidity and by ongoing dredging of the navigational channel and maintenance dredging, which creates a depth discrepancy between the shallow upstream area and the depended harbor and fairway area of the estuary (Pein et al., 2019; Pein et al., 2023; Sohrt et al., 2021; Weilbeer et al., 2021; Winterwerp et al., 2013). The Elbe transports around 82 kt of nitrogen in the form of nitrate into the estuary every year (Johannsen et al. 2008). The port region is a hotspot for nitrification, which further increases the amount of nitrate that reaches the North Sea via the estuary (Dähnke et al., 2022; Sanders et al., 2018). In the deepened zones in the harbor, however, there is a change in the nitrogen pattern, as the phytoplankton sinks, light is limited and dies off. The ammonium concentrations in this region rise, as do ammonium assimilation processes. Strong heterotrophic processes also occur in this region as also in the maximum turbidity zone downstream (Dähnke et al., 2022; Kamjunke et al., 2023).

The Oder Estuary

The Oder up to Swinemünde has a length of 898 km and a total catchment area of 122,511 km^2, which transports $16872*10^6$ m^3 of water per year into the estuary. This water also transports 70 kt of nitrogen into the estuary every year (Korth et al., 2013; Voss et al., 2005). Its estuary, the Szczecin Lagoon, is an oligohaline estuary with only a small connection to the Baltic Sea. However, it is a special case in the classifications, as it is also a non-tidal estuary and therefore lacks two of the major factors that characterize a "classic" estuary (Hagy et al., 2013; Paturej, 2008). The Szczecin Lagoon is a shallow area and is separated from the large lagoon on the eastern side by a dredged shipping channel. This channel also provides the largest proportion of flow through the lagoon. The lagoon is well mixed because of very shallow water depth and wind-induced currents, which is why there is hardly any stratification of the water masses (Schernewski et al., 2001; Wolnomiejski et al., 2013). The Great Lagoon is not discussed further in this study, as no sampling could be carried out there. A smaller proportion of the water that passes through the lagoon flows through the Achterwasser into the Greifswald Bodden, a partially enclosed area with somewhat deeper regions. From the Szczecin Lagoon and the Greifswald Bodden there are access points to the Oder Bight. The Oder Bight is the connection between the estuary and the Baltic Sea via the Oder Bank. Despite strong measures to reduce nutrient inputs, the area of the Szczecin Lagoon remains highly eutrophic (McQuatters-Gollop et al., 2009). However, a relatively high retention time of the water in the lagoon comes into play here, which ensures that a large proportion of the nitrate is assimilated by the phototrophic phytoplankton, especially in spring (Hellemann et al., 2017). This in turn promotes the sedimentation of material at the bottom and substrate-bound nitrification and denitrification, which means that a large proportion of the nitrate transported by the river can be removed in the lagoon before it reaches the Baltic Sea (Asmala et al., 2017). This creates the character of a coastal filter that most of the lagoon regions on the coast of the Baltic Sea have (Carstensen et al., 2020). Nevertheless, the processes in the sediments release nutrients and at the same time deplete oxygen, sometimes exceeding the rates of oxygen production, which would mean that the system could have a propensity for anoxic conditions if the shallow water column in the Szczecin Lagoon were not continuously mixed (Schernewski et al., 2001; Kache et al., sub).

The Baltic Sea

The Baltic Sea itself can be seen as an inland sea of 393 000 km^2, with a mean depth of a 54 m and with little exchange to the North Sea and thus the Atlantic. However, it also has some of the characteristics of estuarine systems, because with have regions with high salinity influence and regions with near freshwater character (Snoeijs-Leijonmalm et al., 2017). It is divided into several basins, mostly separated by shallow sounds or sills (Elmgren et al., 2015). The Central Baltic Sea is permanently stratified, with an upper layer of low salinity water (6-8 PSU) and a more saline deep water layer (10-14 PSU) separated by a strong halocline. Strong environmental gradients create a variety of habitats with different biota in different sub-basins (Szymczycha et al., 2019). However, this stratification favors the spread of oxygen minimum zones, particularly in deep basins such as the Gotland and Bornholm Basins (Carstensen et al., 2014). The western Baltic Sea and Belt Sea are shallower than 30 m. Wind-driven hydrodynamics interact with changes in the two-layer exchange flow of more saline bottom water from the Kattegat and surface outflow from the central Baltic Sea through the Danish Straits. Salinities are relatively high compared to the central Baltic Sea. Its special morphology and characteristics make the Baltic Sea a special ecosystem. The Baltic Sea is characterized by a high variability of nitrogen processing processes. The western Baltic Sea is dominated by diatoms (Neumann, 2010; Wasmund et al., 2011), which take up the nitrogen entering the Baltic Sea from terrestrial sources namely in the form of nitrate (Kuliński et al., 2022; Reckermann et al., 2022; Voss et al., 2005). In the central Baltic Sea, massive cyanobacterial blooms dominate, especially in the summer months (Kahru et al., 2020; Karlson et al., 2015; Wasmund, 1997). These fix nitrogen from the air for growth. This dichotomy in new N-sources entering the Baltic Sea also leads to isotope mixing patterns, as ^{15}N-enriched nitrate dominates the Western Baltic Sea and coastal areas, while ^{14}N-enriched nitrate dominates in the open Baltic Sea (Korth et al., 2014; Voss et al., 2005).

1.3 Traditional and new biogeochemical proxies to characterize an estuarine food web

There are several approaches to understand the flow of mass and energy or the mere position of organisms in aquatic food webs in estuarine systems such as those mentioned above. One of these include stomach analyses of various organisms in order to determine the trophic position on the basis of the prey organisms. The disadvantage of this method is that it can only cover very short periods of time and there is a certain potential for error, as some components are quickly digested and can therefore over-represent other prey organisms (da Silveira et al., 2020). Another approach includes metabarcoding and DNA analyses of food components or fecal examinations (Hardy et al., 2017; Siegenthaler et al., 2019; Whitaker et al., 2019). This approach is somewhat more long-term and more qualitative, but it cannot directly indicate trophic positions of organisms (da Silveira et al., 2020). Both approaches also have the disadvantage that nitrogen sources cannot be identified and thus the flow of different nitrogen species in the food web and the associated benefits cannot be resolved. For these reasons it was decided for this dissertation to follow a third, stable isotope-based approach to unravel changes in the trophic structure and nitrogen supply of food webs, which includes the analyses of stable nitrogen and carbon isotopes in bulk samples as well as to carry out a compound-specific stable nitrogen isotope analysis of the amino acids in this tissue. With the help of the ratio of the stable nitrogen isotopes ^{15}N and ^{14}N, the $\delta^{15}N$ value, the path of nitrogen in the food web can be traced by analyzing organism's tissue. The first calculations of the position of an organism in the food web, the TP, could already be made on the basis of analyses of total nitrogen or bulk nitrogen (Post, 2002). This required a measured value of the base of the food web, one of the target organism and a factor that represents the fractionation of the nitrogen in the system (Post, 2002). Following this approach, the trophic structure, the nitrogen sources as well as the carbon sources for numerous ecosystem has been unraveled (Besser et al., 2022; Chikaraishi et al., 2010; Steffan et al., 2015). A challenge namely in aquatic ecosystems is to find the correct source value at the base in order to calculate the TP. With the advent of component specific analysis of nitrogen isotopes in amino acids, CISA-AA, this became easier (McClelland et al., 2002). Within the amino acids, a distinction is made between the source amino acids, the trophic amino acids and the metabolic amino acids. Source amino acids are very little fractionated and discriminated within the reactions in the organisms, which is why their $\delta^{15}N$ value quite accurately reflects the $\delta^{15}N$ value of the primary producers at the base of the food web and can therefore be used as an N-source proxy. These include phenylalanine, serine, lysine and tyrosine and glycine. In contrast to these are the trophic amino acids glutamic acid, alanine, arginine, valine, leucine, isoleucine, proline and aspartic acid, some of which are strongly fractionated within the organisms and are enriched with a maximum of 8‰ per trophic transfer (Chikaraishi et al., 2009; McMahon et al., 2016). Finally, there are the metabolic amino acids, such as threonine, for which it is not yet clear exactly which pattern they show (Mompeán et al., 2016). With the help of the trophic AA, source AA and an organism specific trophic discrimination factor (TDF), the flow of nitrogen and thus the position of an organism in the food web can now be measured empirically using only the target organism (Bradley et al., 2015; Karlson et al., 2024; Loick-Wilde et al., 2019; McMahon et al., 2015). With the help of the calculated TPs, the structure of the food web can be resolved and thus also the flow of energy from its base to the top. However, it is important to note that CSIA-AA analyses can never considered alone ("Fretwells Law" in Kendall et al. (1998)), and should always be accompanied by other research, such as stomach content analyses (SCA), nutrient analyses, population analyses and others to not led to misinterpretations. However, CSIA-AA can play a central role in representing the functional biodiversity of an ecosystem and thus also in understanding when trophic lengthening can occur and how these events change the ecosystem. To clarify how different stressors affect the food web, three estuaries or brackish water systems were selected in this study that have different characteristics and are therefore well suited for comparison.

In order to better understand the complex states and processes in aquatic food webs and ecosystems, further proxy variables were used soon after the introduction of stable isotope analyses in ecology. These are largely based on the isotope ratios, but also on the concentrations of nitrogen and carbon in the measured sample.

➔ Two different proxies can be used in aquatic food webs to determine whether a phytoplankton or zooplankton sample is contaminated with the other sample type or whether degraded organic material or fecal pellets are present. One is the C to N ratio, which can be used to determine how far a sample deviates from the Redfield ratio of 6.6, for example. If the samples have higher C:N ratios, it is more likely that they are phytoplankton (Steinberg et al., 2008). This proxy can be combined with the $\Delta\delta$ - values of the $\delta^{15}N$ values of Ala and Thr normalized by the sources AA value of Phe. A distinction can be made between zooplankton and phytoplankton or fecal pellets and microbial degraded organic material, as Thr as a metabolic amino acid shows relatively low $\delta^{15}N$ values in processes within animal organisms. Several studies have shown that animal and plant organisms and their waste products can be distinguished in this way (Doherty et al., 2021; McMahon et al., 2016; Mompeán et al., 2016).

➔ Carbon sources (for example in phytoplankton) in aquatic ecosystems can be tracked via the $\delta^{13}C$ values in a sample. In addition, in transitional waters with a gradient in salinity, the $\delta^{13}C$ values of a sample can be used as a proxy for the mean salinity in which the organism resided during the turnover period (Fry, 2002; Kerr et al., 2007).

➔ Especially in estuarine systems under high stress due to turbidity, where autotrophic phytoplankton is at a disadvantage, heterotrophic processes can occur. In order to be able to track and show these processes, another proxy is used that is based on the scattering behavior of the trophic amino acids (McCarthy et al., 2007). It is called ΣV and can be used to detect the uptake of freshly synthesized amino acids or to show bacterial or eukaryotic heterotrophy and re-synthesis of amino acids.

➔ Finally, another proxy factor can be used for the detection of heterotrophic processes. This is based on the nitrogen isotopic fractionation among amino acids during de novo synthesis. This proxy can then be used to distinguish de novo synthesized sources from autotrophic assimilated sources in a sample (Yamaguchi et al., 2017).

1.4 Hypotheses

Due to their different characteristics but similar stressors, the three estuaries and brackish water systems Elbe Estuary, Oder Estuary and Baltic Sea are ideal for clarifying the processes behind any changes in functional biodiversity and how trophic lengthening can influence the mass and energy flows in estuarine food webs. Based on that I want to clarify the following hypotheses in the course of this dissertation:

I. In the Elbe estuary, heterotrophic processes and multiple stressors lead to quantifiable changes in the trophic structure of the pelagic and benthic food webs up to the key species smelt and terns, while the food webs in the Oder estuary are still undisturbed.

II. In the Baltic Sea estuary, trophic lengthening in the pelagic but not the benthic food web is based on long chain-forming, nitrogen-fixing cyanobacteria in the central Baltic Sea and is sustainable enough to affect cod from the central Baltic Sea.

III. Changes in the quality of nitrogen for primary production, if associated with a change in the importance of the microbial system at the base of the food web, is a common mechanism for trophic lengthening in estuarine food webs.

Clarifying these hypotheses can be a crucial step towards a better understanding of functional diversity and productivity of aquatic and in particular estuarine ecosystems. This is necessary in order to ultimately enable the protection of such systems, but also to optimize their use by humans and make them sustainable.

2. Materials and Methods

In order to test my hypotheses, a large sampling campaign was carried out within the BluEs project during which a number of different organisms were sampled in the respective estuarine food webs and subsequently analyzed using stable isotope analyses (Tab. S1). In the course of the project, interdisciplinary co-operations were also used to gain further in-depth knowledge about estuarine ecology.

2.1 Study Areas

Within the framework of the BluEs Project, comparative sampling for this dissertation took place in three estuaries under high anthropogenic and climate stress to identify trophic lengthening in their food webs and common environmental factors regulating it. The three study areas included: 1. the Elbe estuary, 2. the Oder estuary, and 3. the brackish Baltic Sea. As described above, they were selected because they have three very different ecosystems with different biotic and abiotic factors (Tab. 1).

Table 1: The most important abiotic and biotic characteristics of the three sampled areas Oder and Elbe estuary and the Baltic Sea.

Factor Type	**Factor**	**Oder Estuary**	**Reference**	**Elbe Estuary**	**Reference**	**Baltic Sea**	**Reference**
Abiotic (Phys)	salinity gradient [‰]	3.2 - 7.9	Korth et al. (2013)	0.5 - 31.4	Dähnke et al. (2008)	2-13 (Central Baltic)	Kniebusch et al. (2019)
	tidal Range [m]			3.66	Hamburgerhafen (2020)		
	catchment area [km2]	118,840	Korth et al. (2013)	148.268	Boehlich et al. (2008)	1.7 x 106	Nehring et al. (2003)
	maximum Depth	~3.8 m, channel: 10.5 m	Radziejewska et al. (2008)	27.5 m, channel: ~16 m	Mahavadi et al. (2024)	459 m	Kautsky et al. (2000)
Abiotic (Chem)	nitrate δ^{15}N [‰]	7-14.9	Korth et al. (2013)	8.2-16.2	Dähnke et al. (2008)	3.6 ± 1.0	Korth et al. (2014)
	N catchment per year	60 - 70 kt	Voss et al. (2006) Korth et al. (2013)	82 kt	Johannsen et al. (2008)	830 kt nitrate and 926 kt fixation	Voss et al. (2005)
	mean C:N ratio	6.9 ± 0.7	Korth et al. (2013)	7.5 - 8.7	Deutsch et al., (2009)	6.9 ± 1,2	Naumann et al. (2024)
Biotic	key fish species	flounder and ruffe	Winkler (2004)	smelt, twaite shed and herring	Thiel et al. (2001); Thiel et al. (1995)	cod and herring	Barz et al. (2024)
	chlorophyll Conc.	18- 25.8 µg l	Deutsch et al., (2009)	100-180 µg l	Dähnke et al. (2008)	12.9 mg l	Wasmund et al. (2018)
	POC Conc.	60 - 120 µmol l	Deutsch et al., (2009)	190 µmol l	Dähnke et al. (2008)	22.2 µmol l	Naumann et al. (2024)

2.2 Seasonal sampling campaigns in the Elbe and Oder estuaries

Within the Blue Estuaries project, several sampling campaigns relevant for this thesis were carried out in the Elbe and Oder estuaries during spring, summer, fall and winter in 2021 (Tab. S1).

2.2.1 Sampling in the Elbe estuary

Sampling in the Elbe estuary took place at a total of 5 stations, starting with the Mühlenberger Loch (ML), Schwarztonnensand (ST), Brunsbüttel (BB), Medemgrund (MG) and the island of Neuwerk (NW) (Fig. 5), water samples, mesozooplankton samples and isolated fish samples were taken at the first three stations on board of the research vessel "Ludwig Prandtl" of the Helmholtz-Zentrum Hereon Geesthacht, using 100µm Apstein net (zooplankton) or 200µm round net (fish) and on board water pumps (water/POM samples). All other fish samples, in addition to those from Medemgrund, were caught by the fishing vessel "Ostetal" using stow net for both tides. After catch evaluation which involved species determination, length and weight measurements, the collected fish samples were directly frozen on board of the fishing vessel. Subsequently, the frozen specimens were stored at a temperature of -20 °C within dedicated freezers. These stations were sampled in May/June/July/ August 2021 and November 2021. On the Island of Neuwerk, feather samples of terns (*Sterna paradisaea* and *Sterna hirundo*) were collected during the breeding season (June 2021), which could be clearly assigned to an individual bird. This timing was chosen because to assure that the sampled birds had been feeding exclusively in the estuary region in recent months, or had provided their young with food exclusively from this region, in order to avoid any falsification of the data by other isotope signatures from regions that the birds visited on their long journeys.

Figure 5: Sampling stations for the Elbe sampling campaign. The stations were located in the Mühlenberger Loch (ML), near the island Schwarztonnensand (ST), Brunsbüttel (BB), Medem Grund (MG) and the island Neuwerk (NW).

2.2.2 Sampling in the Oder estuary

A total of 3 stations were sampled in the Oder estuary. The Szczecin Lagoon and the Greifswald Bodden were sampled either with the working boat "Klaashahn" or from land from the pier at Lubmin, depending on weather conditions (Fig. 6). Water samples were taken using hand scoops and zooplankton and fish larvae samples were caught using a 100 µm Apstein net or a 200 µm round net. In addition, sediment and benthos samples were taken from the top 10cm of the sediment using a Van Veen grab. On land, the water samples were filtered through 0.2 µm Wattmann$^{®}$ glass fibre filters for the EA-IRMS analyses and through 0.2 µm polycarbonate filters for the GC-C-IRMS analyses. The zooplankton samples were partially freed from contamination using a light trap. The benthic organisms were searched out of the sediment and determined at species level. All samples were then frozen at -20C.

The Oder Bight was sampled by the research vessel “Elisabeth Mann Borgese”. Water samples were taken using a crane sampler with an integrated CTD probe. The samples were then filtered through 0.2 µm GF or PC filters. The zooplankton samples were taken using a 100µm WP2 net through multiple hauls, all of which were then transferred to a light trap. The sediment and benthos samples were taken using a Van Veen grab, sorted over a sieve table and transferred to sample containers. All samples were then frozen at -20C.

Sampling in the Oder estuary took place in May, July/August, October 2021 and February 2022. The fish samples were acquired from local fishermen over this period and stored frozen.

Figure 6: Sampling stations for the sampling campaign in the Oder Estuary. The stations were located in the Szczecin Lagoon (SH), the Greifswalder Bodden (GB), the Oder Bight (OB) and the island Greifswalder Oie (GO).

2.3 Fish sampling in the Baltic Sea

The following subchapter and the later subchapter “2.6.1 Trophic Positions calculation for cod and flounder” are part of the manuscript "Trophic lengthening triggered by filamentous, N_2-fixing cyanobacteria disrupts pelagic but not benthic food webs in a large estuarine ecosystem" by M. Steinkopf, U. Krumme, D. Schulz-Bull, D. Wodarg and N. Loick-Wilde. This article is published in Ecology and Evolution, 2024, 14(2), e11048, DOI: 10.1002/ece3.11048. My own share to this work is 90%, which includes sampling conception, partial sampling, analysis of the fish samples in the laboratory, manuscript conception and preparation of text and images, which were then reviewed by the co-authors.

The fishes originated from four cruises to the western Baltic Sea (ICES subdivision (SD) 22) and central Baltic Sea (SD 24-25) in January/February 2019 and 2020 (Fig. 7, Table S2). In the management area SD 24-25 offshore spawning European flounder *Platichthys flesus* and the coastal spawning Baltic flounder *Platichthys solemdali*, a recently described new species (Momigliano et al., 2018); are present, with an estimated share (from survey data of 2014 and 2015) of approximately 85% and 15%, respectively (Bergenius-Nord et al., 2023). Presently, species assignment is only possible using genetics. Here, we assume that all flounder were *P. flesus*.

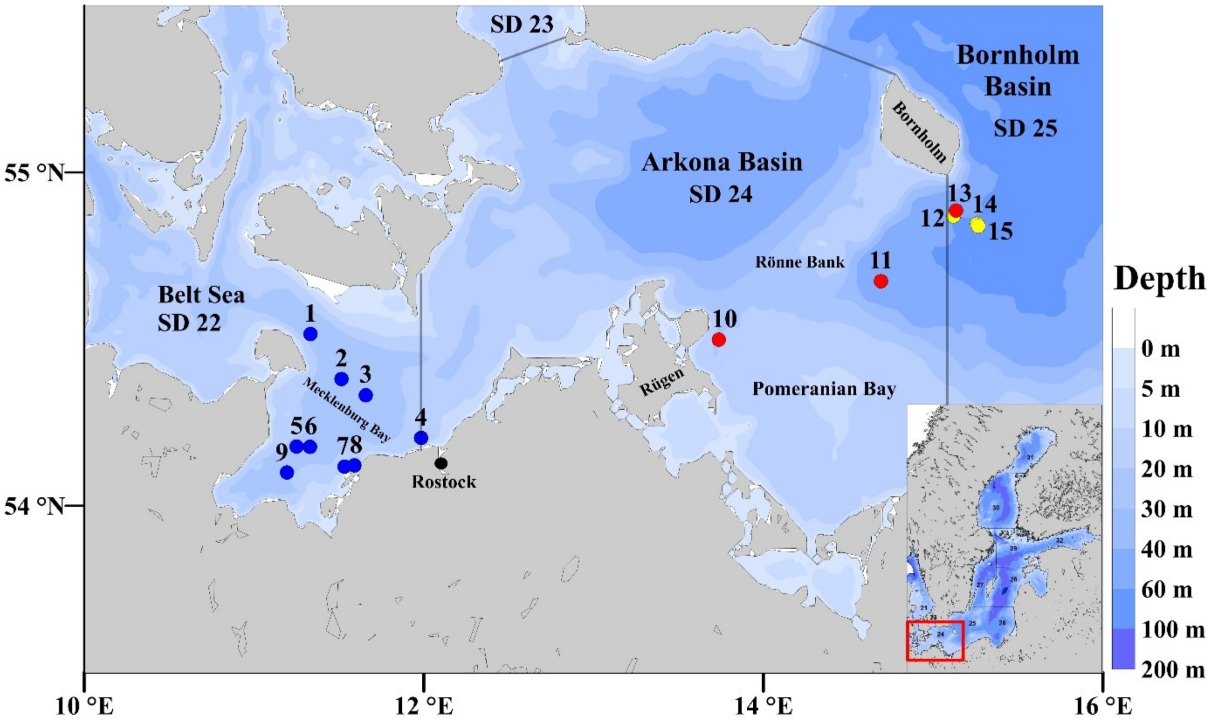


Figure 7: Sampling stations for Baltic cod and flounder in the western (blue) and central (red and yellow) Baltic Sea. Western Baltic Sea stations were located in the Mecklenburg Bay as part of the Belt Sea in 2020. Central Baltic Sea stations were located near Rügen and the south-western Bornholm Basins in 2019 (red) and 2020 (yellow). Inlay map shows the entire Baltic Sea. From (Steinkopf et al., 2024).

The fish were caught by bottom trawl, with a Bacoma cod end (2019) and a TV30 #520 trawl gear (2020) following the standards of the Baltic International Trawl Survey (ICES, 2017). On board, the fish were killed following German regulations, identified to species level, counted, and measured. The otoliths were extracted and a sample of white muscle tissue was taken from behind the third dorsal fin of 30 cod individuals and from the tail muscle of 21 flounder individuals. All samples were frozen immediately at −20°C for later stable isotope analyses. All fishes were sampled within a certain size range (cod: 20-40 cm, flounder 20-30 cm), to ensure comparability between the two areas and be sure that there is no major length or age effect in the trophic position calculations.

Cod and flounder otoliths were embedded, sliced and aged using the standard procedures for the two species in the Baltic Sea region. Age determination revealed an age range for cod (median length 30.5 ± 5.0 cm) of 1-4 years (median: 2 years) and for flounder (median length 25.0 ± 5.0 cm) from 2-4 years (median: 3 years), respectively (Table S2 from Steinkopf et al. (2024)).
Table S1 comprises the different variables and samples collected during the different sampling campaigns in each ecosystem used in this dissertation.

2.4 Samples Preparation and Derivatization

The frozen samples were either cleaned mechanically and with distilled water to remove surface contaminants, freeze dried (Christ Alpha 1-4), and then ground and homogenized for further processing or dried at 60˚C without further processing (Filters). For compound-specific stable nitrogen isotope analyses of amino acids (CSIA), ~10 - 20 mg of each dried sample was transferred into a heat-resistant borosilicate vial, mixed with 5 ml of 6M HCl solution and 1 ml of internal standard (trans-4 (amino methyl)-cyclohexane carboxylic acid), and hydrolyzed for 24 h at 110°C. The samples were then filtered through cellulose-acetate filters, dried under a nitrogen flow at 50°C, and then derivatized to TFA-isopropyl amino esters (Hofmann et al., 2003; Silfer, 1991), which included an additional purification step using a chloroform-phosphate buffer solution (Veuger et al., 2005). The derivatized samples were dissolved in 500 µl of methylene chloride and stored in GC-vials at −20°C until analyzed as described below.

2.5 Bulk and compound specific isotope measurements

The measurements of the bulk $\delta^{13}C$ and bulk $\delta^{15}N$ values were carried out according to Loick et al. (2007), using Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS) analyses of the samples (Thermo Finnigan Delta Plus + Thermo Flash EA 1112). For this purpose, the dried and powdered samples were weighed (~0.5 mg per sample), packed in tin boats and measured by EA-IRMS. Calibration for the total carbon determination was done daily with an acetanilide standard (Merck). All isotope abundances are expresses in δ notation as follows: $\delta X (‰) = [(R_{sample}/R_{standard}) - 1)] * 10^3$, where X is ^{13}C or ^{15}N, and R is the $^{13}C:^{12}C$ or $^{15}N:^{14}N$

ratio. The internal laboratory reference gas for the C-analyses was ultrapure CO_2 or N_2, which was calibrated against the materials from the International Atomic Energy Agency (IAEA), for carbon: NBS 22 (mineral oil $\delta^{13}C = -29.74$ ‰) and USGS 24 (graphite $\delta^{13}C = -15.99$ ‰) and nitrogen: IAEA-N1 ($(NH_4)_2SO_4$, $\delta^{15}N = 0.538$ ‰), IAEA-N2 ($(NH_4)_2SO_4$, $\delta^{15}N = 20.343$ ‰) and IAEA-NO3 (KNO_3, $\delta^{15}N = 4.613$ ‰). In addition, peptones (Merck) were analyzed as in-house standards after every sixth sample run. The analytical error for stable isotope ratios indicated by the peptone standards was less than ± 0.2 ‰ for carbon isotopes.

The TFA-isopropyl-derivatized samples were analyzed for their content of $\delta^{15}N$ in 13 amino acids; an external standard (16 AA) was also included. The 13 amino acids were: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), tyrosine (Tyr), and lysine (Lys). The concentrations of cysteine (Cys), arginine (Arg), and methionine (Met) in the samples were below the qualitative detection limit of the measurement device and could therefore not be determined in the samples. During the initial hydrolysis step, glutamine and asparagine were converted into glutamic acid and aspartic acid, respectively, such that they were considered together as Glu + Gln (referred to herein as Glu) and Asp + Asn (Asp) (Brault et al., 2019).

Amino-acid-specific $\delta^{15}N$ values were measured using an isotope ratio mass spectrometer (IRMS, Thermo Finnigan GmbH, MAT 253 MS, Germany) connected via a ConFlo IV interface unit to a gas chromatograph combustion periphery (GC-C). The latter consisted of a gas chromatograph and a combustion oven (Thermo Scientific Trace 1310 GC, Italy; Thermo Scientific, GC Isolink, Germany). The separation column in the GC consisted of a non-polar column coated with 5% phenyl-polysilphenylenesiloxane (BPX5, 60 m, 0.32 mm inner diameter, film thickness of 1µm; SEG Analytical Science, Ringwood, Victoria, Australia). For each run, 2 µl of sample was injected via a PTV injector in splitless mode. The temperature program was as follows: initial temperature 50°C, hold for 1 min, heat at 12°C/s to 120°C, and hold for 17 min, then heat at 3°C/s to 180°C and hold for 10 min, then heat at 5°C/s to 200°C and hold for 6 min and finally heat at 10°C/s to 250°C and hold for 7 min. Each sample was injected and measured at least three times using helium (He) as the carrier gas. The standard deviation from three runs was usually < 1.0 ‰ for all 13 amino acids.

2.6 New Isotope Proxies to characterize Estuarine Food Webs

2.6.1 Trophic Positions calculation for cod and flounder

Since the calculation of trophic positions of vertebrates has become increasingly differentiated for different species in recent years, several TP formulas were used and partly also adjusted to the ecosystem specific populations in this dissertation.

There are currently four CSIA-based TP models available that are suitable to estimate the TP of vertebrates (Bradley et al., 2015; Brault et al., 2019; Germain et al., 2013; Nielsen et al., 2015). They were tested and partly modified as described below to identify, which TP model is most suitable for cod and flounder, respectively. Specifically, CSIA data of cod and flounder from the western Baltic Sea were chosen to validate and calibrate the CSIA-based TP models based on the assumption that the CSIA-based TPs at this reference site should be close to bulk stable isotope analysis (SIA) based TP estimates from the western Baltic Sea and ideally also close to the global mean TP value for cod of 4.1 ± 0.2 and for flounder of 3.1 ± 0.2 from Fishbase.com (Froese et al., 2024). The rational for this is that the TP of cod and flounder from the western Baltic Sea should not be largely impacted by any trophic lengthening in the mesozooplankton compartment (Loick-Wilde et al., 2019), since filamentous, N_2-fixing cyanobacteria and the massive microbial system triggered by them are largely absent in the western Baltic Sea. The SIA-based TP values for cod and flounder from the reference site were calculated based on bulk $\delta^{15}N$ data from literature (Mittermayr et al., 2014; Mohm, 2014;2018) after Post (2002) as follows:

$$TP_{Bulk} = \gamma + \frac{\delta^{15}N_{higher\,consumer} - \delta^{15}N_{base}}{\Delta_n} \qquad \text{(Equation 1)}$$

Where γ is the TP of the benthic or planktonic primary consumer (e.g. $\gamma = 2.0$ in zooplankton in the western Baltic Sea or $\gamma = 2.7$ in zooplankton in the central Baltic Sea), $\delta^{15}N_{base}$ is the measured value of the benthic or planktonic primary consumer, $\delta^{15}N_{higher\,consumer}$ is the measured value of the benthic or planktonic higher consumer (e.g., shore crab, herring, sprat, cod) and Δ_n is the enrichment in $\delta^{15}N$ of 3.4‰ per trophic level (Post, 2002).

The weighted mean values of the groups of source and trophic AAs (Eq. 2) and their errors (Eq. 3) were calculated as follows:

$$AA_{wmean} = \frac{\sum_i^n p_i \times \delta^{15}N_i}{\sum_i^n p_i} \qquad \text{(Equation 2)}$$

$$\sigma = \pm \frac{1}{\sqrt{\sum_i p_i}} \qquad \text{(Equation 3)}$$

Where p is the weighting factor based on standard deviation of three injections per AA, and σ is the error for the weighted mean values for the group of AAs.

For testing the different TP models, different sets of trophic and source AA $\delta^{15}N$ data (Tab. 2) of the cod and flounder samples from the western and central Baltic Sea were added either into Eq. 4 according to Brault et al. (2019), Bradley et al. (2015), and Nielsen et al. (2015), or into Eq. 5 according to Germain et al. (2013):

$$TP_{wmn} = \left[\frac{\left(\delta^{15}N_{wmt} - \delta^{15}N_{wms} - \beta_{\frac{Trp}{Src}} \right)}{TDF_{AA}} \right] + 1 \qquad \text{(Equation 4)}$$

$$TP_{multi-TDF} = \left[\frac{(\delta^{15}N_{wmt} - \delta^{15}N_{wms} - TDF_{fish})}{TDF_{plankton}} \right] + 2 \qquad \text{(Equation 5)}$$

The $\delta^{15}N_{wmt}$ and $\delta^{15}N_{wms}$ are the $\delta^{15}N$ values of the model-specific trophic and source AAs. $\beta_{Src/Trp}$ represents the difference between the $\delta^{15}N$ values of the trophic and source AAs for primary producers (TP = 1.0). TDF_{AA}, TDF_{fish}, and $TDF_{plankton}$ are the average $\delta^{15}N$ increases in trophic AAs relative to source AAs per trophic level (Glibert, Altabet, et al., 2019; McMahon et al., 2015). Instead of the TDF value for seals (also called TEF_{Seal} in the reference) in the model of Germain et al. (2013), the TDF_{AA} value for teleost fish from Bradley et al. (2015) was applied, therefore we refer to this model as Germain-modified (Tab 2). Further, we added a modified TP model after Brault et al. (2019), for which the trophic AA Ile was exchanged by the trophic AA Pro, therefore we refer to this model as Brault-modified (Tab. 2). The variables for all TP-models are summarized in Tab. 2. The standard error (SE) in the TP estimations, computed by propagation of the analytical error in the individual amino acid determinations, typically did not exceed 0.2 TP.

For cod, exclusion criterion for any of the respective TP-models was an extreme drop in the calculated TP values below a TP of 4.0 and towards a TP of 3.0 based on CSIA data of cod from the western Baltic Sea. Cod in the western Baltic Sea cannot reach TP values around or below 3.0, as their food base (from stomach content analyses) consisted at least of carnivorous animals (Funk et al., 2021), which makes cod a secondary carnivore and a TP below 3.0 is thus not realistic. This criterion resulted in the exclusion of the TP models after Germain et al. (2013), a modified version of Bradley et al. (2015), and Nielsen et al. (2015), while the TP model of Brault et al. (2019) yielded a mean cod TP value closest to the western Baltic mean TP of 4.1 ± 0.2 (Fig. 8). We then further improved the TP result after Brault et al. (2019) for cod from the western Baltic to become congruent with the western Baltic mean TP value for cod (Fig. 8, Tab. 2), based on calculated TP values from literature SIA data as well as stomach content analyses and model data from fishbase (Froese et al., 2024; Mittermayr et al., 2014; Mohm, 2014;2018), by a simple exchange of the trophic AA Ile with the trophic AA Pro (Tab. 1). The TP model modified after Brault et al. (2019) thus resulted in the best TP estimation for cod from the western Baltic Sea 4.1 ± 0.5 (Fig. 8, Tab. 2). Notably, independent from the model applied, the TP of cod from the central Baltic Sea was always significantly higher than the TP of cod from the western Baltic Sea.

Table 2: Summary of the five TP models tested for calculating the TPs of cod and flounder. wms and wmt are the different amino acids (AAs) used for the calculation of the weighted mean δ^{15}N values of the trophic (wmt) and source (wms) AA, $\beta_{\mathrm{Src/Trp}}$ is the difference between the δ^{15}N values of the trophic and source AA for primary producers (in ‰), $\mathrm{TDF}_{\mathrm{AA}}$, $\mathrm{TDF}_{\mathrm{fish}}$, and $\mathrm{TDF}_{\mathrm{plankton}}$ (all in ‰) are the trophic discrimination factors which give the average δ^{15}N increases in trophic AA relative to source AA per trophic level. Highlighted in bold are variables that were modified from the original model.

Model ID	**TP-Equation**	**wms**	**wmt**	**$\beta_{\mathrm{Src/Trp}}$**	**TDF Plankton**	**$\mathrm{TDF}_{\mathrm{AA}}$**	**$\mathrm{TDF}_{\mathrm{Fish}}$**	**Constant**	**Reference**
Brault	1	Phe Ser Gly	Glu Ala Ile	3.1		4.5		1	Brault et al. (2019)
Germain-modified	2	Phe	Glu		7.6		**5.7**§	2	Germain et al. (2013)
Bradley	1	Phe Ser Gly	Glu Ala, Ile	3.6		5.7		1	Bradley et al. (2015)
Nielsen	1	Phe Ser Gly	Glu Ala Ile	2.9		5.9		1	Nielsen et al. (2015)
Brault-modified	1	Phe Ser Gly	Glu Ala **Pro***	3.1		4.5		1	This study

Within the flounder samples, the TP patterns calculated from the five TP models were somewhat different. The modified model after Nielsen et al. (2015), which uses the weighted mean source and trophic AA values, resulted in a mean TP value of 3.4 ± 0.3 for fish from the western Baltic Sea (Fig 9, Tab. 2). This value corresponds best to the western Baltic mean TP of flounder based on the calculated TPs based on SIA literature values (Mittermayr et al., 2014; Mohm, 2014;2018) and matches also the stomach content analyses and model TP from fishbase (Froese et al., 2024). All other TP models resulted in TP estimations either higher or lower than the western Baltic mean TP reference value (Fig. 9). The TP model after Nielsen et al. (2015) thus resulted in the best TP estimation for flounder from the western Baltic Sea.

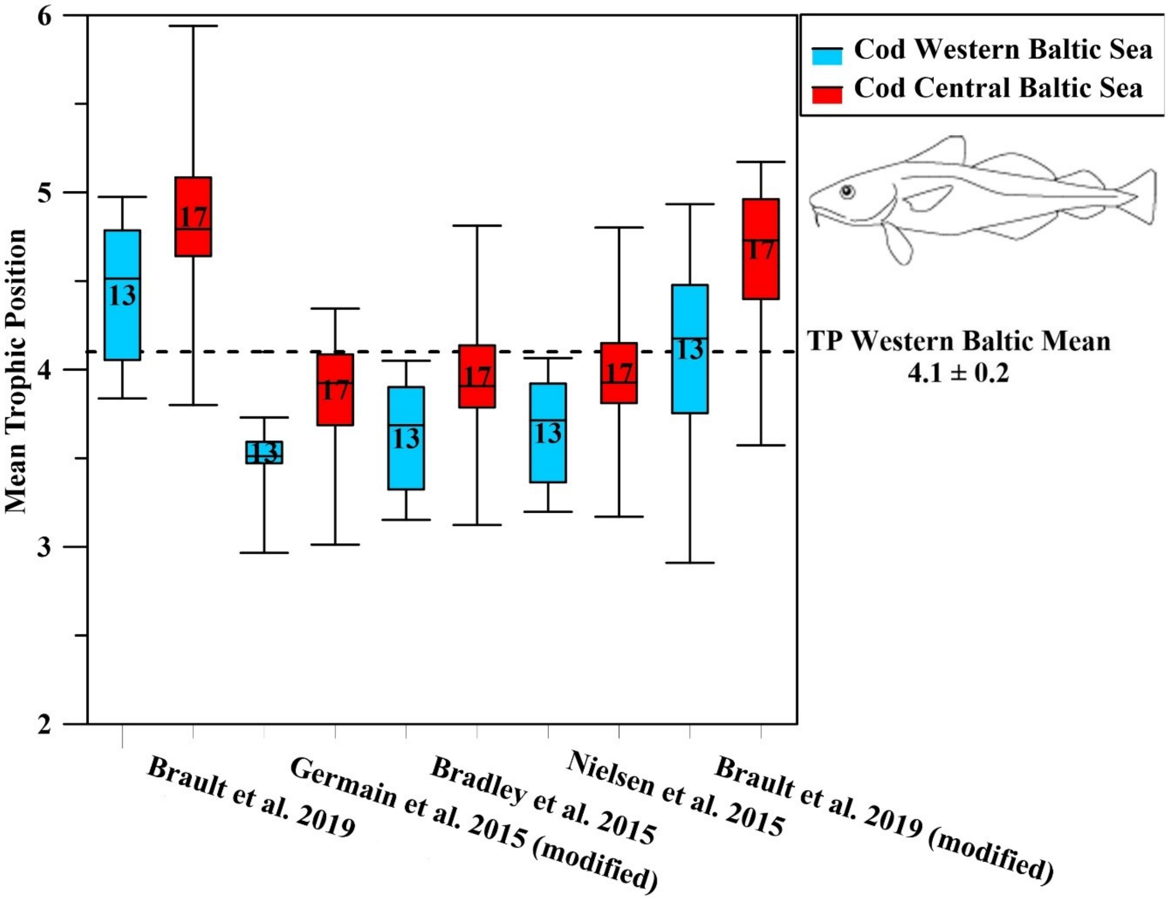


Figure 8: Calculated mean TP of cod from the western Baltic Sea (in blue, n = 13) and the central Baltic Sea (in red, n = 17) according to five different CSIA-based TP models for vertebrates. The western Baltic mean TP of cod (dotted line) is based on calculated TP values based on SIA data from literature (Mittermayr et al., 2014; Mohm, 2014;2018) (Tab. 2).

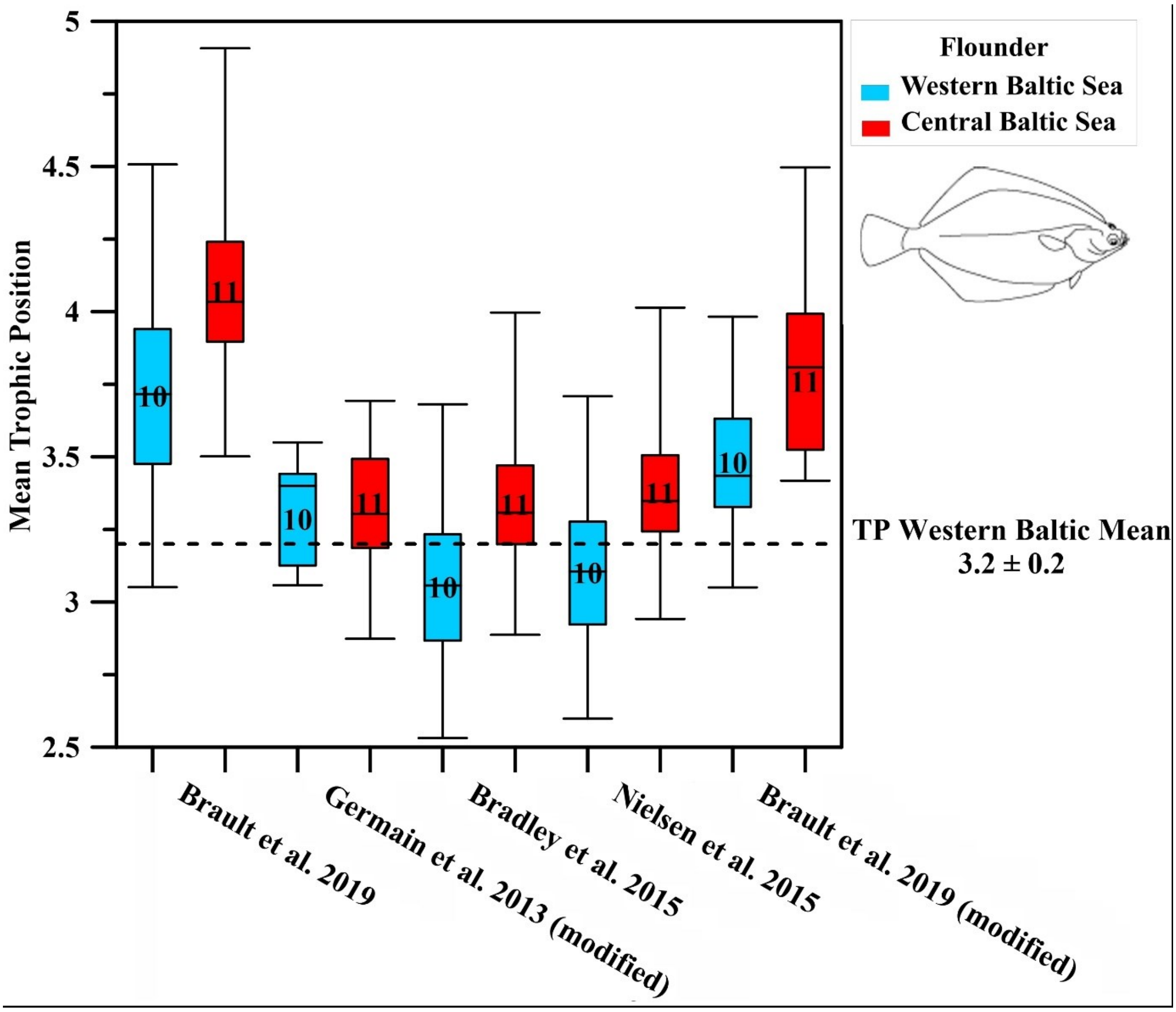


Figure 9: Calculated mean TP of flounder from the western Baltic Sea (in blue, n = 10) and the central Baltic Sea (in red, n = 11) according to five different CSIA-based TP models and compared to the western Baltic mean TP of flounder based on calculated TP values based on SIA data (dotted line, Mittermayr et al. (2014); Mohm (2014;2018), Table 2).

Interestingly, in contrast to cod no significant differences in TPs were found for most CSIA-based TP model between flounder from the western and central Baltic Sea, except for the one after Bradley et al. (2015).

It is beyond the scope of this paper to identify why the best fitting TP estimations for cod and flounder from the western Baltic Sea compared to their reference TP mean were generated by two different TP models. However, an underlying physiological mechanism for this result may include species-specific differences in fat and protein anabolism and catabolism (Bradley et al., 2015; Germain et al., 2013; McMahon et al., 2016).

2.6.2 Trophic position calculations for organisms of the Elbe and Oder

For **zooplankton** and **POM** samples the $TP_{Glu/Phe}$ formula after Chikaraishi et al. (2009) were used and for all other organisms **(fish and seabirds)** the weighted means of the sources AA:

Phe, Gly, Ser and the trophic AA: Glu, Ala, Ile and Glu, Ala, Pro were used to calculate the TP (Bradley et al., 2015). The weighted means and their errors were calculated as in Steinkopf et al. (2024), see Eq. 2 and Eq. 3.

The two established TP formulas from Steinkopf et al. (2024), were also used to test the different TDF models for Elbe and Oder (Eq. 4 and Eq. 5).

The $\delta^{15}N_{wmt}$ and $\delta^{15}N_{wms}$ are the $\delta^{15}N$ values of the model-specific trophic and source AAs. $\beta_{Src/Trp}$ represents the difference between the $\delta^{15}N$ values of the trophic and source AAs for primary producers (TP = 1.0). TDF_{AA}, TDF_1, and TDF_2 are the average $\delta^{15}N$ increases in trophic AAs relative to source AAs per trophic level (Glibert, Altabet, et al., 2019; McMahon et al., 2015). The standard error (SE) in the TP estimations, computed by propagation of the analytical error in the individual amino acid determinations, typically did not exceed 0.5 TP.
The trophic position of the organisms of Elbe and Oder estuary were calculated using species-specific and tissue-specific TDFs. For **POM/phytoplankton** and **zooplankton**, the conservative formula according to Chikaraishi et al. (2009) with a TDF of 7.6 and a β-value of 3.4 was chosen. For **smelt**, a TDF of 5.3 was calculated from the ratio of zooplankton and smelt and used together with a β-value of 3.4. For the **zander**, the TDF of 4.5 and β-value of 3.1 were used for higher vertebrates according to Brault et al. (2019). For the whiting in the study, the Multi-TDF calculation with the TDF value of 5.3 calculated in this study for fish was used together with the TDF of 4.5 according to Brault et al. (2019). For the **terns**, the review by Stephens et al. (2023) was used to search for TDF values of seabirds fed with pelagic fish or crustaceans and then a TDF of 2.65 according to Cherel et al. (2005) and a β-value of 3.4 were used. The selection of the TDF values for the calculations was subject to special rules for limit values of the respective calculated TP values. These were as follows:

No specific limits were established for POM and zooplankton, as the original TDF and β-value were already adapted to zooplankton and their diet.

For the fish, reference data from Fishbase (Froese et al., 2024) and from the analysis of stable isotopes were used to calculate a global mean TP for zander, smelt and whiting (Kopp et al., 2009; Timmerman et al., 2020). This global mean TP was then used to validate the different TP models including different TDF values to find the best species-specific TP equation (Tab. 3).

Table 3: Reference TP data based on Fishbase (Froese et al., 2024) and when available stable isotope and stomach analysis data (Kopp et al., 2009; Poiesz et al., 2021; Timmerman et al., 2020) used to calculate global mean TP values for smelt, zander and whiting

Species	TP Fishbase	STDV Fishbase	TP Isotopes	STDV Isotopes	TP SCA	Literature	Global mean TP	STDV
Osmerus eperlanus	3.5	± 0.42	3.4	n.a.	3.93	Froese et al. (2024) Poiesz et al. (2021)	**3.6**	**± 0.2**
Zander lucioperca	4.0	± 0.78	4.2	± 0.1	n.a.	Froese et al. (2024) Kopp et al. (2009)	**4.1**	**± 0.1**
Merlangius merlangus	4.4	± 0.2	3.9	± 0.25	n.a.	Froese et al. (2024) Timmerman et al. (2020)	**4.1**	**± 0.2**

Subsequently, the TPs were calculated with the respective potential TP equations and the respective mean TPs as well as upper and lower limits were compared and the best fitting equation was selected.

2.6.3 Degradation Status of amino acids

For the representation of the degradation status of amino acids and the potential presence of heterotrophy in higher trophic levels, the ΣV proxy introduced by McCarthy et al. (2007) was used (Eq. 6):

$$\sum = \frac{1}{n}\sum Abs_{(\chi_{AA})} \qquad \text{(Equation 6)}$$

Where χ (deviation) of each Trp-AA = [$\delta^{15}N_{AA}$ - AVG $\delta^{15}N$ (Ala, Asp, Glu, Ile, Leu, Val and Pro)], and n = the total number of Trp-AA used in the calculation (McCarthy et al., 2007).

2.6.4 Mixotrophy test and two step phytoplankton validation

In order to detect potential mixtrophy in the sampled POM samples, the apparent fractionation factors ε Phe/Glu and ε Phe/Ala values were calculated according to Yamaguchi et al. (2017) (Eq. 7):

$$\varepsilon_{PHe/TrAA} = 1000 \times (\frac{\delta^{15}N_{Phe}+1000}{\delta^{15}N_{TrAA}+1000} - 1) \qquad \text{(Equation 7)}$$

It was then tested whether the sampled POM samples were phytoplankton or whether these samples were otherwise contaminated. A two-step procedure was used for this purpose (Doherty et al., 2021; Yamaguchi et al., 2017). In the first step, the C:N ratio was compared with the C:N Redfield ratio to see whether the POM samples were within the phytoplankton ratio. In the second step, the differences of the $\delta^{15}N$ values of Ala and Phe as well as Thr and Phe were compared and the POM samples were assigned to the compartments degraded OM, Fecal Pellets, Zooplankton and Phytoplankton according to Doherty et al. (2021).

2.7 Statistical analysis

The statistical analyses in this dissertation were carried out using RStudio® version 2023.06.2+561. The graphical processing of the data was carried out using Golden Software Grapher 9 for the figures and Golden Software Surfer 11 for one map of the individual sampling areas. For the PCAs carried out and comparisons with abiotic factors in the Elbe, additional data from the FGG Elbe data platform were consulted.

3. Results and Discussion

3.1 Heterotrophy and trophic lengthening in the Elbe estuary

The results of this chapter are part of the manuscript “Environmental Stressors and the Disruption of Pelagic Food Webs in the Elbe Estuary” by Markus Steinkopf, Jesse Theilen, Leonie Enners, Ralf Thiel, Dirk Wodarg, Matthias Gehre, Steffen Kümmel, Iris Liskow, und Natalie Loick-Wilde. This manuscript is in preparation for Limnology and Oceanography. My share in this manuscript constitutes 90% and includes preparation of samples for bulk and compound specific isotope analysis in the lab, the discussion of the results, was well as writing of the manuscript.

3.1.1 Anthropogenic Stressors of the Elbe estuary

A large part of the Elbe estuary is dominated by the port of Hamburg and the associated anthropogenic stressors such as the expansion of port facilities, deepening of navigation channels and shipping. Back in the 1980s, the estuary was already in an extremely poor ecological condition, which also had an extreme impact on fish stocks, such as smelt, caused by untreated sewage and supply lines. However, these problems were successfully combated by advanced sewage treatment plants and general regulations on discharges into the Elbe (Bergemann et al., 2010; Pätsch et al., 2010). As a result, the Elbe recovered. Nowadays, however, the Elbe is once again characterized by nutrient loads which promote eutrophication in the German bight (Dähnke et al., 2008; Dähnke et al., 2022; Schulz et al., 2023) and, above all, high turbidity levels (Pein et al., 2023). However, these do not stem exclusively from organic sources, but are largely due to the deepening of the Elbe, the last of which began in 2019 and was completed in 2022 (HPA, 2024). Since then, however, maintenance dredging has had to be carried out (Wasserstraßen- und Schifffahrtsamt Elbe-Nordsee), as sediments are repeatedly brought in by the enormous tidal wave and thus fill up the harbor and the fairway. This results in enormous turbidity values, which sometimes reach values of over 800 NTU at the maximum turbidity zone between Brunsbüttel and the river island of Schwarztonnensand ("Datenportal der FGG Elbe," 2024). The Elbe estuary is classified as autotrophic upstream of the harbor zone. However, with the beginning of the dredged part, where the water depth suddenly increases, the current velocity increases and the turbidity rises (Burchard et al., 2004; Kerner, 2007; Pein et al., 2023), the estuary becomes increasingly heterotrophic, as the phytoplankton sinks there and is no longer competitive due to the light limitation (Kamjunke et al., 2023). It dies and sinks to the bottom, where it promotes nitrification. These processes draw oxygen out of the system and thus ensure the formation of anoxic zones (Geerts et al., 2017; Schöl et al., 2014), especially in the warm summer months when stratification sometimes occurs. Further downstream, the system partially stabilizes and nitrate again plays a more important role, but here the role of autotrophic phytoplankton is less important (Dähnke et al., 2022; Kamjunke et al., 2023; Sanders et al., 2018).

3.1.2 Results

The results of this chapter are based on the materials and methods described in detail in the Elbe estuary-specific subchapters 2.1.1 and 2.2.1 as well in the more general materials and methods subchapters 2.4 – 2.7.

Bulk $\delta^{15}N$ and $\delta^{13}C$ values

The bulk $\delta^{15}N$ measurements showed large fluctuations in the zooplankton from 4.9‰ as the smallest value to 16.4‰ as the highest value (Fig. 10). Zander and smelt show small indications of increasing $\delta^{15}N$ values with increasing salinity (smelt with $R^2 = 0.634$, $p = 9.863e*10^{-17}$, and zander with $R^2 = 0.809$ and $p = 2.992e*10^{-06}$, respectively). The salinity also reflects the respective station. Whiting show solely marine $\delta^{13}C$ values and stay in a quite narrow range in $\delta^{15}N$ values from -17.47‰ to -18.73‰, which match the $\delta^{15}N$ particle values from the station BB (Dähnke et al., 2022). The terns, sampled on the island of Neuwerk showed solely marine $\delta^{13}C$ values while showing a huge scattering in their $\delta^{15}N$ values from 9.5‰ to 18.7‰ (Fig. 10). An exception are three data points with very low $\delta^{13}C$ and $\delta^{15}N$ values, which we further treated as outliers.

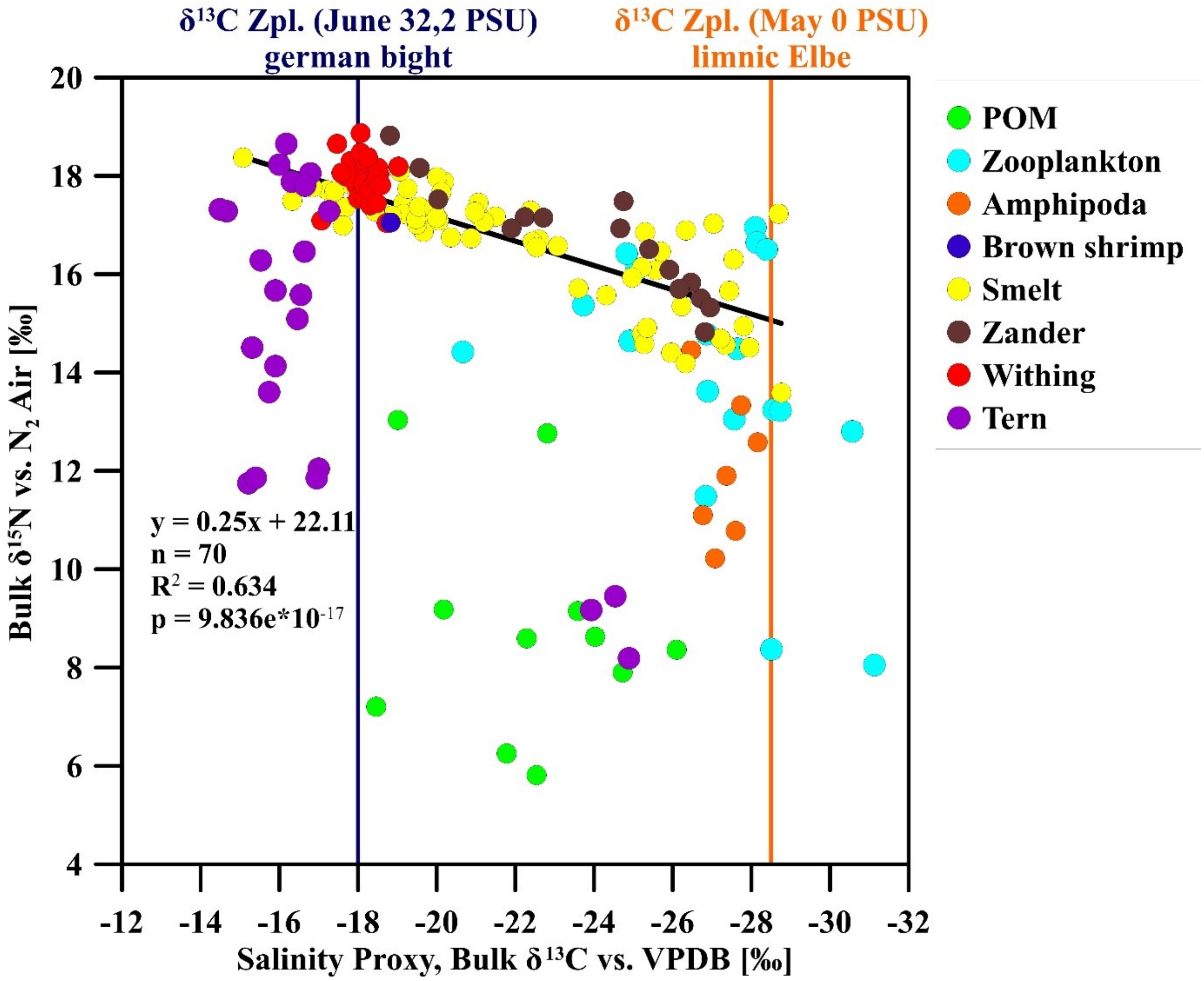


Figure 10: Bulk $\delta^{15}N$ values vs. salinity proxy (bulk $\delta^{13}C$ values) of sampled POM, zooplankton, amphipods, brown shrimp, smelt, zander, whiting and terns from the Elbe estuary. Note the reversed order of the x-axis in order to better reflect the geographic extension of the salinity gradient of the Elbe river from the limnic harbor (minimum $\delta^{13}C$ values) to the marine realm of the North Sea (maximum $\delta^{13}C$ values).

Environmental influence on the Elbe food web structure

Within the data of the component-specific analysis of amino acids, the zooplankton and phytoplankton remain decoupled from the gradient in their $\delta^{13}C$ values towards the more marine stations (Fig. 11). The smelt samples show high TP fluctuations from omnivorous values of TP 2.5 to second level carnivorous TPs of 4.5, whereby the majority of the lower TPs are found in the middle $\delta^{13}C$ values of the estuary, while the extremes are found at the respective end or beginning of the estuary. The zander remain relatively constant across the stations with TP values from 4.0 to 4.4 apart from two outliers with TP values of 4.6 and 4.9. The measured whiting shows a similar pattern as in the bulk analyses. They remain in the marine part of the estuary and show quite similar TPs without major outliers, nevertheless, the difference between their highest and lowest value is 1.1 TP. The terns, on the other hand, show an enormous dispersion in their TPs (Fig. 11), ranging from 3.5 to almost 5.7, while they continue to show only marine $\delta^{13}C$ signatures, with the exception of one outlier that showed very low $\delta^{13}C$ values of 24.54‰, similar to the bulk measurements.

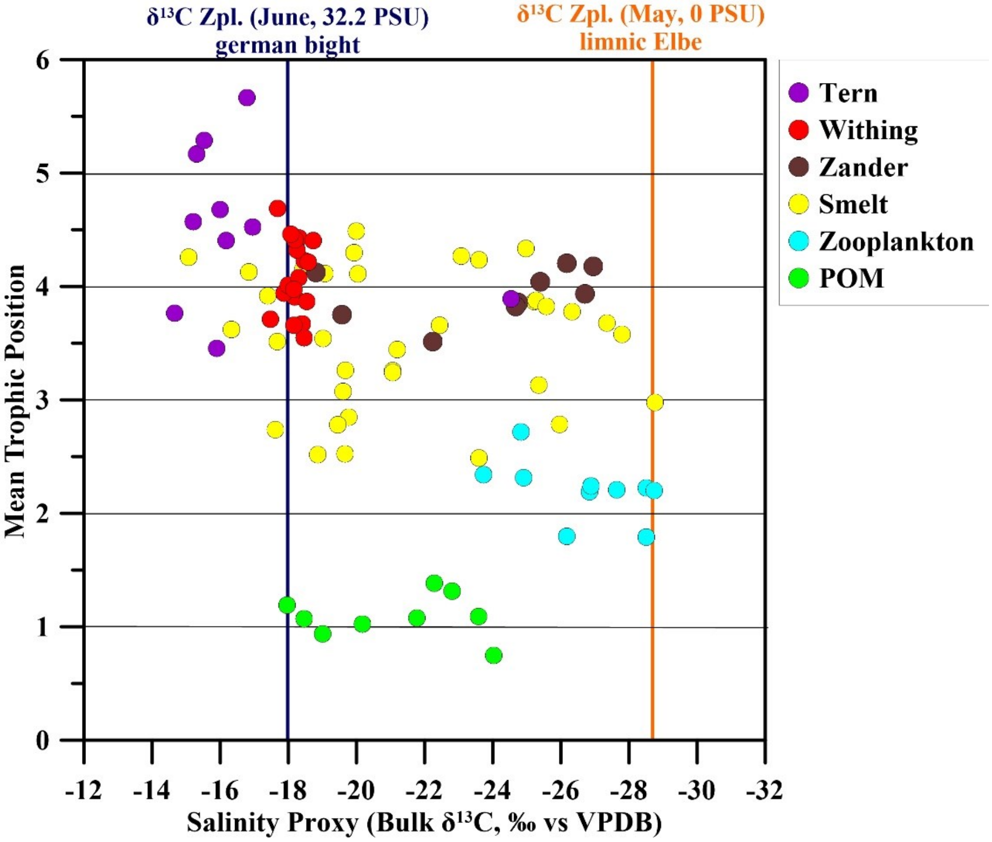


Figure 11: Calculated trophic positions of phytoplankton, zooplankton, smelt, zander, whiting and terns against the salinity proxy based on the bulk $\delta^{13}C$ values. Colored lines indicate salinity thresholds. Note the reversed order of the x-axis as in Fig. 9.

To explain the decreasing and increasing TPs in smelt, the values were plotted separately against the $\delta^{15}N$ source AA values as a proxy for the nitrogen source (Fig. 12). This shows a decrease in TPs in smelt, zander and whiting with increasing $\delta^{15}N$ source AA values.

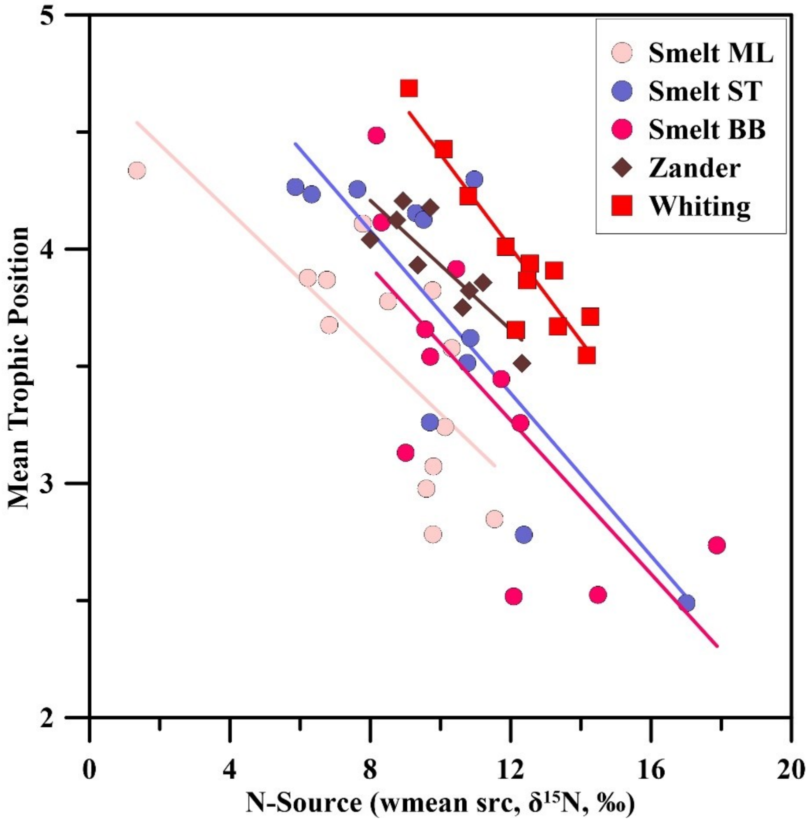


Figure 12: Calculated TPs of individual smelt, zander and whiting from the different stations of the Elbe estuary, plotted against the N-source proxy based on the δ^{15}N values of the weighted mean source amino acids (Phe, Ser, Gly). Regression Lines indicate tendencies within the different sampled species.

Degradation Status of amino acids

The ΣV proxy shows few data points indicating autotrophic synthesis of AA (ΣV of 0.5-1) while the proportion of freshly ingested AA (ΣV of 1-1.5) is particularly high in whiting. The majority of data points in zooplankton, fish (except whiting) and terns clearly indicate a heterotrophic microbial influence at the base of the food web (Fig. 13) that is transmitted further (ΣV 1.5 - ≥2).

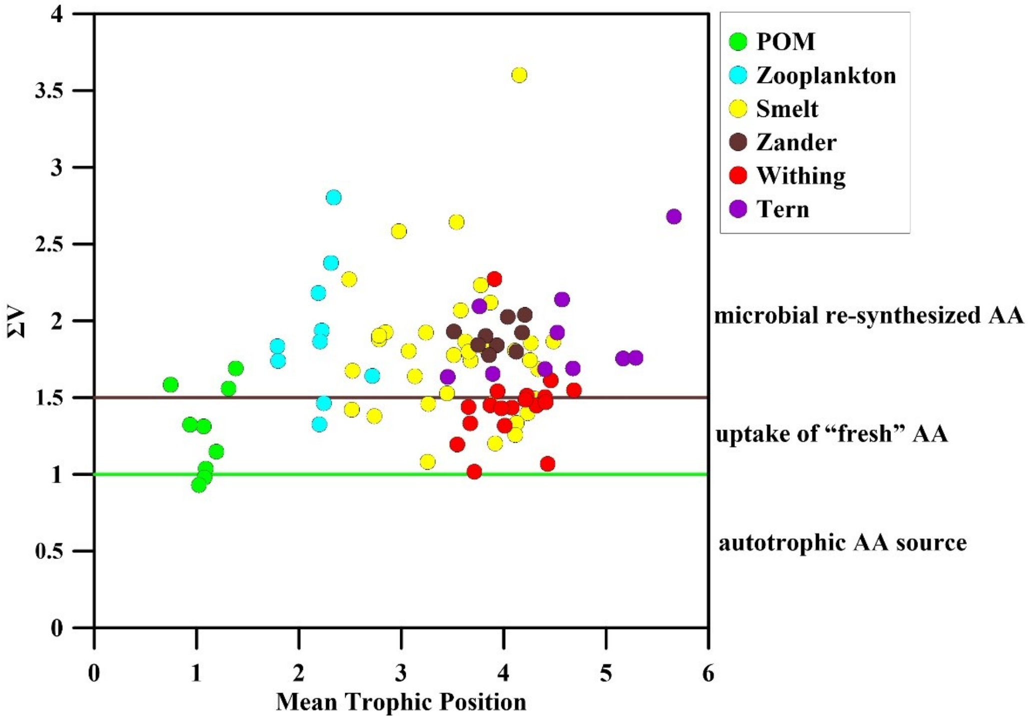


Figure 13: Mean trophic position against the proxy for microbial re-synthesis and heterotrophy (ΣV) for POM, zooplankton, smelt, zander, whiting and terns. The lines show threshold values for autotrophic synthesis, uptake of fresh AA and heterotrophic re-synthesized AA.

However, as there is a high scattering of smelt from ΣV 1.1 to 3.6, these were plotted again separately with the sampled stations (Fig. 14). This makes it easier to separate the individual groups and shows slightly significant differences (t-test, $p = 0.04$, without outlier 0.001) between the smelt from station BB (1.1 - 1.9, outlier 2.6) and the smelt from station ML (1.5 - 2.2, outlier 2.6). The smelt from station ML showed increased values for heterotrophic influences, while the smelt from station BB showed increased values for the intake of fresh AA.

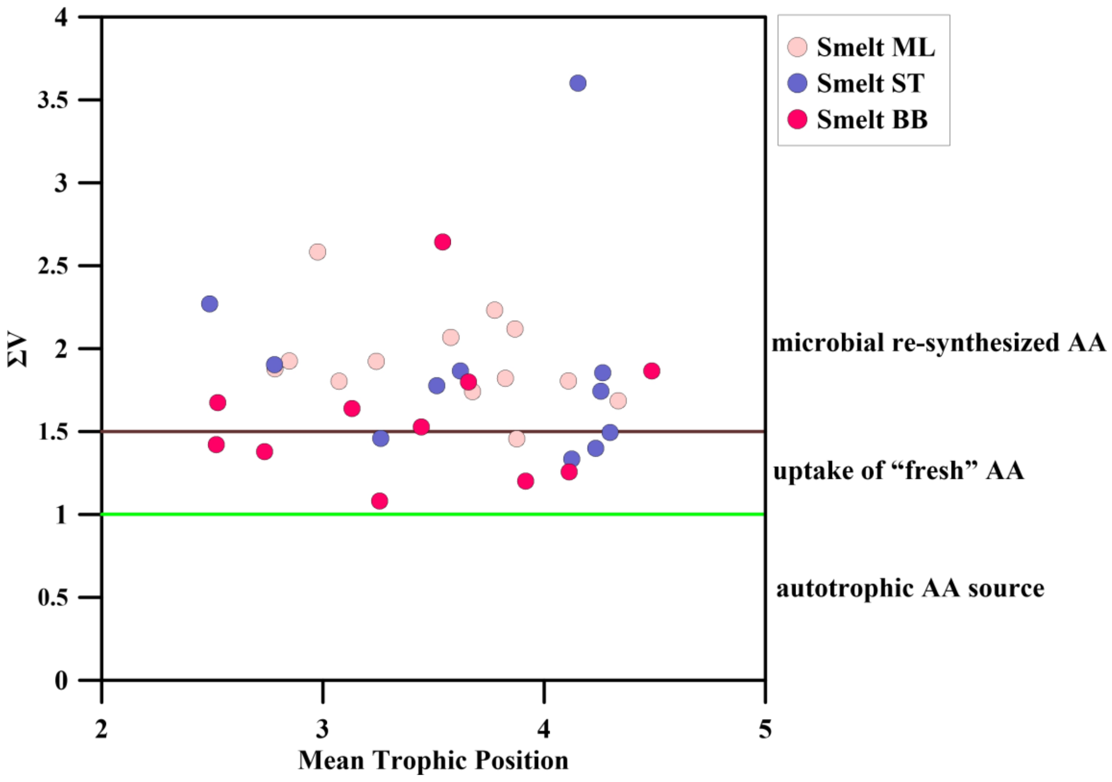


Figure 14: Mean trophic position against the proxy for microbial re-synthesis and heterotrophy (ΣV) for smelt, differentiated by their sampling station. The lines show threshold values for autotrophic synthesis, uptake of fresh AA and heterotrophic re-synthesized AA.

Mixotrophy analysis

As some of the TP calculations for the sampled POM samples from the Elbe resulted in relatively high TP values, additional calculations and plots were created to determine if there were signs of mixotrophy within the samples. The calculation of the ε Phe/Glu value for two samples from the Mühlenberger Loch and for Schwarztonnensand resulted in values below -5.5 (-5.7 in May for ML and -6.2 in May for ST) and thus indications of osmotrophy (Uptake of dissolved organic compounds by osmosis). The E Phe/Ala value showed values below -4 for three samples (-5.4 and -5.2 at ML in May and July and -5.9 at ST in May) and thus show clear signs of phagotrophy (Fig 15).

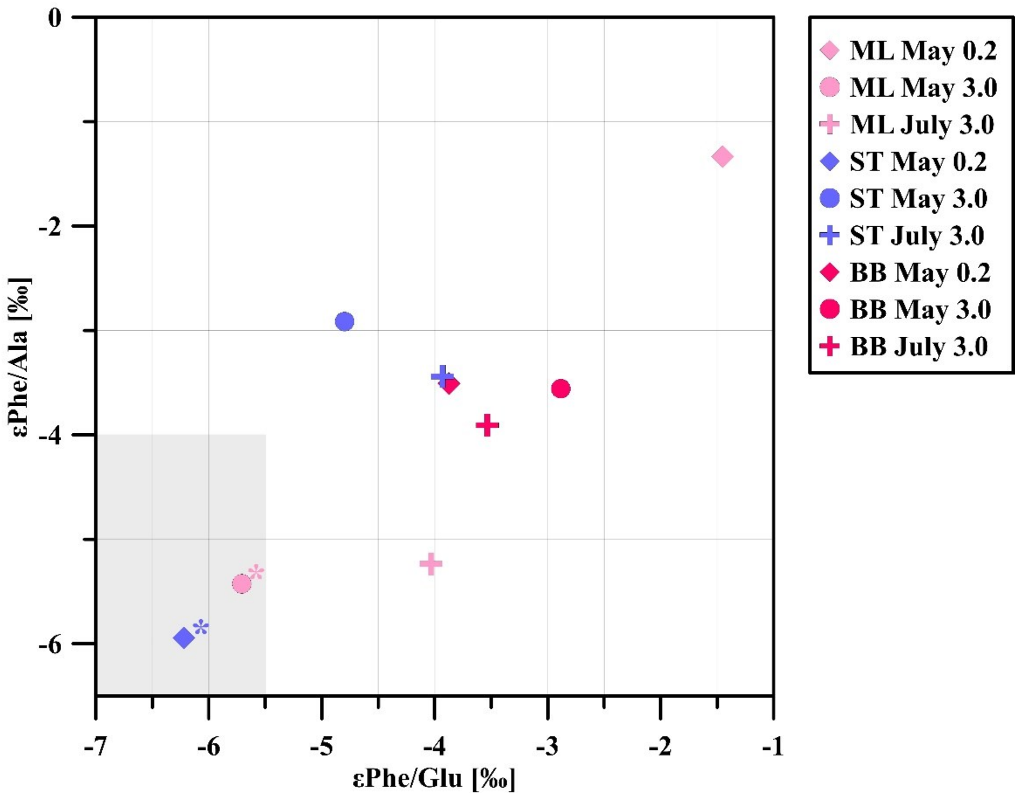


Figure 15: Apparent isotope fractionation of the two trophic amino acids (glutamic acid + 394 glutamate, Glu; alanine, Ala) relative to the source amino acid (phenylalanine, Phe) showing deviations from the values expected for autotrophs due to contributions of protist grazing (εPhe/Ala < −4‰) or use of ambient of amino acids by bacteria or phytoplankton (εPhe/Glu < −5.5‰) in the shaded area. Potential mixotrophic samples are marked with an asterisk.

A two-step procedure was used to determine whether the POM samples tested were phytoplankton. It was shown that all values of the POM samples were within a range of C:N = 8.08 - 14.23 and thus all were above the threshold value of 6.6 (Fig 16).

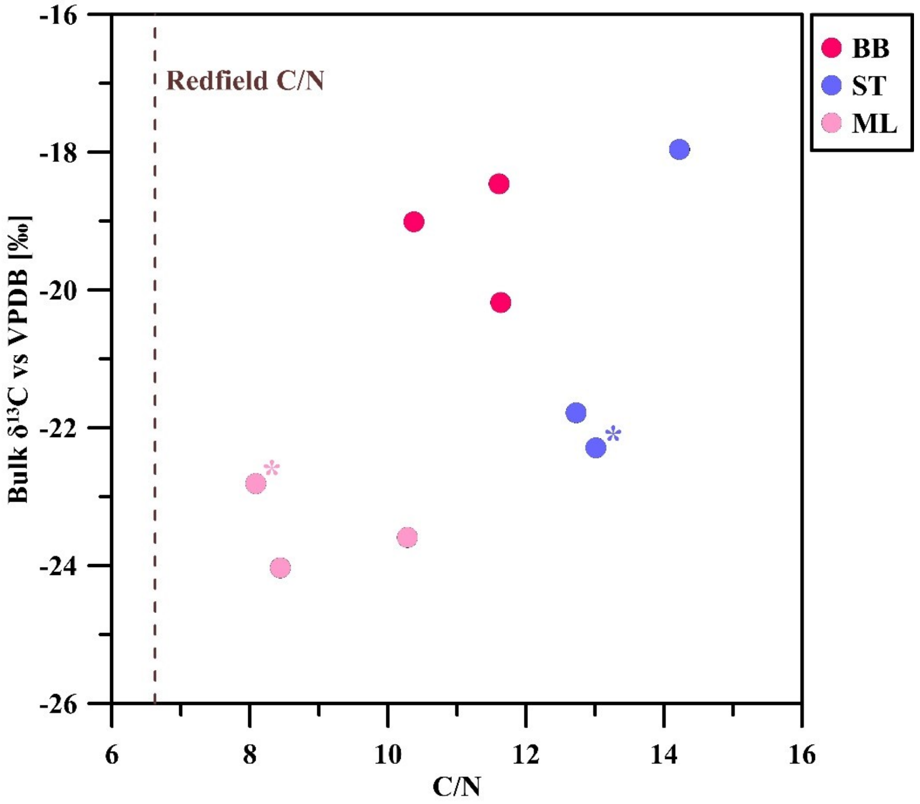


Figure 16: First plot of the two step validation process of the POM samples from the Elbe estuary as a proxy for phytoplankton. The bulk carbon isotopes vs the C:N ratio. Dashed Line indicates the C:N Redfield ratio of 6.6.

The second step showed that most analyzed POM samples from the Elbe were within the range for phytoplankton within the 5 possible compartments defined by Doherty et al. (2021). One exception was the 2 µm BB sample with a Thr-Phe value of -7.2 which may indicate contamination with fecal pellets (Fig. 17).

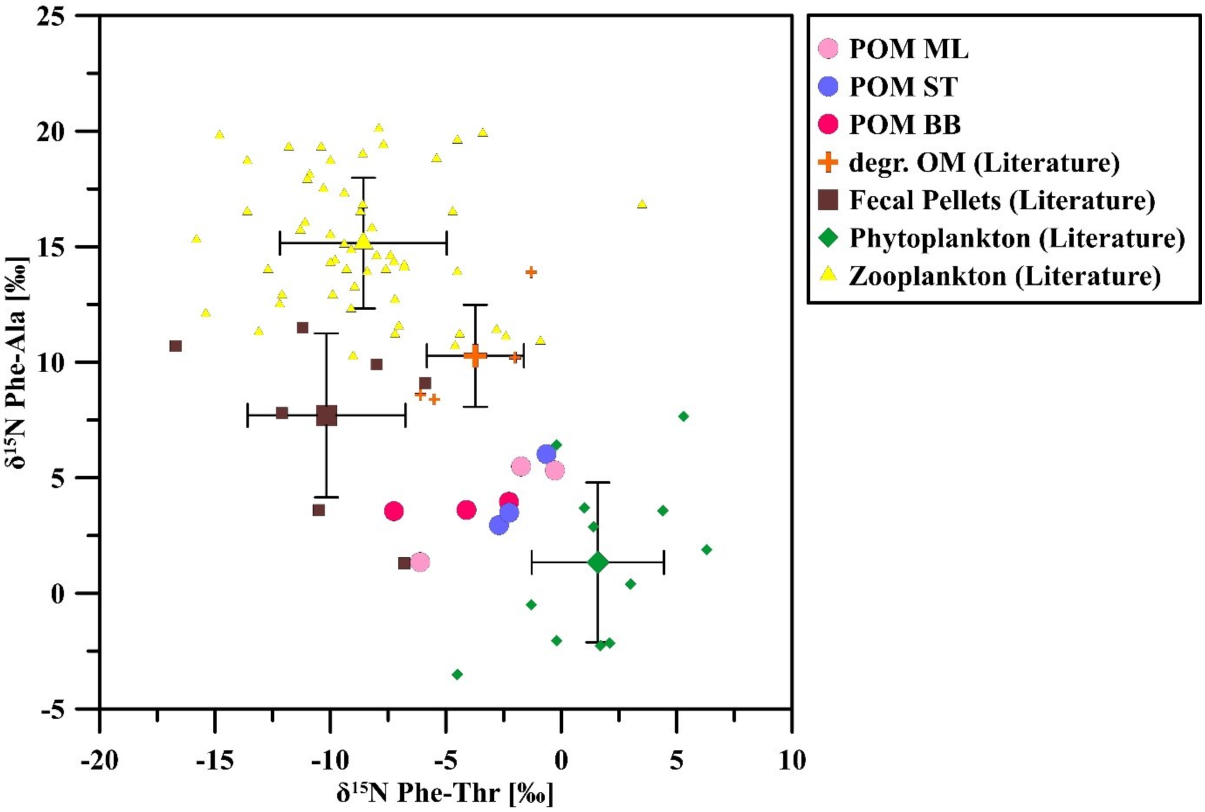


Figure 17: Plot of the difference of the δ^{15}N values of Ala and Phe vs the difference between Thr and Phe in the approximation proposed by Doherty et al. (2021) for differentiating different compartments in seston (phytoplankton, microbial degraded particulate organic matter, fecal pellets, and zooplankton). Literature values, mean values and standard deviations of degraded OM, Fecal Pellets, Phytoplankton and Zooplankton are provided from Doherty et al. (2021).

Influence of environmental factors on trophic positions

Seasonal data for surface temperature, turbidity, nitrate, - nitrite, - phosphate- and ammonium concentrations for the year 2021 were extracted from the FGG Elbe portal determine whether and to what extent abiotic factors have an influence on changes in the trophic positions of organisms in the Elbe. These data were analyzed together with the data for the trophic position in a principal component analysis to see which of the variables can be summarized in factors (Fig.18).

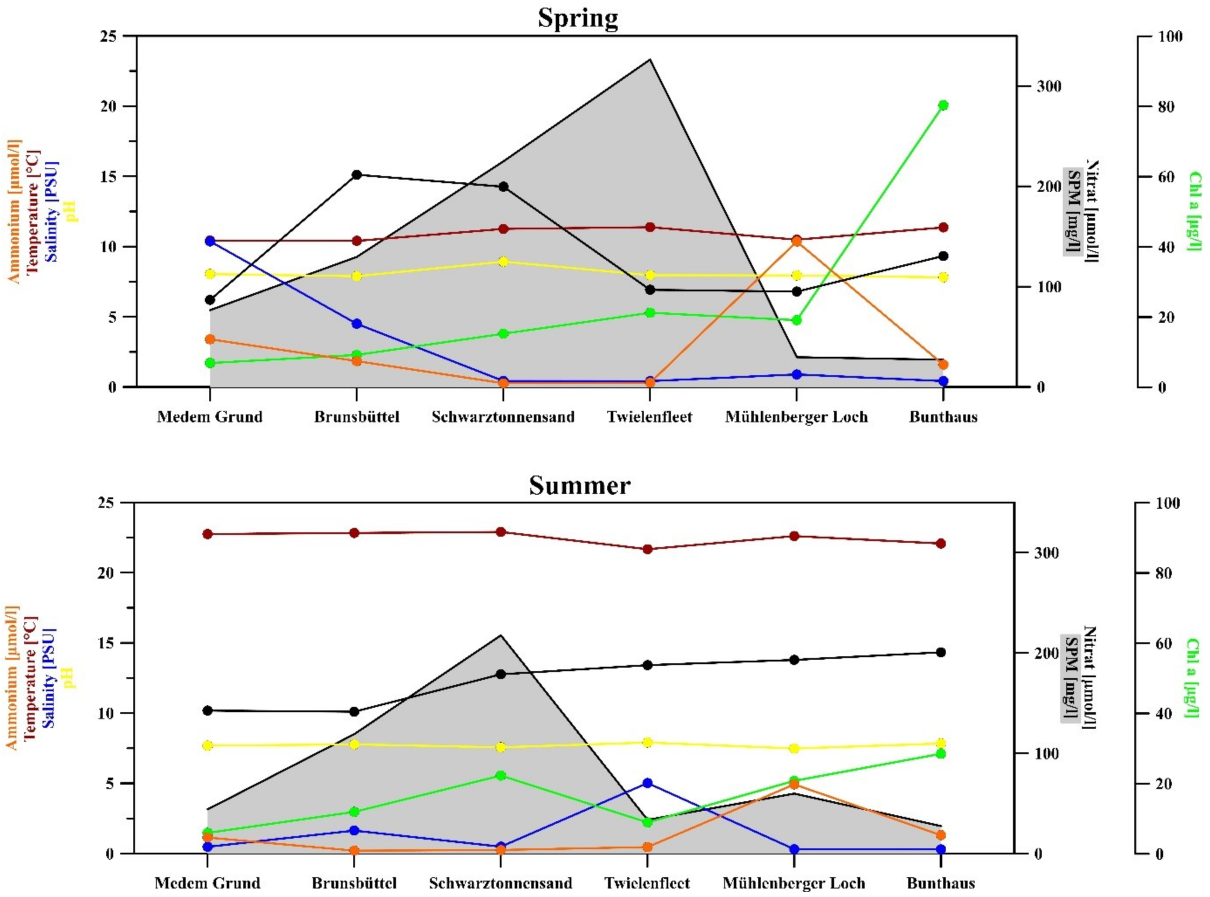


Figure 18: Plots of the abiotic variables nitrate-, ammonium-, chlorophyll a-, suspended particulate matter concentration, temperature, pH and salinity, along six station in the Elbe estuary in spring and summer 2021. Data obtained from the FGG Elbe data portal.

A principle component analysis was carried out to obtain an initial overview of the relationships between the sampled fish species and the abiotic variables used. PC1 explained 47.6% and PC2 27.4% of the variability (Fig. 19). This showed a clear grouping of the samples in summer and fall due to seasonal differences in temperature and oxygen conditions. Smelt and zander were grouped in opposite positions to whiting samples along PC1, which was mainly defined by the variables salinity, ammonium and nitrate.

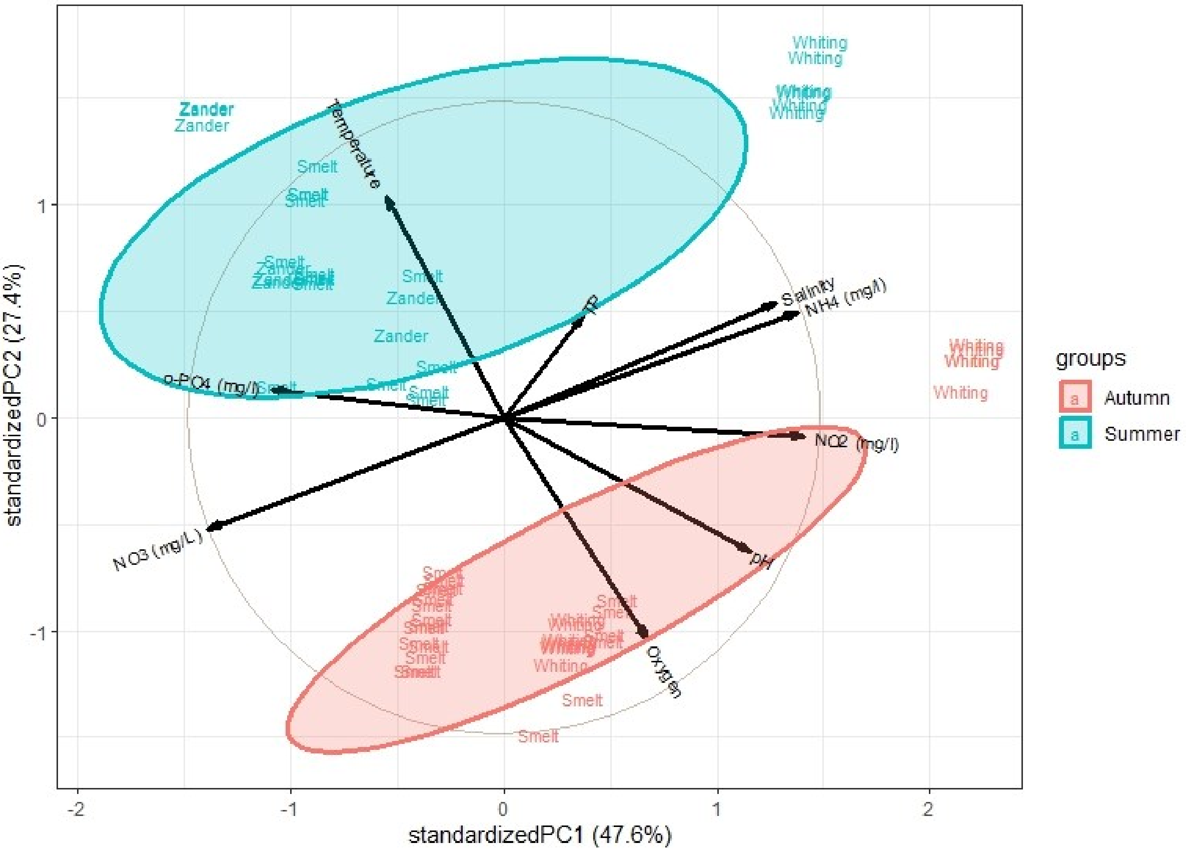


Figure 19: PCA biplot of the seasonal abiotic variables and the trophic positions of the sampled smelt, zander and whiting. The abiotic data were obtained from the FGG Elbe data portal.

Given the key function of smelt for the Elbe estuary, a refined PCA was carried out using higher resolution data from different age stages of smelt individuals from the Elbe. Accordingly, the $\delta^{15}N$ and $\delta^{13}C$ bulk values and the $\delta^{15}N$ values of phenylalanine and the weighted mean source AA from different age groups of smelt were included in the analysis. (Fig. 20).

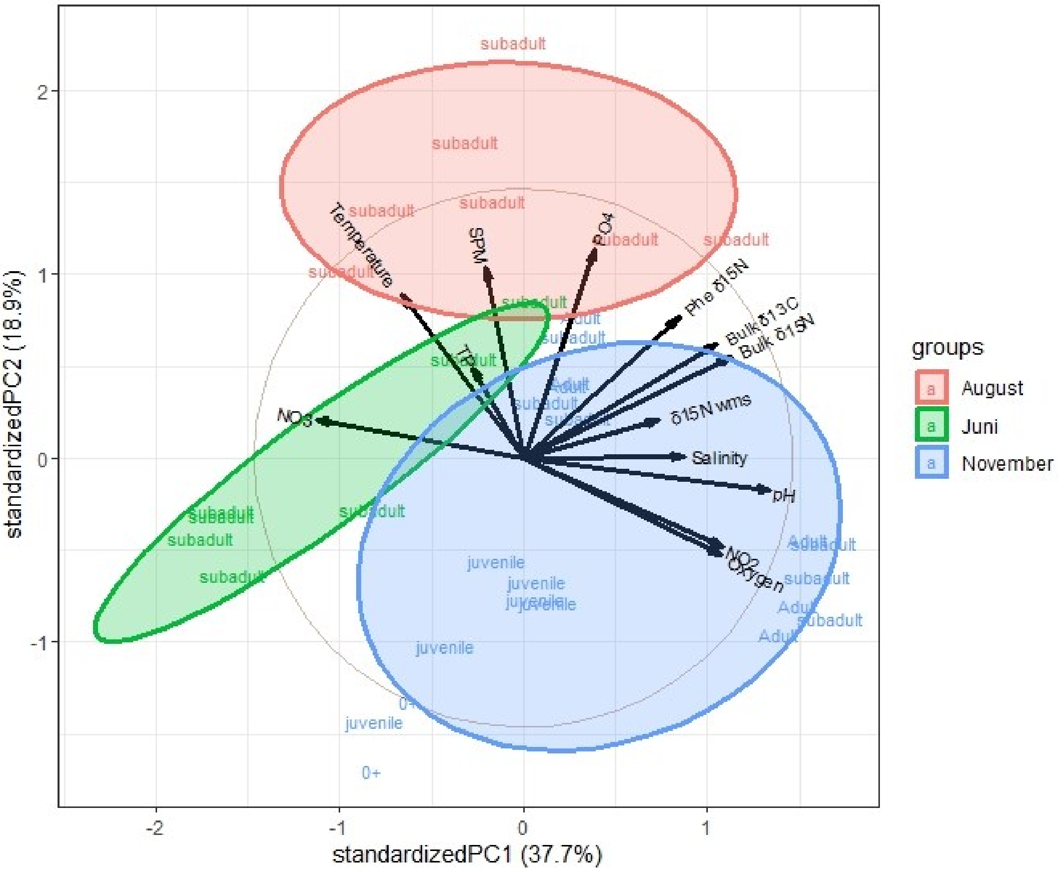


Figure 20: PCA biplot of the TP of smelt and the data of environmental factors salinity, temperature, pH, SPM-, phosphate-, oxygen-, nitrate-, and nitrite concentration as well as the $\delta^{15}N$ bulk, $\delta^{15}N$ Phenylalanine and $\delta^{15}N$ weighted mean source amino acid values and $\delta^{13}C$ bulk values. The environmental data were obtained from the FGG Elbe data portal. The single data points show the also labeled after age of the fishes and the ellipses summarizes monthly data sets.

Also this PCA showed a clear division between the seasons along PC2, but even between the individual months according to temperature and oxygen. There is also a orientation of individuals along the PC1 defined by nitrate, salinity, as well as bulk $\delta^{15}N$ and $\delta^{13}C$. The slightly positive relationship between salinity and bulk $\delta^{13}C$ in the fish samples is also evident here ($R^2 = 0.173$, $p = 0.0009$).

Correlation of abiotic factors and trophic position

A correlation analysis including nutrient data, other abiotic variables, as well as $\delta^{15}N$ and $\delta^{13}C$ bulk and the $\delta^{15}N$ source amino acid values and the trophic positions of smelt (Fig. 21), showed a clear correlation between the TPs of the smelt only with the weighted mean source AA value ($p < 0.01$). There were also noteworthy correlations ($p < 0.05$) between temperature and oxygen, nitrite and nitrate concentration, bulk $\delta^{15}N$ values and nitrate concentration and pH value with salinity and nitrite.

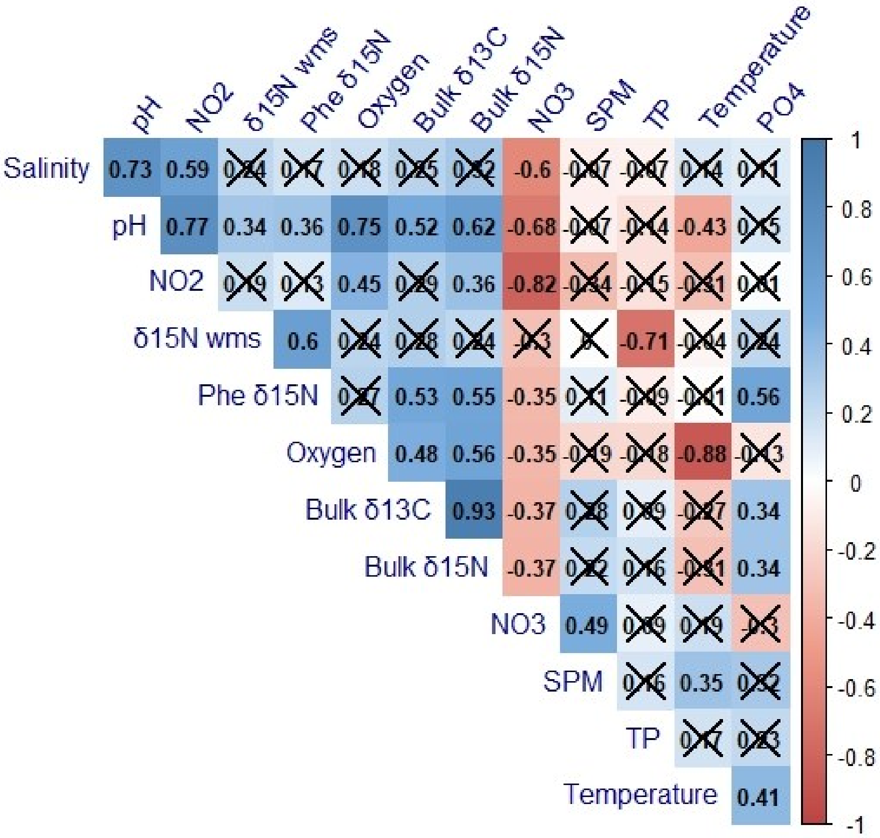


Figure 21: Correlation matrix and heat map of the abiotic factors of the Elbe estuary as well as the trophic position, $\delta^{15}N$ and $\delta^{13}C$ bulk and the $\delta^{15}N$ source amino acid values of the sampled smelt, sampled in summer and autumn 2021. The numbers in the boxes and their color show the correlation coefficient (blue = positive, red = negative). The boxes marked with a cross show values outside the significance level of 0.05, while the other boxes show significant correlations. Abiotic data were obtained from FGG Elbe data portal.

3.1.3 Discussion

The Elbe Estuary has been a place of continuous change for several decades. Starting with the purification of wastewater in the 1980s (Bergemann et al., 2010), which ensured that fish stocks especially smelt, and recovered (Thiel et al., 1995). However, as the port of Hamburg continued to expand and the size of the container ships continued to grow (HPA, 2024), the first deepening of the shipping channel had to be carried out as early as the 1990s (HPA, 2024). This continued throughout the 2000s and in the late 2010s the estuary was increasingly deepened and maintenance dredging is still being carried out today, both in the harbour basins and in the estuary's navigation channel (Boehlich et al., 2019; Hein et al., 2021). Further problems arise from continuing eutrophication, pollution, shipping traffic and tourism (Heise et al., 2024). All of these factors have either a direct or indirect effect on the food webs of the Tidal Elbe ecosystem, in addition to the very diverse environmental conditions that individual organisms face in the course of the estuary.

Eutrophication as a driver of changes in the food web

The Elbe and especially the harbour region in Hamburg, as well as some sections beyond, are still regarded as a major sink for nitrate, acting as biological reactors in which many different processes take place (Dähnke et al., 2022; Deek et al., 2013; Sanders et al., 2018). The drivers here are highly reactive organic particles that also contribute to turbidity in the later sections of the estuary (Dähnke et al., 2022). In particular, I am focussing on the last section of the harbour and the subsequent Mühlenberger Loch. Upstream of the harbour region, the estuary is characterised by high chlorophyll a concentrations and is mainly phototrophic, where the nutrients of the eutrophic Elbe play a major role in phytoplankton growth (Dähnke et al., 2022). The morphology of the Elbe changes with the beginning of the harbour basins, which were increasingly deepened. The current velocity increases, as does the water depth. The particles sink very quickly here and the photosynthetic capacity decreases (Kamjunke et al., 2023). The turbidity increases, mainly due to inorganic particles that have been washed up. This leads to light limitation for the autotrophic phytoplankton, which is deprived of its dominance (Dähnke et al., 2022; Sanders et al., 2018). The system changes from mainly autotrophic to heterotrophic. Other reactions increasingly take centre stage, mainly nitrification and denitrification (Norbisrath et al., 2022; Sanders et al., 2018). The fresh organic matter from the river with low C:N values as well as high PN and PC contents, that entered the estuary was rapidly degraded in the Port region (Dähnke et al., 2022; Schulz et al., 2023), causing the oxygen saturation and the pH value to fall (Kamjunke et al., 2023). In the course of the estuary to the mouth of the North Sea, the nutrient concentration and phytoplankton stabilise, albeit at a low level, with salinity and oxygen concentration increasing (Dähnke et al., 2022; Kamjunke et al., 2023). A key component to be considered in such variable systems as the Elbe estuary are the $\delta^{15}N$ values of the particles (POM or SPM) which act as drivers of the processes (Dähnke et al., 2022). Tracking these particles in the food web can reveal consequences of eutrophication or changes in trophic position (TP). This is because a change in the $\delta^{15}N$ values of these particles can be an indication of a change in the most important nitrogen source in the system. Such changes from, for example, nitrate to more reduced forms such as ammonium can be an indication of an increased occurrence of mixotrophic organisms (Glibert et al., 2016; Sommer et al., 2002). In the Elbe, we find high fluctuations in the TPs of organisms, especially in the smelt analysed, with the highest TP values in and around the Mühlenberger Loch. Importantly, these elevated TPs decrease when the $\delta^{15}N$ values of the source AA increase. The source AA in turn reflect the nitrogen signal of the base of the food web because they are influenced by the $\delta^{15}N$ values of the particles. Interestingly, we see both low bulk $\delta^{15}N$ particle and low source AA $\delta^{15}N$ values in the harbour region and in the Mühlenberger Loch, which increase as the estuary progresses. The TPs of the smelt, but also of the other fish, decrease with increasing $\delta^{15}N$ source AA values towards the North Sea. The pattern together thus identify two contrasting areas in the estuary: A) The region in the harbour and in the area downstream of it, which was characterised by heterotrophy, high nutrient turnover, low oxygen concentration, low bulk $\delta^{15}N$ in particle, low source AA $\delta^{15}N$ values in smelt and high TPs in smelt. And B) the region further downstream behind the Mühlenberger Loch up to Brunsbüttel and Cuxhaven, characterised by higher salinity, higher Chl a values (Dähnke et al., 2022), low source AA and particle $\delta^{15}N$ values and low TPs of the organisms in the food web. However, this general pattern applies with a few exceptions, namely in smelt. Although this species shows higher TPs in mainly in individuals caught at the Mühlenberger Loch, individuals were also caught in Brunsbüttel with TPs of up to 4.5, which is one TP unit above the average TP value of 3.5 from Froese et al. (2024). Thus, there appear to be two possible mechanisms in the Elbe estuary that influence food web structures. A first underlying explanation for these pattern in the Mühlenberger Loch was provided by the ΣV values of the smelt, which were significantly higher in the Mühlenberger Loch compared to the station near Brunsbüttel. This shows a clear signal of extensive microbial re-working of amino acids that extends into the smelt. In contrast, in large parts of the samples from Schwarztonnensand and Brunsbüttel, the ΣV values mainly point to a fresh source of amino acids. This points to a more heterotrophic system at the Mühlenberger Loch (Kamjunke et al., 2021) that was sustainable enough to influence the food web up to the level of smelt. The microbial food web in this area is characterized by nitrifiers, whose nitrification significantly impacts the N-budget by doubling the nitrate load of the river (Dähnke et al., 2008; Dähnke et al., 2022; Sanders et al., 2018). The $\delta^{15}N$ particle values show a change in the primary nitrogen source from nitrate to ammonium and the concentration of primarily autotrophic big phytoplankton like diatoms is decreasing (Kamjunke et al., 2023).

Phytoplankton is rather dominated by smaller autotrophs like picocyanobacteria, which are mainly grazed by ciliates and flagellates (Glibert et al., 2016; Sommer et al., 2002; Yamaguchi et al., 2017). This also changes the trophic structure of the microbial food web in favor organisms that can also adopt mixotrophic lifestyles thus they can switch between photo- and phagotrophy (Glibert et al., 2016; Sommer et al., 2002; Yamaguchi et al., 2017). Our data indicate that this indeed happened in the Mühlenberger Loch according to enhanced TPs above 1.0 (TP = 1.3) of the POM samples and $\varepsilon_{Phe/Glu}$ and $\varepsilon_{Phe/Ala}$ values below -4‰ and -5.5‰ respectively. Further, dominance of mixtrophic flagellates in this area around km 633, i.e. at the Mühlenberger Loch, in 2021 was confirmed by metabarcoding analysis (Martens et al., 2024). These organisms have enormous advantages through phagotrophy, especially in areas of the Elbe limited by turbidity, which can cover energy requirements better than pure autotrophy (Martens et al., 2024; Sommer et al., 2002). However, as some of the POM samples show, this leads to the trophic position of the phytoplankton or producers being lengthened by around 0.5 TP units, which in turn can affect all other subsequent levels in the food web. However, we were not able to prove that this will also elevate the mesozooplankton TPs as too many of the mesozooplankton samples were contaminated by POM. In the smelt in the Mühlenberger Loch, however, we could clearly see an increase in the TPs in conjunction with a decrease in the $\delta^{15}N$ particle values. Unfortunately, we were not able to prove that this also elevated the TPs in mesozooplankton as too many of the mesozooplankton samples were contaminated by POM. In smelt from the Mühlenberger Loch, however, there was a clear increase in TP in conjunction with a decrease in the $\delta^{15}N$ particle values compared to the downstream areas. Further, there was a correlation between the source AA $\delta^{15}N$ values and the TPs in smelt as well as between the source AA $\delta^{15}N$ values in smelt and $\delta^{15}N$ SPM values. In addition, there was a clear influence of microbial-resynthesis according to elevated ΣV values in smelt from the Mühlenberger Loch. Together these lines of evidence suggest that the microbial food web at Mühlenberger Loch was an important component of the food web relevant for smelt This has fatal consequences for the smelt population in the Elbe, because although the smelt in the area of the Mühlenberger Loch can still access a large proportion of copepods (BluEs, 2024), feeding in a food web that is trophically extended by an extensive microbial food web inevitably leads to less energy reaching the smelt as they have moved up a trophic level. In summary, the enhanced TPs of smelt at Mühlenberger Loch must be the result of the above described changes at the dietary base. However, this may also be one of the adjustments needed to give some of the smelt population a chance to stabilise again. However, this would require ensuring that the harbour and the Mühlenberger Loch lose their heterotrophic and reactive properties, which will prove difficult due to the ongoing expansion of the harbour (HPA, 2024). However, if this is to be achieved, massive re-naturalisation measures would have to be carried out and the nutrient load from the Elbe would have to be further reduced (Pein et al., 2021).

The smelt and the turbidity

As already mentioned, the Mühlenberger Loch is not the only region in the Elbe where elevated TPs were found in some of the smelt caught. TPs of 4 and higher were also found along the Schwarztonnensand and Brunsbüttel stations in individuals which, based on the $\delta^{13}C$ values, can also be clearly assigned to the regions with elevated salinity values and must have spent at least a longer period of time there. The estuarine maximum turbidity zone is located exactly between Brunsbüttel and Schwarztonnensand, which is also a hotspot for nitrification and a source of nitrate, although much lower than concentration of nitrate in the harbour area (Dähnke et al., 2022). In the course of the deepening and dredging works in the shipping channel as well as the harbour areas, the turbidity was increasingly exacerbated (Kerner, 2007). The high nitrification rates are caused by particle-bound nitrifiers and the fact that phototrophic phytoplankton, who compete with nitrifiers for ammonium, cannot tolerate the high light limitation and increased salinity and increasingly die off or are exchanged by organisms with mixotrophic tendencies (Martens et al., 2024; Sommer et al., 2002). However, turbidity can have effects on more than just phytoplankton and microorganisms. Zooplankton and fish can also be affected by high turbidity levels. Turbidity can have a particularly negative effect on fish and fish larvae. This is also the case for smelt in estuaries around the world. However, only above a certain level. As Illing et al. (2024) was able to confirm in their study, European smelt have an optimum level of turbidity in the water at which it can even be to their advantage. Turbid water is therefore a disadvantage for any piscivorous predators that see smelt and their larvae as prey (Pekcan-Hekim et al., 2013; Snow et al., 2018; Vollset et al., 2011). It offers the smelt an excellent hiding place in its important spawning grounds. The lower predation pressure also reduces the stress level of the animals, which also has a positive effect on the survival rate (Illing et al., 2024). On the other hand, turbidity can also have the aforementioned negative effects on smelt. For example, Hasenbein et al. (2013) showed that delta smelt in California's estuaries shows a reduced feeding rate at a turbidity levels of around 250 NTU, leading to starvation and weakening of populations in areas with increased turbidity. Illing et al. (2024) have shown that European smelt in the Elbe also exhibit increasingly lower survival rates and reduced feeding activity at turbidity levels of 300 NTU and above. Especially since the last major deepening of the channel and the associated maintenance dredging, there are regions in the Elbe estuary, where the turbidity continues to rise above the threshold value of 300 NTU ("Datenportal der FGG Elbe," 2024). The areas between Schwarztonnensand and Brunsbüttel, where the maximum turbidity zone is located and where turbidity values where well above 800 NTU in 2021, are particularly worth mentioning ("Datenportal der FGG Elbe," 2024). The high turbidity values could also explain the change in the feeding behaviour of the smelt, as BluEs (2024) showed, there was a change in the main food organisms from the smaller copepods, which still dominate at the Mühlenberger Loch, to larger gammarids or even mysids and other larger Crustacea, which are still easier to catch even at higher turbidity values. This would explain the wide range of TP values of the smelt, as gammarids in particular often feed on an omnivorous level (Dehedin et al., 2013; Pellan et al., 2016), while mysids and *Crangon crangon*, for example, show an omnivorous or carnivorous diet (Oh et al., 2001; Westheide et al., 1996). Thus, both the very low TP values and the very high TP values in smelt at Schwarztonnensand and Brunsbüttel can be explained without any sign of sustained heterotrophy at the food base. If the situation of the enormous turbidity values within the Elbe does not improve and further maintenance dredging is carried out, the system will remain in an unsustainable state and the turbidity will continue to remain at this high level (Kerner, 2007; Pein et al., 2023). Therefore, the TPs of the smelt will remain higher than it needs to be for a sustainable population. In combination with the trophic lengthening based on mixotrophy in the harbour area and the Mühlenberger Loch, it will continue to have enormous negative effects on the survival rates of smelt larvae as well as sub adult and adult smelt, as they are anadromous migrants and depend on passing through these areas in the Elbe to reach their spawning grounds. Just as the larvae and juvenile animals also migrate downstream in the course of their ontogenesis (Thiel et al., 1995) and thus also have to migrate through the affected areas again and often stay there for longer periods of time, as the $\delta^{13}C$ values of all age classes' show. The increased TPs due to turbidity and trophic lengthening can be sustainable enough to keep the energy level of smelt populations in the Elbe Estuary low and thus also reduce the spawning stock biomass and the survival rate of juveniles. In order to stop this, the Elbe estuary would have to undergo extensive restoration measures, such as the opening of oxbow lakes to allow meandering and the restoration of shallow riparian areas to further reduce current velocities but also to slow down the force of the flood flow, as this provides more volume to balance out this force and restore the actual estuarine character as it was before these oxbow lakes were cut off (Pein et al., 2023). This would lead to the flood current and ebb current being equalised. In the long term, this would promote the removal of dissolved sediments from the estuary and thus improve the turbidity caused by inorganic particles.

Feeding ecology of terns in the Elbe estuary

The various species of terns that live in and around the Elbe estuary are also directly linked to the smelt in the Elbe (Hennig et al., 2016). In particular, I focussed here on the Arctic and common terns, which occur on the islands of Neuwerk, Scharhörn and Nigehörn, which lie in the immediate outer area of the Elbe estuary. The terns are record holders among migratory seabirds and cover thousands of kilometres on their journeys (Egevang et al., 2010). Nevertheless, the breeding success of the population in the Elbe estuary depends on the local conditions, as the animals have to provide their young with food from the surroundings of their breeding area (Dänhardt et al., 2011). However, there are differences between the Arctic tern and the common tern. In 2015, the common terns favoured smelt as their main prey organism in the Elbe estuary with almost 100%, while the Arctic terns prefer a more balanced diet with equal proportions of smelt and herring and a higher proportion of invertebrates (Hennig et al., 2016). The terns are restricted to a relatively small hunting radius of between 4 and 6 kilometres around their breeding grounds. Internationally, the terns are not listed as endangered (IUCN, 2024), but on the basis of the German red list (Grüneberg et al., 2015) they are listed as endangered and the common terns even as threatened with extinction. Populations have been in decline for several years, partly due to a redistribution of breeding pairs from other islands to Neuwerk, but also due to other factors that influence the populations, such as breeding success, food supply, predation and human influence (BluEs, 2024) It has long been suspected that the decline has a lot to do with the decline of smelt in the estuary, as the numbers of terns continued to fall with the collapse of smelt populations in the Elbe (BluEs, 2024). However, my data showed something different for the Arctic tern. The $\delta^{13}C$ values almost consistently showed a marine dietary source originating from the Wadden Sea or at least the downstream end of the estuary, with three exceptions, where the $\delta^{13}C$ signatures must have originated from approximately the centre of the estuary. The majority of the species sampled (all but one of which were Arctic terns) thus must have hunted in marine waters and fed their young from these areas. In marine waters, terns appear to actively select prey organisms and only feed high quality food to the young and mating partners, while low-quality food is eaten on site (Dänhardt et al., 2011). In the tern samples from 2021, the $\delta^{15}N$ values of source AA and bulk $\delta^{15}N$ were largely decoupled from those of the fish species in the inner estuary, assuming an enrichment of 3.4‰ per trophic level. The bulk $\delta^{15}N$ values in the birds were much lower than those of the stint, which indicates that the terns on Neuwerk had feed on a different, largely marine food source. The highly variable TP values can be explained by terns that fed on a broader dietary base including *Crangon crangon* and various fish species, as has been observed in other studies (Dänhardt et al., 2011). This means either that the decline in Arctic terns does not coincide with the decline in smelt or that the decline in smelt has already affected the terns on Neuwerk to such an extreme extent that they have inevitably had to switch to another food source in the marine area, even if this is of poorer quality and/or lower quantity. However, the significantly increased ΣV values in terns indicated for a dietary source that was characterised by microbial re-synthesis but which did not originate from the harbour region. The widely scattered TP values also indicated a rather diverse food source. For example, the high ΣV values may have come from the diet of detrivores crustaceans such as *Crangon crangon* which forage in the sediments, and the elevated TP values in the terns may be due to the incorporation of fish from a higher trophic level than smelt such as whiting, which indeed were very abundant in the estuary region in 2021 (BluEs, 2024). Whiting, are larger, piscivorous, gadoid mesopredators that can show trophic positions of 4.4 to 4.7 thus well above the TP of smelt (Fig 11, Froese et al. (2024). Further studies will be necessary to determine exactly which food source the terns of Neuwerk rely on. This may provide a further indication of why the TP values vary so much and why the populations ultimately continue to decline over time.

Stressors and Trophic structures of the Elbe estuary

The Elbe estuary has been characterised by changes since the first human settlements. However, these only became particularly influential in the period from the last century to the present day. In particular, the discharge of waste water, heavy over-fertilisation (Bergemann et al., 2010; Dähnke et al., 2022) and the ongoing expansion of the waterways to provide enough space for the increasing size-classes of ships (HPA, 2024). All of these factors have had an impact on the ecosystem and the populations of animal and plant species. The latest analyses show how strongly stressors affect the food web in some cases. Here, too, as signs of trophic lengthening became apparent caused by changes at the base of the food web and mixotrophy or by the introduction of new species. These scenarios have also been observed in other estuaries around the world and require intervention in order to find the quickest possible ways to stabilise food webs and ecosystems. Analyses from an estuary in California have shown that this can work if appropriate measures (e.g. reduction of nutrient intake or reduction of turbidity) are implemented (Glibert et al., 2022). For the Elbe estuary, too, there is still a period of time in which something can be changed, changed, as there still are smelt in the estuary and terns on its islands.

3.2 Trophic structures in the Oder estuary

3.2.1 Nitrogen turnover in the Oder estuary

Most of the nutrients carried by the river Oder as the second largest drain into the Baltic Sea (Korth et al., 2013; Voss et al., 2006) and those that enter the estuary from diffuse sources, initially remain in the Szczecin Lagoon for some time because of the long residence time of water in the lagoon (BluEs, 2024). The majority of the processes are driven by nitrate assimilation by phytoplankton during a large spring bloom (Hellemann et al., 2017). When this bloom begins to die, its particles sink to the bottom and nitrification and bottom-bound denitrification occur (Bartoli et al., 2021). A large amount of nitrogen is lost through these processes. As a result, a significant less amount of nitrogen from these processes will ultimately reach the central Baltic Sea. These estuarine systems therefore act as coastal filters and retain a large proportion of the nitrogen that would otherwise have entered the Baltic Sea directly without further transformation (Asmala et al., 2017). However, whether this stability of the nitrogen regime in the Oder estuary causes a stability of the food web has not yet been further investigated.

3.2.2 Results

The results of this chapter are based on the materials and methods described in detail in the Oder estuary-specific subchapters 2.1 and 2.2.2 as well in the more general materials and methods subchapters 2.4 – 2.7.

Bulk $\delta^{15}N$ and $\delta^{13}C$ values of the Oder estuary

The bulk δ^{15}N and bulk δ^{13}C data in the Oder are quite widely distributed. The POM and zooplankton data range from -31.06‰ to -18.13‰ in the bulk δ^{13}C values. But also in the bulk δ^{15}N values from 3.12‰ to 18.17‰ (Fig. 22).

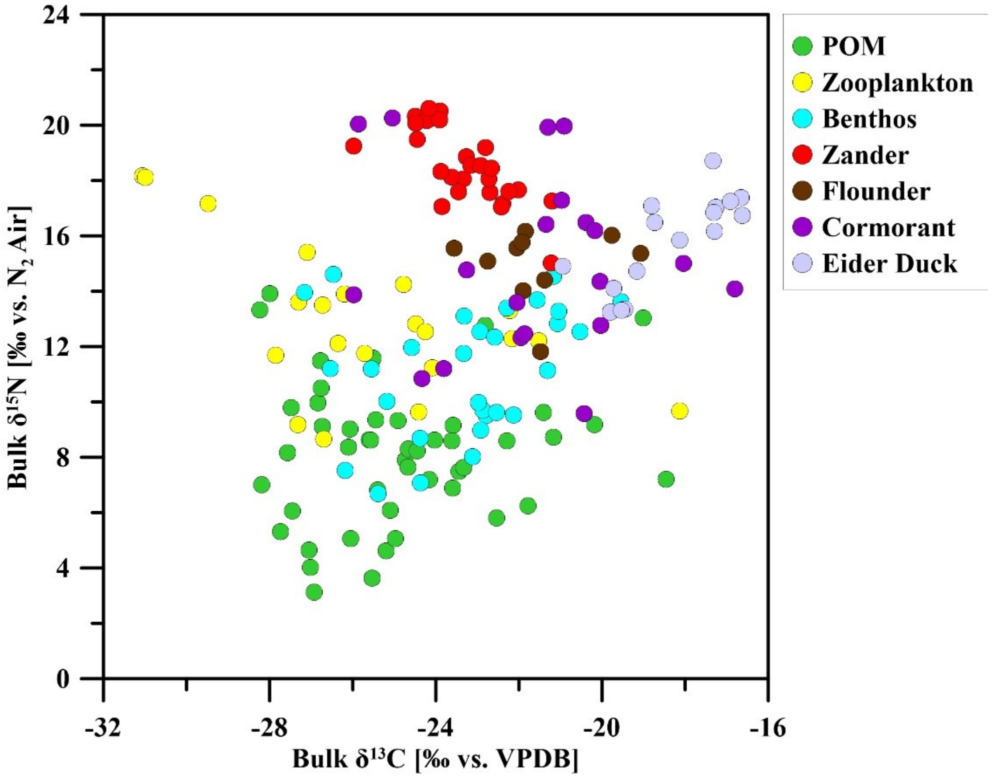


Figure 22: Bulk $\delta^{15}N$ values vs. salinity proxy (bulk $\delta^{13}C$ values) of sampled POM, Zooplankton, Benthos, Zander, Flounder, Cormorant and Eider Duck from the Oder estuary.

The calculated TP values of the sampled organisms in the Oder estuary were all within the TP values shown in the literature (Froese et al., 2024; Kiljunen et al., 2020; Loick-Wilde et al., 2019; Mittermayr et al., 2014) for this region or neighboring regions (Fig 23). There were no significant differences within the individual organism groups.

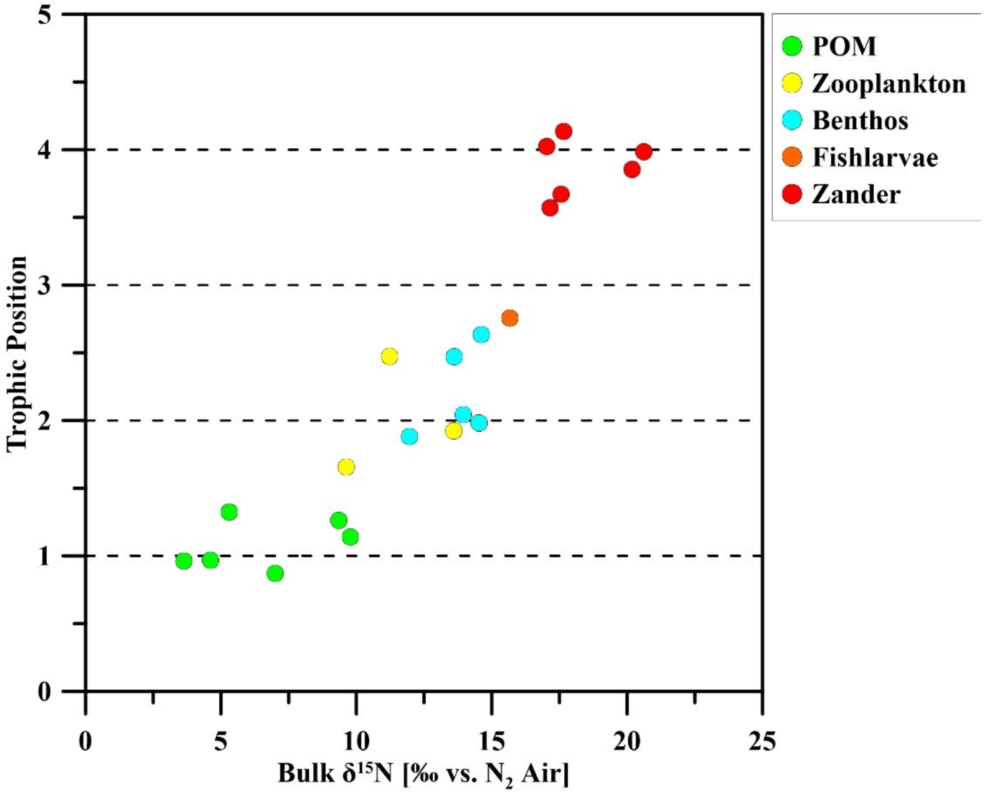


Figure 23: Calculated trophic position of POM, zooplankton, benthos, fish larvae and zander samples form the Oder estuary plotted against the nitrogen source proxy (bulk δ^{15}N). In both zooplankton and benthos, samples with very low TP (< 1.5) hat to be excluded, indicating that they were contaminated by POM or phytoplankton.

3.2.3 Discussion

Nitrogen sources of the Oder estuary

The POM samples of this study clearly reflect the δ^{15}N nitrate values of the spring bloom (4-14‰) and do not vary significantly along the δ^{13}C salinity proxy. It is also very clear that the subsequent parts of the food web also show the signal of the same nitrate (Korth et al., 2013), assuming an enrichment of about 3.4‰ per trophic step (Chikaraishi et al., 2009; DeNiro et al., 1981; Minagawa et al., 1984; Post, 2002). The nitrogen assimilated by the spring bloom is therefore actively processed and transported through the food web. An exception seem to be some of the seabirds, especially eider ducks, which show a higher δ^{13}C signature, but this can be explained by the fact that their core area is defined as relatively small (Beuth et al., 2017) This would mean that they do not stray too far from the mussel beds near the Greifswalder Oie and thus rarely come into contact with the spring blooms in the Szczecin Lagoon. The situation is similar with some cormorants, but due to their ecology as fish eaters they still cover a wide radius, even within the Baltic Sea, and can therefore also "carry off" other signatures (Grémillet et al., 1998). The zander sampled show a fairly uniform pattern in their signatures, which are somewhat decoupled from the rest of the sampled organisms, suggesting that we do not match their actual food source with our measurements, which seems logical, as their prey consists mainly of smaller fish that have not been sampled (Froese et al., 2024). The sampled flounders showed slightly elevated $\delta^{15}N$ source values compared to the animals measured in the central Baltic Sea (Steinkopf et al., 2024), indicating that the animals in the Oder estuary must have mainly fed on mussels that filtrated POM and phytoplankton from the spring bloom. This would also mean that we cannot find the strong fractionation signals here that we found in some of the flounders in the central Baltic Sea. However, further studies are needed here in order to precisely determine the sources of amino acid values and the trophic positions of the flounders and ultimately to be able to compare them with those from the central Baltic Sea. The analysed mussel species also clearly show the signal of the phytoplankton bloom while some of the measured poly- and oligochaetes in the benthos show signs of fractionation caused by the processes in the sediment like nitrification and denitrification (Bartoli et al., 2021). In summary, it can be said that the food web in the Oder estuary is strongly characterised by the spring bloom in the phytoplankton and the associated assimilation of nitrate. This reservoir of nitrogen appears to be sustainable enough to maintain the system in its current state.

Trophic structures of the Oder estuary

The few compound-specific nitrogen isotope samples of amino acids analysed show a clear picture of the Oder estuary. We found a well-structured food web based on the spring bloom in the phytoplankton, which can be fed on very well by the zooplankton and thus mass and energy can be transported further up the food web. Surprisingly, we found no signs of trophic lengthening in the pelagic food web, despite the enormous eutrophic conditions in this area. The trophic positions of the organisms are stable at a level expected from literature data (Froese et al., 2024; Kiljunen et al., 2020; Loick-Wilde et al., 2019; Mittermayr et al., 2014). This indeed confirms part of my first hypothesis, according to which the Oder estuary is largely not characterised by trophic lengthening. A further indication of this is the food web that continues to rely on nitrate, which can utilise the nitrogen introduced by river nitrate very efficiently and so there is no sustained change in the primary N source that would trigger changes in the functional diversity at the base of the food web (Glibert et al., 2016). On the one hand, this finding may show, that the estuarine ecosystem of the Oder is fairly stable for the moment and can continue to maintain its function as a coastal filter (Asmala et al., 2017). This is especially true for the Szczecin Lagoon, as it has a very shallow water depth and a wind-driven mixed layer with high oxygen concentrations. In view of the continuing eutrophication of the water body via the Oder and other diffuse sources, as well as ongoing climate change that leads to higher surface temperatures and extreme events, which leads to the fact, that the estuary remains at a tipping point (Conversi et al., 2015). One such trigger could be a prolonged period of calm winds and heatwaves, which may occur more frequently due to climate change (Frölicher et al., 2018; Laufkötter et al., 2020). The higher temperatures that follow, together with a lack of mixing of the water masses and emerging stratification, can lead to oxygen depletion and subsequent anoxia ((Kache et al. sub.). This in turn would promote anaerobic processes and thus lead to a change in nitrate turnover. Similar to the Elbe, this scenario would also be predestined for heterotrophy and associated changes in the food web structure. This could lead to the estuary to lose its function as a coastal filter (Asmala et al., 2017). This in turn will have enormous consequences for the Baltic Sea, as even more nutrients will now reach the central Baltic Sea and exacerbate the existing problems there (Carstensen et al., 2014; Eglite et al., 2019; Loick-Wilde et al., 2019; Savchuk, 2018; Steinkopf et al., 2024). For this reason, it is necessary to take appropriate measures now to protect coastal areas and estuaries along the Baltic Sea. In addition, further studies with compound-specific analyses in amino acids should be carried out on other organisms such as seabirds, smaller planktivorous fish species like smelt, three-spined stickleback, herring or sprat and fish larvae to see whether the food web is as stable in its other parts, as our data suggest.

3.3 Cyanobacteria induced trophic lengthening in the central Baltic Sea

The results of this chapter are part of the manuscript "Trophic lengthening triggered by filamentous, N_2-fixing cyanobacteria disrupts pelagic but not benthic food webs in a large estuarine ecosystem" by M. Steinkopf, U. Krumme, D. Schulz-Bull, D. Wodarg and N. Loick-Wilde. This article is published in Ecology and Evolution, 2024, 14(2), e11048, DOI: 10.1002/ece3.11048. My own share to this work is 90%, which includes sampling conception, partial sampling, analysis of the fish samples in the laboratory, manuscript conception and preparation of text and images, which were then reviewed by the co-authors.

3.3.1 Introduction

Blooms of filamentous, N_2-fixing cyanobacteria (FNCs) are predicted to increase in the future due to rising sea surface temperatures, increased seasonal stratification but also due to nearshore eutrophication, namely phosphorous (Hallegraeff, 1993; Joehnk et al., 2008; Kahru et al., 2020; Paerl et al., 2008; Viitasalo et al., 2022; Wurtsbaugh et al., 2019). Hot spots of coastal FNCs occur around the globe, e.g. in the Pacific off Australia (Bolch et al., 1999; Ani et al., 2023; Bell, 2021; Hallegraeff et al., 2021), the Atlantic off the Canary Islands (Benavides and Aristegui, 2020), the southern South China Sea off Vietnam (Tang et al., 2004), the Arabian Sea off India (D'Silva et al., 2012), or the Baltic Sea (O'Neil et al., 2012) but sometimes also in the Mediterranean Sea (Rahav and Bar-Zeev, 2017). FNCs are not inherently toxic, but they are rarely grazed upon directly. Importantly, FNCs change the phytoplankton species composition so that availability of directly palatable autotrophic species decreases in favour of a massive heterotrophic microbial system. Thus, in an ecosystem FNCs can cause a directly measurable increase in the TPs of mesozooplankton, we call trophic lengthening (Paerl, 1988; Mulholland, 2007; Loick-Wilde et al., 2019). Since FNCs are not top-down controlled and remain in surface waters due to their robust gas vacuoles even after microbial degradation has started (Loick-Wilde et al., 2018; Mulholland, 2007), carnivory, rather than herbivory, dominates in FNC-affected mesozooplankton communities, particularly during aging and decaying FNC blooms (Loick-Wilde et al., 2019; Weber et al., 2021).

As evidence for trophic lengthening in FNC-affected mesozooplankton communities is mounting, there is a growing need for understanding the effects on the productivity of higher trophic levels like fish. So far it is unclear, whether FNCs and the associated trophic lengthening in mesozooplankton, caused by an increasing influence of the microbial system, also affects the TPs of commercially important fish species like cod and flounder. Any increase in the TPs of fish species would have massive negative consequences for the stock productivity because trophic lengthening directly reduces the efficiency of energy transport to fish (Paerl, 1988; Mulholland, 2007; Reichle, 2023).

The TP of fish and the inorganic nitrogen source (N source) supporting fish production can now be determined empirically from different field locations using compound-specific analysis of stable nitrogen isotopes in amino acids (CSIA). The strength of CSIA lies in providing information on both TP and N sources from a single fish sample, which is achieved with a comparison of the $\delta^{15}N$ values of two different groups of amino acids (AA), the source AAs and the trophic AAs. While trophic AAs are enriched in ^{15}N by ~8.0‰ per trophic transfer (Chikaraishi et al., 2009), the $\delta^{15}N$ of source AAs remain nearly unchanged when the AA is transferred through the food web and thus they reflect the isotopic composition of the primary producers (N-source measure) (Chikaraishi et al., 2010; McClelland and Montoya, 2002; Steffan et al., 2015). With the $\delta^{15}N$ ratio of both amino acid groups and the trophic discrimination factor (TDF) that is specific for different groups of organisms, the TP of vertebrates like fish can now be calculated empirically with an accuracy of 0.1-0.2 units (Bradley et al., 2015; McMahon et al., 2015; Brault et al., 2019).

Sea areas where nitrogen from N_2 fixation (diazotroph nitrogen) is an important new N source for biological production have in common that the isotopic signature of nitrogen in thermocline nitrate, which is the other important new N source in the ocean besides N_2, uniquely reflects the impact of N_2 fixation on a habitat or ecosystem (Sigman and Fripiat, 2019). This is the case e.g. in the subtropical Pacific (Casciotti et al., 2008), subtropical Atlantic (Knapp et al., 2005), or central Baltic Sea (Voss et al., 2005; Korth et al., 2014). These areas have in common that the $\delta^{15}N$ value of thermocline nitrate is around 3.6‰ or lower. The strong N-source proxy quality of the $\delta^{15}N$ of phenylalanine and other source amino acids allow for an end-to-end quantification of inorganic nitrogen sources like diazotroph nitrogen or thermocline nitrate fuelling new production up to the trophic position of top carnivores (McMahon and McCarthy, 2016; Ohkouchi et al., 2017; Glibert et al., 2019).

Stable isotope data, when considered alone, can lead to misinterpretation as the same isotopic value can reflect different processes (see "Fretwell's Law" in Kendall and Caldwell, 1998). To ensure that evidence from stable isotopes is useful, patterns in stable isotope data must be supported by other oceanographic, biogeochemical, and population ecology information from the ecosystem (Kendall and Caldwell, 1998; Fry, 2006). A well-studied example and model for the biogeochemical and ecological effects of FNCs is the Baltic Sea (Elmgren, 2001; Voss et al., 2005; Reusch et al., 2018). The Baltic Sea is a postglacial, semi-enclosed, heavily exploited, microtidal, brackish-water Sea that experiences numerous anthropogenic pressures, especially eutrophication (Conley, 2012; Voss et al., 2011). Despite high nutrient loads, during the midsummer period, the dissolved nitrate pools in surface waters are largely depleted, but not the phosphorus pools. Under these conditions, cyanobacteria are competitively superior to other algae leading to massive blooms of unpalatable FNCs that frequently occur in the central Baltic Sea (Kahru et al., 2020; Karlson et al., 2015; Wasmund, 1997). Their N_2 fixation is the second largest source of new N for the Baltic Sea, adding 370-926 kt N yr^{-1} into the central Baltic Sea during summer (Voss et al., 2005). The largest new N source for the Baltic Sea is anthropogenic N, e.g. from terrestrial run-off via rivers like the Odra or Vistula. Rivers transport on average 830 kt N yr^{-1}, mainly as nitrate, into coastal waters around the entire Baltic and the relatively shallow western Baltic Sea, especially in winter and spring (Voss et al., 2005; Kuliński et al., 2022; Reckermann et al., 2022). The spatial and temporal dichotomy of N_2 fixation and anthropogenic N inputs between the central and western Baltic Sea go along with the other contrasting ecological and biogeochemical differences, which are highly relevant to understand the dynamics in the trophic position and nitrogen supply of adult fish species in the two areas of the Baltic Sea:

1. Phytoplankton quality: Unpalatable FNCs dominate the new production in the central Baltic Sea in summer (*Nodularia spumigena*, *Aphanizomenon* sp.), whereas palatable phytoplankton like diatoms (namely *Cerataulina bergonii, Rhizosolenia* spp., *Skeletonema marinoi*) and dinoflagellates dominate new production in the western Baltic Sea during spring and fall (Neumann, 2010; Wasmund et al., 2011; Zettler et al., 2020; Dutz et al., 2022).
2. TP of mesozooplankton: Omni- and carnivorous mesozooplankton with a TP of 2.5 to 3.0 dominates in the central Baltic Sea due to unpalatable FNC blooms and the associated massive heterotrophic microbial system, while herbivorous mesozooplankton with a TP of 2.0 dominates in the western Baltic Sea and nearshore areas given that the phytoplankton is palatable (Loick-Wild et al., 2019).
3. $\delta^{15}N$ values in water-column-nitrate and sediments: In the central Baltic Sea, N_2-fixation causes low $\delta^{15}N$ values of 3.6 ± 1.0‰ in thermocline nitrate and surface sediment particles. In the western Baltic and coastal areas, anthropogenic N causes high $\delta^{15}N$ values of 7.2 ± 0.9‰ to 7.9 ± 1.8‰ in thermocline nitrate and surface sediment particles (Voss et al., 2005;

Korth et al., 2014). Here we used the δ^{15}N of the source amino acids in samples from adult cod and flounder to dedicate the samples to a habitat either largely influenced by N_2 fixation or inputs of anthropogenic N. Specifically, the δ^{15}N of source amino acids were used to identify how tightly the fish are associated with a thermocline nitrate pool that is largely influenced by either of the two N sources (N_2 or anthropogenic N).

Altogether, this makes the Baltic Sea an ideal model ecosystem to test the hypothesis that trophic lengthening in mesozooplankton due to a massive heterotrophic microbial system triggered by FNCs is transferred also to organisms from higher trophic levels like fish that are associated with the pelagic and benthic food webs.

To verify this hypothesis, our analysis focuses on individuals of cod and flounder from populations in the western and central Baltic Sea. Both species rely on new production of phytoplankton for growth, but depending on the location of their population, they rely on very different food webs to receive the energy. In the Baltic, flounder mainly use shallow coastal areas and only migrate into deeper waters for spawning in the first quarter of the year. They use relatively small foraging areas (e.g. Dando et al., 2011) and mainly feed on bivalves (Haase et al., 2020). Since bivalves unselectively filter-feed on any sedimenting phytoplankton, including cyanobacteria, flounders are unlikely to be affected by FNC triggered trophic lengthening in more selectively-feeding mesozooplankton, as most of the emerging microbial food web around FNCs will not sink down as the FNCs do after some time (Mulholland et al., 2007). Therefore this key part of trophic lengthening should not appear in the benthic food web. Cod is a flexible predator preying on invertebrates and fish and can move large distances. In the western Baltic, the cod diet is dominated by the common shore crab *Carcinus maenas* (Funk et al., 2021). In contrast, cod in the central Baltic Sea mainly feed on pelagic fishes like *zooplanktivor*ous sprat and herring (Kulatska et al., 2019; Haase et al., 2020). It is likely that cod from the central Baltic Sea display a higher TP than cod from the western Baltic due to both, different foraging strategies and trophic lengthening in mesozooplankton triggered by FNCs, as both processes are limited to the central Baltic (Loick-Wilde et al., 2019).

Here, for the first time, we can distinguish between spatial and species-specific changes in the inorganic N source supporting the fish production in relation to changes in trophic structure in pelagic and benthic food webs in the Baltic Sea. We use δ^{15}N-AA measurements from samples of demersal fish species differently associated with the pelagic and benthic food webs in their areas to resolve a major issue concerning the impact of trophic lengthening triggered by FNCs on marine and estuarine food webs.

3.3.2 Results

The results of this chapter are based on the materials and methods described in detail in the Baltic Sea-specific subchapters 2.1.3 and 2.3 as well in the more general materials and methods subchapters 2.4 – 2.7.

Bulk $\delta^{13}C$ in cod and flounder

The bulk $\delta^{13}C$ values in cod and flounder from the central Baltic Sea were significantly lower than the values of conspecifics from the western Baltic Sea (t-test, cod: p= 0.1e-03; flounder: p=1.7e-05, Fig. 24). However, flounder covered an overall larger $\delta^{13}C$ range (-23.5‰ to -17.2‰) compared to cod (-21.7‰ to -19.3‰), both in the western and central Baltic Sea.

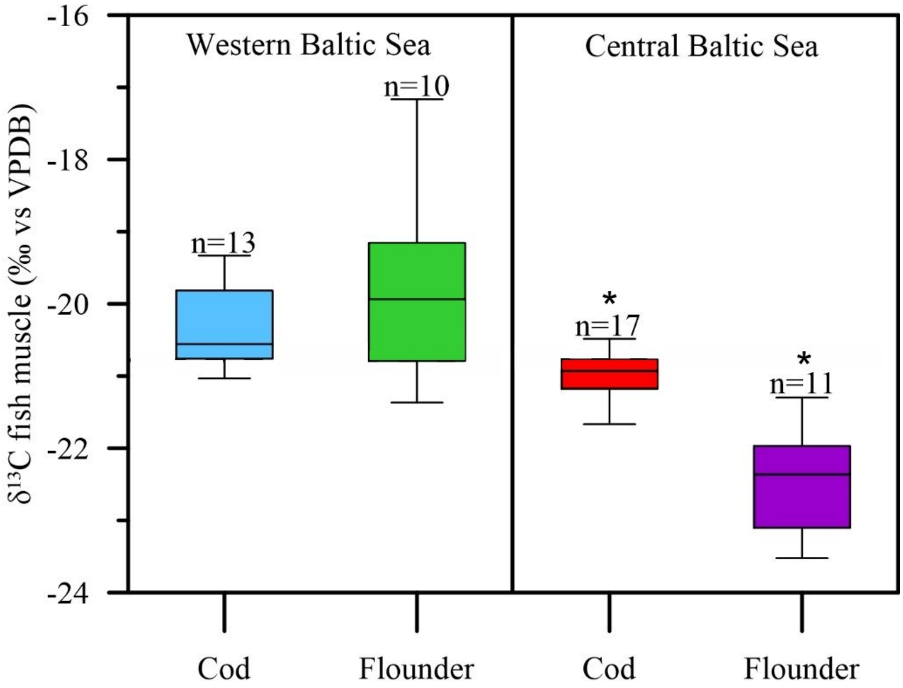


Figure 24: Average $\delta^{13}C$ values of cod and flounder muscle tissue samples from the western and central Baltic Sea. Number of samples are given above each group (Steinkopf et al., 2024).

Environmental influence of benthic and pelagic food web structure

The N source measure $\delta^{15}N$ of source AAs and the food web structure measure TP from the best fitting TP model were identified for both fish species and plotted against each other (Fig. 25). Interestingly, $\delta^{15}N$ values of the source AAs of cod varied strongly and indirectly with TP ($r^2 = 0.63$). This is in contrast to flounder, which displayed no significant relationship between TP and the N source measure ($r^2 = 0.26$).

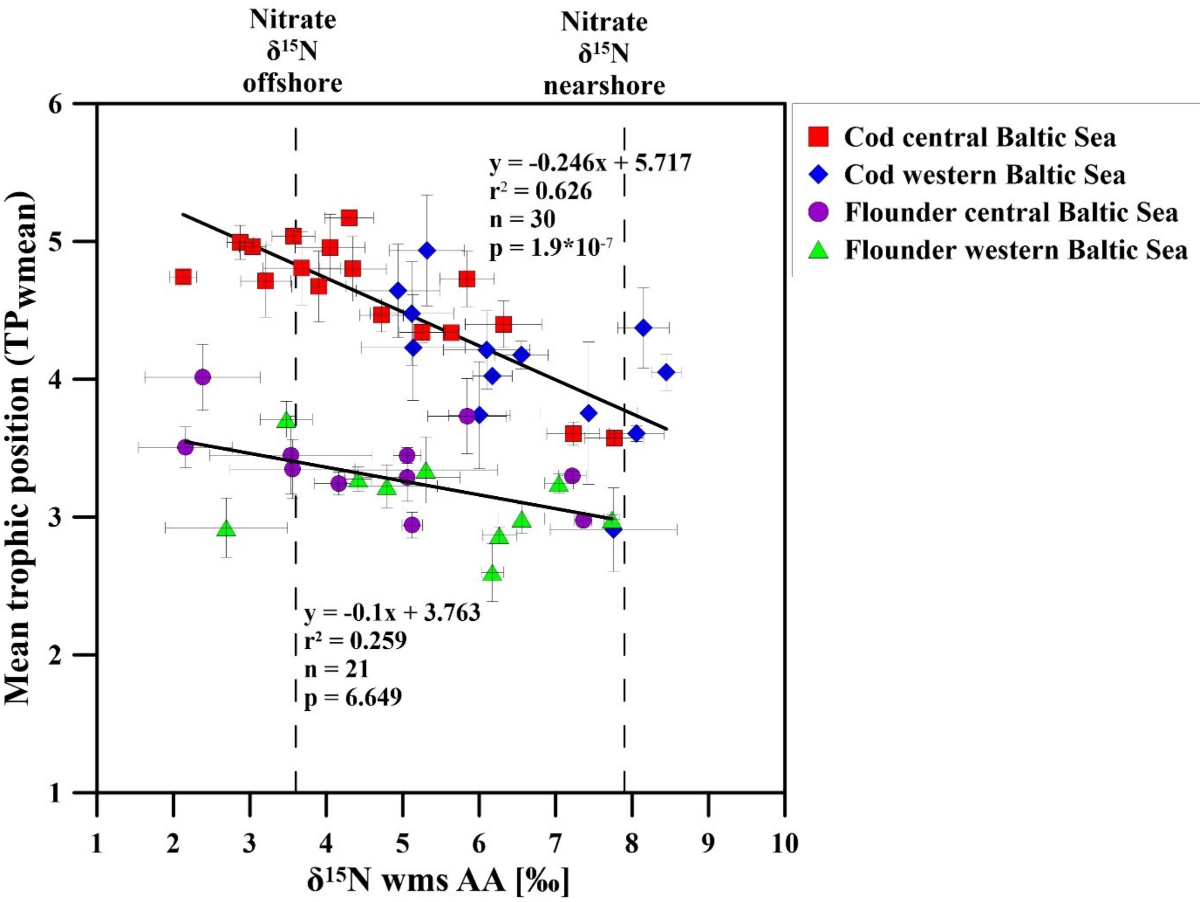


Figure 25: Mean trophic position (TP_{wmean}) of cod and flounder as a function of the nitrogen source proxy $\delta^{15}N_{wms}$ AA ($\delta^{15}N$ values of the weighted mean source amino acids, in ‰) from the western (blue: cod, green: flounder) and central (red: cod, purple: flounder) Baltic Sea. Dashed lines: $\delta^{15}N$ nitrate values influenced by N_2 fixation (offshore central Baltic Sea) and by anthropogenic nitrogen (nearshore western/coastal Baltic Sea). Vertical/horizontal error bars indicate uncertainties in TP and the wms AA (Steinkopf et al., 2024).

Furthermore, a clear difference in the N source measure $\delta^{15}N_{wms}$ was found in cod, with significantly lower $\delta^{15}N_{wms}$ values of cod in the central than in the western Baltic Sea (t-test, p = 7.4e-06, Fig. 25). Specifically, in the central Baltic, $\delta^{15}N_{wms}$ values of cod ranged from 2.1‰ to 7.8‰, with a mean of 4.6‰ (sd ± 1.5, n = 17). For cod in the western Baltic Sea, the $\delta^{15}N_{wms}$ values ranged from 4.9‰ to 8.4‰, with a mean of 6.8‰ (sd ± 1.3, n = 13). The $\delta^{15}N_{wms}$ values of cod from the central Baltic closely resembled the $\delta^{15}N$ endmember values for food webs with major N_2 fixation (nitrate $\delta^{15}N$ offshore, Fig. 25), while in the western Baltic they reflected major anthropogenic N inputs (nitrate $\delta^{15}N$ nearshore, Fig. 25).

The difference in the N source measure was accompanied by a clear difference in the TP of cod with significantly higher TP values for cod in the central Baltic Sea than in the western Baltic Sea (t-test, p = 0.004, Fig. 25). The mean TP for cod from the western Baltic Sea was

4.1 ± 0.5 (n = 13), which was, as calibrated, very close to the SIA-based western Baltic TP mean of 4.1 ± 0.2 (B Deutsch et al., 2006; Mittermayr et al., 2014; Mohm, 2014;2018) and also congruent to the global mean TP of cod (Froese and Pauly, 2022), but with a standard deviation twice as high as previously reported. Cod from the western Baltic Sea thus showed no impact of trophic lengthening but a higher plasticity in the TP value than the global mean. In strong contrast, the mean TP of cod from the central Baltic Sea was much higher at 4.6 ± 0.4 (n=17) and thus clearly above the global mean of 4.1 ± 0.2 (Froese and Pauly, 2022). In summary, the TP estimates robustly showed that the mean TP of cod from the central Baltic Sea was significantly 0.5 TP units higher than the TP of cod from the western Baltic Sea.

In flounder, differences in the N source measure $\delta^{15}N_{wms}$ between the two areas did not largely affect the TP. Interestingly, the $\delta^{15}N_{wms}$ values in flounder varied widely and had a similar range (Fig. 25), both in the western Baltic (range: 2.7-7.7‰; mean: 5.6 ‰ ± 1.5 ‰, n = 10) and in the central Baltic (range: 2.2-7.4‰; mean: 4.5‰ ± 1.8‰, n = 11). Thus, unlike cod, no direct assignment of individual flounder to the two areas was possible based on the N source measure $\delta^{15}N_{wms}$. The TP of flounder also did not significantly differ between areas (t-test, p = 0.06) and the mean TP value of 3.1 ± 0.3 was similar to the western Baltic mean TP value of flounder (Mittermayr et al., 2014; Mohm, 2014;2018) Froese and Pauly, 2022).
A difference between flounder from the western and the central Baltic Sea only emerged when TP was plotted against the salinity proxy $\delta^{13}C$ (Fig. 26).

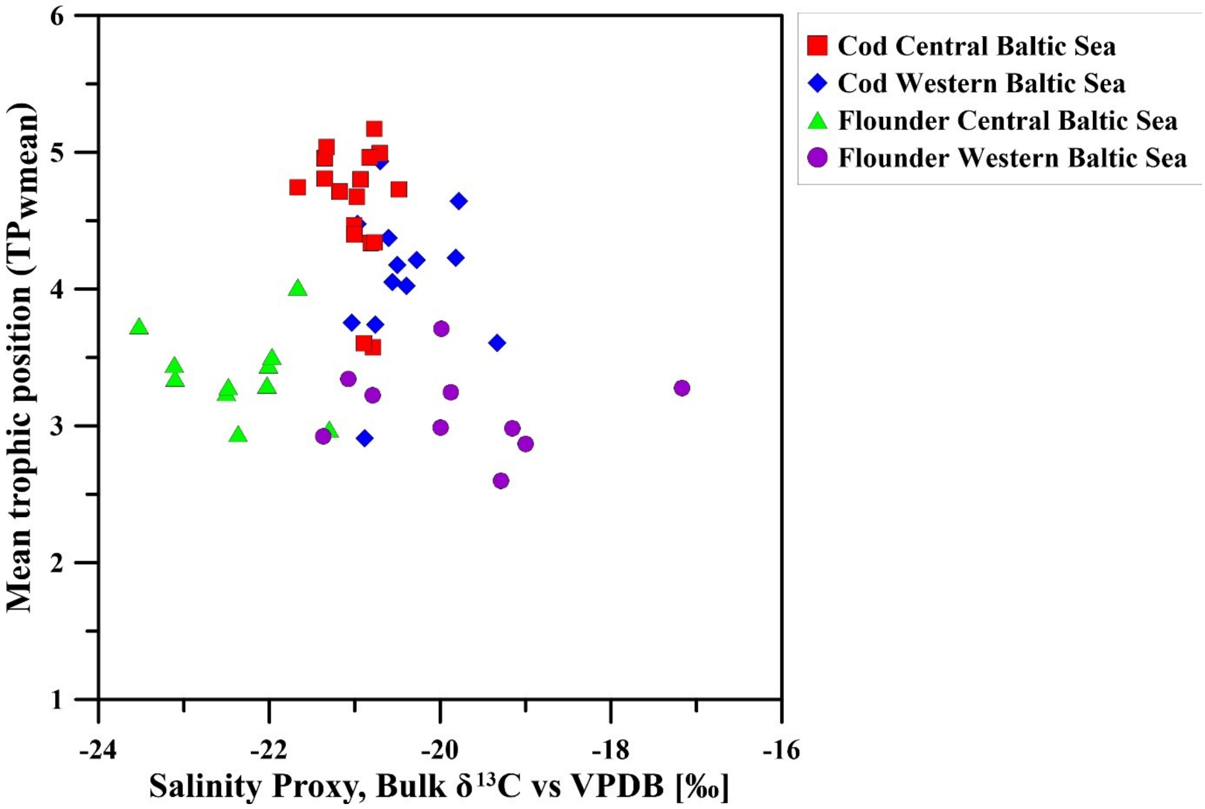


Figure 26: Mean trophic position (TP_{wmean}) of cod and flounder as a function of bulk $\delta^{13}C$ (in ‰) from the western (blue: cod, green: flounder) and central Baltic Sea (red: cod, purple: flounder (Steinkopf et al., 2024)).

3.3.3 Discussion

The recent quantification of trophic lengthening in mesozooplankton triggered by FNCs from the central Baltic Sea revealed a potentially important process of energy loss for commercial fish production, but the complexity and long turnover times of adult fish pose significant challenges for quantifying any impact of FNCs on their TP. We applied TP models based on empirical amino acid nitrogen stable isotope data to determine the N source and TP of individual fish from two Baltic Sea areas differently impacted by FNCs. Our data provide the first direct measurements of the N source and the TP in flounder and cod showing that trophic lengthening triggered by FNCs causes a significant increase in the TP of cod in the central Baltic Sea, while flounder, although caught in the same trawl hauls, is not affected.

New nitrogen source and role of export production for fish in the Baltic Sea

The majority of cod caught in the Mecklenburg Bay had clearly relied on the isotopically heavier, shallow-water food web of the western Baltic with anthropogenic N inputs and high $\delta^{15}N$ values in thermocline nitrate. In contrast, the majority of cod caught in the Arkona and

Bornholm Basins were linked to the isotopically lighter pelagic food web of the central Baltic with new N inputs from a thermocline nitrate pool that is rather impacted by diazotroph N.

Different to thermocline nitrate, diazotroph nitrogen per se was no important N source for adult cod in the central Baltic Sea. Here we define diazotroph N as inorganic and organic N species that directly originate from the process of N_2 fixation in cyanobacteria. Diazotroph N can have the molecular structure of inorganic N like ammonium or nitrate as well as of organic N forms like amino acids. Diazotroph N in any molecular form has in common that its isotopic $\delta^{15}N$ signature is 0‰ or lower as in case of diazotroph phenylalanine, which has a $\delta^{15}N$-Phe of -3.6‰ in N_2-fixing marine cyanobacteria (McClelland et al., 2003; Eglite et al., 2018). In Fig. 25, only four out of 17 cod individuals from the central Baltic Sea had $\delta^{15}N_{wms}$ values somewhat below 3.6‰ pointing to a weak share of diazotroph N in their tissues, while the majority had $\delta^{15}N_{wms}$ values close to the endmember value of 3.6‰ as for thermocline nitrate in the central Baltic Sea (Korth et al., 2014). Thus, no imprint of diazotroph nitrogen but of thermocline nitrate originating from the remineralization and nitrification of compounds originating from N_2-fixing cyanobacterial biomass (Korth et al., 2014) was found in cod from the central Baltic Sea. Finding the temporally integrated isotope signal of thermocline nitrate impacted by diazotroph N rather than of diazotroph N per se in the animals is not surprising. The share of diazotroph N in cod juveniles and larvae may be higher, but cod individuals analyzed in this study were older than one year and must have experienced multiple spring and summer blooms based on both, diazotroph nitrogen and thermocline nitrate originating from diazotroph N, in their lifetime.

In summary, the adult cod from the central Baltic Sea that were analyzed in this study showed no significant imprint of diazotroph nitrogen in their tissues but rather of thermocline nitrate originating from diazotroph nitrogen. Future CSIA studies have to show, how much diazotroph nitrogen gets incorporated by cod larvae and juveniles.

The lowest and highest values $\delta^{15}N_{wms}$ occurring in both groups of cod suggested that some cod from the central Baltic Sea had spent time feeding near the coast and thus relied on a diet from the isotopically heavier nearshore food web with less N_2 fixation but more eutrophication impacts on N sources (Voss et al., 2005; Korth et al., 2014). This can be the case in the Pomeranian Bay and other nearshore areas along the southern Baltic coast as suggested by recent recaptures of cod (Hüssy et al., 2020). Similarly, some cod caught in the western Baltic may have been exposed to FNC-influenced isotopically lighter food web components which could be due to eastern Baltic cod foraging in areas normally inhabited by western Baltic cod (e.g. McQueen et al., 2019). In order to reduce uncertainty originating from a lack of individual stock assignment in an area affected by stock mixing, genetic analyses may be considered in the future and cod should preferentially be sampled further east of Bornholm. This could facilitate that mainly those cod will be analyzed that are part of the pelagic food web of the central Baltic Sea. A future simultaneous stomach content analysis of the sampled animals could also help to access the role of benthic and pelagic food in the diet of the sampled animals, which could influence the TP values.

Isotope mixing of thermocline nitrate with the isotopic imprint of either anthropogenic nitrogen or diazotroph nitrogen can account for the major trends in cod $\delta^{15}N$ source AA values, but not in flounder, which showed a wide range of N source proxy data for both areas. In flounder, transient processes like isotopic fractionation associated with the uptake of nitrate by phytoplankton must also be considered to understand the $\delta^{15}N_{wms}$ values. The wide range of flounder $\delta^{15}N_{wms}$ values can be explain by the interaction of nitrate uptake by phytoplankton and export production. Export production is the amount of organic material produced in the ocean by new production that is not recycled (re-mineralized) before sinking within a few hours to days (depending on water depth) from the euphotic zone to the bottom, where it becomes available to the benthic food web.

Isotope theory specific for nitrate-based phytoplankton blooms predicts that during new production in phytoplankton enzymatic processes can produce different patterns of $\delta^{15}N$ variation over time as the initial substrate pool of nitrate is consumed e.g. during spring bloom or when nitrate is injected into a system, for example by a river plume or upwelling (Montoya, 2007). If sufficient nitrate is still present at the beginning of a bloom, the lighter ^{14}N nitrate is preferentially taken up, resulting in a 5-10‰ lower $\delta^{15}N$ value in phytoplankton compared to the $\delta^{15}N$ in nitrate (Montoya and McCarthy, 1995; Waser et al., 1998). As the bloom progresses and the phytoplankton takes up ^{14}N-nitrate with priority, the $\delta^{15}N$ of the residual nitrate increases, which in turn leads to an increase in the $\delta^{15}N$ of the developing seston. If there is little sedimentation of seston during a short bloom as part of export production, or if little phytoplankton is grazed by herbivores such as copepods, the $\delta^{15}N$ of seston converges against the $\delta^{15}N$ of the initial nitrate pool available for growth. If significant losses occur due to sedimentation or grazing, the current ^{15}N signal from the phytoplankton will be found in the sinking seston and in the seston-filtering macrozoobenthos, such as bivalves, or in the mesozooplankton. If these loss processes are high, the $\delta^{15}N$ of seston in the euphotic zone can exceed and overshoot the initial $\delta^{15}N$ of nitrate. Thus, the isotopic perturbation associated with a bloom can propagate to both, the pelagic and benthic food webs, potentially producing both higher and lower isotopic signals in particles relative to the initial $\delta^{15}N$ in nitrate. Macrozoobenthos nitrogen and zooplankton nitrogen are converted more slowly than seston, so the magnitude of isotopic perturbation decreases with TP and the temporal pattern of $\delta^{15}N$ fluctuations may be offset between trophic levels. If either of these disturbance signals in the phytoplankton biomass persists long enough relative to the turnover times of the macrozoobenthos or zooplankton biomass, these nitrate fractionation signals will propagate through the food web to the bivalves and zooplankton as well as higher trophic levels like fish. The extent of this signal propagation, in turn, provides a qualitative measure of the progression of the fractionation process in the nitrate pool, reflecting the time course of the energy flow characterizing the benthic or pelagic communities.

Applying these concepts to our data set, we might expect to see both low and high $\delta^{15}N_{wms}$ readings in fish reflecting different stages of nitrate uptake at sites with large nitrate inputs. Indeed, we found very low $\delta^{15}N_{wms}$ values in flounder at sites with high nitrate availability in the western Baltic Sea, but not in cod (Fig. 25). Because $\delta^{15}N_{wms}$ values below the nitrate value of 7.9‰ may originate from thermocline nitrate originating from diazotroph biomass, by fractionation in the early stages of isotopically heavy nitrate consumption, or from recycling (Montoya et al., 1992; 2002), we cannot fully elucidate the origin of these low $\delta^{15}N_{wms}$ flounder signatures. That being said, the contextual clues of high nitrate concentrations and low $\delta^{15}N_{wms}$ in flounder without corresponding low $\delta^{15}N_{wms}$ in cod points to a transient process related to e uptake of isotopically heavy nitrate as the primary cause. This assumption is strengthened by the fact that also in the central Baltic Sea, where in contrast to the cod, very high $\delta^{15}N_{wms}$ values were found in the flounder in addition to the low $\delta^{15}N_{wms}$ values, which can be explained by isotope fractionation signals during nitrate uptake that has been transferred into flounder, but not into cod.

This assumption is supported by $\delta^{15}N$ values of total nitrogen in seston from sediment trap samples collected at 35 m depth in the Arkona Basin (Fig. 27).

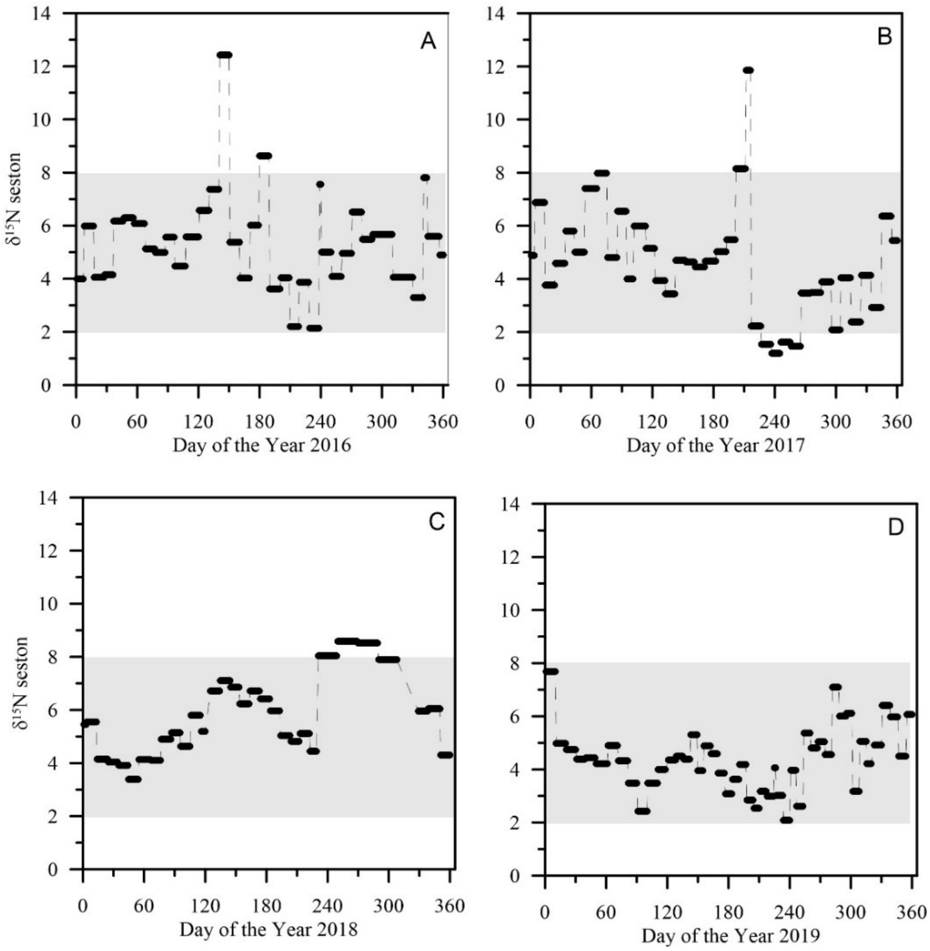


Figure 27: Isotopic signature of total nitrogen ($\delta^{15}N$ in ‰) in seston from a sediment trap at 35 m depth in the central Arkona Basin in 2016-2019 (Wasmund et al., 2017; 2018; 2019; Zettler et al., 2020). Shaded in grey is the $\delta^{15}N_{wms}$ value range between 2-8 ‰ found in flounder (see Fig. 25). From Steinkopf et al. (2024)

Specifically, the 2- to 4-year-old flounder caught in 2019 and 2020 and measured here have the same range of $\delta^{15}N_{wms}$ values as export production in the form of $\delta^{15}N$ of seston from the Arkona Basin in their years of growth from 2016-2019. Except for a few outliers in seston, $\delta^{15}N$ values in both, the seston and flounder varied between 2-8‰ (Figs. 25 and 27).

In summary, since flounder mainly feed on seston incorporating bivalves, the most likely explanation for the wide $\delta^{15}N_{wms}$ ranges in the flounder from both areas is that their $\delta^{15}N_{wms}$ values, just like the $\delta^{15}N$ of seston in the sediment trap samples, reflect isotopic fractionation in export production. Since the cod follows a completely different foraging strategy and feeds on considerably higher TPs than the flounder, it receives a smoothed N-source signal without perturbation from isotope fractionation. The δ^{15}Nwms values in cod are rather characterized by the isotopic mixing of thermocline nitrate, which is formed from anthropogenic nitrogen in the western Baltic Sea and from diazotroph nitrogen in the central Baltic Sea.

Cyanobacteria and trophic lengthening in the pelagic but not the benthic food web

Cod in the central Baltic Sea fed on organisms from a significantly higher TP than cod in the western Baltic. The difference corresponded to 0.6 TP units, if all cod individuals from the central Baltic Sea were included. This is a conservative estimation given that it also includes animals with $\delta^{15}N_{wms}$ values above the $\delta^{15}N$ endmember value of 3.6 ± 1.0‰ for thermocline nitrate and surface sediment particles from the central Baltic Sea (Korth et al. 2014) that likely are migrators to coastal waters. Excluding any potential migrators, the difference in TP between cod from the western and central Baltic Sea increases to 0.8 TP units.

Interestingly, other studies have also found increased TP values in the food web of the central Baltic Sea (Kiljunen et al., 2020) compared to TP values for the same species/compartment from the food web of the western Baltic Sea (Mittermayr et al., 2014, Tab. 4). The higher TP values in herring and sprat from the central Baltic Sea (Kiljunen et al., 2020) are particularly interesting here, as they are the missing link between zooplankton and cod. The higher TPs in cod from the central compared to the western Baltic Sea cannot be explained by differences in prey alone. We calculated the TP of cod from the western and central Baltic Sea based on bulk $\delta^{15}N$ values from literature and estimated their TP for different types of diet to compare them with the CSIA-based TPs of cod (Table 4, Fig. 28).

Table 4: Averages and ranges of published bulk $\delta^{15}N$ values and the calculated and published TP values of cod and their key dietary organisms from the pelagic and benthic food webs of the western and central Baltic Sea. Calculation of TP values after Post (2002) on the basis of published bulk $\delta^{15}N$ values for different assumed food preferences (pelagic, benthic, mixed = mix of both) and of CSIA-based TP values from this study. For the central Baltic Sea, additional theoretical TP values for cod with a pelagic food preference were calculated excluding the effect of trophic lengthening on the mesozooplankton compartment (TP of primary consumer of 2.0 rather than 2.7). Values from this study in bold. From Steinkopf et al. (2024).

Site	**Organisms**	**δ^{15}N bulk [‰]**	**δ^{15}N bulk [‰]**	**Number**	**Size class**	**Trophic Position**			**δ^{15}N Analysis**	**Reference**	**TP**
		Mean ± STDV	**Range**	**Individuals/ Hauls**	**cm**	**Pelagic**	**Benthic**	**Mixed**			**Theoretical** Pelagic
Western Baltic Sea	Copepods	6.7±0.9	5.6-7.7	8 Hauls	>0.01	2.0±0.2	n.a.	n.a.	CSIA	Loick-Wilde et al. 2019	n.a.
	Copepods	7.1 ± 1.3	6.3-8.1	1490	>0.015	2.0±0	n.a.	n.a.	SIA	Mittermayr et al. 2014	n.a.
	Mussels	8.5±0.2	8.1-8.7	182	n.d.	n.a.	2.0±0.3	n.a.	SIA	Mittermayr et al. 2014	n.a.
	Shore Crab	10.5 ± 1.4	9.1-11.9	4	n.d.	n.a.	3.3±0.3	n.a.	SIA	Mittermayr et al. 2014	n.a.
	Herring	12.3±1.9	8.0-16.5	20	8-20.5	3.6±0.4	n.a.	n.a.	*after Post 2002*	Mohm 2014, Mohm 2018	n.a.
	Sprat	11.9±0.8	10.6-13.0	10	6.5-13.5	3.5±0.2	n.a.	n.a.	*after Post 2002*	Mohm 2018	n.a.
	Flounder	11.9±0.8	10.9-13.2	9	20-32	n.a.	3.0±0.2	n.a.	*after Post 2002*	Mohm 2014, Mohm 2018	n.a.
	Flounder	11.7±1.1	9.27-13.2	11	20-30	n.a.	**3.4±0.3**	n.a.	CSIA	this study	n.a.
	Cod	14.6±0.2	14.2-14.8	3	16.5-25	4.3±0.0	3.8±0.0	4.0±0.0	*after Post 2002*	Mohm 2014, Mohm 2018	n.a.
	Cod	15.1±0.0	14.6-15.6	10	35-39	4.4±0.0	3.9±0.0	4.2±0.0	*after Post 2002*	Deutsch & Berth 2006	n.a.
	Cod	12.7±1.2	9.9-14.9	13	20-35	n.a.	n.a.	**4.1±0.5**	CSIA	this study	n.a.
Central Baltic Sea	Copepods	4.2±0.3	2.1-4.8	12 Hauls	>0.01	2.7±0.2	n.a.	n.a.	CSIA	Loick-Wilde et al. 2019	2.0
	Copepods	7.1±1.4	5.7-8.5	5 Hauls	>0.015	2.7±0.3	n.a.	n.a.	SIA	Kiljunen et al. 2020	n.a.
	Mussels	8.6±1.9	6.7-10.5	19	n.d.	n.a.	2.0±0.5	n.a.	SIA	Kiljunen et al. 2020	n.a.
	Saduria	10.1±1.4	8.7-11.5	4	n.d.	n.a.	3.0±0.4	n.a.	SIA	Kiljunen et al. 2020	n.a.
	Herring	12.3±1.4	10.9-13.7	45	17.9±4.1	4.0±0.5	n.a.	n.a.	SIA	Kiljunen et al. 2020	n.a.
	Sprat	11.8±1.2	10.6-13.0	21	11.8±1.4	4.3±0.4	n.a.	n.a.	SIA	Kiljunen et al. 2020	n.a.
	Flounder	12.8±1.5	11.6-16.0	8	22-30	n.a.	3.2±0.4	n.a.	*after Post 2002*	Mohm 2014, Mohm 2018	n.a.
	Flounder	11.4±1.4	9.1-13.7	11	20-30	n.a.	**3.1±0.3**	n.a.	CSIA	this study	n.a.
	Cod	13.1±0.6	11.6-13.9	98	23-40	4.9±0.1	4.0±0.1	4.5±0.1	*after Post 2002*	Mohm 2018	3.9±0.1
	Cod	12.0±0.4	11.1-12.6	10	34-39	4.6±0.1	3.7±0.1	4.1±0.1	*after Post 2002*	Deutsch & Berth 2006	4.2±0.1
	Cod	12.4±0.8	11.2-14.4	17	20-38	n.a.	n.a.	**4.6±0.4**	CSIA	this study	n.a.

Although cod from the central Baltic Sea feed in some parts on benthic food such as *Saduria entomon* (Kulatska et al., 2019), purely benthic or mixed benthic and pelagic diet of cod from the central Baltic Sea would lead to similar low TPs as in cod from the western Baltic Sea (Fig. 8). Further, a purely pelagic diet of western cod would not lead to elevated TPs as we found for cod from the central Baltic Sea (Fig 28). Ruling out any trophic lengthening effect by assuming a TP of 2.0 rather than of 2.7 for zooplankton (called theoretical diet type in Fig. 28) would also lower the TP of cod in the central Baltic Sea down to western Baltic Sea TP levels. Fig. 28 reveals that only the SIA-based TP calculation for cod including both, a TP of 2.7 for zooplankton and a high proportion of pelagic herring and sprat in the diet, reaches the high TP values in cod as estimated from the CSIA-based TP approach. This shows that trophic lengthening has an important influence on cod from the central Baltic Sea. Yet, without a small share of benthic diet and/or times of growth without trophic lengthening in the pelagic food web (e.g. during the spring bloom of diatoms), cod from the central Baltic Sea would probably have even higher TP values than those found in this study.

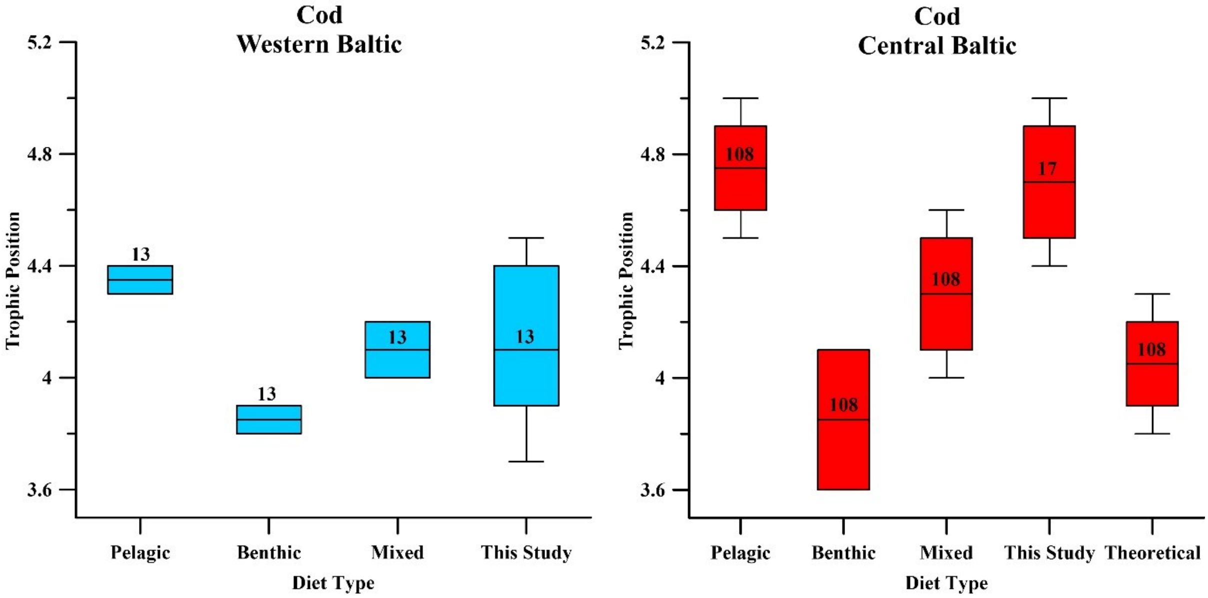


Figure 28: Boxplot of the SIA-based TP values of cod from the western and central Baltic Sea calculated after Post (2002) from literature data for different assumed food preferences (pelagic, benthic, mixed = mix of both) and of CSIA-based TP values (Table 4). For the central Baltic Sea additional theoretical TP values for cod with a pelagic food preference were calculated excluding the effect of trophic lengthening on the mesozooplankton compartment (TP of primary consumer of 2.0 rather than 2.7, Table 2). The high TPs in cod from the central Baltic Sea from this study only can be explained by a preferential pelagic diet including the effect of trophic lengthening. See text for more details. From Steinkopf et al. (2024)

Higher TPs of cod from the central Baltic Sea than of cod in the western Baltic robustly demonstrate the long-lasting effect of a trophically longer food web affected by an FNCs induced influence of the microbial system. Given the strong correlation of TP with the N source measure $\delta^{15}N_{wms}$ in cod (Fig. 25), the underlying mechanism must be related to the dominance of FNCs, which are largely unpalatable and thus not subject to direct grazing (Wannicke et al., 2013; Motwani et al., 2018; Loick-Wilde et al., 2012). Instead, a functionally more complex food base of protists, flagellates, ciliates, bacteria, viruses, and other microorganisms gains a more important influence with the unpalatable cyanobacteria (Sheridan et al., 2002; Eigemann and Schulz-Vogt, 2019). However, this also leads to a feedback loop in late summer, as the microbial system mediates the nutrients of the now dying and sinking cyanobacteria back into the system, which can support new phytoplankton growth. Also some of these organisms, namely microzooplankton like flagellates and ciliates, form now an additional trophic level in the pelagic food web when they serve as a dietary source for otherwise mainly herbivorous or maximally omnivorous mesozooplankton, which thus becomes carnivorous (Loick-Wilde et al., 2019). In the central Baltic Sea, this is especially true for *Temora longicornis*, a copepod species that predominantly feeds on flagellates and ciliates during cyanobacterial blooms and reaches a TP of 3.0 (Eglite et al., 2018; Loick-Wilde et al., 2019; Dutz et al., 2012). The food chain is lengthened accordingly and the effect is transferred to higher trophic levels. Interestingly, sprat and herring, which are widespread in the Baltic Sea and which in case of sprat mainly feed on *T. longicornis* (Möllmann et al., 2004; Bernreuther et al., 2018), also have higher TPs in the central Baltic Sea (Kiljunen et al., 2020) compared to the western Baltic Sea (Tab. 4). Ultimately, sprat and herring is eaten by cod in the central/eastern Baltic Sea, which increases the TP of cod from ~4.0 to near 5.0 as shown in this study (Fig. 29).

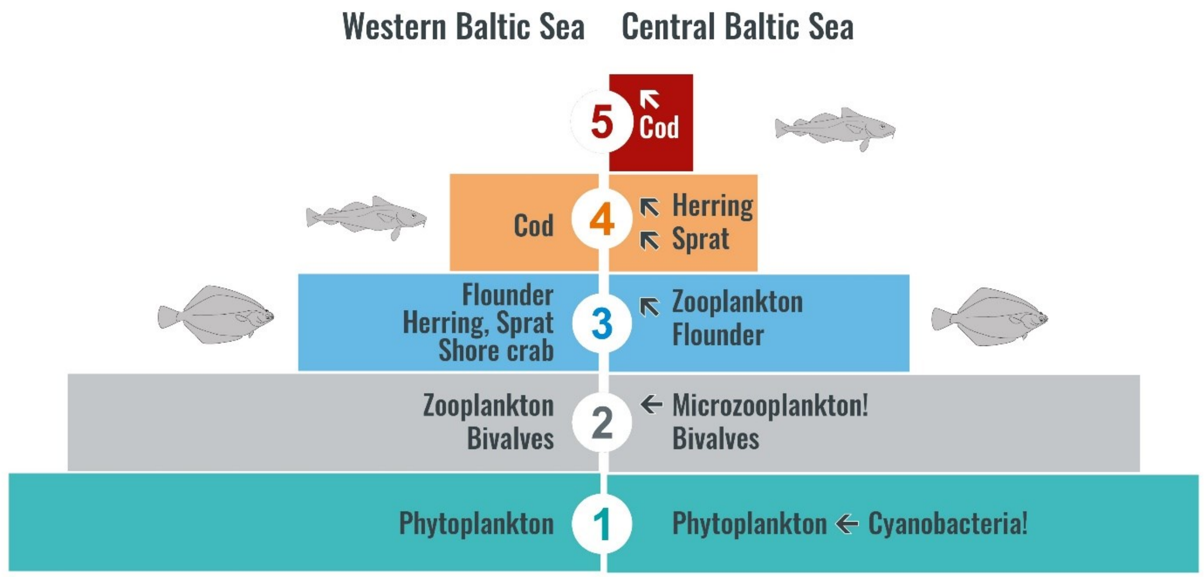


Figure 29: Trophic pyramid including the trophic positions (TPs) of key organisms of the pelagic and benthic food webs in the western and central Baltic Sea. Only the pelagic food web in the central Baltic is affected by trophic lengthening due to the growth of filamentous, N_2-fixing cyanobacteria and associated microzooplankton (←!), which together cause increased TPs (↖) up to the level of cod. The benthic food web including flounder is not affected by trophic lengthening in neither area. From Steinkopf et al. (2024)

This clearly shows how trophic lengthening at the base of the pelagic food web can change the TP of its higher secondary consumers. The cyanobacteria are the trigger of this effect, but not the general effector. The general effector is the microbial system including microzooplankton, which can modulate (cause or negate) a trophic lengthening through increasing or decreasing importance within flows and feedback loops in the food web. The microbial structure exists in every aquatic system and changes in the trophic flow through it (e.g. caused by FNCs) can cause trophic lengthening. In the central Baltic Sea, the trigger for massive microbial systems are FNCs. However, massive microbial systems can also be caused by other factors such as eutrophication (Dähnke et al., 2008) or oxygen minimum zones (Fernández-Urruzola et al., 2023).

In flounder, there were no significant differences or trends in the mean TPs between the two areas and the scatter of the individual TPs of flounder was lower than in cod. This suggests that flounder has a narrower diet composition and is decoupled from trophic lengthening in the pelagic food web. Mesozooplankton is the central pivot in pelagic food webs which enables the transfer of mass and energy from the phytoplankton to the higher trophic levels including cod. For flounder, mesozooplankton is of minor importance because flounder almost exclusively feed on bivalves (Haase et al., 2020). Bivalves closely reflect the δ^{15}N signal in seston from exported new production and transfer it into the $\delta^{15}N_{wms}$ of flounder. This highlights a major difference in the functioning of the benthic compared to the pelagic food web of the central Baltic Sea: Without the transfer of mass and energy via mesozooplankton, flounder is unaffected by the trophic lengthening that disrupts the energy transfer in the pelagic food web (Fig. 30) and leads to a higher TP in cod (Fig. 29).

FNC-caused disruption of the pelagic but not the benthic food webs

Trophic lengthening in a food web inevitably leads to losses of thermal and chemical energy at higher trophic levels. Following the second law of thermodynamics, a pyramid of energy can never be inverted since it accounts for the turnover rate of the organisms with energy loss as heat and waste products in all metabolic processes involved (Reichle, 2023). However, in contrast to heat and waste product losses, the associated increases in TP can now be measured directly in organisms using CSIA.

Given a trophic efficiency of about 10%, trophic lengthening results in a massive decrease in biomass production, meaning that instead of the previous 0.1%, only 0.01% of the energy and mass from new production reaches the cod stock in the central Baltic Sea (Fig. 30).

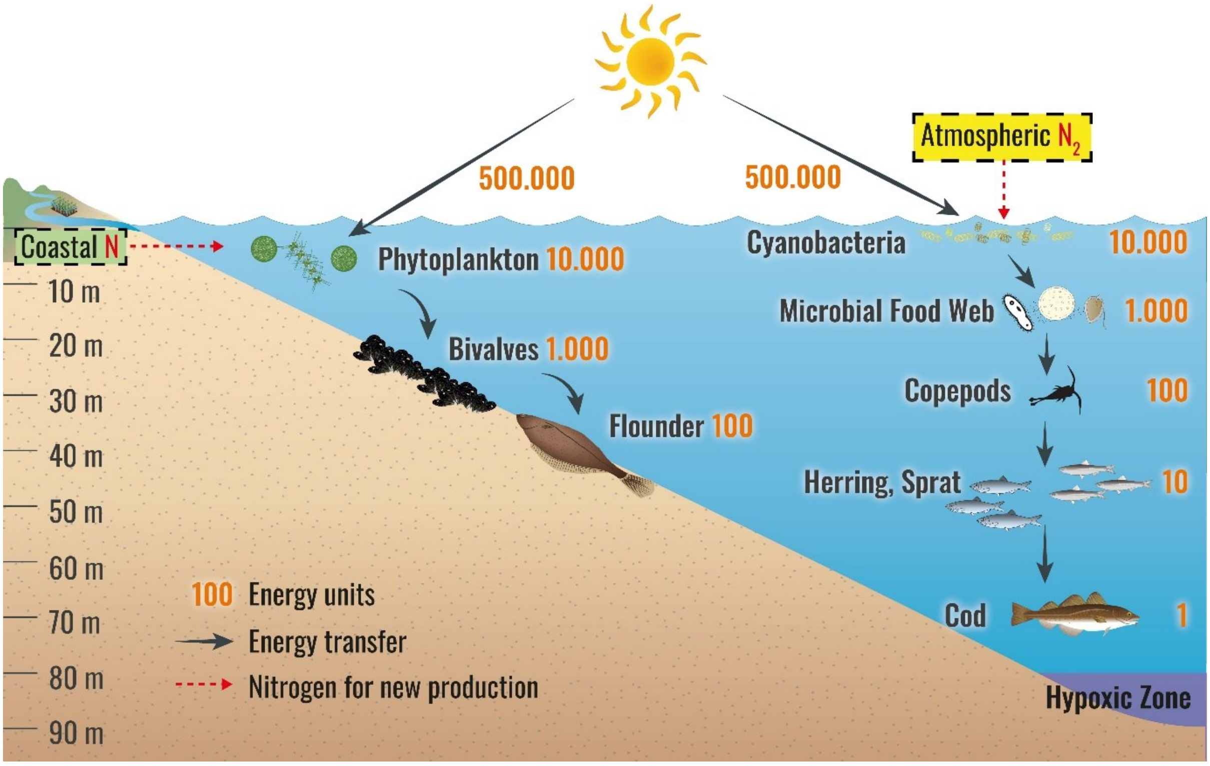


Figure 30: Conceptual view of the energy transfer to benthic and pelagic food webs in the central Baltic Sea including the process of trophic lengthening in the pelagic food web due to an FNC triggered massive microbial food web. Efficiency of energy to phytoplankton due to low conversion efficiencies in photosystems (Melis, 2009). See text for more details. From Steinkopf et al. (2024)

In other words, assuming an input of new nitrogen via N_2 fixation of 370,000 – 926,000 kt N y^{-1} in the Baltic Sea (Voss et al., 2005), only 37.0-92.6 kt N y^{-1} instead of 370-926 kt N y^{-1} of nitrogen of the annual new production would reach the end of the food web and be incorporated in cod and other predators at a TP of 5.0. FNC-triggered an enormous increase in production at times and the production of cyanobacteria is closely coupled to the rest of the food web. However, because there is almost no direct grazing of FNCs, the mass and energy is transported mainly indirectly to zooplankton via the microbial food web including microzooplankton with inevitable large losses of mass and energy as heat and waste products.

In contrast to cod, the TP flounder was not affected, thus the position of flounder in a trophic pyramid (Fig. 29) and the energy transfer (Fig. 30) is not changed by FNC-caused trophic lengthening. Therefore, the missing or massive effect of trophic lengthening on the benthic or pelagic food web, respectively, could explain the stable spawning stock biomass (SSB) of flounder (Bergenius-Nord et al., 2023) and the historic low SSB of cod, respectively, in the central Baltic Sea (Eero et al., 2023).

Past and future impact of trophic lengthening on eastern Baltic cod

FNC-triggered trophic lengthening does not seem to be a phenomenon of the 21st century. FNC blooms are closely linked to eutrophic conditions, namely the supply of phosphorus, which limits N_2 fixation in the Baltic Sea (Voss et al., 2011; Conley, 2012). Anthropogenic eutrophication is not a recent stressor in the Baltic Sea (Andersen et al., 2017; Murray et al., 2019). Analyses of sediment cores revealed that massive blooms of *Nodularia spumigena* and *Aphanizomenon flos-aquae* already occurred at the beginning of the 20th century in the central Baltic Sea (Kaiser et al., 2020). A potential lengthening of the food web due to unpalatable primary producers and accompanied detrimental effects for cod production in this environment may therefore have played a role even before the historic SSB peak of the eastern Baltic cod (EBC) stock in the early 1980s (Eero et al., 2023) since the SSB of EBC also showed remarkable lows in the 1960/70s. Interestingly, the SSB peak of the EBC in the early 1980s coincided with lower sea surface temperatures and a lower frequency of FNC accumulations

(Kahru et al., 2020; Kaiser et al., 2020). The SSB decline since the 1990s was accompanied by a rapid increase in the bloom frequency of FNCs and a rise in sea surface temperatures. Moreover, the historical decline in EBC SSB between the 1980s and 1990s matches the period when an increase of the EBC TP by 0.4-0.7 units took place and which stayed high in EBC ever since (Karlson et al., 2024). During this period a major biological regime shift took place in the central Baltic Sea (Möllmann et al., 2008; 2009; Alheit et al., 2005; 2009). One so far unconsidered and unaccounted underlying mechanism for this change could be related to the FNC blooms, which lead to a trophic lengthening of the food chain and a change in the energy transfer (Fig. 29). This is supported by biomass data of copepods, which show that *Pseudocalanus acuspes* was gradually replaced by e.g. *Temora longicornis*, a copepod that has been shown to feed at higher TPs, i.e. it preys upon ciliates and flagellates (Dutz et al., 2010; 2012). CSIA-based TP estimates of central Baltic herring and Baltic sprat are missing but the high SIA-based TP estimates (Kiljunen et al., 2020; Table 4) and much lower level in mean weight-at-age of herring and sprat since the 1990s (Bergenius-Nord et al., 2023) strongly suggest that these small, zooplanktivouros pelagics were also affected by trophic lengthening. This lengthening of the pelagic food web from the mid 1980s was apparently strong enough to eventually reach the EBC stock and negatively affect stock productivity.

3.4 Comparison of three different brackish and estuarine ecosystems

A comparison of the three systems Oder, Elbe and Baltic Sea is generally quite difficult, as they differ quite strongly in their morphology (size, depth, current, stratification), their abiotic properties (salinity, nutrients, pH, temperature, turbidity). Nevertheless, all three are estuarine systems that are exposed to similar stressors (Bartoli et al., 2021; Kennish, 2002; Kennish et al., 2014; Kennish et al., 2023) In this dissertation, I was able to show that the most serious stressors affecting all three systems are, above all, excessive nutrient input and the associated eutrophication, as well as increased temperatures over a longer period of time and extreme events and the associated changes in stratification and oxygen distribution. This is also shown by the results of the PCA, which shows a clear influence of temperature on the oxygen content in the Elbe estuary. There are already large oxygen minimum zones in the Baltic Sea (Carstensen et al., 2019; Conley et al., 2009; Conley et al., 2011)) and seasonal hypoxia in some areas of the Elbe, including in the nursery grounds of smelt (Koll et al., 2024; Pein et al., 2021). The Oder does not yet have to contend with hypoxia or anoxia at times, but this is due to the good mixing and the shallow water depth, as the potential for anoxia is certainly there

(Schernewski et al., 2001; Kache et al., sub.). If there are longer windless phases with high temperatures, this danger also exists in the Oder estuary. Eutrophication continues to be another major stressor in all three systems. However, there is actually a big difference between the three. In the Elbe, the high concentration of nutrients that enter the estuary with the river promotes the growth of phytoplankton in the area in front of the harbour (Dähnke et al., 2021). These in turn die off when the turbidity and water depth in the harbour increase (Kamjunke et al., 2023). The Oder estuary, on the other hand, is highly eutrophic and here too the nutrients promote large blooms of phytoplankton, which can assimilate the nitrate very effectively and thus add it to the nitrogen cycle of the estuary (Hellemann et al., 2017). In this way, some nutrients can be bound in the estuary (Bartoli et al., 2021). The system thus remains quite stable. However, a large proportion of nutrients continue to enter the Baltic Sea. It has also had eutrophic status for a long time (Murray et al., 2019; Stigebrandt et al., 2020). Here, however, the nitrate pool is depleted relatively quickly, which leads to an advantage of N-fixing cyanobacteria. This results in massive blooms (Elmgren et al., 2015; Kahru et al., 2020). However, there are many other stressors that affect the systems, as I have already shown. However, these vary in intensity. For example, turbidity is a huge problem in the Elbe, whereas in the Baltic Sea it is limited to localised events that do not have an effect over very long periods. These events are either caused by human activities (nourishments, dredging, etc.) or by extreme events such as storms (Glück, 2023). There is a high level of organic turbidity in the Oder, particularly during the spring bloom (Voss et al., 2010), but this does not have the same negative impact as in the Elbe. To summarise, it can be said that all three systems react differently to similar stressors or are exposed to different stressors. This in turn means that different approaches have to be taken in order to turn the adjusting setscrews that minimise eutrophication or reduce turbidity in the areas, for example. In general, however, it can be said that all three systems are strongly influenced by humans and that further measures are definitely needed to bring the systems into a stable state or maintain them, especially in view of climate change and the associated higher temperatures and increased frequency of extreme events. To this end, it is probably necessary to further develop projects such as the BluEs project and, through these projects, to bring together leading person from industry, business, politics, administration, tourism and nature conservation to discuss these setscrews and associated possible measures.

4. Conclusion

4.1 Trophic lengthening in estuarine ecosystems

The three estuarine ecosystems studied in this dissertation provided a good overview of how the same stressors affect different systems with different characteristics in different ways. The focus was on the change in the length of a food web, the so-called trophic lengthening. The change in nutrient levels and in particular the nitrogen species at the base of the respective food web play a decisive role when it comes to changes in food web length. It turns out that when trophic lengthening could be detected in a food web, it was always associated with a change in the dominant nitrogen species, either from nitrate to ammonium or from nitrate to N_2 as already proclaimed in literature (Glibert et al., 2016). This change in the N-source in turn triggered a change in the importance of the microbial system, which then became increasingly important for the uptake and transfer of nitrogen to the other levels of the food web (Steinkopf et al., 2024). This greater importance can usually also be due to the fact that the primary producers are restricted in their role (e.g. light limitation) (Martens et al., 2024) or that the dominant primary producer cannot be consumed directly by the zooplankton because they are either too big, e.g. in large FNCs (Loick-Wilde et al., 2019; Steinkopf et al., 2024), or too small (Queiros et al., 2024). This inevitably leads to a redistribution of the transfer ways of energy that no longer distribute directly into the zooplankton, but takes detours via the complex microbial system. Heterotrophic processes such as osmotrophy and phagotrophy in protists can also play a role here, as these processes also lead to a redistribution of energy pathways (Sommer et al., 2002; Glibert et al. 2016). However, all pathways have one thing in common: they increase the general number of levels in the food web (Post, 2002). This ultimately means that considerably less energy reaches the upper levels of the food web (Ryther, 1969; Pauly and Christensen, 1996; Stock et al., 2016). For some populations of mesopredators and apex predators, this can mean that they no longer have enough energy available to maintain their respective populations at a stable level, as has already been shown for cod, smelt and terns. However, this dissertation also showed that although a change in the nitrogen species can be an initial symptom of trophic lengthening, only the combination of this with the change in the importance of the microbial structure can really trigger trophic lengthening. This is important to mention because temporary changes in nitrogen species can occur more frequently in a system without having a long-term impact on the food web. Other simultaneous stressors also play a role here, such as the persistent high turbidity in the Elbe, which is a disadvantage for photoautotrophy (Dähnke et al., 2021; Kamjunke et al., 2023). This knowledge can be used to prevent trophic lengthening before the serious symptoms, such as the loss of mesopredators, even become apparent. If appropriate measures are taken like reduction of nutrient intake via agriculture, renaturation of natural rivers sides or oxbows or reduction of turbidity, even systems such as estuaries that have been seriously affected by anthropogenic stress can recover (Glibert et al., 2022). Therefore, the compound-specific analysis of nitrogen must be further expanded in order to gain even more precise and faster insights into the state of food webs worldwide. It is also necessary to further investigate the TDF in the TP equations, as recent studies have shown that this value is by no means the same for all organisms, but that species and ecosystem specific TDF factors are needed (Bradley et al., 2016; McMahon et al., 2016; Brault et al., 2019; Stephens et al., 2023). Thus the TP calculations can give an even better picture of the structure of food webs. Furthermore, it must be clarified to what extent trophic lengthening has now become a global phenomenon, which is becoming even more important due to climate change and other human influences, as collapses in the production of fish stocks worldwide could be linked to a change in the food web structure of these regions, caused for example by El Niño or a lack of upwelling (Fernandez-Urrozola et al., 2023). Or whether there are other general factors that can trigger trophic lengthening, such as marine heat waves (Gomes et al., 2024) or parasites (Moore et al., 2024). If the here suggested general mechanism that trophic lengthening is caused by changes in the nitrogen source in combination with an increasing influence of the microbial structure can also be demonstrated in other marine and aquatic systems, we will be one step closer to understanding functional diversity and loss of biodiversity.

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Acknowledgements

First and foremost, I would like to thank Dr. Natalie Loick-Wilde for supervising my dissertation over the years and for the advice, suggestions and kind words that have always brought me back on track should I have lost it. I would also like to thank Prof. Dr. Maren Voss for the official supervision of my thesis and for making my PhD studies possible.

I would also like to thank Dr. Uwe Krumme, Ana Fernandez-Carrera, Dirk Wodarg and Iris Liskow, who always supported me with advice and assistance, both in my work in the laboratory, on the measuring equipment and in writing the thesis.

I would also like to thank Sophie Kache, without whom some of the samplings would not have been possible. As well as Robert Mars, Kristian Rose, Frank Pohl, Holger Posselt, Andreas Frahm and Uwe Hehl, who made my participation in the MARNET cruises and the BluEs samplings possible, ensured that the sampling went smoothly and always improved the atmosphere on board when they were there.

I would also like to thank all the project partners of the BluEs project for the good cooperation, the great discussions and the opportunity to be part of the project.

I would also like to thank Prof. Dr. Matthias Gehre and Dr. Steffen Kümmel for the opportunity to spend a few weeks at the UFZ to measure my last important samples. Just like Dr. Katharina Kesy for the opportunity to use the laboratories at the University of Greifswald and the good company.

I would like to thank my girlfriend Katharina Taube, who always stood by my side, even when things weren't going so well and when I wasn't making progress. I would also like to thank my family for their support in all situations over the last few years and especially my brother Tim Steinkopf, who has been a great help to me on many occasions with his artistic skills. Last but not least, I would like to thank all my friends who have accompanied me along the way, as well as the staff of the IOW and Thünen Institute and the crews of the research vessels Elisabeth Mann Borgese, Solea, Clupea and Ludwig Prandtl for their support during sampling and general support.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe. Dazu habe ich keine außer den von mir angegebenen Hilfsmitteln und Quellen verwendet und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen habe ich als solche kenntlich gemacht.

Rostock, 12.09.2024

(Markus Steinkopf)

Supplemental Material

Table S1: List of the Samples, sampling partners how help with the sampling, the sampling year and the resulting variables for the three estuaries Baltic Sea, Oder and Elbe.

Region	Year	Sampling Partner	Sample Type	Variables
Baltic Sea	2019	Thünen Institute for Baltic Sea Fisheries	Cod	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
			Flounder	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
	2020	Thünen Institute for Baltic Sea Fisheries	Cod	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
			Flounder	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
Oder Estuary	2021	Leibniz-Institute for Baltic Sea Research	Zooplankton	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
			POM	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
			Fischlarvae	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
			Benthos	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
	2021	University of Hamburg	Flounder	Bulk δ^{15}N
				Bulk δ^{13}C
			Zander	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
	2021	HAW Hamburg	Benthos	Bulk δ^{15}N
				Bulk δ^{13}C
				δ^{15}N AA
	2021	Verein Jordsand	Eiderduck	Bulk δ^{15}N
				Bulk δ^{13}C
			Cormorant	Bulk δ^{15}N

				Bulk $\delta^{13}C$
Elbe Estuary	2021	Hereon centre Geesthacht	POM	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA
			Zooplankton	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA
	2021	University of Hamburg	Smelt	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA
			Zander	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA
			Withing	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA
	2021	Verein Jordsand	Tern	Bulk $\delta^{15}N$
				Bulk $\delta^{13}C$
				$\delta^{15}N$ AA

Table S2: Individual fish sampled for this study. By species, station, depth stratum, sex (f = female, m = male, n.d. = no data), size (cm), ICES-Subdivision (SD), areas in the Baltic Sea (BS = Belt Sea, AB = Arkona Basin, BB = Bornholm Basin), cruise (SB = FRV “Solea”, C = FRV “Clupea”) and month/year. From Steinkopf et al. (2024)

Species	**Station**	**Depth [m]**	**Sex**	**Size [cm]**	**ICES-SD**	**Area**	**Cruise**	**Month/Year**
Cod	1	20-30	f	35	22	BS	SB774	Feb 20
Cod	2	20-30	f	20	22	BS	SB774	Feb 20
Cod	4	10-20	m	33	22	BS	SB774	Feb 20
Cod	4	10-20	f	29	22	BS	SB774	Feb 20
Cod	4	10-20	m	34	22	BS	SB774	Feb 20
Cod	4	10-20	m	35	22	BS	SB774	Feb 20
Cod	4	10-20	f	34	22	BS	SB774	Feb 20
Cod	4	10-20	f	32	22	BS	SB774	Feb 20
Cod	5	20-30	m	20-30	22	BS	C341	Jan 20
Cod	6	20-30	m	20-30	22	BS	C341	Jan 20
Cod	8	10-20	f	20-30	22	BS	C341	Jan 20
Cod	8	10-20	f	20-30	22	BS	C341	Jan 20
Cod	8	10-20	m	20-30	22	BS	C341	Jan 20

Cod	10	10-20	n.d.	38	24	AB	SB759	Feb 19
Cod	11	50-60	n.d.	23	24	AB	SB759	Feb 19
Cod	11	50-60	n.d.	32	24	AB	SB759	Feb 19
Cod	11	50-60	n.d.	34	24	AB	SB759	Feb 19
Cod	11	50-60	n.d.	36	24	AB	SB759	Feb 19
Cod	11	50-60	n.d.	36	24	AB	SB759	Feb 19
Cod	12	60-70	f	36	25	BB	SB773	Feb 20
Cod	13	60-70	n.d.	29	25	BB	SB759	Feb 19
Cod	13	60-70	n.d.	29	25	BB	SB759	Feb 19
Cod	13	60-70	n.d.	35	25	BB	SB759	Feb 19
Cod	13	60-70	n.d.	36	25	BB	SB759	Feb 19
Cod	13	60-70	n.d.	32	25	BB	SB759	Feb 19
Cod	14	60-70	m	20-30	25	BB	SB773	Feb 20
Cod	14	60-70	f	20-30	25	BB	SB773	Feb 20
Cod	14	60-70	m	20-30	25	BB	SB773	Feb 20
Cod	15	60-70	f	20-30	25	BB	SB773	Feb 20
Cod	15	60-70	m	20-30	25	BB	SB773	Feb 20
Flounder	3	20-30	f	20-30	22	BS	SB774	Feb 20
Flounder	4	20-30	f	20-30	22	BS	SB774	Feb 20
Flounder	7	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	7	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	9	20-30	n.d.	20-30	22	BS	C341	Jan 20
Flounder	13	60-70	n.d.	20-30	24	AB	SB759	Feb 19
Flounder	13	60-70	n.d.	20-30	24	AB	SB759	Feb 19
Flounder	13	60-70	n.d.	20-30	24	AB	SB759	Feb 19
Flounder	13	60-70	n.d.	20-30	24	AB	SB759	Feb 19
Flounder	13	60-70	n.d.	20-30	24	AB	SB759	Feb 19
Flounder	14	20-30	m	20-30	25	BB	SB773	Feb 20
Flounder	14	20-30	f	20-30	25	BB	SB773	Feb 20
Flounder	14	20-30	m	20-30	25	BB	SB773	Feb 20
Flounder	14	20-30	m	20-30	25	BB	SB773	Feb 20
Flounder	14	20-30	f	20-30	25	BB	SB773	Feb 20
Flounder	14	20-30	n.d.	20-30	25	BB	SB773	Feb 20